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Original Research Article

Evaluation of Invasive Prenatal Test Indications and Outcomes at a Tertiary Care Center: Experience from Western India

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Abstract

Background: Prevention of genetic disorders by prenatal detection is now standard antenatal care. We present retrospective analysis of indications and results of invasive prenatal procedures amniocentesis and chorionic villous sampling (CVS) in 284 patients at tertiary care teaching university hospital. **Methods:** Maternal age and indication were obtained. Gestation age for amniocentesis was 16 weeks onward and for CVS was 11-14 weeks. Results were analyzed with respect to indications. **Result:** Out of total 284 invasive procedures, 60.91% were amniocentesis and 39.08% were CVS. Total 50.70% of fetuses were tested for chromosomal anomaly. Most frequent indication was abnormal serum screen test (54.86% of cases). Among remaining 48.94% of cases, procedures were done with indication of family history of single gene disorder. Sample which was tested for chromosomal anomaly 4.35% of fetuses found affected. Most frequent chromosomal anomaly was Down syndrome (33% of cases). On the contrary, samples which were tested for single gene disorder 19.42% fetuses found affected. Most frequent indication was previous child with thalassemia major. **Conclusion:** For detection of Down syndrome, most predictable indication from present study is abnormal serum screen test combined with soft marker in USG. USG detectable major malformation has highest yield in detection of affected fetuses but it needs a syndromic approach like testing for single gene disorder if standard microarray test comes normal.

Key words: Amniocentesis, Chorionic villous sampling,Chromosomal anomaly, Congenital malformation, Prenatal invasive procedure, Single gene disorder

Introduction

Every pregnant woman wants a physically and mentally healthy baby. In the modern medicine era, various noninvasive and invasive diagnostic procedures are

developed to screen the health of unborn baby. Noninvasive procedures like ultrasound or serum screening tests have their own limitation. These are only screening tools. They cannot detect genetic constitution of the baby. Genetic diagnosis helps in genetic counseling and assessment of risk of recurrence of the fetal condition. For this necessity, invasive diagnostic procedures are important part of high risk obstetric care. At first, amniocentesis was performed in 1956 to determine the sex of fetus in utero.[1] Later, in 1966 chromosomal analysis was done from amniotic fluid and abnormal karyotype was given as the first prenatal diagnosis.[2]

Early amniocentesis was done at 11-14 weeks. It had many limitations in terms that amniotic fluid can be withdrawn in a small amount, associated with more complication like leaking from the site of insertion, congenital telepesequinovarus etc.[3] Late amniocentesis is done from 16 weeks onward. This is a safe procedure and adequate amniotic fluid can be withdrawn but the results are late upto 17-20 weeks. So in 1970s chorionic villous sampling(CVS) was developed to get fetal cells. It can be done as early as 11 weeks.[4]

Indications of both procedures are similar. These procedures can be done for fetal karyotype, single gene disorders, enzymes assays, various biochemical tests, fetal infections etc.[5] However, there is preference of one test over the other. In cases where there is family history of genetic disorderand patient has abnormal sonography finding in first trimester, CVS is the test of choice.

We have performed a retrospective analysis in a tertiary care teaching hospital with aim to find out indications and outcome of invasive prenatal procedures.

Material and Methods

This is a retrospective descriptive analytical study conducted on pregnant women those underwent into CVS and amniocentesis, in Medical Genetics department in a tertiary care teaching university hospitalbetween time period from June 2014 to May 2020. The study was approved by institutional ethical committee (IEC).

Patient's Period of Gestation was calculated using her Last Menstrual Period and if menstrual cycles were irregular early dating scan was used to confirm the period of gestation.

We counseled the patients and obtainedinformed written consent before doing CVS or amniocentesis procedure. The clinical details of the patient, age, obstetric history, gestational age, indications, result of the procedure and short term complications were recorded.

Both procedures were done via trans abdominal route.At our center, amniocentesis was done from 16 weeks onward and CVS between 11 to 13 weeks of gestation as day care procedures.

Technique of procedures

Before performing the procedures, first step was to localize the placenta, site of sampling and examination of fetus for viability and any major malformation. Abdomen was prepared with povidone iodine (10%) and draped. Probe was covered with a sterile glove.

Amniocentesis:

No anesthesiawas given during intervention. Twenty gauge spinal needle was used for procedure. Amniotic fluid was aspirated with a 2 ml and two 10 ml syringes applying negative pressure. Initial 0.5-1ml amniotic fluid withdrawn by 2 ml syringe was discarded. Fifteen to 20 ml of amniotic fluid sample was taken. USG scan was done immediately after the procedure to ensure the viability of fetus to mother. During the procedure, we paid attention not to involve a fetal part or cord in fluid pouch. Vertical transplacental passage was used when needed to do so.

CVS: Local anesthesia was given before performing the procedure. About 1 ml of 0.2% lignocaine wasinfiltratedlocally into the abdominal wall at the site of entry. Under USG guidance, 18 G long Spinal Needle was inserted into the abdominal wall, seen traversing through the uterine wall into long axis of placental tissue. With the needle in place, a 20cc syringe containing tissue medium or normal saline was attached to the needle to aspirate the villi. Negative pressure was generated in the syringe and 5-7 to and fro movements were made in the placenta which caused some of the villi to come into medium. Then, medium containing villi was transferred into sterile falcon tube. Tube was examined for finger like white colour villi tissue and sent to the laboratory. After the procedure, USG scan was done to check fetal viability and look for any subchorionichaemorrhage.

Post procedure, patients were allowed to go home after 2 hours of the procedure. Prophylactic antibiotic was given. Rh prophylaxis with Anti D IG was given following each procedure in Rh negative mother. All patients were asked for follow up in case of vaginal bleeding, leaking or miscarriage.

Results

The present study includes total 284 invasive procedures. Out of the 284 procedures, 173 were amniocentesis and 111 were CVS. The mean maternal age was 30.18±5.03years in the amniocentesis and 28.86±5.15 years in CVS group.

Most of the amniocentesis (82.08%) were carried out between 15-20 weeks of gestation age. Eighty nine (80.18%) CVSwere performed between12 and 13 weeks of gestation age. However 17% of amniocentesis and 19.8 % of CVS were done at >20 weeks and between 14-15 weeks of gestation age respectively due to late visit of pregnant women..

We classified the cases according to indications for which procedures were opted (table 1). Total 9 categories were made. Maximum indications for amniocentesis were for abnormal serum marker screening (83/173). Other common indications for amniocentesis were abnormal USG finding (17.92%) and previous child with chromosomal disorder (14.45%). Most of the CVS procedures were conducted in cases with previous family history of single gene disorders (107/111). Most common single gene disorder indication was thalassemia major (59/111). Other single gene disorder indicationswere duchenne muscular dystrophy (DMD) (8.10%), spinal muscular atrophy (SMA)(4.50%) and miscellaneous rare single gene disorder which included GM1 gangliosidosis, osteoporosis pseudoglioma syndrome, MECP2 gene mutation, criglernajjar syndrome etc. (30.63%).

Results of these procedures were tabulated into table 2. Out of 144 fetuses those were tested for chromosomal abnormalities,6were positive for chromosomal anomalies. Other threeshowed benign variation in chromosomal test. Twoout of these 3 fetuses showed robertsonian translocation and one had polymorphism.

In pregnancies with family history of thalassemia major, 17 (24.64%) cases came out to be affected. Only 1 (7.14%) fetus turned out to be affected in 14 pregnancies with family history DMD.

On the comparison of the invasive procedureindications, “USG detected major malformation” and “soft marker with abnormal serum screen” showed highest positive predictive value of 30% and 14.29%respectively for detection of affected fetuses(table 3).Indication of isolated “abnormal serum screen” showed lowest specificity and diagnostic accuracy of 68.8% and 60.92% for detection of chromosomally abnormal fetuses. In the present study, highest odds ratio (4.75; 95% CI: 1.99-11.32) for detection of affected fetuses was obtained when

indication was family history of single gene disorder. This association was statistically significant (p = 0.0004)(table 4).

Table 1: Distribution of indication for amniocentesis and chorionic villous sampling

S. No.	Indication	Amniocentesis (n= 173)	Chorionic villous sampling (n= 111)	Total case (n= 284)
1.	Abnormal serum markers			
	a)Double marker test positive	19	1	20
	b) Triple marker test positive	7	0	7
	C) Quadruple marker test positive	50	0	50
	Borderline NIPT test	2	0	2
2	Soft marker + screen test positive	6	1	7
3	Abnormal USG findings	32	1	33
4	History of previous child with chromosomal disorder	23	0	23
5	Previous child with thalassemia major /parents thalassemia trait	10	59	69
6	Previous child with DMD	5	9	14
7	Previous child with SMA	2	7	9
8	History of previous child with single gene disorder (miscellaneous)	12	35	47
9	Mother had chicken pox at 11 weeks /raised AFP	3	0	3

Table 2: Results of antenatal invasive procedures in term of two broad categories: chromosomal and single gene disorder

S. No.	Indications	Outcome of amniocentesis		Outcome of chorionic villous sampling		Affected/ total
		Affected	Normal	Affected	Normal	
1	Chromosomal disorders	6	134	0	4	6/144
2	Thalassemia	3	7	14	45	17/69
3	DMD	0	4	1	9	1/14
4	SMA	1	1	0	7	1/9
5	Miscellaneous Single gene disorders	3	9	5	30	9/47

DMD-duchenne muscular dystrophy, SMA- spinal muscular atrophy

Table 3: Pretest probability of antenatal invasive procedure indications

	Sensitivity	Specificity	PPV (%)	NPV (%)	Negative LR (95% CI)	Positive LR (95% CI)	Diagnostic accuracy (%)
Two Soft markers in USG	2.9	92.8	5.26	87.55	1.05 (0.98-1.12)	0.41 (0.06-2.96)	82.04
Soft marker + abnormal serum screen	2.9	97.6	14.29	88.09	0.99 (0.93-1.06)	1.23 (0.15-9.87)	86.27
Nonimmunehydrops	-	98.4	-	87.86	1.02 (1.00-1.03)	-	86.62
USG detected major Congenital	8.8	97.2	30	88.56	0.94 (0.84-1.04)	3.11 (0.85-	86.48

malformation						11.47)	
Screen test positive	2.9	68.8	1.27	83.9	1.49 (1.27-1.56)	0.09 (0.01-0.66)	60.92
Previous child with single gene disorder	79.4	55.2	19.42	95.17	0.37 (0.19-0.73)	1.77 (1.42-2.21)	58.10
Previous child with chromosomal disorder	2.9	91.2	4.35	87.36	1.06 (0.99-1.14)	.33 (0.05-2.4)	80.63

Table 4: Odds ratios of antenatal invasive procedure indications

	Total (n)	Affected fetuses (+)	Unaffected fetuses (+)	P	ODDS (95% CI)
Two Soft markers in USG	19	1	18	0.37	0.39 (0.05-3.02)
Soft marker + abnormal serum screen	7	1	6	0.849	1.23 (0.14-10.56)
Nonimmunehydrops	4	0	4	0.878	0.79 (0.04-15.07)
USG detected major Congenital malformation	10	3	7	0.091	3.36 (0.83-13.67)
Screen test positive	79	1	78	0.0082	0.07 (0.01-0.49)
Previous child with single gene disorder	139	27	112	0.0004	4.75 (1.99-11.32)
Previous child with chromosomal disorder	23	1	22	0.27	0.31 (0.04-2.41)

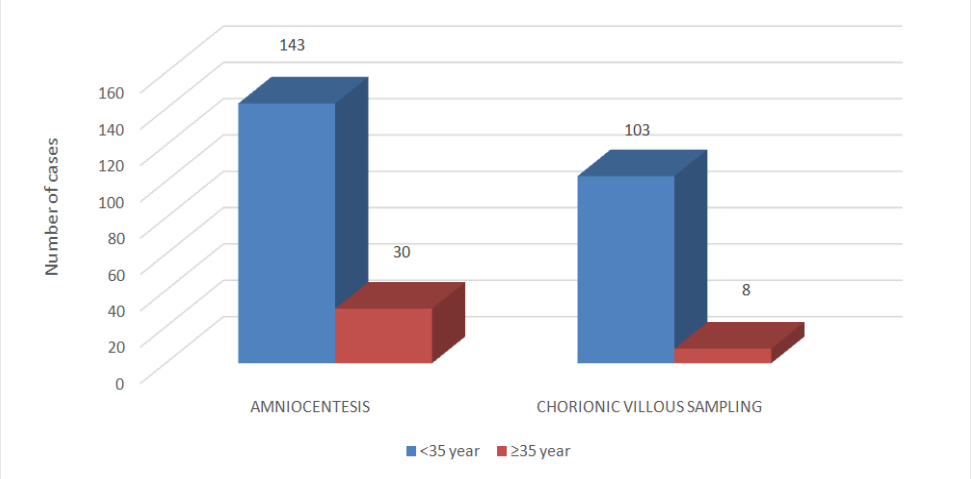

Figure 1: Distribution of cases according to the maternal age

Discussion

This is a retrospective analysis of various indications of prenatal invasive diagnostic procedures and results in terms of affected fetuses. Out of total 284 invasive procedures 60.91% were amniocentesis and 39.08% were CVS. Out of 144 samples which were evaluated for chromosomal abnormality, 4.17% fetuses found affected and it is comparable to various studies in literature which showed detection rate of 3.3% to 4.98%.[6-8]. In

139 samples for single gene disorder, 19.42% fetuses turn out to be affected.

Mean age of mother in present study was less than 35 year. 82% of amniocentesis and 92% of CVS were done before the maternal age of 35 year.[9] This was in contrast toValayatham et al study, whichshowed maximum cases between age group 35-39 years.[10] Major indications were advanced maternal age and fetal anomalies. These findings may be skewed because of the difference in indications and age structure of women population those underwent various noninvasive screening tests. If indication of procedure is based on previous family history of genetic disorders, relatively younger age women need invasive testing to prevent recurrence of the disorder. In the present study, 32.4% amniocentesis and 97.3% CVS were done because of previous family history of chromosomal and single gene disorder. Second reason may be younger age of marriage and child bearing in Indian women.

In Maaitaet al study, advanced maternal age was a major indication for invasive prenatal testing in the initial years of the study.[11] With the availability of high resolution sonography and maternal serum marker test, this indication showed decreasing trend. In the present study, no procedure was done with this indication. Serum screen positivity and abnormal USG finding has replaced the advanced maternal age as an indication for Down syndrome testing. [9,10]

In the present study,50.70% of fetuses were tested for chromosomal anomaly. Among them,most frequent indication was abnormal serum screen test (54.86% cases). The large series of 2185 and 4953 prenatal invasive procedures by Özcan et al and Die et al showed abnormal maternal serum screen as maximum indications in 58% and 50% cases respectively.[12,13] Their results are comparable to the present study.

Serum marker study is an important cost effective noninvasive screening test for prediction of chromosomal abnormality in fetus specially trisomy 21. Dual marker test has a detection rate of 70%. When it is combined with NT, detection rate increases to 85-95% with a false positive rate of 5%.[14,15,16]Associated sonography findings including both soft markers and major malformation has better detection rate for chromosomal abnormality than isolated serum screen test.[17] This fact is further strengthened in the current study, when we compared the indication with results. Isolated abnormal serum screen showed lowest odds ratio0.07(0.01-0.49) with only 1.3% fetuses turned out to be affected. When abnormal serum screen test combined with soft marker in USG, odds ratio increased to 1.23 (0.14-10.56).

In the present study,most frequent chromosomal abnormality was trisomy 21 (33%), followed by trisomy 18 and monosomy X.[18] Indication for invasive testing in cases of trisomy 21 were soft markers in USG and abnormal serum screen test. In cases of trisomy 18 and monosomy X, indications were USG detected major malformation, congenital heart disease and cystic hygroma respectively. Prenatal detection of trisomy 21 is always a challenge. Major USG detectable malformation like congenital heart disease and duodenal atresia are present in only 50% and 3% of cases respectively. In rest of the cases, detection relies on the basis ofsoft markers on high resolution USG and screen tests in contrary to other chromosomal abnormalities where most of the fetuses have USG detectable major malformations. In Bromley et al study, 33% of down syndrome were detected by using second trimester scans, of which only 36% had congenital malformation. Cardiac defect was the most common malformation. On the contrary, soft markers were present in all cases. Thick nuchal fold was the most common soft

marker.Soft markers when present in cluster or associated with major malformation are important predictor of abnormal fetuses specially trisomy 21. Detection rate is low for isolated soft marker without abnormal serum screen and structural malformation.[19,20,21]

Cihan et al and Saatçi et al showed a detection rate of 8.88% and 10.84% of chromosomal abnormal fetus respectively when the indication was USG detected major malformation.[8,9] In contrast,detection rate was as high as 30% with similar indication in the present study. Etiology of USG detected major malformation depends on the type and number of major malformations and type of used genetic test for diagnosis.[22]In cases of multiple malformation, one need to make syndromic association of malformations. For example, in one of the case from present study previous fetus was terminated because of cleft lip/palate without genetic testing. In present pregnancy, there was recurrence of cleft lip/palate, microarray and whole exome sequencing were done. Microarray test turned out to be normal but pathogenic variations were found in MASP1 gene(3MC syndrome-1) in whole exome sequencing.This may explain high yield of affected fetuses in the present study.

Family history of single gene disorder has better odds ratio for detection of truly affected fetuses than family history of chromosomal abnormality. Reason for low diagnostic yield in cases of family history of chromosomal abnormality isdenovo (95%) chromosomal abnormality in affected fetuses. Only 3-4%of the chromosomal abnormalities are inherited from parents. Even inherited, maximum risk of recurrence is 10-15% due to abnormal segregation of chromosome and abnormal gamete formation leading to recurrent spontaneous abortion and intrauterine death. Due to low recurrence of chromosomal disorder,with pretest counseling ,97.22% of cases at risk of chromosomal abnormality, the chosen invasive test was amniocentesis. Because second trimester amniocentesis has lower complications and maternal pain consequently posttest anxiety in comparison to CVS. This low recurrence was explained by results from the present study, only 4.35% of fetuses found affected.

In contrast, risk of recurrence is 25%in single gene disorder. The chosen invasive test was CVS in 76.97% of fetuses at risk of single gene disorder. Early diagnosis relieves parents anxiety. Family history based indications sensitize parents in preconception period for early testing. This high recurrence reflected in the current study where 19.42% fetuses found affected.

Major limitation of the present study is small sample size. Other limitations aresingle center study and its retrospective nature. Single center study may cause case selection bias

Conclusion:

Both isolated serum screen test positivity(PPV-1.27%) and isolated two soft markers in USG(PPV-5.55%) have lower predictive value for truly affected DS fetuses compared to combined soft marker with screen test positivity(PPV-14.29%). Prenatal diagnosis of major malformations in USG always needs a syndromic approach to test for both chromosomal and single gene disorder to get a higher diagnostic yield.Invasive prenatal testing proves beneficial for the couple for early detection of genetic abnormality. It also helps for counseling about risk of recurrence of disorder and cause of sonographic abnormality.

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