REVIEW ARTICLE

Ultrasound Markers of Chromosomal Anomalies in the First Trimester

Ashok Khurana

Consultant in Reproductive Ultrasound, The Ultrasound Lab, C584, Defence Colony, New Delhi, India

Correspondence: Ashok Khurana, Consultant in Reproductive Ultrasound, The Ultrasound Lab, C584, Defence Colony New Delhi-110024, India

ABSTRACT

Chromosomal anomalies are associated with considerable morbidity and mortality. The protocol for identifying these fetuses had for many years included a single clinical criterion of maternal age. Advances in biochemical screening combined with the excellent display of fetal dysmorphology afforded by technological advances in ultrasound equipment have resulted in a paradigm shift in the diagnosis of chromosomal abnormalities in the fetus, from the second trimester to the late first trimester. The accuracy of diagnosis as reported in multiple large series has pushed both screening and diagnostic testing for chromosomal disorders to the window now referred to as the 11 to 13 weeks + 6 days scan. Recent data have shown chorion villus sampling after 10 weeks to be as safe in experienced hands as amniocentesis and this has pushed the advantages of first trimester screening further. Ultrasound parameters for the detection of Down's syndrome in the first trimester include the nuchal translucency (NT) as the most well-defined and studied parameter, evaluation of the nasal bone (NB), frontomaxillary facial (FMF) angle, ductus venosus (DV) flow velocity waveform, tricuspid regurgitation (TR) and fetal heart-rate. Each parameter has well-defined criteria to be fulfilled for accurate quantification. Biochemical parameters that are currently in wide use include PAPP-A and free beta-hCG. Other parameters that the software accounts for are the gestational age assessed by the crown-rump length, maternal age, ethnicity, smoking, IVF and number of fetuses with chorionicity. Combining maternal age, biochemistry, NT and NB between 11 to 13 weeks + 6 days yields a detection rate of 96% with a false positive rate of 5%.

Keywords: First trimester screening, Ultrasound markers, Trisomy 21, Ultrasound genetic screening, Nuchal translucency, Nasal bone, First trimester risk assessment, 11 to 13 weeks + 6 days scan.

INTRODUCTION

Chromosomal anomalies are associated with considerable morbidity and mortality. Trisomy 21, in particular with its attendant's intellectual and physical challenges and long lifespan, places considerable demands on the affected individual, family, society and nation. One of the aims of antenatal care has, therefore, been to identify fetuses with these disorders in order to give parents the option of terminating such pregnancy.

The protocol for identifying these fetuses had for many years included a single clinical criterion of maternal age. All mothers beyond 35 years of age were offered amniocentesis for a fetal karyotype. The fetal loss rate of one in 200, consequent to amniocentesis resulted in a significant loss of normal fetuses for every abnormal fetus identified. Even with the safety of amniocentesis increasing and the loss rate falling to one in 500 to 800, it still remains unjustified to subject all mothers above 35 years of age to the procedure. Additionally, although the incidence of trisomy 21 is higher in older mothers, since most pregnancies occur in the younger age group, the age criterion alone identifies only 30% of affected fetuses. As a consequence, there has been an endeavor to identify criteria to help identify those mothers most likely to benefit from amniocentesis. These criteria are referred as "markers" and include ultrasound findings and biochemical parameters. These constitute "screening" tests. Definitive diagnosis is done by invasive testing, such as amniocentesis between 16 and 20 weeks of pregnancy or by chorion villus sampling between 10 and 14 weeks of pregnancy and these are referred to as "diagnostic" tests. Material obtained by these invasive tests can be assessed by culture and karyotype, fluorescent *in situ* hybridization (FISH) or quantitative fraction polymerase chain reaction (QF-PCR) to identify or exclude the trisomy.

Advances in biochemical screening, combined with the excellent display of fetal dysmorphology afforded by technological advances in ultrasound equipment have resulted in a paradigm shift in the diagnosis of chromosomal abnormalities in the fetus from the second trimester to the late first trimester. The accuracy of diagnosis, as reported in multiple large series[1,2] from various parts of the globe over the past decade and a half has pushed both screening and diagnostic testing for chromosomal disorders to the window now referred to as the 11 to 13 weeks + 6 days scan. This section discusses techniques and clinical implications of ultrasound screening for markers of trisomy in the first trimester.

There are several advantages of early diagnosis apart from the ease and safety of first trimester termination. These include social privacy for the couple, since pregnancies can remain unannounced at this stage, and a fairly lesser degree of parental fetal bonding resulting in an easier situation with reference to coping with a loss. From the perspective of sensitivity as well, first trimester screening far exceeds the second trimester triple test and genetic sonogram. Importantly, nuchal thickening, the cornerstone of diagnosis, may regress by 14 weeks of gestation.

Additionally, the 11 to 13 weeks + 6 days scan offers a fairly good delineation of normal fetal anatomy,[3] identification of several major structural anomalies, confirmation of chorionicity and amnionicity in multifetal pregnancies, and holds great promise for screening of prematurity, pre-eclampsia and neural tube defects.

Recent data have shown chorion villus sampling after 10 weeks to be as safe in experienced hands as amniocentesis, and this has pushed the advantages of first trimester screening further.

Statistical Perspective and Natural History of Chromosomal Abnormalities

Chromosomal anomalies may be numerical or structural and it is the former which lends itself easily to ultrasound recognition. Structural chromosomal anomalies and single gene disorders can be diagnosed only by laboratory evaluation after ultrasound-guided invasive procedures.

The incidence of chromosomal anomalies decreases with gestational age. The incidence of trisomy 21, 18 and 13 increases with maternal age.[4] The incidence of Turner's syndrome and Triploidy does not change with maternal age.

The incidence of chromosomal anomalies is as high as 50 to 60% in abortuses and about 0.7% in newborns. The high rate of early abortions, second trimester abortions, fetal demise, and still births in chromosomally abnormal zygotes accounts for this markedly reduced incidence at birth compared to the antenatal period.

The common aneuploidies at birth include Down's syndrome (trisomy 21), Edward's syndrome (trisomy 18), Patau sydrome (trisomy 13), Turner syndrome (Monosomy X), Triploidy, and sex chromosome disorders.

70 to 80% of trisomy 21 babies are born to mothers who are less than 35 years old. This makes universal screening imperative.

Parameters for First Trimester Screening

Ultrasound parameters for the detection of Down's syndrome in the first trimester include the nuchal translucency (NT) as the most well-defined and studied parameter for evaluation of the nasal bone (NB), frontomaxillary facial (FMF) angle, ductus venosus (DV) flow velocity waveform, tricuspid regurgitation (TR), and fetal heart rate. Each parameter has well-defined criteria to be fulfilled for accurate quantification and these are discussed in the following sections. Additional parameters that have received attention in the literature and used by some groups include maxillary length, ear length, megacystis, flat iliac wings, and early onset growth restriction.

Biochemical parameters that are currently used widely include PAPP-A and free beta hCG.

Other parameters that the software accounts for are the gestational age assessed by the crown-rump length, maternal age, ethnicity, smoking, IVF, and number of fetuses with chorionicity.

Nuchal Translucency

The term 'nuchal translucency' refers to the anechoic stripe, visible just internal to the skin stripe at the level of the back of fetal neck. It is consequent to the subcutaneous accumulation of fluid in the fetal neck in the first trimester. The term 'translucency' is used irrespective of thickness, extent or presence of septations. The incidence of chromosomal abnormalities and structural anomalies is related to the thickness rather than the appearance. The translucency usually resolves in the second trimester, but may persist as a cystic hygroma or nuchal edema.

Chromosomal abnormalities are found in one-third of fetuses; 75% of these are trisomy 21 or trisomy 18, 75% of cystic hygromas have a chromosomal abnormality and 75% of these are Turner syndrome. Thickening of the translucency has a multifactorial cause, including cardiac failure, superior mediastinal compression causing venous congestion, altered composition of extracellular matrix, abnormal or delayed development of the lymphatic system, abnormal fetal lymphatic drainage consequent to decreased fetal movements, and fetal anemia. Consequently, anomalies encountered in fetuses with a thickened nuchal translucency include chromosomal anomalies, cardiac defects, pulmonary malformations, skeletal dysplasias, congenital intrauterine infections, metabolic disorders and hematological disorders.

Several strict criteria are to be met for an accurate assessment of the nuchal translucency. The fetus should be in a true sagittal section. An ideal image includes nasal skin, echogenic tip of the nose, nasal bone, the palate in a rectangular shape, translucent diencephalon in the center, and the nuchal translucency posteriorly in the same image (Fig. 1). It should definitely not include any part of zygoma between nose and palate. Rotation of the head by 10 degrees brings the zygomatic arch into the image (Fig. 2), and further rotation to 15 degrees shows coalescing of the zygomatic bone and palate (Fig. 3). The crown-rump length should range between 45 and 84 mm. Magnification of the image should be such, so as to include only the head and upper-third of the thorax (Figs 4 and 5). The head should be in a neutral position. Hyperextension increases the thickness (Fig. 6) and flexion reduces the thickness (Fig. 7) of nuchal translucency. Care has to be taken to distinguish between the amnion and nuchal skin (Fig. 8). To achieve this, it may be necessary to wait for the fetus to move, ask the mother to cough or make her laugh or tap the maternal abdomen with the transducer. It is also important to exclude the presence of the umbilical cord near the fetal neck. This increases the translucency above the level of the cord. This error may be overcome by waiting for the cord to move off or by measuring both above and below the cord and averaging the two readings. After all these criteria are fulfilled, the anechoic region of the lucency should be measured at its widest part. This should be done with the "+" calipers and not the "x" calipers. This makes it easy to ensure that the placement merges with the white of the margins of the lucency, and is not in the lucent area. Several readings of the translucency should then be taken and the highest should be reported.

The NT increases with gestational age, and therefore it is necessary to interpret it in the perspective of crown-rump length. Normal values (Fig. 9) range from 1.2 to 2.1 mm at 45 mm up to 1.9 to 2.7 mm at 84 mm.[2] A small but definite number of normal fetuses have thickened nuchal translucency. A pregnancy

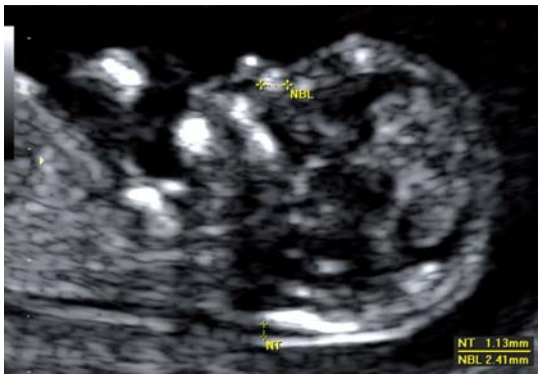

Fig. 1: True sagittal section for the assessment of nuchal translucency, nasal bone and the facial angle. The zygoma is not seen and the magnification is ideal. The neck is in a neutral position and nasal skin, the nasal tip and the diencephalon are in evidence

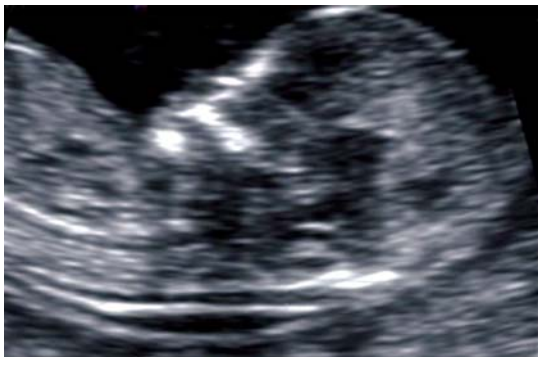

Fig. 2: Inappropriate off-axis study, the zygoma is evident between the nose and the palate

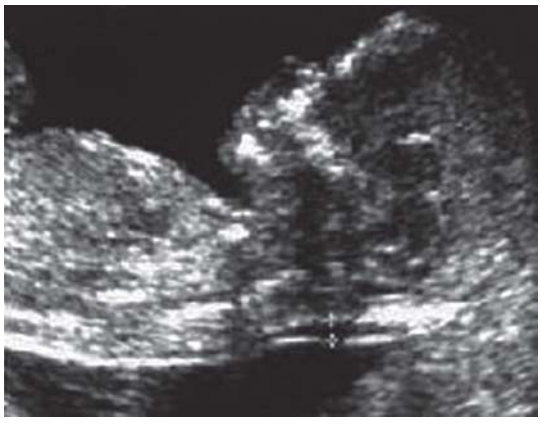

Fig. 3: Inappropriate off-axis section showing the zygoma coalescing with the palate

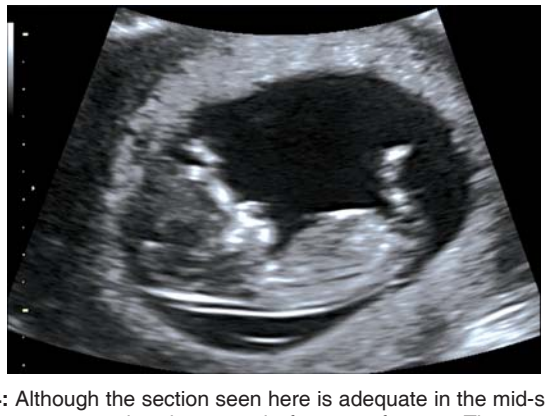

Fig. 4: Although the section seen here is adequate in the mid-sagittal plane, no attempt has been made for magnification. The section is therefore, inadequate

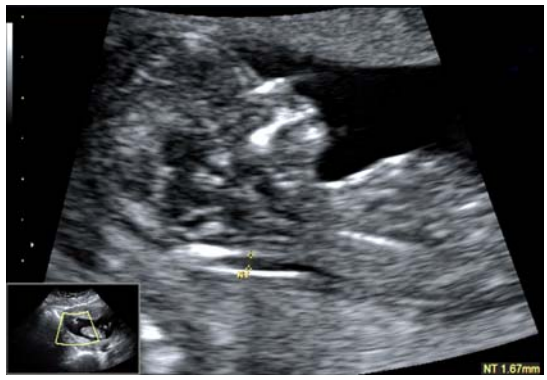

Fig. 5: Appropriately magnified section for assessment of nuchal translucency

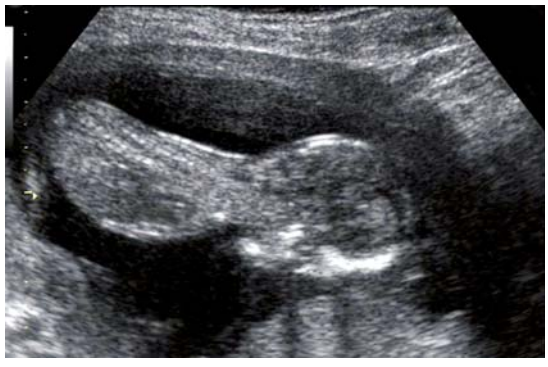

Fig. 6: Hyperextension falsely thickens the nuchal translucency

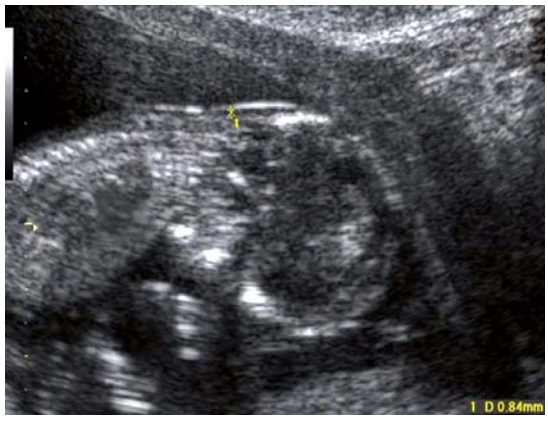

Fig. 7: Flexion results in inaccurately thin measurements of the nuchal translucency

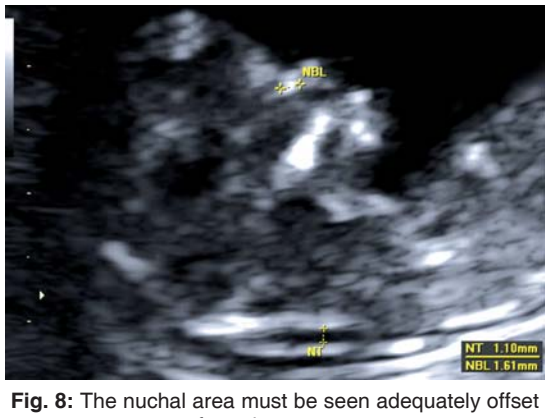

Fig. 8: The nuchal area must be seen adequately offset from the amnion

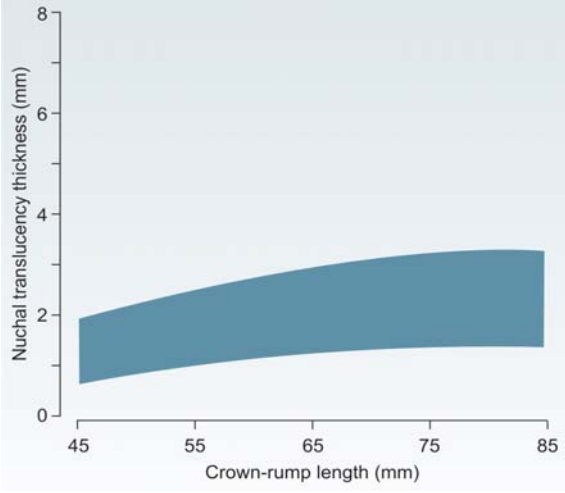

Fig. 9: The nuchal translucency shows an increase in its thickness from 11-14 weeks of gestational age. No random cut off figure is possible to assign for the upper limit and all calculations should be read off in the perspective of the crown-rump length

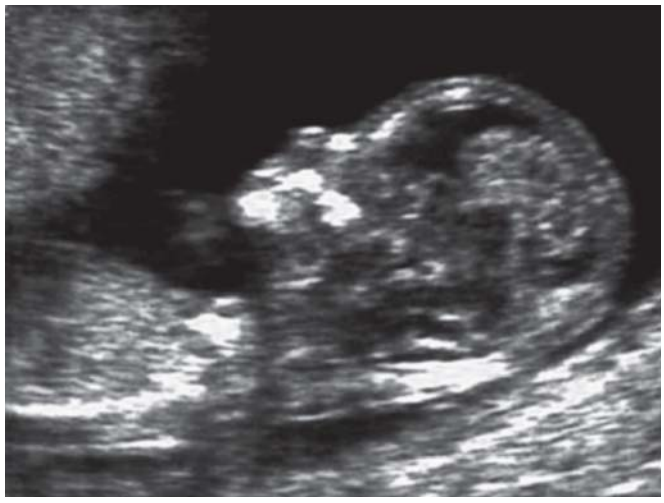

Fig. 11: Appropriate section for visualizing the nasal bone

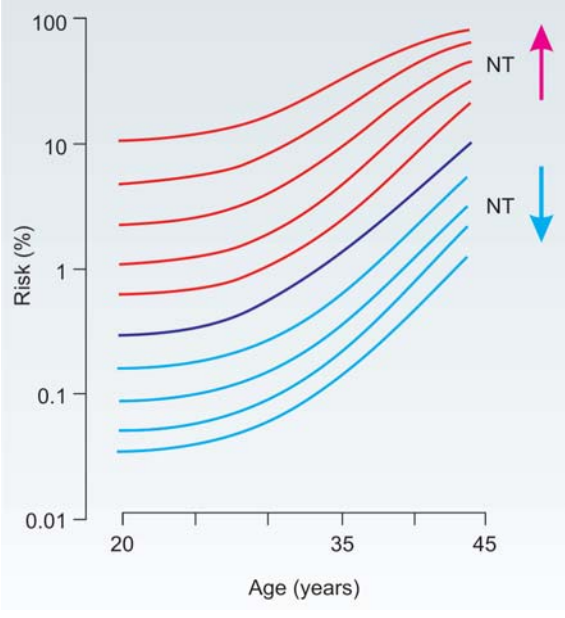

Fig. 10: Graphical representation of the relative thickening of the nuchal area. Higher the deviation from the 50th percentile, higher the possibility of aneuploidies

should, therefore, never be terminated on the basis of this finding alone. In screening, patient-specific risks are obtained by multiplying the maternal age and gestational age related risk (derived from software/charts) by a "likelihood ratio" (LR). This LR, unlike the biochemical value of Multiples of Median (MoM), depends on the difference[5] (delta value in mm) in the measured NT from the median NT for that crown-rump length (Fig. 10). Multiple ethnicity specific charts are available in standard textbooks and free of cost on the internet.

There is no association between thickened NT and maternal age, and therefore these can be combined to enhance the detection rates in a screening program.

Fetal Nasal Bone

The nasal bone is absent or hypoplastic in 69% of fetuses with trisomy 21 in the 11 to 13 weeks + 6 days scan period.[6] It is, therefore, useful to assess it for screening of trisomy 21 during this period. It must be remembered, however, that the nasal bone may be absent or hypoplastic in 1.4% of chromosomally normal fetuses in a significant number of normal Afro-Caribbeans, and that the incidence of absence decreases with gestational age and CRL.[7] The incidence increases with an increase in NT thickness. The nasal bone is absent in 50% of trisomy 18 fetuses and 40% of trisomy 13 fetuses.

Technically, the section for assessment and measurement is the same as for the NT. The transducer should be parallel to the direction of the nose. Three lines are clearly evident in this section as shown in Figure 11. These include the skin represented by the top line, the echogenic nasal bone just below this, which is thicker than overlying skin, and a third line in front of the nose, which represents the tip of the nose. The nasal bone is regarded as present if it is more echogenic than the overlying skin. It is regarded as absent if it is either not seen, or its echogenecity is equal to or less than the skin. Although the Fetal Medicine Foundation does not recommend measuring the nasal bone and assessing it subjectively, some authors have published reference charts.

Assessment of the nasal bone increases the detection rate of trisomy 21 from 90 to 93% and decreases the false-positive rate from 3 to 2.5%.

Fetal Facial Angle

The facial angle is the quantification of the flat facial profile seen in fetuses with trisomy 21.[8] This is also known as the frontomaxillary facial angle (FMF angle). Since the maxilla is small and set back in these fetuses, the angle becomes wider. The angle in the software improves the performance of screening.

The facial angle is increased (> 95th percentile) in 5% of euploid fetuses, 45% of fetuses with trisomy 21, 55% of fetuses with trisomy 18, and 45% of fetuses with trisomy 13.

The facial angle decreases with an increase in CRL, and the software must therefore include the CRL. The angle is

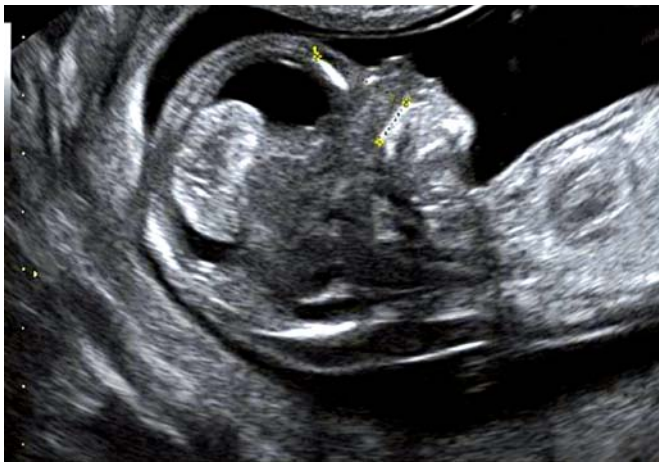

Fig. 12: Delineation of the nasal bone in an appropriate section

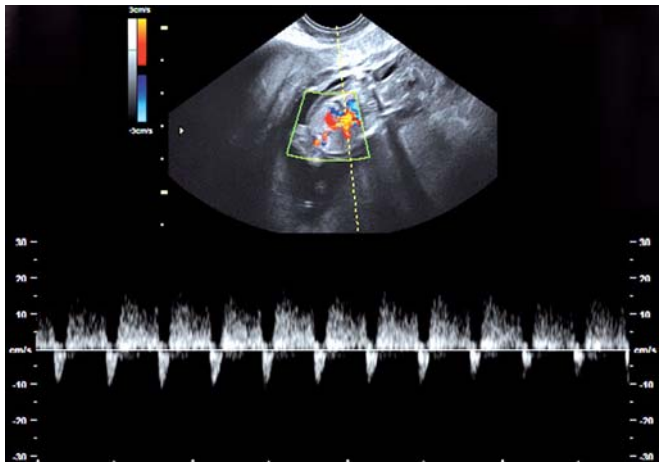

Fig. 14: Abnormal ductus venosus flow velocity waveforms

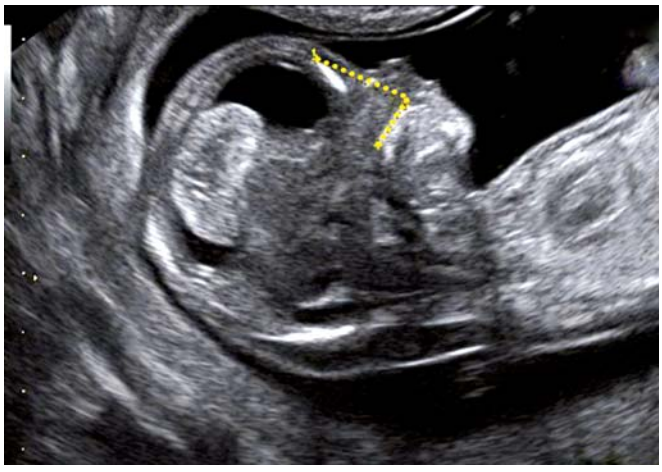

Fig. 13: Appropriate section for assessing the facial angle 14

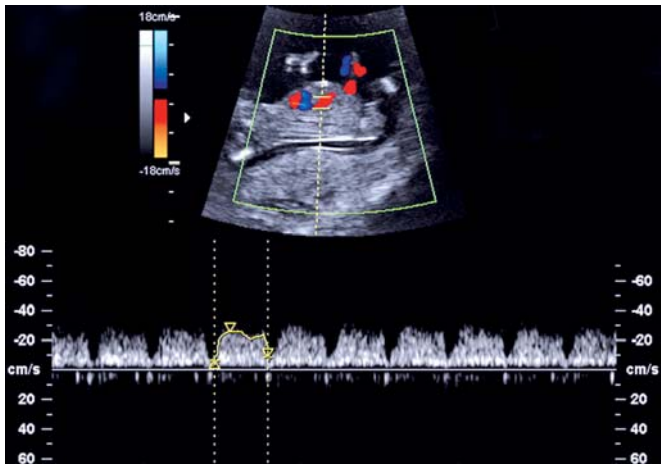

Fig. 15: Normal ductus venosus flow velocity waveforms

measured in the same image as the NT and NB. The angle is measured between a line along the superior surface of the palate and a line drawn from the anterosuperior corner of the maxilla to the anterior surface of the frontal bones (Figs 12 and 13). As a rough guide, the facial angle decreases from about 83 degrees at a CRL of 45 mm to 75 degrees at a CRL of 84 mm. Assessment of the facial angle, in addition to the NT, increases the detection rate of trisomy 21 from 90 to 94% and decreases the false-positive rate from 3 to 2.5%.

Ductus Venosus

Abnormal ductus venosus flow in the 11 to 13 weeks + 6 days scan is associated with chromosomal anomalies, cardiac abnormalities and adverse fetal outcomes.[9] 80% of trisomy 21 fetuses and about 5% of normal fetuses show reversed flow in the 'a' wave (Fig. 14).[10] However, it must be noted, that in about 80% of fetuses with reversed 'a' waves, the pregnancy has a normal outcome.

The Fetal Medicine Foundation recommends the fulfilling of several strict criteria to ensure an accurate quantification. The fetus should be still. The thorax and abdomen should occupy the entire screen. A right ventral mid-sagittal section has to be obtained. Color or power Doppler flow mapping should be used to delineate the umbilical vein, ductus venosus and fetal heart. The sample gate should be between 0.5 and 1 mm. It should be placed in the area of highest aliasing. The insonation angle should be less than 30 degrees. Filter settings should be set at a low range of 50 to 60 Hz. The sweep speed should be high (2-3 cm/s), so that the waveform is widely displayed. The criteria are numerous but must be fulfilled for adequate assessment of the 'a' wave in the flow velocity waveform (Fig. 15).

This marker has a weak correlation with abnormal NT measurements, and therefore serves as an independent marker for improving screening. However, delineation requires operator's skill and time, and this marker, therefore, is being used largely by tertiary centers to fine-tune borderline risks. Inclusion of this marker for first trimester screening improves the detection rate from 90 to 95% and reduces the false-positive rate from 3 to 2.5%.

Tricuspid Regurgitation

Evaluation of tricuspid flow has been shown in recent studies to enhance performance of first trimester screening.[11] The documentation of tricuspid regurgitation increases the risk for trisomy 21 as well as for cardiac defects. The incidence is related to nuchal thickening and decreases with increasing CRL.

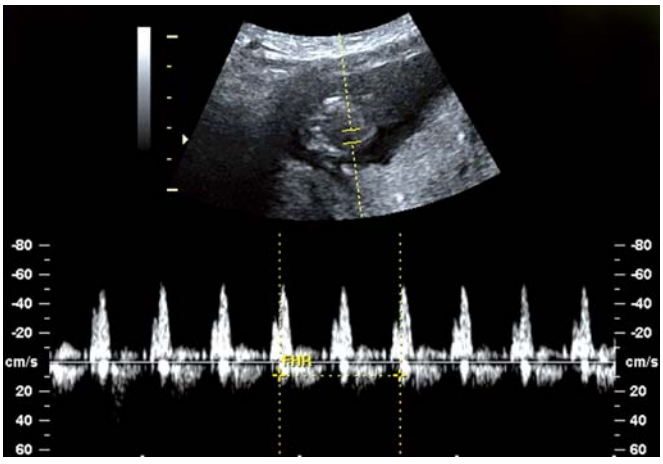

Fig. 16: Normal tricuspid tracings

The fetus should not be moving. An apical 4 chamber view is obtained and magnified, so that the entire screen is occupied by the thorax. Color flow mapping is not used. The insonation angle should not exceed 30 degrees. The sample volume is positioned across the tricuspid valve. The gate should be 2 to 3 mm wide (Fig. 16). The sweep speed should be high: 2 to 3 cm/sec. TR is diagnosed if it is found during at least half of the duration of systole and with a velocity greater than 60 cm/sec. The latter cut-off is important because aortic or pulmonary arterial blood flow can produce a velocity of up to 50 cm/sec at this period of gestation.

Fetuses with TR that have a normal karyotype should be followed up carefully to assess for cardiac anomalies.

Other Parameters

Several other parameters have received attention over the years and are generally not in routine or specialized use.

Underdevelopment of the maxilla is present in 50% of fetuses with trisomy 21. These fetuses have a median maxillary length that is 0.7 mm less than the normal median for crown-rump length. The independent significance of this length is diluted by the observation that there is a very significant association between maxillary length and nuchal thickness, and also between maxillary length and hypoplasia of the nasal bone. It is, therefore, not in routine use.

Trisomy 21 fetuses have a short ear length. However, the degree of deviation from the normal median for CRL is too small for this to be useful. Similar logic exists for femur and humeral lengths during the 11 to 13 weeks + 6 days scan window.

A single umbilical artery shows a sevenfold increase in the risk of trisomy 18, but no such association with trisomy 21.

An abnormal longitudinal urinary bladder length (megacystis) is defined as a length of 7 mm or more. When the length is 7 to 15 mm, the incidence of trisomy 13 and 18 is 20%. In chromosomally normal fetuses, there is spontaneous resolution of megacystis in 90% of cases. When the bladder diameter exceeds 15 mm, the incidence of chromosomal anomalies is 10%. The presence of megacystis increases the likelihood of trisomy 13 and 18 by a factor of 6.7.

In trisomy 21, the fetal heart rate (FHR) is mildly increased and is above the 95th percentile in about 15% of cases. This low incidence erodes its utility in screening. In trisomy 18, the FHR is mildly decreased and is below the 5th percentile in about 15% of cases. In trisomy 13, the FHR is substantially increased and is above the 95th percentile in 85% of cases.

Working of Screening Protocols

Thickened nuchal translucency, absent or hypoplastic nasal bone, and the facial angle represent the quantified equivalent of the classical trisomy 21 features as first described by Langdon Down: A skin that is too large for the body, a small nose and a flat face. Doppler evaluation of the tricuspid valve and the ductus venosus yield characteristics that enhance the performance of screening using maternal age, NT, NB, and biochemistry.

In day-to-day practice, a risk for chromosomal defects is calculated to guide further management. Every pregnancy has a risk for chromosomal defects. The starting point of this calculation is maternal age and gestational age. This is known as a priori risk. This is then multiplied by a likelihood ratio obtained by assessing PAPP-A, free beta hCG, CRL and NT in the background of maternal weight, ethnicity, method of conception, number of fetuses, and smoking.[12] The likelihood ratio for a given ultrasound or biochemical parameter is obtained by dividing the percentage of chromosomally abnormal fetuses by the percentage of normal fetuses with that measurement. This yields a new risk, which then forms the priori risk for calculations using additional parameters.

A risk of one in 50 or more represents a high risk and an indication to offer invasive testing. A risk of one in 1000 or less implies a low risk and these patients are offered a second trimester genetic sonogram at the time of anomalies scan. A risk of one in 51 to one in 999 represents an intermediate risk. These patients undergo reassessment of risk assignment using likelihood ratios from assessment of the NB, facial angle, TR, and DV studies. The new risk is then used for decision making for invasive diagnosis. Various centers use cut-offs of one in 200 to one in 300.

Combining maternal age, biochemistry, NT and NB with 11 to 13 weeks + 6 days scan, yields a detection rate of 96% with a false-positive rate of 5%.

REFERENCES

1. Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal translucency thickness at 10-14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. Lancet 1998;352:343-46.
2. Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. SURUSS Research Group. First and second trimester antenatal screening for Down's syndrome: The results of the Serum, Urine and Ultrasound Screening Study (SURUSS). Health Technol Assess 2003;7:1-77.

3. Khurana A, Dahiya N, Sheikh M. Impact of Newer Ultrasound Technologies on the early diagnosis of fetal congenital malformations. Journal of Perinatal Medicine 2005;33 (Supplement-1):42.
4. Hecht CA, Hook EB. The impression in rates of Down's syndrome by 1-year maternal age intervals: A critical analysis of rates used in biochemical screening. Prenat Diagn 1994;14:729-38.
5. Spencer K, Bindra R, Nix ABJ, Heath V, Nicolaides KH. Delta-NT or NT MoM: which is the most appropriate method for calculating accurate patient-specific risks for trisomy 21 in the first trimester? Ultrasound Obstet Gynecol 2003a;22:142-48.
6. Cicero S, Curcio P, Papageorghiou A, Sonek J, Nicolaides KH. Absence of nasal bone in fetuses with trisomy 21 at 11-14 weeks of gestation: An observation study. Lancet 2001;358:1665-67.
7. Cicero S, Rembouskos G, Vandecruys H, Hogg M, Nicolaides KH. Likelihood ratio for trisomy 21 in fetuses with absent nasal bone at the 11-14 weeks scan. Ultrasound Obstet Gynecol 2004;23:218-23.
8. Borenstein M, Persico N, Kagan KO, Gazzoni A, Nicolaides KH. Frontomaxillary facial angle in screening for trisomy 21 at 11+0 to 13+6 weeks. Ultrasound Obstet Gynecol 2008;32(1): 5-11.
9. Matias A, Gomes C, Flack N, Montenegro N, Nicolaides KH. Screening for chromosomal abnormalities at 11-14 weeks: The role of ductus venosus blood flow. Ultrasound Obstet Gynecol 1998;2:380-84.
10. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol 2004;191:45-67.
11. Falcon O, Faiola S, Huggon I, Allan L, Nicolaides KH. Fetal tricuspid regurgitation at the 11+0 to 13+6 weeks scan: Association with chromosomal defects and reproducibility of the method. Ultrasound Obstet Gynecol 2006;27(6):609-12.
12. Kagan KO, Etchegaray A, Zhou Y, Wright D, Nicolaides KH. Prospective validation of first-trimester combined screening for trisomy 21. Ultrasound Obstet Gynecol 2009;34:14-18.