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ORIGINAL ARTICLE

Prenatal Invasive Testing at a Tertiary Referral Center in India: A Report of 433 Cases Under a Single Operator

Vandana Bansal[1,2] · **Rujul Jhaveri**[3]

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Abstract

Purpose of the Study Chromosomal aneuploidies are major causes of perinatal death and childhood handicap. Awareness about screening and prenatal diagnosis for these disorders among obstetricians and primary care physicians is increasing. Since invasive tests like amniocentesis or chorionic villus sampling (CVS) are associated with a risk of miscarriage these tests should be carried out judiciously in pregnancies considered to be at high risk for aneuploidies and other genetic disorders. The purpose of our study was to examine the patterns, trends and outcomes of the various screening procedures and invasive tests results.

Methodology Retrospective observational study done over a period of 3 years and one month including 433 pregnant women with high risk for genetic disorders undergoing invasive prenatal testing like chorionic villus sampling, amniocentesis or cordocentesis. Data were collected from our department records regarding the maternal age, indication for invasive testing, past obstetric history, family history of genetic syndromes, ultrasound findings in the current sonographic examination and the results of the tests done. Any immediate or late complications of the procedure if any were telephonically addressed.

Results A total of 436 procedures on 433 patients (418 singleton,12 single fetus of twin, 3 both fetuses of twins) were done out of which 281 were amniocentesis(64.4%), 153 were chorionic villus sampling (35.1%) and 2 were cordocentesis(< 1%). Of the 436 procedures, 373(85.5%) were done for positive screening tests for chromosomal aneuploidies and 63(14.4%) were done for previous history of genetic syndromes. The positive predictive value of biochemical marker alone was around 2.7% and higher around 13% for a combined first trimester or a second-trimester screen along with ultrasound abnormalities. The higher the biochemical risk does not translate into higher chance of chromosomal abnormality. Nineteen percentage of fetuses with NT above 95th centile had chromosomal abnormality. Twenty-one percentage of fetuses with absent nasal bone in our study had trisomy 21.

Conclusion Aneuploidy screening is the most common indication for prenatal invasive testing with dual marker combined with nuchal translucency, nasal bone, tricuspid regurgitation and ductus venosus flow providing the best detection rates. The chance of an affected fetus in a patient with aneuploidy screen positive overall is only 6.7%.

Keywords Prenatal test · Amniocentesis · Chorionic villus sampling · Invasive tests · Aneuploidy

Introduction

It is estimated that around 5% of the pregnant population (approximately 30,000 women per annum in the UK) are offered a choice of invasive prenatal diagnostic tests [1]. Chromosomal aneuploidies are major causes of perinatal death and childhood handicap [2]. Awareness about screening and prenatal diagnosis for these disorders among obstetricians and primary care physicians is increasing. With advances in medical science, screening tests have become available for the detection of common genetic disorders and are being offered to all pregnant women, both in public and

Vandana Bansal is an Associate Professor at Nowrosjee Wadia Maternity Hospital, Parel, Mumbai and Director, Dept. Of Fetal Medicine, Surya Mother & Child Hospital, Mumbai; Rujul Jhaveri is a Junior Consultant, Dept. Of Fetal Medicine, Surya Hospital, Mumbai.

✉ Vandana Bansal
drvandana_bansal@yahoo.co.in

Extended author information available on the last page of the article

private sectors in India. Consequently, the confirmation or exclusion of chromosomal disorder for a positive screen test constitutes the most frequent indication for invasive prenatal diagnosis. Since these invasive tests are associated with a risk of miscarriage these tests should be carried out judiciously in pregnancies considered to be at high risk for aneuploidies and other genetic disorders. The purpose of our study was to examine the patterns, trends and outcomes of the various screening procedures and invasive tests results.

Material and Methods

- *Design*: Retrospective observational study
- *Period of study*: 3 years (July 2016 to July 2019)
- *Sampling Unit*: All pregnant women with high risk for genetic disorders undergoing invasive prenatal testing like chorionic villus sampling, amniocentesis or cordocentesis
- *Sample Size*: 433 patients
- *Inclusion Criteria*: Patients who have undergone diagnostic prenatal interventions for:
- – Positive biochemical/ ultrasonographic/ both screening for Chromosomal aneuploidies
- – Previous history of genetic disorders in previous child or familyhistory of genetic syndromes with/ without known mutation
- *Exclusion Criteria*: Interventions for other indications such as fetal reduction, transfusions, fetal therapy or fetal surgery. Patients who refused invasive testing were also excluded.
- *Methodology*: A formal permission for the study was taken from the institutional ethics committee. As a standard workup protocol at our tertiary care fetal medicine center, all patients referred for opinion on fetal diagnostic intervention are re-evaluated for the need for testing, a detailed sonographic examination is done by a single FMF certified fetal medicine consultant to identify any additional soft markers for chromosomal aneuploidies, any other gross congenital anomaly or growth restriction. A detailed consultation with the patient and relatives is then done to explain the need for prenatal invasive testing, its risks and complications, alternative options available with the advantages/disadvantages of one above the other. A written, valid and informed consent is taken before the procedure. All procedures are done transabdominally under sterile aseptic precautions, using 22G spinal needle for amniocentesis and 20G spinal needle for chorionic villus sampling / cordocentesis, under ultrasound guidance using freehand method by a single operator. Post-procedure prophylactic oral antibiotics and progesterone support are administered to all patients and anti-D injection where indicated. All ultrasound examination and invasive procedure were done using transabdominal 3.5–5 MHz or 2–9 MHz curvilinear transducer GE Voluson E8 or E10.

Since it is a retrospective study, data were collected from our department records regarding the maternal age, indication for invasive testing, family history of genetic syndromes, ultrasound findings in the current sonographic examination and the results of the tests done. Any immediate or late complications of the procedure if any were telephonically addressed.

Results

Types of Diagnostic Invasive Tests

During a period of 3 years, a total of 436 procedures on 433 patients (418 singleton, 12 single affected fetus of twin, 3 both fetuses of twins depending on chorionicity and reasons for testing) were done of which 281 were amniocentesis (64.4%) (Fig. 1), 153 were chorionic villus sampling (35.1%) (Fig. 2) and 2 were cordocentesis (< 1%).

Indications for Invasive Testing

Three hundred and seventy-three (85.5%) were done for positive screen tests for chromosomal aneuploidies and 63 (14.4%) for previous history of genetic syndromes. Of the 373 invasive done for positive aneuploidy screening, 183 were done for isolated positive biochemical screening tests with normal fetal structure including NT/ anomaly scan (FTS or dual/triple/quadruple), while 126 were done for positive ultrasound findings where biochemical screen was either not done due to a significant abnormality or the screen was normal (soft markers/ major abnormalities), 46 were

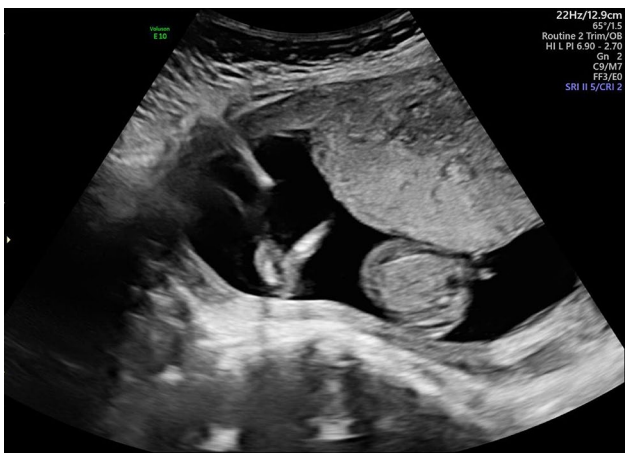


Fig. 1 Amniocentesis

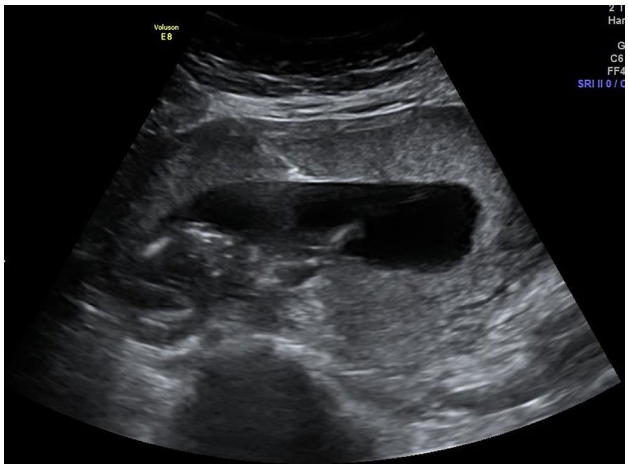


Fig. 2 Chorionic Villus Sampling

done for an abnormal combined ultrasound and biochemical findings and 18 for previous history of Trisomy 21 with normal ultrasound findings (no screen was done for all these patient).

Among 183 women with only positive biochemical aneuploidy screen, distribution of first and second-trimester screening and their true positive values are illustrated in Table 1. In spite of a better screen test (quadruple test) available in the second trimester, there were still 15 cases where a triple test was offered by the obstetrician.

Out of the total 183 invasive tests done for only positive biochemical screening 5 (2.7%) had abnormal FISH and/or karyotype reports, while of the 126 invasive test done for only strong ultrasound markers or anomalies 13(10.3%) had abnormal reports. Among 46 cases where invasive testing was undertaken for combined abnormal biochemical screening and abnormal ultrasound findings, 6(13%) had abnormal karyotype. Only one woman with previous Down's syndrome had a recurrence of Trisomy 21(5%) that was 21/21 Robertsonian translocation. Of the total 373 procedures done where couples came anxious with a positive aneuploidy screen report, 25(6.7%) fetuses had an aneuploidy, while all the other 348 (93.3%) fetuses were normal (Table 2).

Among 25 patients with abnormal karyotype report, 8 had a normal FISH report for 13, 18 and 21 and sex chromosomes. These 8 patients had chromosomal abnormalities other than those detected in the FISH report, although the indication for invasive test in all cases was a screen high risk for trisomy 21or 18. Hence, the importance of full karyotype in all patients who undergo invasive tests. Among these, except for the mosaic trisomy 18 fetus, another fetus with 11p15 and 12p13 translocation with sacrococcygeal teratoma and third fetus with pericentric inversion with bilateral echogenic lungs who terminated, all other 5 cases had inherited the abnormality from one of their normal parent as seen on parental karyotype, hence advised to continue pregnancy. Twenty patients among 373 women with screen positive for aneuploidy actually had a chromosomal abnormality significant enough for termination (5.3%) (Table 3).

Considering sole biochemical risk cut-offs in 183 patients, higher biochemical risk did not translate into higher chance of chromosomal abnormality (Table 4). In the cases

Table 1 True positives among biochemical screening result (*n* 183)

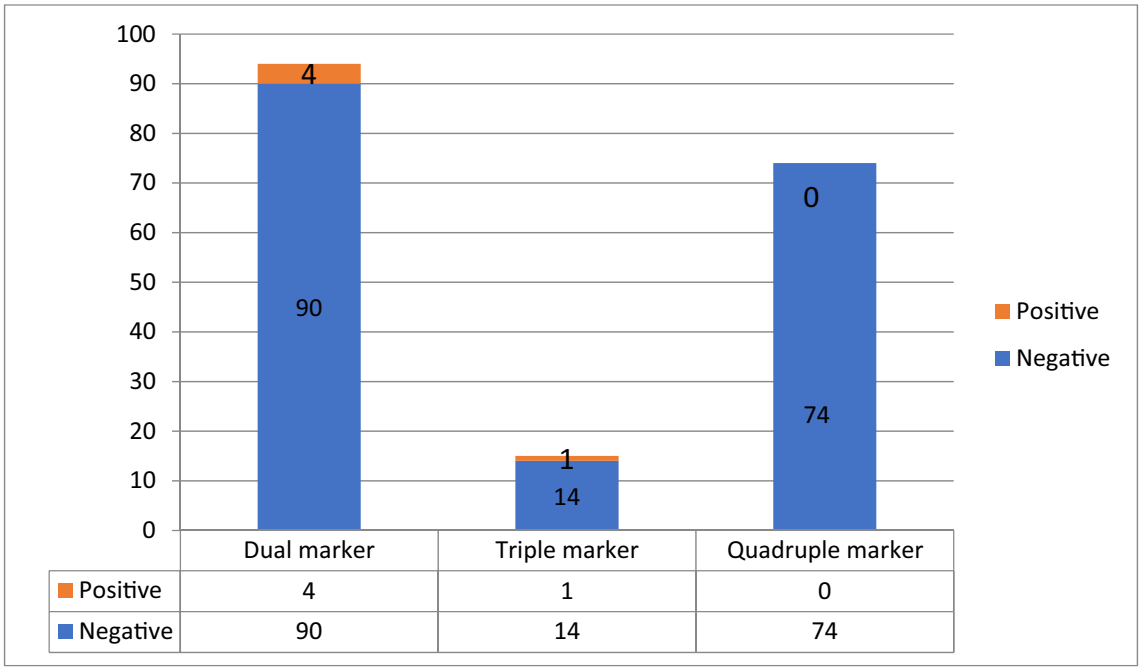


Table 2 True positives among all indications for positive aneuploidy screen (*n* 373)

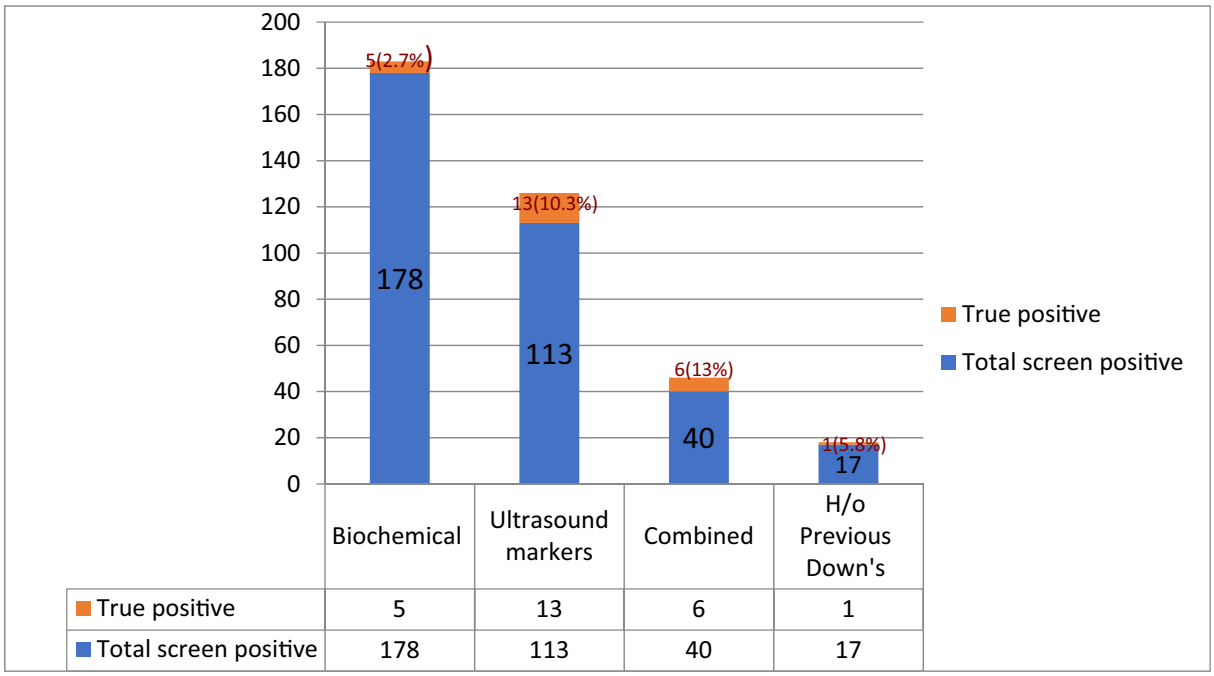


Table 3 Chromosomal abnormalities detected in the karyotype testing (Total 25)

Trisomy 21	12
Trisomy 13	0
Trisomy 18	3
Monosomy X	2
Inversion in Sex chromosome	3
Chromosomal marker of unknown significance	1
Inversions	1
Mosaicism T18	1
Translocations	1
Additional material on sex chromosome	1

done for intermediate risk, either on patient request or association with other soft markers, no cases were abnormal.

Ultrasound and Chromosomal Abnormalities

In patients with ultrasound abnormalities, invasive testing was offered in the following conditions.

- Major single soft marker with high likelihood ratio (LR > 2) like absent/hypoplastic/unossified nasal bone, ventriculomegaly, increased nuchal translucency, increased nuchal fold thickness and aberrant right subclavian artery
- Two or more mild soft markers with low likelihood ratio (LR < 2)
- Major structural abnormality associated with chromosomal aneuploidy
- Early onset fetal growth restriction

Out of these 172 procedures that had been done for positive ultrasound abnormality with or without biochemical screen, 78 cases had only one single strong ultrasound soft marker or major abnormality, while 94 (54%) had multiple abnormalities on ultrasound. 19/172 (11.0%) of these were abnormal on chromosomal analysis.

The highest association with aneuploidy was seen with increased nuchal translucency in the first trimester and absent nasal bone in the first and second trimester. None of the other strong soft markers were associated with chromosomal abnormalities in our study. Among all congenital anomalies, major cardiac defects were most commonly associated with abnormal karyotype (Tables 5,6).

Increased NT and Associated Anomalies

In cases where invasive testing was done for an increased nuchal translucency (*n* = 42), 25% in the 95th to 99th centile group and 13.6% in the > 99th centile group had an abnormal karyotype report (Table 7). At the time of the study, microarray was not routinely available and was not offered as standard of care.

Among all the 42 cases with increased nuchal translucency > 95th centile, 12 cases (28%) had associated other abnormalities. Increased nuchal translucency with or without associated ultrasound abnormalities was associated with

Table 4 True positives as per risk cut-offs

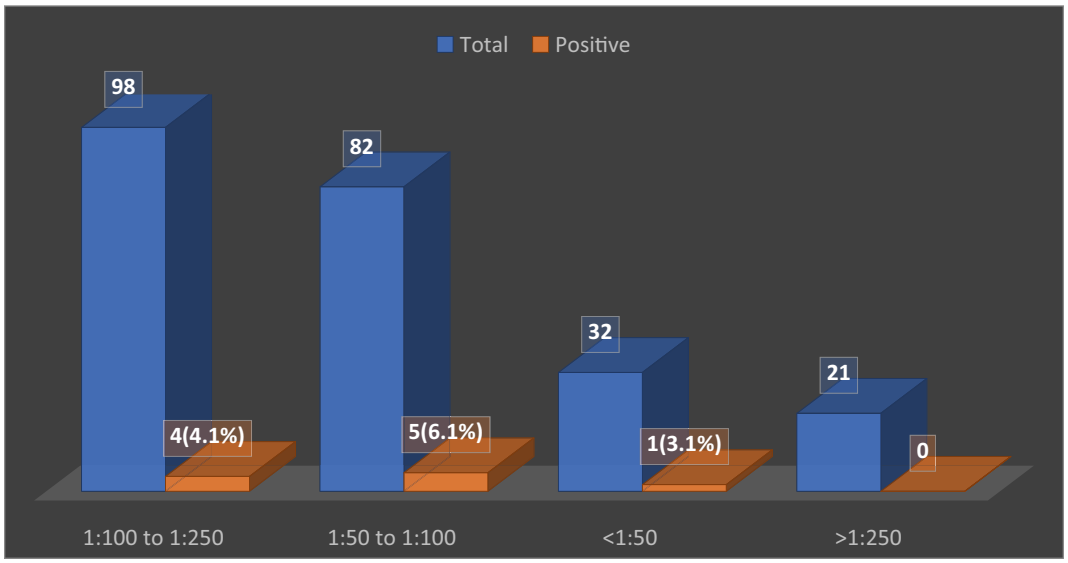
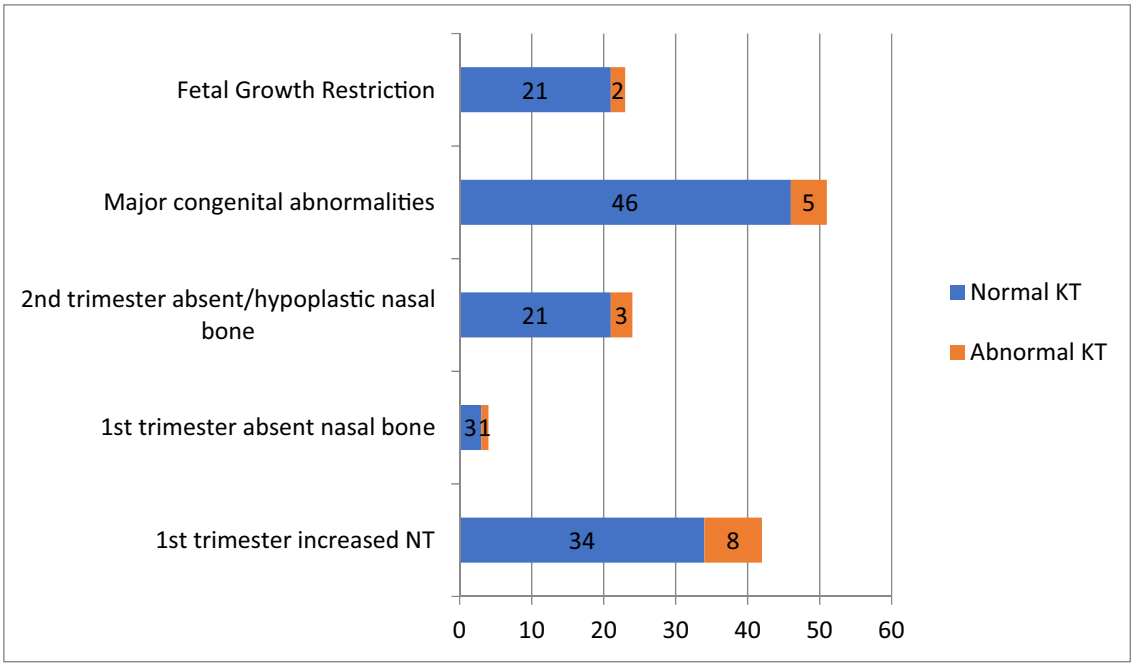


Table 5 Ultrasound markers and their association with aneuploidy



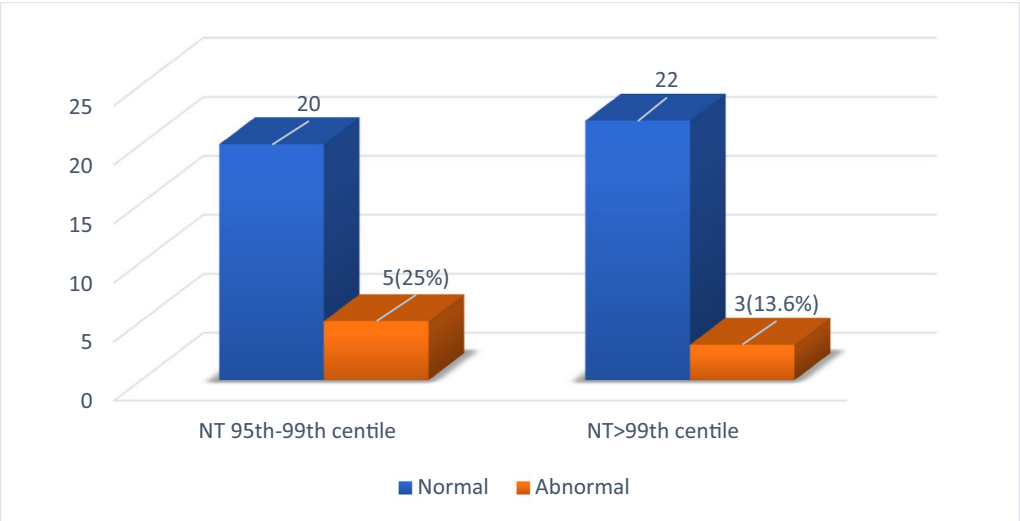
aneuploidy in 19% of cases (8/42). Even among the 30 cases with isolated increased NT, 5 (16.6%) had an abnormal KT report.

Eight patients with increased NT and abnormal KT terminated the pregnancy. Out of the remaining 34 with normal KT, 5 had anomalies with guarded/ poor prognosis, and the couples decided to terminate the pregnancy. In the remaining 29 patients with increased nuchal translucency with normal karyotype with no other major abnormality on anomaly scan, 3 patients had a preterm delivery (all these babies survived and were doing well at follow up), one had an intrauterine fetal death at 30 weeks and 19 patients had an uneventful antenatal course with delivery at term with good neonatal outcome. At further follow up, these 22 babies were doing well in terms of neonatal and infant milestones at 1 year of age. A total of 6 patients were lost to follow up. Thus, in all patients with increased NT where follow up was available, the take home baby rate was 22 out of 36 (61%). In patients with normal structure at anomaly scan and normal

Table 6 Ultrasound abnormalities in all cases undergoing invasive test

Ultrasound Abnormality	Total Cases
(A) Major Single Soft Marker (LR >2)	First trimester- Increased nuchal translucency- 42, Absent Nasal Bone- 4 Second Trimester- Hypoplastic nasal bone- 9, Unossified/Absent nasal bone- 14, Ventriculomegaly - 10, Increased nuchal fold thickness- 4, Aberrant Right Subclavian Artery- 4
(B) Two or more Soft Markers	First Trimester- Ductus venosus abnormalities- 2, Tricuspid regurgitation- 5, Cystic hygroma- 7 Second Trimester- Single umbilical artery- 19, Intracardiac echogenic focus- 11, Pelviectasis- 13, Echogenic bowel- 14, Short long bones- 7, Prenasal edema- 2,Choroid plexus cyst-16
(C) Major Structural Abnormality	51
(D) Early Onset Fetal Growth Restriction	23

Table 7 Correlation with Nuchal Translucency centiles and abnormal Karyotype reports



KT (with only an isolated first trimester increased nuchal translucency), the take home baby rate was 95.6% (22/23).

The Presence or Absence of Nasal Bone and its Impact

Of all first and second trimester fetuses, 19 cases had absent/unossified nasal bone and 9 had hypoplastic nasal bone of which 4 had trisomy 21(14.3%). Two out of these 4 had isolated absent nasal bone as the only marker for invasive testing. None of the fetuses with hypoplastic nasal bone had aneuploidy (Table 8).

Major Congenital Abnormalities and Their Associations

Out of the 51/436 fetuses which had major congenital anomalies known to be associated with aneuploidies, 5 had an abnormal karyotype (9.8%). Three of these 5 fetuses had anomalies involving multiple systems. Decision for continuation/ termination was taken depending on the structural abnormality and the timing of detection. Wherever necessary DNA sample was saved for genetic evaluation later.

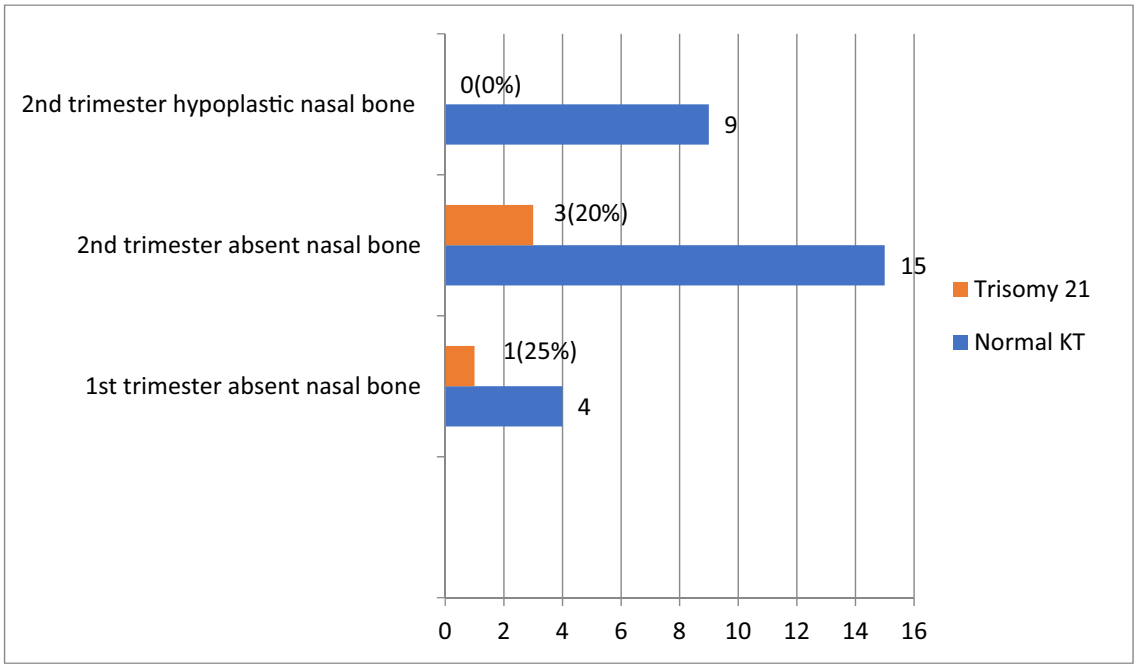
Fetal Growth Restriction and Association with Chromosomal Abnormalities

Invasive test was done in 23/ 436 (5.3%) cases with early onset fetal growth restriction. Nineteen fetuses (82.6%) had associated cardiac, skeletal or central nervous system abnormalities or associated soft markers. There were chromosomal anomalies in only 2 cases (8.7%), both were Trisomy 18. Only 4 patients had isolated early onset intrauterine growth restriction with normal karyotype, TORCH PCR and anomaly scan (17.4%) who continued pregnancy. Out of these 4 only one (25%) fetus carried till near term and was salvaged with strict monitoring with color doppler, while remaining three fetuses (75%) were so severely affected that they did not survive to the age of viability. One case of FGR with ventriculomegaly with calcifications had an amniotic fluid sample positive for Cytomegalovirus infection (4.5%) with normal karyotype report. Hence, out of the total 23 cases of early onset growth restriction, only one (4.5%) survived.

Invasive Testing for Other Genetic Disorders

Out of the total 436 procedures, 63 were done for mutational analysis for cases where either the first baby was affected or parents were affected or carriers for genetic disorders. Invasive testing confirmed fetal affection in 15 of 63 fetuses (23.8%) who underwent termination of pregnancy. In most cases, the previous baby was affected but not evaluated so both the index child and the parental samples were analyzed simultaneously followed by prenatal testing. Although we tested all these fetuses for karyotype also, none of them had an associated chromosomal abnormality.

Table 8 Correlation of Absent or hypoplastic Nasal bone with Trisomy 21



Procedure-Related Complications

Out of the total 436 procedures, 433 were done with a single prick, while 3 required a second prick. None of the cases had any complications like amniotic fluid leakage, chorioamnionitis or fetal losses in the next 4 weeks. Spotting was observed in 3 cases (0.68%) of chorionic villus sampling done on low-lying placenta which settled with conservative measures. Culture failure was seen in 2 cases (0.46%) of chorionic villus sampling done for biochemical screen positive for trisomy 21 where FISH report was normal. No patient required a re-procedure. No procedure-related miscarriage was encountered in our 436 interventions.

Discussion

Down's syndrome is the most common cause of intellectual disability and is responsible for 15–30 percent of such cases [2]. The birth prevalence of Trisomy 21 in India varies from one in 1230 to one in 1362 [3, 4]. Individuals with trisomy 21 may have physical abnormalities such as a cardiac defect but may just present with varying degree of developmental delay, early onset Alzheimer disease, and/or increased rates of leukemia. Malformation scan may show no abnormal findings in about 50% of fetuses with trisomy 21.

Down's screening has matured from initial indications being only advanced maternal age to more refined universal screening tests. The current benchmark for Down syndrome screening involves combined first-trimester screening (c FTS) based on maternal age, the ultrasound marker nuchal translucency (NT) and the biochemical markers free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A). If patient reports in the 2nd trimester, quadruple test along with a genetic sonogram is considered a good screening test. If the risk of trisomy 21 is higher than a specified risk cut-off as determined by the screening program, a diagnostic test is offered to confirm or exclude the presence of trisomy 21 (and other chromosome abnormalities). The most common indication for invasive testing in our study was a fetus at high risk for chromosomal aneuploidy on screening (373/436, 85.5%).

Out of the total 373 procedures done for a positive aneuploidy screen report, 25 (PPV 6.7%) fetuses had an abnormal FISH and/or karyotype report, while all the other 348 (93.2%) couples were reassured of the normalcy of their fetus. In the literature also, only about 5% of the positive high-risk cases actually carry a fetus with T21 giving a positive predictive value of 1 in 20 [5, 6].

Out of the total 183 tests done for only positive biochemical screening, positive predictive accuracy for a first-trimester screen positive was 4.25% (4/94), while for a second-trimester screen was only 1.1% (1/89) in our data. The first-trimester Screening has a detection rate of around 92–95% in the literature [5, 6].

With increased awareness of better available tests with higher sensitivity and detection rates, the uptake of first trimester screening in our study was 94 / 183(51.4%) which suggests that second trimester Downs screening is no longer as popular as in the past. The triple marker is fading out from the basket of screening tests routinely chosen by obstetricians some years back. Looking at trends in population-based Down syndrome screening and invasive prenatal testing with the introduction of first-trimester combined screening in South Australia between 1995 and 2005, Peter R Muller noted a significant decrease in the use of second-trimester maternal serum screening and a corresponding significant increase in first-trimester combined screening [7]. Consequent to early screening, trends in invasive testing by chorionic villus sampling have also increased in comparison to amniocentesis in our subset of patients.

In the first trimester addition of ultrasound markers like nuchal translucency along with ductus venosus, tricuspid regurgitation and nasal bone improve detection rates of first trimester screening from 65% (only biochemistry dual marker) to 92% (including NT) to 96% (all 4 markers) with a false positive of 2.5–5% [5]. Similarly in our study, the risk of a screen report coming as abnormal on invasive testing (Positive Predictive Value), which is the most important question in counseling, is 2.7% for a positive biochemical test alone with normal ultrasound, 10.3% if there are the presence of abnormal significant soft markers / anomalies and increases to 13% for combined biochemical and ultrasound abnormality. It is extremely important to meticulously scan these patients before an invasive test as presences of additional markers improve the yield of an invasive test.

While most studies suggest that a biochemical risk cut off of > 1:250 signifies a high risk for chromosomal aneuploidy, with proportionate increase as the risk increases to > 1:50 [8], in our study we did not find such a proportionate rise in abnormalities with higher risk cut off. The value of the biochemical risk cut off should not decide the chance of a true positive fetus during patient counseling.

Fetuses with increased fetal nuchal translucency (NT) measurement are at elevated risk of chromosomal, cardiac and other structural abnormalities. In our study, the risk of chromosomal abnormality with a nuchal translucency above 95th centile in our study was 19% and increased further to 25% if increased NT was associated with other soft markers or major abnormalities suggesting the need to do a structured protocol based anatomical survey even during the 11–13^{+6} weeks scan using higher resolution ultrasound.

In a study by Bardi [9], et al. 43% of fetuses with NT ≥ 95th percentile had either genetic or structural abnormalities, with rates increasing proportionally to the degree of NT enlargement. In our study also 17 of 42 (40.5%) patients

with increased NT > 95th centile had either a structural or chromosomal abnormality. Even among the 30 cases in our study with isolated increased NT, 16.6% had an abnormal KT report. However, there was no correlation between the severity of NT centiles with fetal abnormality or aneuploidy.

It cannot be re-emphasized that all NT > 95th centile with or without additional markers need to undergo invasive testing for chromosomal abnormalities. Additional structural anomalies detected on a detailed ultrasonographic evaluation will help decision-making, prognostication and allow additional test to be done with the fetal sample.

The prevalence of genetic disorders in these fetuses with increased NT would be much more if microarray was done in all these cases rather than the conventional cytogenetic study [10]. Most studies use an NT cut-off value of 3.5 mm as an indication for Chromosomal Microarray Analysis [11]. An association between increased nuchal translucency thickness (NT) and pathogenic findings on chromosomal microarray analysis (CMA) has not been evaluated in our study because of initial non-availability and the cost constraints. We might have detected more genetic abnormalities if chromosomal microarray was done in all increased NT fetuses in our subset.

Bardi et al. found a positive correlation between increased NT and major non-cardiac structural birth defects in the absence of chromosomal abnormalities [12]. In our case study of 42 fetuses with increased nuchal translucency (> 95th centile), 12 cases also had associated other abnormalities. Patients with increased NT and normal karyotype and normal anomaly scan, the intact survival was 95.6% (22/23) in our study.

Absence/ hypoplasia of Nasal bone is a strong soft marker for aneuploidy with a positive and negative likelihood ratio of 40.0 and 0.71 in the first and second trimester. The prevalence of absent nasal bone is affected not only by the fetal karyotype but also by maternal ethnicity, being higher in Black than in White women [13]. In our series of 28 fetuses with absent or hypoplastic nasal bone, 4 had trisomy 21 (14.3%). First trimester nasal bone was classified as either absent or present, while second trimester was reported as absent if nasal bone was not seen or its echogenicity was less than the overlying skin. Diagnosis of hypoplastic nasal bone was made only in the second trimester if the length of nasal bone was below 5th centile for gestational age. None of the fetuses with hypoplastic nasal bone in our subset had chromosomal abnormality. It appears likely that race and ethnicity have an impact on fetal nasal bone length and taking Indian or Asian cut-off may reduce the false-positive screen for our population. In a study done by Cicero et al. on all patients undergoing amniocentesis between 15 and 22 weeks, hypoplastic nasal bone was found in 0.5% of Caucasians and in 8.8% of Afro-Caribbeans women with chromosomally normal fetuses [14].

Inspite of 51 fetuses in our study having one or multiple major congenital anomalies known to be associated with aneuploidies, only 5 patients had an abnormal karyotype (9.8%). Chromosomal microarray in these cases with structural anomalies might have added some significant copy number variations that have not been evaluated in our study. Surgically correctable major anomalies should not be terminated only on the grounds that they have a strong association with aneuploidy but need to undergo invasive testing to prognosticate and determine recurrence risk.

Survival among early onset fetal growth restriction is very low (1/23) as most would be associated with either a structural anomaly or fetal infection or a chromosomal abnormality or severe placental disease where reaching viability is difficult. Additional association with syndromes and single gene defects would also be important and in these cases fetal DNA must be saved for later evaluation to determine etiology of FGR and plan management in next pregnancy.

Rapid aneuploidy detection using FISH has an inherent limitations that they cannot detect most structural chromosome abnormalities, mosaicism and atypical abnormal karyotype other than trisomy 21,13,18 and monosomy X. The presence of these atypical abnormal karyotype likely to affect phenotype, was encountered in 8 of 25 of our patients with abnormal karyotype report, which was not picked up by targeted FISH testing. Hence, it is extremely important to ask for a full conventional karyotype in all patients who undergo invasive tests. These findings also suggest that women of advanced maternal age, increased NT, abnormal biochemistry or fetus with a structural abnormality have a higher risk of having a fetus affected by atypical abnormal karyotype that will be missed by targeted NIPT or FISH. Hence, patients need to be counseled accordingly when considering NIPT/only FISH for above indications.

The yield of an invasive test is much higher for a single gene disorder than a chromosomal abnormality. The numbers of true positives among all screen-positive for aneuploidy was 25/373 (6.7%) and was much higher 15/63 (23.8%) among those tested for a previous history of genetic syndromes in index child or parents. Due to timely counseling and testing these pregnancies could be terminated as per the couple wishes. It also highlights the fact that in the event of an abnormal child being born, it is important to reach a specific genetic diagnosis using clinical expertise from pediatric sub-specialties (neurologists, geneticists, cardiologists). This would prove invaluable for the subsequent pregnancies for the couple. In the event of neonatal demise blood should be saved for further evaluation.

Invasive Procedures and Their Risk

In a systematic meta-analysis, Akolekar suggested that the procedure-related risks of miscarriage following

amniocentesis and CVS are much lower than previously quoted (0.11% and 0.22%), respectively)[15]. No procedure-related fetal loss was encountered in our 436 interventions.

The ISUOG Practice Guidelines for invasive procedures [6] suggest the risk for fetal loss, amniotic fluid leakage and chorioamnionitis range from 0.1 to 1%, 1–2% and < 1%, respectively following amniocentesis. The risk of amniotic fluid leakage following amniocentesis remains higher up to 24 weeks and spontaneous sealing of the membranes is common. Less experience, multiple attempts, blood-stained amniotic fluid and the presence of fetal abnormalities may increase the risk for fetal loss.

For chorionic villus sampling, complication rates range from 0.2–2% for fetal loss and 10% for vaginal bleeding in literature. Repeated needle insertions and gestational age < 10 weeks increase the risk of fetal loss. The fetal loss rate after transcervical CVS is higher and reported around 2.5%. All CVS in our series were done transabdominally.

In our center, we have performed about 436 procedures in 3 years by a single operator with a rate of > 145 procedures per year with a zero major complication rate. None of the cases had any complications like amniotic fluid leakage, chorioamnionitis or fetal loss in the next 8 weeks in either amniocentesis/ CVS or cordocentesis. In our study, vaginal bleeding was observed in only 3 cases (0.7%) of CVS done on low lying placenta which is much less than reported in the literature. Successful sampling was 100% in single sitting, 99.3% in single attempt. Overall lower complication is documented in our study as compared to that reported in the literature probably because all procedures have been done by a single fetal medicine consultant with vast experience of 20 years with strict protocols for pre- and post-procedure care, in place. Such low complication rate is expected only after a long learning curve.

Failure of amniocyte/ trophoblastic cells to culture is reported after amniocentesis in 0.1% and 0.5% of CVS in literature [16]. Culture failure was seen in 2 of our total cases (0.46%), both of these had undergone chorionic villus sampling for biochemical screen positive for trisomy 21 where FISH report was normal. No patient required a re-procedure.

Limitations of our Study

Chromosomal microarray has not been evaluated in our data of 436 patients due lack of availability in the initial part of the study and cost constraints in a developing country like ours where the entire brunt of paying for prenatal diagnosis is on the individual with no insurance policies or government policies in place to help.

Conclusions

Points we all know from global literature and were substantiated by our study

1. Aneuploidy screening is the most common indication for prenatal invasive testing with dual marker combined with nuchal translucency, nasal bone, tricuspid regurgitation and ductus venosus flow providing the best detection rates.
2. Highest association with aneuploidy was seen with increased nuchal translucency, absent nasal bone and cardiac abnormality.
3. 19% of fetuses with NT above 95th centile had chromosomal abnormality and 5/42 (11%) had major structural anomalies. This may increase once microarray is used as a routine for all invasive test done for increased NT.
4. Among fetuses with major congenital anomalies known to be associated with aneuploidies, only 9.8% had an abnormal chromosomal report on karyotype.
5. Early onset FGR is usually associated with poor outcome (95% in our study). However, the yield of invasive testing for aneuploidy and infection is low (3 / 23). Diligent look for structural anomalies, markers of fetal infection and multi-vessel Doppler assessment must be done. It may be worthwhile to keep DNA saved in all severe early onset FGR for further genetic evaluation in the event of a fetal demise later.
6. There are a significant number of babies with dysmorphism, neuro-developmental delays, cerebral palsy, mental retardation where the chances of recurrence are high (23.8%) which need evaluation prior to conception.
7. FISH alone may not be conclusive and has to be followed up with a full karyotype.
8. CVS and amniocentesis are not associated with any significant increase in the risk of miscarriage over the background risk. There is no evidence that CVS is less safe than amniocentesis. Complication rates can be minimized with expert operators and strict protocols for pre and post-procedure care.

Additional information from the study

1. The chance of an affected fetus in a patient with aneuploidy screen positive overall is only 6.7%.
2. The higher the biochemical risk does not translate into higher chance of chromosomal abnormality.
3. The probability of an invasive test report coming abnormal increases if there are additional ultrasound markers (13%).
4. The positive predictive value of biochemical marker alone was around 2.7% and higher around 13% for a

combined first trimester screen or a second-trimester screen along with ultrasound abnormalities.

5. Twenty-one percentage of fetuses with absent nasal bone in our study had trisomy 21. We need to use lower centile for Indian population to define hypoplastic nasal bone as none of our patients with hypoplastic nasal bone had Trisomy 21.
6. For rare genetic syndromes, evaluation in the neonatal period and collecting neonatal blood for DNA preservation in genetic labs is invaluable for prenatal testing in the subsequent pregnancies.

We routinely use figures and percentages from global publications. This is a large patient data of invasive procedures from India which can be used by clinicians in developing country to counsel women on the odds of invasive report coming true positive and the risk of procedure-related loss.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This was a retrospective observational study involving collection of patient data and hence there was no direct risk to participants. Study has been approved by the hospital ethics committee.

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About the Author

Vandana Bansal Dr Vandana Bansal MD,DGO,DNB,MNAMS, MRCOG,FICOG, FNB(High Risk Pregnancy and perinatology) is presently working as Associate Professor at Nowrosjee Wadia Maternity Hospital and Seth G S Medical College, Mumbai. She is the Director of Dept of Fetal medicine at Surya Mother and Child Hospital. She is the *First Postdoctoral Fellow of National Board (FNB) in India* qualified in the field of High risk pregnancy and perinatology. She is MUHS approved faculty for fellowship program in Fetal Medicine and postgraduate MD/DGO courses. She is the Course Coordinator for ICOG certificate course in Fetal Medicine.

Authors and Affiliations

Vandana Bansal[1,2] · Rujul Jhaveri[3]

Rujul Jhaveri Junior Consultant
rujul28@hotmail.com

[1] Department of Fetal Medicine, Surya Mother & Child Hospital, Santacruz (West), Mumbai, India

[2] Nowrosjee Wadia Maternity Hospital, Parel, Mumbai, India

[3] Dept. Of Fetal Medicine, Surya Hospital, Mumbai, India