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Prenatal Screening Of Foetal Chromosomal Disorders Using Maternal Serum Biochemical Markers And Karyotyping Among Indian Population

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Abstract

Background: Prenatal screening for aneuploidy during the first and second trimesters is a part of obstetric care. Because invasive procedures carry a high risk of miscarriage, these screening tests in high-risk pregnancies for aneuploidies are essential for determining the abnormality. The present study aimed to determine the predictive accuracy of prenatal screening tests and USG soft markers concerning maternal age groups, the incidence of chromosomal abnormalities, and the trends of screening tests chosen by obstetricians for women undergoing prenatal chromosome analysis prenatal screening.
Methods: 2280 Pregnant women were referred for prenatal screening tests in 2019–2020 by a well-established medical diagnostic laboratory, Lifecell International Private Limited. The sample has been analyzed based on the maternal age, screening tests, clinical indicators, karyotype interpretation, and type of chromosomal abnormalities. All the data were examined with the aid of SPSS 16.0 and EXCEL.
Results: 46.40% of women used TMT, 7.28% used DMT, and 3.82% used a combined first-trimester test in the present study. Positive predictive accuracy of the first trimester combined test is the highest (33.33%). Among 2280 women screened positive for aneuploidy based on different prenatal screening procedures, only 149 (6.5%) were found to have an abnormal karyotype, and the remaining 2131(93.5%) had a normal karyotype. USG marker in the absence of biochemical markers can detect considerable aneuploidy risk during the first and second trimesters.
Conclusion: The present study shows that in India second trimester prenatal tests are preferred over first-trimester prenatal tests by obstetricians. It shows that biochemical risk estimated in the first and second trimesters

does not turn into a high likelihood of chromosomal abnormality in the general population. However, these tests can help informed decision-making in high-risk pregnancies based on maternal age and unfavourable obstetric history. Further confirmation through advanced methods like Chromosomal Microarray (CMA), Whole Genome Sequencing (WGS) is warranted for better diagnostic decisions.

Keywords: Chromosomal abnormalities, Clinical indications of aneuploidy, Karyotyping, Prenatal screening, Soft markers of aneuploidy.

Introduction:

In the general population, prenatal screening detects high-risk pregnancies for prenatal illnesses. Non-invasive techniques enable detection over chromosomal aneuploidies such as Trisomy 21, 18, and 13 during early pregnancy [Hixson et al. 2015; Norton et al 2014]. The prenatal screening began with a risk assessment of aneuploidy with maternal age [Cuckle et al. 2016; Summers et al. 2007]. Maternal serum biomarkers and ultrasound soft markers were further included in the screening procedure [Summers et al. 2007]. All pregnant women should be recommended prenatal screening test for aneuploidy, irrespective of maternal age; however, with the growing number of options available for prenatal screening, determining which one is most applicable is gradually becoming complicated. Moreover, false-positive screening tests of maternal serum and ultrasonography, along with a lack of therapeutic alternatives for chromosomal abnormalities, create anxiety among couples. It is crucial for an obstetrician to choose screening tests and diagnostic procedures that are accurate, safe, and can be done throughout early pregnancy so that couples can make informed decisions regarding the continuation of pregnancy. First trimester, maternal serum double marker test (DMT) incorporating pregnancy-associated plasma protein-A (PAPP-A) and free ß-HCG (human chorionic gonadotropin) alone or along with USG (ultrasonography) for nuchal translucency (NT) is an established screening practice in several countries for Down syndrome [Benn et al. 2013]. During the second semester, maternal serum screening tests like a triple marker (TMT) constituting alpha-fetoprotein (AFP), free HCG, unconjugated estriol, and the quadruple marker test (QMT), constituting triple markers along with inhibin A are used alone or in combination with ultrasonography to identify foetuses at risk of autosomal aneuploidies, particularly trisomy 21, 18, and 13 [Cuckle et al. 2016]. Most recent in the screening test category is a non-invasive prenatal test (NIPT), done from mothers' blood to isolate the foetal cell-free DNA to reveal some genetic abnormalities not detected by the multiple marker serum tests [Samura et al. 2020]. Ultrasonography (USG) is the easiest and simplest non-invasive way of detecting aberrant embryogenesis during prenatal screening examinations. However, it is not a very sensitive method for detecting aneuploidy [Sharda and Phakde2007]. USG for detecting malformations is also used for detecting ultrasonographic soft markers as a risk factor for chromosomal abnormalities. Various ultrasonographic markers such as nuchal fold thickening, mild ventriculomegaly, Cystic hygroma, aberrant right subclavian artery, nasal bone hypoplasia echogenic cardiac focus, echogenic bowel, choroid plexus cyst, renal pyelectasis, short femur, and single umbilical artery are known to be associated varying risk of chromosomal anomalies either in isolation or in combination. [Sharda and Phakhde 2007; Meiying Cai 2021]. However, all these prenatal screening tests are only 'risk-assessment-tests,' as only foetal cells can provide a conclusive chromosomal diagnosis. Karyotyping is required for the deterministic diagnosis of chromosomal abnormality. The present study aimed to determine the trends of prenatal screening tests in India and the prevalence and types of chromosomal abnormalities concerning maternal age groups. We have also determined the predictive accuracy of different prenatal screening tests and USG soft markers concerning maternal age.

Material and methods

In this study, amniotic fluid samples from pregnant women were obtained for karyotyping due to the suspicion of a high risk for chromosomal abnormality during first- and second-trimester prenatal examinations. The study was retrospective and was based on medical records. Obstetricians referred them for karyotyping based on their positive screening results for aneuploidy.

Inclusion criteria: All women with foetuses having clinical indications of aneuploidy and who underwent amniocentesis following first and second-trimester prenatal screening tests were included for analysis.
Pregnant women referred by obstetricians based on positive screening tests for aneuploidy and who underwent karyotyping were included. Women's medical records include information on prenatal screening procedures, clinical karyotyping indications, age risk, and karyotype results.

First Trimester	Second Trimester
Biochemical test: Dual markers	Biochemical test: Triple test or Quadruple test
Nuchal translucency (NT) measurement	Biochemical + USG soft markers
Combined test: NT + Biochemical Screening	USG Soft markers
First-trimester USG soft markers other than NT	Integrated: First trimester+Second Trimester

Exclusion criteria: In order to have a representative population, twin pregnancies, IVF pregnancies, and women with incomplete demographic characteristics were eliminated. Due to a lack of information, four women with aneuploidy risk identified by the non-invasive prenatal test (NIPT) were excluded. Records with ambiguous or missing demographic information were also disregarded.
Ethical clearance: In accordance with ICMR norms, the institutional Ethics Committee authorised the current study (*Ref: 137/IRB-IBSEC/SIST2019-2020*).

Karyotyping study

All the Amniotic fluid samples inoculated into 2 ml of Gibco's Amniomax amniocyte cell suspension were incubated at 37 °C with 5 % CO_2 and cultured for 10 to 12 days to karyotyping were subjected as per standardard protocol. In brief, AF sample culture medium inoculated Cells were monitored on daily basis (24 hours) for their growth conditions. Respective amniotic fluid cells were harvested and when multiple clones with numerous doublets mitotic cells were observed under an inverted microscope. Addition of Colcemid solution, a mitotic inhibitor to arrest cells in the metaphase of mitosis. After incubation and centrifugation, the medium is replaced with a hypo-osmotic solution, which leads to cell lysis. Subsequently, the sample is fixed using a methanol-acetic acid fixative, and a few drops of the suspension are placed on a microscope glass slide. The slide is dried, and the chromosomes are stained with Giemsa dye and then can be viewed under a inverted microscope for the cell's karyotype.This technique of producing a visible karyotype through staining chromosome is called Giemsa banding. Five karyotypes were examined, and 15 chromosomal karyotypes were counted.
The cut-off value for aneuploidy risk and indication for amniocentesis was 1/250 based on biochemical tests in the present study.
In the case of ultrasound-based prenatal screening, clinical indications for invasive tests were

- High risk of aneuploidy: The presence of significant single soft markers like absent/ unossified/ hypoplastic/ nasal bone, increased nuchal translucency, ventriculomegaly, increased nuchal fold thickness, and aberrant right subclavian artery
- Moderate risk: echogenic focus on the heart and echogenic bowel
- Low risk: the presence of choroid plexus cyst, single umbilical artery, short humerus/femur, and renal pyelactasis in isolation or with other anomalies

Statistical analysis:

The variables for analysis in the present study were maternal characteristics like maternal age, screening tests, clinical indications, and the karyotype outcome and type of chromosomal abnormalities. All the data were analysed using SPSS 16.0 and MS Excel softwares.

Results:

A total of 2280 pregnant women at high risk for foetal chromosomal abnormality with gestational age ≥ 24 weeks underwent prenatal karyotyping for clinical indications of abnormal foetus determined by first and second-trimester screening tests.
In the present study, TMT was the most preferred method of prenatal screening referred by an obstetrician to 46.40% of pregnant women despite the availability of QMT. It shows that second-trimester maternal serum

tests are used mainly by obstetricians for aneuploidy screening in India. One of the reasons for preference for these tests is their ability to screen neural tube defects in the foetus along with aneuploidy. However, the screening in the second semester could delay the aneuploidy detection and informed decision-making in the case of positive screening for aneuploidy**.** However, for low-risk women for aneuploidy, this could be the preferred method for screening by obstetricians. The second most preferred test referred by 24.52% of obstetricians was Ultrasound/ ultrasonography. The first-semester screening, which includes DMT (7.28%) and combined tests (3.82%), was the least represented screening method for aneuploidy in the present study. Less representation of the first-trimester test could indicate later report of pregnant women to obstetricians and inadequate staff with specialised training for NT measurement for a combined test.

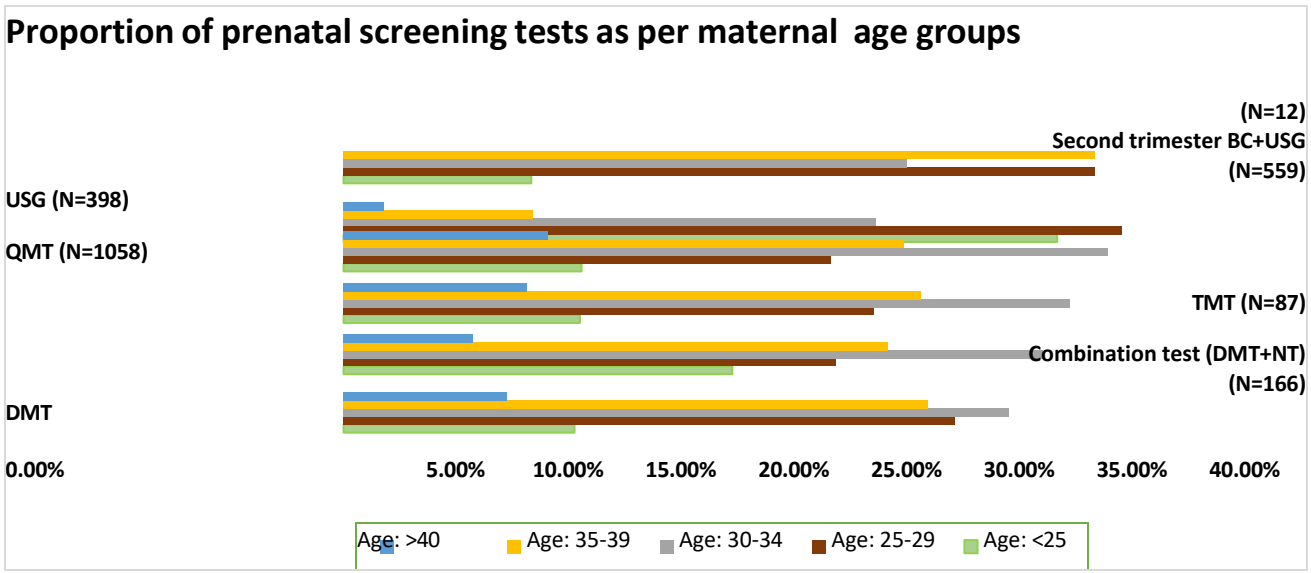

Fig1: Distribution of different prenatal screening tests in maternal age groups.

Table1: shows that in the maternal age group <25, the most preferred screening test for aneuploidy screening was an ultrasound, constituting 48.8% of all the screening procedures. As this group is a low risk for aneuploidy, routine ultrasound in early pregnancy detected the soft markers for aneuploidy. On the contrary, ultrasound is the least preferred method in the high-risk age group 35-39 and >40, and biochemical markers of the second trimester are the most preferred by obstetricians. Less representation of first trimester tests could be due to late reported pregnancy in high-risk maternal age groups.

Table2: The positive predictive value of DMT is 13.86 %, whereas, for the combined test, it increased to 33.3%. It shows that adding NT as a marker for aneuploidy has increased the predictive accuracy of prenatal Down Syndrome. The combined test in the first trimester has the highest predictive accuracy among all the tests, followed by the Combined second-trimester test BC+USG (16.67%), DMT (13.86%), and USG alone (9.8%), TMT (1.89%), QMT (5.66%). TMT has the least positive prediction rate though it is the most widely used test for screening. USG alone has better prediction accuracy than QMT and TMT. If we compare the predictive accuracy of various screening tests, we can see that the predictive accuracy of ultrasound rises significantly in high-risk maternal age groups 35-39 (27.7%) and >40 (30.0%). However, the predictive accuracy of other tests does not vary significantly in different maternal age groups. This shows that ultrasonography markers in high maternal age groups have high positive accuracy for chromosomal abnormalities.

Table 3: Among 2280 women screened positive for aneuploidy based on different prenatal screening procedures, only 149 (6.5%) were found to have an abnormal karyotype, and the remaining 2131(93.5%) had a normal karyotype. No trend was observed when comparing the positive prediction accuracy of the screening tests in different maternal age groups. It was highest at 8.5% in maternal age group <25, followed by 8.1% in maternal age group >40, 7.2% in maternal age 35-39, 6.4% in maternal age 25-29, and 4.8% in the maternal age group 30-34.

Table 4: This table shows the distribution of chromosomal anomalies in screen-positive women with different maternal age groups. Out of the 2280 karyotypes analyzed, 149 (6.5%) had a chromosomal abnormality. Among these abnormal karyotypes, 29% include deletion, inversions, multiple anomalies, Robertsonian translocation, and translocation, which cannot be detected with current non-invasive prenatal testing methods. Thus, only 71% of abnormalities are currently detectable using screening tests. Trisomy 21 accounted for

53.7% of the abnormal karyotypes. Though women were found to be screened positive for aneuploidy and were subjected to karyotyping for the confirmation of screening results, several other structural abnormalities were detected along with aneuploidies. The table above shows the various chromosomal abnormalities and their detection rate in screen-positive women for aneuploidy. The detection rate of trisomy 21(Down's syndrome) was the highest (3.5%). Other trisomies detected were trisomy 18 (N=13) with a detection rate of 0.57%, trisomy XXX (N=1) with a detection rate of 0.04%, and trisomy XXY (N=5) with a detection rate of 0.21. Three cases of triploidy, four cases of monosomy (i.e., 45 XO, Turner Syndrome), and 5 cases of mosaicism were detected. Besides suspected aneuploidies, there were structural abnormalities such as Robertsonian translocation and translocations.

Clinical indications of ultrasound abnormalities for invasive tests were as follows:

- High risk of aneuploidy: The presence of significant single soft markers like absent/ unossified/ hypoplastic/ nasal bone, increased nuchal translucency, ventriculomegaly, increased nuchal fold thickness, and aberrant right subclavian artery.
- Moderate risk: echogenic focus in heart and echogenic bowel.
- Low risk: choroid plexus cyst, single umbilical artery, short humerus/femur, and renal pyelectasis present in isolation

Soft markers were present in isolation as well as in combination. In the present study, combinations of more than one major soft marker or moderate soft marker were rare. When a major/ moderate soft marker was present in combination with a low-risk marker, analysis was based only on the major soft marker. However, there were clinical indications for karyotyping based on the isolated minor soft markers as well.

Table5: Absent/ossified/hypoplastic nasal bone was the prominent clinical indication 245(53.15%) for karyotyping in ultrasound-based prenatal screening for aneuploidy. This was followed by nuchal translucency 47(10.2%) in the absence of a dual marker biochemical test. Echogenic cardiac foci comprised 37(8.03%) cases, followed by choroid plexus cyst (5.42) and single umbilical artery 25(5.42%). Despite being a major indication, Nuchal fold thickness was present in 5.21 % of cases indicated for karyotyping. There were 98 clinical indications based on ultrasound which does not include the above soft markers. In these cases, either some minor soft markers were present in multiples, foetus indicated for IUGR (foetal growth retardation), or multiple structural anomalies were observed. We have analyzed only the cases with major/moderate soft markers and minor soft markers in isolation as clinical indications for aneuploidy. Any other indications present in 98 cases were not analyzed due to the complexity of the data.

In small numbers, some first-trimester soft markers such as ductus venosus, cystic hygroma, and aberrant right subclavian artery were also present as clinical indications for aneuploidy. In USG based prenatal screening procedure, there were 47 cases of isolated increased NT, out of which 5 cases were detected as an abnormal karyotype. This included one translocation 46, t(5;12) (q32;p13)[20], one Robertsonian translocation 46 rob(14;21)(q10;q10),+21[20], 2 Trisomy 21 and one case of Trisomy 18. Among screened positive 245 cases of absent/hypoplastic/ossified nasal bone as a clinical indication, there were 21 cases of trisomy 21, 5 karyotypes with Robertsonian translocation, one with translocation, two mosaics, and one 47 XXY, and one with multiple anomalies, 6, rec (4)? del (4) (q21q24)inv(4) (p14q25)[20]. Among 21 cases of increased nuchal fold thickness, only one karyotype was found abnormal with translocation 46, t (9;12;18) (q11;q13;q11.1). Among 12 cases with isolated ventriculomegaly as an indication, none of the karyotypes was abnormal. Among 16 cases of echogenic bowel, one karyotype with structural polymorphism with pericentric inversion in chromosome 9, (46, inv(9) (p11q13)[20]) was reported. Out of 37 cases of echogenic cardiac foci, one abnormal karyotype with T21 was observed. Minor soft markers in isolation or multiples were also indicated for the karyotyping. These included single umbilical artery (N=25), Renal Pyelectesis(N=10), choroid plexus cyst (N=25), and Aberrant right subclavian artery (N=12), absent ductus venosus (N= 4), and Short, long bones (N= 3). Minor soft markers had only two abnormal karyotypes where a single umbilical artery was present with a choroid plexus cyst, and both the karyotypes had Trisomy 18.

Moreover, there were 6 cases of cystic hygroma, 3 of which were found to have abnormal karyotype with two instances of Turner syndrome 45XO and one Trisomy 18. Maternal age with abnormal karyotype was <25 in 2 cases and between 35-39 in one case. There were 4 cases of abnormal ductus venosus; however, all were found to have normal karyotype in the present study. There were 11 isolated cases of aberrant

subclavian artery and one with ossified nasal bone, which was found to have an abnormal karyotype [46, rob (14;21) (q10;q10)+21[20]]. No case of tricuspid regurgitation was indicated in the present study.
Table6: In the present study, the highest predictive accuracy among the soft markers was of the absent nasal bone, with a positive predictive accuracy of 12.65%. Nuchal translucency is done in the first semester, along with a dual marker test. However, in the present study, only NT measurements indicated karyotyping with an optimistic prediction accuracy of 10.64%. Nuchal fold thickness, a major soft maker for aneuploidy, has a detection rate of 4.17% in the present study. Ventriculomegaly is also a major soft marker, but in this study, out of 12 indications of ventriculomegaly, none had an abnormal karyotype. Moderate soft markers in echogenic cardiac foci and echogenic bowels had a positive predictive accuracy of 2.70 and 6.25 respectively. Though minor soft markers in isolation are not indicated for karyotyping, in the present study, many pregnant women with these isolated markers underwent invasive testing with normal karyotype results.

Discussion:

Screening for Down syndrome has progressed from initial indications of advanced maternal age to sophisticated universal prenatal screening tests conducted during pregnancy's first and second trimester. If trisomy 21 is higher than the screening program's risk cut-off, an invasive diagnostic test is offered to confirm or rule out trisomy 21 or any other chromosomal anomaly. Invasive testing was indicated for pregnant women with the foetus at risk of chromosomal aneuploidy on ultrasound, biochemical screening, and a combination of ultrasound and biochemical risks. This study shows that first-trimester prenatal screening tests are not as popular as second-trimester tests in South India. Moreover, TMT is preferred over QMT by obstetricians. One of the reasons for preference for second-trimester tests could be their ability to screen neural tube defects in the foetus along with aneuploidy. However, the screening in the second semester could delay the aneuploidy detection and informed decision-making in the case of positive screening for aneuploidy**.** However, for low-risk women for aneuploidy, this could be the preferred method for screening by obstetricians.24.52% of prenatal screening tests for aneuploidy in the present study included Ultrasound/ ultrasonography in isolation.

The first-semester screening that is DMT (7.28%) and combined tests (3.82%) was the least represented in the present study. A study by Vandana Bansal and Rujul Jhaveri claimed that awareness of first-trimester screening has increased, and second-trimester screening tests for Downs syndrome are no longer as prevalent as earlier in Western India. Obstetricians allegedly favour second-trimester biochemical tests in the current scenario with the introduction of the first semester combined test. Muller PR et al. 2007 reported that for aneuploidy screening in South Australia between 1995 and 2005, there was a substantial decrease in the application of second-trimester maternal serum tests and a subsequent considerable preference for first-trimester combined screening. But in our study, we have found that obstetricians still prefer second-trimester screening tests over first-trimester screening tests.

Another reason for this result in our study could be the lack of awareness among pregnant women, which leads to reporting late prenatal check-ups. Less representation of the first-trimester test also indicates less understanding among obstetricians about these tests and inadequate staff with specialized training for NT measurement for a combined test. Though if we compare the positive predictive value of first-trimester screening tests with second-trimester screening tests, a vast difference in accuracy is found. DMT has predictive accuracy of 13.86 %, whereas TMT and QMT only have a predictive accuracy of 1.89% and **5.66%**. Adding NT as a marker to DMT in combined screening tests increased the predictive accuracy to 33.3%. Thus, it is very crucial to carefully screen pregnant women for the presence of additional soft markers for clinical indications of aneuploidy before counselling for invasive tests. An earlier study reported similar results as the addition of ultrasound markers like NT, accompanied by tricuspid regurgitation, ductus venosus, and nasal bone to DMT improved the detection rates of aneuploidy from 65% for dual marker) 92% with NT, and 96% with all four soft markers, with a 2.5–5% false positive rate [Wald N et al. 2003].
Among all the prenatal screening tests, the combined test (BC+USG) in the first trimester has the highest predictive accuracy, 33.0%, followed by the Combined second-trimester test (16.67%). Though the second-trimester biochemical tests are more prevalent, they are reported to have the least positive predictive accuracy (i.e., for TMT (1.89%) and QMT (**5.66%**). The first trimester DMT has positive predictive accuracy of 13.86%, which is more than based on ultrasound alone (i.e., 9.8%). Our study's combined predictive accuracy of screening tests is 6.53% for detecting an abnormal karyotype. This has reduced to 3.5 % for Down Syndrome in the present study. In the previous studies, approximately 5% of the screen-positive high

pregnancies reported having a foetus with Down syndrome [Wald N et al. 2003; Malone et al. 2005]. If we compare the predictive accuracy of various screening tests, we can see that the predictive accuracy of ultrasound rises significantly in high-risk maternal age groups 35-39 (27.7%) and >40 (30.0%)(table2) . However, predictive accuracy of other tests does not vary significantly in different maternal age groups. This shows that ultrasonography markers in high maternal age groups have high positive accuracy for chromosomal abnormalities. In the present study, out of the 2280 karyotypes analyzed, 149 (6.5%) had a chromosomal abnormality, thus indicating that biochemical risk and USG findings during prenatal screening do not turn into a high likelihood of chromosomal abnormality in the general population. Nevertheless, these tests can improve informed decision-making in high-risk pregnancies based on maternal age and unfavourable obstetric history. Of these 149 abnormal karyotypes, 29% included structural chromosomal anomalies, deletion, inversions, multiple anomalies, Robertsonian translocation, and translocation, which cannot be detected with current non-invasive prenatal testing methods. Only 71% of abnormalities are currently detectable using screening tests. Table 3 shows the distribution of chromosomal abnormalities in different maternal age groups. Earlier studies show that common aneuploidies, detectable by non-invasive prenatal testing, comprise a higher percentage of abnormal results in older women[Norton et al., 2014; Loane et al., 2013].

In our analysis, we could not analyse this comparative data as the pregnant women were from the general population at risk. Only a fraction of women with advanced age >40 was present in the analysis. There was no case-control group for comparative analysis. In the present study, ultrasonography-based clinical indications for aneuploidy included detection of first-trimester and second-trimester soft markers. First-trimester soft markers indicated in the present study were feta nuchal translucency (NT), ductus venosus (DV), absent/ hypoplastic nasal bone (NB), Cystic hygroma, and aberrant right subclavian artery. Nuchal translucency (NT) measurements are done in the first semester, along with a dual marker test. However, in the present study, only NT measurements were considered an indication for karyotyping with a positive prediction accuracy of 10.64%. Increased NT was reported in earlier studies as a potent prenatal marker for Trisomy 21 with detection rates between 63% - 77% with a 5% false-positive rate [Wapner et al. 2003; Snijders 1998]. NT is commonly elevated in foetuses with a range of other abnormalities, such as trisomy's 13 and 18, Turner syndrome, and triploid, besides structural malformations including congenital heart deformities, skeletal dysplasia, diaphragmatic hernia, and foetal anaemia [Long et al. 2021; Souka et al. 2005].

This study reported six cases of cystic hygroma during first-trimester USG screening, three of which were found to have abnormal karyotype with two cases of Turner syndrome 45XO and one Trisomy 18. Maternal age with abnormal karyotype was <25 in 2 cases and between 35-39 in one case. The literature reported that cystic hygroma is a significant finding during the first-trimester USG scan. In 50-80% of cases, it is associated with chromosomal abnormalities [Yakıştıran et al. 2020]. The foetus needs to be monitored for the presence of structural abnormalities in case of a normal karyotype. We have also analysed the present study's predictive accuracy of various first and second-trimester soft markers. The highest predictive accuracy among the soft markers was of the absent nasal bone, with a positive predictive accuracy of 12.65%. Several studies have supported the relationship of absent/ ossified/ hypoplastic nasal bone with foetal aneuploidies [Kim et al. 2018] since the first reported study of its association with Down syndrome by [Cicero et al. 2001]. The absent/ ossified/ hypoplastic nasal bone is found in approximately 50% to 60% of Trisomy 21 foetuses and 6% to 7% of normal foetuses [Moreno-Cid et al. 2014]. Nasal bone can be visualized as a soft marker in the first and second trimester of USG screening. There were 4 cases of abnormal ductus venosus; however, all were found to have normal karyotype in the present study. Ductus venosus is a soft marker for aneuploidy detected in the first trimester [Mavrides et al. 2002].

There were 13 cases of aberrant subclavian artery; out of these, only one co-present with ossified nasal bone was found to have an abnormal karyotype [46, rob(14;21) (q10;q10)+21[20]]. The aberrant right subclavian artery is a rare anatomical abnormality that affects about 0.5–1.5 percent of the population. It is more common in patients with chromosomal anomalies, particularly trisomy 21. Even though it is an anatomical finding that remains consistent throughout the pregnancy, its value in identifying aneuploidies during the first trimester has received little research (Martínez-Payo et al., 2022]. In addition to first-trimester ultrasound markers, prenatal screening can be aided by second-trimester ultrasound soft markers. Second-trimester ultrasound outcomes allow the detection of ultrasound soft markers, which are absent in early pregnancy [Koet al 2022]. These soft markers are also present in normal foetus but found in higher frequency in the

foetus with chromosomal anomalies. These markers are imprecise, often transient, and can be easily identified throughout the second-trimester ultrasonography [Nyberg et al. 2001; Ko et al. 2022].

Various second semesters soft markers indicated in the present study included absent nasal bone, nuchal fold thickness (NFT), ventriculomegaly, echogenic intracardiac foci (ECF), echogenic bowel (EB), long-short bone, renal pylectasis, choroid plexus cyst (CPC), single umbilical artery (SUA). Nuchal fold thickness (NFT), a major soft maker for aneuploidy, has a detection rate of 4.17% in the present study. The NFT is also defined as nuchal edema in the second trimester and is a non-structural marker with suitable sensitivity and specificity [Kazemi et al. 2018]. The existence of echogenic bowel (EB) during second-trimester ultrasound screening is a significant finding indicating abnormal development [Sal et al. 2021]. EB is a moderate soft marker with a positive predictive accuracy of 2 6.25% in the present study. It is associated with aneuploidy, especially trisomy 21, cardiac abnormality, and congenital malformations [SAL et al. 2021.
Another moderate soft marker, echogenic cardiac foci, had a positive predictive accuracy of 2.70% in the present study. EIF is reported to have a likelihood ratio between 1.8 to 5.4 for isolated cases of EIF, which indicates Down syndrome risk of twofold to five-fold [Özsürmeli et al. 2020]. Ventriculomegaly is a major soft marker [Sharda and Phakde 2007], but in this study, out of 12 indications of ventriculomegaly, none had an abnormal karyotype. Minor soft markers such as CPCs, abnormally short- long bones, SUA, and renal pyelectasis were also found to indicate karyotyping in the present study. In some cases, foetal aneuploidy was detected with these isolated minor soft markers, indicating their relevance in detecting the aneuploidy (Table 1-6). There were 25 cases of CPCs in the present study, and only one foetus was detected with trisomy 18 on karyotyping. Several studies report its association with Trisomy 18 [Shah 2018; Sharma et al. 2019].

CPCS does not change the risk of trisomy 21 if detected over absolute risk. Though detection of CPC requires a complete evaluation of foetal hands for likely clenched fist and overlapping digits and to eliminate trisomy 18 risk. [Bronsteen et al. 2004]. In 1998, Chitty et al. examined the significance of CPCs in a standard population and proved that the existence of CPCs magnifies the risk of aneuploidy 1.5 times, predominantly of trisomy 18.

Three isolated cases of Long-short bones were detected in the foetus after sonography; all of them had a normal karyotype. Individuals with Down syndrome can have. Benacerraf et al. (1991) first investigated the occurrence of abnormally short- long bones associated with Downs syndrome. In this study, ten foetuses were detected to have isolated renal pyelectasis as an indication of karyotyping. Foetuses with substantial renal pyelectasis (>10 mm) are at risk for structural abnormalities that necessitate postnatal assessment. In 1990, Benacerraf et al. (1990) proposed a link between renal pyelectasis and aneuploidy, mainly Down syndrome. Chudleigh et al. assessed the aneuploidy prevalence in a foetus with the isolated occurrence of moderate renal pyelectasis to be 0.33% and 2.2% in women <36 years old and >36 years age, respectively [Chudleigh et al. 2001]. As an isolated soft marker and clinical indication for aneuploidy, SUA was detected in 25 cases in the present study; two foetuses were detected with Trisomy 18. Study conducted by Ebbing et al. in Norway found a strong association of SUA with trisomy 13 and 18 [Ebbing et al. 2020]

The present study is based on a large sample of pregnant women with the risk of aneuploidy in a foetus who underwent prenatal screening tests at multiple centres. Being a sizeable multicentre sample, it was possible to calculate the frequency and accuracy of various prenatal diagnostic tests and extrapolate the results to the south Indian population. The limitation of the study includes a lack of information regarding the number of women sampled at each centre for aneuploidy risk. This data could help us find the sensitivity and specificity of each prenatal screening procedure and the soft markers studied in the present work. Moreover, we didn'thave the follow-up data of the pregnant women to evaluate the pregnancy outcome in women with a normal foetus and those with abnormal fetuses who decided to continue the pregnancy. This study also revealed chromosomal polymorphism, including normal variants in heterochromatin and pericentric inversions. Some of these polymorphisms are implicated in infertility and disease susceptibility [Collodel et al., 2006; Sivakumaran et al., 1997]. However, this data was not presented in this paper as the study's goal was to analyze chromosomal anomalies in the foetus. This study emphasizes that if the pregnancies are precisely monitored by non-invasive screening during the 1st and 2nd trimesters in the general population, it is possible to detect the high-risk pregnancies early enough for informed decision making.

Conclusion:

The present shows that first-trimester prenatal screening tests that as DMT, combined test, and USG alone, are less represented despite a high predictive accuracy either due to lack of awareness or inadequate competent staff for first-trimester combined screening. This shows a need to popularise these tests and enhance first-trimester USG expertise to exploit the high predictive accuracy of these tests. This will also offer the couples sufficient time for informed decision-making in case of a positive result. This study provided the predictive accuracy of screening tests and soft markers in different maternal age groups(Table1). This study shows a considerable risk of chromosomal anomalies in the younger age group. This study also found that soft markers detected during routine USG examination during pregnancy can detect a significant risk of aneuploidy.

This study maintains that prenatal screening tests in the general population reduce the burden on more invasive prenatal diagnostic tests, which are more risky, expensive, and laborious. Moreover, it strengthens the likelihood of reducing the load of prenatal invasive cytogenetic tests if the pregnancies are precisely monitored by non-invasive screening during the 1st and 2nd trimesters. This study shows that in women with a positive prenatal screen for aneuploidy who followed diagnostic testing, many structural chromosomal abnormalities were detected, which cannot be diagnosed by non-invasive prenatal testing and screening procedures. This is vital information to be considered by obstetricians while dealing with high-risk mothers for foetal chromosomal abnormalities.

Abbreviations used:

AFP Alpha-fetoprotein CPCs Choroid Plexus Cysts DMT Double marker test DV Ductus venosus
EIF Echogenic intracardiac foci HCG Human chorionic gonadotropin NB Nasal Bone
NIPT Non-invasive prenatal test NT Nuchal Translucency
PAPP-A Pregnancy-associated plasma protein-A QMT Quadruple marker test
TMT Triple marker test USG Ultrasonography

Table1: Prenatal test distribution as per maternal age groups

	Age: <25	**%**	**Age: 25-29**	**%**	**Age: 30-34**	**%**	**Age: 35-39**	**%**	**Age: >40**	**%**
DMT	17	4.68	45	7.55	49	7.13	43	8.87	12	8.05
BC+USG first semester	15	4.13	19	3.19	27	3.93	21	4.33	5	3.36
TMT	111	30.58	249	41.78	341	49.64	271	55.88	86	57.71
QMT	42	11.57	86	14.43	135	19.65	99	20.41	36	24.16
USG	177	48.76	193	32.38	132	19.21	47	9.69	10	6.71
BC+USG Second trimester test	1	0.28	4	0.67	3	0.44	4	0.82	0	8.05

Table2: Prediction accuracy of screening tests in different maternal age groups

Test	**Karyotype Outcome**	**Age: <25** (N=363)	**Age: 25-29** (N=596)	**Age: 30-34 (N=687)**	**Age: 35-39 (N=485)**	**Age: >40** (N=149)
DMT (N=166) Positive prediction accuracy (%)	Abnormal(N=23)	2	7	8	4	2
	Normal (N=143)	15	38	41	39	10
	13.86	11.76%	15.56%	16.33%	9.3%	16.67%
Combination DMT+NT (N=87) Positive prediction %	Abnormal(N=29)	6	6	7	8	2
	Normal (N=58)	9	13	20	13	3
	33.33%	40.0%	31.58%	25.9%	38.09%	40%
TMT (N=1058) Positive prediction %	Abnormal(N=20)	6	2	2	7	3
	Normal (N=1038)	105	247	339	264	83
	1.89%	5.4%	0.80%	0.58%	2.6%	3.5%
QMT(N=398)	Abnormal(N=22)	2	7	8	3	2
	Normal (367)	40	79	127	96	34
Positive prediction %	**5.66%**	4.8%	8.13%	5.9%	3.0%	5.6%
Combination of BC+USG Second trimester test (N=12) Positive prediction %	Abnormal(N=2)	0	2	0	0	0
	Normal (10)	1	2	3	4	0
	16.67%	NA	100%	0	0	NA
USG (N=559)	Abnormal(N=55)	17	14	8	13	3
	Normal (N=504)	160	179	124	34	7
Positive prediction %	9.8%	9.6%	7.3%	6.1%	27.7%	30.0%

Table 3: Prediction accuracy of screening tests as per maternal age group age:

Age groups	**Age: <25** (N=363)	**Age: 25-29** (N=596)	**Age: 30-34 (N=687)**	**Age 35-39 (N=485)**	**Age: >40** (N=149)	**Total (2280)**
Abnormalities	31	38	33	35	12	149
Positive prediction rate	8.5%	6.4%	4.8%	7.2%	8.1%	6.53%

Table 4: Distribution of chromosomal abnormalities in different maternal age groups

	Chromosomal anomalies No	Chromosomal anomalies %	Age: 25 (N=363)		Age: 25-29 (N=596)		Age: 30-34 (N=687)		Age: 35-39 (N=485)		Age: >40 (N=149)	
			No.	% Abnormality in screen - positive women	No.	% Abnormality in screen-positive women	No.	% Abnormality in screen-positive women	No.	% Abnormality in screen-positive women	No.	% Abnormality in screen-positive women
Deletion (Fig .9)	2	2 (0.09%)	0	0	1	0.17	1	0.15	0	0.00	0	0.00
Inversions (Fig.1)	16	16(0.70%)	4	1.102	4	0.67	4	0.58	2	0.41	2	1.34
Monosomy	4	4(0.18%)	3	0.826	1	0.17	0	0.00	0	0.00	0	0.00
Mosaic	5	5(0.22%)	1	0.275	1	0.17	1	0.15	1	0.21	1	0.67
Multiple Abnormality	2	2(0.09%)	0	0.000	1	0.17	1	0.15	0	0.00	0	0.00
Robertsonian translocation(Fig .8)	8	8(0.35%)	4	1.102	2	0.34	0	0.00	1	0.21	1	0.67
Translocation(Fig .4)	10	10(0.44%)	2	0.551	4	0.67	1	0.15	2	0.41	1	0.67
Triploid(Fig .7)	3	3(0.13%)	2	0.551	0	0.00	1	0.15	0	0.00	0	0.00
Trisomy 21(Fig.3)	30	30(3.50%)	13	3.581	17	2.85	22	3.20	24	4.95	4	2.68
Trisomy18(Fig .2)	13	13(0.57%)	1	0.275	4	0.67	1	0.15	5	1.03	2	1.34
Trisomy XXX(Fig .6)	1	1(0.04%)	0	0.000	1	0.17	0	0.00	0	0.00	0	0.00
Trisomy XXY(Fig .5)	5	5(0.22%)	1	0.275	2	0.34	1	0.15	0	0.00	1	0.67

A follow-up to the karyogram of the chromosomal anomalies can be seen below.

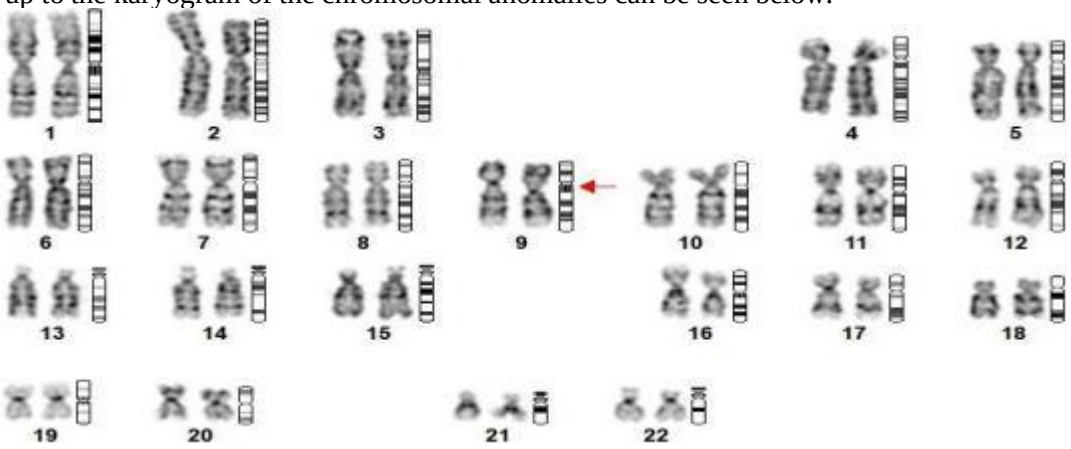

Fig.1: A karyotype of an individual with pericentric inversion in chromosome 9, involving the regions p11 and q13. 46,X?,inv(9)(p12,q13)[ISCN 2020].This heteromorphism is considered to be a polymorphic variant and has been found in both individuals

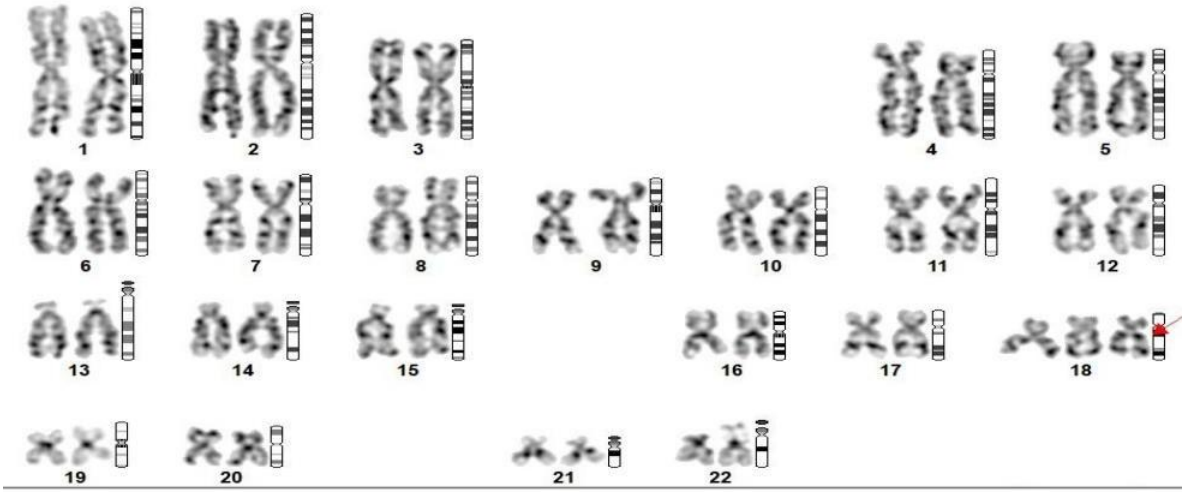

Fig.2: Amniocytes revealed the presence of an additional copy of chromosome 18, suggestive of trisomy 18. 47,X?+18[ISCN 2020] this is indicative of Edward syndrome.

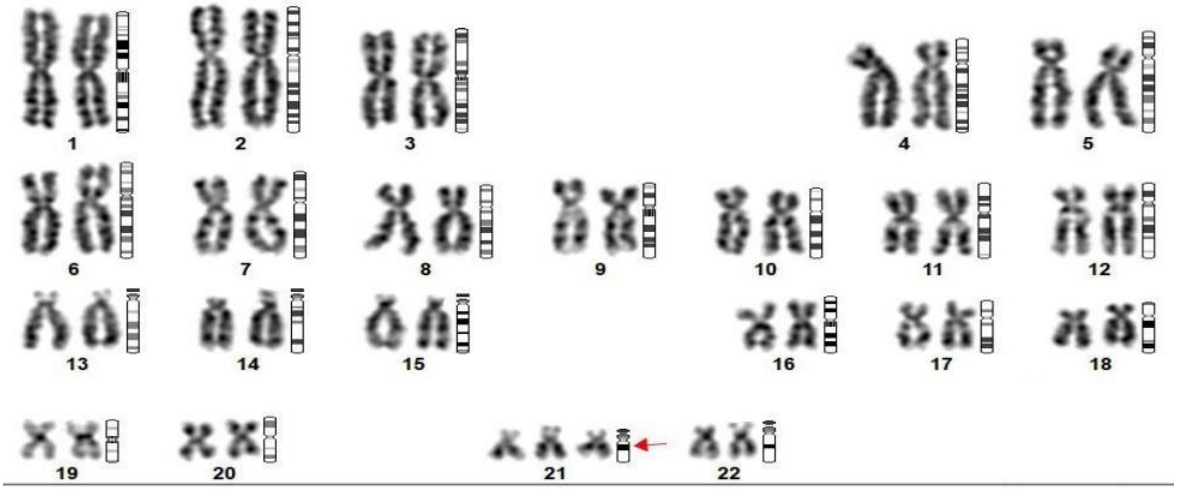

Fig.3:Amniotic cells ran additional, copy of chromosome 21, suggestive of trisomy 21.47,X?,+21[ISCN 2020]

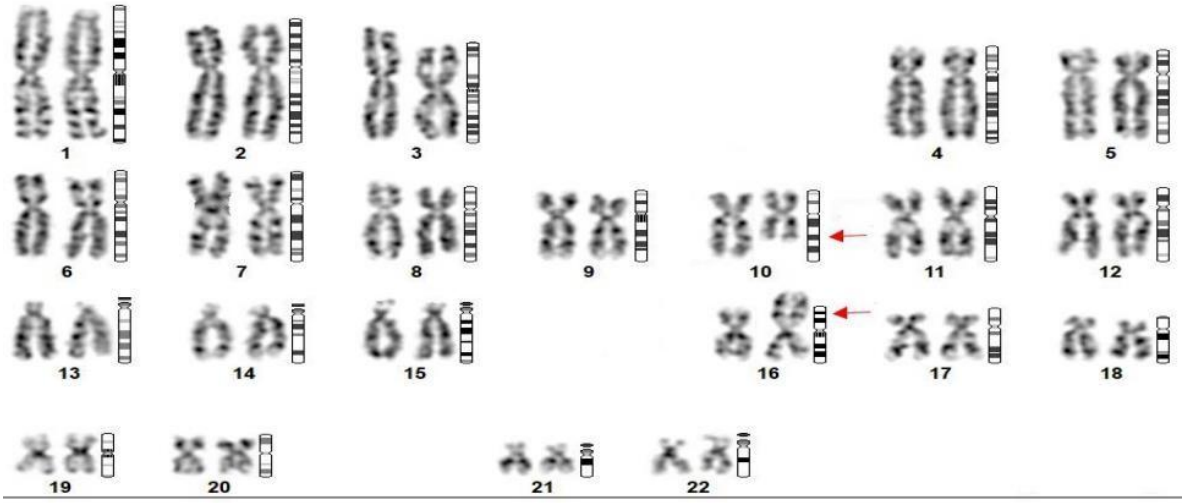

Fig.4: Chromosomal analysis of cultured amniocytes revealed a translocation between the long of one of the chromosomes 10 and the short arm of one of the chromosomes 16 involving the regions q22 and p11.2 respectively that appears to be balanced. 46,X?,t(10;16)(q22;p11.2)mat[ISCN 2020]. This karyotype in the fetus is a maternally inherited translocation.

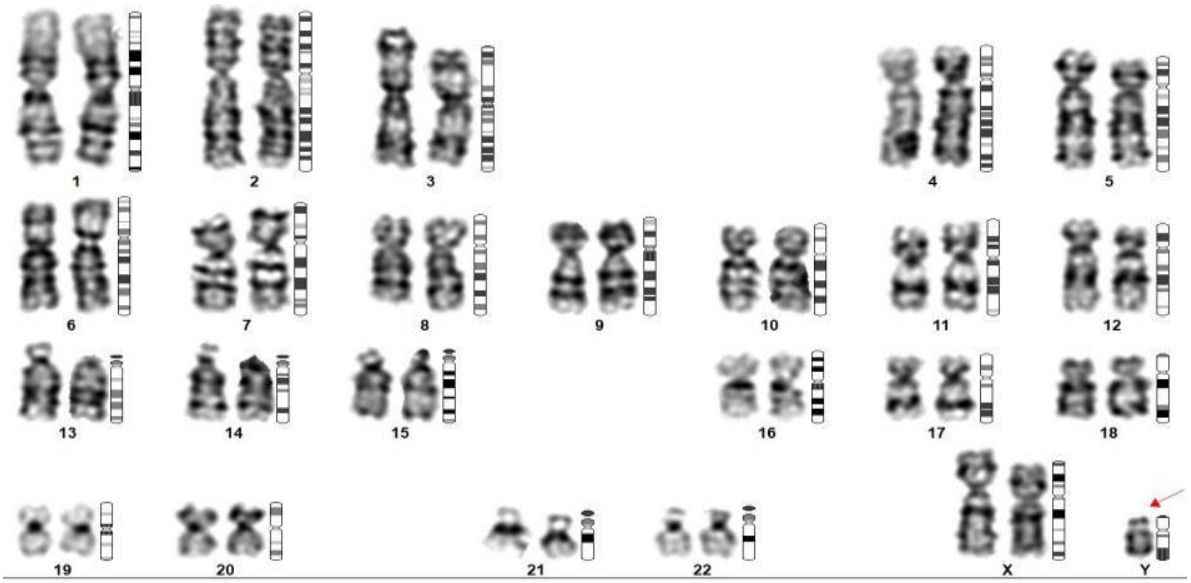

Fig.5: Amniocytes revealed the presence of an additional copy of chromosome X. 47,XXY[ISCN 2020].This karyotype is indicative of Klinefelter syndrome.

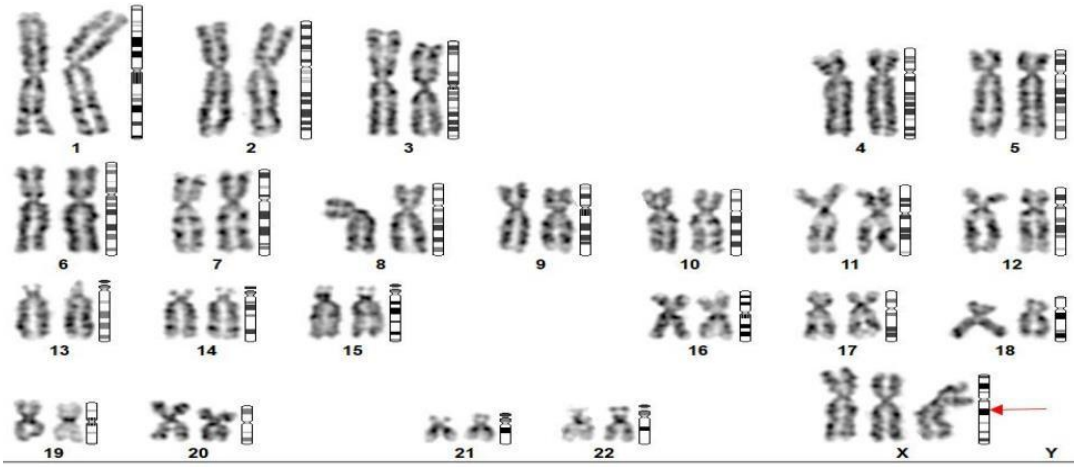

Fig.6: Chromosome analysis revealed cells showed presence of an additional copy of chromosome X and this is indicative of Trisomy X. 47,XXX[ISCN 2020].

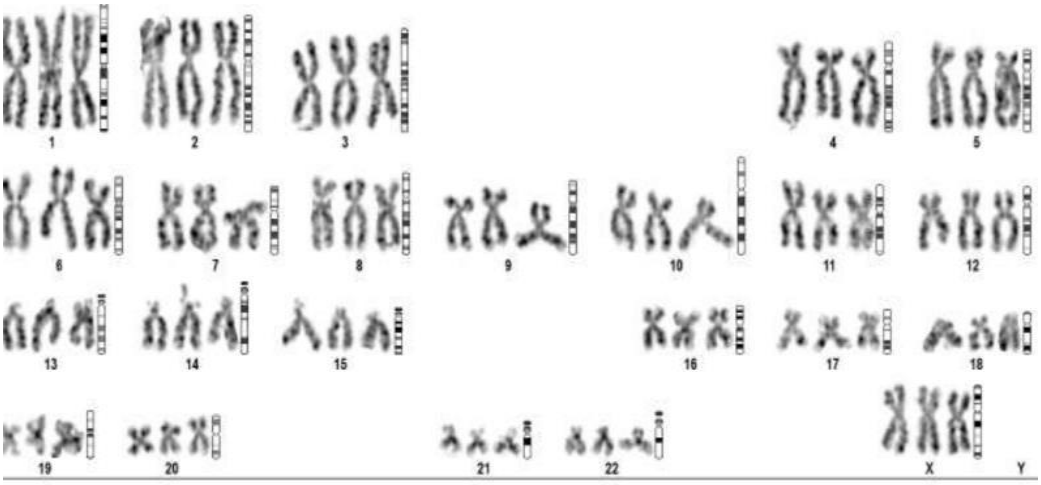

Fig.7: Chromosome analysis revealed the presence of 69 chromosomes which is a condition termed as triploidy. 69[ISCN 2020]. Most triploidic fetuses are stillborn.

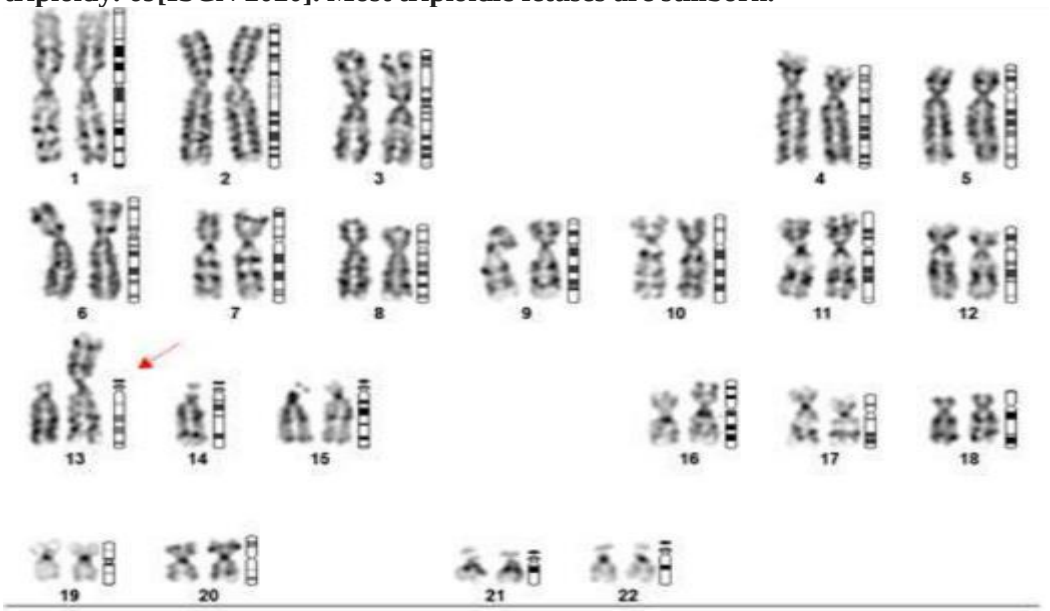

Fig.8: Chromosomal analysis of cultured amniocytes revealed a Robertsonian translocation involving chromosome 13 and chromosome 14 which appears balanced and resulting in a chromosome count of 45. Balanced translocations in has no effect on development or general health because no genes have been lost or gained.45,rob(13;14)(q10:q10)[ISCN 2020]

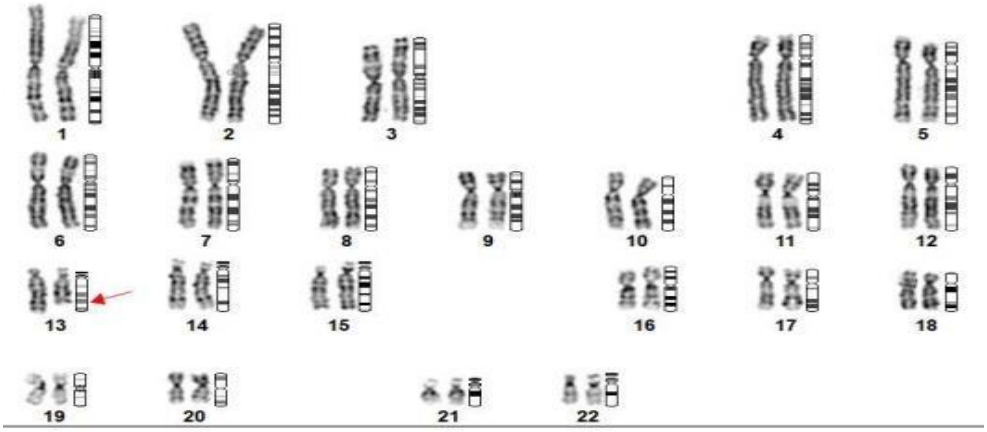

Fig.9: There is presence of a deletion on one of the chromosomes 13 involving the region q22.This appears to be an unbalanced rearrangement .In order to establish whether the rearrangement has resulted in loss or gain of genes that may impact the clinical outcome recommendations have been provided. 46,der(13),del(?13)(q22)[ISCN 2020]

Table 5: % Distribution of clinical indications based on USG for karyotyping

	Clinical indications	**Number**	**%**
First-trimester major soft marker	NT	47	10.20
	Absent NB	245	53.15
	Cystic hygroma	6	4.03
	Ductus venosus	4	2.68
	Aberrant right subclavian artery	13	2.82
Second-trimester major soft marker	Nuchal fold Thickness ventriculomegaly	24	5.21
		12	2.60
Second-trimester moderate soft marker	Echogenic bowel Echogenic cardiac foci	16	3.47
		37	8.03
Second-trimester minor soft marker	Single umbilical artery Choroid plexus cyst Renal Pylectasis Short, long bone	25	5.42
		25	5.42
		10	2.17
		3	0.65

Table 6: Predictive accuracy of USG soft markers in different maternal age groups

UGC indication for Invasive test		overall accuracy	Age: <25	Age: 25-29	Age: 30-34	Age: 35-39	Age: >40
Increased NT (N=47)	Abnormal (N=5)		2	1	1	0	1
	Normal (N=42)		8	9	18	6	1
	Positive predictive accuracy	10.64	20	10	5.26	NA	50
Cystic hygroma	Abnormal (N=3)		2	0	0	1	0
	Normal (N=6)		0	2	1	0	0
	Positive predictive accuracy	50.00					
Absent nasal bone(N=245)	Abnormal (N=31)		10	8	3	8	2
	Normal (N=214)		70	86	41	12	5
	Positive predictive accuracy	12.65	12.5	8.51	6.82	40	28.57
Increased nuchal fold thickness (N=24)	Normal(N=1)		0	0	0	1	0
	Abnormal(N=23)		13	6	2	1	0
	Positive predictive accuracy	4.17	NA	NA	NA	100	NA
Ventriculomegaly(N=12)	Abnormal (N=0)		0	0	0	0	0
	Normal (N=12)		3	6	3	0	0
	Positive predictive accuracy	NA	NA	NA	NA	NA	NA
Echogenic Cardiac foci(N=17)	Abnormal (N=1)		1	0	0	0	0
	Normal (N=36)		25	14	8	2	1
	Positive predictive accuracy	2.70	3.846154	NA	NA	NA	NA
Echogenic Bowel(N=16)	Abnormal (N=1)		0	1	0	0	0
	Normal (N=15)		4	2	8	1	0
	Positive predictive accuracy	6.25	NA	0%	NA	NA	NA

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