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Original Research Article

Invasive genetic testing in pregnancy: experience from a tertiary care center

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ABSTRACT

Background: Evaluation of outcome of pregnancies with high risk first trimester screening or abnormal ultrasonographic findings and complications of amniocentesis in second trimester and chorionic villous sampling in first trimester in a tertiary care hospital in India.

Methods: This is a retrospective study in a tertiary care hospital from 2015 to 2017. All antenatal patients underwent combined nuchal translucency scan and dual screen ratio and who detected to have high risk for trisomy, they underwent amniocentesis/ CVS. These procedures were done at minor OT in our hospital, taking aseptic precaution and under ultrasound guidance. First trimester combined screening included a detail nuchal translucency scan as per the international society of ultrasound in obstetrics and gynecology (ISUOG) guidelines and also biochemical screening done with serum beta human chorionic gonadotrophin (HCG) and pregnancy-associated plasma protein A (PAPP-A) in same genetic lab. Combined risk scoring done as high risk or low risk. Low risk patients without any genetic abnormality in previous obstetric history were followed as normal pregnancy. Results, complications and outcome of invasive genetic testing in pregnancy was observed. At our center CVS was done mostly for single gene disorder.

Results: Out of 179 patients who underwent amniocentesis, total abnormal chromosomal were 14 (7.82%). The most common abnormality was trisomy 21 (4.46%). The other abnormalities were trisomy 18 (1.67%), trisomy 13 (1.11%) and triploidy XXY (0.55%). CVS was done for Nieman Pick disease, androgen insensitivity syndrome, both parents thalassemia minor and ultrasound abnormality detected early in pregnancy. For single gene disorder mutation identified in index case or in parents and same mutation looked in to fetus by chorionic villus sampling (CVS). For Nieman Pick disease, androgen insensitivity syndrome and both parent thalassemia minor, fetus detected to have heterozygous for same and nonpathogenic. Two patients underwent CVS for ultrasound abnormality out of which one detected to have trisomy 18 and other had loss of 2.1 Mb on Ch 22 in 22q11.21 region.

Conclusions: Combined first trimester screening with nuchal translucency scan and dual screen ratio is an efficient method of screening with high sensitivity and low false positive rates. In our study prevalence of trisomy is slightly greater than other studies because number of patients were less and if we increase the number of patients probably we will have a prevalence data of trisomy similar to other studies which has been done for aneuploidy in fetus.

Keywords: Amniocentesis, Chorionic villous sampling, Trisomy

INTRODUCTION

Amniocentesis and chorionic villous sampling are invasive procedure done during pregnancy and amniocentesis is

most commonly performed invasive test. In amniocentesis 20 or 22 gauge spinal needle introduced in amniotic cavity guided by ultrasound. Chorionic villous sampling commonly performed between 11 to 13 weeks by using 18

gauge spinal needle in which placental villi obtained from placenta.

There are two layers outer chorion and inner amnion which surrounds fetus. Amniotic cavity filled with amniotic fluid is surrounded by amnion. Amnion can be seen as a thin line in pregnancy by ultrasound. As the period of gestation progresses the amnion completely obliterates the chorion, which is usually occurs by 12 to 14 weeks. Till the membranes are fused we can see a 3 mm membrane in cavity between the two layers involving 50% of the amniotic cavity.¹

Amniocentesis is usually done beyond 15 weeks. Attempting amniocentesis in early pregnancy causing tenting of amniotic membrane and higher chances of amniotic fluid leakage. If amniocentesis is done before fusion of membrane then there are high chances of failure to prick amniotic membrane and dry tap hence increasing number of pricks and complications.²

Indication of invasive genetic testing in pregnancy: increased risk of aneuploidy in first trimester combined screening, abnormal genetic sonography like even one major soft tissue marker or two minor soft tissue marker or previous history of aneuploidy in fetus or baby or history of balanced translocation in parents; increased risk for genetic diseases in family like any autosomal recessive disease with both parents carrier or x-linked recessive disease; maternal transmittable disease like TORCH infections; to assess fetal lung maturity in late gestation; and therapeutic amniocentesis or amnioinfusion in poly and oligohydramnios respectively.^{3,4}

There is no absolute contraindication of procedure in pregnancy. Relative contraindication of procedure are infections and patients are on oral anticoagulation. Oral anticoagulation should be stopped 48 to 72 hours before the procedure and patient may be shifted to low molecular weight heparin. The complications related to amniocentesis is as follows- the fetal loss rate associated with amniocentesis on an average is 0.11%, the loss is 0.56% within 28 days, 0.09% within 42 days; amniotic fluid leak upto 1% to 2%, and usually associated with spontaneous sealing of membranes; there is a 2% to 3% risk of vaginal bleeding; an estimated 2.6% risk of fetomaternal haemorrhage; there is minimal chance of introduction of skin bacteria into the amniotic cavity, the risk of chorioamnionitis and uterine infections is less than 0.1%; and the procedure increases the risk of preterm, preterm premature rupture of membrane, oligohydramnios.^{5,6}

There is a minimal chance of fetal injury, including ocular, cutaneous injuries. The risk of talipoequinovarus is higher with early amniocentesis and increases when there is amniotic fluid leakage.

The procedure is done under continuous ultrasound guidance. Apart from an ultrasonography machine, the

following equipment is required: sterile swabs and drapes, syringe 2 ml and 10 ml, needle 20 gauge to 22 gauge for amniocentesis and 18 gauge for CVS, containers for collection and sample transport, and 5% povidone-iodine solution for part preparation.

In this era of advanced medical technology, each parent has the right to have a healthy and chromosomally normal newborn. Although even if we have achieved the technology of invasive testing in pregnancy, not everywhere can this be facilitated. Therefore the concern council should develop genetic laboratories and genetic counselors, and train more doctors for these testing even in smaller cities so that patients can be aided.

METHODS

This is a retrospective study done at Command Hospital Chandigarh from July 2017 to June 2020.

Ethical approval has been taken from ethical committee of the hospital.

Patients included in this study were regular antenatal patients who were willing for testing and follow-up. The patients who were not convinced and were unwilling for further testing and follow-up were not included in this study.

Every antenatal patients attending outpatient department (OPD) at our hospital evaluated for any family history of genetic disorder or history of any affected previous child on first visit. We are following universal first trimester combined screening in form of dual screen and nuchal translucency nasal bone (NT NB) scan so each patient underwent dual marker screen ratio and detail NT NB scan between 11 to 13⁺⁶ weeks of period of gestation and later anomaly scan at 18 to 20 weeks period of gestation as per international society of ultrasound in obstetrics and gynecology (ISUOG) guideline. High risk patient identified and planned for amniocentesis or chorionic villus sampling (CVS). CVS was done mainly for single gene disorder. All patients who detected to have high risk in combined screening or having relevant structural abnormality they underwent amniocentesis.

Firstly, couples were counselled about the risk and abnormality and also for invasive prenatal testing and related complications with it. Written and informed consent were taken from patient before procedure.

Procedures were conducted in OPD after all prerequisites and samples were sent for genetic testing. Before each procedure patients were given injection hydroxyprogesterone caproate 500 mg and IV antibiotic single dose.

All procedure of amniocentesis was done after 15 weeks and preferably after 17 weeks to minimize complications. After confirming the prerequisites and once the

preparation is complete, the procedure is commenced. A 20 gauge or 22 gauge spinal needle has been introduced in the amniotic cavity under continuous ultrasound guidance. A firm entry into the amniotic cavity is recommended to prevent the tenting of the amniotic membrane. Once entry into the cavity is confirmed, amniotic fluid is slowly aspirated. The initial 1 ml to 2 ml of amniotic fluid is discarded because it has the highest chance of maternal cell contamination. Approximately 18 ml to 20 ml of amniotic fluid is required for karyotype testing. The needle is removed after adequate amniotic fluid has been obtained. Entry into the amniotic cavity through the placenta should generally be avoided because it increases the chances of bloody tap, especially in Rh-negative women. Apart from amniotic fluid 5 ml maternal blood also taken to rule out maternal cell contamination in both amniocentesis and CVS.

All CVS was done between 11 to 13 weeks by using 18 gauge spinal needle attached with 50 ml syringe through three way connector for making suction. Under aseptic condition and under ultrasound guidance, needle introduced in placenta and suction made by 50 ml syringe and placental villi obtained for genetic testing. This placental villi has been sent in culture media to genetic laboratory.

After the procedure (both in amniocentesis and CVS), fetal cardiac activity is confirmed. Post procedure injection anti-D given in women with Rh-negative pregnancy. All patients advised to take rest post-procedure and observed for two to three hours in ward before sending home.

RESULTS

A total of 179 amniocentesis were performed during the study period. The most common range of maternal age was 35-39 years old (40.78%) (Table 1). The most common range of gestational age performed amniocentesis between 16-18 weeks (46.92%) (Table 2). Most of the amniocentesis procedure was performed by trained fetal medicine specialist and few were done by general obstetrician also. The most common indication for amniocentesis was due to detection of high risk in combined screening which included dual marker screening and NT, NB scan as per ISUOG guideline (88.82%). The other indications were couple at risk of ultrasound abnormality in fetus (6.1%), advance maternal age (2.2%), previous trisomy child (1.1%) and balanced translocation in parents (0.55%) (Table 3).

The results of abnormal chromosome by amniocentesis were 14 cases (7.82%). The most common abnormality was trisomy 21 (4.46%). The other abnormalities were trisomy 18 (1.67%), trisomy 13 (1.11%), triploidy XXY (0.55%) (Table 4).

179 patients underwent amniocentesis and three patients who were having ultrasound abnormality detected in first trimester they underwent CVS for karyotyping of fetus so

total sample size became 182 (179 amniocentesis, 03 CVS). If we combine both amniocentesis and CVS then total abnormal chromosome were 16 (8.79%). The most common abnormality was trisomy 21 (4.39%), trisomy 18 (1.64%), trisomy 13 (1.09%), one triploidy XXY (0.54%), one loss of 2.1 Mb on ch 22in 22q11.21 region (0.54%) (Table 7).

Table 1: Maternal age.

Age (in years)	Number	Percent
20-24	13	7.26
25-29	38	21.23
30-34	42	23.46
35-39	73	40.78
40-44	12	6.70
>45	1	0.55
Total	179	

Table 2: Gestational age at time of amniocentesis.

Gestational age (weeks)	Number	Percent
14-15	32	17.87
16-18	84	46.92
19-21	13	7.26
22-24	8	4.46
>24	42	23.46

Table 3: Indication for amniocentesis.

Indication	Number	Percent
Advance maternal age	4	2.23
Previous child trisomy	2	1.11
Family history of trisomy	1	0.55
Previous child thalassemia major	1	0.55
High risk for combined screening (dual screen and NT NB scan)	159	88.82
Ultrasound abnormality	11	6.14
Balanced translocation carrier	1	0.55

Table 4: Result of chromosomal study.

Result	Number	Percent
Normal chromosome	165	92.18
Abnormal chromosome	14	7.82
Numerical abnormality		
Trisomy 21	08	4.46
Trisomy 18	03	1.67
Trisomy 13	02	1.11
Triploidy XXY	01	0.55

Out of 179 patients who underwent amniocentesis, none had developed fever, per vaginal bleeding and chorioamnionitis. Only one patient who came from far and while going back at home on same day she developed

leaking per vaginum and came back. She was under conservative management but she had spontaneous second trimester abortion after 48 hours. So, complication rate at our centre was 0.55% and which is less than 01%. None of

cases reported with fetal injury during procedure. All patients having chromosomally abnormal fetus underwent second trimester abortion at our centre after counselling of patients.

Table 5: Complication of amniocentesis.

Complication	Number	Duration from procedure	Outcome
Maternal blood contamination due to anterior placenta	02 (1.1%)		Culture could not be performed Result given by FISH
PPROM	01	48 hours	Spontaneous abortion
Fever	Nil	-	-
Chorioamnionitis	Nil	-	-
Fetal injury	Nil	-	-

Table 6: Indication result and complication of CVS done at our center.

S. no.	History	Age in years	Parents abnormality	Result	Outcome	Complication
1	Prev child had Nieman Pick disease and died	26	Both parents were heterozygous for NPC gene	Fetus was heterozygous for NPC gene	Healthy male baby	Nil
2	Prev two child had androgen insensitivity syndrome	28	Both parents were heterozygous for AR gene	Fetus was heterozygous for AR gene	Healthy male baby	Nil
3	Prev child thal major	24	-	Mutation not identified in this fetus	Healthy female baby	Nil
4	Semilobar holoprosencephaly with single bone rt forearm in fetus on ultrasonography	36	-	Trisomy 18	Second trimester abortion done	Nil
5	Pericardial effusion, pleural effusion, ascites, cardiomegaly	38	-	Loss of 2.1 Mb on ch 22in 22q11.21 region	Second trimester abortion done	Nil
6	Non immune hydrops, NT 7.9 mm, hypoplastic heart	35	-	Trisomy 18	Second trimester abortion done	Nil

Table 7: Result.

Amniocentesis (179)	CVS for abnormality of ultrasound(3)	CVs for other causes (3)	Complication of amniocentesis	Complication of CVS
a-Trisomy 21-08	a-Trisomy 18-01	a-Done for Neiman Pick disease-01	a-PPROM and spontaneous abortion-01	Nil
b-Trisomy 18-03	b-Loss of 2.1 Mb on ch 22in 22q11.21 region-01	b-Done for Androgen insensitivity syndrome-01	b-Blood stained amniotic fluid-01	
c-Trisomy 13-02		c-Done for both parents Thalassaemia-02		
d-Triploidy XXY-01				

Total chromosomal abnormality-16 (8.79%) out of which trisomy 21-08 (4.39%), Trisomy 18-03 (1.64%), trisomy 13-02 (1.09%), XXY-01 (0.54%), loss of 2.1 Mb on ch 22in 22q11.21 region-01-01 (0.54%); sample size included 179 amniocentesis and 06 CVS (which was done for abnormality in USG and for single gene disorder), total 185, total abnormal chromosome-16.

Indication of CVS are illustrated in Table 6. One patients had history of Nieman Pick disease in previous child who died at age of 3 years. Both partner underwent whole exome sequencing and detected to have heterozygous for

Niemann-Pick disease type C (NPC) gene and fetus also turned out heterozygous and she delivered a healthy male baby.

Second case of CVS, they had a history of androgen insensitivity syndrome in previous two child and had three consecutive induced abortion due to fear of having same. Both partner underwent whole exome sequencing and detected to have heterozygous for AR gene. After chorionic villous sampling fetus also detected to have heterozygous for AR gene and she delivered a healthy male baby at term.

One patient had history of Thal major in previous child and she underwent CVS due to same but fetus was not Thal major.

Three more patients were having ultrasound abnormality and underwent CVS and two of them detected to have trisomy 18 in fetus and one detected to have 22q 11.21 del in fetus and all three patient underwent termination of pregnancy after counselling.

Out of six patients who underwent chorionic villous sampling none of them had any procedure related complications.

DISCUSSION

At our center most of the amniocentesis done for abnormal screening results and which is similar to studies done in other tertiary hospital in Thailand.⁷⁻¹³

The most common gestational age at which amniocentesis was performed was 16-18 weeks to reduce early amniocentesis complication. The prevalence of chromosomal abnormality was 8.79% and prevalence of trisomy 21 was 4.39% which is little higher than other studies done in Thailand.⁷⁻¹³ Higher detection of prevalence of chromosomal abnormality in fetus may be because of low sample size and if we increase the sample size probably we will have a reduced number of prevalence of trisomy.

Out of 179 patients who underwent amniocentesis only one patient had preterm premature rupture of the membranes (PPROM) same day after procedure and spontaneously aborted after 48 hours. So fetal loss was 0.55% and which is more than studies done by Odibo et al in 2008 and Hamprasertpong et al in 2011 were 0.12-0.13%.^{14,15}

Only one patient of amniocentesis had maternal blood contamination in sample due to anterior placenta so culture could not be done and results were obtained by FISH.

Three chorionic villous sampling was done for Nieman Pick disease, thalassemia major, and androgen insensitivity syndrome. Another three CVS was done because of ultrasound abnormality and all three had chromosomal abnormality and underwent termination of pregnancy after counselling. No procedure related complication observed after CVS.

Many hospital in periphery are not doing first trimester screening for each pregnant ladies and sometimes patient goes back with chromosomally abnormal fetus. First trimester combined screening should be provided universally to each and every patients. Doctors should be trained for invasive procedures so that they can provide services at a secondary hospital level also.

Invasive procedure should also be conducted at smaller hospitals for patient benefit. Only invasive diagnostic procedures remains essential to complete genetic diagnosis. Both procedure are relatively safe in experienced hand. Sensitivity of detection with either invasive test is near 100%. Safest invasive procedure is mid trimester amniocentesis while early amniocentesis and CVS are associated with a higher risk of subsequent pregnancy loss. Patient counselling should include an evaluation of the risk associated with each individual procedure.

Being a tertiary hospital these procedures were established for many years and now being conducted by fetal medicine trained doctors. The number of invasive procedure are increasing every year due to awareness of screening and increase in number of ART patients. Most of these procedures are being done at a higher center because of non-availability of resources and genetic lab in secondary centers. However these procedures can be performed at secondary centers with establishing well logistic there for patient benefit otherwise patients have to refer a higher center for small procedures.

CONCLUSION

Combined first trimester screening with nuchal translucency scan and dual screen ratio is an efficient method of screening with high sensitivity and low false positive rates. In our study prevalence of trisomy is slightly greater than other studies because number of patients were less and if we increase the number of patients probably we will have a prevalence data of trisomy similar to other studies which has been done for aneuploidy in fetus. So each and every patient should undergo first trimester combined screening and whoever detected to have high risk should undergo invasive testing. More and more people should be trained for invasive testing, and establishment of genetic laboratories with genetic counsellors. Out of 179 amniocentesis and 6 CVS we picked up 16 chromosomally abnormal fetus.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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