SOCIETY OF FETAL MEDICINE

Society of Fetal Medicine

SFM Bengal Chronicles

Scanning Down the Marker Mesh

Extra Chromosomes Extra Love

Volume 01
Issue 02
26 August 2022

From The President's Desk

Welcome to the second issue of SFM Bengal Chronicles, the quarterly newsletter from the Bengal Chapter. Having effectively dealt with Thalassemia in the first issue, the theme-based publication takes up the second trimester ultrasound markers for Trisomy 21 this time.

Given their subtle nature and not necessarily amounting to congenital anomalies the oft quoted 'soft markers' are full of debates, disputes and disagreements. We still pay heed as they are statistically linked to common aneuploidies, even though many of them have very poor positive predictive values. The findings often initiate a series of further tests only to receive normal reports at the end. The final product of this rather stressful exercise may just be some added anxiety.

The performance of these second trimester markers are not good enough to qualify for a separate screening test by themselves. Yet we use them in tricky situations to draw further reassurance. Used wisely they may enhance the performance of the standard screening tests. However it is important to have a structured approach for their interpretation. Positive and negative likelihood ratios may be more reasonable than the over-simplistic way of labelling someone "high risk" with mere presence of two or more of these markers.

The detection methods for these ultrasound markers are not robust and they represent different levels of predictions too. Nuchal translucency, nasal bone, maternal serum biochemistry etc have set protocols and thereby some standardization may be expected but most of the second trimester markers like echogenic bowels, mild RPD etc may be amenable to significant intra and inter operator variability. Therefore it is all the more necessary to have a clear idea about their identification, interpretation & further management and that's exactly the purpose of this newsletter. It has not been possible to critically appraise every single aspect of these markers individually or collectively but we have attempted to highlight a few clinically relevant issues.

The Bengal Chapter's flagship quarterly image competition was opened to national level this time and the participation was extremely encouraging. We are indebted to Dr Mohit Shah, our beloved President Elect for taking the trouble of going through the wonderful images and choose the best two. We congratulate the winners whose images have been published in this issue but we also thank all participants whose valuable contributions have lifted the standards of this competition to the current levels. We look forward to receiving their beautiful images again and from the other SFM members too.

The Bengal Chapter is extremely grateful to all esteemed authors who have kindly obliged us with their valuable time and expertise. We also thank the SFM Central team for constantly encouraging us with limitless academic activities. Finally, no words is enough to express our sincere gratitude to our print partner, The Conferences International for turning our dreams into reality.

It is heartening to see the standards of fetal scans getting better with every passing day.

Ultrasonically yours

Dr. Kanchan Mukherjee
President, Bengal Chapter
Society of Fetal Medicine

"Know your markers in a correct way"

Dr. Shankar Dey

MS (O&G), Consultant Fetal Medicine Specialist
ULTRACLINIC, Asansol, West Bengal

Down syndrome (trisomy 21) a genetic disorder caused by the presence of all or a portion of a third chromosome 21, is by far the most common and best-known chromosomal disorder in humans and the most common cause of intellectual disability. It is the most common trisomy among live births characterized by intellectual disability, dysmorphic facial features, and other distinctive phenotypic traits.

Fortunately, Down syndrome can be suspected prenatally by combined ultrasound and serologic screening and confirmed by invasive genetic techniques. The methods used for fetal screening are maternal age assessment, imaging markers in the first- and/or second-trimester ultrasounds, maternal serum biochemical testing, and, more recently, analysis of cell-free fetal DNA from maternal plasma. First-trimester detection of fetal major abnormalities, including trisomy 21, is important, because it offers the couples the advantages of early termination of pregnancy with less medical complications, reduced economical costs to the health system, and minor emotional impact of the couple, or they may continue the pregnancy with informed choice.

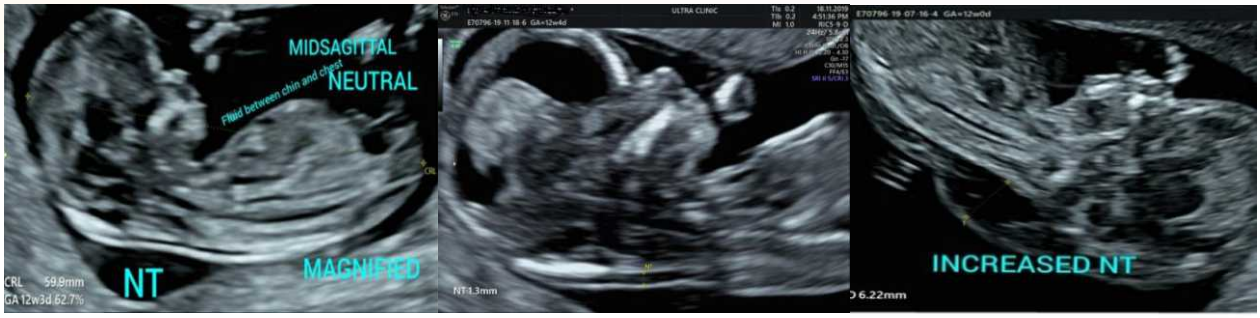
All pregnant women whether they accept or not should be offered screening for aneuploidies. Pregnant women should decide the genetic investigation technique after extensive counselling regarding the advantages, limitations, the sensibility, and false-positive results of every genetic test available.

Ultrasound soft markers are not in themselves abnormalities, but rather ultrasound findings which may indicate an increased risk of underlying abnormalities. Soft markers are most effectively applied in likelihood ratios to calculate the individual risk for a pregnant woman based on her own background risk of aneuploidy. Isolated soft markers have a weak association with DS.
The list of "soft markers" is long and includes, nuchal translucency, nasal bone, ductus venosus, tricuspid flow, fronto-maxillary facial angle and fetal heart rate in the first trimester and in the second trimester they are nuchal fold thickness, short femur, mild hydronephrosis, mild ventriculomegaly, echogenic focus, echogenic bowel, mid phalanx hypoplasia of the fifth digit, brachycephaly, wide pelvic angle, flat profile, pre nasal oedema, macroglossia and hypoplastic or absent nasal bone. Fetal renal pyelectasis, shortened long bones (less than third centile for gestational age) with a shortened femur or/and a shortened humerus, single umbilical artery, aberrant right subclavian artery, and gap sandals toes.
In the presence of soft markers, the risk of Down syndrome is recalculated as new risk = baseline risk x likelihood ratio (LR). The new LR is calculated by multiplying all positive LRs (of markers present) and all negative LRs (of markers absent).
Note: if a single marker is present, then isolated LR is considered. Some of these soft markers will be discussed in a nutshell.

- First trimester:
- In the first trimester of pregnancy the optimal gestational age is 11+0 to 13+6 weeks (crown–rump length (CRL) at least 45 mm upto 84 mm) for the markers described below.

Nuchal translucency:

- Nuchal translucency (NT) is the sonographic appearance of a collection of fluid (septated or not or enveloping the fetus) under the skin behind the fetal neck.
- The incidence of chromosomal and other abnormalities is related to the size, rather than the appearance of NT.
- The translucency usually resolves in majority when followed or it evolves into either nuchal edema or cystic hygromas with or without generalized hydrops in few cases.
- A mid sagittal section of the fetus, where the echogenic nasal bone and rectangular palate are seen separately must be obtained and zoomed such that the fetal head and upper thorax occupy the whole screen.
- The fetus should be in a neutral position, with the head in line with the spine.



- The widest part of translucency must always be measured and the callipers placed ON the line that defines the nuchal translucency - the crossbar of the calliper should merge with the white line of the border, not in the nuchal fluid. It is important to turn the gain down.

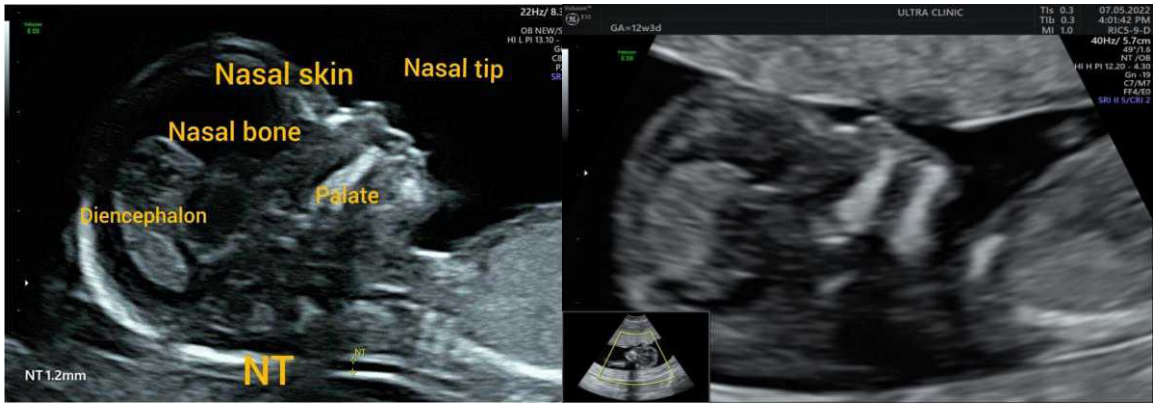
Increased NT is associated with:

- Trisomy 21 and other major chromosomal abnormalities.
- More than 50 fetal defects and genetic syndromes.
- Fetal death.

However, in the majority of cases the NT resolves and the babies are born healthy.

Nasal bone:

- Three distinct lines seen at the level of the fetal nose in a mid sagittal section of the fetus zoomed such that the fetal head and upper thorax occupy the whole screen and the ultrasound transducer parallel to the direction of the nose, gently tilted from one side to the other of the fetal nose.



- The top line represents the skin, the bottom one, which is thicker and more echogenic than the overlying skin, represents the nasal bone whereas a third line in front of the bone and at a higher level than the skin represents the tip of the nose.
- The nasal bone is considered to be present if it is more echogenic than the overlying skin and absent if it is either not visible or its echogenicity is the same or less than that of the skin.

Nasal bone is considered to be absent in:

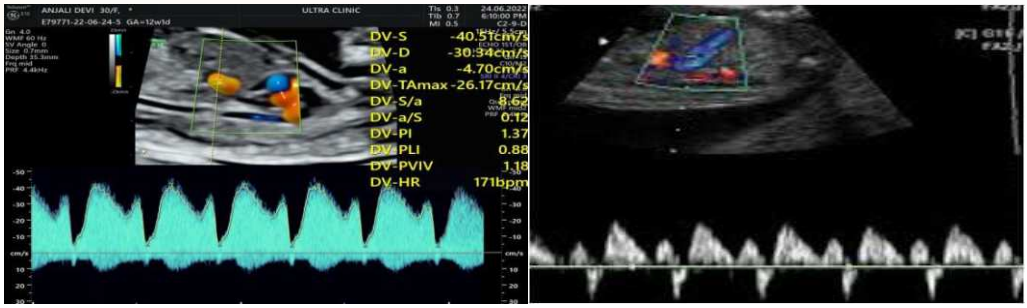
- Euploid fetuses 1-3%
- Fetuses with trisomy 21 60%
- Fetuses with trisomy 18 50%
- Fetuses with trisomy 13 40%

The Ductus Venosus :

It is a short vessel connecting the intra hepatic umbilical vein to the inferior vena cava at its inlet to the heart

· A right ventral mid-sagittal view of the fetal trunk obtained in fetal quiescence and zoomed so that the fetal thorax and abdomen occupy the whole screen.

Colour flow mapping used to demonstrate the umbilical vein, ductus venosus and fetal heart and then the pulsed Doppler placed on the ductus venosus with a small sample gate (0.5-1.0 mm) to avoid contamination from the adjacent veins and placed in the yellowish aliasing area with insonation angle less than 30 degrees, using low frequency (50-70 Hz) filter to allow visualization of the whole waveform and the high sweep speed(2-3 cm/s) so that the waveforms are widely spread for better assessment of the a-wave.



Qualitative assessment of the ductus venosus blood flow is based on the appearance of the a-wave:

- Positive or absent (normal).
- Reversed (abnormal).

Reversed a-wave

Reversed a-wave is found in about:

- Euploid fetuses 3%
- Fetuses with trisomy 21 65%
- Fetuses with trisomy 18 55%
- Fetuses with trisomy 13 55%

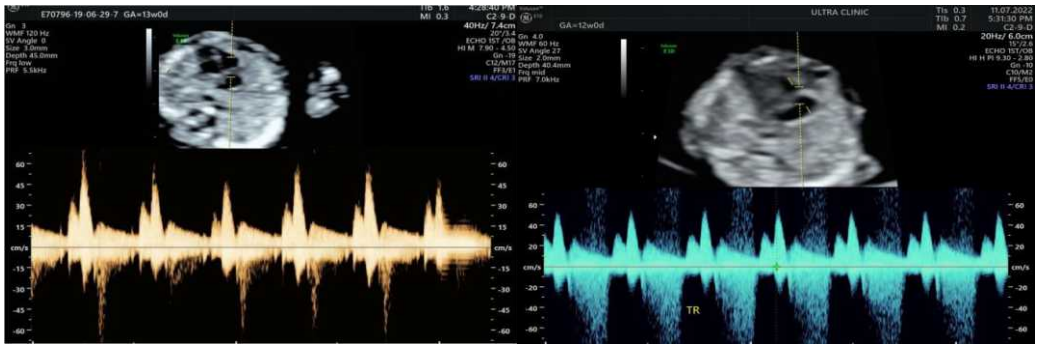
Reversed a-wave is associated with increased risk for:

- Chromosomal abnormalities
- Cardiac defects
- Fetal death

However, in about 80% of cases with reversed a-wave the pregnancy outcome is normal.

Tricuspid flow:

- An apical four-chamber view of the fetal heart is obtained in fetal quiescence and zoomed so that the fetal thorax occupy the whole screen.
- The pulsed Doppler sample gate (2 to 3 mm) positioned across the tricuspid valve and the insonation angle to the direction of flow kept less than 30 degrees from the direction of the inter-ventricular septum with the high sweep speed (2 to 3 cm/s) so that the waveforms are widely spread for better assessment.



- Colour flow mapping should not be used because it is unreliable for the diagnosis of tricuspid regurgitation in the first-trimester.

- The sample volume should be placed across the valve at least three times, in an attempt to access and assess the complete valve.

Normal profile with no regurgitation during systole.
Regurgitation during approximately half of systole and with a velocity more than 60 cm/s.
Tricuspid regurgitation is found in about:

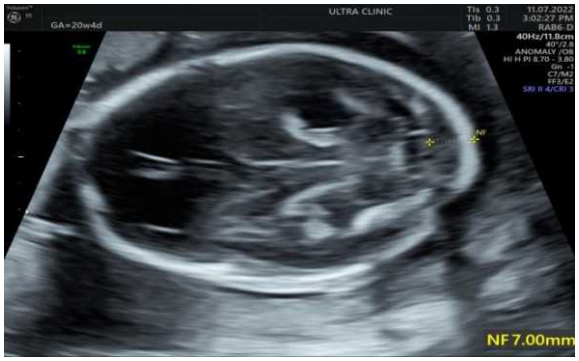
- Euploid fetuses 1%
- Fetuses with trisomy 21 55%
- Fetuses with trisomy 18 30%
- Fetuses with trisomy 13 30%

If there is tricuspid regurgitation it is important that detailed ultrasound examination is carried out to diagnose or exclude major cardiac defects.

Second trimester:

Soft marker: Thickened nuchal fold

Description: Nuchal fold that mea¬sures ≥ 6 mm between 15 and 20 weeks of gestation.
Technique:
Nuchal fold thickness is measured on an axial section through the head at the level of the thalami, cavum septi, pellucidi and cerebellar hemispheres. One calliper should be placed on the outer edge of the skin, and the other against the outer edge of the occipital bone. The ideal angle of insonation is approximately 30o to the horizontal. This plane is less likely to produce a false positive thickened nuchal fold.



Range of likelihood ratios:
- o positive LR: 23.3
- o negative LR: 0.8
- o isolated LR: 3.79

Remarks: One of the most specific markers for trisomy 21, This finding should be rare in fetuses that have had a prior thin nuchal translucency. If the NF is found to be thickened after a thin nuchal translucency, then other possible causes should be considered. Thickened NF may be an early feature of fetal hydrops or cystic hygroma, especially toward the third trimester; however, the risk of karyotypic abnormalities is not reduced.

Soft marker: Absent or hypoplastic nasal bone

Description: Definition of hypoplas¬tic nasal bone varies:
- ≥ 10 or ≥ 11 bipa¬rietal diameter: nasal bone ratio
- ≤ 2.5 mm in length
- < 2.5 gestational age-based percentile
- < 0.75 or ≤ 0.7 MoM

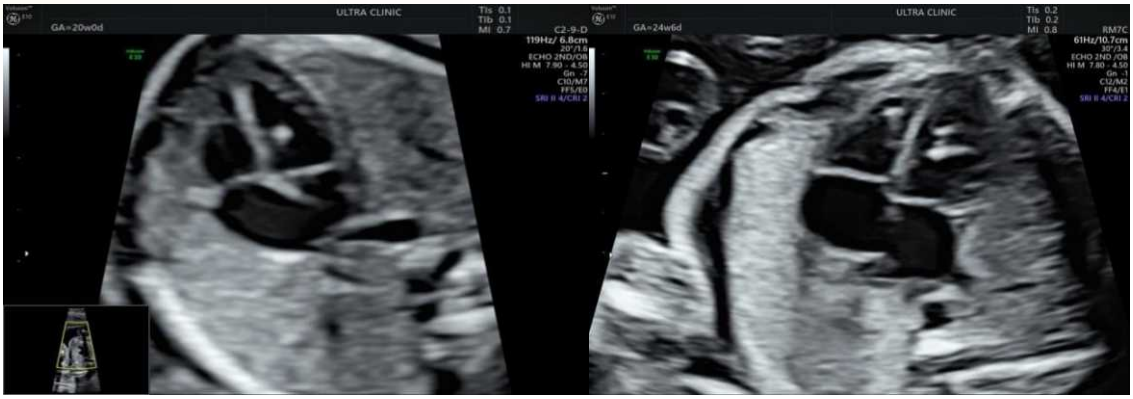
Technique: like in first trimester. It is assessed on a midsagittal view of the fetal face. Ideally, three echogenic lines should be seen.
Range of likelihood ratios:
- o positive LR: 23.3
- o negative LR: 0.46
- o isolated LR: 6.58

Remarks: One of the most sensitive markers for trisomy 21, Can be associated with other genetic syndromes

Soft marker: Echogenic intracardiac focus

Description: Echogenic area < 6 mm in either cardiac ventricle that is as bright as surrounding bone and visualized in at least 2 separate planes

Technique:
They are typically seen as a small bright echoic focus within the fetal heart on a four-chamber view (often as bright as bone). Tissue harmonic imaging should be turned off when evaluating a potential EIF, to avoid false positives. If it is difficult to tell if the EIF is as bright as bone, the gain in the image can be decreased to see which structure disappears first. It is usually single and less than 3 mm. It needs to be differentiated from normal papillary muscle which is not as bright as bone and a moderator band which is situated at the ventricular apex. The majority of echogenic intracardiac foci are unilateral. Out of all the cardiac chambers, the left ventricle is the most frequent in terms of location.



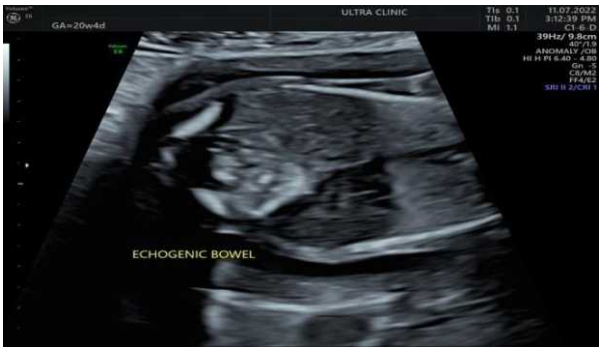
Range of likelihood ratios:

- positive LR: 5.83
- negative LR: 0.8
- isolated LR: 0.95
- Remarks: • Pathogenesis unclear

Do not represent a structural or functional cardiac abnormality
May be present in up to 12% of fetuses with trisomy 21 and associated with trisomy 13
The tightness of the association between an isolated EIF and aneuploidy continues to be debated. Biventricular EIFs are a higher risk for aneuploidy.

Soft marker: Echogenic bowel

Description Echogenic area in fetal bowel greater than or equal to that of surrounding fetal bone, typically the iliac wing
Technique: It is an observation in antenatal ultrasound imaging, in which fetal bowel appears to be bright. To be truly 'echogenic bowel' it must be brighter than bone (e.g. femur, spine), and this should be demonstrated on an image with appropriate gain settings. Tissue harmonic imaging and compound imaging should also be switched off. If there is difficulty discerning whether bowel is as echogenic as bone, one can progressively decrease the image gain to see which structure disappears first. It is most commonly seen in the right lower quadrant of the fetus.



Range of likelihood ratios:

- positive LR: 11.44
- negative LR: 0.9
- isolated LR: 1.65

Remarks:

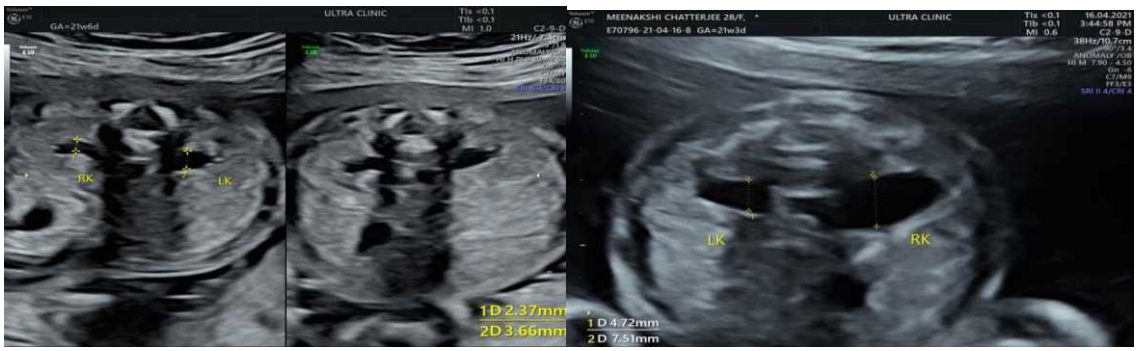
- Echogenic bowel can be a normal variant/isolated finding in up to ~70% of cases

Recognized associations include:

- Intra-amniotic haemorrhage
- Trisomy 21 less commonly associated with trisomy 13, trisomy 18 and Turner syndrome.
- Intrauterine cytomegalovirus infection
- Cystic fibrosis
- Intrauterine growth restriction (IUGR)
- Intrauterine fetal demise (IUFD): ~9 x increased risk
- Particularly if serum alpha-fetoprotein levels are also elevated
- Some data suggests that echogenic bowel occurs before 30 weeks when there is subsequent IUFD
- Meconium peritonitis

Soft marker: Urinary tract dilatation

Description: Fetal pyelectasis refers to the prominence of the renal pelvis in utero
Threshold for diag¬nosis:
• 16-27 weeks of gestation, Antero Posterior Renal Pelvis Diameter ≥ 4 mm
• ≥ 28 weeks of gestation, Antero Posterior Renal Pelvis Diameter ≥ 7 mm



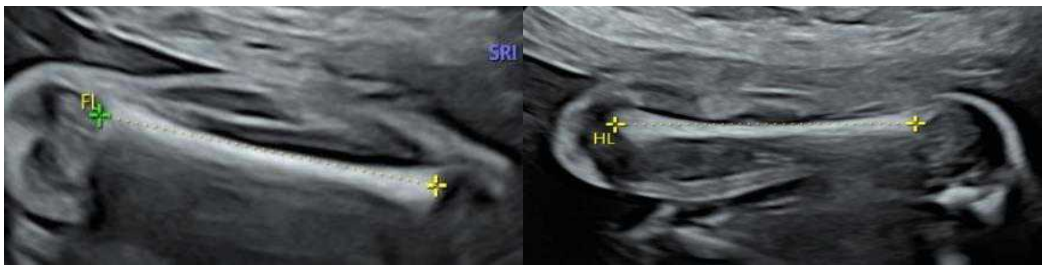
Technique:
Fetal pyelectasis is assessed as an AP measurement of the renal pelvis on an axial plane ultrasound image.
Range of likelihood ratios:
- positive LR: 7.6
- negative LR: 0.9
- isolated LR: 1.08

Remarks: Approximately 17 % of fetuses with trisomy 21 will have pyelectasis. Isolated pyelectasis appears to be a very uncommon finding in aneuploidy. A meta-analysis of soft markers confirmed the lack of significance of this finding. The presence of pyelectasis may actually reduce rather than increase the risk of Down syndrome.
Common patho¬logic causes include vesicoureteral reflux (the most common etiology), ureteropel¬vic junction obstruc¬tion, ureterovesical junction obstruction, multicystic dysplastic kidneys, and posterior urethral valves
Although these patients should be reviewed in the third trimester, the risk with mild pyelectasis is very low. Almost no cases measuring 4mm to 7mm at the second trimester will need surgery.

Soft marker: Shortened long bones (femur, humerus)

Description: Ratio of observed to expected bone length (based on biparietal diameter) for diag-nosis:
- Shortened femur: ratio < 0.92
- Shortened humerus: ratio < 0.90

Alternatively, less than 3rd centile for gestational age
Technique:
Standard technique of fetal long bones measurement like zoomed horizontal image of long bone with measurement of diaphysis only.



Range of likelihood ratios:
- shortened femur
  - positive LR: 3.72
  - negative LR: 0.8
  - isolated LR: 0.61
- shortened humerus
  - positive LR: 4.8
  - negative LR: 0.7
  - isolated LR: 0.78

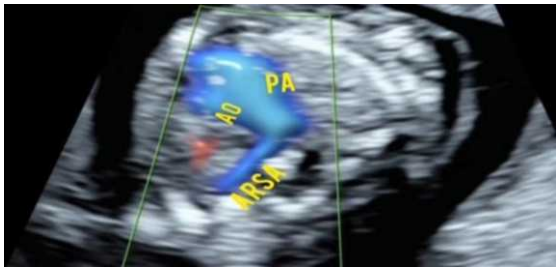
Remarks:
Shortened humerus and femur have both been associated with an increased risk of chromosomal abnormalities. There are many reasons why the long bones may be shortened. If they are severely shortened or abnormal in appearance (for example, with bowing, fractures or reduced mineralisation), then this may be an indication of skeletal dysplasia. Occasionally, shortening of the long bones is a warning sign of early onset intrauterine growth restriction. It would be prudent to review the growth of the long bones in two weeks time.
Almost all studies to date have demonstrated that the humerus is a more reliable discriminator for Down syndrome than the femur. Measurement of humerus length should become part of the routine mid-trimester ultrasound assessment.

Soft marker: Aberrant right subclavian artery (ARSA)

Description: Aberrant right subclavian artery (ARSA) originates from the aortic arch distally instead of brachiocephalic artery as the fourth supra-aortic vessel and follows a retro-tracheal course towards the right arm

- Technique

ARSA can be imaged at the level of three vessel trachea view in the upper mediastinum by putting color Doppler where it can be seen between the trachea and the spine, and runs towards the right arm



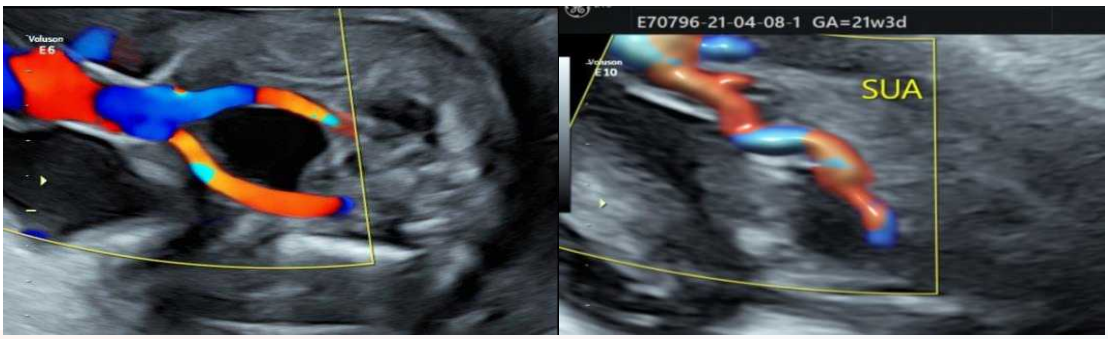
Remarks: Most studies found that isolated ARSA had no clinical significance and did not serve as an invasive prenatal chromosomal test.
Fetuses with ARSA and aneuploidy relevant soft markers, advanced maternal age, and abnormal biochemical screening should undergo amniocentesis.

Soft marker: Single umbilical artery

Description: Umbilical cord containing 1 umbilical artery instead of 2. Single umbilical artery (SUA) results when there is a congenital absence of either the right or left umbilical artery. In the usual situation, there are paired umbilical arteries. For unknown reasons, the absence of the left umbilical artery is much more common (~70%).

- **Technique**

Two vessels within the umbilical cord (one artery and one vein) instead of the usual three (best seen in cross-section). The single artery is often larger in calibre than normal and approaches the diameter of the accompanying vein. Examination of the fetal pelvis will demonstrate only one umbilical artery lateral to the bladder in its course toward the umbilical cord



Remarks: • Co-occurring structural abnormali¬ties most commonly involve the cardio¬vascular and renal systems
Conflicting evidence regarding association with stillbirth or FGR

Soft marker: Choroid plexus cyst

Description Small, fluid-filled struc¬ture within the choroid of the lateral ventricles of the fetal brain

Screen positive report: If not Down's, are we missing out something!

Sadia Mansoor, K Aparna Sharma
Fellow MFM, Additional Professor
Department of Obstetrics and Gynaecology , AIIMS, New Delhi

Overview

1. Introduction
2. Normal Variation
3. Abnormal Variations in markers with Down's Syndrome
4. Other fetal conditions diagnosed with screen positive report
5. Role of biomarkers in prediction of preeclampsia and fetal growth restriction
6. Factors affecting report interpretation

1. Introduction

Aneuploidies are a major cause of perinatal death and childhood handicap. Trisomy 21, 18, and 13, are some of the most common chromosomal anomalies screened in pregnancy and have first-trimester prevalence of approximately 1 in 340, 1 in 1100, and 1 in 3500, respectively. Early detection of pregnancies at high risk for trisomy 21 (Down syndrome) is the primary target of prenatal aneuploidy screening since this is the most common autosomal trisomy among live births. Maternal serum markers during first and second trimester can be used to estimate the pregnant woman's risk of having a fetus with Down's syndrome. This allows the woman to make an informed choice about invasive diagnostic testing, if screened positive. The risk cut- off chosen for test interpretation determines the detection and false-positive rates for the condition. Pregnancy is screen positive if risk is greater than 1:250. Some genetic abnormalities other than Down's syndrome have also been associated with alterations in first- and second-trimester serum markers causing spurious increase in the risk.

2. NORMAL VARIATIONS OF SERUM MARKERS IN PREGNANCY DURING 1ST AND 2ND TRIMESTER

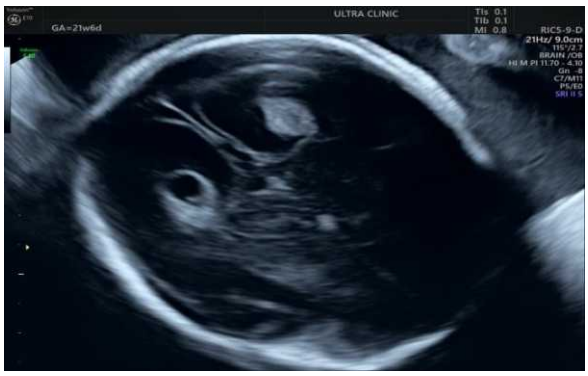
Combined first trimester screening (CFTS) includes the calculation of a composite risk of pregnancy for trisomy 21, 13 and 18 based on maternal age, nuchal translucency (NT) and biochemical serum markers- free/total beta HCG, pregnancy associated plasma protein- A (PAPP-A). It is the best and most economic method of screening offering a high detection rate of 92% at a false positive rate (FPR) of 5%. While, the second trimester screening involves triple
marker test and quadruple marker test with a detection rate of 65% and 70% respectively at 5% FPR.
In an unaffected pregnancy, PAPP-A increases by 30-50% per week between10 - 13 weeks period of gestation. While free/ total beta HCG reaches a peak by 10th week of gestation and declines thereafter at the rate of 5-10% per week. Inhibin A (InhA) exhibits a shallow, U-shaped curve with its nadir at 17 weeks of gestation. In the early second trimester, alpha-fetoprotein (AFP) increases by 15 to 20 percent per week and unconjugated estriol (uE3) by 20 to 25 percent per week.

3. ABNORMAL VALUES OF SERUM MARKERS IN PREGNANCY WITH DOWN'S SYNDROME

Variations have been noted in the biochemical level of serum markers between affected and unaffected pregnancies during 10+3 to 13+6 weeks gestation. These can be used to calculate patient- specific risk for the condition. Median PAPP-A rises from approximately 0.4 multiples of the median (MoM) at 10 weeks to approximately 0.7 MoM at 13 weeks, so the performance of PAPP-A decreases as gestation progresses. While, total/ free beta HCG rises during this period. These two effects tend to cancel each other out so that performance is relatively constant over this indicated time period. In second trimester, levels of AFP and uE3 are, on average, lower by 30 to 35 percent (0.70 to 0.75 MoM) in affected pregnancies. While, levels of total/free beta hCG, and InhA are approximately twice as high (2.0 MoM). The median MoM values of the second-trimester markers in Down syndrome pregnancy are constant over the gestational ages of interest (14+0 to 22+6 weeks). Reasons for altered maternal serum markers in Down syndrome pregnancy are not very well understood. Placental secretory products, such as free beta HCG and InhA are increased in the second trimester of an affected pregnancy. While secretory products synthesized in the combined feto-placental unit like AFP and uE3 are, on an average, low in maternal serum in such cases. Probable explanation for this could be attributed to the compensatory placental hyperfunction in response to poorly functioning fetal tissues.

Technique

- They are typically detected around the 2nd trimester and are seen in the axial plane as sonolucent cysts particularly at the level of atria involving the lateral ventricles.



Remarks: Present in approximately 1%-2% of fetuses in the second trimester

- Present in 30%- 50% of fetuses with trisomy 18

Not considered a structural or functional brain abnormality and nearly all resolve by 28 weeks

Association with T 18 can be negated by showing appropriate hand movement (fingers open) in all mid-trimester ultrasounds

CONCLUSION :

While the detection rate of trisomy 21 by Combined test (NT and serum double marker) is 90% at a false positive rate of 3%, addition of nasal bone improves detection rate to 93% and further increased to 95% if tricuspid flow included, and at the same time decreasing the false positive rate to 2.5%.

The current literature supports a policy of not reporting CPCs or EIF in low-risk women. There are two provisos: the hands must have been seen not to be clenched; and the scan must be of sufficient quality to reasonably expect to detect major anomalies. If views are incomplete or difficult, then a second opinion scan should be sought, not to review the soft marker, but in order to obtain clearer views. Each of the other markers in this article has ramifications beyond the risk of Down syndrome and therefore must still be looked for at the mid-trimester ultrasound.

Dr. Shankar Dey
MS (O&G), Consultant Fetal Medicine Specialist
ULTRACLINIC, Asansol, West Bengal

References :

1 . Significance of Sonological Evaluation of Nuchal Translucency and Correlation with Pregnancy Outcome
M Raksha1, KVN Dhananjaya2, KV Malini3, Aparna Baliga4

2. Society for Maternal-Fetal Medicine
Consult Series #57: Evaluation and management of isolated soft ultrasound markers for aneuploidy in the second trimester
Society for Maternal-Fetal Medicine (SMFM); Malavika Prabhu, MD; Jeffrey A. Kuller, MD; and Joseph R. Biggio, MD, MS

3. . Agathokleous M, Chaveeva P, Poon LC et-al. Meta-analysis of second-trimester markers for trisomy 21. Ultrasound Obstet Gynecol. 2013;41 (3): 247-61. doi:10.1002/uog.12364 - Pubmed citation

4 Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy.
British Medical Journal 1992; 304 doi: https://doi.org/10.1136/bmj.304.6831.867 (Published 04 April 1992)Cite this as: British Medical Journal 1992;304:867

Dr. Shankar Dey
MS (O&G), Consultant Fetal Medicine Specialist
ULTRACLINIC, Asansol, West Bengal

4. OTHER FETAL CONDITIONS DIAGNOSED WITH SCREEN POSITIVE REPORT

There are certain fetal chromosomal anomalies which may be opportunistically picked up on maternal serum screening as they mimic changes in serum markers similar to pregnancy affected with Down's syndrome. These include:

- **Turners syndrome (Monosomy X)** - In first trimester, PAPP-A is generally normal with increased total/free beta HCG and raised NT. In second trimester, pregnancies complicated with fetal hydrops, maternal serum marker pattern is similar to that observed in Down's syndrome, i.e, relatively low uE3 (median 0.61 MoM) and elevated total/free beta HCG (median 5.05 MoM). InhA levels are elevated (median 3.91 MoM) with a low to normal AFP (median range 0.8 to 1.0 MoM). In pregnancy not complicated with fetal hydrops, all maternal serum markers are on lower side.
- **Triploidies** (Type 1 and 2) – Maternal screening for trisomy 21, 18, and 13 results in detection of 84 percent of these cases at a 3.1 percent FPR. Type 1triploidy is of paternal origin and is characterized by cystic placenta with raised InhA and beta HCG and low uE3 levels. Type 2 triploidy, which is maternal in origin, has a small fetus and placenta. Maternal serum levels of uE3 are extremely low with reduced concentration of free beta HCG and InhA.
- **Smith- Lemli Opitz syndrome (SLOS)**:It is an autosomal recessive defect in cholesterol biosynthetic enzyme, C7-reductase, that leads to intellectual disability, poor growth, and a variety of phenotypic abnormalities (microcephaly with intellectual disability, characteristic facies, hypospadias, and polysyndactyly). It is more prevalent in Ashkenazi Jews and north European population.The second-trimester marker pattern associated with SLOS is a very low uE3 level (median 0.21 MoM) because the steroid precursors required for estriol synthesis are deficient in the fetus, with only modest reductions in AFP (median 0.72 MoM) and HCG (median 0.76 MoM). Maternal serum screening for SLOS with AFP, uE3, and HCG is very efficient, with a detection rate of approximately 60 percent at a 0.3 percent false-positive rate.

5. ROLE OF BIOMARKERS IN PREDICTION OF PREECLAMPSIA AND FETAL GROWTH RESTRICTION

Shift in the screening of Down's syndrome from second to first trimester of pregnancy has identified series of early biophysical and biochemical markers of impaired placentation. Maternal serum PAPP-A, which is one of the components of dual screen, in combination with uterine artery pulsatility index (Ut PI), mean arterial pressure (MAP) and placental growth factor(PLGF) at 11-13 weeks can be used to predict the risk of early- onset pre-eclampsia (PE). The risk of consequent fetal growth restriction is also higher in these pregnancies as compared to late- onset ones. In chromosomally normal pregnancies, low PAPP-A level (< 0.4 MOM) has good association with development of PE. The performance of PAPP-A alone in prediction of PE is modest with detection rate of approximately 44% at less than 34 weeks and 37% at less than 37 weeks (95% CI) with a 5% FPR. This performance is greatly enhanced on addition of Ut PI, MAP and PLGF with detection rates reaching approximately 93% and 61% (95% CI) at less than 34 and 37 weeks respectively at 5% FPR.

Some studies have also identified second trimester serum analytes and their association with PE and small for gestational age (SGA) fetuses. Meta-analysis has shown high InhA level
(>2.79MOM) has correlation with development of PE while low AFP level (<10th centile for gestational age) with pregnanacies subsequently developing SGA.

6. FACTORS TO CONSIDER FOR TEST REPORT INTERPRETATION

Following factors need to be kept into consideration before interpreting the results of screening:

- **Maternal age -** Older women have a higher a-priori risk, they are more often screen positive than their younger counterparts.
- **Prior pregnancy history** - Recurrence risk of aneuploidy is 1% if a woman has had a prior Down syndrome pregnancy (non-inherited type). It is higher for younger than for older women.
- **Maternal weight** - Serum marker concentrations decrease as maternal body weight increases because the additional blood volume dilutes the amount of marker that is present. Hence each marker should be adjusted according to body weight leading to substantial change in individual result.
- **Maternal race** - Concentrations of maternal serum AFP, PAPP-A, total/ free beta HCG are increased and InhA levels are decreased in Black versus White women.
- **Multiple gestation** - In first-trimester combined testing, it is possible to assign a separate risk to each fetus based on their NT measurements. Serum concentrations of each marker are approximately twice as high in twin versus singleton pregnancies, except uE3, which is only 1.6 or 1.7 times higher. Since the individual contribution of each twin to the maternal serum marker levels cannot be determined, prenatal screening in dizygotic twin pregnancies is not as efficient as in singleton pregnancies. Screening monozygotic twins is likely to perform as well as in singleton pregnancies. With correction of MoM values in twin gestations, a screening result can be generated that assumes each fetus contributes equally to the marker levels. However, a precise risk estimate cannot be given because of this assumption and because the actual marker levels in twin pregnancies with a Down syndrome fetus or fetuses are not known with certainty.

Serum screening results of higher order pregnancies cannot be reliably interpreted and hence not advisable.

- **Previous false positive results** - There is an increased risk of a false-positive result in a subsequent pregnancy, which could be as high as 20%. Adjustments are made that will minimize the problem of recurrent false-positive results in all women according to their values in the previous unaffected pregnancy.
- **Assisted reproductive techniques** - IVF and ICSI can affect serum marker levels to mimic the pattern associated with Down syndrome. Maternal HCG and InhA levels tend to be increased, uE3 and PAPP-A levels are decreased. Without adjustment of these MoM values, the screen-positive rate for such pregnancies is approximately twice the expected rate in normal population.16 Similar adjustments of free beta hCG and Inh A levels may be required for pregnancies resulting from oocyte donation, ovulation induction or intrauterine insemination.
- **Cigarette smokers** - AFP and InhA levels are higher and uE3, free beta HCG and PAPP-A levels are lower in smokers than in non-smokers. Adjustments are made irrespective of the number of cigarettes smoked per day.
- **Others** – Alterations in serum concentrations have also been noted with respect to maternal parity, fetal sex, maternal systemic lupus erythematosus, renal insufficiency/ hemodialysis, anti-retroviral therapy.

Dr. Aparna Sharma
Hon National Secretary
Society of Fetal Medicine

Dr. Sadia Mansoor
Fellow, MFM
AIIMS, New Delhi

References :

1. Cuckle H, Benn P. Review of epidemiological factors (other than maternal age) that determine the prevalence of common autosomal trisomies. PrenatDiagn 2021; 41:536.
2. Harrison G, Goldie D. Second-trimester Down's syndrome serum screening: double, triple or quadruple marker testing? Ann Clin Biochem. 2006;43:67–72.
3. Palomaki GE, Messerlian G, Canick JA. A summary analysis of Down syndrome markers in the late first trimester. In: Advances in Clinical Chemistry, Makowski G (Ed), Academic Press, 2007. Vol 43, p.177.
4. Qin QP, Christiansen M, Nguyen TH, et al. Schwangerschaftsprotein 1 (SP1) as a maternal serum marker for Down syndrome in the first and second trimesters. PrenatDiagn 1997; 17:101.
5. Canick JA, Knight GJ, Palomaki GE, et al. Low second trimester maternal serum unconjugated oestriol in pregnancies with Down's syndrome. Br J ObstetGynaecol 1988; 95:330.
6. Lambert-Messerlian GM, Saller DN Jr, Tumber MB, et al. Second-trimester maternal serum inhibin A levels in fetal trisomy 18 and Turner syndrome with and without hydrops. PrenatDiagn 1998; 18:1061.
7. Benn PA, Gainey A, Ingardia CJ, et al. Second trimester maternal serum analytes in triploid pregnancies: correlation with phenotype and sex chromosome complement. PrenatDiagn 2001; 21:680.
8. Palomaki GE, Bradley LA, Knight GJ, et al. Assigning risk for Smith-Lemli-Opitz syndrome as part of 2nd trimester screening for Down's syndrome. J Med Screen 2002; 9:43.
9. Craig WY, Haddow JE, Palomaki GE, et al. Identifying Smith-Lemli-Opitz syndrome in conjunction with prenatal screening for Down syndrome. PrenatDiagn 2006; 26:842.
10. Poon LC, Nicolaides KH. First-trimester maternal factors and biomarker screening for preeclampsia. Prenat Diagn. 2014;34(7):618-627.
11. Morris RK, Cnossen JS, Langejans M, Robson SC, Kleijnen J, Ter Riet G, et al. Serum screening with Down's syndrome markers to predict pre-eclampsia and small for gestational age: systematic review and meta-analysis. BMC Pregnancy Childbirth. 2008 Aug 4;8:33.
12. Morris JK, Mutton DE, Alberman E. Recurrences of free trisomy 21: analysis of data from the National Down Syndrome Cytogenetic Register. PrenatDiagn 2005; 25:1120.
13. Watt HC, Wald NJ, Smith D, et al. Effect of allowing for ethnic group in prenatal screening for Down's syndrome. PrenatDiagn 1996; 16:691.
14. Prats P, Rodríguez I, Comas C, Puerto B. Systematic review of screening for trisomy 21 in twin pregnancies in first trimester combining nuchal translucency and biochemical markers: a meta-analysis. PrenatDiagn 2014; 34:1077.

Adding more markers: Are we really detecting more or Increasing interventions

Dr. Seetha Ramamurthy Pal

Down Syndrome affects 1 in 1200 live births and is a disease that necessitates significant societal, financial and legal support. Aneuploidies are a major cause of perinatal death and childhood handicap and hence screening for fetal chromosomal defects should be offered and available to all pregnant women as part of their antenatal care. Fortunately, Down syndrome can be suspected antenatally by combined ultrasound and serologic screening and confirmed by invasive techniques.1

Traditionally screening was offered only to women above 35 years as biochemical screening did not exist and invasive techniques carried a higher risk of miscarriage. However, majority of babies with Down syndrome are born to mothers younger than 35 years and this created interest in developing less invasive screening programs based on assessment of serum and Ultrasound markers that have shown association with Down syndrome. A number of soft markers have been discovered and identified over the years to improve screening, increase detection rate and reduce false positive rate. In the late 1980s, biochemical screening at 16 weeks of maternal serum Alpha-fetoprotein, (lower levels found in T21 fetuses)2, and later HCG and Unconjugated Estriol (Triple test) was added with a sensitivity of 70% at a 5% False positive rate. Subsequently the addition of Inhibin A (Quadruple screen) was introduced with a sensitivity of 81% (FPR-5%).3 The 1990s saw a major breakthrough when Prof Kypros Nicolaides identified a powerful ultrasound marker, the Nuchal Translucency thickness in the first trimester4. Increased NT measurement in isolation alone can detect 80% of fetuses with Downs and other aneuploidies for a FPR of 5%. This in combination with maternal age, and serum testing of beta HCG (Increased in T21 fetuses) and PAPP-A levels (decreased in T21 fetuses) was proposed as a screening method with a detection rate of 90%5.

Ultrasound markers in the second trimester, also called 'soft markers' were further introduced as a screening strategy. These markers are sonographic, structural, non- specific and sometimes transient signs which can be indicative of Fetal aneuploidy. The various second trimester markers are fetal ventriculomegaly (lateral cerebral ventricles > 10mm), Hypoplastic or absent nasal bone, increased nuchal fold thickness (> 6mm), Echogenic bowel, Aberrant right subclavian artery, Short femur and humerus, renal pyelectasis (> 4mm), echogenic intracardiac focus, choroid plexus cysts, single umbilical artery and increased sandal gap.

Historically, there were two main strategies to try to give a more proper risk assessment of Down syndrome. The first used a simple index scoring system having ≥2 as positivity criterion, and a score of 1 is assigned for the soft marker (excluding nuchal fold ≥6 mm, which scores 2) . The second was a Bayesian method, named age-adjusted US risk assessment or AAURA, which considers the a priori maternal age-specific risk combined with a quantitative likelihood ratio. In the presence of a previous risk of 1:200 or greater, the test was considered positive. Each marker has a specific likelihood ratio (LR) which when present would raise the baseline risk of Down syndrome by a factor equal to its positive LR. This approach identified 75% of fetuses with Down syndrome and was used as a primary screening test or used to refine the initial screening test when couples did not opt for invasive testing.

With advances in ultrasound resolution and skills, this was extended to the first trimester to identify ultrasound markers in the first trimester to provide for early detection and diagnosis. Absent or hypoplastic nasal bone, reversed 'a' wave in the ductus venosus, Tricuspid regurgitation found in 60, 66 and 55% of fetuses with Down syndrome were identified as highly sensitive and specific first trimester markers increasing the detection rate to about 95%. New markers like Increased flow in the hepatic artery, Fronto-maxillary facial angle, (angle between the upper surface of the palate and the frontal bone, larger in T21 fetuses), Fronto nasal fold thickness and the Frontonasal fold/Nasal bone length ratio (constant ratio in euploid fetuses) have also been proposed to improve detection rate.

Though many serum and ultrasound markers have been introduced, not all of them are strong and sensitive markers. The Triple test with its low detection rate does not qualify to be an effective screening test. Nuchal translucency in addition with absent NB, 'a' wave reversal in Ductus venosus and Tricuspid regurgitation are very strong markers but they can be found in Euploid fetuses as well and their reliability depends to a great extent on the availability of skilled professional with appropriate training of the operator and strict adherence to the standard protocol in measurement.

Additionally, new markers like hepatic flow artery, FMF angle are technically difficult to be reproducible, need strict image acquisition criteria, increasing the chances of erroneous measurements. With regards to second trimester soft markers, though they were originally introduced to improve detection rate, with the advent of cfDNA, their role has been downsized. In the event of multiple soft markers, ACOG recommends a detailed fetal anatomic ultrasound examination, diagnostic testing and genetic counseling11. Isolated markers result in only a small effect on modifying the risk of Down syndrome, however certain soft markers

are very predictive of Down syndrome and should be considered significant enough to warrant invasive testing. These include Absent nasal bone, mild ventriculomegaly and Increased Nuchal fold. Diagnostic testing should not be recommended to patients with an isolated soft marker in the setting of a negative NIPT result12. Also, looking for soft markers of trisomy 21, should not be performed in women with a normal NIPT result due to its high false-positive rate and poor positive predictive value13.

In conclusion, the addition of new markers has the potential to increase detection rate if each marker is identified correctly, interpreted and dealt with a different management approach but can definitely increase interventions otherwise. The need of the hour is to improve training of professionals to be more skilled in identifying the strong markers and be more conversant in interpreting the analytes so as to avoid unnecessary interventions and anxiety for the couple.

Dr. Seetha Ramamurthy Pal
Consultant Fetal Medicine, Apollo Hospital, Kolkata
Vice President, SFM Bengal Chapter

References :

1. American Congress of Obstetricians and Gynecologists (ACOG). (2007, May 7). ACOG's screening guidelines on chromosomal abnormalities.

2. Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. American Journal of Obstetrics and Gynecology. 1984;148:886-894

3. Sheldon TA, Simpson J. Appraisal of a new scheme for prenatal screening for Down's syndrome. BMJ. 1991;302:1133-1136

4. Nicolaides KH. Nuchal translucency and other First-trimester sonographic markers of chromosomal abnormalities. American Journal of Obstetrics and Gynecology. 2004; 191:45-67

5. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn. 2011 Jan;31(1):7–15

6. Benacerraf, B.R. The Role of the Second Trimester Genetic Sonogram in Screening for Fetal Down Syndrome. Semin. Perinatol. 2005, 29, 386–394

7. Fetal medicine. Research Publications

Time to revisit the concept of nasal bone as an aneuploidy marker

Dr. Lakshmy Ravi Selvaraj
Director. Shri Lakshmi Clinic and Scan Centre, Krishnagiri.

Nasal bone as an aneuploidy marker

Evaluation of the fetal nasal bone (NB) is a powerful tool in the prenatal screening armamentarium for Down syndrome. The last two decades have witnessed significant breakthrough research in this domain. The initial studies reported non visualization of the nasal bone in prenatal ultrasonography in 69% of cases of trisomy 21 in the first trimester. [1] Hence inclusion of the nasal bone in routine screening for aneuploidy appeared very promising.

Inclusion of NB in the first trimester along with other soft markers like tricuspid regurgitation and ductus venosus has been reported to reduce false positive rate to about 2-3% [2]. The meta-analysis by Agathokleous et al. reported absent NB as the most sensitive marker in second trimester with the highest likelihood ratio of 6.58 [3]. Though the nasal bone can be assessed in both first and second trimester by prenatal ultrasound, its diagnostic value and its exact role as a method of risk assessment in clinical practice is yet to be standardised. The technical expertise required to evaluate the fetal nasal bone in the first trimester limits the widespread application of this approach as a universal screening tool.

Standard technique of visualising the nasal bone

In first trimester, the nasal bone is assessed in the same midsagittal section that is taken to obtain the nuchal translucency measurement. Under appropriate magnification, the three distinct lines should be assessed.[4] The first two lines proximal to the forehead are horizontal and parallel to each other resembling an ‘‘equal sign’’. The top line represents the skin and the bottom one, which is thicker and more echogenic than the overlying skin, represents the nasal bone as shown n Figure 1. A third line, almost in continuity with the skin, but at a higher level, represents the tip of the nose. NB was considered present when echogenicity of the second line was greater than that of the first skin line. It is ideal to evaluate the nasal bone when the fetal CRL is in the range of 65–74 mm to avoid false positives.

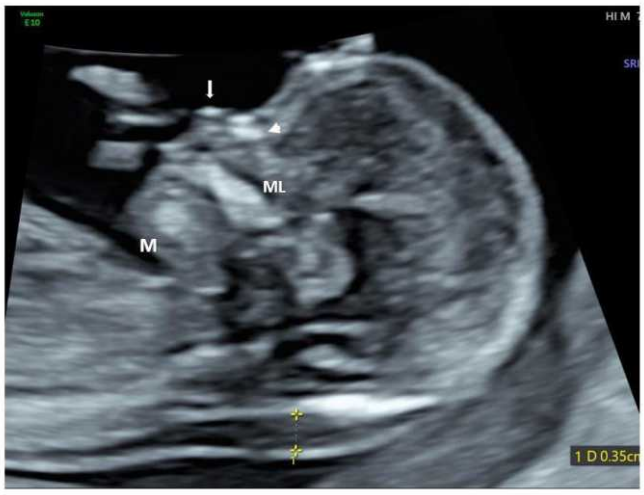


Figure 1: Anatomic landmarks to be considered while imaging the nasal bone in first trimester. Arrowhead points to nasal bone ; Arrow points to skin tip; ML-Maxillary line; M- Symphysis menti of mandible

In the coronal plane, the nasal bones can be identified at the upper tip of the retronasal triangle as two paired small echogenic structures completing the apex of the triangle. [5] When the nasal bone line appears as a thin line less echogenic than the overlying skin and the nasal bone status is indeterminate in the midsagittal section, retronasal triangle view can be incorporated to confirm presence of nasal bone.

In mid trimester, a true midsagittal facial profile is required for assessment of the nasal bone. It is ideally done with the fetal neck in neutral position. Evaluation of the nasal bone should not be done with the fetal neck in extension to avoid spurious results. The anatomic landmarks which can be considered for identifying a true midsagittal section of the face are as follows: the echogenic skin tip of the nose, the rectangular appearance of the maxillary line and the triangular or dot shaped appearance of the symphysis menti of the mandible. (figure 2)

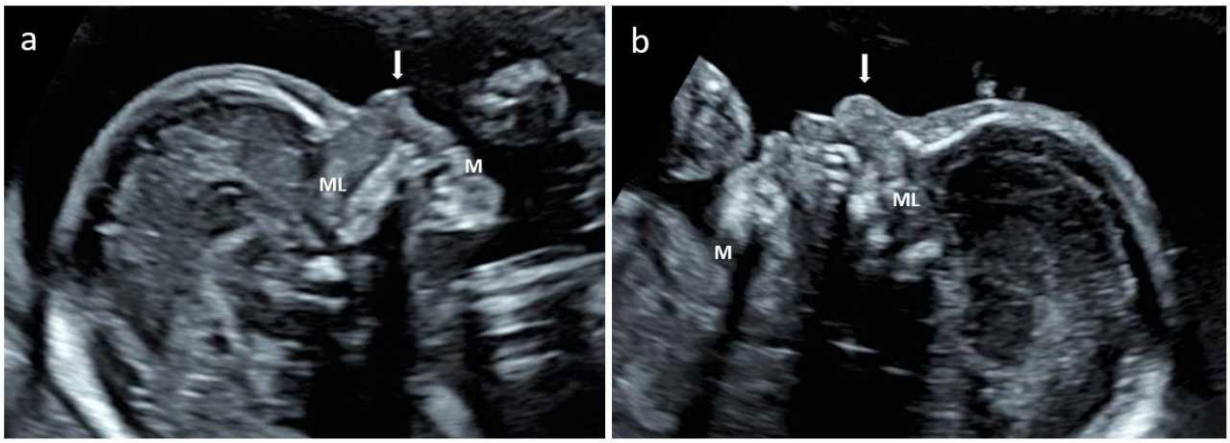


Figure 2: Anatomic landmarks to be considered while imaging the nasal bone in mid trimester. a) Unossified nasal bone b) Hypoplasia of nasal bone. Arrow points to skin tip; ML-Maxillary line; M- Symphysis menti of Mandible

The true definition of hypoplastic nasal bone

Various criteria has been defined to identify hypoplasia of the nasal bone based on measurements such as BPD: nasal bone ratio, gestational age-adjusted nasal bone length, or a single cut-off definition. NB hypoplasia was defined either as a ratio of the BPD/NB >11 or nasal bone length <0.75 MoM for the gestational age.[6] Sonek et al established the normal ranges for nasal bone length measurements based on 3537 fetal cases and the 2.5th centile for nasal bone length has been described across 11-40 weeks of gestation. [7] A single cutoff value of 2.5 mm has been proposed by Cicero et al. [8] Nasal bone length is influenced by maternal ethnic origin and the incidence of nasal bone absence and hypoplasia is higher in the normal Afro-Caribbean and Asian fetuses. [9] Various population specific charts are also available which define normograms for nasal bone length. A consensus is yet to be arrived on the ideal methodology to define nasal bone hypoplasia and standardisation on the definition of NB hypoplasia to be incorporated into routine clinical practice.

Fiascos in implementing nasal bone as a first trimester marker in universal screening programme

Though nasal bone (NB) assessment in first trimester has been proposed to improve the efficiency of combined first trimester screening, unfortunately, the uncertainty of NB visualisation at this early gestational age limits its utility. Further factors like high BMI, a CRL between 45 to 65 mm, the interobserver variation involved limits the consistency of reporting NB.

Indeterminate nasal bone status in first trimester can be attributed to delayed maturation of NB in normal fetuses or compromised image resolution in women with high BMI. Reproducible results are heavily dependent upon technical requirements and operator expertise which are often available only in referral centres.

Malone et al concluded that first-trimester nasal bone evaluation was not a useful test for population screening for trisomy-21 and added little to first-trimester NT screening.[10] The technical limitation and the operator expertise involved significantly limit the usefulness of this aneuploidy screening technique.

Inclusion of NB to risk assessment in all cases in first trimester might falsely increase the number of screen positive cases. However, its inclusion in intermediate risk group alone after doing NT and serum testing might be beneficial. Otherwise nasal bone assessment can be confined only in midtrimester and a new risk is obtained which is modified from the first trimester combined screening results. This approach appears to be the ideal way of screening which eliminates the confounding factors of visualising nasal bone in first trimester.[11]

Scope for the future

The technique used for the assessment of nasal bone must be standardised and there should be no ambiguity in determining the marker status. A uniform standardised method to assess nasal bone hypoplasia has to be defined in our population. Routine combined screening with maternal age, nuchal translucency and serum biochemistry in first trimester serves as a robust method for universal screening. If nasal bone has to be included in risk prediction for wide spread screening purpose which involves less technical expertise, then ideally NB assessment for risk prediction should be taken up in second trimester. It appears to be the right time to revisit the concept of the role of nasal bone assessment in first trimester as an aneuploidy marker in routine clinical practice.

Dr. Lakshmi Ravi Selvaraj
Director, Shri Lakshmi Clinic & Scan Centre
Krishnagiri

References :
1.Cicero S, Curcio P, Papageorghiou A, Sonek J, Nicolaides K. Absence of nasal bone in fetuses with trisomy 21 at 11–14 weeks of gestation: an observational study. Lancet. 2001;358:1665–7

2.Nicolaides KH. Some thoughts on the true value of ultrasound. Ultrasound Obstet Gynecol. 2007;30:671–4.

3. Agathokleous M, Chaveeva P, Poon LC, Kosinski P, Nicolaides KH. Meta-analysis of second-trimester markers for trisomy 21. Ultrasound Obstet Gynecol. 2013;41:247–61.

4. Cicero S, Longo D, Rembouskos G, Sacchini C, Nicolaides KH. Absent nasal bone at 11– 14 weeks of gestation and chromosomal defects. Ultrasound Obstet Gynecol. 2003;22:31–5.

5. Adiego B, Martinez-Ten P, Illescas T, Bermejo C, Sepulveda W. First-trimester assessment of nasal bone using retronasal triangle view: a prospective study. Ultrasound Obstet Gynecol. 2014;43:272–6

6. Odibo AO, Sehdev HM, Stamilio DM, Cahill A, Dunn L, Macones GA. Defining nasal bone hypoplasia in second-trimester Down syndrome screening: does the use of multiples of the median improve screening efficacy?. American journal of obstetrics and gynecology. 2007 Oct 1;197(4):361-e1.

7. Sonek JD, McKenna D, Webb D, Croom C, Nicolaides K. Nasal bone length throughout gestation: normal ranges based on 3537 fetal ultrasound measurements. Ultrasound Obstet Gynecol. 2003 Feb;21(2):152-5.

8. Cicero S, Sonek JD, Mckenna DS et-al. Nasal bone hypoplasia in trisomy 21 at 15-22 weeks' gestation. Ultrasound Obstet Gynecol. 2003;21 (1): 15-8.

9. Prefumo F, Sairam S, Bhide A, Penna L, Hollis B, Thilaganathan B. Maternal ethnic origin and fetal nasal bones at 11–14 weeks of gestation. BJOG. 2004;111:109–12

10. Malone FD, Ball RH, Nyberg DA, et al. First trimester nasal bone evaluation for aneuploidy in the general population. Obstet Gynecol. 2004;104:1222–8.

11.Lakshmy SR, Shobana U, Rose N. Sensitivity of nasal bone as aneuploidy marker—first trimester versus second trimester assessment. Journal of Fetal Medicine. 2017 Sep;4(3):10917.

DV, TR: Downs syndrome marker or cardiac function markers (opinion)

Dr. Khurshid Alam

In this era of innovative technology and sophisticated prenatal screening techniques (like NIPS) in adjunct with advanced knowledge base and plethora of evidence- based publications, the conundrum is whether ductus venosus((DV) and tricuspid regurgitation (TR) should predominantly be used as marker for Downs syndrome or are they downstream effects of abnormal cardiac function. For years, I have wondered and discussed this with my colleagues and senior stalwarts

Before giving my perspective, I would like to throw light on few landmark articles in this regard. J. M. Martinez et al (2010) in his study concluded that the use of DV blood flow assessment increased early detection of CHD by 11% with respect to the use of NT measurement alone. In case of normal NT and no other structural anomalies, the predictive value of finding of absent/reverse DV at the 11–14 weeks scan to detect CHD is about 1/20. If increased NT is also found, the risk increases to about 1/10.

A retrospective study conducted by R. Stressig et al concluded second-trimester ultrasound screening for trisomy 21 based on maternal age with additional assessment of the ductus venosus, tricuspid blood flow and the fetal nasal bone in otherwise normal-appearing fetuses is only marginally better than screening by maternal age alone.

In 2014, Marcin Wiechec et al concluded that TR in combination with other markers is the strongest predictor for aneuploidy. TR, as an isolated parameter, is a poor screening tool, both, for all and for each individual chromosomal abnormality and congenital cardiac defects.

In 2017, a systematic review and meta-analysis by Carolina Scala et al was conducted to establish the predictive accuracy of TR for CHD. Their conclusion was that detection of TR in the first trimester increases the risk of CHD. However, isolated TR in the first trimester does not seem to be a strong predictor for CHD. If additional markers, such as reversed a-wave in the DV or increased NT, are present or there is suspicion of major CHD, early fetal echocardiography should be offered.

In 2019, Natasa Karadzov Orlic et al, in their article, concluded that in chromosomally normal fetuses without non-cardiac anomalies, addition of simple cardiac scan to the combined first trimester screening parameters improves detection of major CHD during first trimester.

G. P. Minnella et al published a retrospective analysis in journal of UOG (2020), in which the author concluded that at 11–13weeks' gestation, measurement of fetal NT and assessment flow across the tricuspid valve and in the ductus venosus can lead to early diagnosis of major heart defect.

In support of my belief about the utility of these 2 markers (DV and TR) to risk stratify Downs syndrome or as indirect marker for underlying cardiac functional/structural abnormality, herein, I present the following viewpoint.

First, to identify or suspect major cardiac abnormality screening by basic three views demonstrating situs, inflows (surrogate of 4 chamber view) and "tick" or "V" sign (surrogate of cross-over of outflow tracts) in 1st trimester is mandatory. In case of **high-risk group,** extended fetal heart examination ("EFHE") composed of 7 views (situs, 4CH, LVOT, RVOT, 3VT, ductal and Ao arch view in 2D and colour) is still the best for cardiac assessment till date. (Baoying Ye et al, Transl Pediatr 2021)

Second, screening for Downs syndrome by adding multiple markers has very limited incremental value to combined first trimester screening detection rate (90% to 93%) but definitely **reduces false positives** (from 5% to 2.5%), thereby reducing invasive procedure (chorionic villus sampling/amniocentesis) rate. In addition, in era of NIPT with structurally normal fetus (in pre NIPT comprehensive ultrasound), these markers have no role in risk calculation.

Finally, it's my conviction, as reflected in scores of literatures with several conflicting statements, elements like DV and TR are losing their sting both as marker for Downs syndrome and cardiac screening tool. Hence, for cardiac screening, emphasis should be only on direct interrogation of fetal heart (3 view or 7 view approach) as stated above and Downs syndrome screening. It may be used as additional markers to bring down false positives but centre-stage should be quality NT and CRL in risk algorithm using FMF accredited software.

Dr. Khurshid Alam
Consultant Fetal Medicine
Fetomat Foundation , Kolkata

Bibliography-

1. J. M. Mart´inez, M. Comas, A. Borrell, et al. Abnormal first-trimester ductus venosus blood flow: a marker of cardiac defects in fetuses with normal karyotype and nuchal translucency. Ultrasound Obstet Gynecol 2010; 35: 267-272.

2. R. Stressig, P. Kozlowski, S. Froehlich et al. Assessment of the ductus venosus, tricuspid blood flow and the nasal bone in second-trimester screening for trisomy 21. Ultrasound Obstet Gynecol 2011; 37: 444–449.

3. G. P. Minnella, F. M. Crupano, et al. Diagnosis of major heart defects by routine first-trimester ultrasound examination: association with increased nuchal translucency, tricuspid regurgitation and abnormal flow in ductus venosus. Ultrasound Obstet Gynecol 2020; 55: 637–644.

Debate topic : Intermediate risk group should be removed from Down syndrome screening : It only mediates more maternal anxiety

Writing for the motion : Dr. Prasanna Roy

Prenatal screen of Down syndrome with maternal serum screening has been established for two decades , leading to a decrease in the prevalence of DS in many developed countries

First-trimester screening combining nuchal translucency thickness measurement and assessment of serum analytes (combined test) is offered to pregnant women during the first trimester.

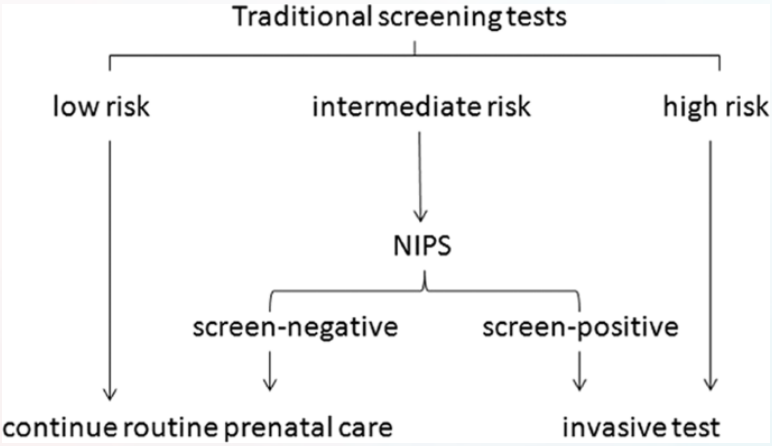
If an intermediate risk (1/101-1/1000) for Down syndrome is identified, women are referred for risk reassessment that includes the use of secondary ultrasound markers (nasal bone, ductus venosus blood flow and tricuspid flow)

The lay public who are the end-users of the screening program may hardly be aware of the differences between a screening test and a diagnostic test, and we confuse them more by putting some of them in the intermediate risk group.

When a couple undergoes a screening test, they expect a concrete conclusion from the treating physician, not a sequential confusion creating risk category labelled as “intermediate’

Therefore the combined test for Down syndrome, should categorize women into high and low risk group only

The purpose of a screening test is only to identify a subset from the screened population to whom the diagnostic test (e.g., chorionic villus sampling/amniocentesis) needs to be offered
So what we are currently following is this flowchart :



But what I think is the correct way of dealing with prenatal screening is this :

Both these flowcharts points to the same tool that we are using to reach a conclusion, when the patient is not in low risk group i.e cf DNA

So when we are using a common tool of cfDNA for both the scenarios, then there is no point including another risk category in our approach to screening.

In fact not all private sector labs are including this "intermediate risk" category in their reports. So, definitely there is no uniformity in generating the reports across all labs. This might result in another confusion.

But all said and done, we cant afford to miss Down syndrome cases in the population. Therefore we should increase our safety net to a number like 1:1000, beyond which lies the low risk group.

Most Indian laboratories that report DS screening tests have fixed a screen-positive cutoff at 1 in 250 without validating the assumption that underpins this cutoff: that the population undergoing screening would distribute itself such that 5% of the population would have a final adjusted risk at or greater than 1 in 250.

The entire performance of any screening test would depend on the cutoff that is considered as screen positive: large-scale data on the distribution of the risks and the risk determinants in our population are distinctly lacking.

Method of screening	Proportion of total population	Proportion of all cases of T21
Maternal age	5% / 20%	30% / 50%
Second trimester serum biochemistry	5%	50-70%
Age, NT, FHR, ß-hCG, PAPP-A (combined)	3-5%	90%
Combined plus additional ultrasound markers	2.5%	95%
Cell free DNA in maternal blood	<0.1%	>99%

Therefore, if we look at the above table, then we can understand that, just by including the additional ultrasound markers, we are getting a DR of 95 %

So if we can do NT at the 1st place, why can't we do a NB, or a DV or a TR for that matter

Finally, we understand that we are moving towards a new era of screening after the introduction of cfDNA in the market, where the initial 1st step will be to do a NT+ cfDNA in the 1st instance itself. This will erase all this confusion regarding risk categorization, and will justify the true meaning of a screening test i. e , on whom to perform an invasive testing ??

Few hospitals and practitioners make an effort to provide meaningful pretest counseling. It is certainly not uncommon to encounter women who have undergone a "brain function test" for their fetus and then undergone termination of pregnancy since the test was abnormal. If the western world is ruing the loss of normal fetuses due to unnecessary invasive tests after screen positivity, we are faced with the shameful situation of losing normal fetuses due to misinformation and lack of information. Therefore, in our Indian scenario, with the so called "busy clinic" , we should avoid putting a pregnant women into such a category of " intermediate risk"

Rather, what we should strive for is to put screening protocols in state sponsored health programmes and most important is to decrease the cost of NIPT, which will help us to alleviate all confusions regarding prenatal screening.

Once NIPT becomes reasonably affordable and easily available, we will not be debating this topic any further in the future.

Against the motion – Dr. Arkyayoti Mukherjee

Screening for common aneuploidies like trisomy 21 or Down syndrome is now a routine practice in today's obstetrics. The expectation from an effective screening method is to be simple, widely available and highly sensitive; so that minimum number of cases are being missed in the population.

Risk-based analysis of the result of such screening tests are done. As, further course of action depends on the risk category, we have to carefully scrutinize the result. The main motto is to increase the detection rate without increasing the number of interventions as well as miscarriages.

If we go through the literature, it is found that, by using the commonly used screening tools (Maternal age, NT, PAPP-A, Beta HCG) detection rate is around 90% with a false positive rate of 5%1, if we perform invasive confirmatory testing only on the 'high risk' group. So, there is definite scope of improvement.

To improve detection rate, either we have to incorporate more testing techniques (Additional ultrasound markers like Ductus Venosus,Tricuspid flow; DR 93-96%;FPR 2.5%)2,3 or increase the number of candidates to be investigated further.

Incorporation of more complex techniques will need more expertise and technology may cause hindrance in the screening of large population and these markers can also be misinterpreted if not done in a proper way. So, the easier way to increase detection rate is by increasing the number of candidates, who will be tested further and here comes the role of 'intermediate risk' group.

The candidates of 'intermediate risk' group should be tested further with non-invasive tests as the immediate next course of action but unlike the 'high risk' group invasive tests are not offered on the first hand. With further testing of this 'intermediate risk' an additional 4-5% cases can be picked up, which is important and much needed performance enhancement. Furthermore, as invasive tests are not offered as the immediate next line of management, there is no increase chance of miscarriage or harm to the fetus.

There are multiple options available in our armamentarium and next line of testing can be chosen carefully by both the treating physician and prospective parents together after proper information sharing and counselling.

Genetic sonogram (negative LR 0.13%)4 and NIPS (NPV <0.1%)5 both are effective options with decent negative predictive values. Invasive tests to be offered only to those cases which fall into 'high risk' category after second line of non-invasive screening test. So, such opportunities should be utilized, otherwise we will miss a considerable number of cases and add worries not only to the prospective parents but also a potential burden to the healthcare system.

If we look into the stress and anxiety related to any screening test result, it is a short-lived problem and can be taken care of by proper information sharing and reassurance. But, if these problems are not addressed properly it can either lead to pregnancy termination or birth of an affected fetus, based on misinterpretation followed by wrong course of management. While interpreting a screening test result, we should always be optimistic but, a note of caution should always be kept in mind.

References:

1. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. Ultrasound Obstet Gynecol 2005; 25: 221–226.

2. Kagan KO, Valencia C, Livanos P, Wright D, Nicolaides KH. 2009c. Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11+0–13 + 6 weeks of gestation. Ultrasound Obstet Gynecol 33: 18–22

3. Maiz N, Valencia C, Kagan KO, Wright D, Nicolaides KH. 2009. Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation. Ultrasound Obstet Gynecol 33: 512–517.

4. Agathokleous M, Chaveeva P, Poon LC, et al. Meta-analysis of second-trimester markers for trisomy 21. Ultrasound Obstet Gynecol. 2013;41(3):247–261

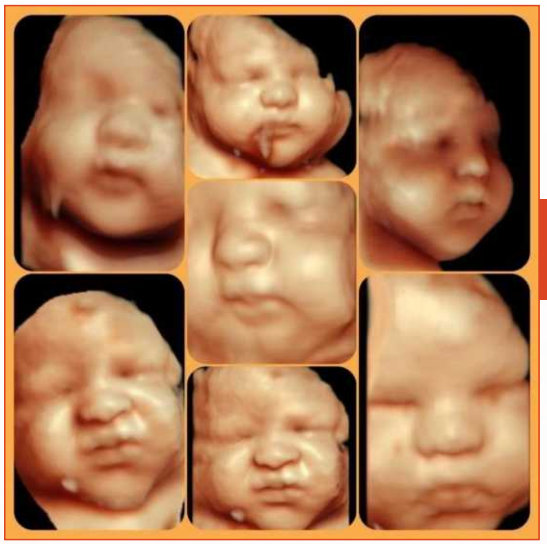
5. Taylor-Phillips S,Freeman K, Geppert J, et al. Accuracy of non-invasive prenatal testing using cellfree DNA for detection of Down, Edwards and Patau syndromes: a systematic review and meta-analysis.BMJ Open 2016;6:e010002.

"Quarterly National Image Contest"
From the Judge's Desk
Dr Mohit Shah

"keep taking keep sharing "

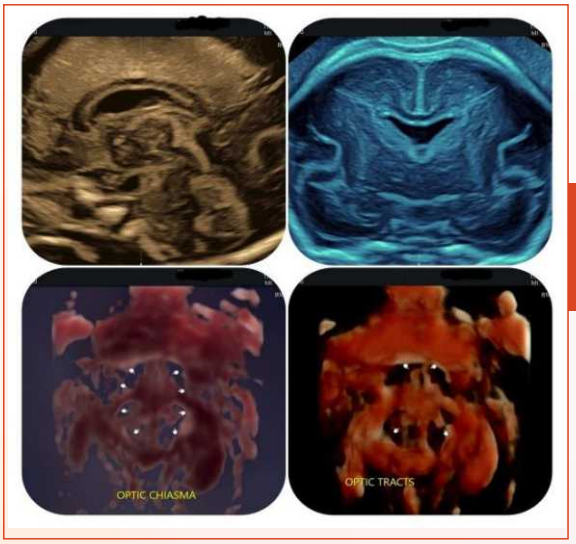
The Bengal Chapter had taken the initiative of holding an image contest for it's members. But this time the contest was opened up at a national level to all members of SFM. We received an overwhelming response from members across all chapters. Only still images were accepted maintaining utmost patient confidentiality. The assessor was blinded to the operator's identities and the scoring was done by our respected judge Dr. Mohit Shah from Mumbai. In his own words he said "Dear friends, it was both a pleasure and honor to judge the image contest. And believe me, it was by no means an easy job. Everyone had to put in so much of efforts to get those images. Congratulations to the winners. Those who didn't make it should not get disheartened and inculcate the habit of taking good images making optimal use of the technology. Our images are our credibility. They talk volumes about your expertise and understanding of the subject. So, I congratulate the members who came up with this idea of the image contest. Wish to get enchanted by more. Keep taking, keep sharing."

This time we had 30 entries and we congratulate our winners



1st prize – Dr. Megha Kamalapurkar (SFM no. 2476)
Kalaburagi, Gulbarga, Karnataka

3D rendered images of fetal face "Mind my emotions"



2nd prize – Dr. Prasanna Roy (SFM no. 2267),
ULTRACLINIC, Asansol, West Bengal

3D VCI & rendered images of a case of agenesis of septum pellucidum, with normal corpus callosum and optic tracts

We thank our esteemed authors who despite their busy schedules spent valuable time to write for our newsletter.

We are grateful to our media partner **Conferences International**, without whose support we couldn't have published this newsletter.