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Study and Importance of Genetic Amniocentesis in Prenatal Diagnosis for High Risk Pregnancies

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Abstract

This study presents chromosomal pattern of 1177 high risk pregnancies referred for amniocentesis. No growth was observed in 12(1.01%) cases. Out of 1165 cases, abnormalities were observed in 85(7.29%) cases. Out of total 85 abnormalities numerical abnormalities were presented in 43 (50.59 %%) cases including, trisomy 21 [34(40%)] trisomy 18 [4(4.70%)] monosomy of one of the sex chromosome and triploidy in one-one (1.18%) case each, and trisomy of sex chromosomes in 3(3.53%) cases. Structural abnormalities were observed in 41(48.23%) cases. The distribution of structural abnormalities includes 9(10.59%) translocations, 20(23.53%) inversions of autosomal chromosomes, 8(9.41%) inversions of one of the sex chromosomes, deletions and duplications in one-one (1.18%)case each and 3(3.52%) cases with derivatives. If the parental karyotype is available at the time fetal karyo the counseling and decision making about termination or continuation of pregnancy may become easier.

Keywords: High risk pregnancies, Chromosomal abnormalities, Prenatal diagnosis.

Introduction

Conception to death, the role of gene has been illuminate by the rapid development in the field of genetics. This is possible due to new discoveries and advancement in genetics with new technologies[1]. Genetic disorders being complex and cause multisystem involvement of the affected individual as well as the family in terms of financial, emotional and social pressure. Genetic disorders also puts burden on the health service of every country[2]. Now it is possible to take the advantage of genetics diagnostic testing which can identify individuals and families at risk through various carriers testing programs[3]. Frequency of chromosomal disorder is frequent. Globally 7.6 million children are born with severe genetic or congenital malformation[4]. Further it is reported that 21,000 babies are born with Down's syndrome, the most common syndrome, every year in India[4]. It is often thought that cytogenetic studies are end studies with no possible treatment[4,5]. Though this is a fact, cytogenetic studies can be successfully used in management of the affected, recurrence risk estimation and in offering various reproductive options to couple[3]. The cell culture from the amniotic fluid was first analyzed by Steel and Berg in 1966[6]. Amniocentesis in prenatal diagnosis is the gold standard method to detect chromosomal abnormalities[7]. Because of it high reliability and safety record it is used in many country as a routine invasive procedure. Though the abortion risk for amniocentesis is 0.5 to 1 % it has the record of lowest fetal loss and embryonic damage as a invasive procedure[8,9].

Material and Methods

The study carried out at Centre for Genetic Health Care (CGHC), Mumbai. Inclusion criteria's for the patients in the study were pregnant women with advanced maternal age, positive maternal serum screening, abnormal ultrasound findings, increased Nuchal translucency, previous affected child, carrier parents etc., to rule out chromosomal abnormalities. Amniotic fluid samples were collected from pregnant women having high risk factors. Cultures were set using the routine amniotic fluid culture procedures at CGHC. Briefly. Dispense collected amniotic fluid sample in two sterile centrifuge tubes. Centrifuge tubes at 800-1000 rpm for 10 minutes. In the laminar flow hood prepare the flask and label it with patient name, number, and date. After centrifugation remove the supernatant, leave 0.5ml of fluid over the pellet. Tap gently with fingers, break up the pellet and add 4 ml of culture medium (Bio AMF-2). Remove the medium with cells by sterile pipette and transfer in the flask. Transfer flask to the CO_2 incubator, loosen the cap and leave it undisturbed at 37^0C with CO_2 supply of 5% for 7-8 days. After 7 or 8 days examine the flask under inverted microscope for colony growth. If sufficient growth of colony is observed, feed the flask with 4ml of fresh medium. After 24 hrs, flask is ready for harvesting. In harvesting the first step is to add 0.1 ml of colchicines to the flask 1 hour before harvesting. Remove the medium containing colchicines from the flask and place in the labeled centrifuge tube. Add 2 ml of Trypsin-EDTA solution to the flask and leave it in incubator at 37^oC for 3 minutes. When the cells are detached, with glass pipette aspire gently and transfer the solution to the centrifuge tube. Centrifuge at 800-1000 rpm for 8 minutes. Decant the supernatant and add 5ml of hypotonic solution, mix well, cover the tube tightly and incubate in the water bath at 37^oC for 12 to 15 minutes. After 12-15 minutes

add 6-8 drops of fixative and mix gently. Centrifuge the tube at 800 rpm for 10 minutes. Decant supernatant, add fresh fixative 5-6 ml and mix well. Repeat step no. 8 and 9 for two washes. Decent supernatant, add 0.5 ml of fresh fixative, mix well. Drop 2-3 drops of the cell suspension on the slide. The droplets should burst and spread evenly on the slide. Drain off the remaining fixative. The back of the slide and label carefully. Age the slides in incubator at 60^{o}C to 70^{o}C overnight. The slides are ready for banding. Slides were analysed at 450 band level and nomenclature as per ISCN[10].

Results and Discussion

Total 1177 cases were enrolled in the study with the high risk factors such as maternal age, positive maternal serum screening, abnormal USG ect. Patients referred for amniocentesis, maternal age was the most common and highest risk factor. Amniocentesis was performed between average gestations age of 16–18 weeks. Out of total 1177 cases, there was no growth in 12 (1.01%) cases. Remaining 1165 cases presented normal karyotype in 1081(92.70%) patients with Chromosomal abnormalities were observed in total 85(7.29%) cases. Out of total 85 abnormalities, 43 (50.59%) cases presented numerical and 41 (48.23%) cases presented structural chromosomal abnormalities. Fetuses carrying numerical abnormalities showed following distributions, 34(40 %) cases of trisomy 21 i.e. Down's syndrome, the most common and highest finding, 4 cases of trisomy 18 (4.70 %) i.e. Edward,s syndrome, 1(1.18%) cases of monosomy of the sex chromosomes i.e. Turner syndrome, one (1.18%) case of triploidy and 3(3.52%) cases of trisomy of the sex chromosomes observed. The chromosomal aberrations of numerical and structural abnormalities are mentioned in the Table-1.

The distribution of structural abnormalities observed are as follows, 9(10.59%) cases with translocation, 20(23.53%) cases with inversion of autosomal chromosomes, 8(9.41%) cases with inversion of one of the sex chromosomes, one(1.18%) case with deletion, one (1.18%)case with duplication and 3(3.52%) cases with derivatives. The distribution of total numerical and structural chromosomal abnormalities in total abnormal cases are presented in the following Figure-1. From the graph it is concluded that trisomy 21 is the highest (40%) numerical chromosomal abnormality observed followed by inversion and translocation. Duplication, deletion monosomy and triploidy are least common (1.18%) abnormalities observed in the study. Total 14 cases were observed in which the parental karyotype was abnormal.

Table-1
Chromosome abnormalities in the parental cases

Type of abnormaities	**Total number of cases**	**Number of cases with chromosomal aberration observed in the study**
Trisomy 21	34	47**,+21 (32), 47,**, 9qh+, +21 (1), 47,**,t(4;6)(q31.3;q25)+21 (1)
Trisomy 18	4	47,**,+18 (4)
Monosomy	1	45,* (1)
Anuploidy	1	69,*** (1)
Trisomy of one of the sex chromosome	3	47,*** (3)
Translocations	9	46,**,t(2;6)(p23;q25),t(10;13)(q11.2;12.3)(1) 46,**,t(5;17))(p13.1;p13)(1), 46,**,t(7;8)(q11.23;p21.3)(1) 46,**,t(8;15)(p10;q10)(1) 46,**,t(9;21)(p13;q22)(1) 46,**,t(2;16)(p23;q13)(1) 46,**,t(8;13)(p21.2;q31) 46,**t(2;17)(p27;p11.2)(1) 46,**t(4;5)(q31.3;q35)(1)
Inv. Of the autosomal chromosomes	20	46,**,inv.(9)(p11;q12)(16) 46,**,inv.(1)(p11;q12(1) 46,**,inv(5)(p11;q11.2)(1) 46,**,inv(5)(p15.3;q13)(1) 46,**,inv(8)(q11.2;q13)(1)
Inv. Of the one of the sex chromosome	8	46,**,inv(*)(8)
Deletion	1	46,**,del(18)(q21.2,q21.2)(1)
Duplication	1	46,**,psu dup(9)(q10;q12)(1)
Derivatives	3	46,**,der(13;14)(q10;q10)(1), 45,**,der(13;14)(q11;q12)(1), 46,**,der(10)t(1;10)(p32;p13)(1)
Total abnormal cases	85	

The classification of fetal karyotype observed is done as follows:

Fetus karyotype same as abnormal karyotype of parents: Total-7 cases observed. The abnormalities observed were inversion, translocation and derivatives.

Fetus karyotype differ from abnormal karyotype of parents: Total-3 cases observed. The abnormalities were derivatives, deletion and translocation.

Fetus karyotype normal from abnormal karyotype of parents: Total-3 cases were observed. In this type parents carries the translocation and inversion type of chromosomal pattern but feruses were normal in karyotype. The details of the correlation of carrier parent as a high risk factor and chromosomal abnormalities observed is listed in Table-2.

Discussion: Prenatal genetic diagnosis using amniocentesis is well established procedure in many countries due to its safety and reliability. Accurate and reliable results give important information to the obstetrician. The results provide useful information to the pregnant women also with high risk factors then in the general population. The results of our study are similar to those of previous studies done before[7]. The world wide risk for amniocentesis is about 1%[9]. The abnormalities observed from 1177 are in 85 cases (7.29%). This high frequency, more then other study done before[8,10], of chromosomal abnormality itself suggests the importance and reliability of prenatal diagnosis. From many study done before it was concluded that Downs syndrome is most common and clinically significant abnormality detected in the prenatal diagnosis[9,11]. In our study Down syndrome was also the most frequent and highest abnormality observed. In this study Down syndrome cases are 40% from the total number of abnormalities and highest one. Inversions of autosomal chromosomes are the second and translocations are the third highest detected anomaly. It has been known that most of the structural abnormality are familial[12]. In our study total 14 cases were there in which the parental karyotype was abnormal. Fetal karyotype results were divided in three category. In first if the fetal karyo is same as parental karyo, in thistype of category, there is no increased risk for phenotypic abnormalities in the child (background risk of 2-3% of course exists). In the second category, the de-novo translocation and structural abnormality detected which is differs from the parental karyo. Studies demonstrate that the risk of birth defects or mental retardation or both in this category is in the range of 6-10%[13]. In third category the fetal karyotype is normal from abnormal parents with no cytogenetic and phenotypic abnormalities.

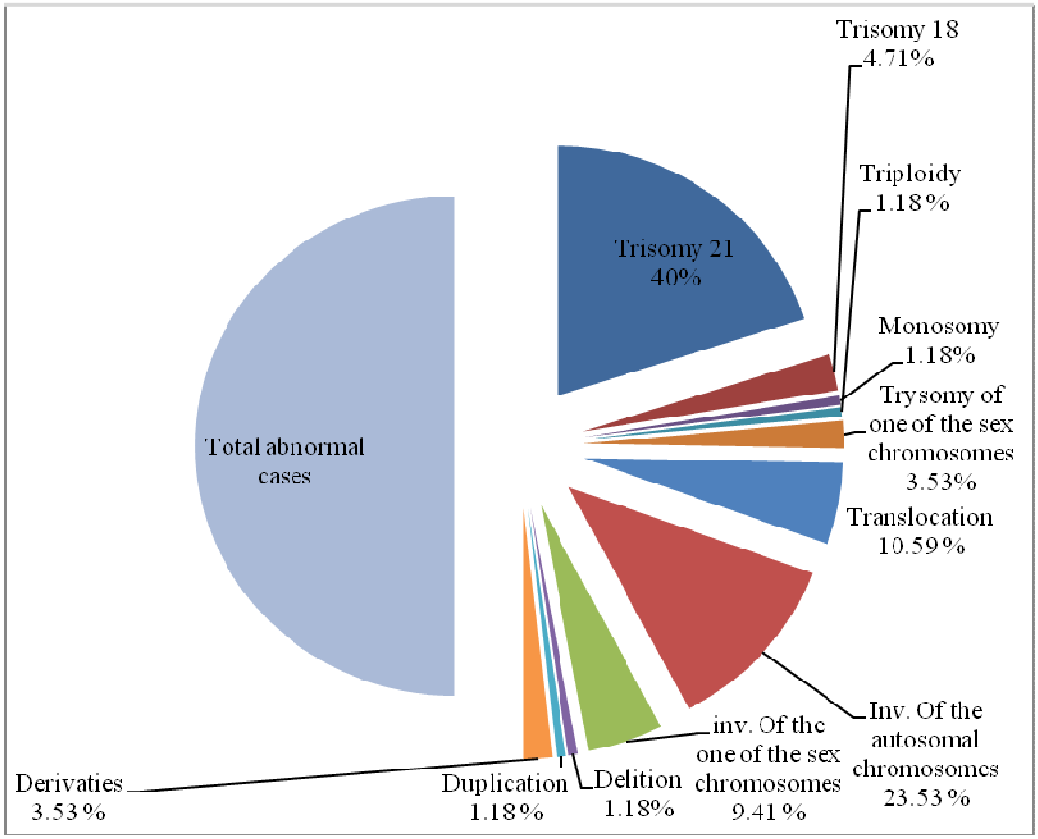

Figure-1
Distribution of chromosomal abnormalities

Table-2
Carrier parents and current chromosomal pattern carring fetus

Carrier parents		Chromosomal patterns carrying Fetus
Father	**Mother**	
Fetus karyo same as abnormal karyo of parents		
46,X,inv(Y), inv(9)	46,XX	46,**,inv(9)(p11;q12)
46,XY,t(2;17)(p27;p11.2)	46,XX	46,**,t(2;17)(p27;p11.2)
45,XY,der(13;14)(p10;q10)	46,XX	45,**,der(13;14)(p10;q10)
46,XY,inv(8)(q11.2;q21.3)	46,XX	46,**,inv(8)(q11.2;q21.3)
46,XY	46,XX,inv(9)	46,**,inv(9)
46,XY,t(4;6)(q31.3;q25.1)	46,XX	46,**,t(4;5) (q31.3;q35)
46,XY	46,XX,t(2;6) (p23;q21)t(10;13) (q11.2;q22.1)	46,**,t(2;6) (p23;q21)t(10;13) (q11.2;q22.1)
Total		7 cases
Fetus Karyo differ from abnormal karyo of parents		
46,XY	46,XX,t(8;21)(p11.2;q12)	46,**,t(8;15)(p10;p10)
46,XY	46,**,t(1;10)(q32;p13)	46,**,der(10)t(1;10)(q32;p13)
46,XY,inv(18)(q11.2;q21.3)	46,XX	46,XY,del(18)(q21.2;q21.2)
Total		3 cases
Fetus Karyo normal from abnormal karyo of parents		
47,XYY	46,XX	46,**
46,XY,t(11;22)(q23;q11.2)	46,XX	46,**
46,XY,inv(Y)	46,XX	46,**
46,XY	46,XX,t(6;11)(q11:q13)	46,**
Total		3 cases
Total (1+2+3)		14 cases

Conclusion

The study suggests the prenatal cytogenetic analysis should be performed in high risk groups along with the parental karyotype. Prenatal diagnosis deals with management and correction of a defect when possible. The decision of a selective termination, when not treatable and post birth management, if the couple decides to deal with the handicapped child.

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