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ORIGINAL ARTICLE

Study of Spectrum of Chromosomal Rearrangements in Recurrent Pregnancy Loss

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Abstract

Introduction Recurrent pregnancy losses (RPLs) are seen in up to 15–20% of all clinically recognized pregnancies, 1–2% of women in general population. Repeated losses are seen in 5–10% of women. The prevalence of chromosomal rearrangements is 6.65% in couples with repeated pregnancy losses. Two to 4% of RPL are associated with parental balanced reciprocal and Robertsonian translocations.

Materials and Methods The study was conducted at a tertiary care hospital in New Delhi, and in total, 204 couples with RPL enrolled in the study.

Results In total, 4490 couples presented to the obstetric clinic, of which 204 (4.5%) couples had repeated pregnancy losses. Cytogenetic analysis was done in 198 couples. Out of total 198 patients, 14 patients (7.1%) had cytogenetic alterations. Most common aberrations observed were structural rearrangements, of which reciprocal translocations were more common. In our study cohort, all the couples had maternal age of ≤ 35 years and all the alterations were seen either in mother or in both parents.

Discussion Our study highlights that cytogenetic alterations not only are common in first trimester miscarriages, but are an important event in miscarriages presenting at later period of gestation and in young mothers as well.

Keywords Robertsonian translocations · Balanced translocations · Unbalanced translocations · Miscarriage

Introduction

Pregnancy loss is an enormous physical, social and emotional burden on a couple which progresses to worry and further discontent if pregnancy loss recurs. Recurrent pregnancy losses are common and are seen in up to 15–20% of all clinically recognized pregnancies. Recurrent pregnancy loss (RPL) is defined as "three or more consecutive pregnancy losses prior to 20 weeks from the last menstrual period" [1]. Successful pregnancy is dependent on multiple factors including genetic and the reproductive tolerance of the couples. When this tolerance is not achieved, it results in the repetitive pregnancy loss with genetically incompatible fetus. The prevalence of spontaneous pregnancy loss is 1–2% of women in general population.

Repeated pregnancy losses are seen in 9–12% of women aged <35 years and increase to 50% after 40 years of age, of which nearly 50% remain unexplained [2]. The risk of miscarriage in subsequent pregnancies is 30% after two losses, compared with 33% after three losses among patients without a history of a live birth [3]. Two to 4% of RPLs are associated with a parental balanced structural chromosome rearrangement, most common of which are balanced reciprocal and Robertsonian translocations. Others include chromosomal inversions, insertions and mosaicism. These abnormalities are responsible for unequal exchange of

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chromosome or genetic content during gametogenesis, leading to partial deletions and duplication in the conceptus.

The prevalence of chromosomal rearrangements has been found to be 2–8% in couples with repeated pregnancy losses as compared to 0.55% of the general population [4]. The prevalence of chromosomal abnormality in product of conception of miscarriage is found to be 50–70% [5] which reemphasizes the need for comprehensive and systematic cytogenetic workup of couples with RPL which remains an uncommon practice till today.

The prevalence of cytogenetic alterations varies widely in different studies. Most of the pregnancy losses during early first trimester are attributed to chromosomal abnormalities of which trisomies are the most frequently detected anomalies (60–80%), followed by triploidies (12.4%), monosomy X (7–20%), tetraploidies (9.2%) and structural chromosome anomalies (4.7%) [5–7]. The mechanism for aneuploidies is predominantly nondisjunction which may be related to advanced maternal age, while structural abnormalities may occur secondary to unequal chromosome segregation during meiosis.

The study brings attention to unequivocal need for cytogenetic workup to save couples from the guilt of being incomplete as they cannot create a viable pregnancy as well as for better understanding of disease biology.

Materials and Methods

The study was conducted at a tertiary care hospital in New Delhi, India, from January 2016 to December 2018. In total, 204 couples with RPL presented at obstetric clinic. We reviewed the cytogenetics findings in all the couples with history of repeated spontaneous miscarriage. Cytogenetic analysis was offered to all the couples, for screening of parental carrier abnormalities when other causes had been ruled out, out of which 198 couples consented for cytogenetic analysis.

A total of 198 couples were investigated for chromosomal abnormalities with detailed case history of consecutive miscarriages or stillbirth or previous history of malformed fetus with multiple congenital malformations. The gestational age at the time of pregnancy loss was estimated by reviewing ultrasound. Parental karyotyping was performed to look for chromosomal defects. Once a cytogenetic cause was found, prenatal diagnosis was offered by amniocentesis and karyotyping. Cytogenetic diagnosis was performed by culturing of amniotic cells or by chorionic villi culture. Minimum 20 GTG-banded metaphase cells were karyotyped and analyzed depending on availability of metaphases as per ISCN 2016. In addition, total numbers of live births and subsequent miscarriages are also being recorded. Dichotomous variables were analyzed using Chi-squared and Fisher's exact test (Table 1).

Statistical analysis of all the data set was performed using Stata 14.0.

Results

In total, 4490 couples presented to the obstetric clinic, of which 204 (4.5%) couples had repeated pregnancy losses. One hundred and ninety-eight couples consented for cytogenetic analysis. Cytogenetic analysis was done in all these couples. Out of total 198 patients (couples), 14 couples or their offsprings (7.1%) had cytogenetic alterations. Most common aberrations observed were structural rearrangements, of which reciprocal translocations were more common. In our study cohort, all the couples had maternal age of $\leq$35 years. Cytogenetic analysis revealed rearrangements in maternal genome in all the couples. In three couples, father also had cytogenetic alteration.

Reciprocal Translocations

Seven families (50%) had reciprocal translocation, of which six were carrier parents and one was child with de novo reciprocal translocation with normal karyotype in parents. Out of six carrier parents, five had children with normal phenotype of which four had normal genotype as well, while one had balanced translocation as was seen in mother. One of the six carrier parents had offspring with recombinant chromosomal rearrangement as 46,X_,rec(15)(15pter$\rightarrow$15q22::5p15$\rightarrow$pter)mat. One of the couples with normal child in this pregnancy had offspring with unbalanced translocation in previous pregnancy where the couple was counseled and termination was planned.

Robertsonian Translocations

Three out of 14 (21.4%) patients had Robertsonian translocations. Chromosome 13 was involved in all of these cases, two of which had involvement of chromosome 14 with chromosome 13 (66.7%), while one had fusion of chromosome 22 with chromosome 13 (33.3%). Among one of the couples, father had additional chromosomal material on short arm of chromosome 21. Children of all three couples delivered phenotypically normal children, of which two had normal genotype, while one had Robertsonian translocation same as that seen in the mother. All three patients had phenotypically normal children. However, one of these patients had similar alteration as seen in the mother.

Table 1 Spectrum of cytogenetic aberrations in couples with repeated pregnancy loss

Case no.	Age (years)	Gravida/parity	POG of miscarriage (weeks)	Clinical spectrum of conceptus	Tests performed	Outcome		Follow-up and course of action	Subsequent outcome
						Mother	Father		
Robertsonian translocation									
2	26	G5 P2 A2	G1–A1 (13 weeks) G2–L1 (survival-1.5 years) G3—IUD (26 weeks) G4–A2 (13 weeks)	G2—polydactyly and heart defect	Karyotype	Robertsonian translocation 45, XX, t(13;22) (q10; q10)	46, XY	Prenatal karyotype	45 XY, *t*(13;22)
7	27	G3A2	A1—12 weeks A2—10 weeks		Karyotype	45, XX, rob (13:14) (q 10;q10)	46 XY, 21 pstr +	Prenatal karyotype	Normal
5	35	P1A5	A–A4—14 weeks A5–IUD (34 weeks)	–	Karyotype	Robertsonian translocation 45, XX, rob (13;14) (q10;q10)	46, XY	Prenatal karyotype	Normal
Reciprocal *translocations*									
1	31	P1A1	14 weeks	Multiple congenital anomalies	Karyotype	46 XX, *t*(1:6) (q32;P23)	46, XY	USG follow-up, prenatal FISH	No translocation
3	22	G3 A2 L0	A1—13 weeks A2—10 weeks		Karyotype	46 XX, *t*(10:13) (q23:q22)	46, XY	Prenatal karyotype	Normal
4	28	P1 A1	A1—20 weeks	Multiple congenital abnormalities	Karyotype	46 XX *t*(5;15) (q13;q15)	46,XY	Prenatal karyotype	Normal
10	26	P1L0A3	G1–A (8 weeks) G2–P (Died after birth) G3–A (8 weeks) G4–A (8 weeks)	G2–IUGR	Karyotype	46,XX (5;15) (p15, q22)	46,XY	Prenatal karyotyping	Termination 46,X_, rec(15) (15pter→15q22::5p15→pter) mat
14	24	A4	G1–A1 (13 weeks) G2–A2—ectopic pregnancy G3–A3 (20 weeks) G4–A4 (anembryonic pregnancy)	–	Karyotype	46, XY inv(6) (p23q27)	46, XY	Prenatal microarray	Normal
6	29	G1	G1 triple screen positive	USG scan normal	Karyotype	46, XX	46, XY	Prenatal karyotype	Balanced translocation 46, X_,*t*(1;16)(p36.3;p11.2) Microarray normal

Table 1 (continued)

Case no.	Age (years)	Gravida/parity	POG of miscarriage (weeks)	Clinical spectrum of conceptus	Tests performed	Outcome		Follow-up and course of action	Subsequent outcome
						Mother	Father		
12	32	G2P1A0	G1–P Global developmental delay	47, XY, mar+	Karyotype	*t*(9:10)(q22.1:p15.1)	46,XY	Prenatal karyotyping microarray	46, XX, *t*(9,10) (q13;p11.2) No copy number variation
Unbalanced translocation									
8	28	P0A3	A1—16 weeks A2—14 weeks A3-IUD (23 weeks)	Multiple malformations	Karyotype	46,XX dup (9)(q12), 9qh+,	46, XY	Prenatal karyotype	Normal
9	34	P1A3	G1–A (17 weeks) G2–A2 (12 weeks) G3–IUD (30 weeks) G4 IUD (28 weeks)	G3 Pierre Robin syndrome G4–TOF/VSD Multiple anomalies	Karyotype—normal FISH for 22q deletion	46, XX No del at 22q11	46, XY	Microarray	17p11.2 439 kb deletion
11	28	P0A2	G1–A (12 weeks) G2—molar pregnancy	–	Karyotype	46,XX fra(16)(q22),22pss(7)/46, XX,22PSS [43]	46, XY	Counseling	Normal
13	29	P0A2	G1–A (12 weeks G2–A (18 weeks)	–	Karyotype	46, XX, 6q [1] /46, XX [30]	46XY 15p+(satellite)	Prenatal FISH, microarray	Normal

Unbalanced Translocation

Four families (28.5%) had unbalanced translocation with chromosomal gain or loss, out of which three couples had chromosomal alteration. Chromosomal analysis revealed that two of these three couples had chromosomal rearrangements in both the parents. One of the offsprings with karyotypically normal chromosomes had loss of 439 kb on 17p11.2 (Smith–Magenis syndrome). The couple had four previous miscarriages, including one with Pierre Robin syndrome and one with multiple congenital anomalies including congenital heart defects. The couple was counseled, and termination was planned. Two of the couples where both the parents had chromosomal rearrangements had normal phenotype and genotype in present offspring after previous 2–3 miscarriages. One patient, who presented with positivity for triple screen, was found to have balanced translocation in the offspring.

Discussion

We, in India, have scarcity of data on prevalence and spectrum of cytogenetic alteration in repeated pregnancy losses. The study was carried out to bring increased awareness of the genetic role in recurrent miscarriages. Our study supports the previous finding of higher prevalence of genetic alterations in couples with pregnancy losses. Though it highlights that cytogenetic alterations are common not only in first trimester miscarriages, they are important in miscarriages presenting at later period of gestation as well. We found higher frequency in second and third trimester pregnancy losses as well.

In the present study, all the mothers presenting with RPL were young (< 35 years), hence challenging the perception of more prevalence of cytogenetic alterations with higher maternal age.

Robertsonian translocations (ROBs) are the most common structural abnormality in general population with overall incidence of Robertsonian translocations of approx 1/1000 newborns [8, 9]. Though all human acrocentric chromosomes are capable of participating in the formation of Robertsonian translocations, chromosomes 13 and 14 are most commonly involved [10]. In those with RPL, ROBs involving chromosomes 13 and 14 followed by 13 and 22 were reported to be more common [11]. Similar findings were noted in our study as well. Homologous ROBs are predominantly de novo in occurrence, while heterologous ROBs are inherited from carrier parent [10, 12]. In our study as well, the heterologous ROB was inherited from mother.

Reciprocal translocations were most common and were seen 42.8% of couples (6/14) in our study. Some cytogenetic derangements were noted. One child was found to have reciprocal translocation where mother had increased risk on triple screen (AFP, unconjugated estriol and beta hCG) for Down syndrome which denotes false positivity, further reemphasizing the need to carry out cytogenetic studies and karyotyping in particular. The most commonly involved chromosomes were chromosomes 5, 6, 10 and 15. Reciprocal translocation of chromosomes 5 and 15 was seen in two patients though at different band positions. One of the offsprings in the couple showed recombinant chromosome. Chromosome 6 had pericentric inversion and translation with chromosome 1 in one patient each. Chromosome 10 had reciprocal translocation with chromosomes 10 and 13 each. In the literature, reciprocal translocations have been reported in approximately 50% of patients [13]; similar prevalence was noted in our study as well.

Unbalanced translocations were seen in 03 out of 14 patients (21.4%). In one of these, although both husband and wife had cytogenetic rearrangement, the present offspring had normal rearrangement. One couple was karyotypically normal and had small deletion in offspring. It is little surprising that individuals with microscopic unbalanced translocations were presented with some abnormality. Couples with unbalanced translocations found it difficult to conceive than to have miscarriages. Scrutiny of the cases revealed that one such case had heteromorphic variation of pericentric inversion of chromosome 9, and the other two were the presence of extrachromosomal material at short arm of acrocentric chromosome.

Of the total 14 couples, nine (69.2%) couples had normal outcome in subsequent pregnancies. Out of four couples, whose subsequent pregnancies had cytogenetic alterations, two had similar rearrangement as seen in one of the parents, one had recombinant chromosome, while one was incidental finding where chromosomal analysis was done because mother was triple screen positive. One couple underwent cytogenetic workup as previous product of conceptions showed features suggestive of Pierre Robin syndrome and another one had multiple congenital anomalies.

To conclude, structural cytogenetic rearrangements are very common in couples with repeated pregnancy losses, most common of which were found to be sporadic reciprocal translocations in our study. For Robertsonian and sporadic reciprocal translocations, karyotyping remains the procedure of choice for chromosomal analysis. However, for submicroscopic deletions or duplications, microarray is required to be performed.

We recommend that all the patients with more than two pregnancy losses at any period of gestation should undergo routine parental cytogenetic analysis with karyotyping

followed by microarray where clinical suspicion of involvement of genetic factors is high. The challenge is to identify the abnormality early to save the couple from agony of pregnancy loss. Complete genetic workup will facilitate appropriate genetic counseling to these couples.

Compliance with Ethical Standards

Conflicts of interest There are no potential conflicts of interest to declare.

Human and Animal Rights Research involved human participants for workup of miscarriages, and no animal trials were involved.

Informed Consent Written informed consent was obtained from all the patients.

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