RESEARCH ARTICLE

Prenatal Aneuploidy Screening and Diagnosis—Its Evolution and Trends: A 3-year Analysis in a Fetal Medicine Center

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Abstract

Introduction: After the ACOG guideline in 2007 recommending that all women, regardless of age, should be offered aneuploidy screening before 20 weeks of gestation. This protocol in the name of the fetal aneuploidy screening program was slowly introduced in various Indian hospitals. This observational study was performed to analyze population-based trends of prenatal testing (serum screening and invasive testing) for aneuploidy over 3 years (2017–2019).

Materials and methods: A retrospective single-center study was carried over a period of 3 years (January 2017 to December 2019). This hospital was a tertiary care hospital with fetal medicine unit that had approximately 3,000 annual births. Analysis of data of all pregnant women undergoing prenatal testing before 20 weeks of gestation was collected in the following subheads: (1) aneuploidy screening data, (2) invasive testing data [amniocenteses and chorionic villus samplings (CVSs)], and (3) tertiary care hospital birth statistics from January 2017 to December 2019.

Results: Over a period of 3 years, aneuploidy screening was accepted by the target population and at present >85% target population undergo aneuploidy serum screening. Annual numbers of invasive prenatal tests climbed steadily from 2017 to 2019. The proportion of invasive testing performed for abnormal serum screening (ASS) increased steadily from 51% in 2017 to 72% ($p < 0.05$) in 2019. While the indications abnormal ultrasound finding (AUS) showed a steady decline over the same timeline but an indication of previous baby affected with aneuploidy (PBAA) remained in the same range. By 2019, the most common indications for invasive tests were positive ASS (72%) and AUS abnormality (15%). The diagnostic yield of all invasive tests for a major chromosome abnormality over a 3-year study period was 4.7%. The rate of CVS to amniocentesis rose to 17.5% in 2019 from 4.6% in 2017. Fewer complications of invasive tests were observed as compared to previous studies.

Conclusion: The study demonstrates a rise in aneuploidy serum screening and its acceptance in the pregnant population. Abnormal serum screening is the main indication of prenatal invasive testing. This study also adds to the safety profile of invasive testing.

Keywords: Amniocentesis, Chorionic villus sampling, Down syndrome, Noninvasive prenatal test, Prenatal diagnosis, Prenatal screening.

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Introduction

The story of aneuploidy screening started in the early nineteenth century when Dr Lionel Penrose proposed a significant association between increasing maternal age and the birth of a Down syndrome child (aneuploid).[1] Later in 1970 with the development of cytogenetic techniques for analysis on cultured amniocytes, all women aged >36 years were offered an amniocentesis to diagnose fetal aneuploidy. Soon after this, ultrasound-guided chorionic villus sampling (CVS) and amniocentesis made its way into clinical practice and they provided genetic material for prenatal aneuploidy testing.[2] This modality exposed women to high rates of invasive testing and failed to capture the majority of aneuploid pregnancies in the younger population. The appreciable miscarriage rate after invasive testing of 0.5–1.2% was another significant drawback of this approach.[3]

With the evolution of maternal serum biomarkers, such as BHCG, AFP, and unconjugated estriol as a surrogate marker of aneuploidy, the use of maternal serum screening (MSS) in the 1990s opened the door of aneuploidy screening to women of all ages in the second trimester. In the past two decades, combined first-trimester screening, using ultrasound measurement of the fetal nuchal translucency (NT) and maternal serum markers, has become the mainstay of the aneuploidy screening program as it allows earlier diagnosis compared with all other MSS protocol also has, but has higher detection rates for trisomy 21 [85–95% for a fixed false-positive rate (FPR) of 5%].[4] Next-generation sequencing using maternal cell-free DNA (cffDNA) has changed the landscape of prenatal screening. This test outperforms all other methods of screening for trisomy 21, 18, and 13 with reported sensitivities and specificities of almost 99% for trisomy 21.[5] It has been proved statistically that cffDNA will reduce prenatal invasive testing by 88% if used as a secondary screening test in all women identified as high risk by serum screening.[5,6] In tandem with these developments in screening, advances in molecular genetics have led to a better assessment of the fetal genome in detail.[7,8] With the development of chromosomal microarrays (CMA) has led to an increase in prenatal diagnostic yield, particularly in fetuses with structural abnormalities.[9,10]

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This study aimed to analyze the trends in serum screening and invasive diagnostic testing since 2017 with respect to the evolution of prenatal screening programs and changing indications for prenatal testing in our setup. Secondary analysis was to analyze the results of invasive procedures, indications for invasive prenatal testing, prenatal diagnosis of aneuploidy, the diagnostic yield of invasive tests, and complications of invasive testing.

Materials and Methods

Before the year 2017, serum testing for aneuploidy screening was carried out on hospital-based protocols. We introduced a protocol for serum testing for aneuploidy screening in our tertiary care hospital for all our antenatal patient patients which included dual marker and NT/nasal bone (NB) scan for patients who reported during the first trimester (11–13+6 weeks) and quadruple marker to patients who reported during the second trimester (14–19 weeks). All recruited antenatal patients underwent anomaly scans during 18–20 weeks of gestation. During the last 6 months of the study, the patients were counseled regarding cffDNA-based noninvasive prenatal test (NIPT) also and a few of them agreed to underwent the same. Indications for prenatal invasive testing were categorized into three groups:

- Abnormal serum screening (ASS)—this group included patients with a high risk of aneuploidy in a combined test or quadruple test. A combined test or quadruple test is considered high risk for trisomy 21 if the risk is >1:250 and for trisomy 18/13 if the risk is >1:200. The sensitivity of the combined test is 89% for an FPR of 4.5% and the quadruple test is 79% with an FPR of 6.5%.[11]
- Abnormal ultrasound group (AUS)—this group included patients with a fetal abnormality detected by ultrasound (one major: all major structural abnormalities of fetus or presence of two soft markers).
- Previous baby with aneuploidy and single gene disorder (PBAASGD)—this group included a patient who has the previous baby affected with aneuploidy and single gene disorder

Whenever patients matched with any one of the above categories, they were offered diagnostic invasive testing in the form of CVS or amniocentesis. Exclusion from the study was multiple pregnancies and prenatal testing done for maternal request. A patient who opted for cffDNA NIPT after serum screening was excluded from the study.

The types of genetic tests included conventional G-banded karyotype, fluorescent *in situ* hybridization (FISH). Results of chromosome analyzes were categorized as normal or abnormal. The abnormal group was further categorized by type of chromosome abnormality, "major" or "minor". Major chromosome abnormalities included all cases of autosomal and sex chromosome aneuploidy, unbalanced rearrangements, polyploidy, and level III mosaics. Minor chromosome abnormalities were balanced translocations and other rearrangements and confined placental mosaicisms. If CVS report came as placental mosaicism, patients were offered amniocentesis to confirm the diagnosis. In case of autosomal or sex chromosome abnormality, patients were counseled and all of them chose to terminate the pregnancies. Results from January 1, 2017, to December 31, 2019, were obtained. Local institutional ethical committee approved the study protocol.

Statistical Analysis

Data from the hospital were collected and analyzed. Our hospital has approximately 3,000 births annually and in 2019 had a median maternal age of 30.5 years. Only diagnostic procedures performed at <20 weeks of gestation were included. Annual statistics on live births in our hospital were obtained to estimate uptake rates of screening and invasive testing. Diagnostic yield was defined as a percentage of the abnormal cases with single, combined, or all indications. Time-series regression analysis was performed for a variance to evaluate the significance of trends and Chi-square tests to compare rates, using SPSS for Windows (SPSS Inc., Chicago, IL, USA). For the test of significance, a p value of <0.05 was considered significant.

Results

Over the period of 3 years, 9,566 women were offered serum screening tests (combined test + quad test) and out of these 5,641 women consented for it while 3,925 opted out. The cumulative acceptance of screening among all those offered was 59%. The total number of births year-wise was 2017 (n = 3,020), 2018 (n = 3,030), and in 2019 (n = 3,050). However, the number of serum screening tests increased each year from 2017 to 2019 as shown in the serum screening uptake rate (number of screening tests as a percentage of total births per year) from the start of aneuploidy screening, i.e., from 40% in 2017 (n = 1,208), 60% in 2018 (n = 1,810) to 86% (n = 2,623) in 2019 (Fig. 1).

A total of 272 women were offered invasive testing and 93% (n = 253) women opted for it while 7% (n = 19) opted out during the 3-year study period. Annual numbers of invasive testing increased steadily from the commencement of prenatal invasive testing in 2017 (n = 74), peaked in 2019 (n = 94) ($p > 0.05$) as shown in Figure 2. In 2019, 94 women underwent prenatal invasive testing (14 CVS and 80 amniocenteses), representing the highest number of invasive prenatal procedures recorded in 3 years.

Major changes in the common indications for invasive diagnostic testing occurred during the study period as shown in Figure 3. In 3 years, the proportion of invasive testing performed for ASS increased steadily from 51% in 2017 to 72% ($p < 0.05$) in

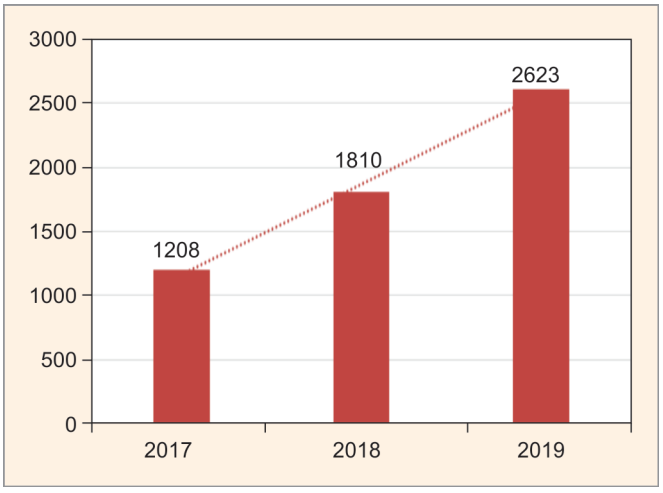

Fig. 1: Total number of aneuploidy serum screening

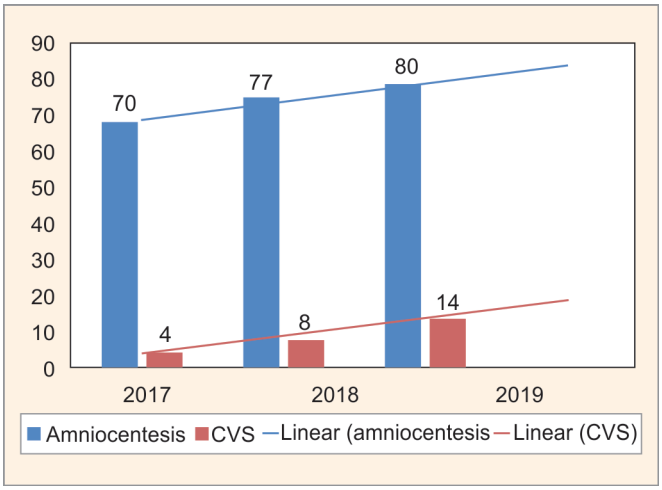

Fig. 2: Annual number of invasive testing

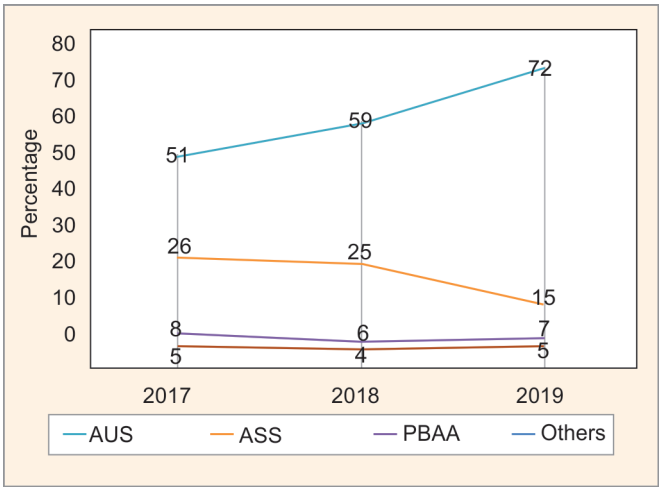

Fig. 3: Indications of invasive testing

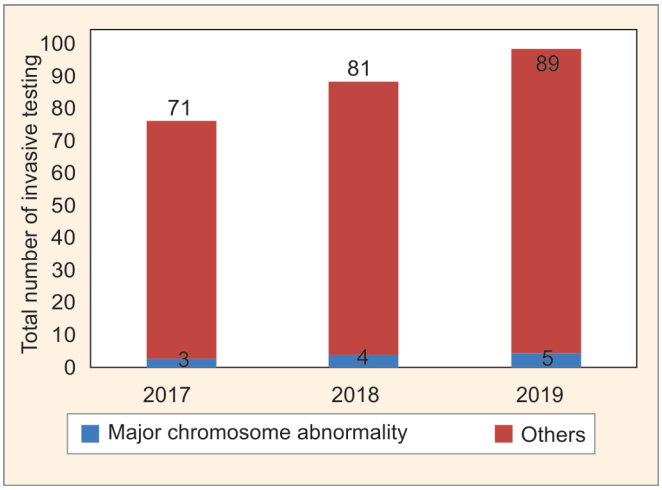

Fig. 4: Diagnostic yield

2019 which was incommensurate with other published studies.[12] While the indications AUS showed a steady decline over the same timeline but an indication of PBAA remained in the same range. By 2019, the most common indications were positive ASS (72%) and AUS abnormality (15%), reflecting major changes in prenatal screening strategies over the same period.

Also, the proportions of invasive testing performed for single-gene disorders, history of chromosome rearrangements, or previous baby with a chromosomal abnormality, which remained in the range of 4–5% during the study period.

The diagnostic yield of all invasive testing finding a major chromosome abnormality (total number of major chromosome abnormality divided by the total number of invasive testing) over a 3-year study period was 4.7% (12/253), the highest rate was in the year 2019 with *p* value <0.01. In Figure 4, the diagnostic yield of all invasive tests finds a major chromosome abnormality year-wise, i.e., 4% (2017), 4.7% (2018), and 5.3% (2019). When compared with annual births, rate the number of invasive testing represent a non-significant increase from 2.4% in 2017 to 3.08% in 2019 ($p > 0.05$). The rate of CVS to amniocentesis rose to 17.5% in 2019 from 4.6% in 2019 which clearly demonstrates the acceptability of first trimester combined screening.

The number of major chromosome abnormalities detected per year that rose over the study period was statistically insignificant. Trisomy 21 remains the most common condition detected, consistently constituting approximately one-half of all major chromosome abnormalities as shown in Table 1.

The complication of invasive prenatal testing is shown in Table 2. Out of 253 invasive testings, only one patient had an abortion within 2 weeks of the procedure and the karyotype later came as trisomy 21.

Discussion

Our tertiary care hospital populations are unique as it is pan India data not limiting to only one geographical part of India. Before the introduction of serum screening, aneuploidy screening was based on advanced maternal age and ultrasound marker. But after the introduction of a modern and efficient screening program, we were able to identify pregnancies at high risk of aneuploidy 4.8% (272/5,641, number of people offered invasive testing divided by number of people undergoing serum screening). This study shows a high diagnostic yield of 4.7% with 4.4% rates of invasive testing with almost nil complication in our patients. An average invasive procedure of 21 was performed for each diagnosis of a major chromosome abnormality. This study reaffirms improving detection rate for fetal anomalies with a decrease in procedure-related risks after the introduction of aneuploidy screening in our center.

Also, the uptake of serum screening program after IEC (information, education, counseling) increased in the targeted population to the current level of 86% of all live birth in the year 2019. This improvement in diagnostic yield retrospectively reaffirms the effectiveness of screening strategies. Further data emerge from analyzing the trends of indication for invasive testing that ASS report forms the major bulk of indication while abnormal ultrasound indication has shown a steady decline. The second most common indication with respect to diagnostic yield was abnormal ultrasound abnormality consistent with other population-based studies.[12] Chorionic villus sampling group has almost 50% indication from the previous baby affected with single gene disorder and previous baby with aneuploidy. Strengthening of serum screening program leading to 100% coverage of pregnant population will further enhance diagnostic yield. In the light of the introduction of cffDNA-based NIPT as a screening tool with higher sensitivity

Table 1: Abnormal chromosomal results of invasive tests

Chromosomal pattern	*2017*	*2017 USG*	*2018*	*2018 USG*	*2019*	*2019 USG*
Trisomy 21	1/74	Absent nasal bone	2/85	1. Nil 2. Atrioventricular septal defect	3/94	1. Nil 2. Echogenic bowel
Trisomy 18	1/74	Choroid plexus cyst	1/85	Nil	1/94	Choroid plexus cyst
Trisomy 13	0/74		0/85	NA		NA
Other autosomal trisomy	0/74	NA	0/85	0/85	0/94	NA
Sex chromosome aneuploidy	0/74	NA	1/85	Nil XXY Chromosome	0/94	NA
Unbalanced rearrangement	0/74	NA	0/85	NA	1/94	Unbalanced translocation of chromosomes 7 and 12
Total	2/74		0/85		5/94	

Table 2: Complications of invasive tests

Complication	*Amniocentesis (237)*	*CVS (26)*	*Total invasive test (253)*
Pain	4	3	7
Syncope	0	0	0
Leaking per vaginum	0	0	0
Chorioamnionitis	0	0	0
Miscarriage (<2 weeks after procedure)	1	0	1

and specificity for aneuploidy screening, the rate of invasive testing can be brought down. A major change is anticipated in the future due to the widespread use of cffDNA-based NIPT for aneuploidy screening, with experience with CMA interpretation and compilation of copy number variant phenotype databases.[13] However, the cost of NIPT is a constraint to apply it as a screening tool. These small data on the complication of invasive testing also add to the safety profile of invasive testing in the hands of an experienced operator incommensurate of a recent meta-analysis by Akolekar et al.[14] *Further ACOG guidelines for screening for fetal chromosomal abnormalities 2020 emphasize that* prenatal genetic screening (serum screening with or without NT ultrasound or cffDNA screening) and diagnostic testing (CVS or amniocentesis) options should be discussed and offered to all pregnant women regardless of maternal age or risk of chromosomal abnormality. After review and discussion, every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing. And if screening is accepted, patients should have one prenatal screening approach, and should not have multiple screening tests performed simultaneously.[15]

The major strength of the study is the pan India population, completeness of data, data of all invasive testing over the past 3 years, and their follow-up. These results emphatically demonstrate the impact of screening strategies on the indication of invasive testing, rate of invasive testing, and diagnostic yield in the last 3 years. This study also confirms low complication rates of invasive testing. Only standard karyotype was taken into consideration and a report of CMA was not put into data analysis was a limitation of our study. Certain data were not complete due to privacy reasons and lack of timely data collection showing the importance of data collection as an important and most important tool for any scientific study. The data of NIPT were also excluded from this data set. The addition of CMA report data and data of NIPT would have thrown further light on changing scenarios of prenatal screening and diagnosis.

Conclusion

This study clearly illustrates the role of information, education, and counseling in increased uptake of prenatal serum screening for aneuploidy. The diagnostic yield of invasive testing also increased due to the increase proportion of pregnant women undergoing aneuploidy screening. Also, the safety profile of invasive testing was further strengthened. Population-based aneuploidy screening, USG detection of anomalies, invasive testing outcome, and follow-up is vital cog of any prenatal screening program.

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