

## Occurrence of Chromosomal Alterations in Recurrent Spontaneous Abortion Couples: A Case-Only Study from Kashmir, North India

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### Abstract

The present study was proposed to unveil the incidence and pattern of chromosomal abnormalities in recurrent spontaneous abortion couples of Kashmir, North India. A total of 71 couples within the age group of 24 to 42 years and having history of two or more recurrent spontaneous abortions were included in the study. Peripheral blood lymphocyte cultures were set for each subject according to standard protocol and chromosomal analysis was carried out on well spread metaphases by the help of Cytovision software Version 3.9. The incidence of chromosomal abnormalities in spontaneous abortion couples of this region was found to be 7.75% that include numerical (1.40%) as well as structural (7.75%) chromosomal abnormalities. Both males (2.11%) and females (5.63%) possessed chromosomal aberrations that comprised balanced translocations (4.22%), duplications (0.70%), deletions (0.70%) and inversions (2.11%). Besides, We report three unique balanced translocations viz., t(1;3)(q24.3;p25)(1 case); t(6,16)(p11;q23)(1 case) and t(7;14)(p13;q12)(2 cases). that have not been found elsewhere in the literature. We conclude from the present study that chromosomal alterations do occur as an etiology in the RSA couples of Kashmir and their incidence is consistent with many reports around the world. The precise molecular characterization of the unique breakpoint regions reported in our study could help in identification of new genes involved in recurrent spontaneous abortions. The study being the first of its kind in this part of the world forms the basis for further studies of the couples of this region with recurrent spontaneous abortions.

### Introduction

Miscarriages are the most common complication of pregnancy, affecting approximately 15% of all clinically recognized pregnancies in the general population. It is estimated that more pregnancies are lost spontaneously than are actually carried to term [1-2]. Most of the miscarriages are sporadic and non-recurrent, and are often caused by chromosome abnormalities in the fetus [3]. Recurrent spontaneous abortion (RSA) is often defined as the occurrence of three or more consecutive, clinically detectable pregnancy failures before the 20th week of gestation [4-6]. However, some studies have also included patients with two miscarriages [7,8].

There are numerous factors that may cause RSA, but the underlying problem often remains undetected. Although much work has been done to identify the underlying mechanisms, the cause of miscarriage can be identified in only about 50% of cases. The known causes of RSA include chromosomal and metabolic abnormalities, uterine anomalies and immunologic factors [9,1]. Cytogenetic screening of couples with RSA has revealed that parental chromosomal abnormalities occur in either partner in 5-7% of couples with RSA, while the rate in the normal population is approximately 0.2% [10].

Uterine defects, such as uterine anomalies and fibroids, can predispose to miscarriage by affecting implantation [11,12]. Subnormal hormone production or abnormal endometrial response to circulating steroid hormones is one another cause of miscarriages [9,13]. Besides, several other maternal endocrinological abnormalities such as uncontrolled diabetes, high androgen levels, hyperprolactinaemia, thyroid dysfunction, and obesity have been implicated as etiological factors for RSA [14,15,16,17,18,19]. Among all, the genetic factors causing RSA are, however, difficult to study because the fetus is lost at an early stage of development and is therefore difficult to examine. Consequently, most of the studies conducted on RSA, are based on studying the couples experiencing the miscarriages. As the identification of the underlying factors is crucial for the development of more successful treatment and improvement of the outcome of

future pregnancies in couples experiencing RSA, the present study was conducted to identify chromosomal alterations in couples with RSA referred to our centre for cytogenetic analysis.

### Methodology

#### Subjects

A total of 71 couples (142 subjects) within the age group of 24 to 42 years were evaluated in this study. All the couples were recruited from the patient group referred by clinicians with a history of two or more pregnancy losses during the year 2013 and 2014. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans. An informed consent was taken from each patient as per the norms of Institutional Ethics Committee. The history of the patients was noted in a predesigned standard format to study the inheritance pattern. All the necessary information of the couples, their offspring and parents was noted down.

#### Lymphocyte cultures

Peripheral venous blood samples were collected in heparinized vacutainers from each patient and aseptically transferred into sterile

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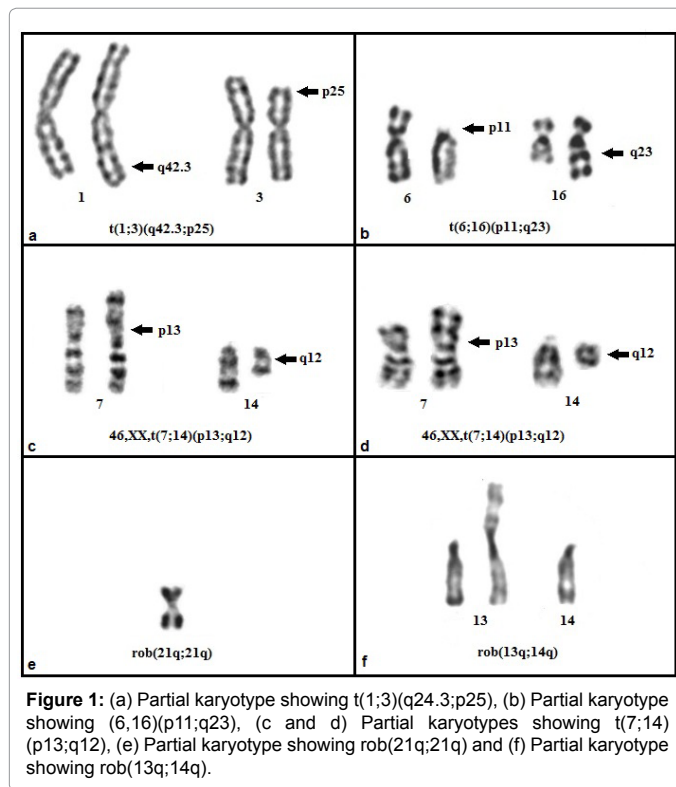
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culture tubes with 5-8ml of RPMI-1640 medium (Sigma, Michigan, USA), supplemented with L- glutamine, 10% fetal bovine serum (Himedia Labs, India), Penicillin - streptomycin solution (Invitrogen, California, USA) and phytohaemagglutinin (Himedia Labs, Mumbai, India). Lymphocyte cultures were set according to modified standard

protocol [20]. Parallel cultures were set for each sample. The culture tubes were marked accordingly and incubated in a CO<sub>2</sub> incubator for 72 hours. 50µl colchicine was added to each culture tube at the completion of 70 hours to arrest the cells at metaphase. After 72 hours of incubation, the cell suspensions were centrifuged for 10 minutes at 1000 rpm. The supernatant was discarded and the pellet was treated with hypotonic solution (0.075M KCl) by gentle flushing and cyclomixed. The centrifuge tubes were incubated again at 37°C for 45 minutes. The tubes were again centrifuged carefully at 1000 rpm for 15 minutes. The supernatant was removed and 5-8 ml of freshly prepared pre-chilled Cornoy's fixative was added to the pellet while mixing on cyclomixer. The tubes were allowed to stand overnight and washed with freshly prepared pre-chilled Cornoy's fixative repeatedly for 3-4 times. GTG banding was performed and the slides were stained with 1% Giemsa stain [21].



### Karyotyping

Karyotyping was performed with the help of Cytovision software Version 3.9 (Applied Imaging, Michigan, USA) on well spread G-banded metaphase plates. At least 30 metaphases were examined for each subject to rule out any chromosomal anomaly and mosaicism.

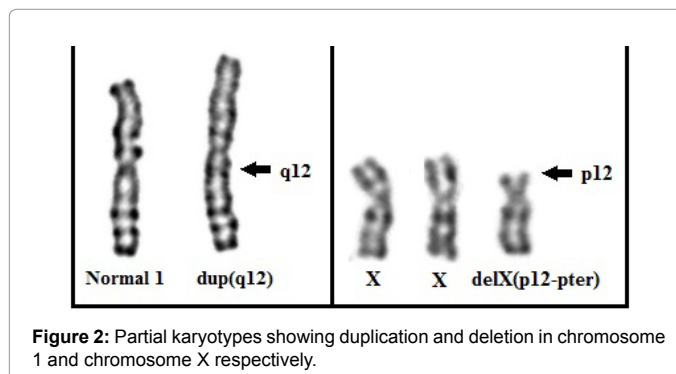
### Results

A total of 142 cases (71 couples) were evaluated for karyotyping analysis to rule out the possible chromosomal anomalies as an etiology for their history of recurrent spontaneous abortions. Eleven cases (7.75%) were found to have chromosomal abnormalities including numerical ( $n=2$ , 1.40%) as well as structural ( $n=11$ , 7.75%) chromosomal abnormalities. Among these both male partners ( $n=3$ , 2.11%) and female partners ( $n=8$ , 5.63%) possessed chromosomal alterations that comprised balanced translocations ( $n=6$ , 4.22%) (Figure 1), duplications ( $n=1$ , 0.70%), deletions ( $n=1$ , 0.70%) (Figure 2) and inversions ( $n=3$ , 2.11%). The karyotype particulars of couples are shown in Table 1.

The incidence of chromosomal alterations in recurrent abortion couples of the present study, excluding the advance maternal age females, females with Toxoplasma/Rubella/Cytomegalovirus/Herpes Simplex Virus (TORCH) infections, intrauterine malformations, hypothyroidism and abnormal immunological profiles was found to be 11.22%.

### Discussion

Chromosomal anomalies are known to be the single most common cause of spontaneous abortion. About 50% of spontaneously expelled abortuses have been found to have chromosomal abnormality as revealed by previous studies [22]. Several studies have been carried out to determine the frequency of chromosomal aberrations among couples with repeated fetal loss and it has been reported that about 3-5%



S. No	Cytogenetic grade	Karyotype	
		In females (no. of cases)	In males (no. of cases)
1	Normal Karyotype	46,XX (63 cases)	46,XY ( 68 cases)
2	Balanced Translocations	46,XX, t(1;3)(q24.3;p25)(1 case)	46,XY,t(6,16)(p11;q23)(1 case)
		46,XX,rob(13q;14q) (1case)	46,XY/46,XY,t(7;14)(p13;q12)(1case)
		46,XX,rob(21q;21q)(1 case)	
		46,XX/47,XXX/	
3	Duplications	46,XX,t(7;14)(p13;q12)(1case)	
4	Deletions	46,XX, dup(1q12) (1 case)	
5	Pericentric Inversions	47,XXX, delX(p12-pter) (1 case)	
		46,XX, inv9(p12;q12)(2 cases)	46,XY, inv9(p12;q12) (1case)

**Table 1:** Karyotype particulars of couples referred for cytogenetic analysis (Number 71).

couples with recurrent spontaneous abortions have one partner with a cytogenetic abnormality [23-25]. In the present study chromosomal abnormalities were observed in 7.75% of couples which is markedly higher than these reports. However, Niroumanesh et al. (2011) reported that 12% of the patients with the history of recurrent miscarriages have chromosomal abnormalities [26]. In a North-Indian population, Dubey et al (2005) reported 2%(31/1484) cases of the recurrent abortions to have a chromosomal anomaly which is quiet low as compared to our study [27]. In another study by Iravathy et al (2006) chromosomal abnormalities were reported in 16.6% (10/60) of the patients [28]. The differences in the frequency of chromosomal abnormalities reported in isolated studies may be due to the variation in the number of subjects and their selection criteria.

The female subjects of the present study, having advanced maternal age, TORCH infections, intrauterine malformations, hypothyroidism and abnormal immunological profile as an independent etiology for recurrent abortions, if excluded raises the incidence of chromosomal alterations in the studied patients to 11.22% which is higher than that reported earlier [23-25].

However, the percentage of patients having chromosomal abnormalities is almost similar as reported elsewhere [26].

Both numerical as well as structural chromosomal anomalies were observed in the subjects of our study, however, 4.22% were found to have translocations. Translocations are known to be the most common type of structural chromosomal abnormalities including both balanced as well as unbalanced translocations [29].

Balanced translocations account for the largest percentage of these karyotype abnormalities. All the translocations observed in the present study were found to be balanced-type that include reciprocal translocations (n=5, 3.52%) and Robertsonian translocations (n=1, 0.70%).

Reciprocal (non-Robertsonian) translocations are one of the most frequently occurring human chromosomal aberrations and have a frequency of about 7% in couples with recurrent miscarriages. These rearrangements are twice more common in females than males [30]. However, in the present study balanced translocations were evenly present in males and females. Besides, Robertsonian translocations were found in female partners of two couples. Couples carrying balanced chromosomal rearrangements can produce abnormal gametes with unbalanced chromosomal rearrangement during gametogenesis and transfer this abnormality to their foetus, which may result in either RSA or congenital abnormalities [31,32]. This could be the possible cause of RSA in couples of our study harbouring balanced translocations.

We report three unique chromosomal translocations in four different subjects of our study that have not been found elsewhere in literature viz., t(1;3)(q24.3;p25)(1 case); t(6,16)(p11;q23)(1 case) and t(7;14)(p13;q12)(2 cases). The presence of t(7;14)(p13;q12) in one male and one female partner of two different couples indicates that the breakpoints involved must have a significant role in the etiology of RSA that need to be ascertained by their molecular characterization.

In our study, three (4.22%) of the male partners were found to harbor structural chromosomal aberrations. The structural chromosomal problems in men often lead to low sperm concentrations, abnormal sperms or teratozoospermia male infertility, and therefore, a reduced likelihood of pregnancy and increased miscarriage [33]. However, sperm analysis of these males with structural chromosomal abnormalities was not available.

Numerical chromosomal aberrations are less frequently encountered among couples with repeated abortions. Those aberrations are usually in the form of sex chromosomal aneuploidy, and they occur in a very low frequency of less than 0.15% of cases [34]. Two of the female subjects (1.40%) of our study had numerical abnormality. One was found to have 46, XX/47, XXX/46, XX, t (7; 14) (p13; q12) mosaic karyotype and the other 47, XXX karyotype. However, the second female had a deletion (delXp12-Xpter) in the extra X chromosome. Previously Malla and co-workers in a case report have discussed de novo Xp terminal deletion as a rare one and as a possible cause of recurrent abortions in a triple X female [35].

Pericentric inversion of chromosome 9 reported in our study has been implicated in various studies to recurrent spontaneous abortions and bad obstetric history [36,37]. Besides, it has been found that females with a pericentric inversion have a 7% risk of abnormal live-born infants and males carry a 5% risk [22]. Although pericentric inversions of chromosome 9 are said to be common in general population, they need to be considered in recurrent pregnancy loss to determine future risk and better genetic counseling and management [38,39].

The karyotype particulars of the products of conception of the couples of our study were however, not available. However, the inheritance pattern of the chromosomal anomalies that were found in the couples was explained to them as a plausible cause of recurrent miscarriages. Besides, the associated risk of recurrence of spontaneous abortions in these couples was explained in their post-test genetic counseling sessions routinely done at our centre.

In addition to the chromosomal alterations among the subjects of the present study, twelve females partners (16.90%) had deranged hormonal profile (hypothyroidism), four females (5.63%) had abnormal immunological profile (Lupus/Anti-nuclear antibodies/Antiphospholipid antibodies positive) and six female subjects (8.45%) were positive for TORCH infection. Intrauterine malformations/ovarian cysts were reported in four (5.63%) female subjects. However, all of them had normal karyotypes (46, XX). The spontaneous abortions in these females could possibly be due to their abnormal hormonal and immunological profiles, TORCH infection and intrauterine conditions, if the other etiologies like gene mutations are excluded.

Eighteen (25.35%) of the female partners of studied couples were found to have advanced age (35 years and above) which is reported to be an independent risk factor for miscarriage [40-42]. Besides, the sharp increase in the rate of miscarriage in women of advanced maternal age has been attributed to increasing rates of aneuploidy in association with older oocytes [43,44]. In the present study, two females (11.11%) of the advanced age group were found to have abnormal karyotypes. Considering advanced maternal age as an independent risk factor, the incidence of chromosomal alterations in the RSA couples of our study was calculated to be 8.87% which is higher as reported elsewhere in the literature [23-25].

## Conclusion

The results of the present study conclude that chromosomal alterations do occur as an etiology in the RSA couples of Kashmir in almost the same range as that reported by several other workers at isolated places. However, we report for the first time three unique cases of chromosomal translocations associated with RSA. Precise molecular characterization of the breakpoint regions in these unique translocations can be helpful in identification of new genes or genes involved in RSA and also help in better understanding of molecular mechanism underlying these alterations. Besides, it establishes the

cause of the fetal losses and helps in genetic counseling. Cytogenetic analysis, therefore, should be mandatory for all the couples with reproductive failures. The carriers of such abnormalities should be informed about the risk of the birth defects in their offspring due to de novo submicroscopic rearrangements. Adequate genetic counseling strategies should also be offered which could allow the couples to make an informed reproductive decision regarding subsequent pregnancies. The study being the first of its kind in this part of the world forms the basis for further studies of the couples of this region with RSA.

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