Original Article

Prevalence of Cytogenetic Anomalies in Couples with Recurrent Miscarriages: A Case–control Study

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Abstract

Background: About 15%–20% of couples get affected by recurrent miscarriages (RM) and chromosomal abnormality in one partner affects 3%–6% of RM couples. **Aims:** The present study aimed to determine the prevalence of cytogenetic anomalies in couples with RM. **Settings and Design:** A case–control study was undertaken, in which 243 couples who had experienced 2 or >2 miscarriages were investigated for chromosomal abnormalities and compared with 208 healthy, age-matched control couples who had at least one healthy live born and no history of miscarriages. **Material and Methods:** Peripheral blood (PB) lymphocytes were cultured using PB-Max Karyotyping medium (GIBCO) for chromosomal analysis and 20 metaphases were analyzed for each individual. **Statistical Analysis:** Student's *t*-test was used for statistical evaluation and $P < 0.05$ was considered statistically significant for all instances. **Results:** The current study revealed 3.1% RM cases showing structural chromosomal aberrations, of which balanced translocations and Robertsonian translocations constituted 66.7% and 26.7% cases, respectively, while inversions constituted 6.7% abnormal RM cases. Polymorphic variations were observed in 1.9% RM patients and 1.2% controls as well. However, the number of abortions were significantly more ($P = 0.027$) in male carriers of balanced translocations as compared to female carriers in the RM group. There was no significant difference for age ($P = 0.539$) between RM women and control women. **Conclusions:** Although similar studies exist in literature, our study is the first of its kind from our region that has compared the chromosomal anomalies between the RM group and the control group. We observed 3.1% of balanced translocations and an increased number (though nonsignificant) of polymorphic variations and satellite associations in the RM group as compared to the control group.

Keywords: *Chromosomal abnormalities, heteromorphism, pericentric inversions, polymorphic variations, recurrent miscarriages, Robertsonian translocations*

Introduction

Human reproduction has been considered as an inefficient phenomenon as only 1/3 of conceptions result in live births.[1,2] Recurrent miscarriage (RM) is defined as a distinct disorder characterized by the loss of two or more clinical pregnancies. Approximately, 15%–20% of couples get affected by this complication of pregnancy.[3] It has been estimated that 70% of all pregnancies fail to reach term and out of these 50%–60% end within the first trimester of pregnancy.[4] The known etiological factors such as uterine malformations, antiphospholipid antibodies, parental chromosomal abnormalities, endocrine and immunological factors responsible for repeated miscarriages account for only 20%–50% of RM

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cases[5] while etiology for rest of the RM cases remains unexplained.[6]

Chromosomal abnormality in one partner has been estimated to affect 3%–6% of RM couples which is ten times higher than the general population.[7] Different studies have reported a varying frequency of 3%–8% for carriers of chromosomal aberrations among RM couples.[8-12] When chromosomes segregate during meiotic division, the presence of balanced chromosomal rearrangements leads to an unbalanced chromosomal constitution in the carrier's gametes which ultimately results in conditions such as spontaneous abortions, stillbirths or malformations.[13] The most commonly observed cytogenetic abnormalities include balanced translocations and inversions which do not have any effect on the phenotype of the carrier but attributes to a 50% risk in the fetus to have an unbalanced chromosomal constitution.[14]

Heterochromatin region on chromosomes is comprised of highly repetitive sequences of DNA that do not encode proteins and are hence, considered as normal variations.[15] In individuals with polymorphic variations, heterochromatin seems to have no functional or phenotypic effect.[16] However, some studies have suggested their cellular roles in various clinical conditions, including infertility.[17] Apart from chromosomal anomalies, heteromorphism in chromosomes is also observed in cases of RM. Polymorphic heterochromatic variations in chromosome 1, 9, and 16 have been found to be associated with infertility. Furthermore, heterochromatin regions are commonly seen on short arms of acrocentric chromosomes and regions of Y chromosome as well.[18-20] The present case–control study aimed to determine the prevalence and types of chromosomal anomalies and polymorphic variations in RM couples and their comparison with healthy control couples.

Material and Methods

The current study evaluated 243 RM couples and 208 ethnicity and age-matched healthy control couples for cytogenetic abnormalities. The inclusion criterion for RM couples involved two or more consecutive pregnancy losses and for controls, at least one live birth and no history of miscarriages. The referred cases were examined thoroughly and detailed clinical and obstetric histories were recorded in prepared proformas. Information about their family history of abortions was also recorded in the form of pedigree. Written informed consent was taken from all study participants. Age and number of miscarriages were noticed for all participants of the study. Patients were screened to rule out inherited thrombophilia, antiphospholipid syndrome, incompetent cervix, uterine anomalies, Factor V Leiden mutation, infections, endocrinological imbalance, and any other known cause of RM. Metaphase chromosome preparations were made from peripheral blood (PB) lymphocyte cultures set up by adding 0.5 ml of heparinized blood sample in 5 ml of PB-Max Karyotyping medium (GIBCO). GTG (G-banding by Trypsin using Giemsa stain) banded chromosomes were karyotyped using computerized image analysis software (Cytovision 3.0) and International System for Human Cytogenetic Nomenclature, 2013. At least, 500–550 band resolution level was maintained. Twenty metaphases were analyzed and five metaphases were karyotyped for each individual. The same criterion was used for the assessment of polymorphic variations in cases as well as in controls. Furthermore, nucleolar organizing regions staining was used to validate the increased satellite lengths in few cases.

Statistical analysis was done using SPSS software, version 20 (SPSS, Inc., Chicago, IL). Difference between two groups was determined using Student's *t*-test and for all instances, $P < 0.05$ was considered statistically significant. This study was approved by the Institutional Ethics Committee.

Results

The present case–control study evaluated 243 RM couples and 208 healthy control couples for chromosomal aberrations. The data are expressed in terms of mean and standard deviation (SD). Age of the participants in RM group ranged from 21 to 48 years (mean = 32.4, SD = 4.4), whereas in control group, the range was 25–46 years (mean = 31.3, SD = 4.2). In the RM group, the mean age of females was found to be 31.1 years (SD = 4.3) and that of males was 33.9 years (SD = 4.3). While in control group, the mean age of females was 30.8 years (SD = 4.9) and of males was 33.5 years (SD = 4.7), respectively. No significant difference was observed for age between RM women and fertile control women ($P = 0.539$). Moreover, mean age at menarche was 13.1 years (SD = 1.0) and number of miscarriages ranged from 2 to 10 (mean = 3.1, SD = 1.3) for females in the RM group. However, a higher frequency (41.9%) of women with 2 miscarriages followed by 3 miscarriages (27.9%) and 4 miscarriages (20.6%) was identified in the RM group [Table 1]. Furthermore, we recorded a family history of abortions in 4.3% of RM cases through their pedigree analysis. No consanguinity was observed in the investigated couples. In RM group, women were categorized into two groups. One group ($n = 206$)

comprised of RM women of age 35 or <35 years, whereas the second group (n = 37) consisted of RM women above the age of 35 years. In the current study, we did not observe any significant difference for number of abortions (P = 0.269) and age at menarche (P = 0.1703) between these two groups.

Chromosomal aberrations were detected in a total of 15 individuals (10 females and 5 males) in the RM group, whereas no chromosomal abnormality was observed in the control group. These included 10 (66.7%) cases of balanced translocations, of which 5 (50%) were females and 5 (50%) were males. While 4 (26.7%) cases showed Robertsonian translocations of which all were female carriers. Furthermore, one (6.6%) case of inversion in chromosome 22 was observed in RM group [Table 2]. However, partners of these 15 cases of RM showing structural chromosomal anomalies had normal chromosomal constitution.

Table 1: Number of abortions in recurrent miscarriages women in the present study

Number of abortions	RM women, *n* (%)
2	102 (41.9)
3	68 (27.9)
4	50 (20.6)
5	13 (5.3)
6	3 (1.23)
7	5 (2.1)
10	2 (0.82)
Total	243 (100)

RM=Recurrent miscarriages

Interestingly, a phenotypically normal couple with nonconsanguineous marriage was reported where the male partner (aged 31 years) showed a complex translocation involving three chromosomes 46, XY, t(2;8;14)(q33;p22;q23.3) [Table 2] while chromosomal analysis of the wife (aged 27 years) showed normal karyotype (46,XX). This couple was married for 9 years and the husband was reported with oligozoospermia. Table 3 describes the frequency distribution of males and females as carriers of chromosomal anomalies in RM group. Furthermore, between male and female carriers, no significant difference (P = 0.635) for age was noticed but number of abortions in male carriers were significantly more (P = 0.027) than in female carriers [Table 4].

Polymorphic variations were seen in 9 (1.9%) RM cases and 5 (1.2%) controls as well [Table 5]. However, there was a nonsignificant difference (P = 0.282) between the RM group and the control group. These polymorphisms included variations in length of heterochromatin region, size of satellites and length of stalks of acrocentric chromosomes and pericentric inversion of Y chromosome and few observed polymorphic variations are shown in Table 6. In RM group, 4 (44.4%) were females and 5 (55.6%) were males while in control group, 3 (60%) were males and 2 (40%) were females. In RM group, only one male was reported showing pericentric inversion of Y chromosome as well as increased heterochromatin region in q arm of chromosome 1. On the other hand, in control group also, one female was seen with 2

Table 2: Chromosomal aberrations in recurrent miscarriages cases

Karyotype	Number of cases	Age (years)/sex	Number of abortions
Robertsonian translocation			
45,XX, rob(13;22)(q10;q10)	1	31 (female)	4
45,XX, rob(13;21)(q10;q10)	1	30 (female)	4
45,XX, der(13;14)(q10;q10)	2	32 (female), 24 (female)	4, 3
Balanced translocation			
46,XY, t(1;12)(q32.1;q24.11)	1	34 (male)	2
46,XX, t(1;13)(q44;q31.2)	1	28 (female)	3
46,XX, t(1;16)(p36.1;p13.1)	1	27 (female)	2
46,XY, t(6;17)(p23;q23)	1	28 (male)	4
46,XX, t(3;4)(p23;q21.3)	1	29 (female)	2
46,XY, t(5;13)(q31;q14)	1	30 (male)	10
46,XX, t(13;18)(q14.3;q21.33)	1	25 (female)	2
46,XY, t(7;18)(q31.2;p11.3)	1	28 (male)	5
46,XX, t(11;22)(q24;q11)	1	37 (female)	4
46,XY, t(2;8;14)(q33;p22;q23.3)	1	31 (male)	7
Inversions			
46,XX, inv(22)(q12).3q13.3)	1	30 (female)	3
Total	15		

Table 3: Gender distribution of abnormal karyotype

Gender	Balanced translocation (%)	Robertsonian translocation (%)	Inversion (%)	Total
Male	5 (50)	0	2 (66.7)	7
Female	5 (50)	4 (100)	1 (33.3)	10
Total	10	4	3	17

Table 4: Comparison of age and number of abortions between male and female carriers

	Male carriers	Female carriers	*P*
Age (years)	30.2±2.49	29.3±3.71	0.635
Number of abortions	5.6±3.049	3.1±0.875	0.027

Data is expressed as mean±SD and evaluated by Student's *t*-test. SD=Standard deviation

Table 5: Polymorphic chromosomal variants in cases and controls

Variants	Number of cases	Number of controls	Age (years)/sex
46,XX,15cenh+	1		32 (female)
46,XY,15pstkstk	1		32 (male)
46,XX,15ps+	1		25 (female)
46,XY,13ps+		1	31 (male)
46,XX,1qh+	2		35 (female), 35 (female)
46,XY,1qh+	2		42 (male), 26 (male)
46,XY,22ps+		1	33 (male)
46,XX,9qh+		1	28 (female)
46,XY,21ps+		1	31 (male)
46,XX,9qh+,14ps+		1	28 (female)
46,X, inv (Y) (p11.2q11.2),1qh+	1		36 (male)
46,X, inv (Y)	1		32 (male)

polymorphic variations (9qh+ and 14ps+). Chromosomes 1 and 9 were frequently seen to show increased heterochromatin region (qh+) in 35.7% of cases showing polymorphic variations. Furthermore, 35.7% and 7.1% cases of polymorphic variations were found to represent variations in the size of satellites (ps+) of all acrocentric chromosomes (13, 14, 15, 21, and 22) and length of stalks (pstkstk) of chromosome 15, respectively. Of these, pericentric inversion of Y chromosome was reported in 2 cases which constituted 22.2% of RM cases with polymorphic variations. A comparison of occurrence of polymorphic variations between the RM group and the control group is shown in Figure 1. Furthermore, satellite associations in 36 (7.4%) RM cases and 23 (5.5%) controls were seen in the present study. However, we observed a nonsignificant difference (P = 0.08) for satellite associations between the RM group and the control group. Moreover, no numerical chromosomal anomaly was seen either in RM cases or controls. The overall frequency distribution of different cytogenetic findings observed in our study is shown in Figure 2.

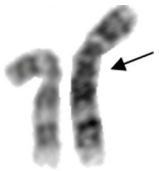
Discussion

The most likely mechanism involved in the pathogenesis of RM is a multifactorial mode of inheritance. Mechanisms such as skewed X chromosome inactivation, genomic imprinting, single gene mutations, chromosomal instability, and sperm chromosome abnormalities are believed to explain idiopathic pregnancy losses.[21] In general, 1 in 6 couples experiences difficulties in the outcome of a pregnancy.[22] Approximately, 15%–20% of all human pregnancies end up in spontaneous

Table 6: Few of the observed polymorphic variations in our study

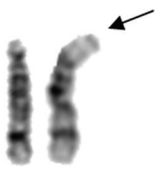
Increased heterochromatin of q-arm (qh+)

1qh+

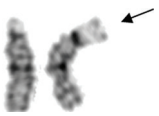


9qh+

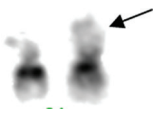
Increased satellites of acrocentric chromosomes



14ps+



15pstkstk



21ps+

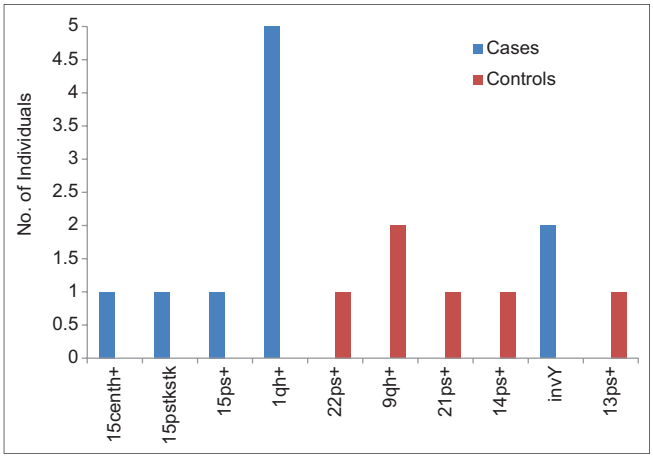


Figure 1: Comparison of polymorphic variations between recurrent miscarriage cases and controls

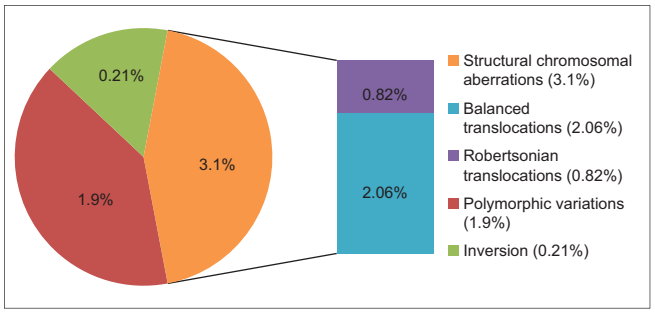


Figure 2: Cytogenetic findings in the recurrent miscarriage group in the present study

abortions. Of this, 50% of the abortions are caused due to chromosomal abnormalities in the developing fetus.[23] The frequency of chromosomal abnormalities in spontaneous abortions is such that majority (95%) cases have numerical anomalies, of which 60% show trisomy, 20% have monosomy X, and the remainder (15%) is polyploidy especially triploidy.[24,25] Although the frequency of chromosomal anomalies in RM couples varies among different populations, still it is found to be higher when compared to general population (0.3%–0.4%).[26,27] The carriers of balanced reciprocal translocation are at the risk of meiotic nondisjunction. During first meiotic division, the mispaired translocated chromosomes eventually result in different forms of segregation leading to aneuploidy of the translocated chromosomes.[28]

In the present study, we observed 3.1% structural chromosomal aberrations in the RM group. Our results are consistent with other studies who have reported this frequency to be 3.35%[8] and 3%–4%.[29,30] Various other studies[9,31] have observed variable frequency ranging from 6% to 12% which is quite higher than that observed in our study. The variation observed in these frequencies can be attributed to differences in sample size and criteria used for participant selection in these studies.

In this study, no numerical chromosomal aberration was observed. Although different proportions of numerical and structural chromosomal aberrations have been observed in different studies[24,32,33] still, a higher proportion of structural anomalies is normally seen as compared to numerical aberrations in all of them.

In this study, the most common structural aberrations were translocations and further, a higher proportion of balanced translocations (66.7%) were observed as compared to Robertsonian translocations (26.7%). Similarly, higher proportions of balanced (46%) in comparison to Robertsonian (18%) translocations have been reported by Tunç *et al*.[34] Our findings are in concordance with the previous findings[10,24,30,35] reported in the literature. In this study, chromosomes 1, 4, 8, and 13 were frequently involved in balanced translocations and out of this, chromosome 1 alone accounted for 30% of the translocation cases. Dutta *et al*.[8] have also observed chromosome 1 to be frequently involved in translocation cases. The size of the chromosome segment, frequency of the breakpoints and their positions play an important role in reproduction. In case of translocations, breakpoints are nonrandom, especially in couples with bad obstetric history.[36] In carriers of balanced translocations, errors occurring during meiosis-I and II lead to the formation of unbalanced gametes which further results in partial trisomy and partial monosomy of the regions involved in rearrangement.[37]

In the present study, number of affected females is 9 (64.2%), whereas males are 5 (35.7%) in number when balanced translocations and Robertsonian translocations are considered together. However, balanced translocations when considered alone, 1:1 ratio of affected females to males is observed. Our study is in agreement with the majority of studies[31,38,39] which have reported a higher number of females with balanced chromosomal translocation as compared to the male carriers. Dutta *et al*.[8] in their study reported that female partners affected with translocation were seen in 18 cases (66.66%) while males were affected only in 9 cases (33.33%). These findings are in concordance with other reports which have described the association of maternal chromosomal constitution and RM.[40,41]

In this study, 4 (0.82%) cases of Robertsonian translocations were seen and interestingly, all affected individuals were females. In general population, its frequency has been reported to be 0.1% as compared to 1.1% in couples with recurrent pregnancy loss. Of all cases of Robertsonian translocation, chromosome 13 and 14 alone accounts for 75% of the cases.[42] In this study

also, chromosome 13 was involved in all the 4 (100%) cases while chromosome 14 accounted for 50% of the abnormal cases. Our finding is consistent with other studies[34] who have reported Robertsonian translocations involving chromosomes 13 and 14 in 58.3% of their cases and where women constituted majority (83.3%) of the cases. Between male and female carriers, the current study depicted a significant difference (P = 0.027) for number of abortions which lies in contradiction to Fan *et al*.[39] who have reported significantly more number of abortions in female carriers (mean = 3.21) in comparison to male carriers (mean = 2.6).

Moreover, in our study, 9 cases of polymorphic variations in the RM group and 6 in the control group were seen. Further, female-to-male ratio was 5:4 in RM cases and 2:3 in control group. Acrocentric chromosomes (13, 14, 15, 21, 22), chromosomes 1 and 9 were commonly seen to show polymorphic variations. Satellite polymorphic variants (ps+ and pstkstk) (42.9%) and qh+ (35.7%) heteromorphism constituted a major part of polymorphic variants documented in our study. Similarly, De la Fuente-Cortés *et al*.[35] have also reported qh+ and satellite polymorphisms to be 30.9% and 22.15%, respectively. Chopade *et al*.[43] have documented 29 cases (16 males and 13 females) of chromosomal heteromorphisms in acrocentric chromosomes with a frequency of 10% in males and 8.12% in females. On evaluating 842 cases of RM and infertility, Madon *et al*.[17] reported a frequency of polymorphic variants as 28.2% in males and 17.19% in females. Statistically significant increase in the frequency of chromosomal variants in infertile women (28.3% vs. 15.16%) and infertile men (58.68% vs. 32.55%) as effect of epigenetics on phenotype has been observed by Minocherhomji *et al*.[18] On the other hand, Brothman *et al*.[44] concluded these common cytogenetic variants as heteromorphisms which do not have any clinical significance. Several studies have suggested that these heteromorphisms play a role in spindle attachment and chromosome movement, meiotic pairing, and sister chromatid cohesion.[45]

Satellite associations among different acrocentric chromosomes were observed in 36 (7.4%) RM cases and 23 (5.5%) controls in our study. According to Anuradha *et al*.[46] these satellite associations might predispose chromosome to nondisjunction, and hence, lead to translocations as a significant increase of satellite associations in the RM couples has been reported in their study. Furthermore, 3 cases of inversion were reported in our study. Of these, 2 men were showing pericentric inversion of the Y chromosome while 1 woman had an inversion in chromosome 22. Pericentric inversions have been found to be associated with RM. During crossing over in meiotic division, inversions may lead to deletion or duplication of a chromosome segment.[47] Posam and Sabnis,[48] reported 1.2% of cases with pericentric inversion of Y chromosome in their study. Inversion of Y chromosome is considered to be a chromosomal heteromorphism without any clinical significance and does not affect sperm production.[49,50] The clinical significance of polymorphism of various chromosomes has always remained debatable but cytogeneticists should consider these variations as they play an important role in reproductive failure.[51] Although number of cases reported with inversion Y and polymorphic variations in different chromosomes in RM is scanty, still its presence in RM should not be ignored and considered for further molecular level investigations.

Conclusions

Our study has depicted 3.1% structural chromosomal aberrations and 1.9% polymorphic variations among RM couples. Our results are in agreement with previously published studies. Further, number of abortions was significantly higher in male carriers of balanced translocations as compared to female carriers in the RM group. However, we did not find any significant difference for polymorphic variations between the RM group and the control group. Furthermore, this study presented a limitation of small sample size to produce conclusive results regarding polymorphic variations in RM cases. Thus, cytogenetic investigation of couples with RMs and genetic counselling for the carriers of balanced translocations to monitor their future pregnancies are strongly recommended.

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Conflicts of interest

There are no conflicts of interest.

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