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Cytogenetics of Recurrent Spontaneous Abortions: A Study of 250 Products of Conception

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ABSTRACT The study conducted on two hundred fifty (250) Products of Conception samples to investigate the cytogenetic cause of recurrent abortion. Products of Conception samples are studied using conventional method Karyotyping and FISH is performed when samples failed to grow in culture. Chromosomal anomalies were observed in 31 cases. The researchers observed autosomal trisomy and monosomy including monosomy X. Mosaic cell lines were observed with XY/XYY, XX/XX,+21 and XY/XXY,+18 chromosome complement. A case of 46,XX,der(13;14)(q10;q10),+14 and a triploidy was detected. The rate of chromosomal anomaly as per the maternal age was: 50 percent in 30-35 years and 30 percent in 25-30 years. A statistically significant difference was observed in the frequency of abnormalities in recurrent abortions as compared to spontaneous abortions ($p<0.05$). The cytogenetically normal cases need to be study at the submicroscopic level for genome wide analysis to unravel genetic aberration responsible for the abortion and thus useful for recurrence risk assessment and preimplantation genetic diagnosis.

INTRODUCTION

Recurrent pregnancy loss or recurrent spontaneous abortion (RSA) is one of the major health issues globally. This is classically defined as the occurrence of three or more consecutive abortions; however, the American Society of Reproductive Medicine has recently re-defined recurrent pregnancy loss as two or more abortions. Pregnancy loss is a clinically recognized pregnancy, involuntarily ending before 20 weeks of gestation (Sheth et al. 2013). About 10 percent to 15 percent of clinically recognized pregnancies results in Spontaneus Abortions (SA) and the total pregnancy loss is estimated to be 30 percent to 50 percent of all conceptions (Rai and Regan 2006; Stephenson and Kutteh 2007). A majority of miscarriages that occur before 10 weeks of gestation are due to chromosomal aneuploidies arising from new non-disjunctional events, which are more frequent in very early miscarriages (Sierra and Stephenson 2006). In spontaneous abortions, the majority of chromosomal anomalies (95%) are numerical. About 60 percent are trisomies, 20 percent are monosomy X and another 15 percent are polyploidy, especially triploidy (Warburton et al. 2004). Advanced maternal age is one of the major risk factors of spontaneous abortions and recurrent miscarriages (Choi et al. 2014). Structural chromosomal aberrations can also be a cause of pregnancy loss. Presence of balanced chromosomal aberration in one of the parents may result in abnormal segregation of the chromosomes at meiosis, aneuploid gametes that can be responsible for pregnancy loss (Farcas et al. 2007). Balanced translocation carriers are reported to not have recognizable phenotypic expressions but have more risk of recurrent spontaneous abortions or children with serious birth defects due to unbalanced chromosome complement (Kunwar and Bakshi 2016). Factors other than chromosomal aberrations include hormonal imbalances, hematological and immunological disorders, anatomical abnormality, infections, environmental exposures, etc. (Jauniaux et al. 2006).

Cytogenetic analysis of a spontaneously aborted conceptus provides valuable information for patients. Chromosomal analysis for this requires culture and karyotype of chorionic villi or fetal tissues (Jobanputra et al. 2011). However, culture failure, microbial infection of the sample, maternal cell contamination and poor chro-

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mosomal preparations often hampers conventional karyotyping with an overall failure rate of 21 percent (Jia et al. 2015). In case of culture failure, FISH can be informative and it is found to be a reliable diagnostic method for clinically important chromosome aneuploidy (Jobanputra et al. 2002).

In this study, the researchers studied Products of Conception from recurrent miscarriage or spontaneous abortion to identify abnormalities by Karyotyping or aneuploidy of chromosome 13, 15, 16, 18, 21, X and Y by FISH in case of culture failure.

Objective

The spontaneous and recurrent abortions are reported to be linked with chromosomal imbalance that increases with older parental age. The researchers' objective is to study the frequency and distribution of chromosomal abnormality abortus tissue in parents as per the age groups with history of spontaneous and recurrent miscarriages.

MATERIAL AND METHODS

Sample Selection

The previous history of involuntary abortion was an important sample selection criteria. The patients, their close relatives, or their clinicians gave a written consent while collecting the sample. Fetal tissue parts were selected for the culture to prevent maternal cell contamination. About two hundred and fifty products of conception (POC) samples were cultured for karyotype analysis to ascertain the chromosomal aberrations. Simultaneously, uncultured specimens were saved (in case of culture failure) for FISH to identify the common numerical aneuploidies. The gestational age of the pregnancy demise was determined based on the ultrasound findings.

Products of Conception (POC) Culture and Karyotype Analysis

The POC samples were transported in sealed and sterile sample collection vial containing RPMI media with antibiotics at room temperature. The samples were then processed for culture. Abortus material (preferably villi, skin tissue from upper thigh, toe tissue or Placenta) was washed, enzyme treated and cultured in T-Flask in Amniomax media and harvesting was performed on samples after the proper confluency of the culture appeared using standard cytogenetic methods (Jobanputra et al. 2002).

Interphase Fluorescence in situ Hybridization (I-FISH)

Interphase FISH was performed on uncultured cells using a set of FISH probes for chromosomes 13, 15, 16, 18, 21, 22 and X, Y to ascertain the common aneuploidies (Jobanputra et al. 2002)

RESULTS

About 78 (31.2%) samples were cultured successfully and their karyotype is analyzed. Interphase FISH was performed on 154 cases, which failed to grow in culture. Eighteen cases yielded no result due to unsupportive tissue condition to grow while being transported. The age group of the mother was categorized into four groups of 20-25, 26-30, 31-35 and above 35 years. Out of 250 cases, 31 abnormal cases (12.4%), 201 normal (80.4%) and 18 cases (7.2%) with no results observed (Tables 1 and 3). Age group 31-35 years shows maximum number of abnormal cases, that is 16 (50%), followed by 26-30 years with 7 (30%) abnormal cases (Table 2). Different chromosomal anomalies were identified in the study which consisted of 09 monosomy X (29%), 01 mono-

Table 1: Showing the percentage distribution of total cases

Cases	*Normal cases*	*Abnormal cases*	*No results*
Percentage	81	12	7

Table 2: Frequency of abnormal cases according to age group

Age group	*Percentage*
Less than 25 years	6
26- 30 years	23
31-35 years	52
36 years or above	19

somy 21 (3%), 05 trisomy 21 (16%), 04 trisomy 16 (12%), 02 trisomy 18 (6%), 01 each trisomy 13 (3%), and 15 (3%), 02 trisomy 20 (6%), 01 case with triploidy (3%), 03 mosaic XY/XYY (3%), XY/XXY,+18 (3%) and XX/XX,+21 (3%) (Table 4). Out of 31 abnormal findings, one case showed structural abnormality 46,XX,der (13;14)(q10; q10),+14 and the rest thirty showed numerical abnormality. Seven (22.5%) chromosomal anomalies were detected by karyotype and interphase FISH (Table 4). Gestational age of the aborted fetus ranges from 8-16weeks. The least and greatest maternal age range was 23 and 39 years. The success rate of POC culture was 31.2 percent. The statistically significant difference was observed in the frequency of abnormalities in recurrent abortions as compared to spontaneous abortions ($p<0.05$).

DISCUSSION

Recurrent miscarriage has remained a challenging reproductive problem for the patient and clinician. Cytogenetic study provides key genetic information regarding cause of pregnancy loss. Knowing the chromosomal cause of preg-

Table 3: Comparative distribution of total cases based on age groups

Age	*Comparative groups*	*Total*	*Normal*	*Abnormal*	*TNP*
20-25 years	RSA	33 (14%)	28 (13%)	2 (6%)	3 (17%)
	Spontaneous abortions	16 (6%)	14 (7%)	0	2 (11%)
	Total	49 (20%)	42 (20%)	2 (6%)	5 (28%)
26-30 years	RSA	54 (21%)	45 (22%)	4 (13%)	5 (28%)
	Spontaneous abortions	37 (15%)	32 (15%)	3 (10%)	2 (11%)
	Total	91 (36%)	77 (37%)	7 (23%)	7 (39%)
31-35 years	RSA	44 (18%)	34 (18%)	9 (29%)	1 (5.5%)
	Spontaneous abortions	35 (14.0%)	27 (13%)	7 (23%)	1 (5.5%)
	Total	79 (32%)	61 (31%)	16 (52%)	2 (11%)
36 and above	RSA	16 (6%)	13 (7 %)	2 (6%)	1 (5%)
	Spontaneous abortions	15 (6%)	8 (5%)	4 (13%)	3 (17%)
	Total	31 (12%)	21 (12%)	6 (19%)	4 (22%)
	Total cases	250 (100%)	201 (100%)	31 (100%)	18(100%)

Table 4: Number and percentage distribution of abnormal cases

Types of chromosomal abnormality	*No. of cases*	*Percentage*	*Karyotype*	*FISH*
Monosomy 21	1	3		1
Monosomy X	9	29		9
Trisomies:				
13	2	6		2
15	1	3	1	
16	4	13		4
18	2	6	1	1
20	2	6	2	
21	5	16	1	4
Rearrangements: 46,XX,der(13;14)(q10;q10),+14	1	3	1	
Mosaic:				
XY/XYY 70%	1	3		1
XY/XXY,+18 60%	1	3		1
XX/XX,+21 65%	1	3		1
Triploidy	1	3	1	
Total Cases	31	100	7 (22.5%)	24 (77.5%)

nancy loss is psychologically important to overcome grief and loss. This study was focused on the frequency and distribution of abnormal cases in different age groups with spontaneous and recurrent miscarriages. Understanding the genetic information of these aborted fetuses will help improve genetic counseling to the patients. Available reports of the study by (Rubio et al. 2005) have confirmed that chromosomal abnormalities are the cause of spontaneous and recurrent miscarriages. Fifty percent of the miscarriages are associated with cytogenetic abnormalities including trisomy, polyploidy and monosomy X (Lauritsen 1975; Hassold 1986; Kalousek et al. 1993). Most frequently associated abnormality with recurrent spontaneous abortion is autosomal trisomy. In the present study, autosomal trisomy accounts for 49 percent of the total abnormal cases, which include trisomy of chromosome 13, 15, 16, 18, 20 and 21. This study shows 5 cases of trisomy 21 followed by another often-found autosomal trisomy 16 (4 cases). Trisomy 16 is another common abnormality found in recurrent spontaneous abortions, which concurs with previous reports (Ljunger et al. 2005; Rolink et al. 2010). The actual reason behind the diversity of trisomy is still a puzzle to be answered. Other non-cytogenetic factors may be responsible for the miscarriages with a normal karyotype. The effect of maternal age has specific correlation with certain autosomal aneuploidies, but not with sex chromosome aneuploidy. This citation from previous study (Hyde et al. 2015) is also consistent with the present study, as 29 percent of all the abnormalities detected comprises of monosomy X. Presence of monosomy X is uniformly distributed among all the ages ranging from 19-39 years. Another non-existing abnormality is the presence of autosomal monosomy, an important reason for fetal demise. Apart from monosomy X this study also shows the presence of monosomy 21 in one case with recurrent abortions. Apart from aneuploidies another numerical abnormalities reported here are triploidy and presence of mosaic cell lines.

Women with more than 36 years of age show increase in the frequency of euploid miscarriages (Stephenson et al. 2002) which is consistent with the present study, as fifty seven percent of the normal cases have maternal age between 19 years to 30 years. Increase in the frequencies of miscarriage is one of the important phenotypic features among the carriers of balanced translocations with unbalanced exchange of gametes. In comparison to available literature, in this study, the researchers observed only one case for rearranged chromosome 46,XX,der (13;14)(q10;q10),+14. Here parental karyotype was performed and mother was found to be a carrier for Robertsonian translocation, 45,XX,der (13;14)(q10;q10). (Sismani et al. 2008) reported the association of balanced translocations showing abnormal phenotypes with causative cryptic chromosomal imbalances. As the frequency of abnormal embryonic chromosome complement is significantly higher in recurrent abortions as compared to those in spontaneous abortions ($p<0.05$).

CONCLUSION

In case of spontaneous abortions with the abnormal embryonic chromosome complement the recurrence risk is higher hence the cytogenetic study is important. This can unravel causative genetic aberration; hence can be useful for pre-implantation genetic diagnosis in future pregnancies. The lower rate of cytogenetic abnormality in this study than reported earlier, could be due to non-genetic factors not ruled out owing to incomplete clinical history and lack of awareness. In addition to karyotype or FISH including microarray can improve, the yield of genetic abnormalities.

RECOMMENDATIONS

The study at submicroscopic level using molecular cytogenetic technique like MLPA for targeted gene analysis and microarray or sequencing for genome wide analysis is recommended for the cases, which were normal at cytogenetic level. This will unravel genetic aberrations and thus genetic counselling can be more effective.

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