original article

The pattern of chromosomal abnormalities in recurrent miscarriages: a single center retrospective study

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BACKGROUND: Chromosomal abnormalities are more common in first trimester recurrent miscarriages (RM). Chromosomal anomalies affect approximately 2%-8% of couples with RM.

OBJECTIVES: Evaluate the spectrum and the frequencies of chromosomal anomalies in RM.

DESIGN: A retrospective hospital record-based descriptive study.

SETTING: A tertiary care center in Turkey.

PATIENTS AND METHODS: We studied couples with RM between October 2020 and January 2022. Relevant family and medical history, clinical examination and the results of karyotype were statistically analyzed.

MAIN OUTCOME MEASURES: Prevalence and types of chromosomal aberrations in couples with RM.

SAMPLE SİZE: 362 couples with a history of RM

RESULTS: Among the 362 couples, 14 cases (3.86%) had chromosome abnormalities. Eight cases (57.14%) were structural anomalies and six cases (42.86%) were numerical chromosomal aberrations. We found five balanced translocations (67.5%) and three Robertsonian translocations (37.5%). The prevalence of polymorphic variants was 51/362 (14.1%).

CONCLUSIONS: This study supports the conclusion that clinicians should understand the importance of chromosome analysis in these couples and direct them to karyotyping after two abortions in order to exclude the possibility of a genetic cause of RM.

LIMITATIONS: Single-center study and retrospective.

CONFLICT OF INTEREST: None.

Spontaneous recurrent miscarriage (RM) is defined as two or more consecutive pregnancy losses before the 20 weeks of gestation. Approximately 15%-20% of couples are affected by this condition.[1] The etiology of RM is often uncertain and may involve several factors including uterine malformations, thrombophilia, immunological, endocrinological disorders, infectious, environmental and parental or fetal chromosomal abnormalities.[2] In 50% of the couples with RM, the specific cause remains unexplained and is regarded as idiopathic or unexplained RM.[3] Parental chromosomal abnormalities have been estimated to affect 3%-5% of cases. A varying frequency of 3%–8% has been reported for carriers of chromosomal rearrangements among RM couples.[4] Chromosomal abnormalities can determine failure of reproduction, and for this reason genetic analysis can play an important role in an infertility investigation. Chromosomal factors alone account for 2%-14% of male infertility and as much as 10% of female infertility.[5,6] These chromosomal abnormalities can be numerical or structural. The most common structural cytogenetic abnormalities include balanced reciprocal or Robertsonian translocations and inversions that contribute to conditions such as spontaneous abortions, stillbirths or malformations.[7,8] The aim of this study was to determine the types and frequencies of chromosomal abnormalities in Turkish couples with RM.

PATIENTS AND METHODS

This retrospective study was done in couples with RM who were offered chromosomal analysis from 1 October 2020 to 1 January 2022. The study was approved by the Ethics Committee of Eskisehir Osmangazi University (Protocol No: 2022-38). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. The study size was based on the number of available records. The inclusion criterion for RM couples involved two or more recurrent miscarriages in first trimester. A detailed medical history, age and number of miscarriages were noted for all participants of the study. The women were screened for multiple thrombophilic gene variants. Ultrasonography and hysterosalpingography were used for the detection of possible abnormalities of the genital tract. Patients were evaluated to rule out antiphospholipid syndrome, infections, endocrinological and autoimmune disorders, and any other known cause of RM. Peripheral blood samples in heparinized injectors were processed for karyotyping. Phytohaemagglutinin (0.1 mL) was used for the stimulation of cell proliferation and cultured for 72 h at 37°C, in RPMI 1640 medium. Colchicine was added to the cultures. G-banded karyotyping was performed with trypsin–giemsa banding procedure. The karyotypes were evaluated according to the recommendations of ISCN 2015 (International System for Human Cytogenetic Nomenclature 2015). Twenty metaphases were analyzed and five metaphases were karyotyped for each patient. The application was extended for at least fifty metaphases for suspicious situations (e.g., mosaicism).

RESULTS

We retrieved the records of 362 couples (724 individuals) with recurrent pregnancy loss (RPL) and who met other inclusion criteria (**Table 1, Figure 1**). Among the 362 couples, 14 cases of chromosomal abnormalities (3.9%) were detected. Eight cases had structural abnormalities and six cases had numerical aberrations (**Table 2**). Out of 8 cases, 5 had balanced translocation and 3 had the Robertsonian translocation. Chromosome analysis images of patients with balanced translocations are shown in **Figure 2**. Five female cases and 1 male case had numerical sex chromosome anomalies (**Table 3**). Three of the numerical anomalies (50%) were monosomy X mosaicism and in two cases (33.3%), the combination of monosomy-trisomy X mosaicism was detected. One case (16.7%) had the combination of mosaic monosomy, trisomy and tetrasomy X. In addition, there were 51 (51/362, 14.1%) cases who have heteromorphic chromosomal variations. The most frequent polymorphic variant was inv9 with nine cases (**Table 4**). Eight individuals had 1qh+, six cases had 9qh +, six cases had 16qh + and nine cases had Yqh+/Yqh- . Fifteen cases had satellite increments including 13, 14, 15, 21 and 22 chromosomes (**Table 4**). The appearance of heteromorphic chromosomes detected in this study is shown in **Figure 3**.

DISCUSSION

Spontaneous RM occurs in approximately 15% of couples.[9] Currently, there are many accepted non-genetic etiologies for RPL. These include untreated hypothyroidism, uncontrolled diabetes mellitus, certain uterine anatomical abnormalities, and antiphospholipid antibody syndrome. Other probable etiologies include hereditary and/or acquired thrombophilias, immunological abnormalities, infections, and environmental factors.[3,4,10] Despite the volume of research in the area of RPL, only in 50% of the cases is the cause identified.[5,8] Although the frequency of chromosomal anomalies in RM couples varies

Table 1. Demographic and clinical characteristics of the study group (n=362 couples).

Mean (range) maternal age	29.1 (18- 46)
Mean (range) paternal age	32.4 (20-54)
Median (range) number of miscarriages	2 (2-7)
Number of miscarriages	
2 miscarriages	202 (55.8)
3 miscarriages	116 (32.0)
4 miscarriages	28 (7.7)
5 miscarriages	13 (3.6)
6 miscarriages	1 (0.3)
7 miscarriages	2 (0.6)

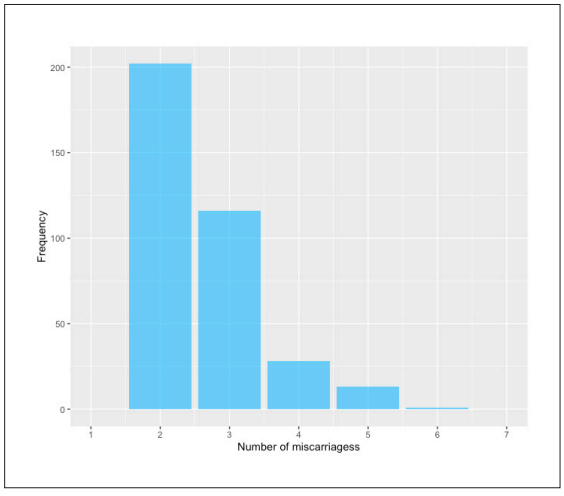

Figure 1. Frequency distribution of miscarriages.

Table 2. Chromosomal abnormalities among 362 couples.

Reciprocal translocation	5 (1.4)
Robertsonian translocation	3 (0.8)
Aneuploidy	6 (1.7)
Polymorphic variants	51 (14.1)
Total	65 (18)

Data are n (%).

Table 3. Structural and numerical chromosome abnormalities.

Structural chromosome abnormalities
46,XX, t(5;9)(q13;q32)
46,XX, t(10;11)(p11.1;q24.2)
46,XX, t(15;17)(q26.1;q21)
46,XX, t(12;15) (p13;q11.2)
46,XY, t(10;18)(p13;q21.1)
45,XX, der(13;14)(q10;q10)
45,XX, der(13;15)(q10;q10)
45,XY, der(13;15)(q10;q10)
Numerical chromosome abnormalities
45,X[4]/46,XX[56]
45,X[6]/46,XX[54]
45,X[9]/46,XY[75]
45,X[6]/47,Xxx[4]/46,Xx[40]
45,X[1]/47,XXX[3]/46,XX[56]
45,X[2]/47,XXX[5]/48,XXXX[3]/46,XX[50]

Table 4. Polymorphic chromosomal variations (n=51).

Variants	Number of cases
46,XX, 1qh+	5
46,XY, 1qh+	3
46,XX, 9qh+	1
46,XY, 9qh+	4
46,XX, 16qh+	4
46,XY, 16qh+	1
46,XY, 13pss+	1
46,XX, 14pss+	1
46,XX, 15pstk+	2
46,XY, 15pstk+	1
46,XX, 15pss+	1
46,XY, 15pss+	1
46,XX, 21pss+	2
46,XY, 21pss+	1
46,XX, 21pstk+	2
46,XY, 22pss+	1
46,XY, 21 pss+, 16qh+	1

according to different populations, chromosomal abnormalities are detected in 2.7%-13.9% of couples on average.[11] In a study by Kalotra et al, the rate of chromosome anomalies in couples with recurrent miscarriages was 3.1%.[6] Dutta et al detected this ratio as 6.7%.[12] Mozdarani et al reported the highest percentage (13.9%) in this condition.[13] Stephenson et al observed chromosomal aberrations in 2.7% of RMs, the lowest percentage in the literature.[14] In the present study, we found that the incidence of chromosomal abnormalities was 3.9%, which is similar to previous studies (**Table 5**).

Table 4. Polymorphic chromosomal variations (n=51).

Variants	Number of cases
46,XX, 9qh+, 21 pss+	1
46,XY, Yqh +	3
46,XY, Yqh -	6
46,XX, inv9 (p12;q13)	3
46,XY, inv9 (p12;q13)	6

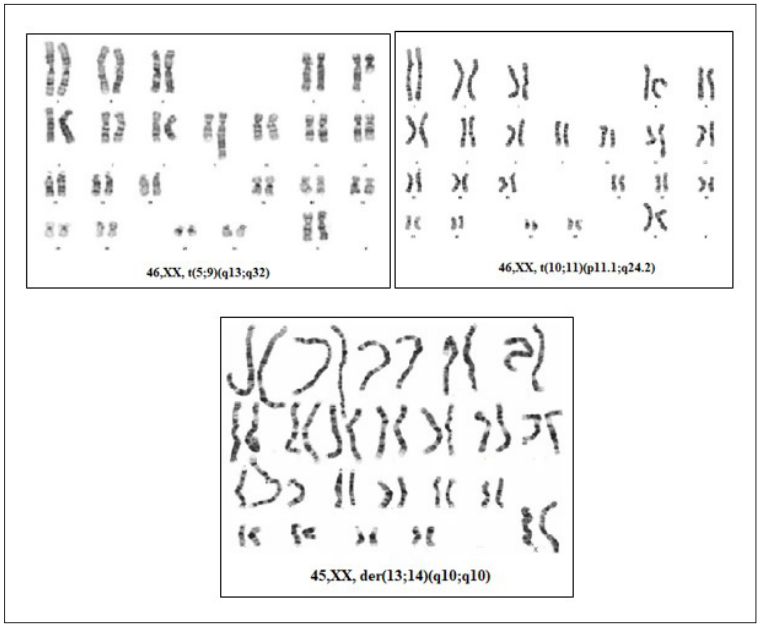

Figure 2. Examples of balanced structural chromosomal abnormalities in this study.

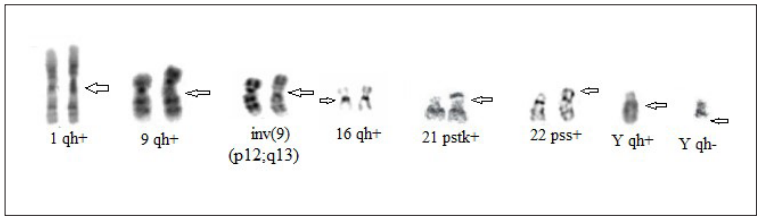

Figure 3. Types of polymorphic variants.

Many previous studies show that structural chromosomal abnormalities are more common than numerical abnormalities.[15] In our study, we observed 2.2% structural chromosomal aberrations (**Table 2**). Indeed, in our study, Robertsonian translocations were detected in 1.4% of couples, followed by reciprocal translocations (0.8%). The variation observed in these rates may be explained by differences in sample size and inclusion criteria used in the selection of participants in these reports. Reciprocal translocations are the most commonly balanced chromosomal abnormalities shown in couples with RM. There was a relationship between such chromosomal abnormalities and other gynecological complications such as fetus with congenital anomalies, infertility, and in vitro fertilization failures.

In this study, we found numerical chromosomal aberration in 6 (1.7%) patients (**Table 2**). The sex chromosomal aneuploidy (especially X chromosome) is the most common numerical chromosomal aberration in couples with RM. Mosaic Turner syndrome is the most common numerical chromosomal anomaly in female individuals with RM.[16] In this present study, the most common chromosomal variants were such as heterochromatic polymorphisms and satellite increments. In our RM group, polymorphic variation was defined in 51 (14.1%) of the patients. Polymorphic variations were commonly seen in acrocentric chromosomes (13, 14, 15, 21, 22) and the other chromosomes such as 1, 9 and Y. Satellite polymorphic variants (pss+ and pstk) (15/51), qh+ (23/51) and -qh (6/51 for Y chromosome) heteromorphism constituted a major part of polymorphic variants documented in our study. On the other hand, we found inv (9) in nine cases (9/51, 17.6%). Pericentric inversion of chromosome 9 was found in 6 men and 3 women (**Table 4**). Pericentric inversions are detected with a frequency of 1%-3% in the general population.[17,18] Pericentric inversions were associated with RM. Ueharas et al demonstrated that inv (9) was linked with infertility and spontaneous recurrent abortions.[19] The inversions can result in unbalanced deletion or duplication of a chromosome segment during crossing over in meiotic division.[20] The significance of the heteromorphism variants is a subject of controversial. For example, most clinicians consider inv(9) to be clinically insignificant.[12] Anuradha et al have reported that the satellite heteromorphisms might be predispose to nondisjunction, and lead to a significant increase in translocations related to satellite

associations in couples with RM.[21] We observed nine men who had heterochromatin variants of the Y chromosome in which three were Yqh+ and six were Yqh-. Men without sperm anomalies were excluded from this study. The importance of the presence of the Y chromosome variant is also speculative. Although Genest et al considered that Yq+ might be the cause of habitual abortions while Rodriquez et al reported that Yq+ was not associated with birth failure or recurrent miscarriages.[22] Kalantari et al concluded that Y chromosome heteromorphism did not directly affect the sperm count and male infertility.[23] Boronova et al detected that the polymorphic variants of Y chromosomes were linked with reproductive failure in females.[24] Previous reports showed that the prevalence of a heterochromatin polymorphism was 1.9%–15.8%.[25,26] In our series, heterochromatin polymorphism was detected in 51 (14.1%) individuals with RPL. The frequency of heterochromatin polymorphisms in our study was consistent with the literature. Also, chromosomal abnormalities are frequently described in human infertility. The incidence of chromosomal abnormalities in male infertility is 1.1%-7.2%, and the incidence of chromosomal anomalies in women is 10.0%-16.28%,[5,6,27,28] so a thorough understanding of chromosomal abnormalities is essential, both to reduce the burden of infertility and to prevent recurrent miscarriages.

We found that 51 couples (14.1%) were normal polymorphic variants, the most common chromosomal heteromorphism in individuals with RM that was higher in males than females (29/23). However, the effect of chromosomal variants on reproductive problems continues to be considered benign as it is also common in the healthy population.

In conclusion, RM is common in healthy indicuduals capable of reproduction. Chromosome analysis is an important step in etiological research in couples with RM. Structural and numerical chromosomal abnormalities can be considered as risk factors for RM. Since the most common heterochromatic variants in our patients are also seen frequently in the healthy population, it is highly unlikely that they play a role in the etiology of miscarriage. A limitation of our study is that the patients could not be compared with a control group. Further studies in different populations are needed to determine the possible association of chromosome heteromorphisms with RM.

Table 5. Frequencies of chromosomal abnormalities in previous studies.

Authors	Number of couples	Frequency of chromosomal abnormalities (%)
Hanif et al[2]	32	9.3
Azim et al[3]	300	5.3
Goud et al[4]	380	6.84
Niroumanesh et al[9]	100	13.0
Yildirim et al[11]	300	8.7
Dutta et al[12]	1162	3.35
Mozdarani et al[13]	221	13.9
Stephenson et al[14]	1893	2.7
Soltani et al[15]	608	3.54
Alibakhshi et al[16]	570	11.5
Caglayan et al[25]	336	4.0
De la Fuente-Cortés et al[26]	158	7.6
Present study	362	3.86

Authors' contributions

AK wrote the manuscript and revised it. HK, SG supervised and designed the study. HK, SG ollected and assembly of data. AK analyzed the data. All authors read and approved the submitted version.

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