Chapter

Prenatal Screening of Aneuploidies

Madhavilatha Routhu and Shiva Surya Varalakshmi Koneru

Abstract

Chromosomal abnormalities includes 1) abnormalities in number of chromosomes which are known as aneuploidies and 2) structural defects like translocations and deletions. In this we will discuss about Aneuploidies The incidence of Aneuploidy is around one in 200 live births. Aneuploidy increases with advancing maternal age. Fetal aneuploidy has been associated with significant pregnancy complications such as growth restriction, congenital malformations and perinatal deaths. Several Major developments are happened in prenatal screening of Aneuploidy especially the introduction of first trimester screen with Nuchal thickness and fetal cell free DNA in maternal plasma and identification of ultrasound markers and biochemical screening in second trimester. In this chapter we will discuss about what are trisomies, why "Down syndrome" is important to detect prenatally, history of "Down syndrome", advances in screening methods biochemical as well as sonographic markers in first and second trimester and the criteria to get those markers. What are the features of trisomy 21, trisomy18 and trisomy13.

Keywords: Aneuploidies 1, "Down syndrome" 2, ultrasound markers 3, Nuchal translucency 4

1. Introduction

Aneuploidies are Trisomy21 ("Down syndrome", T21), Trisomy18 (Edward syndrome, T18), trisomy13 (Patau syndrome, T13), monosomy (turner syndrome, monosomy) and triploidy. "Down syndrome" is more focused than other aneuploidy due to Trisomy 13 and 18 are lethal, do not have very long-term consequences, and almost all cases have major structural abnormalities and can be identified on the basis of these features. Where as in T21 the ultrasound and laboratory findings are subtle and nonspecific. Special effort has to be made to identify these nonspecific features and analyse their importance. Identification of T21 is based on these subtle abnormal structures i.e., ultrasound markers and abnormal biochemistry (low PAPP-A and raised β-HCG). The abortion rate in monosomy X is 98% and Edwards is 86% whereas "Down syndrome" is only 30%. Not only this Downs is the commonest congenital cause of mental disability with long life span and need life-long family support. The incidence is 1in 800 pregnancies. Downs can lead to considerable ill health, although some individual may have only mild problems and can lead relatively normal lives. Having baby with "Down syndrome" is likely to have significant impact on family life. There is currently no known cure. A significant number of parents would opt for terminating such a pregnancy or if they want to continue prior information would benefit for

preparing for such a baby. Downs occur due to non-disjunction type (Errors in meiosis). Translocation type and mosaic type which is rare.

2. History

In 1862 & 1887 Langdon Down noted that common characteristics of patients with trisomy 21 are skin deficient in elasticity, giving the impression of being too large for the body, and face is flat, broad and destitute of prominence. The cheeks are roundish and extended laterally. The eyes are obliquely placed, and internal canthi more than normally distanced from one another. The palpebral fissure is very narrow. The tongue is long, thick and much roughened. The nose is small. In 1987 B Benacerraf [1], told that this loose skin can be seen in mid trimester scan at 20 weeks as a thickening of skin at the back of neck in axial view of skull in trans cerebellar plane which was defined as nuchal fold. After 5 years it was realized that the excess skin of individuals with Down's syndrome can be visualized by ultrasonography as increased nuchal translucency in the third month of intrauterine life [2]. About 75% of trisomy 21 fetuses have increased nuchal translucency (NT) and 60–70% have absent nasal bone.

2.1 History of screening methods

Aneuploidy increases with advancing maternal age. So, increasing the maternal age increases the risk. in the early 1970s, the screening was based only on the association with advanced maternal age. In late 1980s not only maternal age but also found that the concentration of various fetoplacental products in the maternal circulation has taken into account for screening. At 16 weeks of gestation the median maternal serum concentrations of alpha-fetoprotein (AFP), un-conjugated estriol (μE3), human chorionic gonadotropin (HCG) (free- β and total) and inhibin-A in aneuploidy are sufficiently different from normal to allow the use of combinations or some or all of these substances to select high risk group. This method is more effective than maternal age alone. It can identify about 60–70% of the fetuses with T21. In1990s, screening by a combination of maternal age and fetal NT thickness at 11–13 + 6 weeks of gestation was introduced. This method shown to identify about 75–80% of affected fetuses for a screen-positive rate of about 5%. There by,

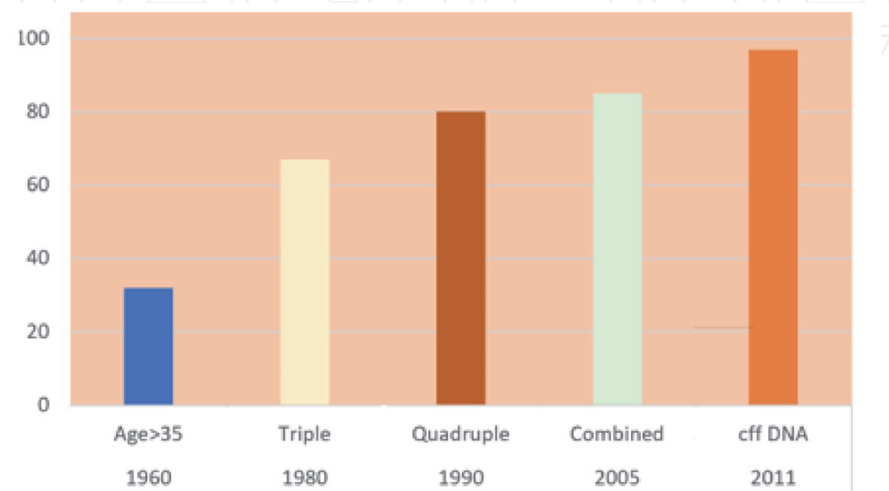

Figure 1.
Aneuploidy screening Approach: observed Detection rates.

maternal age was combined with fetal NT and maternal serum biochemistry (free β-HCG and PAPP-A) in the first-trimester to identify about 85–90% of affected fetuses. In 2001, it was found that 60–70% Trisomy 21 fetuses were associated with non-visualized nasal bone. Inclusion of nasal bone and the other ultrasound markers to NT and biochemistry for the screening procedure increase the detection rate in to more than 95% in first trimester with a screen positive rate of 2.5% (**Figure 1**). Furthermore, introduction of one-stop clinics for assessment of risk (OSCAR) which is a new method of biochemical testing, where with-in 30 min of taking blood sample, made it possible to assess the risk [3, 4].

3. Type of screening tests

"Down syndrome" can be diagnosed during pregnancy. Diagnostic tests are invasive and have an inherent miscarriage rate, however, small they are also expensive. Screening tests can identify a large number of patients who would benefit from diagnostic testing thus reducing risks and costs. Screening tests by definition, cannot identify all accepted pregnancies. First trimester screening is far more effective than later screening. Aneuploidy screening should be offered to all the pregnant women.

Screening tests that are performed in the first and second trimesters include integrated, sequential and contingent screening. The basic types are 1) first trimester combined screening the components in this are Nuchal translucency (NT), PAPP-A and β-HCG. The detection rate is 85–95%. If you add nasal bone and other ultrasound features to this the detection rate increases 93–96%. 2) Triple test the components are β-HCG, MS-AFP and unconjugated Estriol. The detection rate is only 60–65%. 3) Quadruple test β-HCG, MS-AFP, unconjugated Estriol and inhibin A. the detection rate is 70–75%. 4) Penta screen includes hyper glycosylated HCG in addition to quadruple test. If patient come for screening in first trimester, first trimester combined screening is advised, if she comes at 14-20 weeks quadruple test, if she comes at both first and second trimester integrated test is best for screening (**Table 1**).

Integrated test:-Integrate the First trimester PAPP-A, Free β-HCG and NT analyte screening followed by a second trimmester Quad screen and receives a single

Methods of screening	**Detection rate**	**False-positive rate**
Maternal age(MA)	30%	5%
First trimester		
MA+ fetal nuchal translucency(NT)	75–80%	5%
MA+ serum free β-hCG and PAPP-A	60–70%	5%
MA + NT + free β-hCG and PAPP-A (combined Test)	85–95%	5%
Combined Test+ nasal bone or tricuspid flow or ductus venosus flow	93–96%	2.5%
Second trimester		
MA + serum AFP,hCG, μE3(triple test)	60–65%	5%
MA + serum AFP,free β-hCG, μE3, inhibin A(Quadruple test)	70–75%	5%
MA + NT + PAPP-A(11-13 weeks) + quadruple test	90–94%	5%
Nicolaides KH. Screening for fetal aneuplodies at 11t013weeks.Prenat Diagn 2011;31:7–15.		

Table 1.
Methods of screening and its detection rate.

screen test result. The detection rate of this test is 90–94%. Limitations includes the withholding of first trimester screening test results until the second trimester which delay the management option.

Sequential screening: - these are two types one is stepwise another one is contingent model. These methods were developed to maintain a high detection rate. in step wise sequential model it can be achieved by using the combined first and second trimester screening approach while also reporting the patients first trimester screening test risk, which allows for earlier management options. If first trimester test result is higher than lab derived positive screening cutoff, we can offer them the diagnostic test or NIPT, and the screening protocol is discontinued. If the patient has a lower risk can counseled and proceed to quad screening in the second trimester. Sequential screening has a detection rate of 91–93% with a positive screening test result rate of 4–5% [5–7].

Contingent model classifies aneuploidy risk as high, intermediate or low on the basis of first trimester screening test results. High risk patients are offered cell free DNA screening or diagnostic testing with CVS and for low risk women further screening or testing is not recommended. Only those with intermediate risk are offered second trimester screening.

4. Method of sequential screening

Every woman has a risk that her fetus has a chromosomal abnormality.

4.1 Standard first trimester aneuploidy screening

to calculate the individual risk, the clinical information which is necessary to take into account the background or a priori risk, depends on maternal age, weight the ethnicity (in terms of south Asian, east Asian, south east Asian black or Caucasian), IVF, number of fetuses diabetes and smoking. This information should be combined with ultrasound information and biochemistry. Which is based on crown rump length, NT, PAPP-A, free β-HCG. Then make calculation by a series of factors or likelihood ratios, which depend on the results of a series of screening tests carried out during the course of the pregnancy to determine the patient-specific risk. A priori risk established by maternal age has been adjusted successfully by NT screening. This has been one of the most important elements of aneuploidy screening as it resulted in a significant reduction in unnecessary invasive testing on pregnant women with advanced maternal age. If you add rest of the ultrasound features like nasal bone, ductus venosus and tricuspid regurgitation which can increase the rate of detection.

4.2 Standard genetic sonogram aneuploidy screening

Genetic sonogram has been used to screen for Aneuploidy by using specific findings. In this approach seeks major structural abnormalities and minor ultrasonographic soft markers. These Soft markers are minor ultrasound abnormalities, considered as variants of normal, they do not constitute a structural defect. Presence of Soft markers are indicative of an increased age adjusted risk of an underlying fetal aneuploidy or some non- chromosomal abnormalities. So, these are also a priori risk. Detection of soft markers increase the risk for aneuploidy by constant proportion (likelihood ratio LR). Absence of these markers lower the risk (Negative predictive value NPV). These were decided after a meta-analysis study of second trimester markers for trisomy21 [8], (**Table 2**).

Marker	LR+(95%CI)	LR-(95%CI)	LR isolated marker*
Intra cardiac echogenic focus	5.83(5.02–6.77)	0.80(0.75–0.86)	0.95
Ventriculomegaly	27.52(13.61–55.68)	0.94(0.91–0.98)	3.81
Increased nuchal fold	23.30(14.35–37.83)	0.80(0.74–0.85)	3.79
Echogenic bowel	11.44(9.05–14.47)	0.90(0.86–0.94)	1.65
Mild Hydronephrosis	7.63(6.11–9.51)	0.92(0.89–0.96)	1.08
Short humerus	4.81(3.49–6.62)	0.74(0.63-o.88)	0.78
Short femur	3.72(2.79–4.97)	0.80(0.73–0.88)	0.61
ARSA	21.48(11.48–40.19)	0.71(0.57–0.88)	3.94
Absent or hypoplastic nasal bone	23.27(14.23–38.06)	0.46(0.36–0.58)	6.58

Table 2.
Meta-analysis of 2nd trimester markers for trisomy21-M. Agathokleous et al.

Every time a test is carried out the *a priori* risk is multiplied by *the likelihood ratio* of the test to calculate a new risk, which then becomes the *a priori* risk for the next test [9].

If a systematic second- trimester ultrasound examination demonstrates the absence of all major defects and markers, there is a 7.7fold reduction in risk for trisomy 21. Detection of any one of the markers during the scan should stimulate the sonographer to look for all other markers or defects. Post-test odds for trisomy 21 is derived by multiplying the pre-test odds by the positive LR for each detected marker and the negative LR for each marker demonstrated to be absent.

In Sequenitial screening first do the first trimester combined screening test identify the risk based on this risk if it is high risk do the invasive procedure (CVS) or NIPT. If there is false positive and false negetive results then you need to combine with quadraple test and sequentially calcuate the risk as the false positive rate is very very low.

5. Biochemical markers

First trimester markers are pregnancy associated plasma protein A (PAPP-A), Free β Human chorianic gonadotropin (β-HCG) where as second trimester markers are Alpha fetoprotein (AFP) Unconjugated oestriol (μE3), Total human chorianic gonadotropin (HCG) and inhibin-A.

The PAPP-A level is low in T21 which is about half of euploid pregnancies. β-HCG levels are double that of unaffected pregnancies. The concentrations of these markers vary with gestational age. In first trimester PAPP-A increases and free β-HCG decreases. In second trimester AFP and μE3 increase HCG and inhibin-A will decreases before 17 weeks after that it may increas. The measurements of these markers may vary between laboratories. In account of this variation the concentration of each marker is expressed as multiple of median for unaffected pregnancies of the same gestational age (MoM).

6. First trimester sonographic markers

provision of a high-quality first trimester screening service significantly enhances the autonomy of pregnant women [10].

6.1 Nuchal translucency (NT)

The gestation should be 11–13 + 6 weeks and the fetal crown–rump length should be 45–84 mm. Criteria for the Standardized Measurement of the Nuchal translucency at 11–13 + 6 weeks are- fetus must be in the midsagittal plane. The image must be magnified so, that it is filled by the fetal head, neck and upper thorax, the magnification should be as large as possible and each slight movement of the callipers should produce only a 0.1 mm change in the measurement. The fetal neck must be in neutral position, it should not be flexed, and not hyperextended. Amnion must be seen separate from NT line. The margins of NT edges must be clear enough for proper placement of the callipers (**Figure 2**). The + callipers on the ultrasound must be used to perform the NT measurement. Electronic callipers must be placed on the inner borders of the nuchal line space with none of the horizontal crossbar itself protruding into the space and the callipers must be placed perpendicular to the fetal long axis. Measurement must be obtained at the widest space of the NT. Cord round the neck may be present in 5–10% of cases which may produce a falsely increased NT. In such cases, the measurements of NT above and below the cord are different so, the average of these two measurements should be appropriate for calculating risk. One of the studies involving 96,127 pregnancies, at a crown rump length of 45 mm the median and 95th centile was 1.2 and 2.1 mm and the crown rump length of 84 mm were 1.9 and 2.7 mm [11]. The average NT in aneuploidy is about 2.5 mm above the normal median for crown-rump length. In Turner syndrome, the median NT is about 8 mm above the normal median.

6.2 Nasal bone (NB)

It may be present, absent or hypoplastic. In the normal fetus between the 11th and early 12th week of gestation, the nasal bone may appear poorly ossified or absent [12]. In such cases, it is recommended to repeat the measurement one week later [12]. Nasal bone hypoplasia is calculated as BPD/NBL ratio if >11 than hypoplasia. Several studies have demonstrated a high association between absent nasal bone at 11–13 + 6 weeks and trisomy 21, as well as other chromosomal abnormalities [13]. Criteria for the Standardized Measurement of the Nasal Bone at 11–13 + 6 weeks are mid sagittal view of face with the magnification of the image should be such that the fetal head and thorax occupy the whole screen. Mid sagittal face is defined by the presence of the echogenic tip of the nose and rectangular

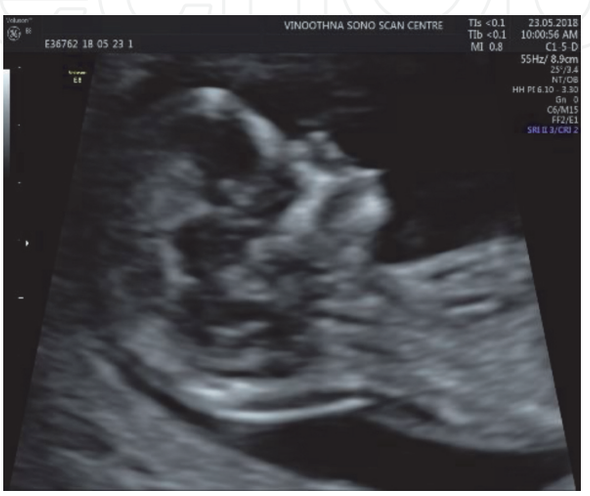

Figure 2.
Normal NT and nasal bone.

shape of the palate anteriorly, the translucent diencephalon in the center, and the nuchal membrane posteriorly. Minor deviations may cause non-visualization of the tip of the nose and visibility of the zygomatic process of the maxilla. The ultrasound transducer should be parallel to the direction of the nose and it should be gently tilted from side to side to ensure that the Nasal bone is seen separate from the skin (**Figure 2**). The echogenicity of NB should be greater than the overlying skin. Three distinct lines are noted in nasal bone demonstration: the first two lines are horizontal and parallel to each other where the top line represents the skin and bottom line is the NB. Third one represents the tip of the nose. When the NB line appears as a thin and less echogenic than the overlying skin, which suggests that the NB is not yet ossified, and it is classified as being absent (**Figure 5**) [12].

6.3 Ductus venosus (DV)

Criteria for the Standardized Measurement of DV at 11–13 + 6 weeks are the magnification of the image should be such that the fetal head and thorax should occupy the whole screen. Right ventral mid sagittal view of fetal trunk should be obtained. Color flow mapping of umbilical vein DV and fetal heart should be

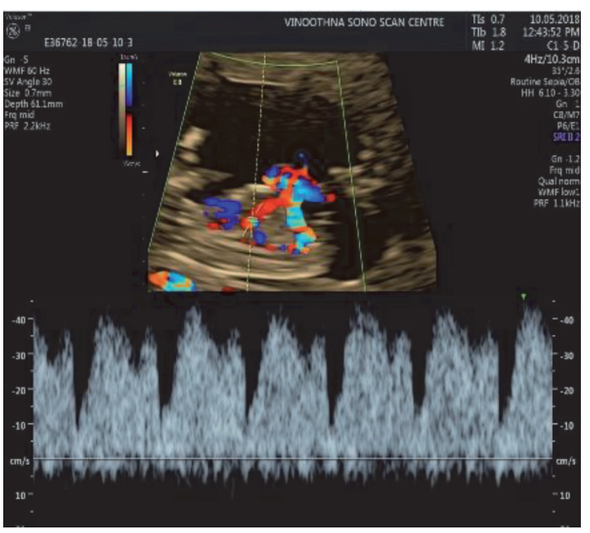

Figure 3.
Normal ductus venosus.

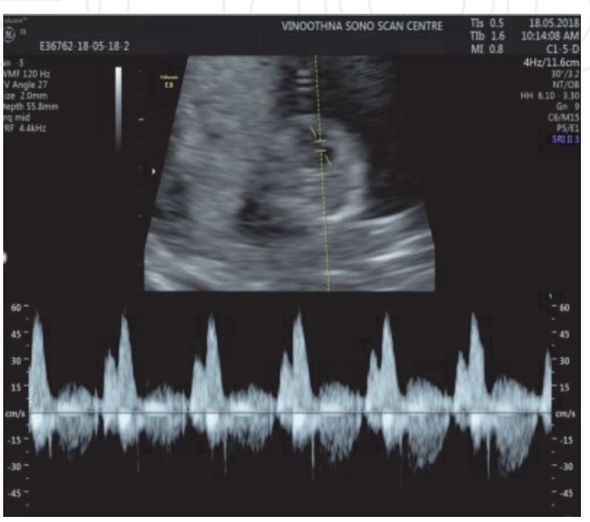

Figure 4.
Normal tricuspid valve.

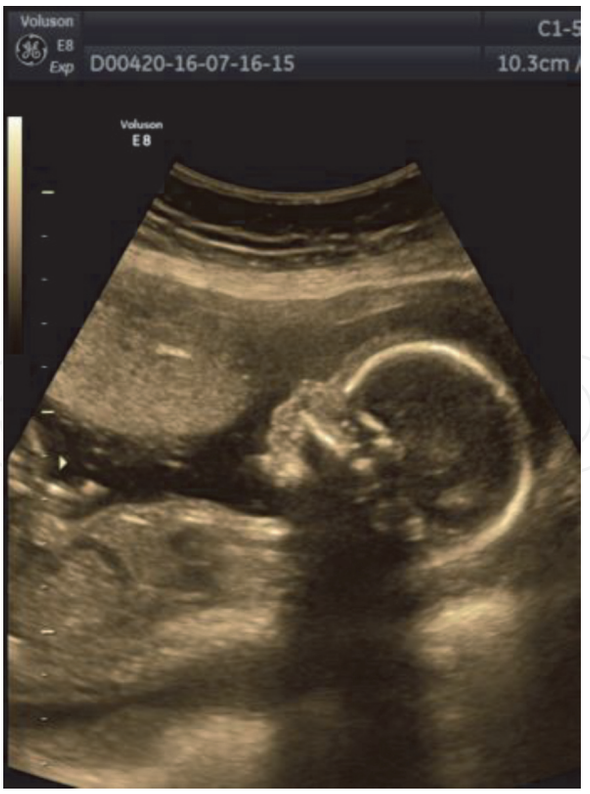

Figure 5.
Absent nasal bone.

demonstrated. Pulse doppler sample volume should be small (0.5–1.0 mm) and it should be placed in the yellowish aliasing area. Insonation angle should be less than 30degrees [12]. The filter should be set at a low frequency (50-70 Hz). Sweep speed should be high (2-3 cm/s) so that the waveforms are spread allowing better assessment of the A wave (**Figure 3**). Ductus venosus shows biphasic wave form with low pulsatility and antegrade flow in the diastolic components (a wave) throughout cardiac cycle. Normal ductus venosus Doppler waveforms show a positive a-wave, whereas the presence of an absent or reversed a-wave defines abnormal ductus venosus waveforms. The presence of high pulsatility or reverse flow of the a-wave in the first trimester increases the risk for chromosomal anomalies, cardiac defects, and the occurrence of twin-twin transfusion syndrome in monochorianic twins. Abnormal flow in the ductus venosus in about 80% of trisomy 21 fetuses and in about 5% of chromosomally normal fetuses [13].

6.4 Tricuspid Valve

Color and pulsed Doppler examination across the tricuspid valve is commonly used in the first trimester to assess for the presence of tricuspid valve regurgitation (TR). The presence of TR in the first trimester has been associated with chromosomal abnormalities [14, 15]. In the first trimester, TR is found in less than 5% of chromosomally normal fetuses, in more than 65% of fetuses with trisomy 21, and in more than 30% of fetuses with trisomy 18 [14]. Interrogation of other cardiac valves with color or pulsed Doppler is reserved for fetuses at risk for valve obstruction or when a cardiac malformation is suspected. Criteria for tricuspid valve evaluation at 11–13 + 6 weeks are- image should be such that the fetal thorax occupies most of the image (**Figure 4**). heart should be in apical position. Sample volume should be 2-3 mm should be positioned across the tricuspid valve with an angle should be less than 30 degrees from the direction of the interventricular septum. Significant TR is

defined when regurgitation is more than half of the systole with velocity of >60 cm/s. The sweep speed should be 2-3 cm/s so that the wave forms are widely spread for better assessment. The tricuspid valve could be in sufficient in one or more of its three cusps, so, therefore the sample volume should be placed across the valve at least three times in an attempt to interrogate the complete valve [12].

6.5 Hepatic artery

It has been reported that high peak velocities in the hepatic artery are present in the first trimester in fetuses at risk for trisomy 21.

7. Second trimester soft markers

They are absent nasal bone, Aberrant subclavian artery, ventriculomegaly, increased Nuchal fold, Echogenic bowel loops, mild hydronephrosis, echogenic intra cardiac foci, short femur short humerus, choroid plexus cysts, single umbilical artery.

Major or minor abnormalities are found in about 75% of fetuses with trisomy 21 and in 10–15% of chromosomally normal fetuses. The Genetic sonogram is a targeted ultrasound looking for major abnormalities as well as minor markers for aneuploidy. Over the years these minor markers are being looked into and things like widened pelvic angle sandal gap deformity is going out of favour and is getting replaced by ARSA, pre nasal thickness and FMF angle. Absence of these markers decreases the risk of downs by around 70–80% but does not completely rule out Downs and hence Absence gives additional reassurance to the patient.

In first step when a soft marker is identified thoroughly search for other soft markers and structural abnormalities. In second step calculate the risk of aneuploidy based on likelihood ratios. This risk is calculated against background risk based maternal age alone or in combination with First trimester combined screening or second trimester quadruple test.

7.1 Increased nuchal fold

In second and third trimesters of pregnancy, abnormal accumulation of fluid behind the fetal neck can be known as nuchal cystic hygroma or nuchal edema. In about 75% of fetuses with cystic hygroma, there is a chromosomal abnormality and, in about 95% of cases, the abnormality is Turner syndrome. Chromosomal abnormalities are found in about one-third of the fetuses of nuchal edema and, in about 75% of cases, the abnormality is trisomy 21 or 18. Edema is also associated with fetal cardiovascular and pulmonary defects, skeletal dysplasia, congenital infections and metabolic and haematological disorders; The positive LR is 23.3 and negative LR is 0.8. Nuchal index is considered by some, because this is associated with gestational age. Nuchal index is (mean nuchal fold/mean BPD) x100 where the value of 11 or greater has a sensitivity of 50% and specificity of 96% (**Figure 6**).

7.2 Aberrant right subclavian artery (ARSA)

occurs in 0.5to 1.4%. four vessels arise from the aortic arch where the right subclavian artery arises from distal part of the aortic arch and courses behind the oesophagus and trachea to the right upper arm (**Figure 7**). ARSA is present in 1% of euploid fetuses and 24% of trisomy 21. ARSA is associated with other conotruncal

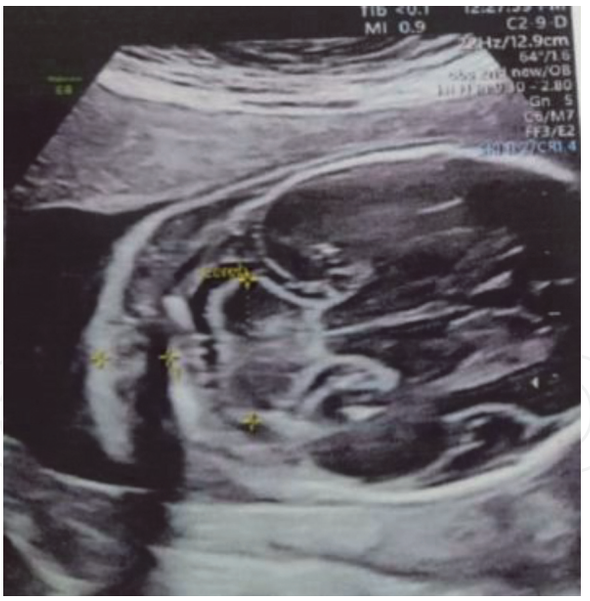

Figure 6.
Nuchal oedema.

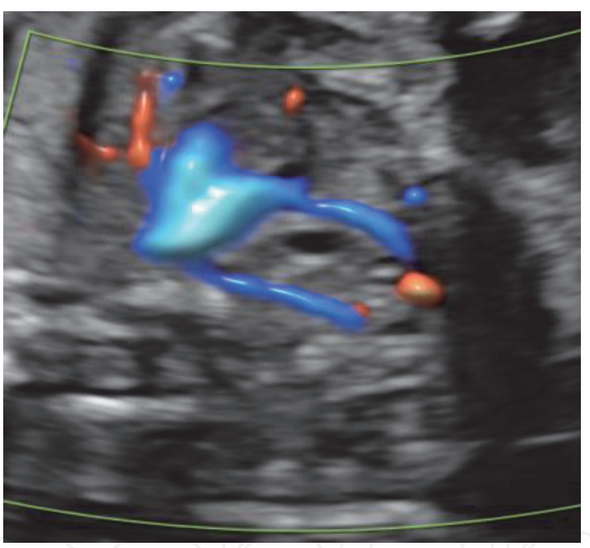

Figure 7.
ARSA.

anomalies increases the risk of microdeletion 22Q11 and other syndromes. The positive LR is 21.5 and negative LR is 0.71. when it is isolated LR is 3.9 times.

7.3 Echogenic bowel loop

This may be due to Swallowed blood, Cystic fibrosis or maternal infections. It may be also associated with congenital malformations of the bowel more so of upper GI lesions. And other perinatal complications, including fetal growth restriction. We have to also look for Ascites and bowel dilatation. Diagnosis of echogenic bowel should be confirmed by low frequency transducer, reduced Gain and without use of harmonics. Echogenicity should be equal to or more than bone (**Figure 8**). Grade 2 similar to bone echogenicity Grade 3 is more than bone. The positive LR of this is 11.4 and negative LR is 0.9.

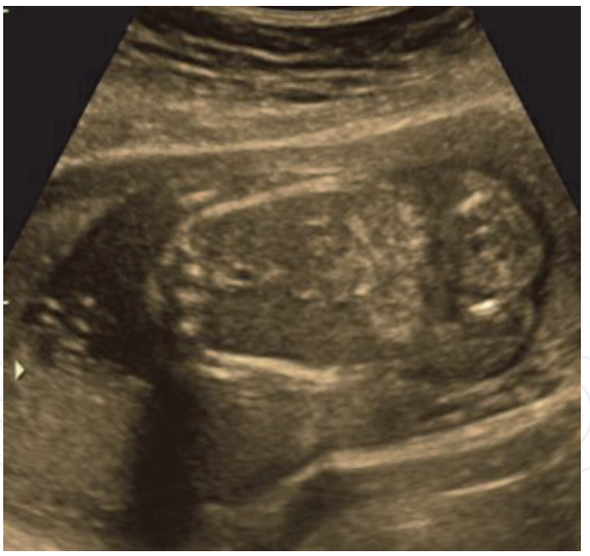

Figure 8.
Echogenic bowel loops.

7.4 Short femur/short Humerus

Short Femur and humerus is when the measurement is below 5th percentile for gestational age or measured/expected ratio < 0.9. The positive LR is 3.72 and negative LR is 0.8. regarding short humerus is the humerus measuring <2.5% or measured/expected ratio < 0.89. The Positive LR is 4.81 and negative LR is 0.74.

7.5 Echogenic intracardiac focus (EICF)

usually noted at region of papillary muscle 88% in Lt ventricle, 5%in rt. ventricle and 7% in biventricular. The echogenicity should be comparable to bone. Grading of EICF - Grade 2 similar echogenicity of bone and grade 3 more denser than bone (**Figure 9**). EICF in RV, biventricular, multiple and bright EICF are more associated

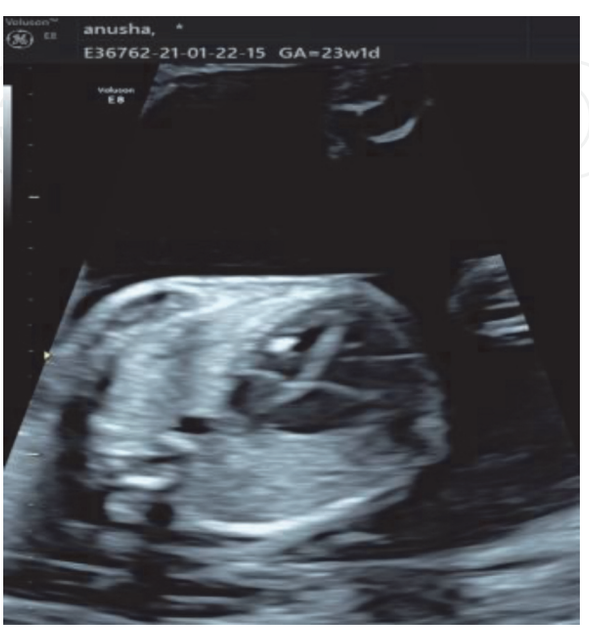

Figure 9.
EICF.

with aneuploidy, when compared to solitary LV EICF. The positive LR is 5.83 and negative LR is 0.8.

7.6 Mild ventriculomegaly

Normal ventricular measurements are <10 mm. If it is defined as mild ventriculomegaly when measurement is between 10 and 15 mm. (**Figure 10**). The overall prevalence of chromosomal defects in fetal ventriculomegaly is about 10% and the commonest chromosomal defects are trisomies 21, 18, 13 and triploidy. The positive LR is 27.52and negative LR is0.94.

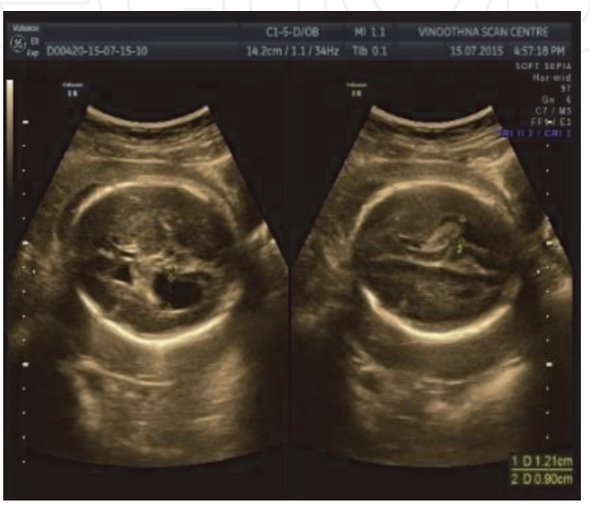

Figure 10.
Mild ventriculomegaly.

7.7 Mild hydronephrosis

pelvic AP diameter measuring >4 mm and it should be measured in transverse section in 12 clock or 6 clock position. The positive LR is 7.6 and negative LR is 0.92 (**Figure 11**).

There are other soft markers also those doesn't have any likely hood ratio but they are important and common in our practise but they are not a part of screening

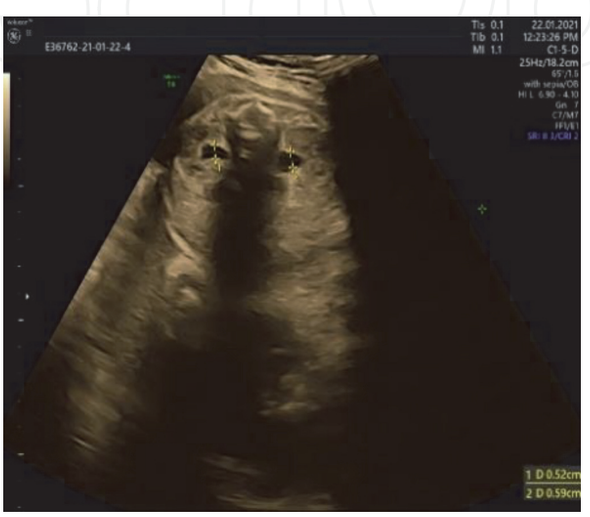

Figure 11.
Pyelectasis.

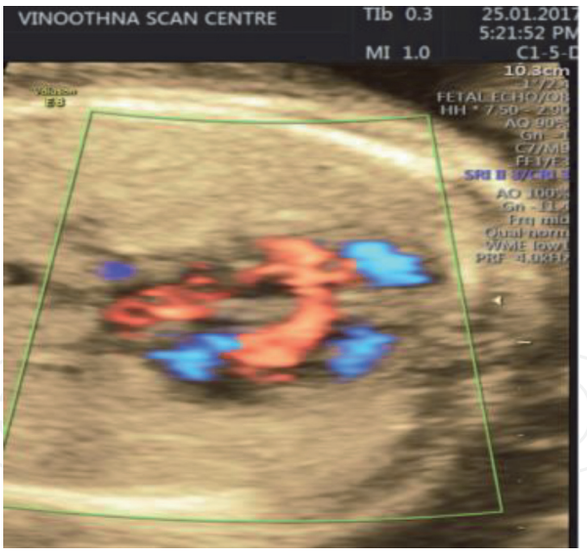

Figure 12.
Small membranous VSD.

protocol. They are the choroid plexus cysts and single umbilical artery, sandal gap toes, short ears, clinodactyly, increased iliac angle. Not only this Duodenal atresia and small membranous VSD (**Figure 12**) is also be associated with aneuploidy [16].

7.8 Choroid plexus cysts

they may be round or oval. May be unilateral or bilateral. They may be large or small. Commonly seen between 16 and 21 weeks by 23 week start undergoing regression. After 25–26 weeks uncommon to see. More commonly associated with trisomy 18. LR for trisomy 18 when isolated is 1.1–1.5.

7.9 Single umbilical artery

No strong association with aneuploidy. Usually associated with fetal cardiac, renal anomalies and oesophageal atresia (**Figure 13**).

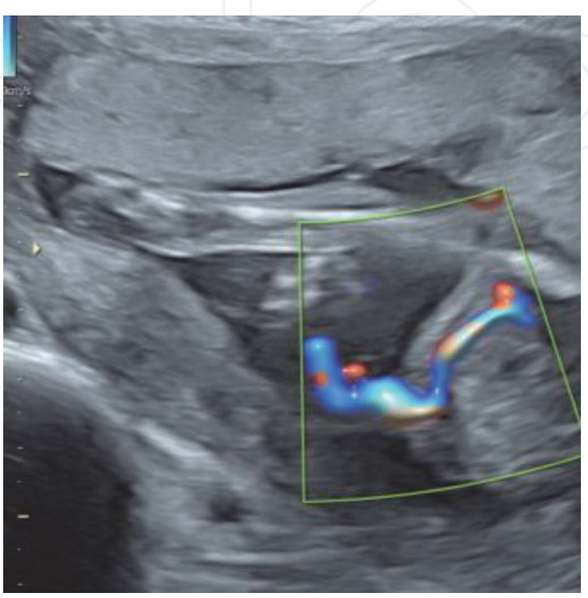

Figure 13.
Single umbilical artery.

7.10 Pre nasal thickness

In normal fetuses, the pre nasal thickness is small and the nasal bone is relatively long, resulting in a ratio of approximately 0.6 [17]. In trisomy 21 fetuses in the first trimester, the prenasal thickness increases, whereas the nasal bone length decreases, resulting in a ratio > 0.8 [17].

8. Non-invasive prenatal testing (NIPT)

Other names for NIPT are NIPS- non-invasive prenatal screening, cfDNA- cell free DNA. The test is based upon the presence of fetal cell-free DNA in the maternal circulation. Placental cell apoptosis releases into the maternal circulation as small DNA fragments (150-200 bp) that can be detected from >7 weeks of gestation [18]. It is estimated that about 2–20% of circulating cfDNA in the maternal circulation is fetal in origin [18]. So, about 1 in 10^3–10^7nucleated cells in maternal blood are fetal which can be enriched to about 1in 10–100 by techniques such as magnetic cell sorting (MACS) or fluorescence activated cell sorting (FACS) after attachment of magnetically labelled or fluorescent antibodies on to specific fetal cell surface markers. However, with the use of fluorescent *in situ* hybridization (FISH) and chromosome specific DNA probes it is possible to suspect fetal trisomy by the presence of three-signal nuclei in some of the cells of the maternal blood enriched for fetal cells. On the basis of currently available technology, examination of fetal cells from maternal blood is more likely to find an application as a method for assessment of risk. The sensitivity of NIPT is comparable to serum screening. Analysis of fetal cells from maternal blood is both labour intensive and requires highly skilled operators whereas in biochemical screening which is relatively easy to apply for mass population screening. The half-life of cfDNA is short and is typically undetectable within hours after delivery [19]. the detection rate for T21 is at 99% for a false-positive rate of 0.16% [20, 21]. Detection rate for T18 is at 97% for a false-positive rate of 0.15% [20]. The use of NIPT is rapidly expanding and is now being offered as the primary screening test in pregnancy. Even if the NIPT test has an excellent detection rate for T21, T18, and T13, other aneuploidies remain missed [22–24]. NIPT is a screening and not a diagnostic test so, caution should be used when NIPT is incorporated in the genetic evaluation of fetal malformations. Low fetal fraction is noted in High body mass and sampling before 10 weeks of gestation.in some laboratories fetal fraction <4% are considered too low to report a result which is often referred as a “no call “result. NIPT results depends on duration of gestation, number of fetuses and whether the fetus is live or not. For confirming number, gestational age and viability needs ultrasound examination before going for NIPT. If its low-risk population the positive predictive value of NIPT is low. False positive in NIPT are in placental mosaicism, vanishing Twin, maternal sex chromosome abnormality and Neoplasia. Even if NIPT is true positive it can-not distinguishes aneuploidy derived from translocation or disjunction type which is needed to know the recurrence risk for this again needs diagnostic test. Not only this the women who has no call report result needs comprehensive ultrasound evaluation and diagnostic tests because low fetal fraction may be associated with increased risk of aneuploidy.

9. Invasive fetal testing

1) **Chorionic villous sampling** should be done at 10–15 weeks. and overall fetal loss is 1%. This test can be done trans abdominal/trans vaginal approach and this

procedure should be done under ultrasound guidance and the sample is Trophoblast cells. Result comes within 48–72 hrs. Randomized studies have demonstrated that the rate of fetal loss following first-trimester transabdominal chorionic villus sampling is the same as with second-trimester amniocentesis. There is an association between chorionic villus sampling before 10 weeks to fetal transverse limb abnormalities, micrognathia and microglossia. It is therefore imperative that chorionic villus sampling is performed only after 11 weeks by appropriately trained operators.

2) **Amniocentesis** should be done at 15–20 weeks. In this we introduce needle inside the amniotic cavity to extract the amniotic fluid. Sampling cells are amniocytes, fetal dermal fibroblasts. Karyotype results take 7–10 days, and overall fetal loss is 0.5%.

3) **cordocentesis** (per cutaneous umbilical blood sampling) which should be done at >18-20 weeks. Under ultrasound guidance needle should be introduced into the cord near the placental insertion. Sampling should be done from umbilical vein. Sampling cells are fetal blood cells sampled from umbilical vein and overall fetal loss is 1.5–3%. In a randomized study, 4,606 low-risk, healthy women, 25–34 years old, at 14–20 weeks of gestation, were randomly allocated to amniocentesis or ultrasound examination alone [25]. The total fetal loss rate in the patients having amniocentesis was 1% higher than in the controls. The study also reported that amniocentesis was associated with an increased risk of respiratory distress syndrome and pneumonia. Randomized studies have demonstrated that after early amniocentesis i.e., around 10–14 weeks of gestation the rate of fetal loss is about 2% higher and the incidence of talipes equinovarus is 1.6% higher than the first-trimester chorionic villus sampling or second-trimester amniocentesis. It was apparent that amniocentesis carried a risk of miscarriage and this in conjunction with the financial cost implications, meant that prenatal diagnosis could not be offered to the entire pregnant population.

10. Sonographic and biochemical features of Aneuploidy

10.1 Trisomy 21

Factors that is associated with an increased risk of "Down syndrome" are higher maternal age, a parental translocation involving chromosome 21, previous child with T21, significant ultrasound findings and a positive screening test result. In pregnancies with T21 fetuses, the maternal serum concentration of free β-HCG is about twice (about 2MoM) as high and PAPP-A is reduced to half (about 0.5 MoM) compared to euploid pregnancies. Although NT measurement alone identifies about 75–80% of T21 fetuses, the combination of NT with maternal biomarkers in the first trimester increases the T21 detection rate to 85–95%, while keeping the false-positive rate at 5%. AFP is decreased in T21.

In addition to NT, other sensitive first trimester ultrasound markers of T21 include absence or hypoplasia of the nasal bone (60–70%), increased impedance to flow in the ductus venosus (about 80%), tricuspid regurgitation, cardiac malformations (atrioventricular septal defect) with or without generalized edema, aberrant right subclavian artery and echogenic intracardiac focus. Increased fronto maxillary fascial angle (short maxilla in 25%), renal pylectasis and echogenic bowel loops are also soft markers for "Down syndrome" (**Table 3**) (**Figures 14–18**).

In second trimester scan the soft markers in Trisomy 21 are nasal hypoplasia, increased nuchal fold thickness, intracardiac echogenic foci, echogenic bowel, hydronephrosis, shortening of the femur and more so of the humerus. It may also be

	Trisomy21	Trisomy18	Trisomy13	Triploidy	Turner
Ventriculomegaly	+	+	+	+	
Holoprocencephaly			+		
Choroid plexus cyst		+			
Dandy walker complex		+	+		
Fascial cleft		+	+		
micrognathia		+		+	
Nasal hypoplasia	+				
Nuchal edema	+	+	+		
Cystic hygroma					+
Diaphragmatic hernia		+	+		
Cardiac defect	+	+	+	+	+
Exomphalos		+	+		
Duodenal atresia	+				
Esophageal atresia	+	+			
Renal defects	+	+	+	+	+
Short limbs	+	+		+	+
Clinodactyly	+				
Overlapping fingers		+			
polydactyly			+		
syndactyly				+	
Talipes		+	+	+	
Fetal growth restriction		+		+	+

Source: Snijders and Nicolaides 1996, Nicolaides et al. 1992.

Table 3.
Common chromosomal defects in fetuses with sonographic abnormalities [9, 26].

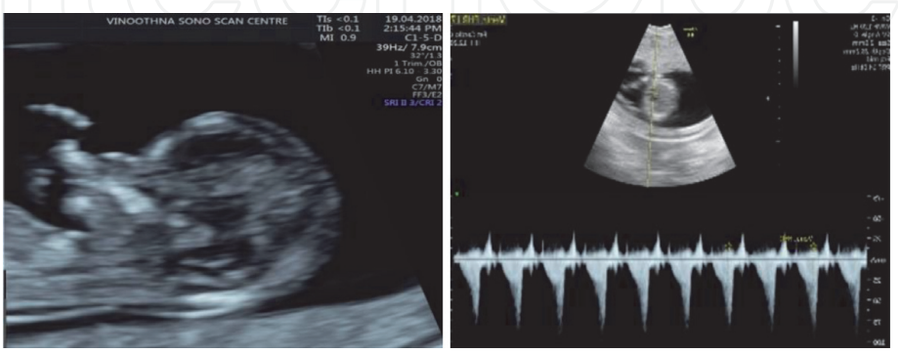

Figure 14.
T21 Fetus of 12 weeks 3 days showing normal NT with AFNB and Tricuspid regurgitation.

associate with cardiac defects, duodenal atresia, sandal gap and clinodactyly or mid-phalanx hypoplasia of the fifth finger. Trisomy 21 is found in about 40% of cases of duodenal atresia.

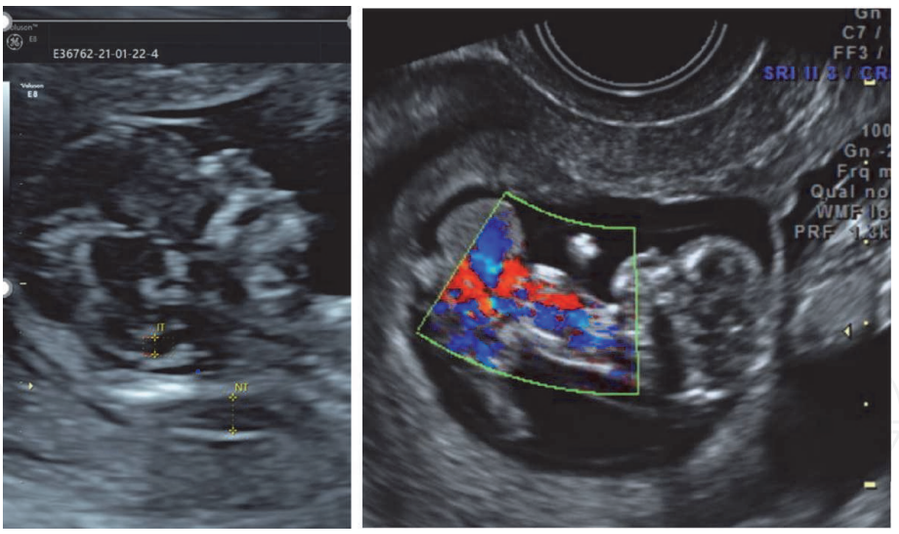

Figure 15.
T21 fetus of 13 weeks 5 days showing increased NT with Omphalocele.

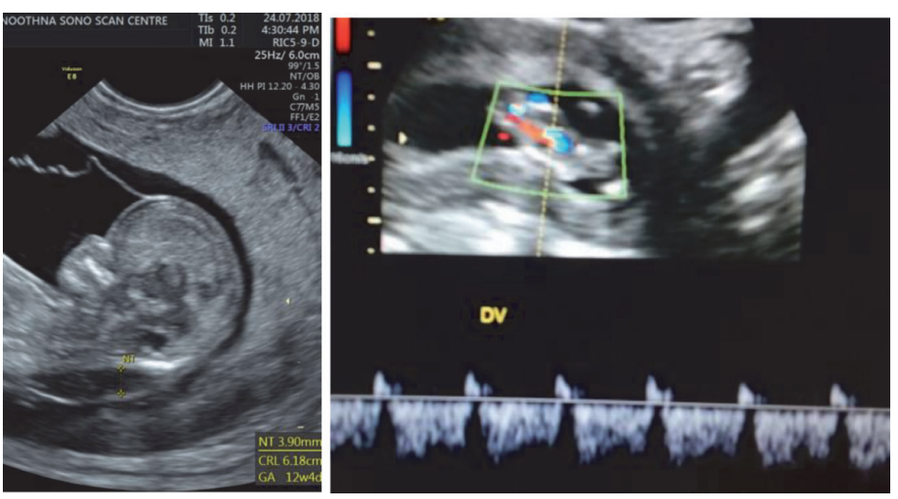

Figure 16.
T21 fetus showing Increased NT with dilated posterior fossa and reverse flow in ductus venosus.

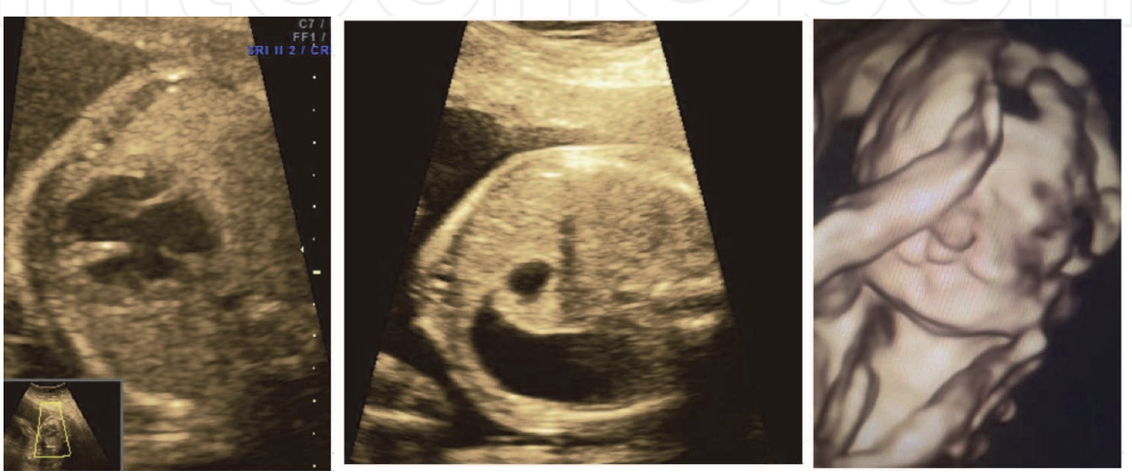

Figure 17.
T21 with Atrioventricular septal defect with duodenal atresia(double bubble sign) and cleft lip with palate.

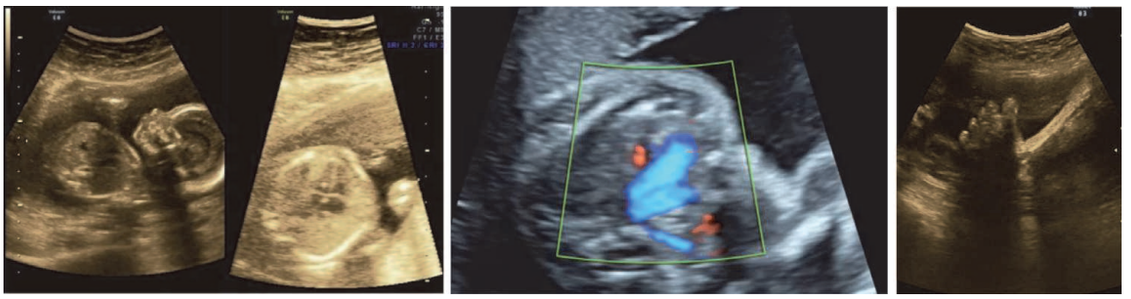

Figure 18.
T21 with Absent nasal bone with EIC, ARSA and club foot.

10.2 Trisomy 18 and Trisomy13

Thickened NT is a common first trimester findings in Aneuploidy. In T18 and T13, NT median values were shown to be 5.5 and 4.0 mm, respectively [16, 27]. Reduced PAPP-A value in both trisomies noted with a median value of 0.2 MoM for T18 and 0.3 MoM for T13. Free β-HCG values are decreased whereas it is increased in T21. In T18 and T13 median values of free β-HCG 0.2 MoM and 0.5 MoM, respectively. T18 or T13 is often first suspected by the presence of typical ultrasound features, rather than by biochemical screening (**Figures 19–25**). single umbilical artery is found 80% fetuses with T18 and in about 3% of chromosomally normal fetuses [28]. There is 7fold increased risk of T18 associated with single umbilical artery noted. Presence of megacystis After taking into account maternal age and fetal NT the increases the likelihood for trisomy 13 or 18 by a factor of 6.7.

Presence of exomphalos in association with T18 in first trimester is 60% compared about 30% at mid gestation and 15% in neonates. Trisomy 13 and Turner syndrome are associated with tachycardia, whereas in trisomy 18 and triploidy there is fetal bradycardia [29]. pulsatile flow in the umbilical vein is noted in 90% of fetuses in T18 and T13 where as 25% of chromosomally normal fetuses. The prevalence of chromosomal defects in Dandy walker -complex is about 40%, mainly in trisomies 18, 13 and triploidy.

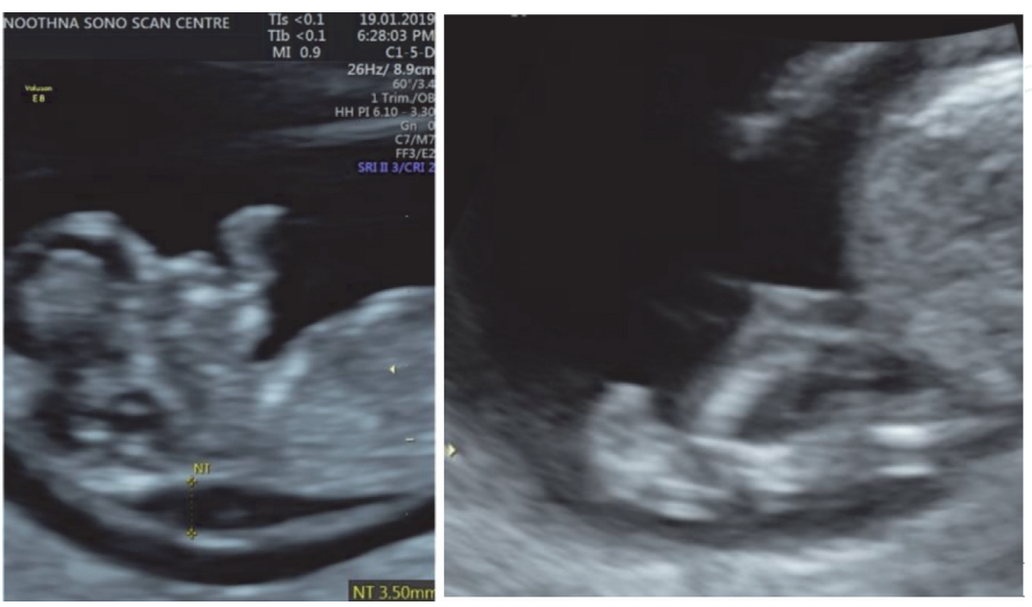

Figure 19.
T18 12 weeks 1 day showing increased NT, absent nasal bone, cleft lip and palate and Congenital talipes equinovarus.

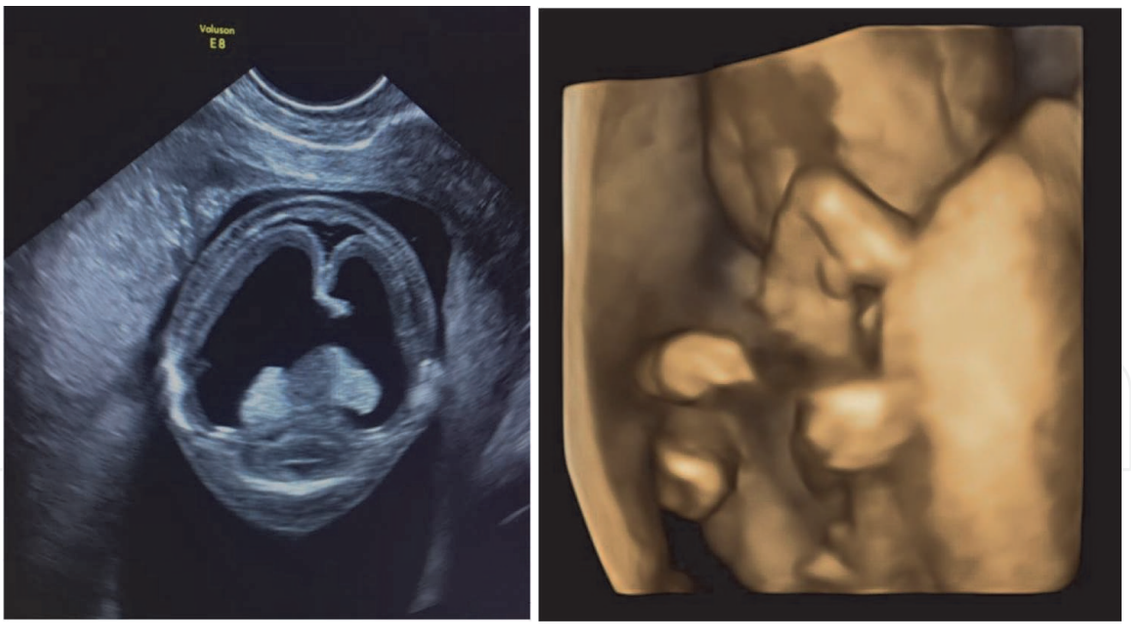

Figure 20.
T18 fetus of 15 weeks gestational age with Holoprocencephaly and radial ray abnormality.

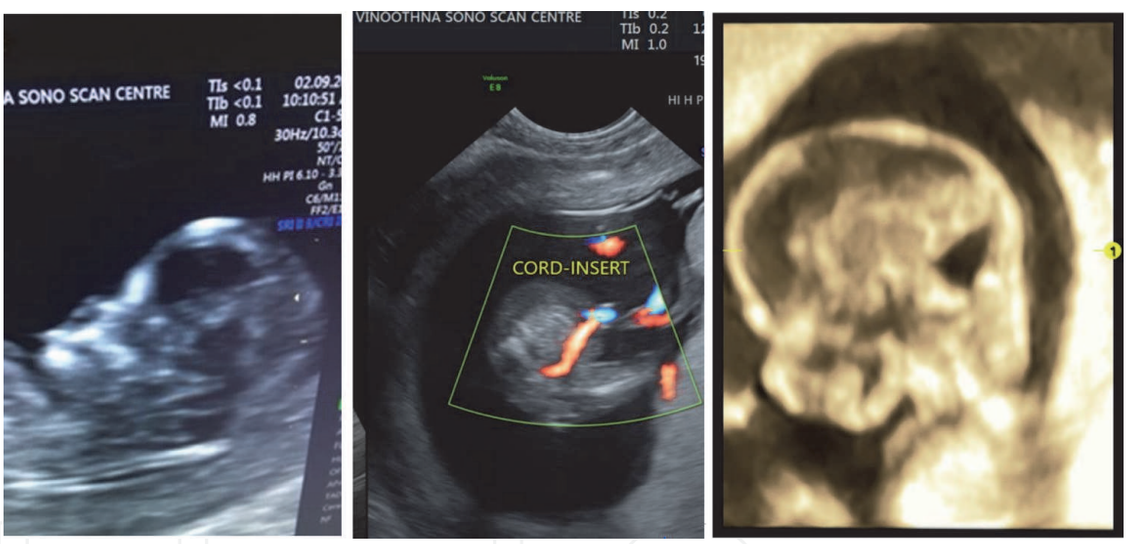

Figure 21.
T18 fetus showing normal NT with dilated posterior fossa and single umbilical artery at 13 weeks 2 days followup 3D at 16 weeks 4 days with vermian rotation and incread Brainstem vermian angle.

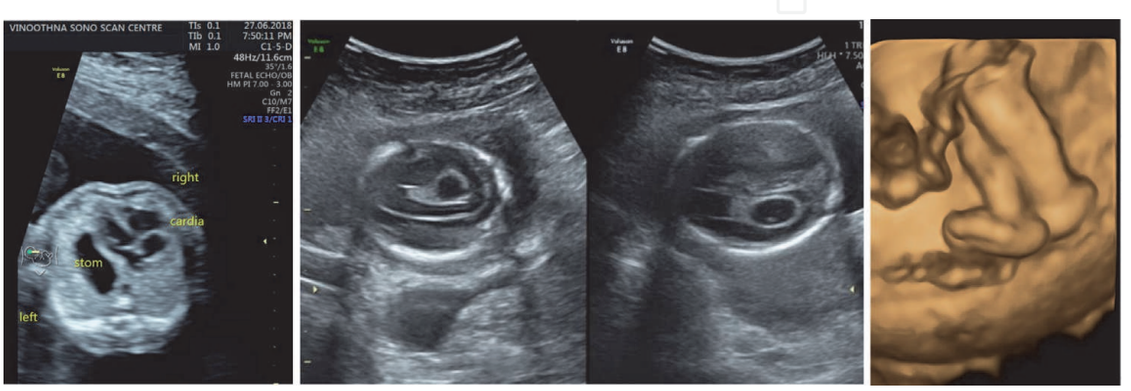

Figure 22.
Fetus of T18 showing Diaphragmatic hernia, choroid plexus cysts and bilateral rocker bottom foot at 21 weeks 5 days gestation.

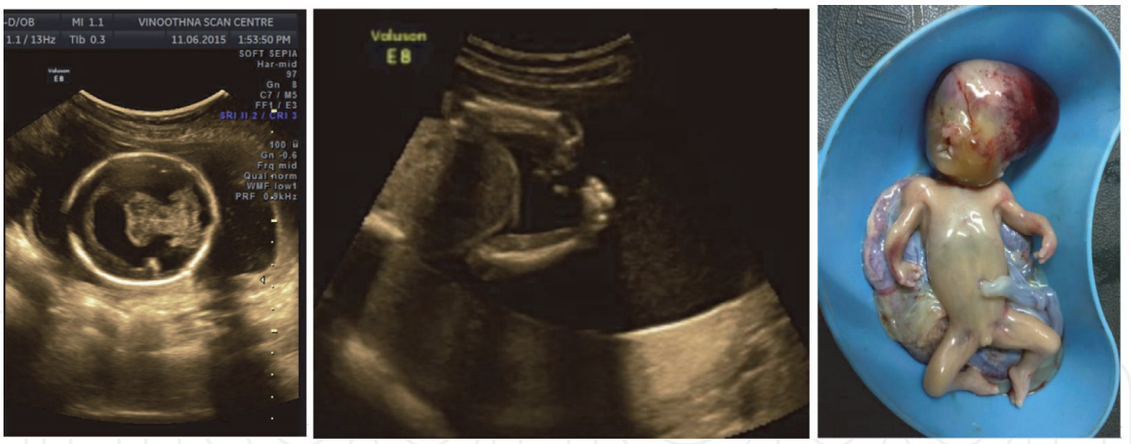

Figure 23.
15 weeks 5 days fetus of T13 showing holoprocencephaly, club hands and aborted fetus showing midline cleft with proboscis anophthalmia and bilateral club hands.

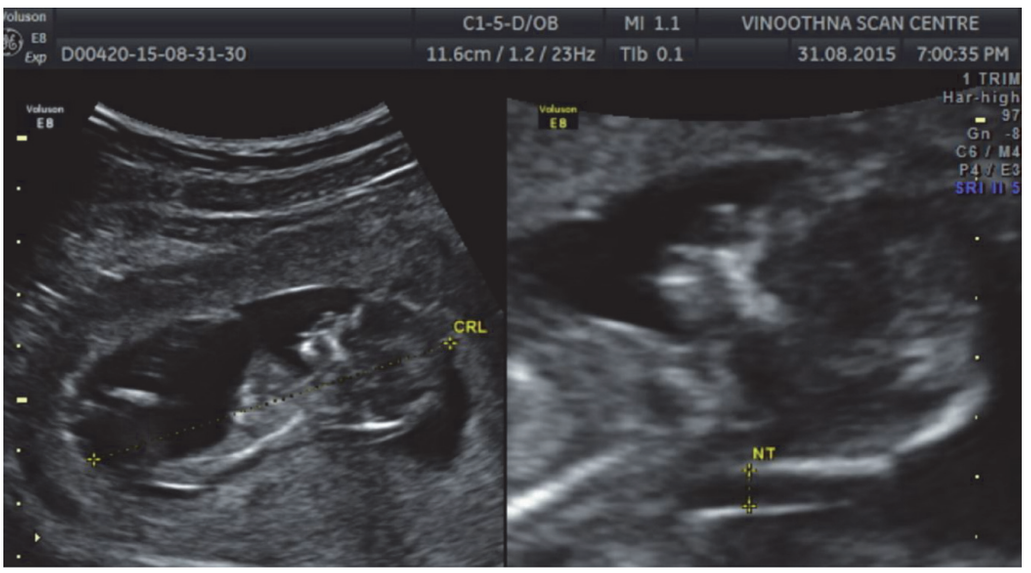

Figure 24.
Megacystits with increased NT of 12 weeks 1 day T13 fetus.

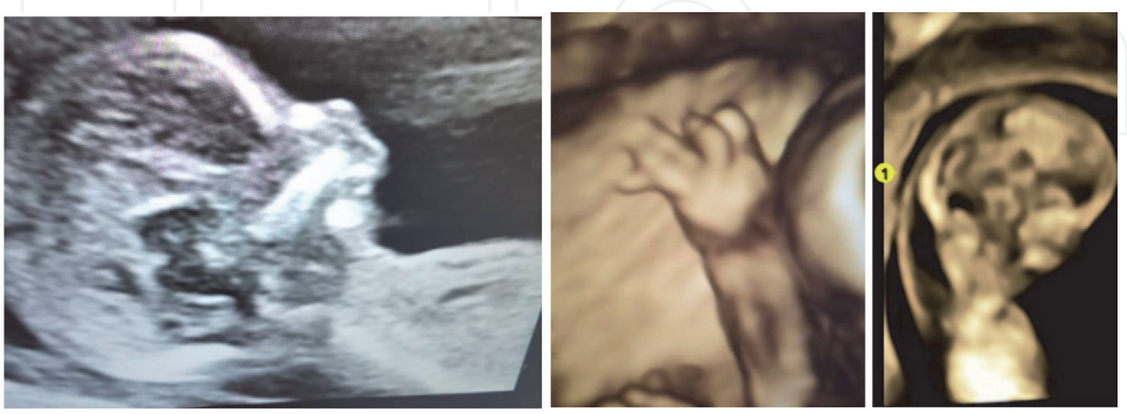

Figure 25.
15 weeks 3 days fetus showing micrognathia with polydactyly and syndactyly. In another fetus of 14 weeks 2 days 3D showing increased NT with posterior fossa dilatation and micrognathia in T13 cases.

20% 0f diaphragmatic hernia is associated with chromosomal defects mainly withTrisomy18. Heart abnormalities are found in more than 90% of fetuses with trisomy 18 or 13 and 40% of those with trisomy 21 or Turner syndrome. 30% and 15%

cases of Exomphalos at mid gestation and in neonates are associated with Chromosomal defects, mainly trisomies 18 and 13. The prevalence of chromosomal defects is four-times higher when the exomphalos sac contains only bowel than in cases where the liver is included. Prenatally 20% of oesophageal atresia cases are associated with chromosomal defects, mainly trisomy 18. Polydactyly is associated with trisomy 13, overlapping fingers, Talipes and rocker bottom feet are associated with trisomy 18. Usually, Trisomy 18 and triploidy are associated with moderately severe growth restriction whereas trisomy 13, Turner syndrome with mild growth restriction and in trisomy 21 growth is essentially normal [30]. In second trimester scan Trisomy 18 is associated with strawberry-shaped head, choroid plexus cysts, absent corpus callosum, enlarged cisterna magna, facial cleft, micrognathia, nuchal edema, heart defects, esophageal atresia, diaphragmatic hernia and usually exomphalos with bowel only in the sac. The other associated findings are single umbilical artery, renal abnormalities, echogenic bowel, myelomeningocele, growth restriction and shortening of the limbs, radial aplasia, overlapping fingers and talipes or rocker bottom feet.

Trisomy 13 is associated with microcephaly, holoprosencephaly, facial abnormalities, cardiac abnormalities, exomphalos, enlarged and echogenic kidneys and post axial polydactyly.

11. Monosomy X (turner syndrome)

NT has a median value of 7.8 mm [16] and has often been described as a cystic hygroma (**Figure 26**). The occurrence of monosomy X is not related to maternal age. Typically, lymphatic disturbances in turner syndrome are not limited to the neck region but involve the whole body including the presence of skin edema, hydrothorax and ascites. Generally Normal Nasal bone is present in fetuses with monosomy X [31]. Normal maternal serum-free β-HCG (1.1 MoM) and low PAPP-A is noted (0.49 MoM) [32]. Typical sonographic features in monosomy X includes large nuchal cystic hygromas, generalised edema, mild pleural effusions and ascites, cardiac abnormalities like left ventricular outflow tract obstruction, fetal tachycardia and renal anomalies such as the presence of horseshoe kidneys.

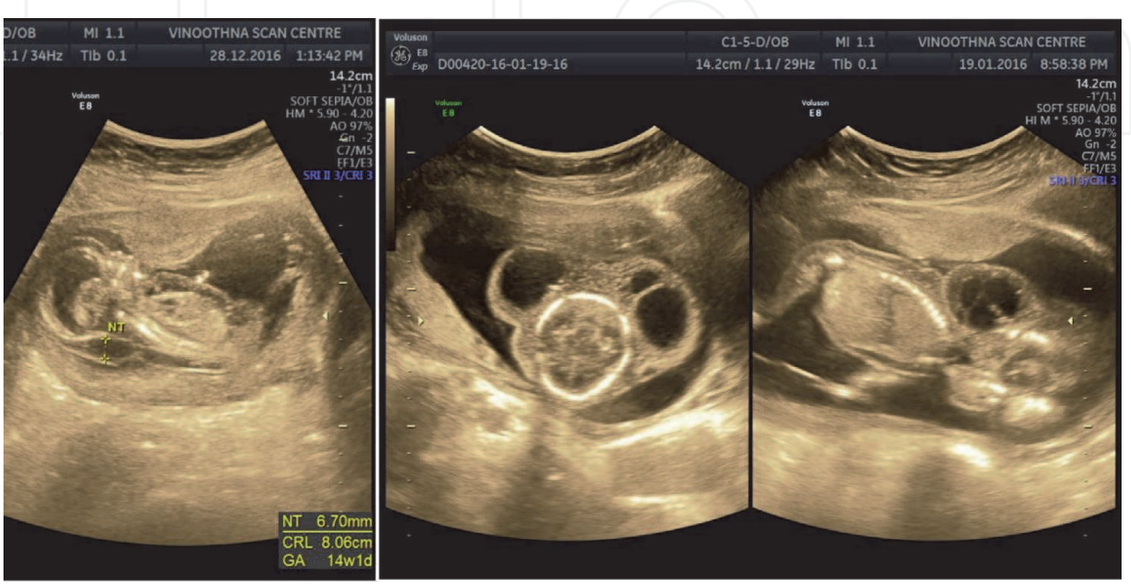

Figure 26.
2 different cases of turners syndrome with generalised edema and cystic hygroma.

11.1 Triploidy

In triploidy, there is a complete additional haploid set of chromosomes resulting in 69 chromosomes in each cell instead of 46 chromosomes. The additional haploid set can be of paternal or maternal origin. The "paternal" type is called diandric triploidy and the "maternal" type is called digynic triploidy. These two types show different features, which can be often differentiated on ultrasound. The typical pattern of diandric triploidy includes the presence of a normally grown fetus with molar placenta, whereas in digynic triploidy, severe growth restriction is noted with a small but not molar placenta. Profile of biochemistry is different in both types due to these placental differences. Diandric triploidy is associated with increased maternal serum-free β-HCG and mildly decreased PAPP-A and in digynic triploidy which is associated with markedly decreased maternal serum free β-HCG and PAPP-A. Significantly short CRL with marked difference in size between the abdominal and head circumference, typically of more than 2 weeks of gestational age [33] which is a pathognomonic sign of digynic triploidy (**Figure 27**). In second trimester scan Triploidy where the extra set of chromosomes is paternally derived is associated with a molar placenta and the pregnancy rarely persists beyond 20 weeks. When there is a double maternal chromosome contribution, the pregnancy may persist into the third trimester (**Figure 27**). Commonly there is mild

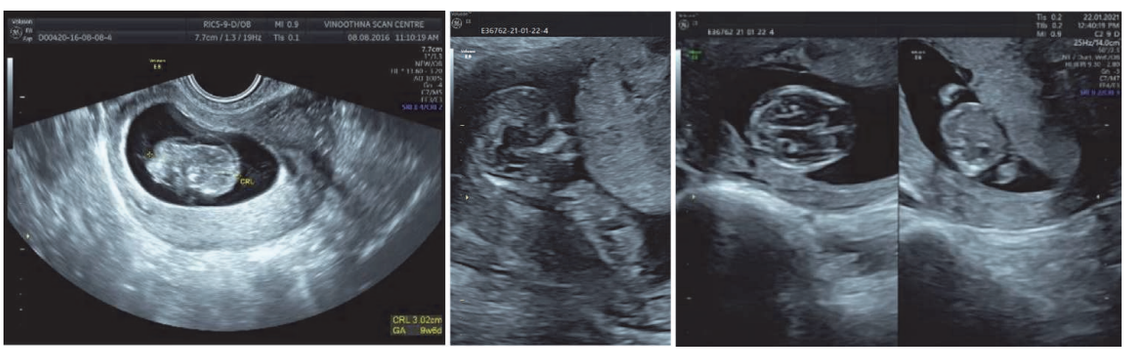

Figure 27.
Two fetuses of Digynic Triploidy showing short CRL with size difference in abdominal head circumference.

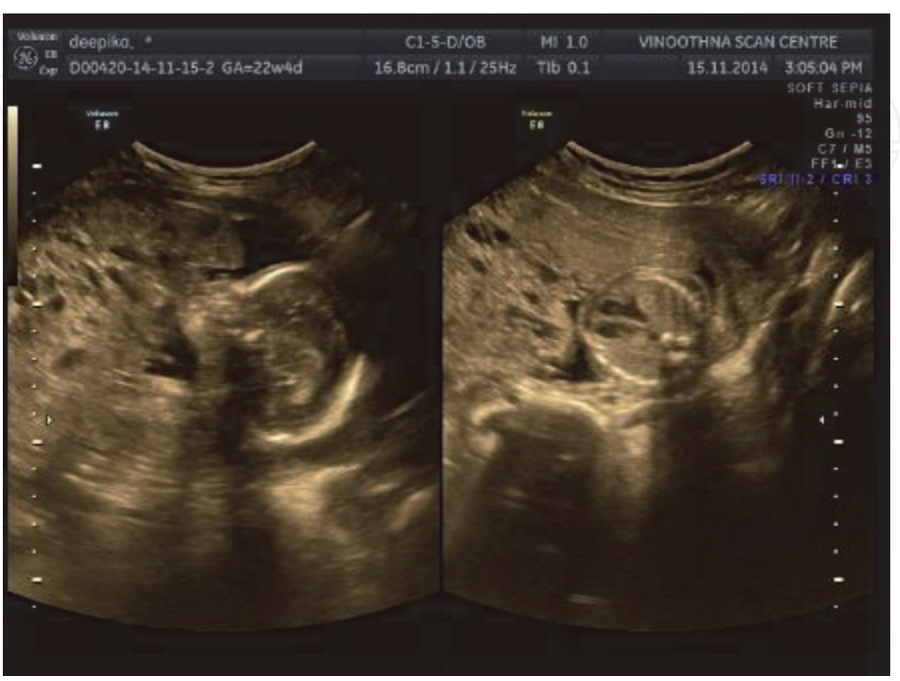

Figure 28.
Live fetus at 22 weeks 4 days with Molar changes in placenta in a diandric triploidy.

ventriculomegaly, micrognathia, cardiac abnormalities, myelomeningocele, syndactyly, and 'hitch-hiker' toe deformity (**Figure 28**).

12. Risk assessment in first and second trimester

The risk for trisomies in women who have had a previous fetus or child with a trisomy is higher than the one expected on the basis of their age alone.

when we have only CRL, NT, maternal age without biochemical markers there are calculators where we can enter these measurements, we get the risk assessment for downs at the time of birth- Pregnancy calculators- EDD. We can do same thing with only 2nd trimester markers without biochemical or first trimester screen results for this we will take the LR+ value of each marker present and LR- values of all absent markers and multiple all of these to get the LR for combination [8].

Instead if we find any soft markers we enter the same into the excel sheet provided by [8] M. Agathokleous et al. Excel sheet for downs.

Meta- analysis of second-trimester markers for trisomy21 [8] M. Agathokleous et al., ultrasound obstet Gynecol 2013;41:247–261.

For example:-.

when we get the measurements, we apply the same into the calculators and get the risk assessment for downs at the time of birth. It is given as in 1 in ————————.

>1in 19(high risk): offer invasive testing.

>1in 50(high risk): offer NIPT/Invasive testing.

<1in 1000(Low risk): Back to routine second trimester genetic sonogram.

1in 50-1in 999(intermediate risk): Assess NB, DV, TR and recalculate risk+/-NIPT.

New cut-of risk for downs as 1:250, borderline between 251 and 1000, and less risk if <1:1001.

First trimester between 11 and 13 weeks 6 days scan evaluate NT, nasal bone along with Tricuspid valve regurgitation, a wave in Ductus Venosus and other major structural defects. Not only this detail cardiac evaluation should be done. If there is no abnormality repeat scan at 18–22 weeks may be recommended. In the second trimester scan look for soft markers, if there is any marker or abnormality detailed anatomy scan and echocardiography. In case of most isolated markers including intra cardiac echogenic focus, echogenic Bowel, mild hydronephrosis and short femur, there is only a small effect on modifying the pre-test odds.

All these are only screening protocols they are not diagnostic so, fetal karyotyping option is aways open to either risk groups.

Previous affected Pregnancy.

In women who had a previous pregnancy with trisomy 21, the risk of recurrence in the subsequent pregnancy is 0.75% higher than the maternal and gestational age-related risk for trisomy 21 at the time of testing. Recurrence is chromosome specific. If a previous pregnancy is T21 the result will be classified as screen positive regardless of level of screening markers. Risk is calculated which takes account of a women's age at the time of her previous pregnancy with "Down syndrome" for the risk calculation.

"Down syndrome" may be non-disjunction type (95%) where there is a recurrence rate of 1% where as in translocation type like (21–21) if either parent is carrying same type of translocation then there is 100% rate of recurrence.

If there is h/o prior affected downs child screening test is not reassuring her so, better to go for direct invasive testing if she comes at first trimester go for CVS.

In Twin gestation.

Dichorionic twin- Free β-HCG and PAPP-A levels are nearly twice as high as singleton. Calculate the risk for each fetus based on maternal age and fetal NT. If one fetus the NT is increased look for other markers. Detection rate is 75–80%.

In monozygotic twins' risk is same as singleton pregnancies.

In monochorionic twin pregnancies raised NT is an early manifestation of TTTS. So, false positive rate will be increased. Free beta HCG and PAPP-A levels are lower than dichorionic twin to twin transfusion syndrome as well as for chromosomal abnormality.

Calculate the risk of each fetus based on NT, serum biochemistry and then the average risk between the two fetuses is considered as whole.

No method is accurate for screening of fetal aneuploidy as it is in singleton pregnancy.

Appropriate Models for aneuploidy detection:

- Age (not recommended).
- CRL & NIPT (Ideal for first trimester, misses advantages of first trimester scan and expensive)
- Age, CRL & NT (skill)
- Age & Biochemistry (poor detection rate)
- Age + CRL + Maternal factors +NT + PAPP-A + HCG (combined test)
- Age + Maternal factors + CRL + NT + Additional markers + Biochemistry (enhanced sensitivity and low FPR but need time and skill)
- First trimester combined test + second trimester Quad (sequential or integrated)
- First trimester Quad: Age + historical factors + PAPP-A + βHCG + PIGF +AFP (risk for pre-eclampsia and NTD)
- First trimester Penta: Combined test + Nasal bone + AFP + DIA + PIGF (high detection rate and low FPR).

13. Conclusion

In the economically privileged patient first trimester screening should include an 11–14 weeks complete assessment with first trimester combined screen, PIGF and NIPT. For population screening is by combined screening. Woman with positive screen test result should be counselled and offered the option of diagnostic testing. Those who have a negative test results should be counselled regarding their lower adjusted risk. Even if a woman has low risk results, she may choose diagnostic testing later in pregnancy whenever there is fetal anomalies or markers on follow-up sonography.

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Author details

Madhavilatha Routhu[1*] and Shiva Surya Varalakshmi Koneru[2]

1 Kakatiya Medical College, Warangal, India

2 Shiva Hospital, Warangal, India

*Address all correspondence to: madhaviradiologist@gmail.com

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