"COMPARISON OF SONOGRAPHICALLY DETECTED FETAL ABNORMALITIES WITH KARYOTYPING BY AMNIOCENTESIS: CROSS SECTIONAL STUDY "

Dissertation submitted to

THE TAMIL NADU DR.M.G.R.MEDICAL UNIVERISTY

CHENNAI

In Partial Fulfillment of the Regulations

for the Award of the degree

M.D. DEGREE EXAMNATION

BRANCH VIII RADIODIAGNOSIS

MADRAS MEDICAL COLLEGE

&

RAJIV GANDHI GOVERNMENT GENERAL HOSPITAL,

CHENNAI -600 003.

THE TAMIL NADU DR.M.G.R.MEDICAL UNIVERISTY

CHENNAI -600 003. TAMIL NADU

MAY-2020

BONAFIDE CERTIFICATE

Certified that this dissertation is the bonafide work of DR.R.HARINI on **"COMPARISON OF SONOGRAPHICALLY DETECTED FETAL ABNORMALITIES WITH KARYOTYPING BY AMNIOCENTESIS: CROSS SECTIONAL STUDY"** during her MD(RADIODIAGNOSIS) in Barnard Institute of Radiology in the academic year of 2017-2020 at the Madras Medical College and Rajiv Gandhi Government General Hospital,Chennai-600003.

PROF.DR.K.MALATHY MDRD,PhD,
Head of the department,
(Guide for the study)
Barnard Institute of Radiology,
Madras Medical College &
Rajiv Gandhi Government General Hospital,
Chennai – 600 003.

PROF.DR.RAVI MD, DMRD
Director,
Barnard Institute of Radiology,
Madras Medical College &
Rajiv Gandhi Government General Hospital,
Chennai – 600 003.

Prof. Dr. R.JAYANTHI., M.D., F.R.C.P (Glasg)
DEAN
Madras Medical College &
Rajiv Gandhi Government General Hospital,
Chennai – 600 003.

DECLARATION

I, certainly declare that this dissertation titled **"COMPARISON OF SONOGRAPHICALLY DETECTED FETAL ABNORMALITIES WITH KARYOTYPING BY AMNIOCENTESIS: CROSS SECTIONAL STUDY"** represent a genuine work of mine. The contribution of any supervisors to the research are consistant with normal supervisory practice, and are acknowledged. I, also affirm that this bonafide work or part of this work was not submitted by me or any others for any award, degree or diploma to any other university board, neither in India or abroad. This is submitted to The Tamil Nadu Dr.MGR Medical University, Chennai in partial fulfilment of the rules and regulation for the award of Master of Radiodiagnosis Branch VIII.

DR.R.HARINI

Date:

Place: Chennai

ACKNOWLEDGEMENT

I would like to express my deep sense of gratitude to Dean, Madras Medical College & Rajiv Gandhi Government General Hospital Chennai, for giving me permission to conduct the study in this institution.

With extreme gratefulness, I express my indebtedness to **Prof.Dr.Ravi**, **MD.,DMRD,** Director, Barnard Institute of radiology, MMC & RGGGH, for allowing me to undertake this study on **"COMPARISON OF SONOGRAPHICALLY DETECTED FETAL ABNORMALITIES WITH KARYOTYPING BY AMNIOCENTESIS: CROSS SECTIONAL STUDY"**

I would like to express my deep gratitude and respect to the head of the department and my guide **Prof. Dr. K. Malathy, MDRD, PhD (Radiodiagnosis)** whose advice and insight was invaluable to me. This work would not have been possible without his guidance, support and encouragement.

I was able to carry out my study to my fullest satisfaction, thanks to guidance, encouragement, motivation and constant supervision extended to me, by my beloved **Prof Dr.Kalpana, MD., DMRD**. My sincere thanks for her practical comments and guidance especially at the inception of the study and for her valuable support through out the study.

I am extremely thankful to my former Associate Professor **Dr.GangaDevi MD.,DMRD.,FRCR** for her invaluable contribution, personal attention, constructive cricticism she has made during the beginning of my study.

I am bound by ties of gratitude to my respected professors, Associate Professors, and Assistant professors **Dr.G.Geetha MD , Dr.S.Iyengaran, Dr.Mohideen Ashraf MD, Dr.Saranya DMRD , Dr.Balan DMRD,DR.Dheeba MD,Dr.Karthick MD** in general, for placing and guiding me on the right track from the very beginning of my career in Radiodiagnosis till this day.

I am fortunate to have my postgraduate colleagues for their valuable suggestions, relentless help for shouldering my responsibilities. Simply words cannot express its depth for their unseen contributions. My lovable thanks to my family for their moral support.

I would be failing in my duty if I don't place on record my sincere thanks to those patients who inspite of their sufferings extended their fullest co-operation.

DR.R.HARINI

INDEX

SL. NO	CONTENTS	PAGE
1	INTRODUCTION	1
2	MAJOR FETAL ABNORMALITIES AND SOFT MARKERS OF ANEUPLOIDY	2
3	IMPORTANCE OF ABSENCE OF THE MARKER	20
4	IMPORTANCE OF PRESENCE OF THE MARKER	21
5	ISOLATED VS MULTIPLE MARKERS	23
6	SOFT MARKERS AND ANEUPLOIDY RISK ASSESSMENT	27
7	AMNIOCENTESIS	30
8	KARYOTYPING	34
9	TRIPLE OR QUAD SCREENING FOR BIRTH DEFECTS	45
10	AIM AND OBJECTIVES	48
11	MATERIALS AND METHODS	49
12	REPRESENTATIVE CASES	53
13	STATISTICAL ANALYSIS	61
14	CONCLUSION	72
15	MERITS	73
16	LIMITATIONS	73
17	SUMMARY	74
18	BIBLOGRAPHY	
19	PROFORMA	
20	ABBREVIATIONS	
21	MASTER CHART	
22	ETHICAL COMMITTEE CERTIFICATE	
23	PLAGIARISM CERTIFICATE	

Introduction

INTRODUCTION

The prevalence of Chromosomal abnormalities is very low occurring in 0.1% to 0.2% of live births.[1,2]. "Down's syndrome" (Trisomy 21) is the most common karyotypic abnormality in live-born infants (1 per 800 live births)[3] .The leading cause of mental retardation is Down's syndrome. [4–6]Structural defects and nonstructural abnormalities or "markers" are seen as sonographic findings in fetus with Trisomy 21 . Trisomy 13, trisomy 18, monosomy X, and triploidy are other sonographically detectable aneuploidies.

Maternal age,[2] biochemical markers,[7] amniocentesis [8,9] and prenatal ultrasound are the methods that have been used to identify women at risk of carrying a fetus with trisomy21. Amniocentesis and other invasive tests determine karyotype of the fetus. Fetal mortality rate associated with these invasive procedures is is 0.5% -1.0%.[8,9]

At 18 to 22 weeks, a second-trimester sonogram is routinely done. Sonographic markers of 2 types ,suggestive of aneuploidy are observed in the second trimester . Major structural abnormalities of the fetus comprise the first type of abnormality (Table 1) and many other fetal defects with less significance as possible markers of aneuploidy, which are called soft markers of aneuploidy (Table 1). They are not pathologic themselves and these markers have been used in screening for and adjusting the risk for Trisomy 21 and other aneuploidies.[10,11] Although Soft markers may be seen in the normal fetuses ,

an increased incidence has been noted in fetuses with aneuploidies. These markers of aneuploidy are nonspecific,are often transient, and are readily detected during the second-trimester ultrasound.[12] Thus, morphologic features of Down syndrome in the fetus can be identified by prenatally performed ultrasonography during the second trimester .[13]

Table 1

Major fetal abnormalities and Soft Markers of Aneuploidy

SYSTEM INVOLVED	**MAJOR STRUCTURAL ABNORMALITY**	**SOFT MARKERS**
CENTRAL NERVOUS SYSTEM	GROSSLY DILATED VENTRICLES	CHOROID PLEXUS CYST
	SMALL HEAD CICUMFERENCE (WHEN BIPARIETAL DIAMETER (BPD) < 1ST PERCENTILE AND HP/FL < 2.5TH PERCENTILE) NONUNION OF CEREBRAL HEMISPHERES	
	AGENESIS AND DYSGENESIS OF CORPUS CALLOSUM	
	POSTERIOR FOSSA CYST SUCH AS DANDY WALKER COMPLEX	

SYSTEM INVOLVED	MAJOR STRUCTURAL ABNORMALITY	SOFT MARKERS
MUSCULOSKELETAL	HAND AND FEET ANOMALIES LIKE BONY OR SOFT TISSUE UNION OF FINGERS, CLENCHED FIST, RADIAL RAY HYPOPLASIA,CTEV AND VERTICAL POSITION OF THE TALUS	SHORTENING OF THE LONG BONES
FACE	CLEFT IN THE PALATE ,CLEFT IN THE LIP, SMALL MANDIBLE, LARGE TONGUE, HYPOTELORISM AND HYPERTELORISM, LOW SET EARS, SMALL EAR	–
NECK	CYSTIC HYGROMA	NUCHAL FOLD THICKENING
HEART	ENDOCARDIAL CUSHION DEFECTS, VSD, HLHS, FALLOT'S TETRALOGY	ECHOGENIC INTRACARDIAC FOCUS
GIT	ATRETIC ESOPHAGUS, ATRETIC DUODENUM, SMALL BOWEL OBSTRUCTION, DIAPHRAGMATIC HERNIA AND OMPHALOCELE	ECHOGENIC BOWEL
GENITOURINARY TRACT	MODERATE AND SEVERE HUN, RENAL DYSPLASIA, AND	MILD DILATATION OF RENAL PELVIS

SYSTEM INVOLVED	MAJOR STRUCTURAL ABNORMALITY	SOFT MARKERS
	AGENESIS OF KIDNEYS	
OTHERS	IUGR IN SECOND TRIMESTER, HYDROPS FETALIS	TWO-VESSEL CORD WITH SINGLE UMBILICAL ARTERY

Major abnormalities are observed in < 25% of affected fetuses in most of the studies,[4,14–16] whereas > / = 1 soft markers can be observed in at least 50% of fetuses.[14,17,18] Prenatal ultrasound technique detect the soft markers; second trimester ultrasound can diagnose 50% to 70% of cases of Trisomy 21, 70% to 100% edward's syndrome,[19,20] and 90% to 100% Patau syndrome.[1].

The most common soft markers of aneuploidy are nuchal fold thickening, rhizomelic shortening of limb, mild pyelectasis, echogenic bowel, and echogenic focus in heart and choroid plexus cyst (CPC). There is a great deal of interest in the ultrasound detection of aneuploidy , as evidenced by the large number of publications on this topic. There is wide variation in the studies evaluating the significance of the soft markers of aneuploidy and they show contradictory results. The most common soft markers that are used to screen aneuploidy, ultrasonographic technique for the correct detection and measurement criteria for the detection of soft markers are discussed below.

Thickening of Nuchal fold

Nuchal edema in the second trimester in between 15 and 23 weeks is called as the nuchal fold. It is the first nonstructural marker identified and also it remains the single most predictive sonographic marker.[12] The measurement of nuchal fold is done in the axial plane of the fetal head ,and it should include the cerebellum, occipital bone, and cavum septum pellucidum (Figure 1).

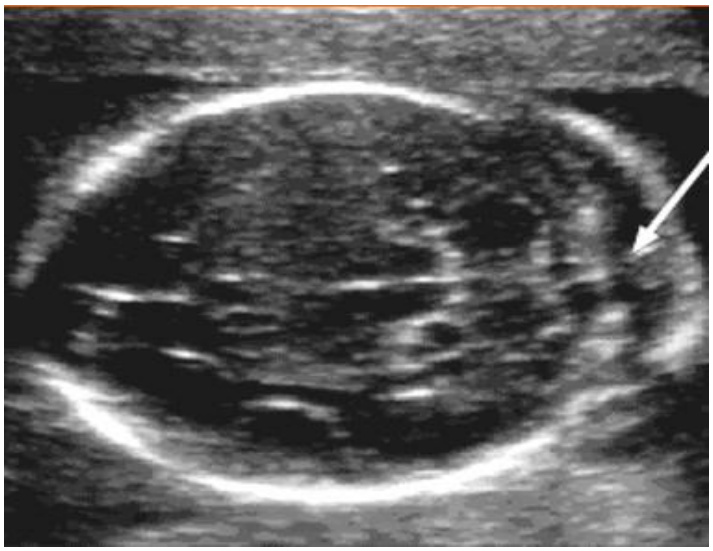


Figure 1 Transverse image of the fetal head showing nuchal fold thickening. Nuchal fold is measured on a transverse image slightly off the biparietal diameter plane during the period of second trimester. The cerebellum, cisterna magna, and occipital bone are the structures to be seen. Soft tissue measurement is taken from the outer echogenic line of occipital bone to the outer echogenic line of skin.

The nuchal fold is to be measured from the outer edge of occipital bone to the outer edge of the skin.[21,22] Nuchal fold thickening of 5 mm is single most cutoff before 20 weeks.[26,27]The gestational age-specific criteria can be used,as the nuchal thickness increases with gestational age normally.[28–30].

Echogenic bowel

Fetal echogenic bowel refers to the presence of hyperechogenecity of the bowel,when compared with the echogenicity of the adjacent iliac bone. [31] The echogenic bowel can be diagnosed when the bowel appears to be at least as echogenic as adjacent iliac bone during the period of second-trimester ultrasound.(Figure 2)

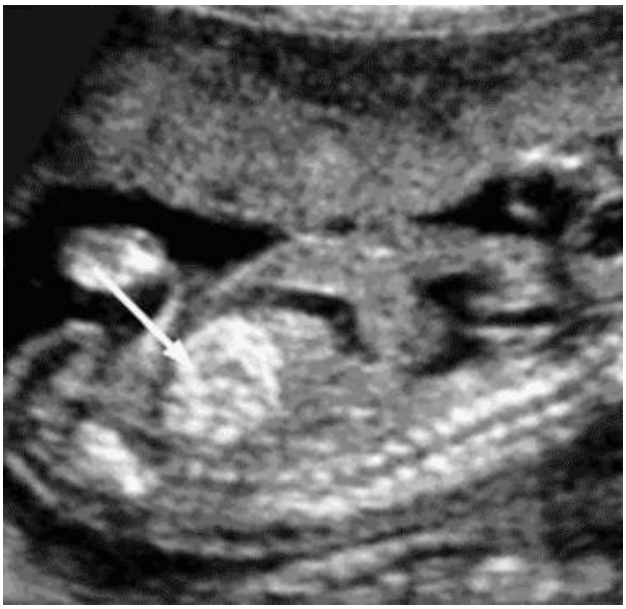


Figure 2 Sagittal image of fetal abdomen showing echogenic bowel.The image should include the fetal bowel, liver and iliac bone for comparison. Echogenic bowel can be diagnosed when the echogenicity of the bowel is equal or more than that of adjacent iliac bone.

In the third trimester ,Echogenic bowel is a relatively commonly encountered finding.[31]The transducer's frequency should be 5 MHz or lower. When the appearance is suspicious of echogenic bowel, the gain of the ultrasound unit need to be gradually lowered to the point when only bone and bowel are visible. Echogenic bowel is classified as focal, multifocal, or diffuse.

There are three grades of the echogenic bowel[32,33], and the echogenicity of the bowel is to be compared with the echogenicity of the iliac crest bone.When the echogenicity of the bowel that is less than the echogenicity of the iliac crest, it is called Grade 1 echogenic bowel ; When the echogenicity of the bowel is equal to the echogenicity of the iliac crest it is called grade 2 echogenic bowel; and When the echogenicity of the bowel is more echogenic than the iliac crest it is called grade 3 echogenic bowel.As the grades go higher (grades 2 and 3),the association of echogenic bowel with aneuploidy and adverse pregnancy outcome becomes strongest .[33]

Echogenic bowel can be diagnosed in 0.2% to 1.4% of all second-trimester ultrasounds.[34] It is associated with normal fetuses, fetuses with trisomy, intrauterine growth retardation (IUGR), bleeding, cystic fibrosis (CF), congenital viral infections, and thalassemia.[31,34–38] The association between echogenic bowel and aneuploidy, particularly trisomy 21,and several studies have demonstated this.[34–37] Theobservation of presence of echogenic bowel in second-trimester ultrasound is an important finding to be noted. A detailed fetal ultrasound following this finding is to be performed, and an amniocentesis to

determine the karyotype, for cytomegalovirus infection (CMV), toxoplasmosis infection, and parvovirus infection are to be recommended. Cystic fibrosis carrier testing is to be done for both parents and maternal serologic testing of Cytomegalovirus and toxoplasmosis should be performed (IgG and IgM).[31] As these fetuses are at risk for Intrauterine growth retardation,follow-up with serial growth scans is recommended,.[31]

Shortened length of Long Bones

Abnormally short long bones are seen in Fetuses with Down syndrome. Biometry of the fetus can be used as a marker for aneuploidy, and it was found that the femur and humerus of fetuses with Down syndrome shows a tendency to be slightly shorter in comparison with the normal controls.The most common method to determine a shortened humerus or femur is by comparing the actual measurement with the expected measurement and is based on biparietal diameter or with any other dating parameter rather than on gestational age. The shortened femur when the measured-to-expected ratio is ≤ 0.91; the shortened humerus is diagnosed when the measured-to-expected ratio is ≤ 0.89[40].It is noted that 24% to 45% of fetuses with Trisomy 21 had short femurs, and it is also noted that 24% to 54% had a short humerus compared to < 5% in the control population [19,40,43]. It is also found that a shortening of the humerus is more predictive than the shortening of the femur[12,44]. The presence of shorteneing of the long bones that involves both the humerus and the femur appears to be less significant than the finding of an isolated shorteneing of the humerus.

Echogenic intracardiac foci

The discrete foci of echogenicity as comparable to the bone seen in the region of papillary muscle in either cardiac ventricle are called Echogenic intracardiac foci [46](Figure 3). If one sees the foci from different angles,one can be sure that one does not wrongly include specular reflections of papillary muscles for EIF[47]. About 1.5% to 4% of pregnancies shows EIF in fetal USG[31,47-50].

A study was done on the potential misinterpretation of an echogenic intracardiac focus [51], and it was found that the rate of true EIF cases as 11 per 200 (5.5%) and the rate of false EIF cases was found to be 34 per 200 (17%). The moderator band, endocardial cushion, and tricuspid valve annulus are the most common locations for identification of spurious EIF[51] and while interpreting the finding of an echogenic focus in the heart, these pitfalls should be borne in mind. In order to avoid false identification and misinterpretation of EIF, ultrasonographic guidelinesare recommended for diagnosing a true intracardiac echogenic focus and it includes:

- EIF should be identified within the ventricle where papillary muscles are situated;
- EIF should be seen from greater than 1 angle;
- EIF should be seen independent of the zone of specular reflection (*Note*: if the suspected EIF lies in this zone, suspicion should be more) and
- EIF should not show an entrance-exit reflection.[51]

Following the detection of an EIF as an isolated finding, a detailed sonographic examination is needed to search for any associated anomalies[31]. The data that are available from low-risk populations indicate that an isolated focus however,does not have any association with an increased risk of Trisomy 21; or, if it is present, that risk is found to be much lesser than the procedure-related loss rates associated with invasive testing like amniocentesis. An isolated echogenic intracardiac focus is considered as a finding that is incidental in a woman younger than 35 years of age, and amniocentesis is not recommended.[31]

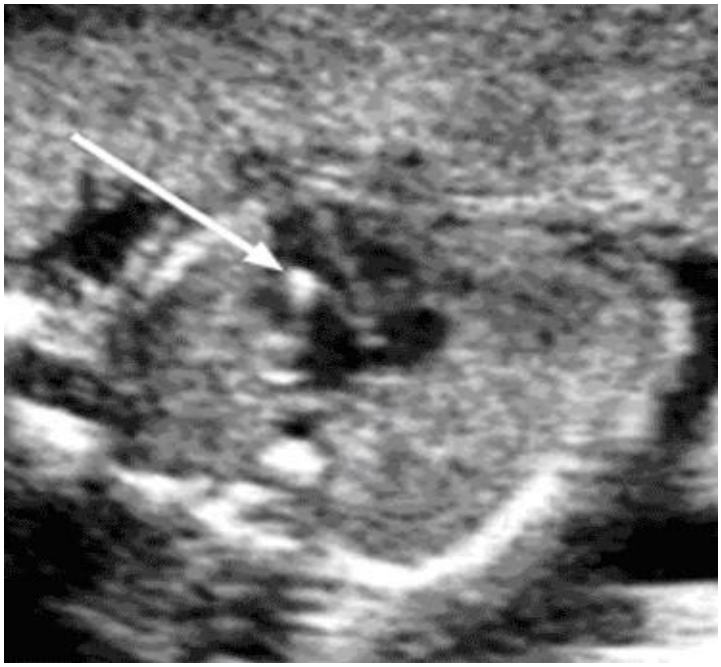


Figure 3 A single echogenic focus on left side of the heart is seen in the Four chamber view of the heart .

A detailed structural survey of the fetus is needed following the identification of the EIF. Amniocentesis need not be indicated in patients who are at low risk ohterwise and have an isolated EIF[47] and in such patients, the performance of amniocentesis is justified with the presence of another major abnormality or minor sign of soft marker with a cardiac echogenic focus.[47]. Isolated EIF in 18 to 34 years aged women was not associated with increased risk for Down's syndrome during the period of midgestation[50].

A study was done on the EIF in a combined total of 21,839 women at low to average risk for trisomy 21 .[52] Among these women, there were 626 fetuses with an EIF as an isolated finding (3%). Only 1 of the 626 with an isolated EIF had Down's syndrome.

Choroid Plexus Cysts

CPCs are seen in about 1% to 2.5 % of normal pregnancies as an isolated finding.Usually no pathologic significance is there when they are seen as an isolated finding.[53–56] CPCs can occur as single or multiple cysts, unilateral, or bilateral cysts. The choroid plexus is seen in the transverse plane of the fetal head and are usually located within the lateral ventricle. A CPC is seen as a well circumscribed anechoic cystic area within the choroid plexus[14] (Figure 4).

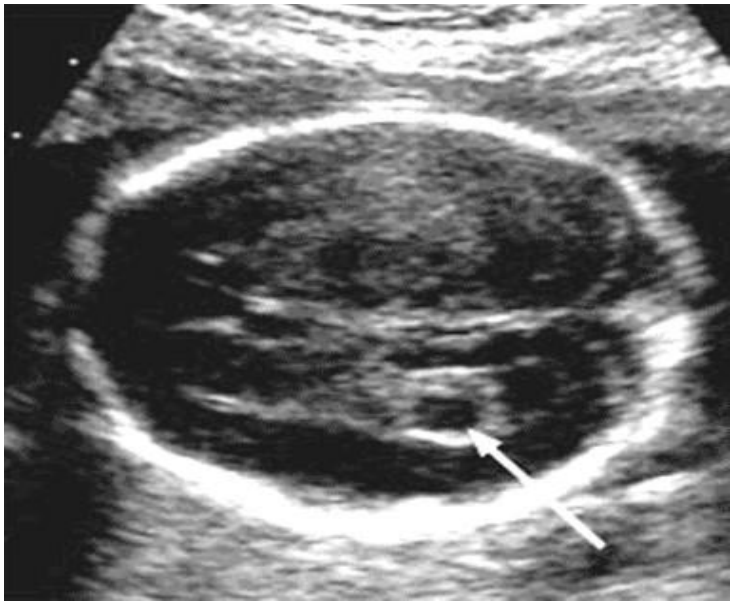


Figure 4 Axial image of the fetal head shows a choroid plexus cyst.

The choroid plexus shows homogeneous echoes,and has an echogenicity similar to soft tissue. When CPC are associated with other anomalies , there is a higher risk for chromosomal defects, especially trisomy 18.[54–58] and the presence of CPCs does not appear to increase the risk of trisomy 21 much above the background risk.[58,59].The fetal hands are to be evaluared in detail for possible overlapping digits and clenched fist to rule out trisomy 18 following the detection of CPC in second trimester ultrasound.[54] In a large multicentric study, the importance of CPCs in an unselected population has been studied in 658 fetuses with CPCs in a total of 101,600 births and a conclusion was made that the presence of CPCs increases the risk for aneuploidy 1.5 times, mainly Edward's syndrome[53].

In a study with 49,435 fetuses between the gestation period of 16 and 25 menstrual weeks, CPC was noticed in 1209 (2.3%), with 1060 cases of CPC being an isolated finding and it was concluded that no fetus with an isolated CPC had trisomy 18[54]. During the period of study ,it was found that 50 cases of Edward syndrome were identified between period of 16 and 25 menstrual weeks and CPCs were detected in half of these fetuses. They made a conclusion that prenatal ultrasonographic identification of CPCs demands an extended anatomic survey of the fetus that includes the fetal hands. If the fetal examination otherwise appears to be normal ,it can be considered that the risk for trisomy 18 is low.[54]

When CPCs are associated with any other antenatally detected anomaly,the probability of a chromosomal abnormality is high and amniocentesis is clearly indicated in these cases to exclude aneuploidy. The predictive value of CPCs is found to be much lower if no other anomalies are detected[60]. The conclusion of the study was that risk of aneuploidy is not related to progression in the size of the cyst as gestation progresses,unilateral or bilateral cysts, and small or large cyst size of the cyst (60% to 80% < 10 mm). CPCs can be regarded as an indication for detailed second trimester ultrasound assessment, rather than invasive testing like amniocentesis.[60]

Mild Pyelectasis

Mild dilation of the renal pelvis of kidneys of the fetus is a common finding during the time of second-trimester ultrasound, with an incidence of 0.3% to 4.5% (average being 1%).[61–64] When the renal pelvis measures more than or equal to 4 mm and less than 10 mm in anteroposterior dimensions in transverse scans of the abdomen, without dilatation of the calyces , in the second trimester, mild pyelectasis is diagnosed (Figure 5).[31]

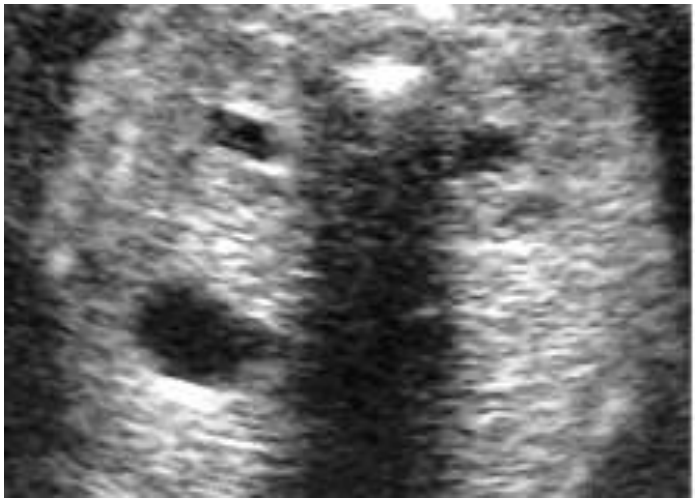


Figure 5 Axial images at the level of the renal pelvis show mild dilatation on both sides . Anterior-posterior diameter of the renal pelvis is to be taken on an axial view.

Fetuses with significant pyelectasis and hydronephrosis ,that is , anteroposterior diameter of renal pelvis ≥ 10 mm, are clearly at risk for having major structural abnormalities and require postnatal evaluation and followup . The possibility of an association of pyelectasis with aneuploidy (primarily Down syndrome)was first raised by Benacerraf and colleagues[63] in 1990; in a high-

risk population group, 25% of fetuses with Trisomy 21 had mild pyelectasis in comparison with 2.8% of fetuses with normal karyotype.[63] In a well known largest published series of fetal pyelectasis [65], they identified 737 fetuses from 101,600 with mild dilatation of renal pelvis; of these 12 (1.7%) had abnormalities in karyotype.

Further, 9 of these 12 fetuses had associated abnormalities in sonography, and 1 mother had advanced maternal age . Only 2 chromosomal abnormalities occurred with the pyelectasis being an isolated finding, in low-risk women (0.3%). It was estimated that the aneuploidy risk in a fetus with isolated mild pyelectasis was 0.33% and 2.2% in women less than 36 years of age and ≥ 36 years, respectively.

The ultrasound findings of 25,586 mainly low-risk, unselected women was reviewed and found 320 cases of pyelectasis with an incidence of 1.25%.[62] 19 of the fetuses with pyelectasis had associated anomalies in the 2nd trimester ultrasound ; pyelectasis was an isolated finding in 301 fetuses. No aneuploidy was found in none of the fetuses identified in this series. The lack of association with aneuploidy when present as an isolated finding was confirmed by many other studies on pyelectasis.[12,66,67]

The results of these studies was that in the absence of any other major structural anomalies or other soft markers or other risk factors for aneuploidy, invasive tests like amniocentesis does not seem to be warranted for isolated mild pyelectasis. Approximately one third to one quarter of fetuses show progression of

pyelectasis,[64,68] and hence the 3rd trimester ultrasound is recommended in order to identify worsening or persistent cases. The persistent pyelectasis or progressive dilatation needs some degree of postnatal evaluation or surveillance.[31]

Ventriculomegaly

Cerebral ventricles are said to be dilated when the measurement across the atria of the posterior horn of lateral ventricles at any period of gestation is >10 mm.The axial plane at the atria of the lateral ventricle and glomus of the choroid plexus.Measurement is to be taken from the inner margin of the medial ventricular wall to inner margin of the lateral wall.

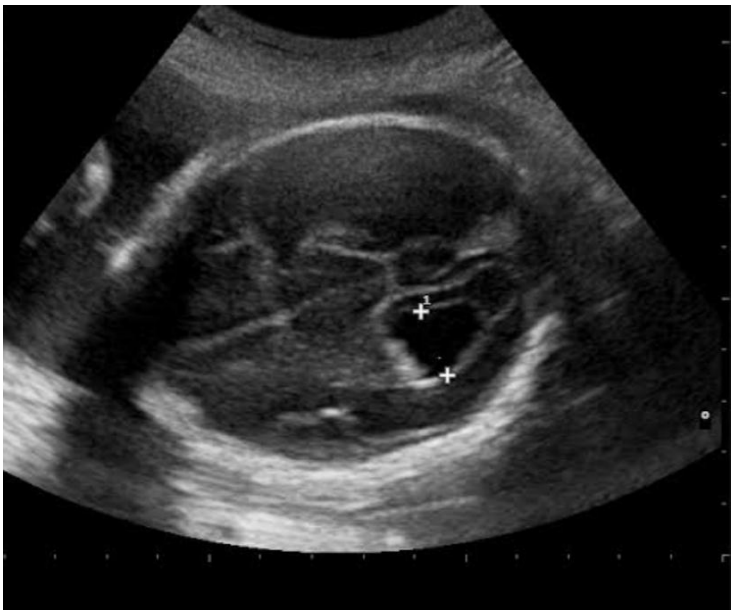


Figure 6 Axial images at the level of the thalamus show mild dilatation of the ventricles .

Holoprosencephaly

It results from incomplete separation of the two cerebral hemispheres.its subtypes includes :

- Alobar Holoprosencephaly
- Semilobar Holoprosencephaly
- Lobar Holoprosencephaly
- Syntelencephaly
- Septo-optic dysplasia
- Central incisor syndrome
- Nonspecific midline dysplasia
- Fronto-nasal dysplasia
- Agnathia-otocephaly

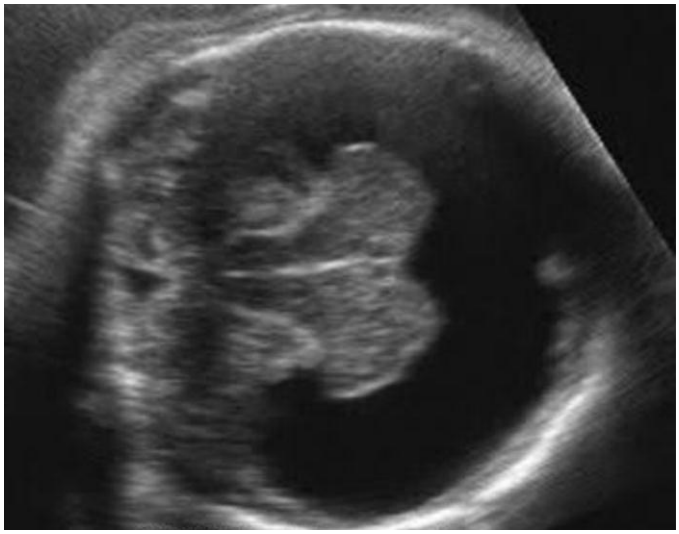


Figure 7 Coronal images show fused thalami and bilateral lateral ventricles and nonvisualisation of falx cerebri.

In Alobar Holoprosencephaly,the thalami is fused and there is a single large posteriorly located ventricle.

In semilobar Holoprosencephaly,the basic structure of the cerebral hemispheres are maintained but are fused most commonly anteriorly and at the thalami associated with agenesis of corpus callosum and aplasia of the olfactory tracts and bulbs.

In lobar Holoprosencephaly,midline abnormalities are more subtle such as fusion of the cingulate gyrus and thalami.The olfactory tracts are absent or hypoplastic.

Microcephaly

As the gestation advances, the diagnosis of microcephaly becomes easier.The small head is best evaluated by using the head circumference(HC).As the shape of the head can be misleading , measurement of the biparietal diameter is helpful in assessing the microcephaly.

Dandy walker complex

It is the triad of

- Hypoplasia of the vermis and cephalad rotation of the vermian remnant
- Cystic dilatation of the fourth ventricle extending posteriorly
- Enlarged posterior fossa with torcular -lambdoid inversion.
- Antenatal sonographic findings include :
- Marked enlargement of the cisterna magna(>/=10mm)

- Complete aplasia of the vermis
- A trapezoid shaped gap between the cerebellar hemispheres
- Antenatal USG may give the appearance of this condition if done before 18weeks since the vermis not being properly formed before that time.

Syndactyly

It is congenital fusion of 2 or more digits.It may be confined to soft tissue or may involve bone.it is often difficult to diagnose on ultrasound.The diagnosis may be suggested if the digits constantly move together.The fingers may appear constantly deformed with complex syndactyly.

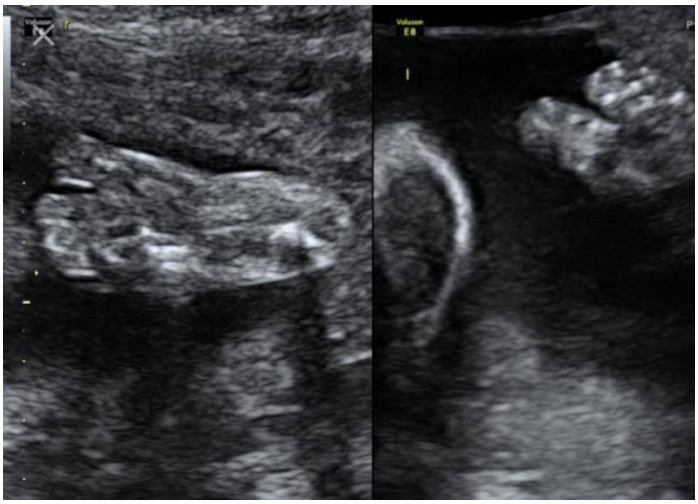


Figure 8 Coronal images of the fetal feet and hands showing fusion of the digits.

Clinodactyly

It is seen as a radial angulation at an interphalangeal joint in the radio-ulnar or palmar planes.It typically affects the 5^{th} finger.

Rocker bottom foot

It is also called as congenital vertical talus and is characterized by a prominent calcaneus /talus and a convexly rounded sole.

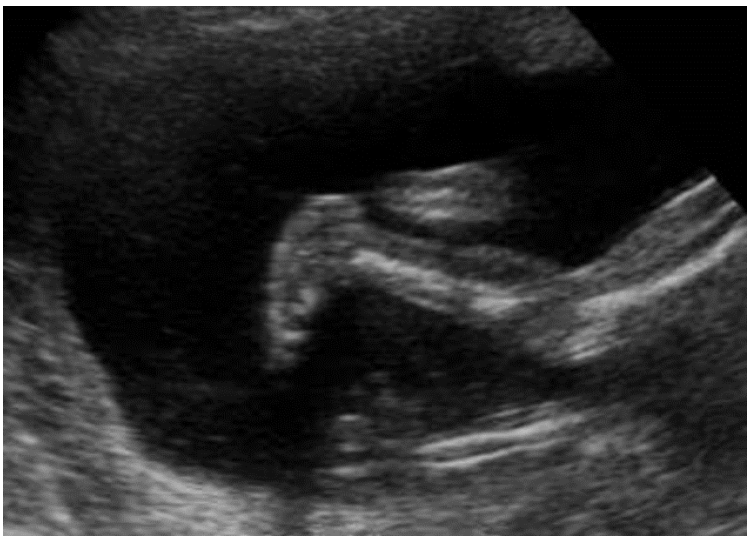


Figure 9 Sagittal images of the fetal feet showing rocker bottom foot .

Importance of Absence of the Marker

A detailed search for sonographic markers of aneuploidy, can be used to identify fetuses at high risk for aneuploidy and, if found to be normal ie, when no sonographic markers are identified,it can be used to provide evidence of a decreased risk for aneuploidy.[1] In a patient with advanced maternal age, the absence of sonographic markers appears to be associated with a decreased risk when compared with the age-related risk. With the second trimester ultrasound being normal, the reported associated reduction in aneuploidy risk has varied from approximately 60% to 83%.[12,69,70] In a survey[71], 72% of maternal-fetal

medicine physicians use ultrasound in second-trimester to adjust aneuploidy risk; the most frequently cited reduction in risk was 50%.

Importance of the presence of marker

Because ultrasound markers are also common among foetuses with normal karyotype, it may not be clear when genetic amniocentesis should be offered. The adjustment of risk secondary to the presence of markers, and the issue of which markers are most significant, remain controversial. In order to identify patients at risk, 2 ultrasound methods have been proposed.

A simple approach have been popularized by Benacerraf and colleagues[72–74], referred to here as the index scoring system (ISS), whereby a score of 2 is assigned for structural defects and nuchal thickening (≥ 6 mm) and a score of 1 is assigned for the ultrasound markers like EIF, echogenic bowel, pyelectasis, short femur, and short humerus. A score of 2 or more is considered to be positive.

Using this method, the authors report a sensitivity of 73% (33 of 45 fetuses) for detecting Down's syndrome , with a false-positive rate of only 4% (4 of 106 fetuses).[73] More recent modifications that also takes maternal age into account(score of 1 for women aged 35 to 39 years and score of 2 for women aged 40 years or older) result in a higher sensitivity (87%), but at the cost of a higher false-positive rate 27%.[74] The importance of including CPCs in this system remains uncertain.[74]

Using a different approach,called the age-adjusted ultrasound risk assessment (AAURA), LRs from ultrasound markers to the *a priori* risk on the basis of maternal age was applied by Nyberg and colleagues[14,75]. This method provides patient-specific risk estimates depending on maternal age, gestational age, and ultrasound findings, although it is more complicated than the ISS and requires computer calculations. By using a threshold of 1 in 200, this method has achieved a sensitivity of 74% (105 of 142) in a high-risk population.[14]

A study was designed by Winter and colleagues[11] to compare the accuracy of the ISS with the accuracy of the AAURA in the prenatal detection of fetal Down syndrome. In this study, 3303 consecutive women with high-risk pregnancies underwent a prospective complete ultrasound examination; each also had genetic amniocentesis. By using a threshold value of at least 2 points to detect trisomy 21, the ISS at its best had a sensitivity of 45.3%, false-positive rate of 4.9%, and LR of 9.3; the positive predictive value in the high-risk population was 13.3%.

Upon Lowering the threshold to 1 point, increase in the sensitivity to 60.4% and increase in the false-positive rate to 15.8% has been noted. Adding points for age the sensitivity increased to 67.9% but also the false-positive rate was increased to 24.3%. Nearly identical results were achieved with AAURA to detect Down's syndrome.

At a 1 in 36 risk threshold, the sensitivity was 43.4% and the false-positive rate was 4.9%; at a 1 in 200 threshold, the sensitivity increased to 69.8% and the false-positive rate increased to 26.1%. Trisomies 18 and 13 were detected with sensitivities of 80.0% and 100.0%, respectively, with either method. The authors concluded that both the modified ISS and AAURA are equivalent in screening for Down syndrome and detect approximately half of all trisomy 21 fetuses at a 5% false-positive rate.

Isolated Vs Multiple Markers

Sonographic markers are considered to be isolated finding when they are not associated with major abnormalities or any other of the markers evaluated. Nyberg and colleagues[12] made the comparison of second-trimester (14 to 20 weeks) sonographic findings in 186 trisomy 21 fetuses with a control group of 8728 consecutive control fetuses through the evaluation of nuchal thickening, hyperechoic bowel, shortened femur, shortened humerus, EIF and renal pyelectasis.

It was reported that an isolated soft marker was the only sonographic finding in 42 (22.6%) of 186 fetuses with trisomy 21 compared with 987 (11.3%) of 8728 control fetuses ($P < .001$). Nuchal thickening ($P < .001$; LR, 11) and hyperechoic bowel ($P < .001$; LR, 6.7) showed the strongest association with trisomy 21 as isolated markers, followed by shortened humerus (LR, 5.1), EIF (LR, 1.8), shortened femur (LR, 1.5), and pyelectasis (LR, 1.5). EIF was the

single most common soft marker in isolation in both affected fetuses (7.1%) and control fetuses (3.9%), but carried a low risk (*P* = .046; LR, 1.8).[12]

164 fetuses with Down syndrome detected by karyotype was studied by Bromley and colleagues[22] . The significance of the sonographic markers as both isolated and nonisolated findings was evaluated and calculated the LRs. The most sensitive sonographic markers for trisomy 21 includes the nuchal fold, short femur, and an EIF.

However, the false-positive rate was also the highest for a short femur and an EIF, resulting in lower LRs. Of all the sonographic markers, the highest LR for trisomy 21 was with the finding of a nuchal fold . A short humerus carried the second highest LR for Down syndrome. A short humerus was identified in 48.7% of fetuses with Down syndrome when compared with 2.1% of control fetuses, yielding an LR of 23.5.

Major structural anomalies were found in 44 (26.8%) of 164 fetuses with Trisomy 21 when compared with 8 (1.2%) of 656 control fetuses, yielding an LR of 22. As isolated findings, the femoral length, pyelectasis, and EIF have low LRs because the prevalence of the isolated markers in the euploid population appears similar when compared with the population with trisomy 21.

As an isolated finding, the highest LR for aneuploidy was retained by the nuchal fold; however, when it was present, it was isolated only 8% of the time. The next highest LR for aneuploidy (5.8)was an isolated short humerus and was isolated just 6% of the time when it was seen. As isolated findings, the femoral length, pyelectasis, and EIF have low LRs because the prevalence of the isolated markers in the euploid population was similar when compared with the population with Down syndrome.

These findings suggest that the markers with the highest LRs for Down syndrome more often presents in clusters with other markers and are present in isolation in only a few instances. It was concluded that the presence of nuchal fold, a major structural anomaly, and a short humerus can be considered sufficient to exceed the commonly accepted threshold for offering amniocentesis.

The suggestion that the presence of several markers that might not be of concern in isolation carries much more importance when they occur in aggregates was putforth by Bromley and colleagues[22]. A LR of 14 resulted with the presence of 2 or more markers and clusters of markers whrn present confer a higher risk of aneuploidy.[22] The same conclusions were made[76] in another study of 104 fetuses with abnormal karyotype; they concluded that the risk for aneuploidy is increased by 12-fold by the presence of multiple markers (≥ 2).

High risk Vs Low risk population

To help improve the sonographic detection of Down syndrome in high-risk women (predominantly pregnant women of advanced maternal age), the soft markers for Down syndrome were originally described, for whom more accurate risk information is needed than that based on age alone before deciding whether or not to undergo amniocentesis.[76] In those women older than 35 years who wish to avoid amniocentesis ,a normal ultrasound scan has been used as evidence for a reduced risk of Down syndrome.[14,18,66,77] For example, approximately 60% reduced risk of Down syndrome was associated with a normal ultrasound scan [14], and for a 40-year-old woman with a normal ultrasound scan the probability of having a fetus with aneuploidy decreases from 19 in 1000 pregnancies to 5 in 1000 pregnancies [18].

Available data suggest that sonographic findings are independent of maternal age and biochemical markers,[78,79] and, therefore, sonographic assessment might be applicable to low-risk patients. However, caution should be exercised in applying LRs to low-risk populations.[12] The importance and optimal course of action in a low-risk patient with a marker on prenatal sonography are controversial and not well established. If an isolated marker with an LR close to 1 is found (eg, a short femur, EIF, or pyelectasis), the patient's risk of having an affected fetus changes only minimally from her *a priori* risk and is probably not clinically relevant.[22]

The procedure should be offered to the patient when prenatal diagnosis is desired ,if a patient at low risk is found to have a thickened nuchal fold, a major anomaly, a short humerus, or an aggregate of markers,as the pattern of findings may result in a high enough LR that the revised risk estimate exceeds the commonly accepted threshold for offering amniocentesis.[22]

As the ultrasound assessment identifies approximately half of fetuses affected with Down syndrome with an acceptable false-positive rate, it is probably most useful in low-risk women younger than 35 years.[14]

Detection of soft markers of aneuploidy is important among high-risk women in whom high sensitivity and positive predictive value are desirable. On the other hand , if any one of a panel of markers is detected in low-risk women, the false-positive rate may be unacceptably high (13% to 17%).[14,18,78,80]

Soft markers and aneuploidy risk assessment

When the risk of aneuploidy is 1/270 or greater ,an individual is considered to be at high risk for fetal Down syndrome, which is the mid-second-trimester prevalence for a 35-year-old woman.[45] When the risk of aneuploidy is 1/270 (at the time of amniocentesis) or greater on the basis of advanced maternal age, maternal serum screening, or both, amniocentesis is generally offered to those individuals.

In order to further refine each patient's individual risk of having an affected fetus, the sonographic markers have provided a method of further evaluating the fetus for morphologic signs of trisomy 21. It is very complex and important to choose which subset of the pregnant population should receive definitive karyotype determination

Commonly encountered aneuploidy are Trisomy 13,Trisomy 18,Trisomy 21.

Trisomy 13 :

The abnormalities encountered include congenital heart disease like hypoplastic left heart syndrome, VSD, holoprosencephaly, microcephaly ,hydrocephalus, enlarged cisterna magna, agenesis of corpus callosum, persistent stapedial artery, spina bifida, IUGR, cleft lip and palate, micropthalmia, anophthalmia, micrognathia, hypotelorism, hypertelorism,cyclopia, Proboscis, polydactyly, rocker bootom foot, clenched hands bladder exstrophy, omphalocele, cryptorchidism, cystic renal dysplasia.

Trisomy 18 :

The abnormalities encountered include congenital heart disease like atrial septal defect, VSD, patent ductus arteriosus, dextrocardia, CPC, agenesis of corpus callosum, Dandy walker malformation, mega cisterna magna, meningomyelocele, spina bifida, IUGR, micrognathia, dolichocephaly, low set ears, cleft lip and palate, cystic hygroma, clenched hands, radial ray abnormalities, absent thumb, rocker bottom feet, club feet, single umblical artery, umblical cord cysts, umblical cord pseudocysts, omphalocele, congenital diaphragmatic hernia, antenatal hydronephrosis, horseshoe kidney.

Trisomy 21:

The abnormalities encountered include VSD, atrial septal defect, atrioventricular septal defects, pulmonary hypoplasia, pulmonary cysts, duodenal atresia,omphalocele,congenital diaphragmatic hernia.

Amniocentesis

Over the past two decades, second-trimester amniocentesis has become a standard procedure for the diagnosis of fetal genetic abnormalities. Cytogenetic, enzymatic, and DNA analyses can be done on cells obtained from amniotic fluid.

In addition, levels of alpha-fetoprotein (AFP) and acetylcholinesterase (AChE) in the amniotic fluid can be measured to diagnose neural tube defects (such as spina bifida and anencephaly) and anterior abdominal wall defects (for example, omphalocele and gastroschisis) prenatally. Thus, amniocentesis is applicable for the prenatal diagnosis of many fetal abnormalities.

Conventional genetic amniocentesis is usually performed between 14 and 20 weeks' gestation ("menstrual weeks") to evaluate a fetus for chromosome abnormalities, neural tube defects, and other detectable genetic and acquired disorders.

Safety of Amniocentesis

The safety of genetic amniocentesis has been addressed by several large collaborative studies. The original trial was sponsored by the National Institute of Child Health and Development5; of 1,040 women undergoing amniocentesis, 3.5% experienced fetal loss after amniocentesis compared with 3.2% of concurrent controls (992 patients). This small difference was not statistically significant. In the United Kingdom, a collaborative trial revealed that the loss rate after amniocentesis was significantly higher than in controls (2.6% for the amniocentesis group compared with 1% for controls).

A common indication for amniocentesis in the United Kingdom group, however, was an elevated maternal serum AFP level, a factor associated with increased fetal mortality. In a later analysis, after subjects undergoing amniocentesis for that reason were excluded, the difference between subject and control groups was reduced to less than 1%.

The increased risk of fetal mortality attributable to amniocentesis may be as high as 0.5%. Finally, we counsel patients that the risks of serious maternal complications or fetal injuries are remote but do exist.

Accuracy

The analysis of amniotic fluid, as well as that of chorionic villi or fetal tissue, entails difficulties that need to be recognized. First, cells obtained by amniocentesis may not grow, or growth may be insufficient to obtain metaphases for cytogenetic analysis. Amniotic cell cultures are usually successful;Trials

comparing chorionic villus sampling (CVS) and amniocentesis revealed the failure to obtain cytogenetic results for patients undergoing amniocentesis to be uncommon (0.1% in the Canadian study and 0.9% in the American study). Second, maternal cells may be inadvertently included in the specimen, thereby creating the possibility of an incorrect diagnosis.

This source of possible error is of greater concern with CVS and percutaneous umbilical blood sampling; in theory, discarding the syringe containing the first milliliter of aspirated amniotic fluid should reduce the chance of maternal cell contamination. A third source of error involves chromosome abnormalities that are not representative of fetal complement.

Such chromosome abnormalities may arise in culture and should be suspected whenever they are restricted to only one of the several culture flasks or clones started from a single amniotic fluid specimen. In fact, cells containing at least one additional structurally normal chromosome are detected in 1% to 2% of all amniotic fluid specimens.

When such cells are confined to a single culture or clone, the phenomenon is termed pseudomosaicism; when they are found in more than one flask or clone, the phenomenon is termed true mosaicism. True mosaicism is found in 0.25% of amniotic fluid specimens, and true mosaicism is confirmed by studies of the abortus or neonate in 70% to 80% of cases.

Although pseudomosaicism is not associated with an increased risk for fetal morbidity or mortality, true mosaicism is associated with an increased risk of phenotypic and developmental abnormalities. A fourth possible problem is that some phenotypes are difficult to predict from the chromosome complement. This is especially the case when an apparently balanced translocation, small inversion, or small supernumerary chromosome is identified. If one of the phenotypically normal parents has the same chromosome aberration, reassurance is generally appropriate.

Apparently balanced de novo structural abnormalities, such as chromosome translocations and supernumerary chromosomes, are associated with about a 10% risk of phenotypic abnormalities. Patients must therefore be made aware that although laboratory failure and cytogenetic discrepancies are now uncommon in amniocentesis, they do occur and may lead the physician to recommend either a second amniocentesis or a different diagnostic test, such as percutaneous umbilical blood sampling, to further evaluate the fetal state.

Indications for Offering Invasive Prenatal Diagnosis

- Increased risk for fetal chromosome abnormalities
- Advanced matemal age (35 yrs at time of delivery)
- Previous offspring with chromosome abnormality
- Parental chromosome abnormality Balanced parental chromosome rearrangement

- Miscarriages (3 or more)
- Fetal structural defects
- Increased risk for mendelian disorders detectable by molecular biologic techniques (sickle cell anemia, cystic fibrosis)
- Increased risk for mendelian disorders detectable by enzyme assays (TaySachs disease)
- Increased risk for polygenic or multifactorial conditions detectable by amniotic fluid analyses (neural tube defects, anterior abdominal wall defects)

Amniocentesis is primarily used by two groups of women: those who are 35 years of age or older at their estimated date of delivery and those who have been found to be at increased risk for fetal neural tube defects or the Down syndrome as a result of maternal serum analyte screening. This screening is performed during the second trimester; accordingly, only amniocentesis is an option for those women who elect invasive prenatal testing after receiving an abnormal screening result.

Karyotyping

Cytogenetics is the diagnostic study of the structure and properties of chromosomes and cell division, which employs various methods, one of them being "karyotyping." It refers to a procedure of photographic representation of a stained preparation in which the chromosomes are arranged in a standard manner. The development of newer techniques such as "karyotyping" has made it possible

to visualize undetected chromosomal anomalies such as small portions of chromosomes and translocations of tiny parts of chromosomes to one another. Because such procedures also enabled each pair of chromosomes to be distinguished individually, it has helped to further our understanding of the chromosomal basis of certain important genetic disorders.

Living beings include variegate species that are distinct from one another. In the same species, every member has his/her own individuality, all owing to the genetic constitution of an organism - the chromosomes, the genes, and the DNA[107]. The blueprint for the formation and maintenance of an organism is provided by the DNA, which is packaged into chromosomes. Chromosomes are the factors, which distinguish one species from one another and which enable transmission of genetic information from one generation to the next. Since a number of genetic abnormalities can be directly related to the chromosomal pattern, the characterization of chromosomes is of considerable diagnostic importance. This can be done by "cytogenetics." It is a photographic representation of a stained preparation in which the chromosomes are arranged in a standard manner and "karyotype" refers to the constitution of chromosomes of an individual[110]. In 1956, Tijo and Levan, Ford and Hamerton found that the normal human somatic cell contains only 46 chromosomes, and that maleness is determined by the presence of a "Y" chromosome, regardless of the number of "X" chromosomes in each cell[111]. The methods they used, with certain modifications, are now being used in "cytogenetic" laboratories to analyze the "karyotypes."

Procedure for Karyotyping

I. Chromosome preparation:

Source of chromosomes - any tissue with nucleated cells undergoing division can be used for chromosomal study:

- Peripheral venous blood - most commonly, the lymphocytes.
- Skin (fibroblasts), bone marrow.
- For fetal chromosome patterns - amniotic fluid cells, chorionic villi.
- 5-10 mL of heparinized venous blood is the most commonly used source. The heparin prevents coagulation, which would interfere with the later separation of lymphocytes[110,111,113].

Culture:

The blood cells are grown in a suitable culture medium containing phytohemagglutinin which, acts as a mitogen, stimulating the T-lymphocytes to divide and agglutinate the red blood cells (RBCs). The commonly used medium has 5 mL culture medium, 1 mL fetal bovine serum, and 0.2 mL phytohemagglutinin. Cultures are incubated at 37°C for 48-72hrs.

Arrest of division:

Mitosis is then interrupted at metaphase with spindle inhibitors such as colchicine (0.01%). Chromosome number, size, and shape at metaphase are species-specific - in nondividing cells, the chromosomes are not visible even with the aid of histologic stains for DNA or electron microscopy. During mitosis and meiosis, the chromosomes condense and become visible in the light microscope.

Therefore, almost all cytogenetic work is done at metaphase[110,111]. The culture is incubated for 45 min. The contents of the vial transferred to a tube and centrifuged at 800 rpm for 5 min.

Suspension in hypotonic solution: Prewarmed hypotonic saline is added to culture. This causes the RBCs to lyse. The osmotic swelling of the lymphocytes results in spreading of the chromosomes. It is incubated at 37°C for 5 min, centrifuged at 800 rpm for 5 min, and the supernatant removed.

Fixation:

A freshly prepared fixative (3 parts of methanol and 1 part of glacial acetic acid) is added. Two changes of fixatives are given at intervals of 45 min.

Slide preparation:

The cells resuspended in fresh fixative and slide are prepared by gently placing a drop of cell suspension on previously cooled cleaned slide and dried followed by staining. [7],[8]

II.Chromosome staining-banding techniques :

Numerous methods are available for identifying chromosomes and preparing karyotypes for diagnosis purposes. Banding patterns became the barcodes with which "cytogeneticists" can easily identify chromosomes, detect subtle deletions, inversions, insertions, translocations, fragile sites and other more complex rearrangements, and refine breakpoints. The ability to analyze chromosomes is dependent on the length of the chromosomes and how well they

are fixed, spread, and stained. When a large number of cells has to be examined for clinical purposes, automatic scanning light microscopy with computer control and analysis can greatly facilitate the identification of chromosomal abnormalities.

A. General techniques: Some human chromosomes may be distinguished on morphological grounds alone, for example, the length of arms and position of primary and secondary constrictions. Autoradiography can also be used, especially for "S" phase identification and identifying chromosomes 4, 5, 13, 14,15,17and18.

Procedure: Suitable tissue preparations with a nuclear emulsion are done in a dark room, after which they are stored in the dark for several weeks and then are photographically developed and fixed. Discrete silver grains can then be seen over the sites that emit radiation; their position indicates sites of incorporation of the radioisotope. Such a preparation is termed as autoradiograph[114,115]. Technique: Add a few drops of stain to the prepared slide, lower coverslip, and apply gentle firm pressure with filter paper or glass rod. Remove excess stain by applying filter paper to the edge of coverslip. Chromosomes stain deep purple. This method is indelible and does not permit destaining and use of subsequent staining methods for banding. It is replaced by the banding techniques. [9],[10]

B. **Banding techniques**: First-banding technique was introduced by Caspersson (1969); it includes G (Giemsa) banding, Q (Quinacrine) banding, R (Reverse) banding, C (centromeric heterochromatin) banding, T (Telomeric) banding, and high resolution (fine) banding.

1) G (GIEMSA) BANDING: It is the most common method, which produces permanent slides that can be studied under a standard light microscope. It produces the same banding pattern as quinacrine with even greater resolution and does not necessitate the use of fluorescence microscopy. It can be used to pair and identify each of the human chromosomes accurately. [12],[13]

Prior to staining, the fixed chromosomes are treated with agents capable of denaturing chromosomal proteins such as proteolytic enzymes (trypsin, most commonly), salts, heat, detergents and urea. G-banding is most consistently produced by pretreatment of chromosomes with trypsin before staining with Giemsa[118,119].

Laboratory procedure: Slides for G-banding should be 1 day at room temperature or overnight at 50-60°C for optimal results.

- Incubate the slide for 20-40 s in 0.025% solution of trypsin in distilled water.
- Rinse thoroughly with phosphate-buffered saline (PBS) or distilled water.
- Stain in 4% buffered Giemsa solution for 5-10 min.

- Rinse slides in distilled water and air-dry.
- When the chromosomes are stained with Giemsa, a DNA-binding dye, after such treatment, G-bands can be seen with a light microscope.
- The G-bands constitute 300-400 alternate dark and light bands, which are characteristic for each chromosome and reflect differential chromosomal condensation[118,119,120,121].

Digital photograph of the entire metaphase spread is taken and both homologs of each chromosome pair are placed side by side in the numerical order for careful band-by-band analysis, which permits identification of relatively subtle changes in banding patterns caused by structural chromosome abnormalities [121].

R-banding:

A pattern that is opposite of G- or Q-banding can be produced by various means and is referred to as reverse (R-) banding. Fluorescent R-banding patterns are produced by dyes with GC base-pair affinity such as chromomycin A3, olivomycin, and mithramycin. It is produced by subjecting slides to high temperatures followed by staining with Giemsa or acridine orange. R-bands have the advantage of staining the gene-rich chromatin, thus enhancing the ability to visualize small structural rearrangements in the parts of the genome that are most likely to result in phenotypic abnormalities.

C-Banding:

C-bands localize the heterochromatic regions of chromosomes. Pardue and Gall (1970) first reported C-bands when they discovered that the centromeric

region of mouse chromosomes is rich in repetitive DNA sequences and stains dark with Giemsa.

T-banding:

This method involves staining the telomeric (end) regions of the chromosomes. Slides are treated with phosphate buffer or Earle's balanced salt solution and then stained using mixed Giemsa solution to produce the t-bands.

CT-banding:

In this method, slides are treated with barium hydroxide to stain both the centromeric heterochromatin and the telomere of chromosomes.

Nucleolar organizing region (NOR) banding: This technique stains NOR located in the satellite stalks of acrocentric chromosomes and house genes for ribosomal RNA. NOR-bands represent structural nonhistone proteins that are specifically linked to NOR and bind to ammoniacal silver. It is useful in clinical practice to study certain chromosome polymorphisms such as double satellites. Resolution cytogenetics provides precision in the delineation of chromosomal breakpoints and assignment of gene loci, greater than with earlier banding techniques. This is achieved by synchronizing the lymphocyte cultures and obtaining more number of cells in prometaphase or even prophase (increasing resolution from 500 to over 1,000 bands in a haploid genome).

Fluorescent *in situ* hybridization (FISH):

This technique allows the visualization of chromosomal location and nuclear location of specific DNA sequences and permits the detection of specific nucleic acid sequences in morphologically preserved chromosomes. It can be performed on either metaphase or interphase cells and involves denaturing genomic DNA.

After banding/staining, the chromosome component of single cells is selected on the basis of representative morphology and chromosome number and photographed under a light microscope. Photographic representation of each individual chromosome is then cut out from such a photomicrograph and arranged so as to construct a "karyotype"[111,122]. Analysis is done either by looking down the microscope under oil immersion or on a photograph and involves:

1. The number of chromosomes is determined: Usually the total chromosome count is determined in 15-20 cells but if mosaicism is suspected, then 30 or more cell counts are undertaken.
2. Chromosomes are assembled as homologous pairs: The homologous chromosomes pairs are arranged in a decreasing order of size to construct a "karyotype."
3. Detailed analysis of the banding pattern of individual chromosomes: This is performed on both members of each pair of homologs in approximately three-five metaphase spreads[111,122].

The banding pattern of each chromosome is specific and shown in the form of a stylized ideal karyotype known as an idiogram.

- If the total count is 46, Group G is identified to diagnose the sex of the individual.
- If the count is more or less, the precise numerical abnormality is identified. Then, any structural abnormality present is identified.
- The chromosomes are usually identified in groups starting with Group G, and then Group D, Group F, Group E, Group A, Group B, and finally Group C.
- Identification of individual chromosomes is possible on the basis of total length of the chromosome, arm ratio, position of secondary constrictions and nucleolar organizers (satellites), subdivision of chromosome into euchromatic and heterochromatic regions, and characteristic banding patterns. [5],[16]

A human somatic cell contains 46 chromosomes, made up of 22 pairs of autosomes and a single pair of sex chromosomes, XX in the female, XY in the male.

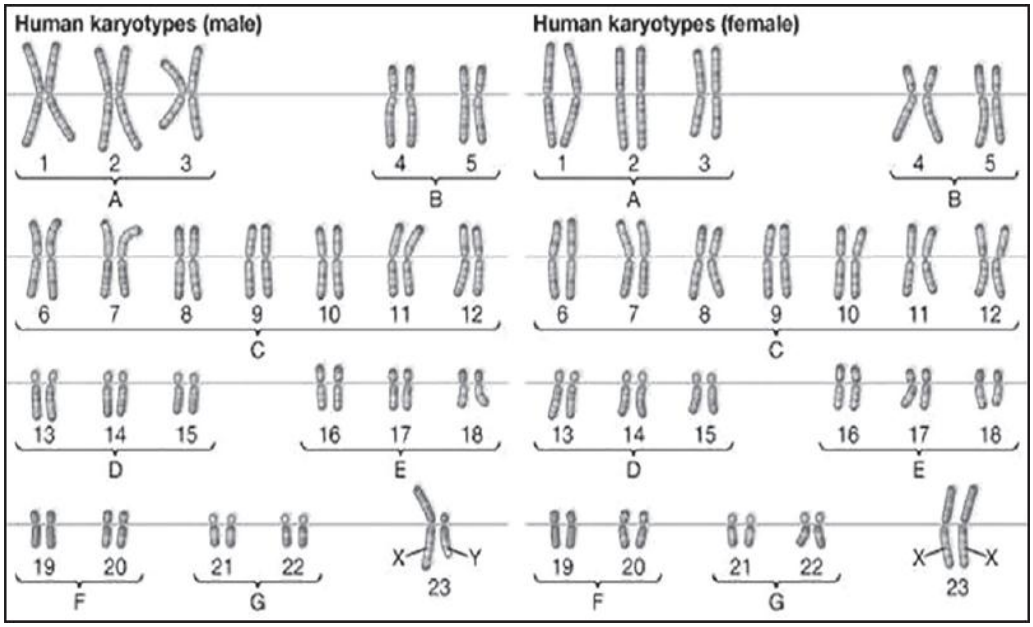


Figure 10 showing normal human karyotype.

Applications:

- Cytogenetic aspect of neonatally discovered congenital abnormalities and prenatal diagnosis.
- Mental retardation and neuropsychiatric disorders.
- Reproductive failure, endocrinology, gynecology, urology.
- Malignancy: Hematology and oncology.
- Mutagenesis: Environmental medicine and industrial medicine. [4],[5]

a. "Karyotyping" in chromosomal abnormalities: Approximately 60% of all spontaneous abortions and 1 out of 200 newborns have some form of chromosomal abnormality, which can be detected by "karyotyping" pre/postnatally.

b. Structural Abnormalities*:* Structural chromosomal rearrangements result from chromosome breakage, with/without subsequent reunion in a different configuration. They can be balanced generally harmless with few effects and unbalanced serious clinical effects. Commonly identified structural abnormalities include translocation, deletion, inversion, ring chromosomes, isochromosomes, mosaicism, and chimerism.

Triple or Quad Screening for Birth Defects

The triple screening measures the amounts of three substances in a pregnant woman's blood: alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), and estriol (uE3). When a test for the hormone inhibin A is added, it's called a quad screening.

The amounts of these hormones help find out the chance that your baby has certain birth defects, such as Down syndrome, spina bifida, or anencephaly. These tests can't show for sure that fetus has a birth defect.A diagnostic test called amniocentesis is needed for confirmation.

The triple or quad screening is usually done at 15 to 20 weeks of pregnancy.

These screening tests look for the amount of:

- Alpha-fetoprotein (AFP).
- Human chorionic gonadotropin (hCG), a hormone made by the placenta.

- Estriol (uE3), a form of estrogen that increases during pregnancy. It is produced in large amounts by the placenta.
- Hormone inhibin A, a protein produced by the fetus and the placenta.

The levels of these substances,along with your age and other factors,to see if fetus has a higher-than-average chance of having a birth defect.

A screening test shows the chance that a baby has a certain birth defect. The accuracy of a screening test is based on how often the test correctly finds a birth defect.

- The triple and quad tests correctly find neural tube defects, such as spina bifida, in 80 out of 100 fetuses who have it and find anencephaly in about 90 out of 100 fetuses. The tests miss spina bifida in 20 out of 100 fetuses who have it and miss anencephaly in 10 out of 100 fetuses.
- The triple test correctly finds Down syndrome in 69 out of 100 fetuses who have it. It misses the condition in 31 out of 100 fetuses.
- The quad test correctly finds Down syndrome in 81 out of 100 fetuses who have it. It misses Down syndrome in 19 out of 100 fetuses.

With the triple or quad test, there is a chance of getting a false-positive test result. This means that the test could show a problem when the baby doesn't have the problem. A false positive may be more likely with the triple screening than the quad screening.A false-positive result can cause stress and lead to unnecessary testing (such as an amniocentesis).

A "positive" result means that there is a higher-than-average chance the fetus birth defect. If the result is "negative," or normal, it means that probably the fetus doesn't have a birth defect.

Cut off value for high risk being lessthan or equal to 1 in 270.

If your result is 1 out of 200 or 1 out of a number less than 200 (such as 1 out of 100),it is positive result and fetus has a higher chance of a birth defect.

Incase of positive test result, the diagnostic test like amniocentesis is needed to find out for sure if there is a problem.

It was noted that the literature is studded with many number of studies on the soft markers of aneuploidy,but most are done on high-risk populations. In order to confirm the value of isolated “soft markers” in low-risk women, prospective studies should be conducted.

Aim and Objectives

AIM & OBJECTIVES

AIM

The purpose of the study is to assess and evaluate for incidence of karyotype abnormalities in fetuses detected to have sonographic abnormalities or triple test positive in high risk mothers.

OBJECTIVES

To determine

- Commonly encountered major fetal abnormalities and soft markers
- Significance of presence and absence of major fetal abnormalities and soft markers
- Occurrence of karyotypic abnormalities with the presence of isolated vs multiple major fetal abnormalities and soft markers
- Significance of markers in high risk and low risk population based on triple screening

Materials and Methods

MATERIALS AND METHODS

This is a prospective cross sectional study with 54 patients. A written "informed consent" was taken from patients before performing Obstetric Ultrasound(As Per PCPNDT Act). Results of first trimester Triple screening was obtained to categorise them into high and low risk group. Screening for fetal anomalies is done in antenatal mothers during 13 to 20 weeks of gestational age. Upon detecting sonographic abnormalities, after counselling and necessary blood investigations, informed consent obtained in Form F And Form G (As Per PCPNDT Act), patients are subjected to amniocentesis, to look for karyotypic abnormalities. Ultrasound guided aspiration of amniotic fluid was done to look for karyotype of the fetus .

Subject selection

Inclusion Criteria

1)Antenatal mothers aged 18 to 38 years,> 16 weeks of gestation with fetal abnormality on USG screening and after triple screening positive .

Exclusion criteria

1) <16 weeks of pregnancy
2) Twin Pregnancy
3) Pregnancy by ART
4) Patient not willing for study
5) severe oligohydramnios

Evaluation of patients

Detailed sonographic evaluation of fetus done using *Hitachi Arietta* USG machine using *1-5MHz curvilinear* probe to look for

- Presence of single or multiple Major fetal abnormalities like Ventriculomegaly, dysgenesis of corpus callosum,CTEV,cleft lip and cleft palate, Endocardial cushion defects,VSD and other complex cardiac anomalies, diaphragmatic hernia ,hydronephrosis and hydrops fetalis.

- Presence of single or multiple soft markers like echogenic bowel,absent nasal bone,mild pyelectasis,thickened nuchal fold,etc.

Technique of USG- Amniocentesis

- Blood Group & Type, HIV/HBV/HCV Status Of The Mother Are Assessed.
- Cefotaxime 1g iv given to AN mother ½ hr prior to procedure.
- Location of placenta is assessed prior to procedure to avoid puncture through the placenta ; hence posterior and lateral location of the placenta are favourable.
- Locate a largest amniotic pocket that is free of fetal parts and umblical cord.
- Under sterile aseptic precautions,under continuous USG monitoring , 15 – 25 ml of amniotic fluid aspirated from the amniotic cavity with precautions

to avoid maternal cells and blood contamination by using 22G spinal needle.

- First few ml of amniotic fluid together with syringe are discarded to avoid maternal and blood contamination.
- Pre and post procedure fetal heart rate are checked.
- Mother instructed to avoid strenuous activity for 24 to 48 hours , to contact physician incase of vaginal bleeding,fever, per vaginal amniotic fluid leak
- Patient advised to review after 2 weeks for karyotyping results.
- Collected sample is sent for cytogenetic analysis.
- Patient reviewed after 2 weeks for amniocentesis result and complications related to procedure.

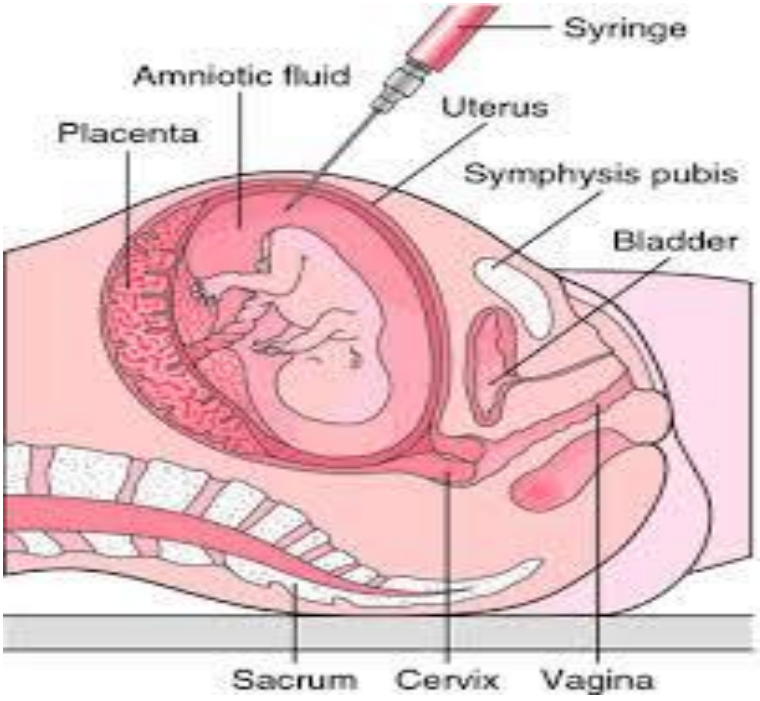


Figure 11 Diagramatic representation of Procedure of Amniocentesis

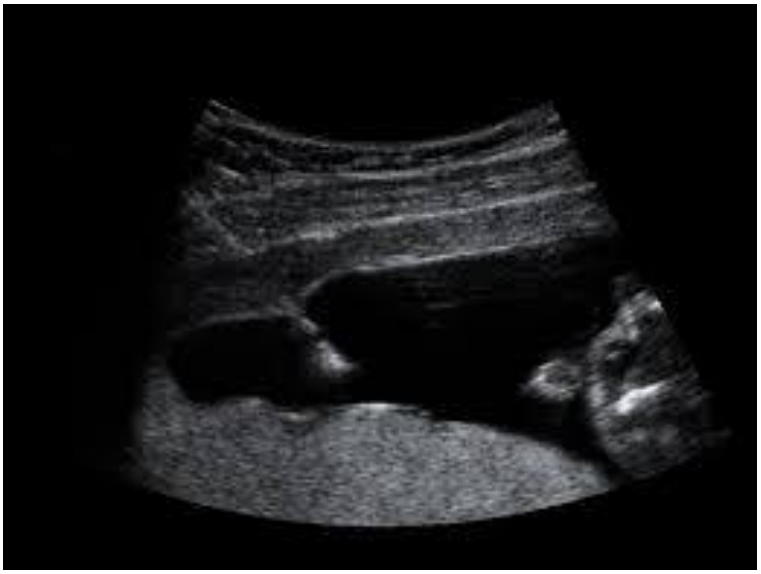
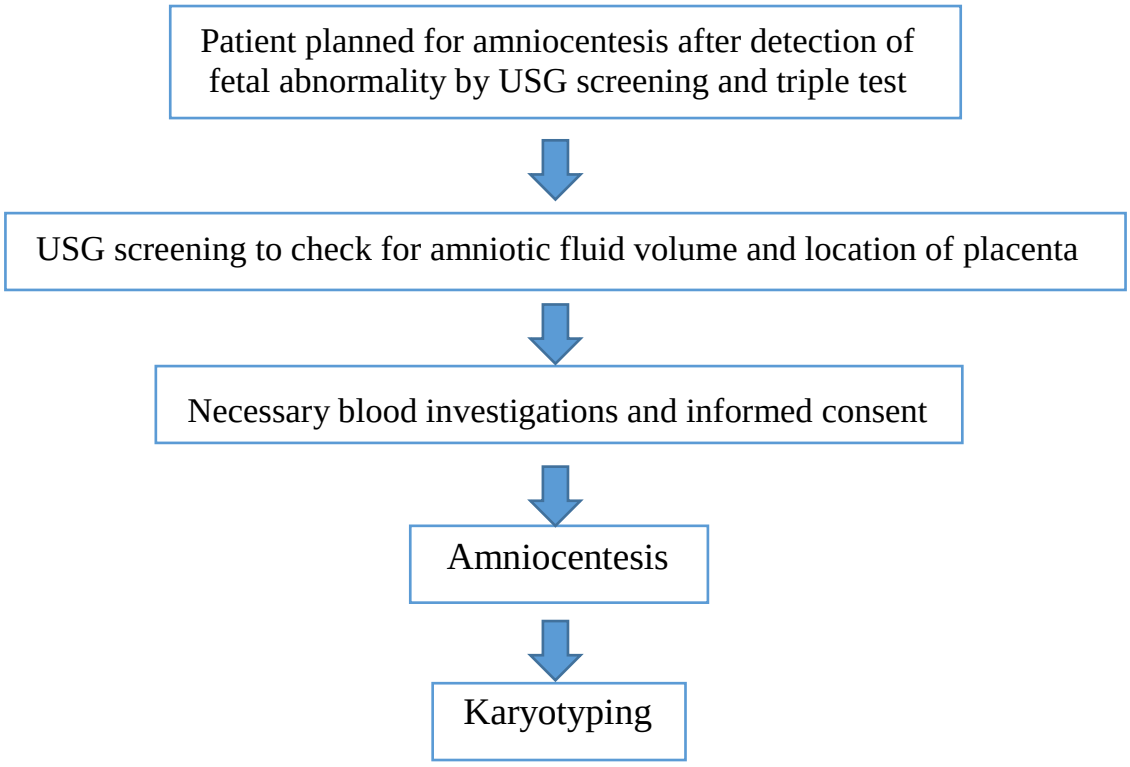


Figure 12 USG showing the procedure of amniocentesis with spinal needle visualised within the amniotic cavity

Line diagram showing the steps involved in the study



Assessment of USG abnormalities and categorisation

- ❖ Ultrasound Findings
  - Presence of single or multiple major fetal abnormality
  - Presence of single or multiple soft markers
- ❖ Triple screening in 1st trimester
  - If Risk </= 1:270,patient is categorized as high risk for trisomy

REPRESENTATIVE CASES

CASE 1 : 31 years aged pt , at 20 weeks of gestation,low risk by triple screening test had bilateral mild pelviectasis on USG screening

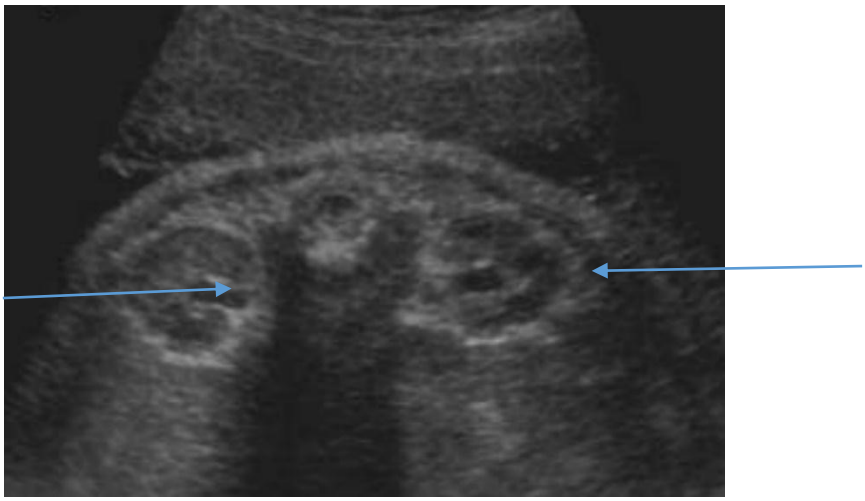


Figure 13 showing bilateral mild pelviectasis in fetus

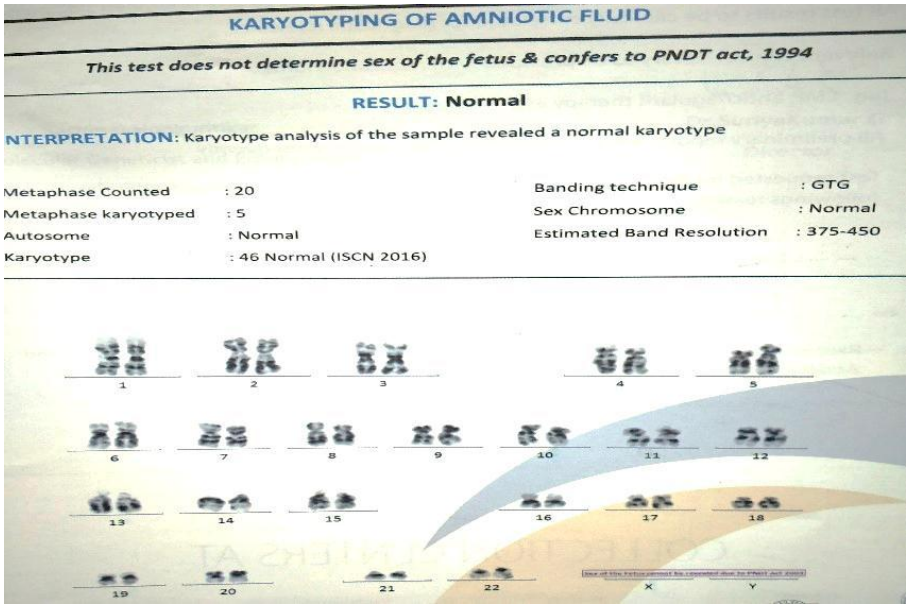


Figure 14 showing the normal karyotype of the fetus by amniotic fluid analysis.

CASE 2 : 17 years aged pt , at 17 weeks of gestation,low risk by triple screening test had limb body wall complex on USG screening

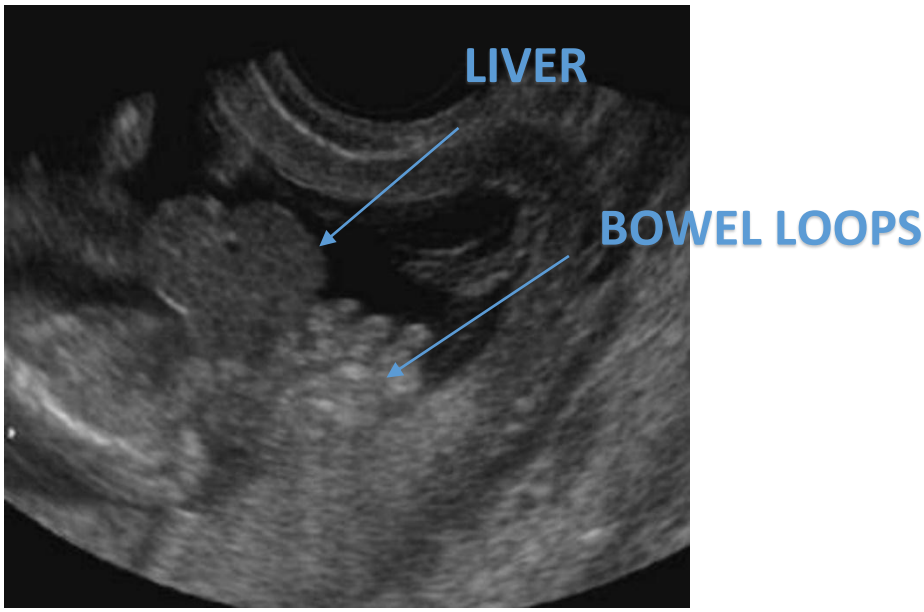


Figure 15 showing herniation of liver and echogenic bowel loops into amniotic cavity

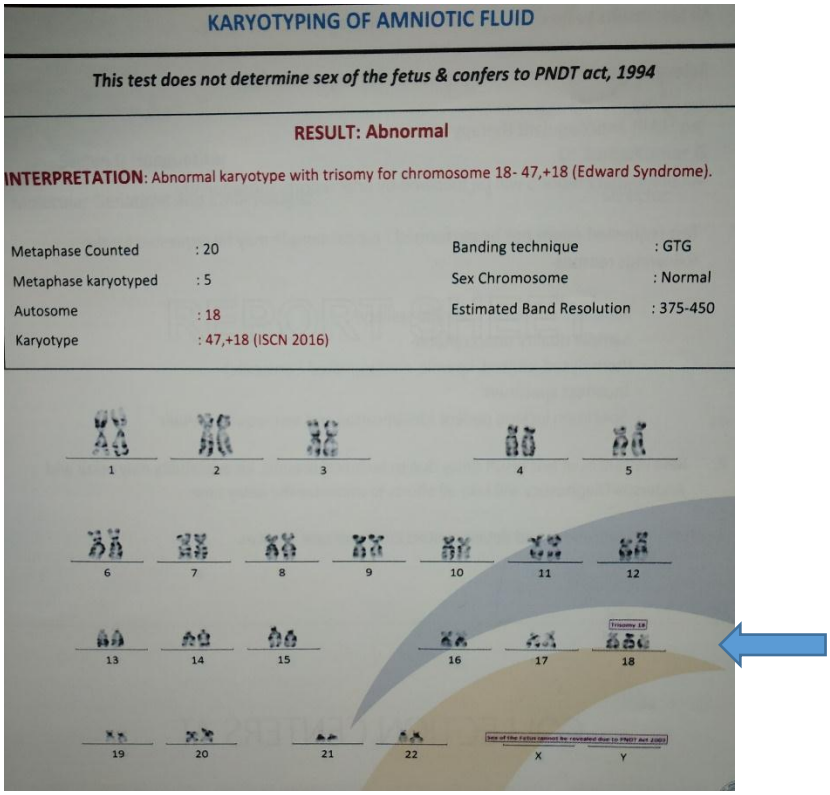


Figure 16 showing the trisomy 18 in the fetus by amniotic fluid analysis.

CASE 3 : 29 years aged pt , at 18 weeks of gestation,low risk by triple screening test had Double outlet left ventricle and unilateral choroid plexus cyst on USG screening

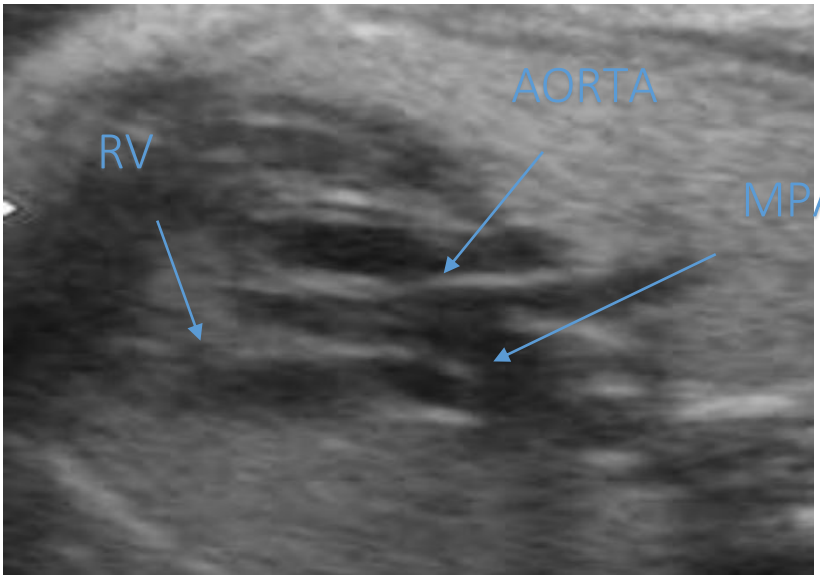


Figure 17 showing both aorta and main pulmonary artery(MPA) arising from the right ventricle(RV).

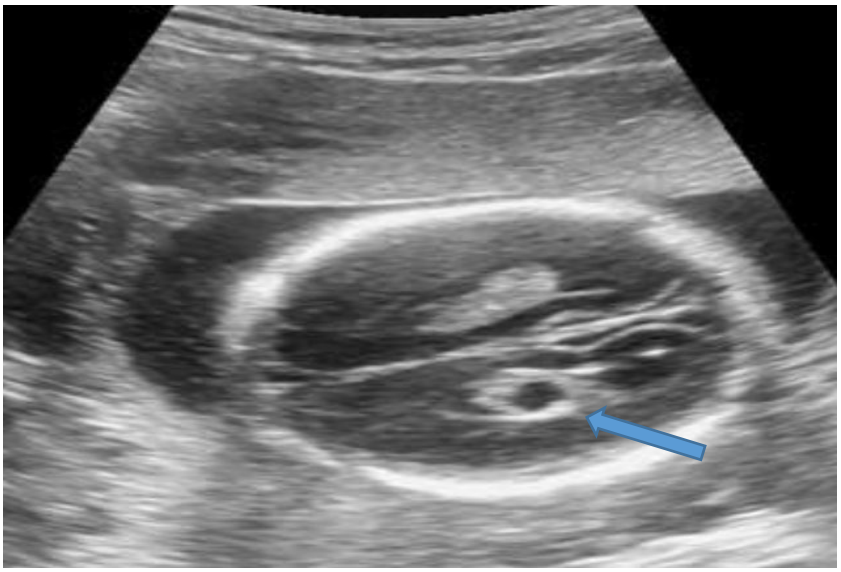


Figure 18 showing choroid plexus cyst within the right lateral ventricle.

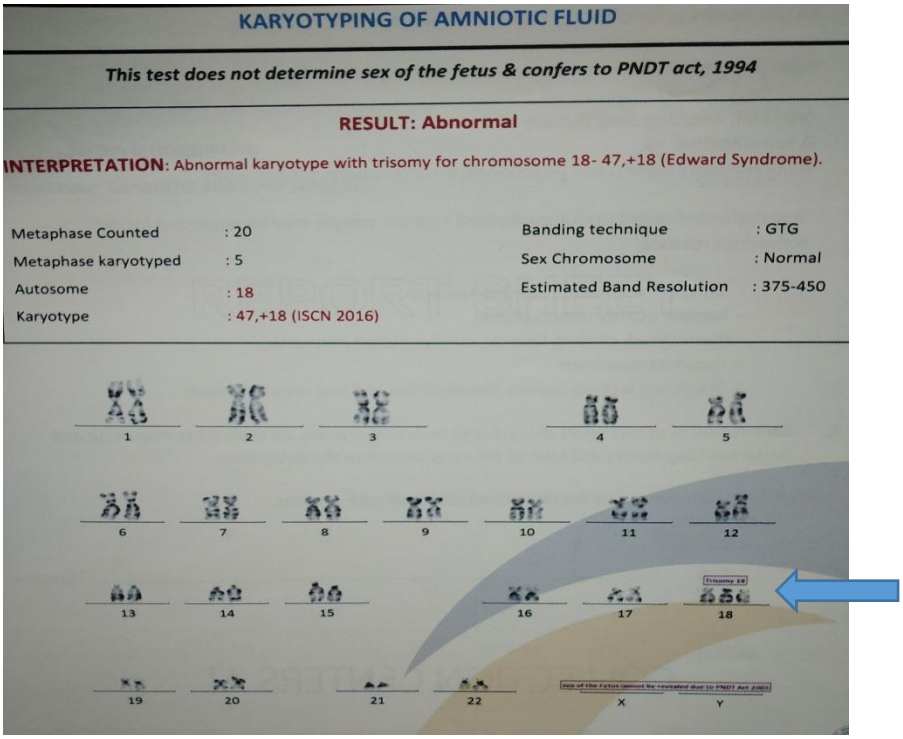


Figure 19 showing the trisomy 18 in the fetus by amniotic fluid analysis.

CASE 4 : 22 years aged pt , at 17 weeks of gestation,low risk by triple screening test had congenital highairway obstruction on USG screening

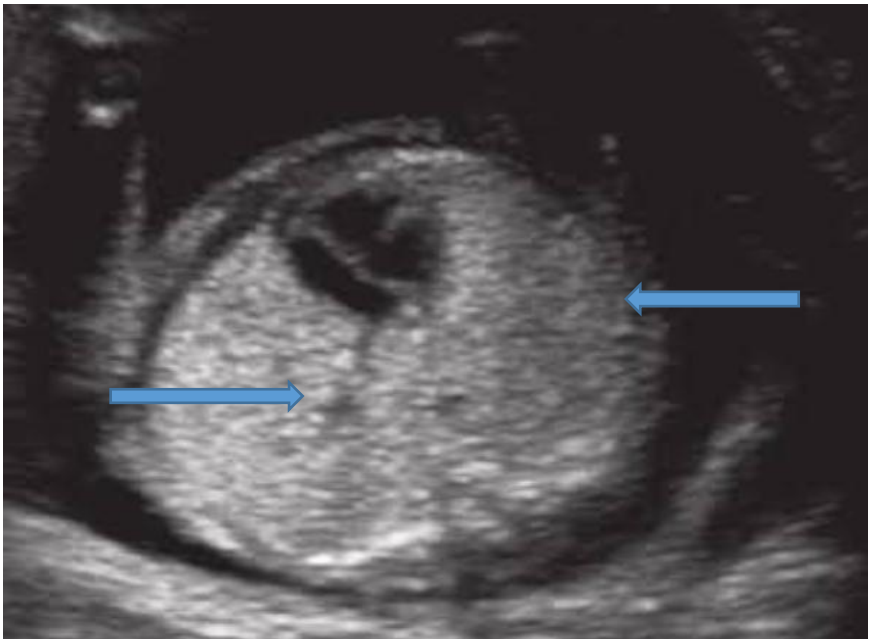


Figure 20 showing bilateral echogenic lungs in a case of congenital high airway obstruction

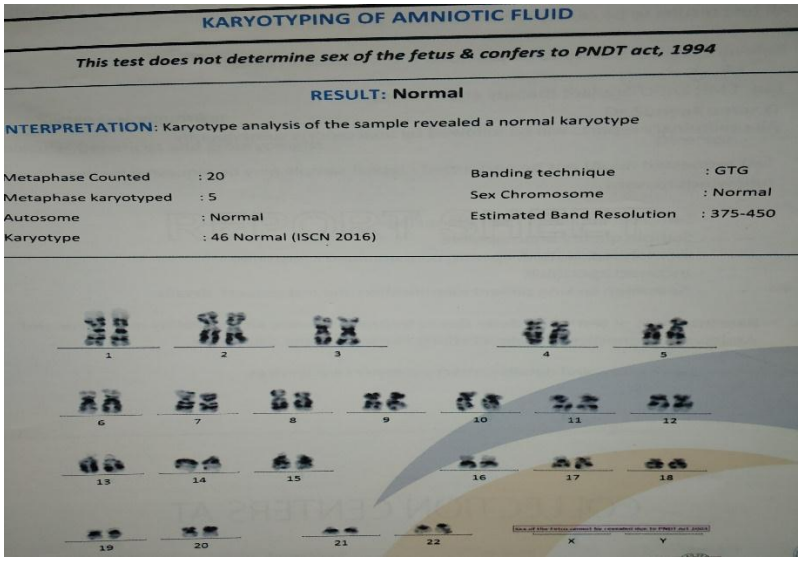


Figure 21 showing the normal karyotype in the fetus by amniotic fluid analysis.

CASE 5 : 23 years aged pt , at 18 weeks of gestation,high risk by triple screening test had absent nasal bone on USG screening

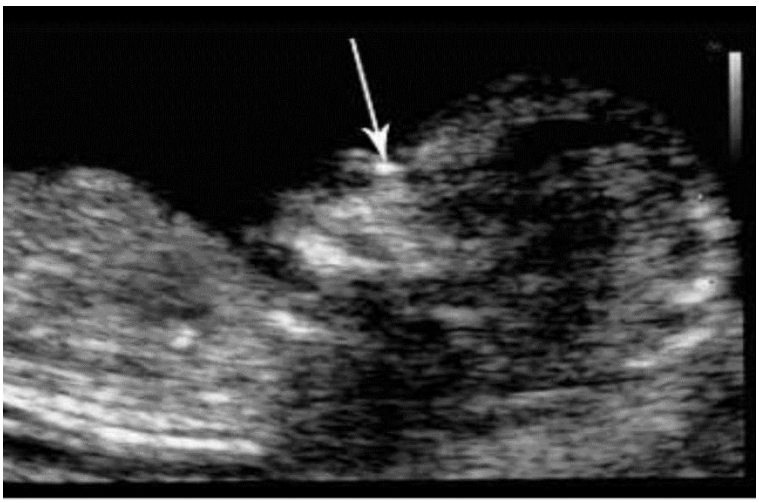


Figure 22 showing normal nasal bone in the fetus

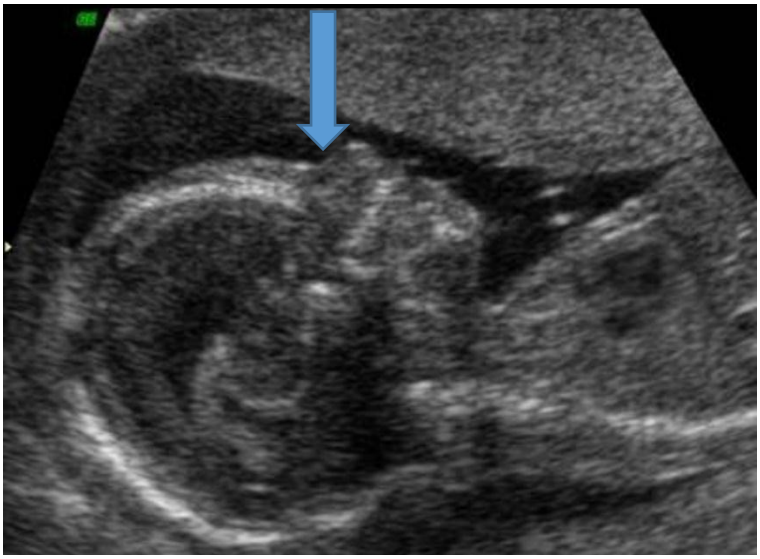


Figure 23 showing absent nasal bone in the fetus

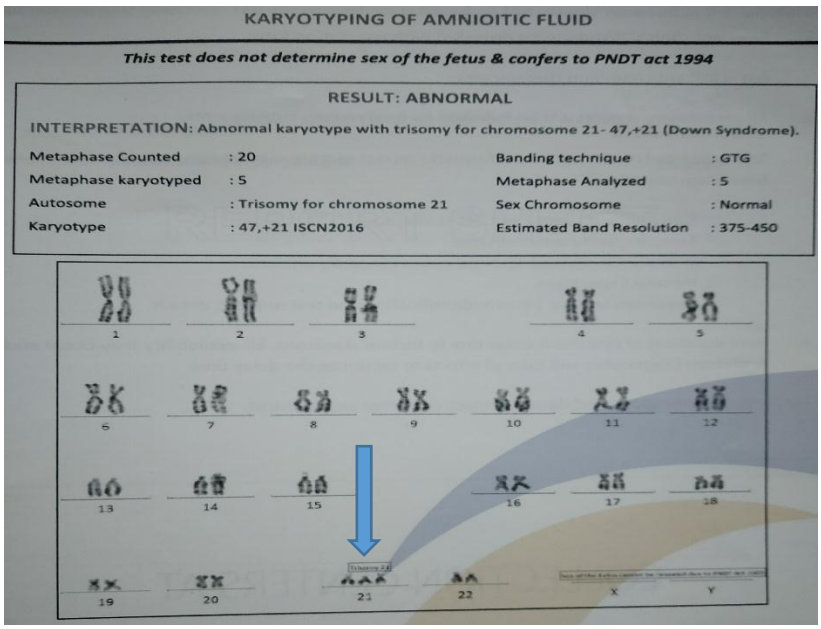


Figure 24 showing the trisomy 21 in the fetus by amniotic fluid analysis.

CASE 6 : 22 years aged pt , at 16 weeks of gestation,low risk by triple screening test had Hydrops fetalis on USG screening

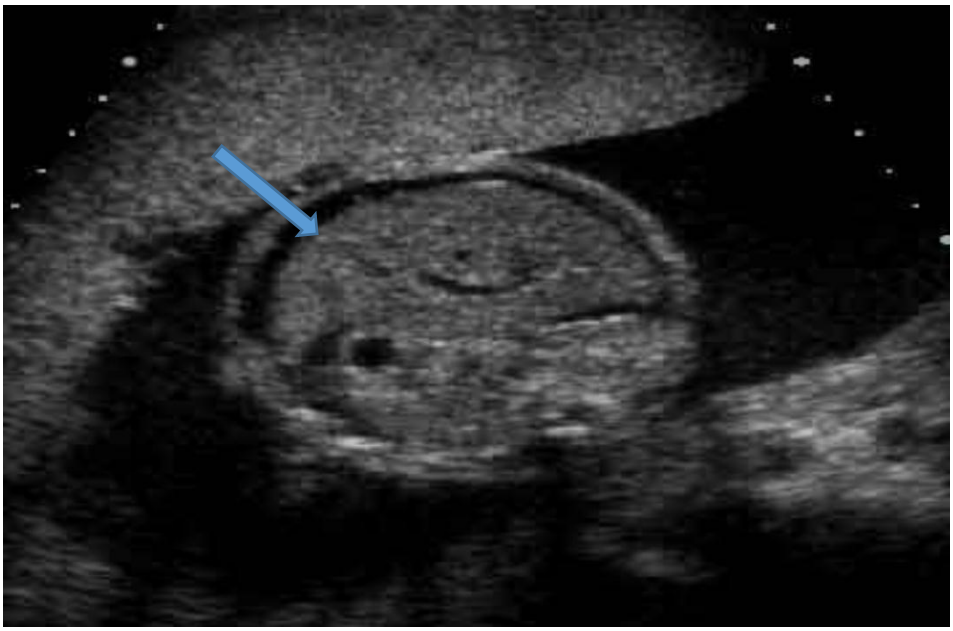


Figure 25 shows ascities in a fetus with hydrops fetalis .

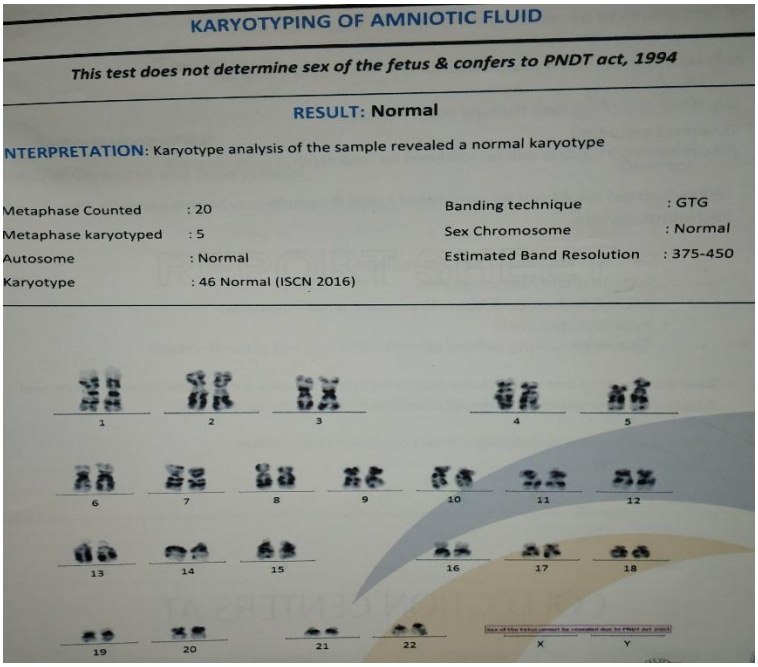


Figure 26 showing the normal karyotype in the fetus by amniotic fluid analysis.

Statistical Analysis

STATISTICAL ANALYSIS

Total number of patients studied is 54.

Age of the patient ranges from 20 to 37 years

Table 2 showing the distribution of gestational age of the patients taken for study

Gestational Age	Frequency (n = 54)	Percentage (%)
16W	8	14.8
17W	13	24
18W	16	29.6
19W	12	22.2
20W	5	9.4

Chart 1 showing the distribution of gestational age of the patients taken for study

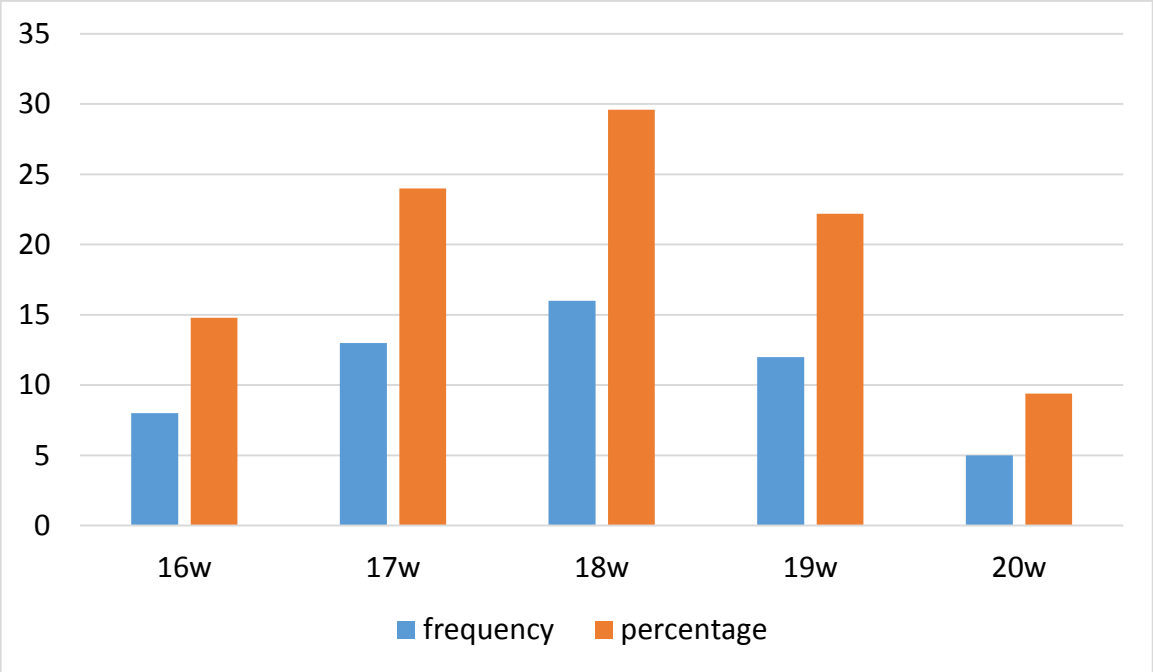


Table 3 showing distribution of major fetal abnormality

Major Abnormality	Frequency (n = 54)	Percentage (%)
Hydrops Fetalis	3	5.5
ACC(Agenesis Of Corpus Callosum)	1	1.8
CHAO(Congenital High Airway Obstruction)	1	1.8
CHD (Congenital Heart Disease)	4	7.4
Flexed Limbs With Limited Movements with club foot	2	3.6
Gross Ventriculomegly	1	1.8
Hypoplastic Cerebellum	1	1.8
Left Cleft Lip And Palate	1	1.8
Limb Body Wall Complex	1	1.8
Rt CDH	1	1.8
Vermis Agenesis	1	1.8

Chart 2 showing distribution of major fetal abnormality

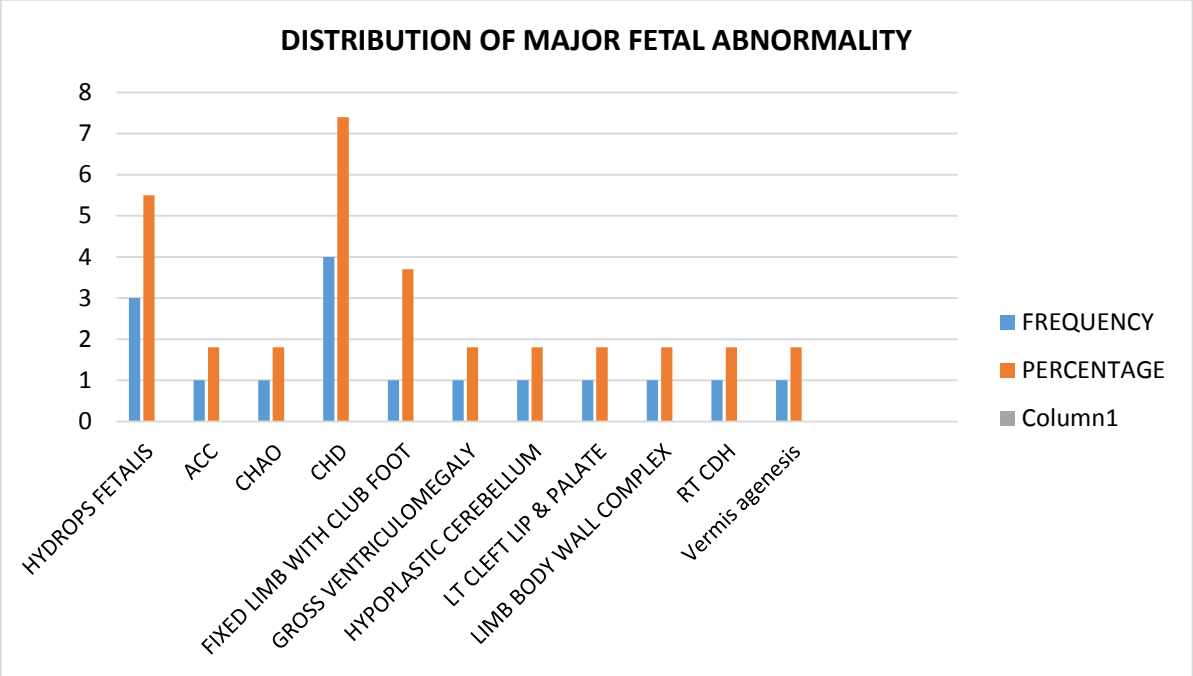


Table 4 showing distribution of soft markers

Soft Markers	Frequency (N = 54)	Percentage (%)
Absent Nasal Bone	21	38.9
Increased NT	21	38.9
Bl Mild Pelviectasis	3	5.6
Bl Choroid Plexus Cyst	2	3.7
Ic Echogenic Foci	2	3.7
Cyst In Neck & Abdomen	1	1.9
Single Umblical Artery	1	1.9

Chart 3 showing distribution of soft markers

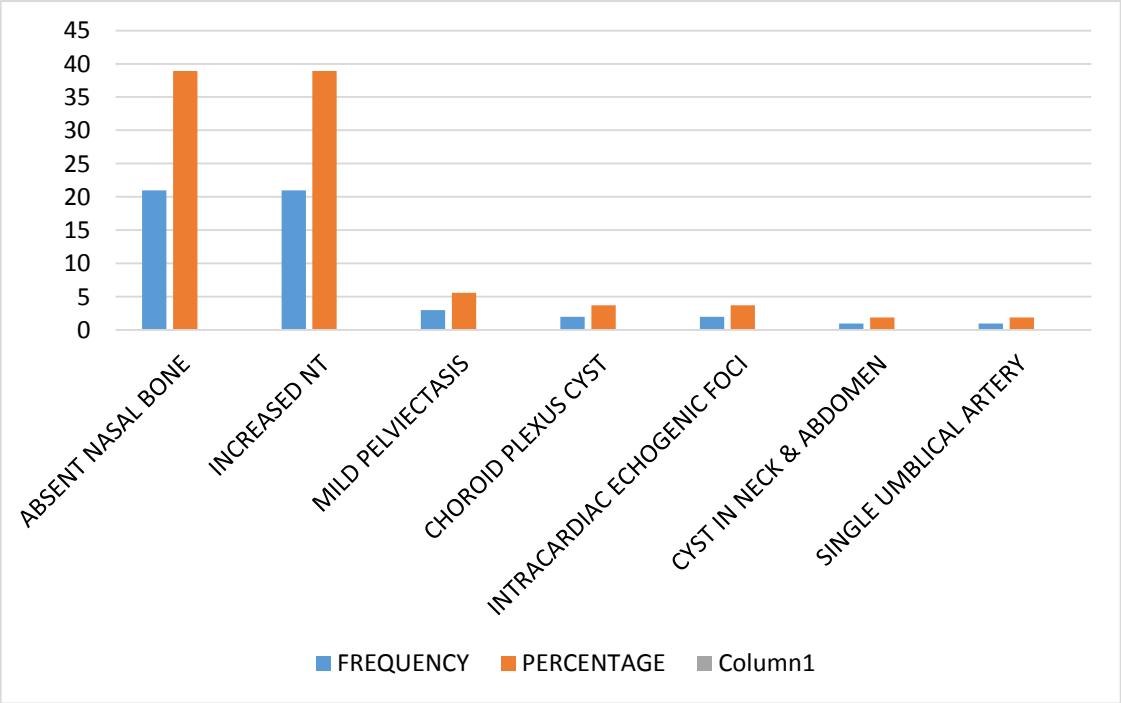


Table 5 showing the distribution of karyotyping

Karotyping	Frequency (n = 54)	Percentage (%)
NORMAL	46	85.2
TRISOMY 18	2	3.7
TRISOMY 21	6	11.1

Chart 4 showing the distribution of karyotyping

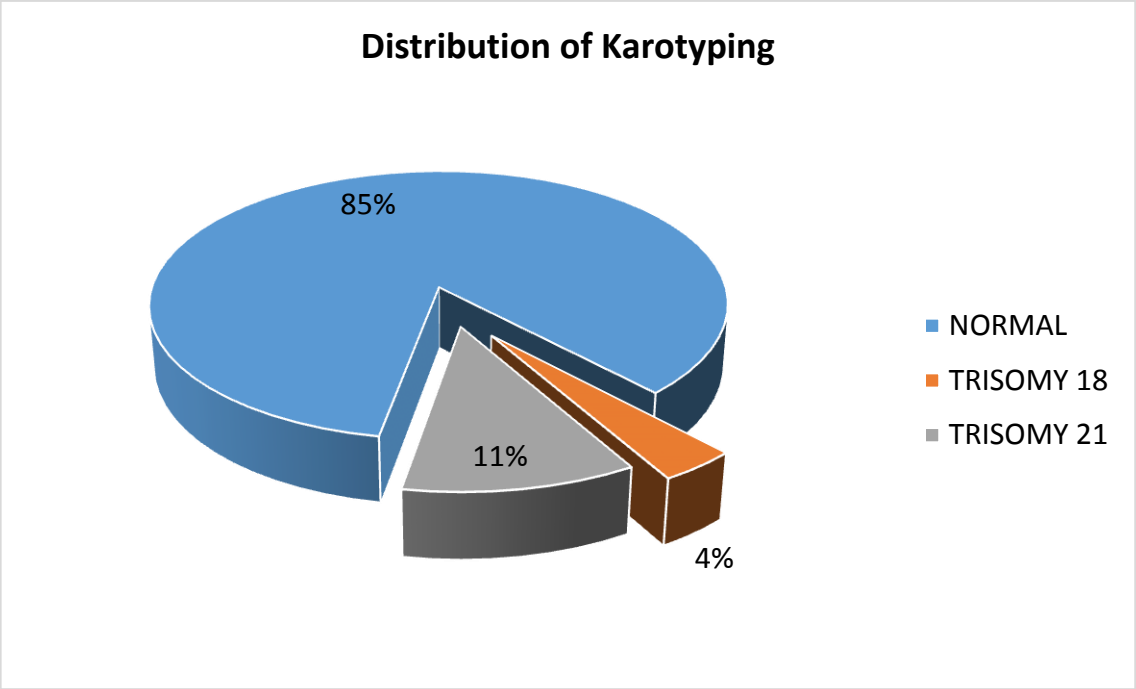


Table 6 showing karyotype of the fetus in the presence Vs absence of major fetal abnormality

Major Abnormality	Karotyping		
	NORMAL (n = 46)	TRISOMY 18 (n = 2)	TRISOMY 21 (n = 6)
Presence of Major Abnormality	8 (17.4)	2 (100)	1 (16.7)
Nil	38 (82.6)	0	5 (83.3)

Chart 5 showing karyotype of the fetus in the presence Vs absence of major fetal abnormality

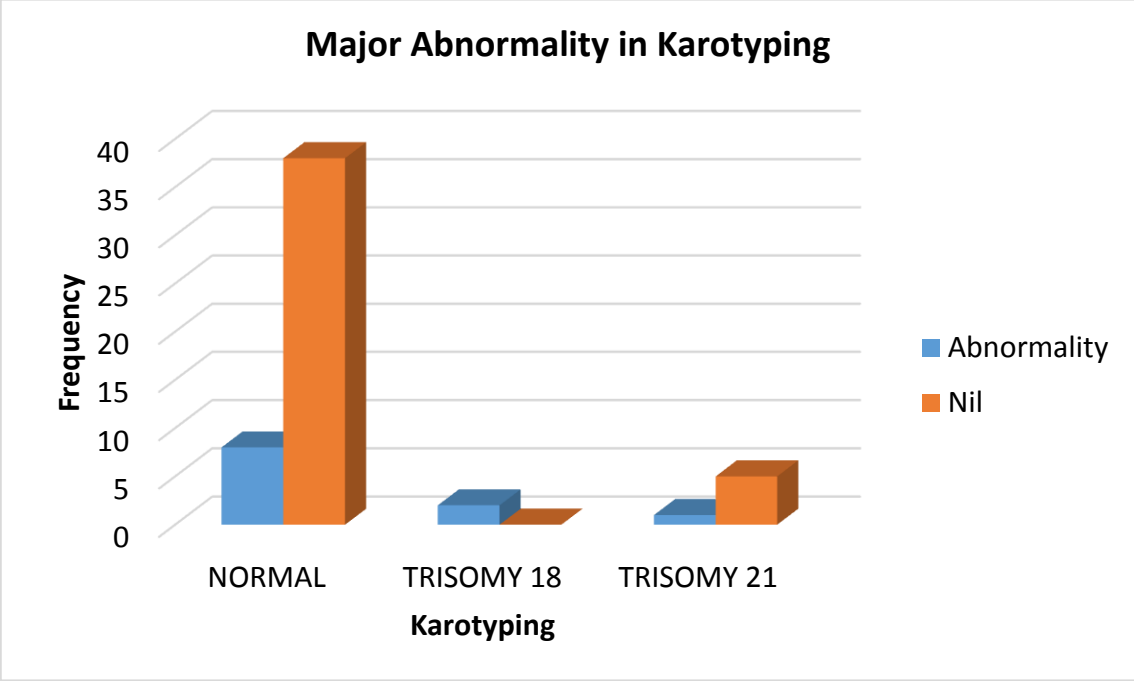


Table 7 showing karyotype of the fetus in the presence Vs absence of soft markers

Soft Maker	Karotyping		
	NORMAL (n = 46)	TRISOMY 18 (n = 2)	TRISOMY 21 (n = 6)
Presence of Soft Marker	41 (89.1)	1 (50.0)	5 (83.3)
Nil	5 (10.9)	1 (50.0)	1 (16.7)

Chart 6 showing karyotype of the fetus in the presence Vs absence of soft markers

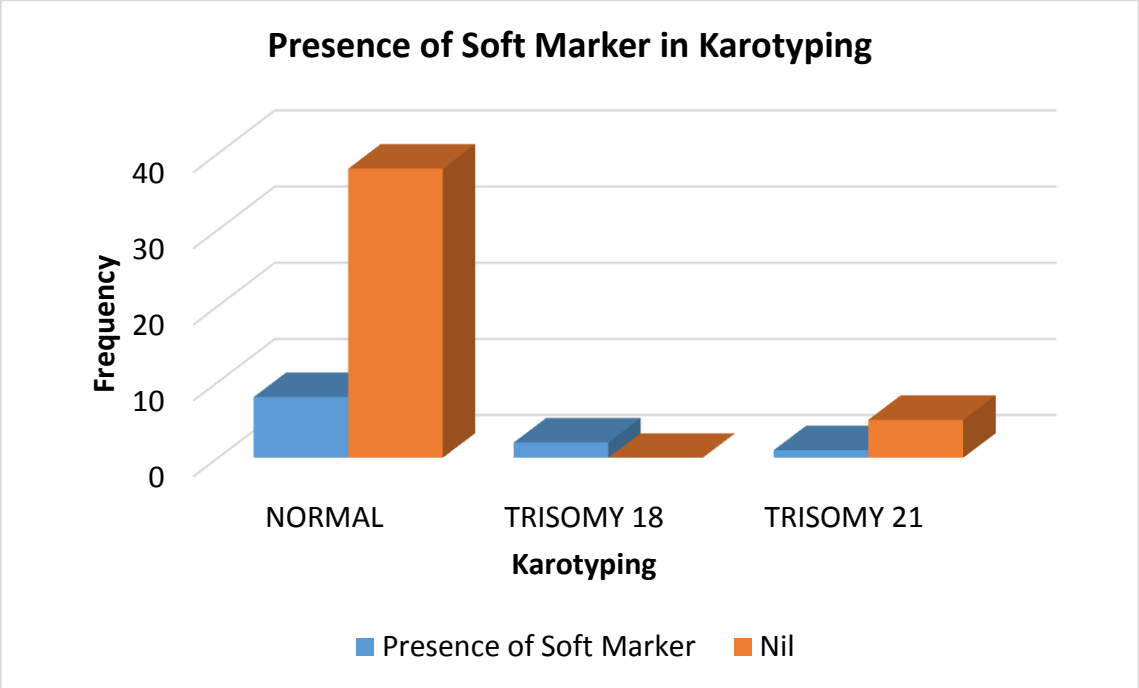


Table 8 showing the karyotype status of the fetuses in the presence of isolated major abnormality,isolated soft markers and in the presence of both.

	Karotyping		
	NORMAL (n = 46)	**TRISOMY 18 (n = 2)**	**TRISOMY 21 (n = 6)**
Presence of only major abnormality	6	2	1
Presence of only soft markers	37	0	4
Presence of both	3	0	1

Chart 7 showing the karyotype status of the fetuses in the presence of isolated major abnormality,isolated soft markers and in the presence of both.

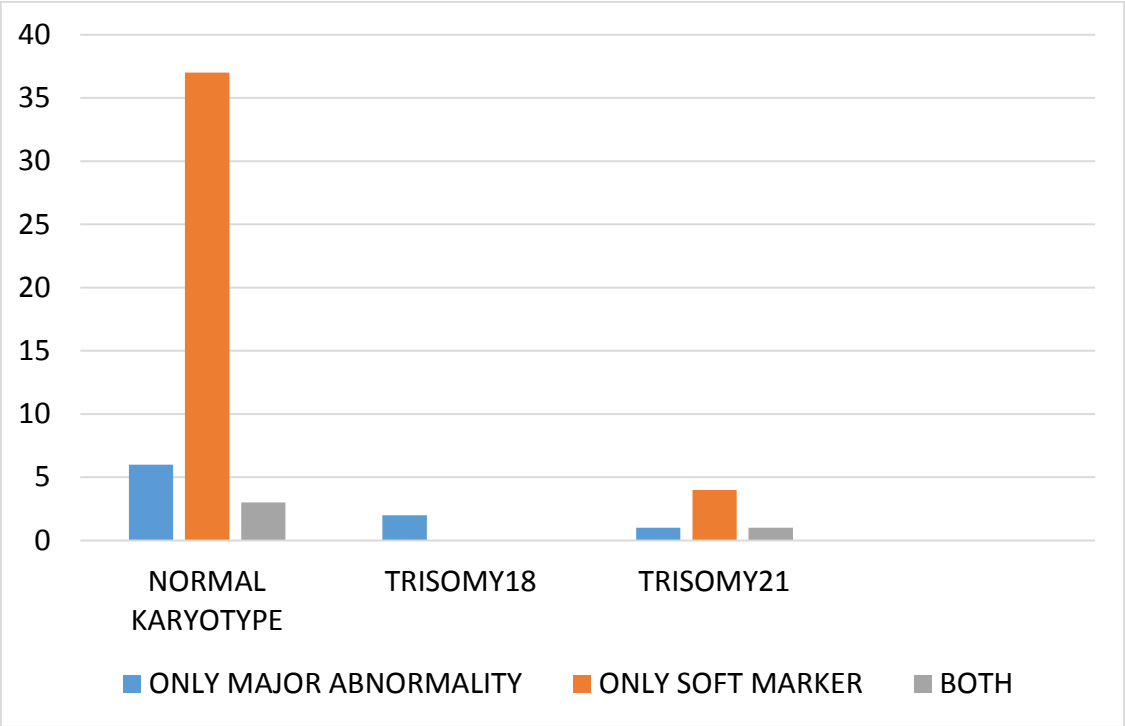


Table 9 showing the karyotype status of the low risk and high risk fetuses

	Karotyping		
	NORMAL (n = 46)	**TRISOMY 18 (n = 2)**	**TRISOMY 21 (n = 6)**
Presence of fetal abnormality in low risk	28	2	4
Presence of fetal abnormality in high risk	18	0	2

Chart 8 showing the karyotype status of the low risk and high risk fetuses.

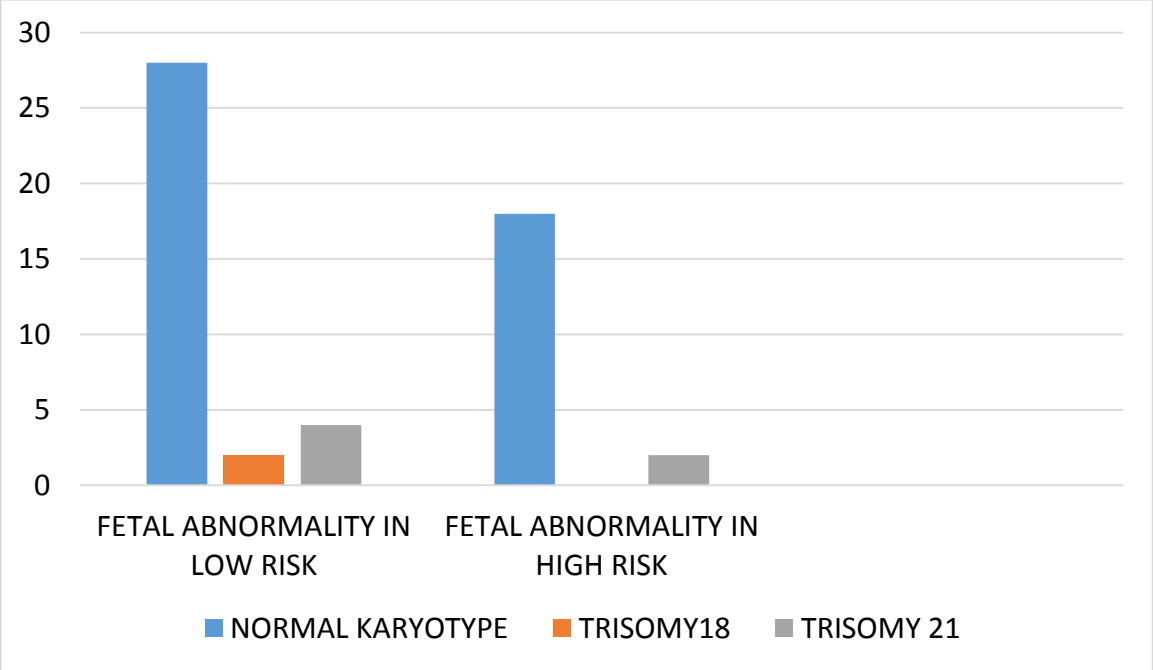


Table 10 Incidence of Karyotyping in with the presence of more than 1 abnormality or soft marker

	Karyotyping		p-value†
	Abnormal N (%)	**Normal N (%)**	
Presence of >1 abnormality or soft marker (n = 8)	3 (37.5)	5 (62.5)	0.050
Presence of 1 abnormality or soft marker (n = 46)	5 (10.9)	41 (89.1)	

† Chi-square test was used to assess the significant difference in the incidence of karyotyping abnormality between subjects with more than 1 major abnormality or soft marker and with the subjects with 1 abnormality or soft marker. The calculate p-value is 0.050 and the incidence of abnormal karyotyping is more in the group with more than 1 abnormality or soft marker (0.375 Vs 0.109).

Therefore, it would be concluded that there is a borderline significant increase in the incidence of abnormal karyotyping in subjects with more than 1 abnormality or soft marker compared to group with 1 abnormality or soft marker

Chart 9 Incidence of Karyotyping in with the presence of more than 1 abnormality or soft marker

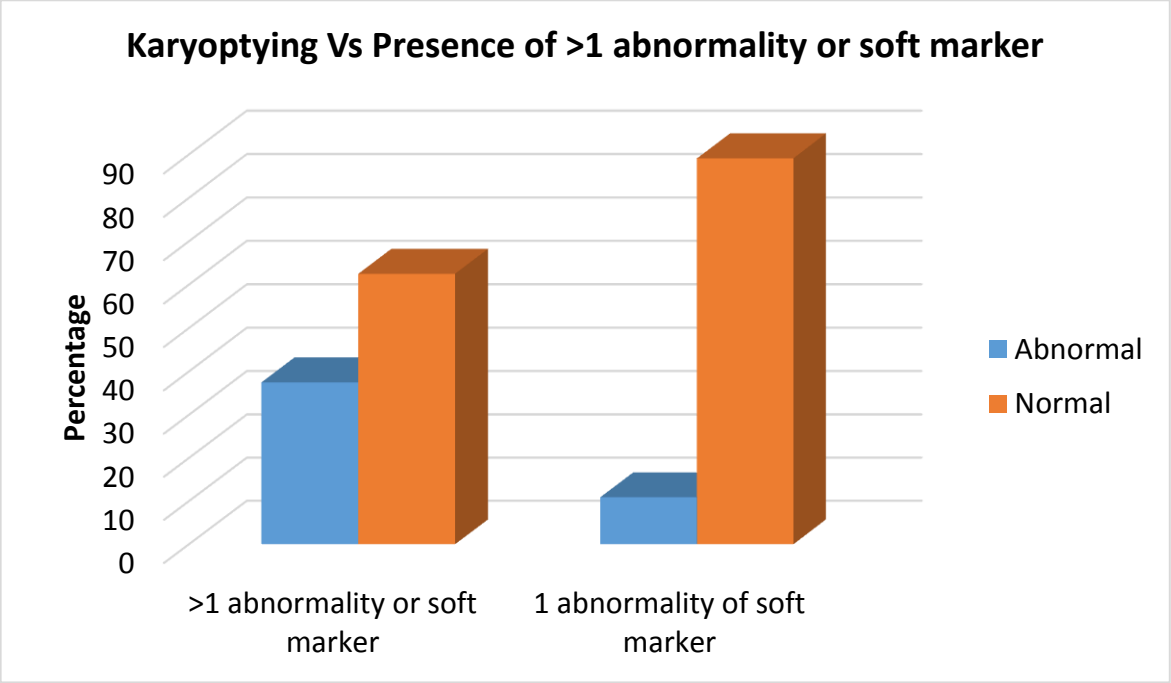


Table 11 & Table 12 showing the sensitivity , specificity , PPV and NPV of major fetal abnormality.

Major Fetal Abnormality	Karyotyping		Total
	Abnormal	Normal	
Major Abnormality	3	8	11
Nil	5	38	43
Total	8	46	54

Table 12

Sensitivity	37.5
Specificity	82.6
PPV	27.3
NPV	88.4

LR + = Sensitivity / 1 – Specificity = 0.375 / 1 – 0.826 = 2.16

LR - = 1 – Sensitivity / Specificity = 1 – 0.375 / 0.826 = 0.76

Table 13 & Table 14 showing the sensitivity , specificity , PPV and NPV of soft markers.

Soft Markers	**Karyotyping**		**Total**
	Abnormal	**Normal**	
Abnormality	6	41	47
Nil	2	5	7
Total	8	46	54

Table 14

Sensitivity	75
Specificity	10.9
PPV	12.8
NPV	71.4

LR + = Sensitivity / 1 – Specificity = 0.75 / 1 – 0.109 = 0.84

LR - = 1 – Sensitivity / Specificity = 1 – 0.75 / 0.109 = 2.29

Conclusion

CONCLUSION

- Commonly encountered major fetal abnormality is congenital heart disease (7.6%); and soft marker is absent nasal bone(38.9%).

- Out of 11 patients ,with major fetal abnormality it is noted that 8(14.8%) have normal and 3(5.5%) have abnormal karyotype respectively ; Out of 43 patients, without any major fetal abnormality , it is noted that 38(70.3%) have normal and 5 (9.2%) have abnormal karyotype respectively and

  Out of 47 patients, with soft markers 41(75.9%) were found to have normal and 6(11.1%) were found to have abnormal karyotype respectively; Out of 11 patients, without soft markers 5(9.2%) were found to have normal and 5(9.2%) were found to have abnormal karyotype respectively.

- Presence of major fetal abnormality and soft marker has a sensitivity of 37.5% and 75%,specificity of 82.6% and 10.8 %,PPV of 27.2% and 12.7% , NPV of 88% and 71.4% respectively.

- The risk of trisomy is 37.5% in the presence of multiple fetal abnormalities and 10.9% with isolated fetal abnormality.

- The risk of trisomy by karyotyping in those with and without increased risk by triple screening is 10% and 11.6% respectively.

Limitations

MERITS

Since most of the studies in literature evaluating the importance of soft markers has been done on low risk and it was stated that in order to confirm the value of isolated “soft markers” in low-risk women prospective studies need to be conducted.

Our study is a prospective study assessing and comparing the importance of soft markers in both low risk and high risk population.

LIMITATIONS

Reviewing the literature, it is noted that the studies on soft markers and aneuploidies were done on a large population involving several thousands. Therefore very low sample size being a major limitation of our study.Most of the analytical methods showed insignifigant results mainly due to inadequate sample size . Comparing the results of our study with studies in the literature also seems inappropriate with this small sample size .

Summary

SUMMARY

- Major fetal anormality has higher specificity and negative predictive value ; soft marker has higher sensitivity and negative predictive value
- Absence of major abnormality and soft marker are associated with reduced risk of trisomy
- Risk of trisomy is higher in the presence of multiple fetal abnormality than with isolated abnormality .
- Presence of fetal abnormality is an independent risk factor for trisomy irrespective of the presence or absence of increased risk by triple screening and invasive testing is mandatory in these patients to rule out karyotypic abnormality.

Aneuploidy cannot be diagnosed or excluded by sonography. Based on a variety of sonographic features,it provides a noninvasive means to adjust the risk of aneuploidy .

The management of each of the fetal abnormality varies but few generalizations can be made. First, the detection of abnormal finding on ultrasound it prompt an immediate detailed ultrasound evaluation of the fetus by an experienced sonographer. Incase there is major fetal abnormality or > 1 abnormal finding on ultrasound, aneuploidy need to be ruled out by amniocentesis.

If a soft marker is detected in isolation, on a second-trimester sonogram, in a patient otherwise considered at low risk for fetal aneuploidy, amniocentesis is not indicated . Sample size being the major limitation of our study , it should be extended to a large population to further evaluate the significance of fetal abnormalities and soft markers in evaluation of aneuploidy.

Biblography

BIBLIOGRAPHY

1. Shipp TD, Benacerraf BR. Second trimester ultrasound screening for chromosomal abnormalities. Prenat Diagn. 2002;22:296–307. [PubMed] [Google Scholar]
2. Adams MM, Erickson JD, Layde PM, Oakley GP. Down's syndrome: recent trends in the United States. JAMA. 1981;246:758–760. [PubMed] [Google Scholar]
3. Thompson M, McInnes R, Willard H. Thompson and Thompson Genetics in Medicine. 5th ed. Philadelphia, Pa: WB Saunders; 1991. [Google Scholar]
4. Nyberg DA, Resta RG, Luthy DA, Hickok DE, Mahony BS, Hirsch JH. Prenatal sonographic findings of Down syndrome: review of 94 cases. Obstet Gynecol. 1990;76:370–377. [PubMed] [Google Scholar]
5. Benacerraf BR, Neuberg D, Bromley B, Frigoletto FD., Jr Sonographic scoring index for prenatal detection of chromosomal abnormalities. J Ultrasound Med. 1992;11:449–458. [PubMed] [Google Scholar]
6. Nicolaides KH, Shawwa L, Brizot M, Snijders RJ. Ultrasonographically detectable markers of fetal chromosomal defects. Ultrasound Obstet Gynecol. 1993;3:56–59. [PubMed] [Google Scholar]
7. MacDonald ML, Wagner RM, Slotnick RN. Sensitivity and specificity of screening for Down syndrome with alpha-fetoprotein, hCG, unconjugated estriol, and maternal age. Obstet Gynecol. 1991;77:63–68.[PubMed] [Google Scholar]
8. The Canadian Early and Mid-trimester Amniocentesis Trial (CEMAT) Group. Randomized trial to assess safety and fetal outcome of early and mid-trimester amniocentesis. Lancet. 1998;351:242–247.[PubMed] [Google Scholar]
9. Tabor A, Philip J, Madsen M, et al. Randomized controlled trial of genetic amniocentesis in 4606 low-risk women. Lancet. 1986;1:1287–1293. [PubMed] [Google Scholar]
10. Benacerraf BR, Gelman R, Frigoletto FD., Jr Sonographic identification of second-trimester fetuses with Down's syndrome. N Engl J Med. 1987;317:1371. [PubMed] [Google Scholar]
11. Winter TC, Uhrich SB, Souter VL, Nyberg DA. The “genetic sonogram”: comparison of the index scoring system with the age-adjusted US risk assessment. Radiology. 2000;215:775–782. [PubMed] [Google Scholar]
12. Nyberg DA, Souter VL, El-Bastawissi A, Young S, Luthhardt F, Luthy DA. Isolated sonographic markers for detection of fetal Down syndrome in the second trimester of pregnancy. J Ultrasound Med. 2001;20:1053–1063. [PubMed] [Google Scholar]
13. Benacerraf BR. Use of sonographic markers to determine the risk of Down syndrome in second-trimester fetuses (editorial) Radiology. 1996;201:619–620. [PubMed] [Google Scholar]

14. Nyberg DA, Luthy DA, Resta RG, Nyberg BC, Williams MA. Age-adjusted ultrasound risk assessment for fetal Down's syndrome during the second trimester: description of the method and analysis of 142 cases. Ultrasound Obstet Gynecol. 1998;12:8–14. [PubMed] [Google Scholar]

15. Stoll C, Dott B, Alembik Y, Roth MP. Evaluation of routine prenatal ultrasound examination in detecting fetal chromosomal abnormalities in a low risk population. Hum Genet. 1993;91:37–41.[PubMed] [Google Scholar]

16. Hill LM. The sonographic detection of trisomies 13, 18, and 21. Clin Obstet Gynecol. 1996;39:831–850. [PubMed] [Google Scholar]

17. Vintzileos AM, Campbell WA, Guzman ER, Smulian JC, McLean DA, Ananth CV. Second-trimester ultrasound markers for detection of trisomy 21: which markers are best? Obstet Gynecol. 1997;89:941–944. [PubMed] [Google Scholar]

18. Nadel AS, Bromley B, Frigoletto FD, Jr, Benacerraf BR. Can the presumed risk of autosomal trisomy be decreased in fetuses of older women following a normal sonogram? J Ultrasound Med. 1995;14:297–302. [PubMed] [Google Scholar]

19. Yeo L, Guzman ER, Day-Salvatore D, Walters C, Chavez D, Vintzileos AM. Prenatal detection of fetal trisomy 18 through abnormal sonographic features. J Ultrasound Med. 2003;22:581–590. [PubMed] [Google Scholar]

20. DeVore GR. Second trimester ultrasonography may identify 77 to 97% of fetuses with trisomy 18. J Ultrasound Med. 2000;19:565–576. [PubMed] [Google Scholar]

21. Stewart T. Screening for aneuploidy: the genetic sonogram. Obstet Gynecol Clin North Am. 2004;31:21–33. [PubMed] [Google Scholar]

22. Bromley B, Lieberman E, Shipp TD, Benacerraf BR. The genetic sonogram - a method of risk assessment for Down syndrome in the second trimester. J Ultrasound Med. 2002;21:1087–1096. [PubMed] [Google Scholar]

23. Benacerraf BR, Barrs VA, Laboda LA. A sonographic sign for the detection in the second trimester of the fetus with Down's syndrome. Am J Obstet Gynecol. 1985;151:1078–1079. [PubMed] [Google Scholar]

24. Benacerraf BR, Frigoletto FD, Jr, Laboda L. Sonographic diagnosis of Down syndrome in the second trimester. Am J Obstet Gynecol. 1985;153:49–52. [PubMed] [Google Scholar]

25. Benacerraf BR, Frigoletto FD., Jr Soft tissue nuchal fold in the second-trimester fetus: standards for normal measurements compared with those in Down syndrome. Am J Obstet Gynecol. 1987;157:1146–1149. [PubMed] [Google Scholar]

26. Gray DL, Crane JP. Optimal nuchal skin-fold thresholds based on gestational age for prenatal detection of Down syndrome. Am J Obstet Gynecol. 1994;171:1282–1286. [PubMed] [Google Scholar]

27. Borrell A, Costa D, Martinez JM, et al. Early midtrimester fetal nuchal thickness: effectiveness as a marker for Down syndrome. Obstet Gynecol. 1996;175:45–49. [PubMed] [Google Scholar]

28. Locatelli A, Piccoli MG, Vergani P, et al. Critical appraisal of the use of nuchal fold thickness measurements for the prediction of Down syndrome. Am J Obstet Gynecol. 2000;182:192–197. [PubMed] [Google Scholar]

29. Bahado-Singh RO, Oz UA, Kovanci E, et al. Gestational age standardized nuchal thickness values for estimating mid-trimester Down's syndrome risk. J Matern Fetal Med. 1999;8:37–43. [PubMed] [Google Scholar]

30. Tannirandorn Y, Manotaya S, Uerpairojkit B, Tanawattanacharoen S, Charoenvidhya D, Phaosavasdi S. Cut-off criteria for second-trimester nuchal skinfold thickness for prenatal detection of Down syndrome in a Thai population. Int J Gynaecol Obstet. 1999;65:137–141. [PubMed] [Google Scholar]

31. Rochon M, Eddleman K. Controversial ultrasound findings. Obstet Gynecol Clin North Am. 2004;31:61–99. [PubMed] [Google Scholar]

32. Nyberg DA, Dubinsky T, Resta RG, Manony BS, Hickok DE, Luthy DA. Echogenic fetal bowel during the second trimester: clinical importance. Radiology. 1993;188:527–531. [PubMed] [Google Scholar]

33. Slotnick RN, Abuhamad AZ. Prognostic implications of fetal echogenic bowel. Lancet. 1996;347:85–87. [PubMed] [Google Scholar]

34. Al-Kouatly HB, Chasen ST, Streltzoff J, Chervenak FA. The clinical significance of fetal echogenic bowel. Am J Obstet Gynecol. 2001;185:1035–1038. [PubMed] [Google Scholar]

35. Al-Kouatly HB, Chasen ST, Karam AK, Ahner R, Chervenak FA. Factors associated with fetal demise in fetal echogenic bowel. Am J Obstet Gynecol. 2001;185:1039–1043. [PubMed] [Google Scholar]

36. Bromley B, Doubilet P, Frigoletto FD, Jr, Krauss C, Estroff JA, Benacerraf BR. Is fetal hyperechoic bowel on second-trimester sonogram an indication for amniocentesis? Obstet Gynecol. 1994;83(5 Pt 1):647–651. [PubMed] [Google Scholar]

37. Berlin BM, Norton ME, Sugarman EA, Tsipis JE, Allitto BA. Cystic fibrosis and chromosome abnormalities associated with echogenic fetal bowel. Obstet Gynecol. 1999;94:135–138. [PubMed] [Google Scholar]

38. Lam YH, Tang MH, Lee CP, Tse HY. Echogenic bowel in fetuses with homozygous alpha-thalassemia-1 in the first and second trimesters. Ultrasound Obstet Gynecol. 1999;14:180–182. [PubMed] [Google Scholar]

39. Benacerraf BR, Nyberg D, Frigoletto FD., Jr Humeral shortening in second-trimester fetuses with Down syndrome. Obstet Gynecol. 1991;77:223–227. [PubMed] [Google Scholar]

40. Nyberg DA, Resta RG, Luthy DA, Hickok DE, Williams MA. Humerus and femur length shortening in the detection of Down's syndrome. Am J Obstet Gynecol. 1993;168:534–539. [PubMed] [Google Scholar]

41. Bahado-Singh RO, Oz AU, Kovanci E, et al. New Down syndrome screening algorithm: ultrasonographic biometry and multiple serum markers combined with maternal age. Am J Obstet Gynecol. 1998;179:1627–1631. [PubMed] [Google Scholar]

42. Benacerraf BR, Cnann A, Gelman R, Laboda LA, Frigoletto FD., Jr Can sonographers reliably identify anatomic features associated with Down syndrome in fetuses? Radiology. 1989;173:377–380. [PubMed] [Google Scholar]

43. Johnson MP, Michaelson JE, Barr M, Jr, et al. Combining humerus and femur length for improved ultrasonographic identification of pregnancies at increased risk for trisomy 21. Am J Obstet Gynecol. 1995;172(4 Pt 1):1229–1235. [PubMed] [Google Scholar]

44. Rodis JF, Vintzileos AM, Fleming AD, et al. Comparison of humerus length with femur length in fetuses with Down syndrome. Am J Obstet Gynecol. 1991;165:1051–1056. [PubMed] [Google Scholar]

45. Smith-Bindman R, Hosmer W, Feldstein V, Deeks J, Goldberg J. Second-trimester ultrasound to detect fetuses with Down syndrome: a meta-analysis. JAMA. 2001;285:1044–1055. [PubMed] [Google Scholar]

46. Stone JL, Eddleman KA, Berkowitz RL. The echogenic intracardiac focus. Contemp Ob/Gyn. 1998;43:73–78. [Google Scholar]

47. Coco C, Jeant P, Jeanty C. An isolated echogenic heart focus is not an indication for amniocentesis in 12,672 unselected patients. J Ultrasound Med. 2004;23:489–496. [PubMed] [Google Scholar]

48. Sotiriadis A, Makrydimas G, Ioannidis JP. Diagnostic performance of intracardiac echogenic foci for Down syndrome: a meta-analysis. Obstet Gynecol. 2003;101:1009–1016. [PubMed] [Google Scholar]

49. Barsoom MJ, Feldman DM, Borgida AF, Esters D, Diana D, Egan JF. Is an isolated fetal cardiac echogenic focus an indication for fetal echocardiography? J Ultrasound Med. 2001;20:1043–1046.[PubMed] [Google Scholar]

50. Anderson N, Jyoti R. Relationship of isolated fetal intracardiac echogenic focus to trisomy 21 at the mid-trimester sonogram in women younger than 35 years. Ultrasound Obstet Gynecol. 2003;21:354–358.[PubMed] [Google Scholar]

51. Winn VD, Joy Sonson BA, Filly RA. Echogenic intracardiac focus potential for misdiagnosis. J Ultrasound Med. 2003;22:1207–1214. [PubMed] [Google Scholar]

52. Filly RA, Benacerraf BR, Nyberg DA, Hobbins JC. Choroid plexus cyst and echogenic intracardiac focus in women at low risk for chromosomal anomalies. J Ultrasound Med. 2004;23:447–449. [PubMed] [Google Scholar]

53. Chitty LS, Chudleigh P, Wright E, Campbell S, Pembrey M. The significance of choroid plexus cysts in an unselected population: results of a multicenter study. Ultrasound Obstet Gynecol. 1998;12:391–397.[PubMed] [Google Scholar]

54. Comstock H. Second-trimester sonography and trisomy 18 - the significance of isolated choroid plexus cysts after an examination that includes the fetal hands. J Ultrasound Med. 2004;23:241–245. [PubMed] [Google Scholar]

55. Ghidini A, Strobelt N, Locatelli A, Mariani E, Piccoli MG, Vergani P. Isolated fetal choroid plexus cysts: role of ultrasonography in establishment of the risk of trisomy 18. Am J Obstet Gynecol. 2000;182:972–977. [PubMed] [Google Scholar]

56. Shields LE, Carpenter LA, Smith KM, Nghiem HV. Ultrasonographic diagnosis of trisomy 18: is it practical in the early second trimester? J Ultrasound Med. 1998;17:327–331. [PubMed] [Google Scholar]

57. Feuchtbaum LB, Currier RJ, Lorey FW, Cunningham GC. Prenatal ultrasound findings in affected and unaffected pregnancies that are screen-positive for trisomy 18: the California experience. Prenat Diagn. 2000;20:293–299. [PubMed] [Google Scholar]

58. Bromley B, Lieberman E, Benacerraf BR. Choroid plexus cysts: not associated with Down syndrome. Ultrasound Obstet Gynecol. 1996;8:232–235. [PubMed] [Google Scholar]

59. Yoder PR, Sabbagha RE, Gross SJ, Zelop CM. The second-trimester fetus with isolated choroid plexus cysts: a meta-analysis of risk of trisomies 18 and 21. Obstet Gynecol. 1999;93:869–872. [PubMed] [Google Scholar]

60. Gupta JK, Cave M, Lilford RJ, et al. Clinical significance of fetal choroid plexus cysts. Lancet. 1995;346:724–729. [PubMed] [Google Scholar]

61. Corteville JE, Kicke JM, Crane JP. Fetal pyelectasis and Down syndrome: is genetic amniocentesis warranted? Obstet Gynecol. 1992;79:770–772. [PubMed] [Google Scholar]

62. Havutcu AE, Nikolopoulos G, Adinkra P, Lamont RF. The association between fetal pyelectasis on second trimester ultrasound scan and aneuploidy among 25, 586 low-risk unselected women. Prenatal Diagn. 2002;22:1201–1206. [PubMed] [Google Scholar]

63. Benacerraf BR, Mandell J, Estroff JA, Harlow BL, Frigoletto F. Fetal pyelectasis: a possible association with Down syndrome. Obstet Gynecol. 1990;76:58–60. [PubMed] [Google Scholar]

64. Ismaili K, Hall M, Donner C, Thomas D, Vermeylen D, Avni FE. Results of systematic screening for minor degrees of fetal renal pelvis dilatation in an unselected population. Am J Obstet Gynecol. 2003;188:242–246. [PubMed] [Google Scholar]

65. Chudleigh PM, Chitty LS, Prembery M, Campbell S. The association of aneuploidy and mild fetal pyelectasis in an unselected population: the result

of multicenter population. Ultrasound Obstet Gynecol. 2001;17:197–202. [PubMed] [Google Scholar]

66. Vintzileos AM, Egan JFX. Adjusting the risk for trisomy 21 on the basis of second-trimester ultrasonography. Am J Obstet Gynecol. 1995;172:837–844. [PubMed] [Google Scholar]

67. Snijders RJ, Sebire NJ, Faria M, Patel F, Nicolaides KH. Fetal mild hydronephrosis and chromosomal defects: relation to maternal age and gestation. Fetal Diagn Ther. 1995;10:349–355. [PubMed] [Google Scholar]

68. Wickstrom E, Maizels M, Sabbagha RE, Tamura RK, Cohen LC, Pergament E. Isolated fetal pyelectasis: assessment of risk for postnatal uropathy and Down syndrome. Ultrasound Obstet Gynecol. 1996;8:236–240. [PubMed] [Google Scholar]

69. Nyberg AS, Bromley B, Frigoletto FD, Benacerraf BR. Can the presumed risk of autosomal trisomy be decreased in fetuses of older women following a normal sonogram? J Ultrasound Med. 1995;14:297–302. [PubMed] [Google Scholar]

70. Vintrileos AM, Uzman ER, Smulian JC, Yeo L, William E, Knuppel RA. Down syndrome risk estimation after normal genetic sonography. Am J Obstet Gynecol. 2002;187:1226–1229. [PubMed] [Google Scholar]

71. Egan JF, Kaminsky LM, DeRoche ME, Barsoom MJ, Bargida AF, Benn PA. Antenatal Down syndrome screening in the United States in 2001: a survey of maternal-fetal medicine specialists. Am J Obstet Gynecol. 2002; 187:1230–1234. [PubMed] [Google Scholar]

72. Benacerraf BR, Nyberg D, Bromley B, Frigoletto FD., Jr Sonographic scoring index for prenatal detection of chromosomal abnormalities. J Ultrasound Med. 1992;11:449–458. [PubMed] [Google Scholar]

73. Benacerraf BR, Nadel A, Bromley B. Identification of second-trimester fetuses with autosomal trisomy by use of a sonographic scoring index. Radiology. 1994;193:135–140. [PubMed] [Google Scholar]

74. Bromley B, Lieberman E, Benacerraf BR. The incorporation of maternal age into the sonographic scoring index for the detection at 14–20 weeks of fetuses with Down's syndrome. Ultrasound Obstet Gynecol. 1997;10:321–324. [PubMed] [Google Scholar]

75. Nyberg DA, Luthy DA, Williams MA, Winter TC. Genetic sonogram: computerized assessment of risk for Down syndrome based on obstetric US findings during the second trimester. Radiology. 1996;201:160.[Google Scholar]

76. Sohl B, Scioscia A, Budorick NE, Moore TR. Utility of minor ultrasonographic markers in the prediction of abnormal fetal karyotype at a prenatal diagnostic center. Am J Obstet Gynecol. 1999;181:898–903. [PubMed] [Google Scholar]

77. Vintzileos AM, Campbell WA, Rodis JF, Guzman ER, Smulian JC, McLean DA. Second-trimester ultrasound (U/S) markers for the detection of

trisomy-21: which markers are best? [abstract] J Ultrasound Med. 1997;16(3 Suppl):66. [Google Scholar]

78. Verdin SM, Economides DL. The role of ultrasonographic markers for trisomy 21 in women with positive serum biochemistry. Br J Obstet Gynaecol. 1998;105:63–67. [PubMed] [Google Scholar]

79. Nyberg DA, Luthy DA, Cheng EY, Sheley RC, Resta RG, Williams MA. Role of prenatal ultrasound in women with positive screen for Down syndrome based on maternal serum markers. Am J Obstet Gynecol. 1995;173:1030–1035. [PubMed] [Google Scholar]

80. Vintzileos AM, Campbell WA, Rodis JF, Guzman ER, Smulian JC, Knuppel RA. The use of second trimester genetic sonogram in guiding clinical management of patients at increased risk for fetal trisomy 21. Obstet Gynecol. 1996;87:948–952. [PubMed] [Google Scholar]

81. Shulman LP, Simpson JL, EliasS: Invasive prenatal genetic techniques, chap 116, In SciarraJ (Ed): Gynecology and Obstetrics,Vol 5. Philadelphia, Pa, JB Lippincott, 1992, pp 1-22 2. SimpsonJL, EliasS: Prenatal diagnosis of genetic disorders,In Creasy RK, Resnick R (Eds): Maternal-Fetal Medicine: Principles and Practice, 2nd Ed. Philadelphia, Pa,WB Saunders, 1989, pp 78-107

82. EliasS, Gerbie AB, SimpsonJL, Nadler HL, Sabbagha RE, Shkolnik A: Genetic amniocentesis in twin gestations. Am J Obstet Gynecol 1980; 138:169173

83. Chueh J, Anderson RL, Goldberg JD, Golbus MS: Prenatal diagnosis in multiple gestation: 20 Years' experience with amniocentesis (Abstr). Am J Obstet Gynecol 1991; 164:420

84. National Institute forChild Health and Development National Registry for Amniocentesis Study Group: Midtrimester amniocentesis for prenatal diagnosis: Safety and accuracy. JAMA 1976; 236:1471-1476

85. Working Party on Amniocentesis: An assessment of the hazards of amniocentesis. BrJ Obstet Gynecol 1978; 85(suppl 2):1-41

86. Simpson JL, Mills JL, Holmes LB, et al: Low fetal loss rates after ultrasound-proved viability in early pregnancy. JAMA 1987; 258:2555-2557

87. Tabor A, Philip J, Madsen M, Bang J, Obel EB, N0rgaard-Pedersen B: Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. Lancet 1986; 1: 1287-1293

88. Canadian Collaborative CVS-Amniocentesis Clinical Trial Group: Multicentre randomised clinical trial of chorion villus sampling and amniocentesisFirst report. Lancet 1989; 1:1-6 89.Rhoads GG, Jackson LG, Schlesselman SE, et al: The safety and efficacy of chorionic villus sampling for early prenatal diagnosis of cytogenetic abnormalities.

90. Simpson JL, Martin AO, Verp MS, Elias S, Patel VA: Hypermodal cells in amniotic fluid cultures: Frequency, interpretation, and clinical significance. Am J Obstet Gynecol 1982; 143:250-258

91. Warburton D: Outcome of cases of de novo structural rearrangements diagnosed at amniocentesis. Prenat Diagn 1984; 4:69-80

92. Benacerraf BR, Greene MF, Saltzman DH, et al: Early amniocentesis for prenatal cytogenetic evaluation. Radiology 1988; 169:709-710

93. Nevin J, Nevin NC, Doman JC, Sim D, Armstrong MJ: Early amniocentesis: Experience of 222 consecutive patients, 1987-1988. Prenat Diagn 1990; 10:79-83

94. Penso CA, Frigoletto FD: Early amniocentesis. Semin Perinatol 1990; 14:465-470

95. Hanson FW, Tennant F, Hune S, Brookhyser K: Early amniocentesis: Outcome, risks, and technical problems at <12.8 weeks. Am J Obstet Gynecol 1992; 166:1707-1711 18. Henry GP, MillerWA: Early amniocentesis. J Reprod Med 1992; 37:396402 19. Henry G, Peakman D, Winkler W, et al: Amniocentesis before 15 weeks instead of chorionic villus sampling for earlier prenatal cytogenetic diagnosis (Abstr). Am J Hum Genet 1985; 37:A219 20. Sandstrom MM, Stryker JM, Frigoletto FD, et al: Early amniocentesis for chromosome analysis: A preliminary experience (Abstr). Am J Hum Genet 1987; 41:A385

96. Burton BK, Nelson LH, Pettenati MJ: False-positive acetylcholinesterase with early amniocentesis. Obstet Gynecol 1989; 74:607-6 10

97. Dunn LK, Godmilow L: A comparison of loss rates for first trimester chorionic villus sampling, early amniocentesis, and mid-trimester amniocentesis in a population of women of advanced age (Abstr). Am J Hum Genet 1990; 47:A273

98. Godmilow L,WeinerS, Dunn LK: Genetic amniocentesis performed between 12 and 14 weeks gestation (Abstr). Am J Hum Genet 1987; 41 :A275

99. Godmilow L,WeinerS, Dunn LK: Early genetic amniocentesis: Experience with 600 consecutive procedures and comparison withCVS (Abstr). AmJ Hum Genet 1988; 43:A234

100. Crandall BF, Hanson FW, Tennant F: Early amniocentesis and amniotic fluid AFP levels (Letter). Lancet 1987; 2:1154

101. Wathen NC, Cass PL, Campbell DJ, Kitau MJ, Chard T: Early amniocentesis: Alphafetoprotein levels in amniotic fluid, extraembryonic coelomic fluid, and matemal serum between 8 and 13 weeks. BrJ Obstet Gynecol 1991; 98:866870

102. EliasS, SimpsonJL, Shulman LP, Emerson D, Tharapel A, Seeley L: Transabdominal chorionic villus sampling for first-trimester prenatal diagnosis. AmJ Obstet Gynecol 1989; 160:879-884

103. Shulman LP, Simpson JL, Felker RE, et al: Transvaginal chorionic villus sampling using transabdominal ultrasound guidance. Prenat Diagn 1992; 12:229234

104. Jackson LG, Zachary JM, Fowler SE, et al: A randomized comparison of transcervical and transabdominal chorionic-villus sampling. N Engl J Med 1992; 327:594-598

105.Brambati B, Oldrini A, Lanzani A, Terzian E, Tognoni G: Transabdominal versus transcervical chorionic villus sampling: A randomized trial. Hum Reprod 1988; 3:811-813

106. MRCWorking Party on the Evaluation of ChorionVillus Sampling: Medical Research Council European Trial of chorion villus sampling. Lancet 1991; 337:1491-1499 32. Firth HV, Boyd PA, Chamberlain P, MacKenzie IZ, Lindenbaum RH, Huson SM: Severe limb abnormalities after chorion villus sampling at 56-66 days' gestation. Lancet 1991; 337:762-763 33. Burton BK, Schulz CJ, Burd LI: Limb anomalies associated with chorionic villus sampling. Obstet Gynecol 1992; 79:726-730 34. Risk of Evaluation ofCVS. Copenhagen, Denmark,World Health Organization/European Regional Office, 1992 35. Froster-Iskenius UG, Baird PA: Limb reduction defects in over one million consecutive livebirths. Teratology 1989; 39:127-135 36. Ledbetter DH, ZacharyJM, Simpson JL, et al: Cytogenetic results from the US Collaborative Study onCVS. Prenat Diagn 1992; 12:317-345 37. Shulman LP, Tharapel AT, Meyers CM, Phillips OP, Simpson JL, EliasS: Direct analysis of uncultured cytotrophoblastic cells from second- and thirdtrimester placentas: An accurate and rapid method for detection of fetal chromosome abnormalities. AmJ Obstet Gynecol 1990; 163(pt 1): 1606-1609 38. BasaranS, Miny P, Pawlowitzki IH, HorstJ, HolzgreveW: Rapid karyotyping for prenatal diagnosis in the second and thirdtrimesters of pregnancy. Prenat Diagn 1988; 8:315-320 39. HolzgreveW, Miny P, BasaranS, FuhrmannW, Beller FK: Safety of placental biopsy in the second and third trimesters (Letter). N Engl J Med 1987; 317:1159

107.Tobias ES, Connor M, Ferguson-Smith M. Essential Medical Genetics. 6 th ed. Wiley Blackwell Science; 1997. p. 57-69 Ciccone R, Giorda R, Gregato G, Guerrini R, Giglio S, Carrozzo R, *et al*. Reciprocal translocations: A trap for cytogenetists? Hum Genet 2005;117:571-82.

108. de Ravel TJ, Balikova I, Thienpont B, Hannes F, Maas N, Fryns JP, *et al*. Molecular karyotyping of patients with MCA/MR: The blurred boundary between normal and pathogenic variation. Cytogenet Genome Res 2006;115:225-30

109.Williams PL. Gray′s Anatomy. 38 th ed. London: Churchill Livingstone; p. 193-211.

110.de Vries BB, Pfundt R, Leisink M, Koolen DA, Vissers LE, Janssen IM, *et al*. Diagnostic genome profiling in mental retardation. Am J Hum Genet 2005;77:606-16.

111.Friedman JM, Baross A, Delaney AD, Ally A, Arbour L, Armstrong L, *et al*. Oligonucleotide microarray analysis of genomic imbalance in children with mental retardation. Am J Hum Genet 2006;79:500-13.

112.Mao X, Lillington D, Child F, Russell-Jones R, Young B, Whittaker S. Comparative genomic hybridization analysis of primary cutaneous B-cell

lymphomas: Identification of common genomic alterations in disease pathogenesis. Genes Chromosomes Cancer 2002;35: 144-55.

113. Mueller RF, Ian D. Young: Emery′s Elements of Medical Genetics. 11 th ed. London: Churchill Livingstone; 2001. p. 84-101.

114. Kumar V, Cotran RS, Robbins SL. Robbin′s Basic Pathology. 7 th ed. Philadelphia: WB Saunders; 2003. p. 120-72.

115. Nora JJ, Fraser FC. Medical Genetics - Principles and Practice. 2 nd ed. Philadelphia: Lea and Febiger Publications; 1981. p. 145-80.

116. Rubin E, Farber JL. Pathology. Philadelphia: JB Lippincott Company; 1988. p. 332-52.

117. Walter JB, Talbot IC. Walter and Israel′s General Pathology. 7 th ed. New York: Churchill Livingstone; 1996. p. 232-51.

118. Schoumans J, Ruivenkamp C, Holmberg E, Kyllerman M, Anderlid BM, Nordenskjöld M. Detection of chromosomal imbalances in children with idiopathic mental retardation by array based comparative genomic hybridisation (array-CGH). J Med Genet 2005;42:699-705.

119. Tompa R, McCallum CM, Delrow J, Henikoff JG, van Steensel B, Henikoff S. Genome-wide profiling of DNA methylation reveals transposon targets of CHROMOMETHYLASE3. Curr Biol 2002;12:65-8.

120. Pinkel D, Albertson DG. Comparative genomic hybridization. Annu Rev Genomics Hum Genet 2005;6:331-54.

121. Cheung SW, Shaw CA, Yu W, Li J, Ou Z, Patel A, *et al*. Development and validation of a CGH microarray for clinical cytogenetic diagnosis. Genet Med 2005;7:422-32.

122. Shaffer LG, Kashork CD, Saleki R, Rorem E, Sundin K, Ballif BC, *et al*. Targeted genomic microarray analysis for identification of chromosome abnormalities in 1500 consecutive clinical cases. J Pediatr 2006;149:98-102.

123. Schaeffer AJ, Chung J, Heretis K, Wong A, Ledbetter DH, Lese Martin C. Comparative genomic hybridization-array analysis enhances the detection of aneuploidies and submicroscopic imbalances in spontaneous miscarriages. Am J Hum Genet 2004; 74:1168-74.

PATIENT INFORMATION SHEET

Name of the Investigator :

Name of the Participant :

We are conducting a study on "**COMPARISON OF SONOGRAPHICALLY DETECTED FETAL DEFECTS WITH KARYOTYPING: CROSS SECTIONAL STUDY"**

- Your cooperation would be valuable for the same
- The privacy of patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part of the study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time. Your decision will not result in any loss of benefits to which you are otherwise entitled.
- The result of the special study may be intimated to you a the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of the investigator Signature of participant

(Dr.R.HARINI)

Date :

PATIENT INFORMED CONSENT FORM

Title of the study: "**COMPARISON OF SONOGRAPHICALLY DETECTED FETAL DEFECTS WITH KARYOTYPING: CROSS SECTIONAL STUDY"**

Name of the Participant: DATE:

AGE: SEX : ID NO:

I have read the information in this form (or it has been read to me).
I have read and understood this consent form and the information provided to me.
I have had the consent document explained to me.
I have been explained about the nature of the study.
I have been explained about my rights and responsibilities by the investigator.
I have been explained that there are no risks associated with my participation in this study.
I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.
I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.
I hereby give permission to the investigators to release the information obtained from me as result of participation in this study. I understand that they are publicly presented.
I have understood that my identity will be kept confidential.
I have had my questions answered to my satisfaction.
I have decided to be in the research study.
By signing this onset it form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

Name :_____ Signature :_____

ஆய்வு தகவல் தாள்

ஆராய்ச்சியின் தலைப்பு :

அல்ட்ராசவுண்ட் மூலம் கண்டறியப்பட்ட கருவில் உள்ள சிசுவின் குறைபாடுகளை, ஆம்னியோசென்டிசிஸ் எனப்படும் பனிக்குடநீர் பரிசோதனையின் மூலம் கண்டறியப்பட்ட குரோமோசோம் குறைபாடுகளுடன் ஒப்பிடுதல் பற்றிய ஆய்வு.

ஆய்வாளர் : **மரு. ரா. ஹரினி**

பங்கேற்பாளர் : **வயது :**

ஆராய்ச்சி மையம் : **பர்னார்டு கதிரியக்க நிறுவனம்,**
இராஜீவ் காந்தி அரசு பொது மருத்துவமனை, சென்னை.

இந்த ஆய்வில் பங்கேற்பதற்காக தாங்கள் அழைக்கப்படுகிறீர்கள். இந்த ஆவணத்தில் உள்ள தகவல்கள் தாங்கள் இந்த ஆய்வில் பங்கேற்க முடிவு செய்துக் கொள்ள உதவும். இதில் ஏதேனும் சந்தேகம் இருந்தால் வெளிப்படையாக கேள்விகளைக் கேட்டு தெரிந்துக் கொள்ளலாம்.

இராஜீவ் காந்தி அரசு பொது மருத்துவமனையில் பர்னார்டு கதிரியக்க நிறுவனத்தில் கர்ப்பிணிப் பெண்களுக்கான அல்ட்ரா சவுண்ட் ஸ்கேன் பிரிவிற்கு வரும் கர்ப்பிணி பெண்களுக்கு ஸ்கேன் செய்து சிசுவின் குறைபாடுகளை, குரோமோசோம் குறைபாடுகளுடன் ஒப்பிட்டு ஆய்வு மேற்கொள்கிறோம்.

அதற்கு உங்கள் பங்களிப்பு எங்களுக்கு பெரிதும் உதவக்கூடும்.

இந்த ஆய்வின் நோக்கம்:

அல்ட்ராசவுண்ட் மூலம் கண்டறியப்பட்ட கருவில் உள்ள சிசுவின் குறைபாடுகளை, ஆம்னியோசென்டிசிஸ் எனப்படும் பனிக்குடநீர் பரிசோதனையின் மூலம் கண்டறியப்பட்ட குரோமோசோம் குறைபாடுகளுடன் ஒப்பிட்டு குறுக்கு வெட்டு ஆய்வு செய்வதாகும்.

ஆய்வு வடிவமைப்பு: குறுக்கு வெட்டு ஆய்வு

ஆய்வு முறைகள்: இந்த ஆய்வில் ஒவ்வொரு கர்ப்பிணி பெண்களுக்கும் அட்ராசவுண்ட் ஸ்கேன் செய்யப்பட்டு, கருவில் உள்ள சிசுவின் குறைபாடுகள் கண்டறியப்பட்டு குறிப்பு எடுக்கப்படும்.

அவ்வாறான குறைபாடுகள் குரோமோசோம் குறைபாடுகளின் வெளிப்பாடாக இருக்கலாம் என்று சந்தேகிக்கும் பட்சத்தில் ஆம்னியோசென்டிசிஸ் முறையில் பனிக்குடநீர் எடுக்கப்பட்டு குரோமோசோம் பரிசோதனை செய்யப்படும்.

இந்த ஆய்விற்காக அல்ட்ராசவுண்ட் ஸ்கேன் செய்யப்படும். மேலும் **Blood Grouping & Typing, HIV / HBV / HCV** போன்ற இரத்த பரிசோதனை மேற்கொள்ளப்படும். இதனை தொடர்ந்து பனிக்குடநீரில் குரோமோசோம் பரிசோதனை செய்யப்படும்.

தங்களது மருத்துவ சிகிச்சை குறித்த தகவல்கள் இரகசியமாக பாதுகாக்கப்படும். ஆய்வின் போதோ அல்லது முடிவுகளை வெளியிடும் போதோ தங்களது பெயரையோ, அடையாளங்களையோ வெளியிடமாட்டோம் என்பதை தெரிவித்துக் கொள்கிறோம்.

இந்த ஆய்வில் பங்கேற்பது உங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்த ஆய்விலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம். இந்த ஆய்வில் பங்கேற்காவிட்டாலும் நீங்கள் வழக்கமான சிகிச்சையை தொடர்ந்து பெறலாம்.

இந்த ஆய்வின் முடிவு தங்களுக்கு ஆய்வின் இறுதியிலோ அல்லது ஆய்வின் போதிலோ தெரியப்படுத்தப்படும்.

ஆய்வாளர் கையொப்பம்

பங்கேற்பாளர் / பாதுகாவலர் கையொப்பம்

தேதி :

சுய ஒப்புதல் படிவம்

ஆராய்ச்சியின் தலைப்பு :

அல்ட்ராசவுண்ட் மூலம் கண்டறியப்பட்ட கருவில் உள்ள சிசுவின் குறைபாடுகளை, ஆம்னியோசென்டிசிஸ் எனப்படும் பனிக்குடநீர் பரிசோதனையின் மூலம் கண்டறியப்பட்ட குரோமோசோம் குறைபாடுகளுடன் ஒப்பிடுதல் பற்றிய ஆய்வு.

பெயர் : வயது : தேதி : உள்நோயாளி எண் :

..... என்பவராகிய நான் இந்த ஆய்வின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக அறிந்து கொண்டேன். எனது சந்தேகங்கள் அனைத்திற்கும் தகுந்த விளக்கம் அளிக்கப்பட்டது. இந்த ஆய்வில் முழு சுதந்திரத்துடன் மற்றும் சுயநினைவுடன் பங்கு கொள்ள சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன். இச்சுய ஒப்புதல் படிவத்தை பற்றி எனக்கு விளக்கப்பட்டது.

இந்த ஆய்வினை பற்றிய அனைத்து தகவல்களும் எனக்கு தெரிவிக்கப்பட்டது. இந்த ஆய்வில் எனது உரிமை மற்றும் பங்கினை பற்றி அறிந்து கொண்டேன்.

இந்த ஆய்வில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில்தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின் வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

இந்த ஆய்வில் கலந்து கொள்வதன் மூலம் என்னிடம் பெறப்படும் தகவலை ஆய்வாளர் இன்ஸ்டிட்யூசனல் எத்திக்ஸ் கமிட்டியினரிடமோ, அரசு நிறுவனத்திடமோ தேவைப்பட்டால் பகிர்ந்து கொள்ளலாம் என சம்மதிக்கிறேன்.

இந்த ஆய்வின் முடிவுகளை வெளியிடும்போது எனது பெயரோ, அடையாளமோ வெளியிடப்பட்டாது என அறிந்து கொண்டேன். இந்த ஆய்வின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்று கொண்டேன்.

இந்த ஆய்விற்காக அல்ட்ராசவுண்ட் ஸ்கேன் பரிசோதனை, **Blood Grouping & Typing, HIV / HBV / HCV** போன்ற இரத்த பரிசோதனை மற்றும் இதனை தொடர்ந்து பனிக்குடநீரில் குரோமோசோம் பரிசோதனைகளை செய்துக்கொள்ள சம்மதிக்கிறேன்.

இந்த ஆய்வில் பங்கேற்கும் பொழுது ஏதேனும் சந்தேகம் ஏற்பட்டால், உடனே ஆய்வாளரை தொடர்பு கொள்ள வேண்டும் என அறிந்து கொண்டேன்.

இச்சுய ஒப்புதல் படிவத்தில் கையெழுத்திடுவதன் மூலம் இதிலுள்ள அனைத்து விஷயங்களும் எனக்கு தெளிவாக விளக்கப்பட்டது என்றும் தெரிவிக்கிறேன் என்று புரிந்து கொண்டேன். இச்சுய ஒப்புதல் படிவத்தின் ஒரு நகல் எனக்கு கொடுக்கப்படும் என்றும் தெரிந்து கொண்டேன்.

பங்கேற்பாளர் / பாதுகாவலர் கையொப்பம் தேதி :

ஆய்வாளர் கையொப்பம் தேதி :

FORM-F

BARNARD INSTITUTE OF RADIOLOGY

RAJIV GANDHI GOVT. GENERAL HOSPITAL AND MADRAS MEDICAL COLLEGE, CHENNAI - 600 003

FORM FOR MAINTENANCE OF RECORD IN CASE OF PRENATAL DIAGNOSTIC TEST / PROCEDURE BY GENETIC CLINIC / ULTRASOUND CLINIC / IMAGING CENTRE

(See Provision to section 4(3), Rule 9(4) and Rule 10 (1A)

Section A : To be filled in for all Diagnostic Procedures / Tests

1. Name and Complete address of the Genetic Clinic / Ultrasound Clinic / Imaging Centre **BARNARD INSTITUTE OF RADIOLOGY CHENNAI.- 3.**
2. Registration No. (Under PC & PNDTAct, 1994) PNA / 1544 / 2001
3. Patient's Name : _____ Age _____
. Total Number of living children: _____
   (a) Number of living sons with age of each living son (in years or months) _____
   (b) Number of living daughters with age of each living daughter (in years or months) _____
5. Husband's / Wife's / Father's / Mother's Name: _____
6. Full Postal Address of the patient with contact Number, if any _____
7. (a) Referred by (full name and address of Doctor(s) / Genetic Counseling Centre) _____
   (Referral slips to be preserved Carefully with form F) (b) Self-Referral by Gynacologist / Radiologist /Registered Medical Practitioner conducting the diagnostic procedures (Referral note with indications and case papers of thepatient to be preserved with Form F) (Self-referral does not mean a client coming to a clinic and requesting for the test of the relative/s requesting for the test of a pregnant woman)
8. Last menstrual period/weeks of pregnancy : _____

Section B : To be filled in for performing non-invasive diagnostic procedures / Tests only

9. Name of the doctor performing the procedure/s:
10. Indication/s for diagnosis procedure (Specify with reference to the request made in the referral slip or in a self-referral note) (Ultrasonography prenatal diagnosis during pregnancy should only be performed when indicated. The following is the representative list of indications for ultrasound during pregnancy) **(Put a "Tick" against the appropriate indication/s for ultrasound)**

i. To diagnose intra-uterine and / or ectopic pregnancy and confirm viability. ii. Estimation of gestational age (dating)

iii. Detection of number of fetuses and their chorionicity

iv. Suspected pregnancy with IUCD in-situ or suspected pregnancy following contraceptive failure / MTP failure. v. Vaginal bleeding / leaking.

vi. Follow-up of cases of abortion. vii. Assessment of cervical canal and diameter of internal os.

viii. Discrepancy between uterine size and period of amenorrhea. ix. Any suspected adnexal or uterine pathology / abnormality.

x. Detection of chromosomal abnormalities, fetal structural defects and other abnormalities and their follow-up

xi. To evaluate fetal presentation and position. xii. Assessment of liquor amnili.

xiii. Preterm labor / preterm premature rupture of membranes.

xiv. Evaluation of placental position, thickness, grading and abnormalities (Placenta praevia, retro placental hemorrhage, abnormal adherence etc.) xv. Evaluation of umbilical cord - presentation, insertion, nuchal encirclement, number of vessels and presence of true knot.

xvi. Evaluation of previous Caesarean Section scars.

xvii. Evaluation of fetal growth parameters, fetal weight and fetal well being. xviii. Color flow mapping and duplex Doppler studies.

xix. Ultrasound guided procedures such as medical termination of pregnancy, external cephalic version etc. and their follow

xx. Adjunct to diagnostic and therapeutic invasive interventions such as chorionic villus sampling (CVS), amniocentesis, fetal blood sampling, fetal skin biopsy, amnio-infusion, intrauterine infusion, placement of shunts etc.

xxi. Observation of intra - partum events. xxii. Medical / Surgical conditions complicating pregnancy. xxiii. Research / Scientific studies in recognized institutions.

11. Procedures carried out (Non-invasive) (put a "Tick" on the appropriate procedure) .

    Ultrasound

    (Important Note: Ultrasound is not indicated / advised / performed to determine the sex of fetus except for diagnosis of sex-linked diseases such as Duchene Muscular Dystrophy, Hemophillia A & B etc.) ii) Any other (specify)
12. Date on which declaration of pregnant woman / person was obtained _____
13. Date on which procedure carried out _____
14. Result of the non-invasive procedure carried out (report in brief of the test including ultrasound carried out) _____
15. The result of pre-natal diagnostic procedures was conveyed to _____ on _____
16. Any indication for MTP as per the abnormality detected in the diagnostic procedures / tests _____

Date :
Place : Chennai

Name, Signature and Registration Number with Seal of Gynaecologist / Radiologist / Registered Medical Practitioner Performing Diagnostic Procedure/s

Section C : To be filled for performing invasive procedure / Tests only

17. Name of the doctor/s performing the procedure/s:_____

18. History of genetic / medical disease in the family (specify):_____Basis of diagnosis ("Tick" on appropriate basis of diagnosis):

    (a) Clinical (b) Bio-Chemical

    (c) Cytogenetic (d) Other (e.g. radiological, ultrasonography etc. - specify)

19. Indication/s for the diagnosis procedure ("Tick" on appropriate Indication/s):

    A. Previous Child / B. Advanced maternal age (35 years)

    Children with: ( ) Chromosomal disorders (ii) Metabolic disorders (iv) Mental Disability C. Mother / Father / Sibling has genetic disease (specify)

    (iii) Congenital anomaly (v) Haemoglobinopathy D. Other (specify)

    (vi) Sex linked disorders (vii) Single gene disorder (viii) Any other (Specify)

20. Date on which consent of pregnant woman / person was obtained in Form G prescribed in PC & PNDTAct, 1994: _____

21. Invasive procedure carried out ("Tick" on appropriate Indication/s):

    i. Amniocentesis ii. Chorionic Villi aspiration iii. Fetal biopsy

    iv. Cordocentesis v. Any other (specify)

22. Any complication/s of invasive procedure (specify)

23. Additional tests recommended (Please mention if applicable)

    (i) Chromosomal studies (ii) Biochemical studies (iii) Molecular studies

    (iv) Pre-implantation gender diagnosis (v) Any other (specify)

24. Result of the procedures/Tests carried out (report in brief of the invasive tests / procedure carried out) _____

25. Date on which procedures carried out: _____

26. The result of pre-natal diagnostic procedures was conveyed to_____on _____

27. Any indication for MTP as per the abnormality detected in the diagnostic procedures / tests _____

கர்ப்பிணியின் ஒப்புதல் கடிதம்

வரிசை எண் :

நான் ..... (கர்ப்பிணியின் பெயர்) எனக்கு அல்ட்ரா சவுண்ட் ஸ்கேன் பரிசோதனை மேற்கொள்ளும்போது, எனது கரு ஆணா, பெண்ணா என அறிய கோரவில்லை என சான்றளிக்கிறேன்.

நாள் :

இடம் : கர்ப்பிணியின் கையொப்பம்

DECLARATION OF THE PERSON UNDERGOING PRENATAL DIAGNOSTIC TEST/PROCEDURE

I, Mrs./Mr_____declare that by undergoing Ultra Sound Prenatal Diagnostic Test, I do not want to know the sex of my foetus.

Signature/Thumb Impression of the person undergoing the Prenatal Diagnostic Test / Procedure

In case of thumb Impression : Identify by (Name)_____Age:_____Sex:_____

Relation (if any)_____Address & Contact No :_____

Signature of a person attesting thumb impression :_____ Date :_____

DECLARATION OF DOCTOR / PERSON CONDUCTING ULTRASONOGRAPHY / IMAGE SCANNING

I, _____ (Name of the person conducting ultrasonography / image scanning) declare that while conducting ultrasonography / image scanning on Ms. _____(name of the pregnant woman), I have neither detected nor disclosed the sex of her foetus to anybody in any manner.

Registration Number with Seal of the Gynaecologist / Radiologist / Registered Medical Practitioner Conducting Diagnostic Procedure

FORM G

[See Rule 10]

FORM OF CONSENT

(For invasive techniques)

I, ... wife/daughter of ..... Age ..... years residing at ..... hereby state that I have been explained fully the probable side effects and after effects of the pre-natal diagnostic procedures.

I wish to undergo the pre-implantation/pre-natal diagnostic technique/test/procedures in my own interest to find out the possibility of any abnormality (i.e. disease/deformity/disorder) in the child I am carrying.

I undertake not to terminate the pregnancy if the pre-natal procedure/technique/test conducted show the absence of disease/deformity/disorder.

I understand that the sex of the foetus will not be disclosed to me.

I understand that breach of this undertaking will make me liable to penalty as prescribed in the Pre-natal Diagnostic Techniques (Regulation and Prevention of Misuse) Act, 1994 (57 of 1994) and rules framed thereunder.

Date
Place

Signature of the pregnant woman.

I have explained the contents of the above to the patient and her companion (Name ..... Address ..... Relationship .....) in a language she/ they understand.

Name, Signature and/Registration number of
Gynaecologist/Medical Geneticist/Radiologist/Paediatrician/
Director of the Clinic/Centre/Laboratory

Date

Name, Address and Registration number of
Genetic Clinic/Institute

SEAL

PROFORMA

STUDY TITLE :

COMPARISON OF SONOGRAPHICALLY DETECTED FETAL DEFECTS WITH KARYOTYPING: CROSS SECTIONAL STUDY

S.NO.

NAME :

AGE / SEX :

OCCUPATION :

ADDRESS :

PRESENTING COMPLAINTS :

CLINICAL EXAMINATION :

USG FINDINGS :

KARYOTYPING RESULT :

ABBREVIATIONS

CPC	-	Choroid plexus cysts
EIF	-	Echogenic intracardiac focus
USG	-	Ultrasonogram
HC	-	Head circumference
CTEV	-	Congenital talipes equina varus
HLHS	-	Hypoplastic left heart syndrome
VSD	-	Ventricular septal defect

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.R.Harini
I Year Post Graduate in MD RD
Barnard Institute of Radiology & Oncology
Madras Medical College
Chennai

Dear Dr.R.Harini.,

The Institutional Ethics Committee has considered your request and approved your study titled **"COMPARISON OF SONOGRAPHICALLY DETECTED FETAL DEFECTS WITH KARYOTYPING: CROSS SECTIONAL STUDY " - NO.05022018**

The following members of Ethics Committee were present in the meeting hold on **06.02.2018** conducted at Madras Medical College, Chennai 3

1. Prof.P.V.Jayashankar :Chairperson
2. Prof.R.Jayanthi,MD.,FRCP(Glasg) Dean,MMC,Ch-3 : Deputy Chairperson
3. Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3 : Member Secretary
4. Prof.N.Gopalakrishnan,MD,Director,Inst.of Nephrology,MMC,Ch : Member
5. Prof.S.Mayilvahanan,MD,Director,Inst. of Int.Med,MMC, Ch-3 : Member
6. Prof.A.Pandiya Raj,Director, Inst. of Gen.Surgery,MMC : Member
7. Prof.Shanthy Gunasingh, Director, Inst.of Social Obstetrics,KGH : Member
8. Prof.Rema Chandramohan,Prof.of Paediatrics,ICH,Chennai : Member
9. Prof. Susila, Director, Inst. of Pharmacology,MMC,Ch-3 : Member
10. Prof.K.Ramadevi,MD., Director, Inst. of Bio-Chemistry,MMC,Ch-3 : Member
11. Prof.Bharathi Vidya Jayanthi,Director, Inst. of Pathology,MMC,Ch-3: Member
12. Thiru S.Govindasamy, BA.,BL,High Court,Chennai : Lawyer
13. Tmt.Arnold Saulina, MA.,MSW., :Social Scientist
14. Thiru K.Ranjith, Ch- 91 : Lay Person

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

6.2.18

Member Secretary – Ethics Committee

URKUND

Urkund Analysis Result

Analysed Document: to check.docx (D57345993)
Submitted: 10/21/2019 9:17:00 AM
Submitted By: hariniramakrishnan10@gmail.com
Significance: 23 %

Sources included in the report:

https://www.medscape.com/viewarticle/518149
https://docplayer.info/93284974-Pertemuan-ilmiah-tahunan-fetomaternal-19.html
https://simdos.unud.ac.id/uploads/file_penelitian_1_dir/6f237da4c7e5749ea902fa139f304a4a.pdf
https://cyberleninka.org/article/n/988057.pdf
https://www.researchgate.net/publication/235655737_Ultrasonographic_soft_markers_of_aneuploidy_in_second_trimester_fetuses
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1681991/
https://www.medscape.com/viewarticle/518149_6
https://www.medscape.com/viewarticle/518149_10

Instances where selected sources appear:

91

CERTIFICATE

This is to certify that this dissertation work titled **"COMPARISON OF SONOGRAPHICALLY DETECTED FETAL ABNORMALITIES WITH KARYOTYPING BY AMNIOCENTESIS: CROSS SECTIONAL STUDY"** of the candidate **DR. R. HARINI** with registration number **201718001** for the award of **M.D. Degree** in the **BRANCH VIII - RADIODIAGNOSIS**. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **23 Percentage** of plagiarism in the dissertation.

Guide and Supervisor sign with seal