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MATERNAL-FETAL MEDICINE

High incidences of chromosomal aberrations and Y chromosome micro-deletions as prominent causes for recurrent pregnancy losses in highly ethnic and consanguineous population

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Abstract

Purpose Recurrent Miscarriages (RM) commonly complicates the reproductive outcome where prominently chromosomal aberrations and molecular factors lead to recurrent miscarriages. We investigated couples with RM for cytogenetic abnormalities and Y chromosome microdeletions in males along with detection of aneuploidies de novo in the product of conception from a highly ethnic consanguineous population (Kashmir, North India) .

Study design Chromosomal analysis was done by Karyotyping on peripheral blood lymphocyte cultures and analyzed by Cytovision software Version 3.9. Microdeletion in Y chromosome was performed by STS-PCR and QF-PCR was used to detect aneuploidy in the product of conception.

Results Of the 380 samples (190 couples) screened for cytogenetic analysis, 50 (13.1%) chromosomal aberrations were detected in both couples. Numerical aberrations were detected in 16.0%, inversions 22%, duplications 16.0% and translocations were found in 26.0% with three unique reciprocal translocations in males. The couples bonded consanguineously had 32% chromosomal changes with a significant difference in chromosomal inversions (37.5% vs. 14.7%) and translocations (37.5% vs. 20.6%) for consanguineous and non-consanguineous group, respectively ($p < 0.05$). Further, translocations and inversions (44.5% and 33.3%) were significantly implicated in couples with a positive family history of RM ($p < 0.05$). Y chromosome deletions were found in 2.1% cases of males.

Conclusion We conclude 15.2% couples affected either by chromosomal or Y chromosome deletions contribute hugely in the diagnosis and management of repeated pregnancy losses. It is recommended that couples that belong to consanguineous and multigenerational group of RM should be considered for cytogenetic and molecular testing after two abortions for successful pregnancy outcomes and management of RM.

Keywords Recurrent miscarriages · Reproductive outcome · Aneuploidies · Consanguinity · Cytogenetic analysis · Kashmir (India)

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Introduction

Recurrent Miscarriages (RM), a common complication of pregnancy is estimated to occur in 10%–15% of pregnancies and about 80% of these miscarriages occur within 2 to 3 months of gestation [1]. Approximately 2% of women suffer two and 0.4%–1% of women experience three consecutive losses [2, 3]. History of one or more first-trimester abortion is related to an increased risk of abortion for the following pregnancy.

RM is a complex multifactorial problem proceeded with endocrine, autoimmune disorders, [4], advanced maternal and paternal age, infections and congenital or uterine

defects in addition to genetic aberrations [5]. Reportedly about 15%–20% of all pregnancies end in spontaneous miscarriages and the chromosomal abnormalities account for cases is nearly 70% [6, 7]. There are reports where studies have depicted the occurrence of recurrent pregnancy losses (RPL) and sporadic miscarriage in families with normal parental chromosomal status [8]. Reports suggest that the frequency of RM increases with elevated risk of subsequent miscarriage.

Any alteration either in the number or structure of chromosomes has a deleterious impact on the expression of a concerned gene that can result in severe physiological and developmental defects. Chromosomal disorders are reported in about 10%–15% of all conceptions [8]. These chromosomal aberrations are the main factors for fetal wastage and may cause birth defects and mental retardation among those pregnancies that survive to full term. Incidence of chromosome number deviation and its structural aberration in RM abortuses accounts for 47.9%, polyploidy 9.8%, monosomy X 8.6%, and 26.8% trisomies for one or more chromosomes [9]. Most unbalanced chromosome aberrations result in severe phenotypes already in early pregnancy and therefore lead to miscarriage. Consequently, the incidence of fetal unbalanced chromosomal abnormalities gradually decreases with the duration of pregnancy to less than 1% among live-born children [10]. Among 50%–70% of all RM cases, cytogenetic aberrations are found in the fetus and the most prominent of these abnormalities is the transmission of an extra chromosome from either parent or polyploidy, loss of X, and translocation events [10, 11]. These chromosomal alterations are inherited or emerge as de novo in the embryo due to aberrant oocyte meiotic division [12]. Either of the parents with a balanced translocation as reciprocal or Robertsonian are considered among the most common abnormalities, found in around 4% of couples with RM. Further, carriers of such translocations confer the risk of producing a chromosomally altered fetus to a frequency of 20%–80%. The other chromosomal abnormalities include para- or pericentric inversions and sex chromosome aberrations [13]. Chromosomal rearrangements dictate the reproductive risk by various factors as the size of chromosome involved, its location, and type of rearrangement carried by either partner [14, 15].

Cytogenetic evaluation of couples with RM has been reported to harbor parental chromosomal aberrations in either partner with a frequency of ~ 7%, while its frequency in the normal population is ~ 0.2% [16]. Accumulating evidences point out that male partner may equally play a pivotal role as a contributing factor in RM for pregnancy losses [17]. The quality and integrity of sperm is very essential for its optimal interactions with the female ovum and its preliminary embryonic growth and development. The genes expressed by the male partners regulate not only the growth and invasiveness of trophoblast cells but aid in placental proliferation [18]. It is known that about half the cases of RM in women still remains unexplored and male factor is now considered one among the many reasons for recurrent pregnancy losses [19]. The microdeletions within 10 Mb AZF portion of Y chromosome are among the most prominent structural defects in the male reproductive problems like infertility, In vitro fertilization failures, testicular histology and now in the RM [20, 21]. Through various investigations, a plausible link has been established that have confirmed the potential connection between specific micro deletions within Y chromosome AZF region (q arm) in males and RM [18, 19, 22]. Therefore, the second objective pertaining to RM was to detect microdeletions using a multiplex STS-PCR within Y chromosome for AZFa, AZFb and AZFc regions in men whose spouses suffered from RM.

Further, detection of aneuploidy in abortuses from the couples helps to explain the genetic cause of RM that are likely inherited or may appear de novo. Certain specific chromosomal trisomies like 13, 18, 21, and sex chromosomes have been seen to induce abortion of the fetus and therefore, result in pregnancy loss. The detection of these aneuploidies by the latest molecular technique by Quantitative florescent polymerase chain reaction (QF-PCR), in which certain short tandem polymorphic marker repeats on known chromosome loci are amplified.

Our population is genetically conserved and people do not prefer to marry outside their ethnic territorial boundaries and thus consanguineous marriages are also very common, a factor that compounds certain genetic disorders. Various investigations have seen that interrelated breeding contributes to increased wastage of fetus through congenital defects and recessive genetic disorders [23]. Evidence reports that the severity of the deleterious effect on embryo mostly occurs in cases where closer the relationship between parents is closer [24]. Hence, consanguinity and genetic disorders, being related to serious health problems, are always considered most important in genetic studies [25].

A substantial number of RM cases occur in our population and we have observed that apart from consanguinity, the pattern of these events observed defies the norm of it being more prevalent in advanced maternal age but are seen in lower age group also (< 30 years). Keeping in view the nature of the strong ethnic population and high incidence of RM, we initiated a cohort study in couples with 2 or more miscarriages to evaluate them for cytogenetic evaluation along with detection of most common chromosomal abnormalities in the product of conception (POC) mostly leading to fetal death by QF-PCR in addition to analysis of microdeletion in Y chromosome by multiplex STS-PCR in male partners to rule out their sole contribution in RM.

Material and methods

Sample collection

A total of 190 female and their corresponding 190 male subjects with two or more than two pregnancy losses were included in this study for evaluation of chromosomal aberrations through conventional cytogenetics. Moreover, all 190 male subjects among the couple were evaluated for Y chromosome micro deletions by multiplex polymerase chain reaction as a male factor responsible for pregnancy losses. Further, 60 tissue samples from the product of conception (abortuses) were examined for chromosomal numerical aberration by Quantitative Florescent Polymerase Chain Reaction. The study was conducted between 2014 and 2018 in Advanced Centre for Human Genetics, SK Institute of Medical Science (SKIMS), J&K (North India). For chromosomal analysis, all the cases were referred from the Department of Obstetrics and Gynecology (SKIMS) and also from various related hospitals of the city. It was ensured that the cases were in strict compliance with the basic diagnostic criteria of RM and all those cases before and/or at 20th week of gestation were included in the study. A comprehensive and detailed pedigree analysis was done to lay emphasis on the cases which have multigenerational history of RM. Clinical evaluation was done and the investigations were collected from all cases. The patient's hormone profile, immunological profile, biochemical and radiological investigations were taken into consideration.

Lymphocyte cell culture for Karyotyping by GTG banding

The peripheral blood cultures for metaphase chromosome preparations from made as per standard cytogenetic protocols. Cytogenetic analysis was done by GTG (G banding using Trypsin and Giemsa). Venous blood (2–3 ml) was obtained in a labeled sterile heprinized syringe. Immediately blood cultures were initiated without any delay and slide preparations were done as per the previous protocol [26]. Freshly collected heprinized blood was cultured RPMI 1640 (containing fetal calf serum and phytohemagglutinin (PHA) (GIBCO, Thermo Fischer Sci.) at 37 °C for 72 h followed by the addition of Cholchicine (0.45 mg/ml). After one hour, the cells were harvested by centrifugation (2000 rpm/10 min) and the cells re-suspended with the remaining solution. Hypotonic treatment (0.075 M KCL) is subsequently done followed by cell concentration and the pellet was re-suspended with remaining hypotonic solution and fixed with solution (3: 1, Methanol: Glacial acetic acid). After required washings and fixation, finally slides were prepared followed by treatment with trypsin. Karyotyping was done with the Cytovision Version 3.9 software (Olympus, Japan).

Quantitative florescent (QF-PCR) for detection of aneuploidy

Quantitative florescent (QF-PCR), a modern molecular technique uses amplification of chromosome-specific repeated DNA sequences known as small tandem repeats (STRs) exploiting di, tri or tetra- nucleotide small tandem repeats (STR) for the detection of prominent chromosomal aneuploidies 13, 18, 21, X and Y in diagnosis recurrent miscarriages. The sample DNA from product of conception (POC) is amplified by PCR using fluorescent primers to help in product visualization and quantified as peak areas of the respective repeat lengths using a DNA analyzer (ABI 3500) with the gene-mapper software.

DNA from tissue of product of conception was using a DNA easy tissue kit (Qiagen GmbH, Germany) according to manufacturers' instructions. The quality of DNA and purity of DNA was checked on spectrophotometer.

QF-PCR technique was performed with a DNA analyzer ABI prism 3500, making use of a commercial multiplex QF-PCR diagnostic kit which could detect trisomy 21, 22, 18, 13, and sex chromosome aneuploidies X and Y (QF-PCR). The Devyser Extend v3 QF-PCR kit contains 27 multiplex markers and sets of short tandem repeats (STRs) that can be used for amplification of selected microsatellites and the amelogenin-SRY (sex-determining region Y chromosome). A 25 µl reaction was set for amplification and it was prepared strictly as per the instruction given in the protocol of the kit. The amplified PCR products were subjected to electrophoresis for separation and analysis by automated genetic analyzer 3500 (ABI, Life Technologies, USA). Gene mapper software in the genetic analyzer was utilized for the interpretation of results. The relative amount of each allele was quantified by determining the ratio of the peak heights or peak areas. A normal diploid sample produced two of each of the somatic chromosomes. For homozygous and heterozygous conditions, specific STR marker of two alleles of a chromosome are detected as two peaks in a 1:1 ratio and one peak respectively. Three peaks in a 1:1:1 ratio and two peaks in a 2:1/1:2 confirm an additional allele that corresponds to the presence of an additional STR marker for extra chromosome (like trisomy).

Y chromosome micro deletion by multiplex PCR

DNA extraction of blood samples from male partners were subjected to DNA extraction using standard phenol/chloroform methods. The purity and concentration of DNA was

estimated at the absorbance of 260 and 280 nm and checked on 1% nuseive agarose gel.

Y microdeletions were evaluated by sequence-tagged sites (STS) using multiplex PCR for regions AZFa, AZFb, AZFc and SRY gene (control) on Y chromosome.

A two-tube multiplex PCR was performed in a final volume of 25 µl containing 50 ng template DNA, 1 × PCR buffer (Biotools, B & M Labs, Madrid, Spain) with 2 mmol/l $MgCl_2$, 0.6 mmol/l of each primer (Genscript, Piscataway, NJ), 50 mmol/l dNTPs (Biotools, B & M Labs), and 1.5 U *Taq polymerase* (Biotools, B &M Labs). The multiplex primers included for tube A to evaluate micro deletions SY84 (AZFa), SY127 (AZBFb) and SY254 (AZFc) whereas tube B included SY 86(AZFa), SY134 (AZFb) and SY 255 (AZFc). The SRY gene was put in both the tubes as control to check the presence of SRY gene bearing these regions. The primer sequences and their interpretation are given in supplementary table S1. For PCR amplification, the standard protocol was used as follows: one initial denaturation step at 94 °C for 7 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, and 30 s of extension at 72 °C, followed by a final elongation cycle at 72 °C for 5 min. Supplementary Fig. 1 is given to show the overall workflow and the sample selection.

Statistical analysis

Different tests for homogeneity of proportions including Chi-square analysis to evaluate survival outcome probabilities were used to determine the significance of the distribution patterns with respect to different clinico-analytical parameters. Statistical analysis was performed by using IBM Statistics SPSS software (Version-23). Statistical significance was set at the level of $p < 0.05$.

Results

A total of 190 couples (380 cases), who suffered pregnancy losses due to recurrent miscarriages were included in this study. Among females with RM, 89 (46.8%) and 101 (53.1%) were seen in the age group of < 30 and ≥ 30 years respectively while on the other hand, 50 (26.3%) in men whose partners suffered RM were < 30 years of age as compared to 101 (73.6%) with age group ≥ 30 years. Among cases with a multigenerational history of RM, there were 35 (18.4%) cases as compared to 155 (81.5%) with a negative history. Affected females who had a family history of RM aggregated to 18.4% as against 21.0% in males. Couples with consanguineous marriage with RM had a frequency of 21.0% versus 79.0% non-consanguineous ones. Number of pregnancy losses in patients with recurrences 2, 3, 4, 5, 6 and 8 were 40.5%, 39.4%, 15.7%, 2.6%, 1.0% and 0.52% respectively. All other details about the patients with RM are given in Supplementary Table S2.

Among the age group < 30 years, frequency of 2, 3, 4, 5 and 6 miscarriages in females was 38 (42.6%), 37 (41.5%), 10 (11.2), and 2 (2.2%) as against males 20 (40.0%), 13 (26.0%), 5 (10.0%) and 1 (2.0%) respectively. In cases of ≥ 30 years, the frequency ratio of two miscarriages among female: male partners was as 39 (38.6%):57 (40.7%), for three miscarriages 38 (37.6%):64 (45.7), for four events 20 (19.8%):20 (14.2%), for five events 3 (4.9%):4 (2.8%) respectively. The ratio of frequency of 2, 3, and 4, miscarriages among the consanguineous and non-consanguineous marriages was observed as 40.9% vs. 39.0% (2 events), 34.1% vs. 42.5% (3 events), 18.2% vs. 15.1% (4 events), respectively (Table 1). Among the pattern of miscarriages in multigenerations, the male and female were observed as to have frequency of two recurrent miscarriages as 42.5% vs. 40%, for three events 28.4% vs. 36.7% and 13.5% vs. 18% for 4 events with rest of the events with almost same frequency (Table 1).

The male partner as a sole contributor to miscarriages was further elucidated by detection of microdeletions in Y chromosome by STS –PCR where we found 4 of the 190 cases with deletion at different regions of AZF aggregating to 2.1% cases of RM (Table 1).

Of the 380 samples (190 couples) screened for cytogenetic analysis, a total of 50 (13.2%) chromosomal aberrations of the varied pattern were detected in both couples (Table 2). Chromosomal numerical aberration were detected in 14.0% where one case each was of 47, XXX (2.0%) and 47, XYY (2.0%) while other subjects had mosaic pattern aggregating to a frequency of 12% (6 of 50) with 46XX/47, XXX accounting for 8% (4 of 50) (Fig. 1A, B). Chromosomal pericentric inversions were found in 22% (11 of 50) cases where inversion 9 chromosome was found in 10% in females [(46, XX, inv(9) (p13 q13)] and 8% in males [(46, XY, inv(9) (p13q13)]. Besides pericentric inversions of chromosome 16 (p11.1q12.2) and 9 (p11, q13) were detected in females each with a frequency of 2%.

Chromosomal translocations were detected with a frequency of 26.0% (13 of 50) involving different chromosomes of varied pattern in both the couples which included 8 (16.0%) in female partners and 5 (10.0%) in males. Two cases were detected as robertsonian translocations (4.0%) 44, XX, *t*(13; 14) (q10; q10) and 45, XY, *t*(13; 1 5) whereas two (4.0%) involved chromosome 7 and 4 as *t*(7; 14) and one each translocation was 46, XX *t*(4:16) (p13.2; q33) and 46, XX, *t*(3; 17) (q29; p11.2). Another female had dual structural change comprising a translocation and deletion in 5q as 46, XX, *t*(3; X) (q21; p11.2), del(5q) (Fig. 1C–F). Three unique reciprocal translocations were found in male partners whose spouses had suffered four events of miscarriage in < 20th week, where one 46 XY, *t*(2; 8) (q31; p11.2) was

Table 1 Demographic features of couples with RM

Maternal parameters	No.	Miscarriages	Events *N* (%)	Paternal parameters	No.	Miscarriages in spouse	Events *N* (%)
Age group							
< 30	89	2	38 (42.6)	< 30	50	2	20 (40.0)
		3	37 (41.5)			3	13 (26.0)
		4	10 (11.2)			4	5 (10.0)
		5	2 (2.2)			5	1 (2.0)
		6	2 (2.2)			6	1 (2.0)
≥ 30 years	101	2	39 (38.6)	≥ 30 years	140	2	57 (40.7)
		3	38 (37.6)			3	64 (45.7)
		4	20 (19.8)			4	20 (14.2)
		5	3 (4.9)			5	4 (2.8)
		8	1 (0.9)			6	1 (0.7)
						8	1 (0.7)
Consanguinity							
Yes	44	2	18 (40.9)	Yes	44	2	18 (40.9)
		3	15 (34.1)			3	15 (34.1)
		4	8 (18.2)			4	8 (18.2)
		5	1/(2.2)			5	1/(2.2)
		6	2 (4.4)			6	2 (4.4)
No	146	2	57 (39.0)	No	146	2	57 (39.0)
		3	62 (42.5)			3	62 (42.5)
		4	22 (15.1)			4	22 (15.1)
		5	4 (2.7%)			5	4 (2.7%)
		8	1 (0.06)			8	1 (0.06)
Family history							
No	155	2	66 (42.5)		150	2	60 (40.0)
		3	44 (28.4)			3	55 (36.7)
		4	21 (13.5)			4	27 (18.0)
		5	5 (3.2)			5	5 (3.4)
		6	2 (1.2)			6	2 (1.3)
		8	1 (0.6)			8	1 (0.06)
Yes	35	2	15 (42.8)		40	2	17 (42.5)
		3	15 (42.8)			3	20 (50.0)
		4	5 (14.2)			4	3 (7.5)
				Y chr del	190		4 (2.1)

detected in a 30 years male and the other two translocations 46, XY, *t*(6; 16) (p11; q24) and 46, XY, *t*(4; 6) (q35; q23) were carried by a 33 years old male Other translocation found from RM cases can be seen in Table 2.

The frequency of duplications in both the couples was detected in16.0% (8 of 50) of different patterns. The duplications were observed more in chromosome Y accounting for 6.0% and 2% each in chromosome 9 and 11 in both the couples. Further, macro Y chromosome was found in four cases (8.0%) of male partners. Duplication seen in two cases was observed in 46, XY males, one with duplication in 11q and other in 9 (pter). The other duplications found in both the partners are listed in Table 2.

Three chromosomal deletions were detected in cases of RM involving chromosome X, 8 and 7 comprising of 6.0%. The study also found a range of chromosomal aberrations that were detected in both couples which included two cases each of 9(qh+), a marker chromosomes (2.0%) in female partners, as can be seen in Table 2. A comprehensive analysis of cytogenetic aberrations was observed in various demographic characteristics as detailed in Table 3. In the case of gender, chromosomal changes were seen in 23 (46.0%) males as against 27 (54.0%) females. Chromosomal duplications were seen significantly observed in males only with a frequency of 30.4%. Translocations of different nature were equally distributed in females

Table 2 Frequency and nature of chromosomal aberration detected in couples with RM

S. no.	Chromosomal aberrations	Karyotype	Gender	No. of cases (%)	Frequency %
1	Numerical	47, XXX	F	1	(2.0)
		47, XYY	M	1	(2.0)
2	Mosaic	46, XX/47, XXX	F	4	(8.0)
		46, XX/47, XXX Marker Chr	F	1	(2.0)
		46XY/47, XY + der(19)	M	1	(2.0)
	Total			8	16
3	Structural				
	Inversion	46, XX, inv(9) (p13q13)	F	5	(10)
		46, XY, inv(9) (p13q13)	M	4	(8.0)
		46, XX, inv(16) (p11.1q12.2)	F	1	(2.0)
		46, XX, inv (9) (p11q13)	F	1	(2.0)
	Total			11	22.0
	Duplication	46, XY, dup(Y) (q+)	M	2	(4.0)
		46, XY dup (11) (q) with macro Y	M	1	(2.0)
		46, XY, Y qh+ macro Y	M	3	(6.0)
		46, XY, macro Y	M	1	(2.0)
		46, X, dup (9) (pter)	M		(2.0)
	Total			8	16.0
	Translocation	46, XX, *t*(13; 14) (q10; q10)	F	1	(2.0)
		45, XY, *t*(13; 15)	F	1	(2.0)
		46, XX *t*(4:16) (p13.2; q33)	F	1	(2.0)
		46XX, *t*(7; 14) (p13; q12)	F	2	(4.0)
		46, XY, *t*(6; 16) (p11; q24)	M	1	(2.0)
		46XY, *t*(2; 8) (q31; p11.2)	M	1	(2.0)
		46, XY, *t*(4; 6) (q35; q23)	M	1	(2.0)
		46, XX, *t*(3; 17) (q29; p11.2)	F	1	(2.0)
		46, XX, *t*(X; 3) (q21; p11.2) del(5q)	F	1	(2.0)
		46, XX, *t*(1; 11) (p34.3; q23)	F	1	(2.0)
		46, XY, *t*(8; 14) (q24.1; q24), inv(9)	F	1	(2.0)
		46, XY, *t*(7; 18) (q32; q21)	M	1	(2.0)
	Total			13	26.0
	Deletions	46, XX, del Xp (p11.1)	F	1	(2.0)
		46, XX, del8(q)	F	1	(2.0)
		46, XY, del(7)p^{14} (^{ter})	M	1	(2.0)
	Total			3	6.0
	Other changes	46, XX, 9(qh+)	F	2	(6.0)
		46, XY, 9(qh+)	M	2	(4.0)
		47, XX/XY (marker)	F	2	(4.0)
		46, XY, 1qh+	M	1	(2.0)
	Total			7	14.0
	Total			50 (13.1%)	(100)

and males (~ 26% each partner). Pericentric inversion ratio was moderately observed high among females than males as 259 as against 17.4% respectively. In case of different age groups, the frequency of chromosomal alterations in < 30 years were 22 (44.0%) vs. 28 (54.0%) for ≥ 30 years. Subjects ≥ 30 years presented with more number of translocations as 9 (25%) as compared to 4 (13.6%) < 30 years and the rest of the changes were marginally equally distributed among the different groups of age (Table 3). When the results were stratified according to number of miscarriages, couples with two, three, four, five and six events had chromosomal abnormalities of 34.0%, 38.0%, 16.0%, 6.0% and 4.0% respectively (Table 3). The frequency and pattern of all types of structural changes

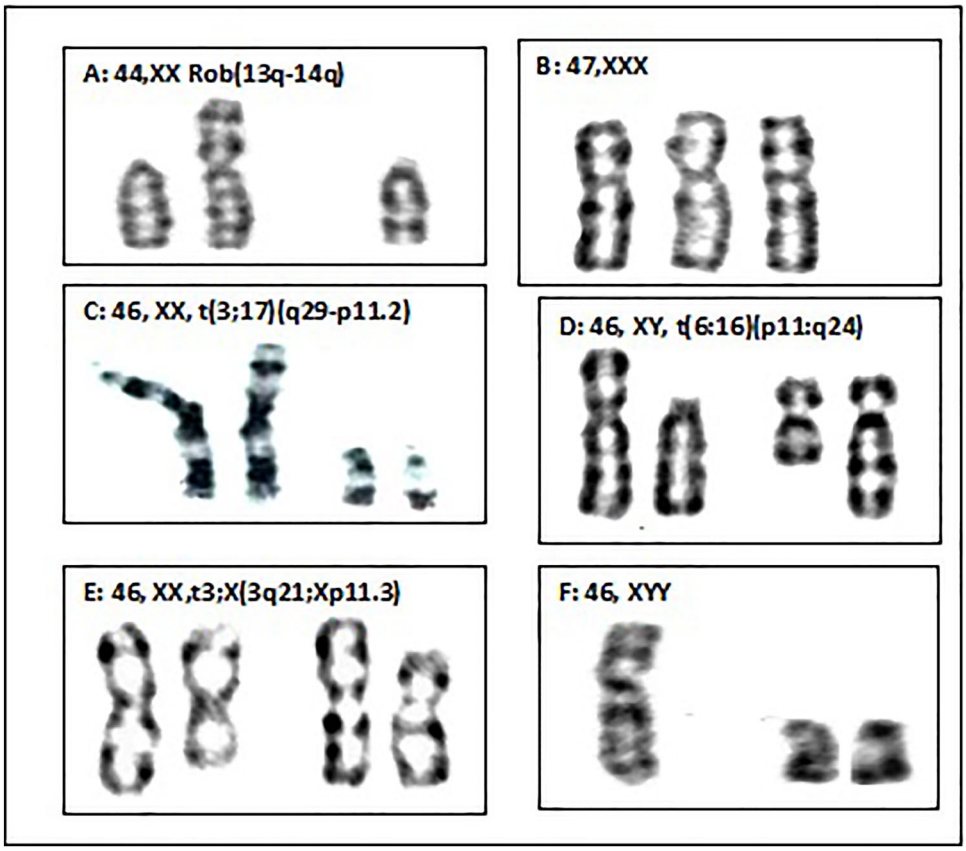

Fig. 1 **A**–**F** Representatives pictures of some prominent chromosomal aberration detected in couples with recurrent miscarriages

observed in number of events were marginally the same except that the pattern of numerical/mosaicism chromosomal was higher in couples with miscarriage events of 2 than 3 (23.5% vs. 15.5%). Translocations were found to increase with each recurrence of miscarriage event as 1% in two events, 21.0% in three events, 50% in four events and finally 75.0% in couples with five events of RM. In case of couples with multi-generation family history RM, the frequency of chromosomal aberrations observed in this study aggregated to 35.0% (18 of 50) as against 64.0% (32 of 50) those subjects with no such history. The type of the chromosomal changes observed among these two groups were closely the same but the frequency of chromosomal inversions were seen more in positive family history than without, 33.3% vs. 15.6%. The hallmark finding was the presence of significantly higher frequency of translocations in couples with a positive family history of RM as 44.5% vs. 15.6% in cases with no history ($p < 0.05$). The couples bonded consanguineously harbored 32% (16 of 50) of chromosomal changes compared to 68% (34 of 50) in non-consanguineously married wherein the difference of frequency pattern of chromosomal aberrations observed were prominent in inversions (37.5% vs. 14.7% respectively) and duplications (6.2% vs. 20.5% respectively). Significantly higher frequency of translocation were seen in consanguineous couples 37.5% vs. 20.6% in non-consanguineous group ($p < 0.05$) as depicted in Table 3.

The study also found six couples, 3.1% (6 of 190) where both partners harbored chromosomal aberrations with different events and other parameters as shown in Table 4. Two cases among these with no family history and consanguineously married had a numerical aberration in female (47, XXX) and male partner has karyotype as 46, XY, 13psat+ while other couple presented with karyotypes as 46, XX, 14ps+ and 46, XY, 9q+. The third couple who were consanguineously married and suffered 6 miscarriage events presented with 46, XX, del 8(q24) and 46, XY, 1(qh+). One couple consanguineously married with three recurrent miscarriages interestingly had the same chromosomal aberration as 46, XY, Inv9 (p13q13) and 46, XX, Inv(9) (p13q13). Lastly, another couple presented with 46, XY, 9(qh+) and 46, XX, *t*(3; 17) (q29; p11.2) karyotypes with three events of miscarriages. Overall four female subjects were observed to harbor dual structural either mosaic or complete chromosomal aberrations. Among them, one case belonged to a female partner who had suffered six events of recurrent miscarriages, was consanguineously married depicted a karyotype as 46, XX, del 8 (q24)/Dicentric *t* (14; 10). Another female consanguineously married had three miscarriage events showed dual chromosomal changes as a deletion in

Table 3 Distribution of various chromosomal changes in different epidemiological characters

S. no.	Parameters	Chromosomal aberrations $n=50$				Frequency n (%)
		Aberration	(n:%)	Aberration	(n:%)	
1	Gender					
	Male	Inversion	4:17.4%	Duplication	7:30.4%	23 (46.0)
		Num /mosaic	2:8.6%	Translocation	6:26.0%	
		Deletion	1:4.3%	Others	3:13.0%	
	Female	Inversion	7:25.9%	Translocation	7:25.9%	27 (54.0)
		Num /mosaic	6:22.3%	Others	4:14.8%	
		Deletion	2:7.4%			
2	Age					
	< 30 years	Inversion	4:18.1%	Duplication	3:13.6%	22 (44.0)
		Num /mosaic	3:13.6%	Translocation	4: 18.1%	
		Deletion	2:9.0%	Others	6:27.2%	
	≥ 30 years	Inversion	7:25.0%	Duplication	5:17.8%	28 (54.0)
		Num /mosaic	5:14.2%	Translocation	9:25.0%	
		Deletion	1:3.5%	Others	1:14.2%	
3	Miscarriage events					
	2	Inversion	4:23.5%	Duplication	3:17.8%	17 (34.0)
		Num/mosaic	4:23.5%	Translocation	2:7.1%	
		Others	4: 23.5%			
	3	Inversion	5:26.3%	Duplication	2:10.5%	19 (38.0)
		Num /mosaic	3:15.5%	Translocation	4:21.0%	
		Deletion	2:10.5%	Others	3: 15.5%	
	4	Inversion	2:25.0%	Translocation	4:50.0%	8 (16.0)
		Duplication	1:12.5%	Num/mosaic	1: 25.0%	
	5	Duplication	1:25%	Translocation	3:75%	4 (6.0)
	6	Duplication	1:50%	Deletion	1:50%	2 (4.0)
4	Family history*					
	Yes	Inversion	6:33.3%	Duplication	1: 5.5%	18 (35.0)
		Num/mosaic	2:11.1%	Translocation	8: 44.5%	
		Deletion	1:5.5%			
	No	Inversion	5: 15.6%	Duplication	7:21.8%	32 (64.0)
		Num /mosaic	6:18.7%	Translocation	5: 15.6%	
		Deletion	2:6.2%	Others	7:21.8%	
5	Consanguinity**					
	Yes	Inversion	6:37.5%	Translocation	6:37.5%	16 (32.0)
		Deletion	1:6.2%	Duplication	1:6.2%	
		Others	1:6.2%	Num /mosaic	1: 6.2%	
	No	Inversion	5:14.7%	Translocation	7: 20.6%	34 (68.0)
		Num /mosaic	7:20.6%	Duplication	7:20.5%	
		Deletion	2:5.8%	Others	6:17.6%	

*Inversions and translocations significant ($p<0.05$)

**Inversions and translocations significant ($p<0.05$)

16 (q24)/ per inversion16. The third case with three events was observed to have a karyotype as 46, XX, del X (p11.1)/ inv(9) (p13q13). Another case of women with multiple abortions had translocation between chromosome 3 and X as 46, XX, *t*(3; X) (q21; p11.2) with 5q deletion (Table 2).

The study detected various chromosomal polymorphic variants across the male and female partners and was mostly seen in chromosome 9 and Y. Prominent among these polymorphic variants like 46, XY, 9(qh+) (4.0%), 46, XY, Y(qh+) (10.7%), and 46, XX, 9(qh+) (6.0%). Besides a male whose spouse suffered six consecutive pregnancy losses was observed to have increased heterochromatin region in chromosome 1(46, XY, 1qh+). There

Table 4 Chromosomal aberration seen in both partners with RM

S. no.	Gender	Age	Family history	Consanguinity	No. of events	Karyotype
1	Husband	≥30	No	Yes	2	46, XY, 13Psat+
	Wife	<30	No			47, XXX
2	Husband	<30	No	Yes	6	46, XY, 1q+
	Wife	<30	No			46, XX, del8(q) (q24)
3	Husband	≥30	No	No	4	46, XY, macro Y (Yh+)
	Wife	≥30				46, XX, 9(h+)
4	Husband	≥30	No	Yes	3	46, XY, Inv(9) (p^{13}; q^{13})
	Wife	≥30				46, XX, Inv(9) (p^{13}; q^{13})
5	Husband	≥30	No	No	3	46, XY, 14Psat+
	Wife					46, XX, 9(h+)
6	Husband	≥30	No	No	3	46, XY, 9(qh+)
	Wife	<30				46, XX, *t*(3; 17) (q29; p11.2)

were some increased satellite regions in few of the chromosome as detailed in Supplementary Table S3.

The male partner as a sole contributor to miscarriages was further elucidated by detection of microdeletions in Y chromosome by STS–PCR where we found four of the 190 cases (2.1%) with deletion at different regions of AZF (Table 5). Two cases were affected by a deletion in S134 region of Y chromosome with normal karyotype 46, XY with a total number of miscarriage events 3 and 4 respectively. Two further cases of Y chromosome deletions were found as SRY deletion and other at S86 (AZFb). When cytogenetic and molecular evaluation was taken together a total of 15.2% ($n=50+4=54$) cases showed abnormalities either in a cytogenetic screening where both the parents contributed for RM (Females: 54.0% and males: 46.0%) and Y chromosome microdeletions where males contributed for RM for 2.1% of cases.

The QF-PCR technique was employed containing 26 STR markers located on chromosomes 13, 18, 21, X and Y on 60 samples of product of conception obtained from abortuses of the females who had suffered RM for aneuploidy detection. These samples corresponded to the same samples of the females who were screened for cytogenetic evaluations. Four different aneuploidies were detected that included trisomies in chromosome 18 (5.0%), 1313 (1.6%), and 21 21 (1.6%), with one POC detected with monosomy X (1.7%). All these female subjects whose POC had aneuploidies were in the age group ≥30 years. Electrophoretogram analysis for 66.7% samples showed chromosome-specific markers that indicated a normal diploid complement of chromosome 13, 18, 21 and normal complement for sex chromosomes (Table 6). Maternal contamination was detected in 4 (6.7%) samples where results could not be interpreted and were inconclusive. All the positive cases of POC with aneuploidy were carrying the normal parental karyotypes.

Discussion

Pregnancy loss due to RM affecting 2%–5% of couples has become a serious reproductive health issue where established cytogenetic abnormalities causes seem to be of vital importance although other factors like uterine anomalies, maternal age, hormonal and metabolic disorders play a role in it. Evidence suggests that a higher frequency of RM (50%–60%) is due to the constitutional consequences of chromosomal aberrations of parental transmission, or can result due to de novo during embryogenesis [6, 7]. Preferential intermarriages, ethnic nature of the population late marriages prevalent in our region are the factors that make the study suitable to look into the genetic and molecular factors that impact both the parents to cause pregnancy losses by RM.

The current study was conducted in three ways from this region: through evaluation of the chromosomal aberrations

Table 5 Y microdeletions in male partners

S. no.	Miscarriage events	Y microdeletion	Karyotype	Frequency%
1	4	S134/AZFb	46, XY	1
2	5	S134/AZFb	46, XY	1
3	3	SRY	46, XY	1
4	3	SY86	46, XY	1
Total				4

Table 6 Detection of aneuploidies by QF-PCR in POC of RM patients

Number = 60	Chromosomal aberrations	No. of cases	Frequency %
Type of aneuploidy	Trisomy 18	3	5.0
	Trisomy 13	1	1.6
	Trisomy 21	1	1.6
	Monosomy X	1	1.6
	Triploidy	1	1.6
Total		7	11.6
Normal		49	81.6
Maternal contamination	Inconclusive	4	6.7

in married couples to get an insight into the prevalence and type of chromosomal alterations either transmitted or occurs de nova that leads to recurrent miscarriages, secondly male partners as sole contributors to RM and finally prenatal POC samples from abortuses. Overall in the current study cytogenetic analysis and Y chromosome deletions were found in 15.2% couples affected with RM. This led us to estimate the subsequent pregnancy outcome and identification of the multigenerational sequence of RM.

Chromosomal abnormalities in couples with RM have been observed to vary across different ethnic regions and populations [27, 28]. Meiotic segregation imbalances can lead to chromosomal rearrangements leading to unbalanced chromosomal abnormalities as inversions, duplications or deletions and translocations that are prime causes of recurrent miscarriages [29]. The clinical outcomes of such unbalances though result in successful implantation but generally are lethal to the fetal deaths in a repeated occurrence [30].

Accumulated evidences from different studies reveal the frequency of cytogenetic aberrations vary from 0% to 21.4% [31]. The frequency reported in the literature varies from 2.9 to 5% [29, 30] except few studies [32, 33] where higher frequencies have been reported. The overall chromosomal alterations detected in this study were marginally high as 13.1% that is possibly keeping in view our region being ethnically conserved with the custom of consanguineous marriages. The scenario seems in semblance with a few reports, in particular, the ones from Iran (11.7%:12.0%; 13.1% our study) [34, 35] and frequency exceeds to the reports from regions like Malaysia [36], Pakistan [37], Saudi Arab [38] (8.9% < 6.7 < 5.3% < 13.1% our study) . All these nations are similar in the behavior of their marital consanguineous pattern. Dubey et al. [39] conducted similar studies from west and central India but the prevalence of the chromosomal defects found was very low as 3.5% and 2%, respectively. A plausible reason for the high frequency found in our region is due to the variations in the occurrence of chromosomal aberrations as seen in selected ethnic populations [39]. Among these population parameters, consanguineous marriages as practiced in our region are customary in various human societies that lead to an increased prevalence of severe genetic disorders [40]. Though the frequency is quite high as compared to the studies done beyond the ethnic boundaries in India, but still a bit higher frequency could be expected as some subtle or submicroscopic chromosomal changes are beyond the scope to be picked up by the cytogenetic method. Sheth et al. [41] found 3.5% and Dubey et al. 2.0% [39] from the Indian subcontinent that are comparably very less than the frequency found in RM cases of our study. The plausible reason seems the ethnic nature of our population where the customary habit of marriages within the relations and even within the geographical boundaries has certainly aggregated the complexity and occurrence of the chromosomal aberrations. Further, Dutta et al. [42] conducted a study on RM cases on a large cohort of 1162 couples from southern India wherein the overall chromosomal defects aggregated to 3.35% which is very less as compared to the frequency of 13.1% in our cases. The high frequency of chromosomal aberration detected in our series of couples may also be due to the strict selection criteria adopted from among subjects who had two or more RM while excluding all the other factors responsible for pregnancy losses such as infection, immunologic and hormonal factors, etc.

It is evident now that most of the RM cases are the fallout of chromosomal aberration in the embryo or fetus which ranges from numerical to structural defects [43]. This study detected numerical aberrations in eight cases (16%) of RM cases which included six cases of mosaic pattern in most of the cell lines. Although numerical chromosomal changes, usually in the form of sex chromosomal aneuploidy are not common events in couples with RM but when present their impact has been always severe on the survival of the fetus. These numerical aberrations have been found in low frequencies in different geographical studies as 0.15% [29] and 0.6% [39] but our study found a marginally higher frequency of 4.0%.

In this study, all the cases of inversions found in RM cases were pericentric involving chromosome nine in 10 cases (90.9%) and one in chromosome 16 (9.1%). On seeing high frequency and unique pattern of inversion 9 chromosome (inv 9) in both couples in RM cases, a number of control samples were put for Karyotyping to look for the

same changes but to our surprise we could hardly observe any such eventuality in healthy individuals. The frequency of inversions as 2.8% found in our cohort cases points to the fact that although considered to be polymorphic [44] but strongly predisposes the couples with RM and has a plausible role in pregnancy losses. Though pericentric inversion of chromosome 9 is found in normal population but the frequency ranges from 1 to 3% [45–47] which is highly specific within the populations. Further, inv(9) has been found to be linked with subfertility as an inversion process may cause complete loss or suppression of the euchromatin chromosome region because of position variegation effect that shows it to be a plausible factor in deleterious impact of chromosome affected leading to significantly high meiotic alterations and aneuploidy rates [47–49]. Further, those pericentric inversions where breakpoints are comparatively in proximity to centromeric region of a chromosome produce severely unbalanced gametes and have shown to be related to pregnancy loss [49, 50]. Considering the presence of inversions in our study was mandatory as other factors responsible for the repeated pregnancy losses were completely ruled out that included immunological dysfunction, infection, environmental or hormonal dysfunction, etc. Although controversial, pericentric inv(9) need to be taken into consideration in pregnancy losses to analyze and manage subsequent pregnancies for better counseling.

In our study, a varied pattern of chromosomal duplications involving different chromosomes was detected in one or both the couples comprising an aggregate of eight cases (2.1%) and prominent among them was seen in Y (1.57%) 11q and 9(pter) (0.26%). Same pattern was also observed by Hemalatha et al. [51] where duplication of chromosome 9q and 1q were significantly associated with bad obstetric history. Mostly majority of the cases were significantly associated with pregnancy losses in the first 10–20 weeks that had duplication either in chromosome 9q or 1q. Other chromosomes that involved duplications in our series of RM samples were chromosome 11 and Y. Various investigations performed across the globe have confirmed the indirect deleterious impact and association with large Y chromosome on higher recurrences of spontaneous pregnancy losses and many other issues of infertility [52–55]. Whether Y chromosome is large due to duplication of heterochromatin or polymorphic regions, Lemos et al. [56] have shown that such Y chromosome harbor cryptic variation that have altered functional properties. Therefore, it is highly recommended to include couples with Y chromosome duplication as a strong candidate for genetic counseling.

A major frequency of chromosomal changes detected in our study included those of duplication or polymorphic regions of some chromosomes and prominent among these include, 9(qh+) and 1qh+. In this context, Purandare et al. [57] depicted that couples of heterochromatic variations to be at increased risk of viable pregnancy outcome and particularly came across almost same heterochromatic variations. Relation of heterochromatic regions with RM is also substantiated by Yuce et al. [58] and Minocherhomji et al. [59]. All the chromosomal duplications found in our study were significantly high in recurrent pregnancy loss cases and we, therefore, strongly recommend these eventualities for offering genetic counseling in couples with RM especially in a region where multigenerational cases of pregnancy loss is very frequent.

Balanced chromosome rearrangements or simply translocations are the most lethal events that have been found in couples with pregnancy wastage due to RM. Chromosomal translocations among structural abnormalities are the most frequent events that cause recurrent abortions carried by either of the parents [60]. A total of 13 cases (3.5% of all cases) harbored balanced translocations in RM couples that involved a range of chromosomes such as 13, 14, 15, 3, 4, 6, 16, 17 and X. Among them, balanced reciprocal translocations were found in eight cases (80.0%) and Robertsonian translocations in two cases (22.0%). A frequency of 26% (13 of 50) counted among all the chromosomal changes detected in the present study is slightly comparable to earlier studies [38, 61–63]. All these cases followed up had subsequent recurrent pregnancy losses and same pattern was observed by Sugiura-Ogasawara et al. [6] whose prediction for a poorer prognosis in carriers of translocation was substantiated with a higher incidence of subsequent pregnancy losses. The hallmark characteristic of balanced translocations is that phenotypic consequences are missing in couples while as there is a very high risk of the carrier of this translocation to produce children with unbalanced chromosomal rearrangements. The risk of RM in couples with balanced translocations is nearly 25%–50%, and 25% with Robertsonian translocation [41]. All the patients in our series with a different set of translocations had a significantly maximum rate of recurrences of abortions within 20th weeks of gestation and, therefore, the kind of event is a very important prognostic factor for assessment of the future fate of pregnancies. It implies that all the couples with balanced translocations need to be emphasized to watch their future pregnancy outcomes by prenatal cytogenetic diagnosis to look for the presence of a chromosomal translocation aberration or else can opt for, in vitro fertilization by selecting chromosomally normal embryos to have successful pregnancy outcome for carriers of structurally aberrated chromosomes.

In the present study, chromosomal deletions were observed in three cases (1.08%) of couples with RM. The frequency of deletions seen in our series are very less although comparable with Dubey et al. [39] but their occurrence has severe clinical consequences owing to chromosomal imbalances which result in gametes with unbalanced

chromosomes that is lethal to the fetal development, thereby often become cause of spontaneous RM [30].

All the demographic features that influence RM due to chromosomal defects were stratified to observe the pattern and consequences of pregnancy losses as given in Table 4. Among gender, male; female ratio of chromosomal aberrations was more or less same 46%:54%. Translocations were seen in almost same frequency in females and males (12.9% vs. 26.0%) and the scenario contradicts earlier studies by Sheth et al. [41, 61] and two other reports from France and Germany [38, 62]. This shows that non-predilection of gender for chromosomal translocations are incompatible with fertility but the lethal effects on growing fetus have clinical consequences for RM in such couples. Although couples with two recurrent pregnancy losses were detected with a higher frequency of 34% (17 of 50) but a significant proportion of chromosomal aberrations were seen in either partners consanguineously married with multigenerational history of RM. Chromosomal duplications (17.8%) were prominent in cases of two miscarriage events followed by inversion, mosaic patterns (23.5% each) and translocations (7.1%). The results clearly demonstrate the importance of considering two events of RMs when the couples are in particular consanguineously married in addition to positive family history. In view of this, we stress upon the need to include such couples for genetic counseling along with cytogenetic analysis.). Our study found an increasing frequency of translocations with each subsequent event of RM in couples (7.1% in 2 events, 21.0% in 3 events and 50.0% in 4 events). Chromosomal translocations depict the most lethal impact among all chromosomal alterations to induce RM and most possibly due to the production of gametes with unbalanced chromosomal defects [64].

Multigenerational history of couples with RM was seen in 76.9% cases while those with consanguineous marriage had a frequency of 23.1% and this scenario seem slightly higher than the global communities with a preference for consanguineous marriage [65, 66]. The rate of intermarriages varies in different ethnic groups, in particular South Asian and Gulf countries [65, 67–71]. Genetic effects of intermarriages may cause the recessive gene to express in an inbred descendent after being cryptic for generations that predispose for the inheritance of genetic disorder [72]. Apart from evaluation of congenital disorders to developmental delay, it impacts nearly 7.5% of all conceptions [73], but on contrary this study found the frequency of chromosomal aberrations in interrelated couples of RM cases significantly higher, 32% (16 of 50). Subhash et al. [74] inclusively conducted an investigation in consanguineous group couples with bad obstetric history where only 3.03% cases harbored chromosomal anomalies in either partner which is very less compared to our population. Further, the present study found that translocations and inversions were significantly implicated in RM couples with a multigenerational history of abortions (44.5% and 33.3% respectively). Similarly among the consanguineous group, chromosomal inversions and translocations were again detected with significantly high frequency. The scenario of these events in the current study is in accordance with the similar events and almost matched frequency detected by Subhash et al. [74] (inversions 37.5% and translocations 25%). It is important to infer that inversions and translocations seem to be important features of changes within ethnically and interrelated marriages. Further, investigations across the globe from different studies have reported a frequency of 1.5%–12.5% [39, 51, 75] but with significantly high frequency (32%) of chromosomal aberrations from our population in consanguineous couples with RM substantiates the unique feature of ethnic nature and conserved genetic pool. Therefore, our preliminary assessment from the study supported by the later one, it seems there is a specific involvement of a similar pattern of chromosomal aberrations in consanguineous groups with RM. Our report stresses upon the need to look keenly for the kind of chromosomal eventualities in intermarried couples with positive family history for RM.

Interestingly, our study found six couples, 3.1% (6 of 190) where both had chromosomal anomalies with all except one who suffered 3 or more repeated miscarriages. Among these cases, couples who were intermarried had severe chromosomal alterations and had semblance for fetal wastage was at around 10th–15th weeks of gestation. The results indicate the essence of chromosomal investigation for couples with RM in particular the region where marriages interrelated are common and should be considered for analysis even when only two miscarriage events cause pregnancy losses. In the backdrop of these results, we emphasize the importance of performing cytogenetic analysis acts as a good genetic tool for providing information about the genetic aspects of RM for the management of repeated pregnancy losses particularly in the backdrop of inbred population. This study also stress upon the need for taking into consideration various demographic factors in particular the inclusion of two recurrent miscarriage events for couples who are consanguineously married for Karyotyping and to be counseled for subsequent management and successful pregnancy outcome.

Y micro-deletion as sole male factor for RM

Recent investigation support the idea that male factors as a sole contributor has a plausible role in RM cases [17] as substantiated by accumulated evidences depicting a potential link of micro deletions of the Y chromosome AZF region [18, 19, 22]. Therefore, it becomes very important to consider the analysis of Y microdeletion unraveling its contribution for pregnancy losses and to predict the subsequent outcome of pregnancies to reach a decisive consent for

treatment options. Therefore, our study included evaluation of Y chromosomal microdeletions in men whose partners suffered RM. The present study found 2.1% cases of men (4 of the 190) with deletions at different regions of AZF in Y chromosome. A number of studies for Y microdeletions in RM carried across the globe have shown discrepancies in incidence that is possible because ethnic variations cause a difference in the etiology of microdeletions. Populations from different ethnic regions have different haplotypes of Y chromosome, which leads to a varied degree of susceptibility and predisposition to Y-chromosomal micro-deletions [76]. The presence of 2.1% mutation in our series clearly defines the role of microdeletions in our population to act as a factor for RM. Similar investigations from Sri Lanka [21], Iran [77] and India [78] could not find any such deletions in RM cases substantiating the involvement of different haplogroups [21, 77, 78]. In contrast, there are studies where micro Y deletions have been found in more of subjects concerned than our report and notable among these are Karaer et al. [19], Agarwal et al. [79] around 32.5%, Said et al. [80], 10% and even Dewan et al. has reported an alarming 82% micro-deletions. Though very controversial at this point of time, but the Y micro deletion seem to have a definite role in the etiology of RM and the technical advancement is needed to study the different haplogroups within Y chromosome within three important regions AZFa, AZFb, and AZFc to discern their role in RM.

Aneuploidy detection in product of conception

One of the major causes of recurrent miscarriages is known to occur due to de nova aneuploidy on fetus. Analysis of the products of conception, therefore, plays a vital role in evaluating the causes of RM and further to manage the risk related to subsequent pregnancy. Our study as part of a third objective randomly chose the product of conception for QF-PCR of RM cases that were found to be normal but were strong suspects for chromosomal aberrations. All the POC cases belonged to the couples who have suffered 4 or more repeated miscarriages with all the other demographical parameters like hormone profile, TORCH profile and immunological profile etc. as normal. Among 60 POC samples studied, the frequency of numerical chromosomal aneuploidies detected were 10.7% which included trisomy 18, 13, 21 and monosomy X with a frequency of 5.3%, 1.6% and 1.6% respectively and the positive cases of POC with aneuploidy were carrying the normal parental karyotypes. Maternal contamination was detected in 4 (6.7%) samples where results could not be interpreted and were inconclusive. In contrast, Guzel et al. [81] comparatively detected very less 1.7% trisomies than 10.7% found in our report. Most of the studies from different regions agree with our report of aneuploidy frequency of around 10–14% in POC of RM samples and notably among these include studies by Diego-Alvarez et al. [82] and Jenderny et al. [83] with 12.5% and 12% respectively. Although embryos that harbor aneuploidy can successfully manage to implant for initiation of pregnancy, but the developmental process of the fetus remains ambiguous till birth. In our study, three POC cases were found to have trisomy 18, one with trisomy 13 and both these trisomies are among the rare abnormalities that are lethal to the fetal development.

Finally, when results of our study are taken together to evaluate their impact on the management of couples with RM, it seems all the parameters studied which include cytogenetic analysis for parents, microdeletions of Y chromosome in males and aneuploidy detection in POC can define the diagnosis and management of repeated pregnancy losses. This investigation also recommends that the consanguineous group should be considered for genetic counseling and testing after two abortions only to help the couples for successful pregnancy outcomes with proper genetic counseling and management of RM [31].

Conclusion

The study first of its kind concludes that the couples affected either by cytogenetic or Y chromosome deletions contribute hugely in the diagnosis and management of repeated pregnancy losses. The prevalence of the chromosomal aberrations seen is quite high particularly due to interrelated marriages as compared to other populations. As a high frequency of genetic alterations was found in couples with two recurrent abortions in the consanguineous group, it is strongly recommended to consider them for cytogenetic and molecular testing after two abortions for successful pregnancy outcomes and management of RM. Further, unique chromosomal structural aberrations detected can augment to characterize the altered regions of chromosomes through functional analysis to unravel the novel genomic involvement in RM cases.

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Author contributions AAP: conceived and designed the work, performed the experiments, manuscript drafting and evaluation of results; UMK, IA, IQ, WZ, and DS: analyzed the data and experimentation; AA and MR: helped to provide samples; DA: manuscript draft editing and logistic support; MHZ and FAD: helped in lab support.

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Declarations

Conflict of interest All the authors declare no conflict of interest.

Ethical approval Human participants involved for performing various procedures were strictly followed in accordance with the ethical standards of the institutional research approval committee in compliance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical approval for this study was duly obtained from the Institutional Ethical Committee of SK Institute of Medical Sciences, J&K, North India (SKIMS Study ref: Protocol IEC-SKIMS Protocol RP 244/2014).

Consent to participate All the patients and control group were voluntary in participation to this study and a written informed consent was obtained from each recruited subject.

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