Original Article

Chromosomal Aberrations in 224 Couples with Recurrent Pregnancy Loss

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Abstract

Background: Recurrent pregnancy loss (RPL) is a major reproductive health issue, affecting 2%–5% of couples. Genetic factors, mainly chromosomal abnormalities, are the most common cause of early miscarriage accounting for 50%–60% of first trimester abortion. **Aim:** To estimate the prevalence and nature of chromosomal anomalies in couples with recurrent miscarriage. **Patients and Methods:** This study included 224 couples with a history of 2 or more abortions. Both partners were karyotyped as part of the primary investigation. Cytogenetic analysis was carried out using the standard method. **Results:** A total of 224 couples with a history of two or more recurrent abortions were enrolled in this study. Chromosomal abnormalities were detected in 26 couples (11.6%) and 28 individuals (6.25%). We found a structural chromosome abnormality in 17/28 patients (60.7%); 12 patients had a reciprocal translocation (42.9%) including one patient with an additional inversion of the Y chromosome, 4 (14.3%) had a Robertsonian translocation, and one patient (3.6%) carried a paracentric inversion of chromosome 2. Numerical chromosome aberrations were detected in 5 patients; three patients (10.7%) with sex chromosome abnormalities and two (7.1%) with a marker chromosome. Six patients (21.4%) showed a heteromorphic variant involving chromosome 9. **Conclusion:** The prevalence of chromosomal abnormalities in couples with RPL is within the range reported worldwide. Cytogenetic analysis should become an integral part of the investigations of couples with at least two pregnancy losses of undetermined etiology.

Keywords: *Chromosomal abnormalities, cytogenetic analysis, recurrent pregnancy loss*

Background

Recurrent pregnancy loss (RPL), defined as two or more clinical, nonnecessarily consecutive, pregnancy losses,[1] is considered a major reproductive health issue, affecting 2%–5% of couples.[2] Established etiological factors include genetic abnormalities, endocrine anomalies, anatomical causes, immune factors, inherited thrombophilic disorders, infective agents, lifestyle and environmental factors.[3,4] Spontaneous abortion is defined as the loss of a clinical pregnancy prior to 20 completed weeks of gestation, or, the loss of an embryo/fetus of <400 g, in case the gestational age is unknown.[5] It is a relatively common event, occurring in 15%–25% of pregnancies, and rises in prevalence with increase in maternal age.[2]

Genetic causes, mainly chromosomal abnormalities, are the most frequent etiological factor of early miscarriage, accounting for 50%–60% of first trimester abortions. Fetal aneuploidy, in which the fetus has an extra or missing autosome or sex chromosome, is the

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most important cause of miscarriage before 10 weeks gestation.[6] The majority of human aneuploidies arise from errors in the first meiotic division of the oocyte.[7]

RPL may occur if one partner carries a balanced reciprocal translocation, a robertsonian translocation, or an inversion. A reciprocal translocation involves the exchange of two-terminal segments of nonhomologous chromosomes, a robertsonian translocation results from the centric fusion of two acrocentric chromosomes, while an inversion involves a change in the orientation of a DNA segment within a chromosome.[8] Due to abnormal segregation at meiosis, carriers of balanced translocations are at an increased risk not only for RPL, but also for the birth of a disabled child.[9] Inversions suppress recombination within the inverted sequence in heterozygotes, which can directly disrupt coding sequences or alter gene expression of adjacent genes or predispose to other rearrangements, mainly copy number alterations and translocations.[10] Balanced inversion carriers experience decreased fertility, higher rates of miscarriage, and have children with multiple congenital anomalies.[11]

Therefore, identifying couples with a chromosomal anomaly would help in providing proper genetic counseling, including prenatal and preimplantation genetic diagnosis (PGD). The study was carried out with the aim of estimating the prevalence and nature of chromosomal anomalies in couples with recurrent miscarriage.

Patients and Methods

The research was reviewed and approved by the Ethics Committee (IORG#: IORG0008812). The minimal sample size was calculated based on a study aimed to detect chromosome abnormalities in couples with RPL and to compare our results with those reported previously. Ghazaey *et al*. (2015) reported that about 11.7% of couples were carriers of chromosomal aberrations. Based on their study, and assuming that 400,000 couples had a history of RPL, 15% out of them had chromosomal aberrations, a minimum sample size of 196 couples with a history of RPL is the enough required sample for estimation of prevalence (cross-sectional) study (Killeen, 2005) (Daniel, 1991), with a significance level of 95% (accepted alpha error of 0.05) and ±5% confidence interval (5% Absolute precision). Sample size per group does not need to be increased to control for attrition bias (Pannucci & Wilkins, 2010).[12-15] This study included 224 couples (448 individuals) with a history of 2 or more abortions, recruited from Human Genetics clinic from November 2015 to October 2019. Informed consents were obtained from all participants after explanation of the purpose of the study. Couples where the female partner reported history of systemic diseases or thromboembolic disorders were excluded from the study. Both partners were karyotyped as part of the primary investigation. Cytogenetic analysis was performed on peripheral blood lymphocytes incubated for 72 h in media enriched with fetal bovine serum and phytohemagglutinin. Twenty-five metaphases were analyzed following Giemsa trypsin banding at 550 band level.[16] Mosaicism was confirmed if a second cell line was present in more than 5% of cells scored.[17] C-banding was used to confirm the presence of inversion or additional heterochromatin in cases of suspected chromosomal heteromorphy.

Statistical analysis of the data

The sample size was calculated according to Charan and Biswas (2013). Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp).Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Chi-square test for categorical variables, to compare between different groups Fisher's Exact correction for chi-square when more than 20% of the cells have expected count less than 5.Student t-test for normally distributed quantitative variables, to compare between two studied groups .F-test (ANOVA) for normally distributed quantitative variables, to compare between more than two groups. Mann Whitney test for abnormally distributed quantitative variables, to compare between two studied groups.[18]

Results

A total of 224 couples with a history of two or more recurrent abortions were enrolled in this study. The mean age of female partners was 28.3 years (range: 16–49 years), whereas the mean age of male partners was 34 years (range: 23–65 years). The number of previous abortions varied from 2 to 16 abortions/couple with a mean of 3.9. We observed no increase in number of abortions with advanced maternal age (P = 0.477), as shown in Table 1.

Consanguineous mating was observed in 123 couples (54.9%). The frequency was higher among couples with normal karyotypes (56.1%) compared to couples with chromosomal aberrations (46.2%), the difference was not statistically significant (P = 0.34). We detected chromosomal abnormalities in 26 couples (11.6%) and 28 individuals (6.25%). We found a structural chromosome abnormality in

Table 1: Relation of number of abortions to maternal age

	Number of abortion			Test of significant	*P*
	2 (*n*=76), *n* (%)	3 (*n*=48), *n* (%)	≥4 (*n*=100), *n* (%)		
Maternal age					
<35	68 (89.5)	41 (85.4)	83 (83.0)	χ^2=1.482	0.477
≥35	8 (10.5)	7 (14.6)	17 (17.0)		

χ^2=Chi square test, *P*=*P* value for comparing between the studied groups

Table 2: Distribution of the chromosomal abnormalities in affected couples

Types	Karyotypes	Frequency (*n*=28), *n* (%)
Numerical abnormalities	mos 47,XXX[3]/46,XX [47]	5 (17.9)
	mos 45,X [23]/46,XX[27]	
	47,XX,+mar	
	mos 47,XY,+mar[3]/46,XY[47]	
	mos 47,XXY[4]/46,XY[46]	
Structural abnormalities		17 (60.7)
Reciprocal translocation	46,XX,t(1;6)(q32.3;q26)	12 (42.9)
	46,XX,t(1;6)(q41;p24)	
	46,XY,t(1;15)(p35;q15)	
	46,XX,t(3;7)(p26;p15)	
	46,XY,t(3;15)(p23;q26.2)	
	46,XX,t(4;6)(q25;q26)	
	46,XY,t(5;18)(q13.1;q12.2)	
	46,X,inv(Y)(p11q11),t(6;7)(q23;q13)	
	46,XX,t(7;11)(q22;q23)	
	46,XX,t(9;11)(q34;q23)	
	46,XX,t(11;22)(q23;q11.2)	
	46,XY,t(11;22)(q23;q11.2)	
Robertsonian translocation	45,XX,der(13;14)(q10;q10)	4 (14.3)
	45,XY,der(13;14)(q10;q10)	
	45,XX,der(14;15)(q10;q10)	
	45,XY,der(21;22)(q10;q10)	
Inversions	46,XX,inv(2)(p11.2p23)	1 (3.6)
Polymorphic variants	46,XX,inv(9)(p11q13)	6 (21.4)
	46,XX,inv(9)(p11q13)	
	46,XX,inv(9)(p11q13)	
	46,XY,inv(9)(p11q13)	
	46,XY,inv(9)(p11q13)	
	46,XY,9qh+	

Table 3: Distribution of the studied couples according to reproductive outcome

	Total studied couples (*n*=224), *n* (%)	Couples with normal karyotype (*n*=198), *n* (%)	Couples with chromosomal aberrations (*n*=26), *n* (%)	χ^2	*P*
RPL only	119 (53.1)	102 (51.5)	17 (65.4)	1.775	0.183
RPL and others	32 (14.3)	29 (14.7)	3 (11.5)	0.181	*P*=1.000^{FE}
RPL and healthy child	73 (32.6)	67 (33.8)	6 (23.1)	1.212	0.271

χ^2=Chi square test, FE=Fisher Exact, *P*=*P* value for comparing between the studied groups, Other=Still birth, neonatal death, infant death, fetal malformation, or dysmorphic child, RPL=Recurrent pregnancy loss

17/28 patients (60.7%); 12 patients had a reciprocal translocation (42.9%) including one patient with an additional inversion of the Y chromosome, 4 (14.3%) had a Robertsonian translocation, and one patient (3.6%) carried a paracentric inversion of chromosome 2. Numerical chromosome aberrations

Table 4: Reproductive outcome in couples with chromosomal anomalies

Karyotypes	Reproductive outcome	Age maternal/paternal (years)
mos 47,XXX[3]/46,XX [47]	3 abortions	26/32
mos 45,X [23]/46,XX[27]	4 abortions	27/28
47,XX,+mar	4 abortions	27/27
mos 47,XY,+mar[3]/46,XY[47]	3 abortions/2 normal children	30/43
mos 47,XXY[4]/46,XY[46]	2 abortions	30/40
46,XX,t(1;6)(q32.3;q26)	9 abortions	25/27
46,XX,t(1;6)(q41;p24)	5 abortions	23/38
46,XY,t(1;15)(p35;q15)	4 abortions	36/39
46,XX,t(3;7)(p26;p15)	2 abortions/dysmorphic infant with 46,XY,der(3)t(3;7)(p26;p15) mat karyotype	33/65
46,XY,t(3;15)(p23;q26.2)	4 abortions/one normal child	28/36
46,XX,t(4;6)(q25;q26)	4 abortions	27/32
46,XY,t(5;18)(13.1q;q12.2)	2 abortions/still birth due to skeletal dysplasia and chest hypoplasia	21/32
46,X,inv(Y)(p11q11),t(6;7)(q23;q13)	5 abortions	18/25
46,XX,t(7;11)(q22;q23)	8 abortions	49/54
46,XX,t(9;11)(q34;q23)	4 abortions	33/36
46,XX,t(11;22)(q23;q11.2)	5 abortions/a girl with 46,XX,t(11;22)(q23;q11.2) aged	39/40
46,XY,t(11;22)(q23;q11.2)	1 day has polycystic kidney and polydactyly/a boy with 46,XY,t(11;22)(q23;q11.2) apparently normal/3 NND	
45,XX,der(13;14)(q10;q10)	4 abortions	27/30
45,XY,der(13;14)(q10;q10)	3 abortions	29/39
45,XX,der(14;15)(q10;q10)	3 abortions/one still birth/one NND with CHD/one infantile death/4 Normal children	35/38
45,XY,der(21;22)(q10;q10)	2 abortions/one NND/one normal child	30/38
46,XX,inv(2)(p11.2p23)	8 abortions/male infant with MCA and 46,XY,rec(2)dup(2)(p15) inv(2)(p11.2p23)mat /one child with normal karyotype	30/32
46,XX,inv(9)(p11q13)	3 abortions	33/49
46,XX,inv(9)(p11q13)	2 abortions/one normal child	20/29
46,XX,inv(9)(p11q13) 46,XY,inv(9)(p11q13)	3 abortions	35/33
46,XY,inv(9)(p11q13)	8 abortions	26/30
46,XY,9qh+	3 abortions	28/28

NND=Neonatal death, CHD=Congenital heart disease, MCA=Major congenital anomalies

Table 5: Distribution of the studied couples according to number of abortions

	Total studied couples (*n*=224), *n* (%)	Couples with chromosomal aberrations (*n*=26), *n* (%)	Couples with normal chromosome complement (*n*=198), *n* (%)	χ^2	*P*
Number of abortion					
2	76 (33.9)	5 (19.2)	71 (35.9)	2.845	0.241
3	48 (21.4)	7 (26.9)	41 (20.7)		
≥4	100 (44.6)	14 (53.8)	86 (43.4)		

χ^2=Chi square test, *P*=*P* value for comparing between the studied groups

were detected in five patients; three patients (10.7%) with sex chromosome abnormalities and two (7.1%) with a marker chromosome. Six patients (21.4%) showed a heteromorphic variant involving chromosome 9 [Table 2]. Identical chromosomal anomalies were present in both partners in 2 couples; one couple showed a balanced translocation between chromosomes 11 and 22 while the second carried an inversion of the heterochromatic region of chromosome 9. Both couples were consanguineous.

Chromosomal aberrations were more frequent in females (16 patients [7.14%]) compared to males (12 patients [5.35%]), the difference was not statistically significant (P = 0.435).

RPL alone as a presenting feature was more common among couples with chromosomal abnormalities (65.4%) than among couples with a normal karyotype (51.5%), however, this difference was not statistically significant (P = 0.183). The frequency of the presence

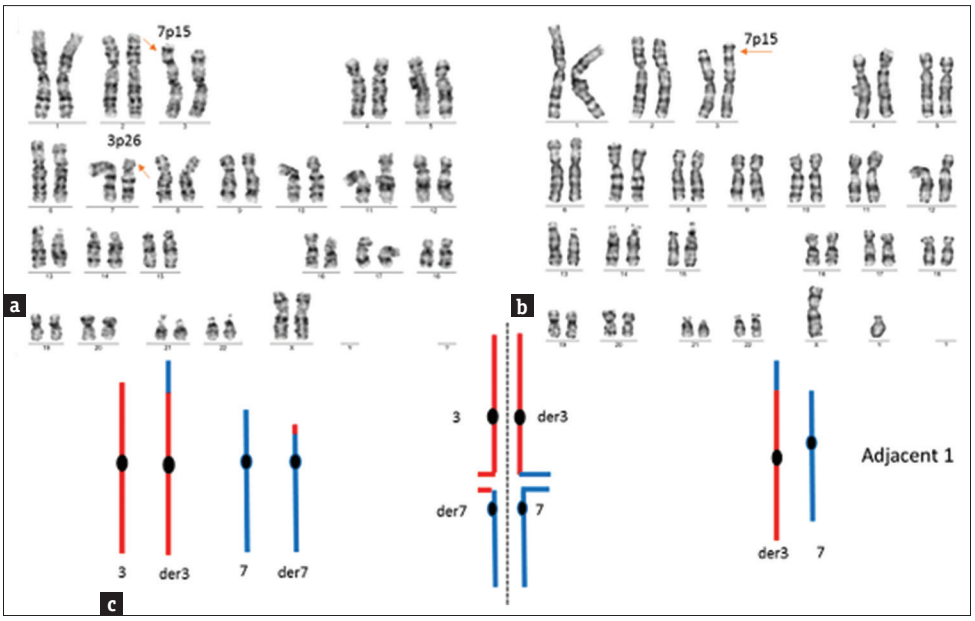

Figure 1: (a): Karyotype of female with 46,XX, t(3;7)(p26;p15), (b): Karyotype of offspring with 46,XY, der(3)t(3;7)(p26;p15)mat, (c): Pachytene diagram of the t(3;7)(p26;p15)

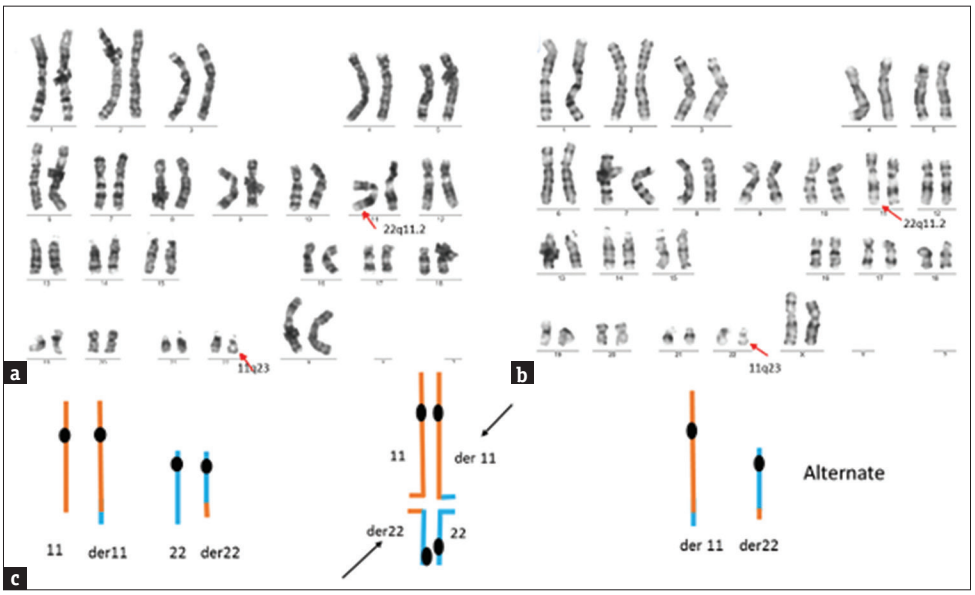

Figure 2: (a): Karyotype of female with 46,XX, t(11;22)(q23;q11.2), (b) Karyotype of offspring with 46,XX, t(11;22)(q23;q11.2)mat/pat, (c) Pachytene diagram of the t(11;22)(q23;q11.2)

of a healthy child was higher among couples who had normal karyotypes (33.8%) compared to couples with chromosomal aberrations (23.1%), the difference did not reach statistical significance (P = 0.271). The reproductive outcome of the studied couples is presented in Tables 3 and 4. Figures 1-3 show the karyotypes of carriers of balanced rearrangements with chromosomally abnormal offspring.

In the present study, more than 50% of couples with a chromosomal anomaly experienced 4 or more abortions, however, this percentage did not reach a statistically significant level (P = 0.241) when compared to couples with normal chromosome complement [Table 5].

Discussion

RPL remains a reproductive challenge both for the clinician and the patient. Carriers of a balanced chromosomal abnormality are at higher risk of generating abnormal gametes leading to stillbirth, recurrent abortions, and the birth of dysmorphic/mentally handicapped infants.[11] Hence, detecting a cytogenetic defect in case of miscarriage may play a significant

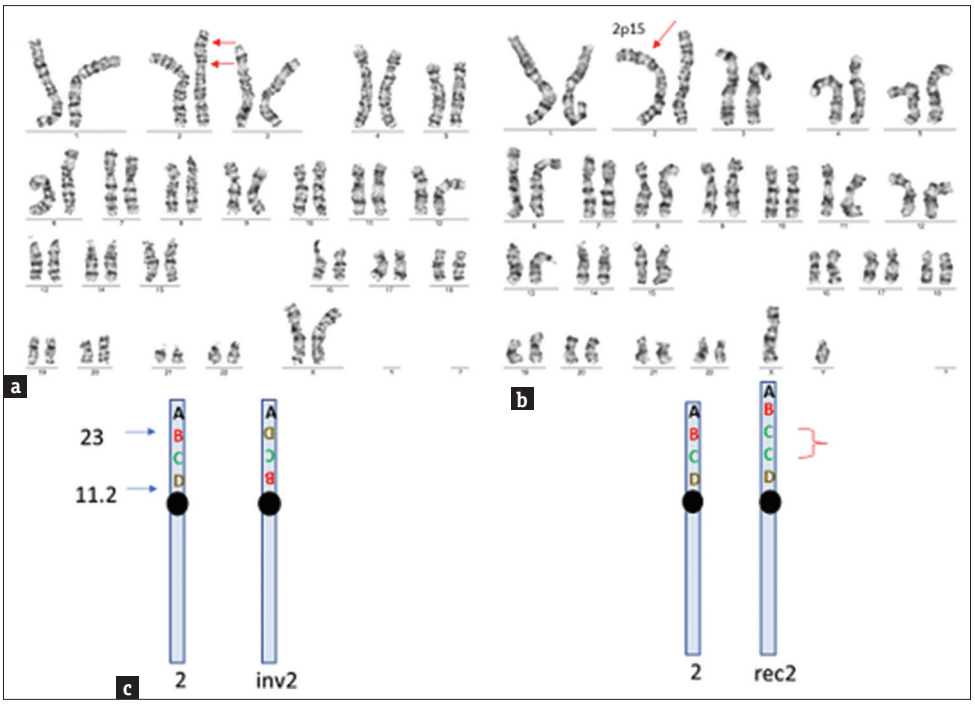

Figure 3: (a): Karyotype of female with 46,XX, inv(2)(p11.2p23), (b): Karyotype of offspring with 46,XY, rec(2)dup(2)(p15)inv(2)(p11.2p23)mat, (c): Schematic diagram of partial karyogram showing the paracentric inversion chromosome 2 with her offspring

Table 6: Comparison of the frequency of chromosomal aberrations in the present study to the literature

	Number of couples studied	Reciprocal translocation	Robertsonian translocation	inversion	Others	Total (%)
Current study	224	11	4	1	10	26 (11.6)
Iran[13]	728	37	7	21	20	85 (11.7)
Saudi Arabia[23]	1074	36	8	11	22	77 (7.2)
Oman[24]	290	-	-	3	3	23 (8)
Egypt[20]	125	7	1	-	-	8 (6.7)
Morocco[25]	238	8	1	4	-	13 (5.45)
Italy[26]	145	4	4	4	2	14 (9.6)
Canada[27]	100	4	3	4	2	13 (13)
Turkey[28]	1510	30	12	9	11	62 (4.1)

role in the management of couples with RPL. In the current study, the prevalence of chromosomal aberrations among couples with RPL was 11.6%, which goes in agreement with both international and national studies[19-21] [Table 6]. The discrepancies between various studies may be attributed to differences in sample size, inclusion criteria, and techniques of cytogenetic studies.[22]

The incidence of chromosomal abnormalities was higher among females (7.14%) compared to males (5.35%); some studies reported such difference to be statistically significant[25,29] whereas others, including the current study, did not find the difference to be of statistical significance (P = 0.435).[30,31] This higher female frequency may be explained by the fact that chromosomal anomalies compatible with fertility in females may be combined with sterility in males.[21,32] Males with chromosomal aberrations were suggested to have lower fertility rate due to poor spermatic motility, abnormal seminal profile with azoospermia or severe oligoasthenoteratozoospermia.[33]

In the present study, more than 50% of couples with a chromosomal abnormality reported 4 or more abortions; however, no significant difference was detected in the number of abortions experienced by couples with a chromosomal abnormality compared to those with normal karyotypes (P = 0.241), which goes in agreement with other published reports.[20,34]

Consanguineous coupling in Egypt is still high, representing 30% of all mating.[35] In the current study, nearly 55% of couples were consanguineous. The frequency of consanguineous mating in the present study was higher among couples with normal karyotype (56.1%) compared to couples

with chromosomal anomalies (46.2%), however the difference was not statistically significant (P = 0.34). Identical chromosomal abnormalities were detected in both partners in 2 consanguineous couples; one with a translocation involving chromosomes 11 and 22, and the other couple had an inversion of the heterochromatin of chromosome 9. This rare event where both partners carry the same chromosomal anomaly has been reported in consanguineous Indian couples.[36] The consanguineous couple where both partners carried the same 11; 22 translocation had two living children. Both children were carriers of the familial translocation, one child had polydactyly and polycystic kidney, and the other was normal. The occurrence of clinical expressions in a balanced translocation carrier may be due to physical interruption of genes or a disturbance in their regulatory environment.[37]

In the present study, structural anomalies were 4 times more frequent than numerical aberrations, which goes in agreement with previous reports.[28,34] Reciprocal translocations were the most commonly identified balanced chromosomal aberrations in couples with RPL, in accord with previous studies.[20,23,25,29] If one partner of a couple carries a balanced chromosomal translocation, the probability of miscarriage is nearly doubled.[38]

Even though carriers of balanced chromosomal rearrangements commonly have a normal phenotype, the probability of generating unbalanced gametes is significant due to complex segregation modes through meiosis.[39] In reciprocal translocation carriers, a quadrivalent arrangement is created at meiosis I via pairing of translocated chromosomes and the two corresponding normal chromosomes. This structure usually undertakes one of three modes of separation: 2:2 (segregation of two chromosomes to one cell and two chromosomes to the other), 3:1 (segregation of three chromosomes to one cell and one to the other) or 4:0 (segregation of all chromosomes of the quadrivalent to one cell and none to the other). Within the 2:2 mode of segregation, chromosomal disjunctions might be alternate, adjacent 1, or adjacent 2. Alternate segregation represents the sole segregation pattern producing gametes with balanced genetic counters: one bearing normal chromosomes while the other carries the balanced translocated chromosomes. Other segregation models will create unbalanced gametes leading to apparent infertility, recurrent abortion, or birth of a phenotypically abnormal offspring with mental retardation or other congenital defects.[40]

In the present study, both adjacent 1 and alternate segregation were observed in the offspring of the carriers of the t(3;7) and t(11;22) respectively. Couples with balanced reciprocal translocation have a 50% risk of RPL and a 20% possibility of having offspring with unbalanced chromosomal rearrangements.[31] The production of unbalanced, balanced, and normal gametes depends on the breakpoints and the chromosomes implicated. The greater imbalance will most probably result in miscarriages, while the slight or less significant imbalance will raise the possibility of having children with unbalanced karyotype. Balanced chromosomal translocations might additionally result in sequence rearrangements of the functional genes that could cause reproductive errors accompanied by RPL.[41]

Robertsonian translocations were less frequently encountered than reciprocal translocations, which agrees with published reports.[20,23,25,29] Robertsonian translocations carry reproductive risks that are dependent on the chromosomes involved and the sex of the carrier. For carriers of the most common Robertsonian translocation der(13;14), the risk for miscarriage is approximately 15%.[42] At meiosis, segregation of trivalent structure may result in nullisomic or disomic gametes for one of the chromosomes involved in the rearrangement and consequently to a zygote with trisomy or monosomy in addition to zygotes with normal chromosome complement or carrying the balanced rearrangement. Zygotes with monosomy are not compatible with life, and most translocation trisomy conceptuses are expected to result in first trimester loss or earlier; however, some survive beyond the second trimester and to term.[43] The risk for trisomy 13 in a carrier of der (13;14) does not exceed 0.4%.[8]

Inversions, both pericentric and paracentric, have been reported in cases with RPL with a frequency lower than Robertsonian translocations,[23,25,29] as observed in the present study. The risk of pregnancy loss in carriers of a chromosome inversion is not known.[34] The couple with a paracentric inversion of chromosome 2, had 8 abortions, a child with a recombinant karyotype exhibiting multiple congenital anomalies as well as a child with normal chromosome constitution. Hypothetically, heterozygous carriers of paracentric inversions do not generate viable unbalanced offspring. During meiosis, the occurrence of crossing-over event(s), within the inversion loop of affected segments, yields one dicentric and one acentric recombinant chromosome, which are both lethal. However, numerous examples of viable recombinant progeny have been reported.[44,45] A number of mechanisms explaining the meiotic creation of recombinant stable chromosomes with duplication and/or deletion have been proposed, including unequal crossover,[46] breakage and reunion of sister chromatids,[44] the abnormal process of

U-loop recombination[47] and breakage of dicentric recombinants.[48] We propose an unusual mechanism, involving breakage and unequal reunion of sister chromatids within the inversion loop, to explain the structure of our patient's recombinant chromosome.

Polymorphic variants including inversion of chromosome 9 and 9qh+, have been observed in the current study in agreement with previous reports.[25,29] Heterochromatic polymorphisms, have been implicated in mitotic instability and a tendency towards an increased risk for aneuploidy.[8]

Genetic counseling is preferably offered before subsequent pregnancy; hence, all choices ought to be discussed, and optimum planning assumed. When a couple presents with RPL, detailed family history should be acquired, as this may present clues about familial chromosomal rearrangement. History of congenital anomalies, infertility, mental retardation, spontaneous miscarriage, or perinatal mortality is substantial since each is characteristic of chromosomal anomalies.

Genetic counseling is vital when a structural genetic factor is recognized as there is a risk of having a child with an unbalanced karyotype. When one of the partners carries a structural genetic abnormality, prenatal diagnosis (through amniocentesis, or chorionic villus sampling)/PGD are possible tools to detect the genetic anomaly in the offspring.[49]

Conclusion

The prevalence of chromosomal abnormalities in Egyptian couples with RPL is within the range reported worldwide. Cytogenetic analysis should become an integral part of the investigations of couples with at least two pregnancy losses of undetermined etiology. Genetic counseling is crucial in the management of couples with RPL. Chromosome abnormalities in couples with repeated abortions are a strong indication for prenatal/PGD, helping a precise reproductive decision considering future pregnancies.

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Conflicts of interest

There are no conflicts of interest.

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