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ORIGINAL ARTICLE

Parental chromosomal abnormalities in individuals with recurrent pregnancy loss

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ABSTRACT

Objective: Chromosomal abnormality is a frequent cause of recurrent pregnancy loss (RPL), which is described as the loss of two or more pregnancies before 24 weeks of gestation. The aim of this study was to investigate the type and frequency of chromosomal abnormalities in couples with a history of RPL.

Material and Methods: Consecutive 400 patients (200 couples) who were referred to our center with the complaint of RPL were included in the study. Routine karyotyping was performed using Giemsa-trypsin-Giemsa (GTG) or Giemsa-trypsin-Leishman (GTL) banding after obtaining a signed informed consent form. The patient data were then retrospectively retrieved.

Results: The median age was 32±6.25 years. Chromosomal abnormalities were detected in 4% of the patients (n=16). Of the 16 patients with chromosomal abnormalities, 9 patients had reciprocal and 1 had Robertsonian translocations. Two had inversions. Two cases had mosaic monosomy X while the remaining two had mosaic trisomy X. No significant association was found between the presence of chromosomal abnormality and age or gender (p>0.05).

Conclusion: The majority of the chromosomal abnormalities causing RPL result from balanced translocations, and other structural or numerical chromosomal abnormalities may also be the cause of pregnancy loss. Parental chromosome analysis is crucial in elaborating the cause of RPL, to provide accurate genetic counseling and preimplantation genetic diagnosis when possible.

Keywords: Chromosomal abnormality, genetic counseling, miscarriage, recurrent pregnancy loss.

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INTRODUCTION

The definition of recurrent pregnancy loss (RPL) differs in different clinical guidelines.[1] However, it is described by the European Society of Human Reproduction and Embryology Preimplantation Genetic Testing (ESHRE-PGT) consortium as ≥2 pregnancy losses before 24 weeks of gestation (including chemical pregnancy) and occurs in 1%–2% of all pregnancies.[1–3]

Chromosomal abnormalities play an important role in early pregnancy losses. More than 50% of early pregnancy losses are due to chromosomal abnormalities. *De novo* nondisjunction events are more common but less repetitive. The incidence of parental chromosomal abnormality carrier status is 2%–4% in couples with RPL.[4] The frequency of pregnancy loss is significantly increased (up to 49%) for the individuals with balanced translocations due to the production of unbalanced gametes.[5] In fact, most of the chromosomal abnormalities causing RPL result from abnormal chromosomal segregation during gametogenesis in parents carrying balanced translocations.[6] This reveals the importance of parental chromosome analysis while investigating the RPL etiology. Moreover, given that RPL is associated with mental disorders that affect the quality of life such as depression and anxiety and that may be associated with social stigma in some cultures, genetic counseling is of utmost importance for couples experiencing RPL.

In this study, we retrospectively evaluated the frequency and types of chromosomal abnormalities in couples with recurrent miscarriages.

MATERIAL AND METHODS

The study protocol was approved by the institutional ethics committee (approval date/no: June 30, 2020/281). Consecutive 400 patients (200 couples: 200 men and 200 women) who were referred to the Department of Medical Genetics between January 1, 2016, and January 1, 2020, with the complaint of RPL were included in the study. RPL was defined as two or more pregnancy losses before the 24th week of gestation. All hematologic (e.g., inherited or acquired thrombophilia), immunologic (e.g., autoimmune diseases), endocrinologic (e.g., thyroid diseases, luteal phase defect, and polycystic ovary syndrome), infectious (e.g., toxoplasma and rubella), and uterine (e.g., congenital abnormalities and adhesions) factors that may be responsible for the RPL etiology had been excluded with relevant examinations and consultations prior to genetic examination.

Genetic counseling was given to all patients, and a signed consent form was obtained. A quantity of 2 cc of blood was collected to heparin tubes from each case. Methotrexate–thymidine synchronization was provided for the cells inoculated on a phytohemagglutinin medium. Metaphases were harvested following a 72-h colcemid treatment. HRB (high-resolution banding) was provided with the GTG (Giemsa-trypsin-Giemsa) or GTL (Giemsa-trypsin-Leishman) technique. Chromosomes were analyzed with an automated optical microscope system in accordance with International System for Human Cytogenetic Nomenclature (ISCN) standards, and karyotype results were obtained.

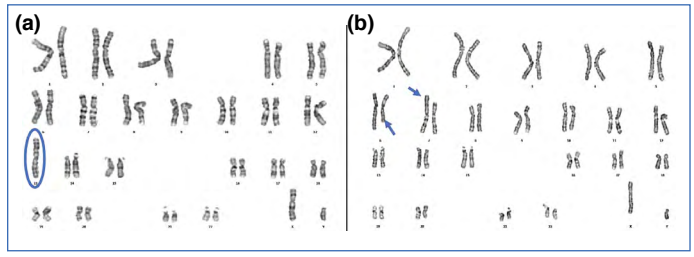

Figure 1: The most common type of chromosomal abnormality was translocation (n=10). Reciprocal translocation was present in 9 of the 10 patients, while only 1 patient had Robertsonian translocation. (a) The karyotype of the case with Robertsonian translocation: 45,XY,der(13;13)(q10;q10), shown in blue circle; GTL banding, banding level 400–450. (b) An example for reciprocal translocation (arrows): 46,XY,t(6;7)(q21;p15), GTL banding, banding level 450.

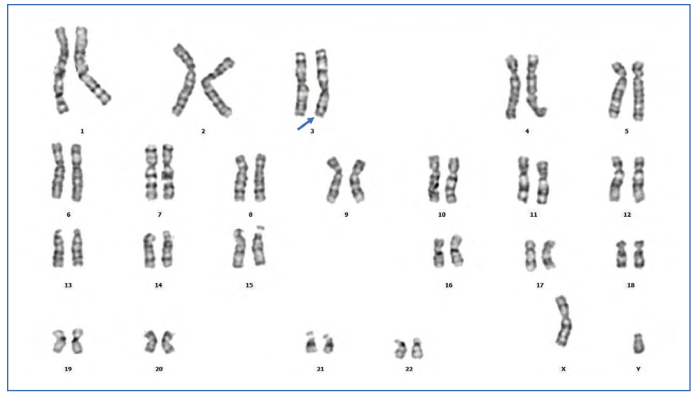

Figure 2: Inversion seen in two cases. Karyotype of the case with 46,XY, inv(3)(p14q24), GTL banding, banding level 450.

Statistical Analysis

Statistical analyses were performed via SPSS version 24. In addition to descriptive analyses, Chi-squared and Mann–Whitney U tests were used to investigate the association between categorical variables. P<0.05 was considered statistically significant.

RESULTS

The median age was 32±6.25 years for the study group (31±6.12 years for women and 33.77±6.09 years for men) (range 18–54 years for women and 22–60 years for men, respectively). Chromosomal abnormalities were detected in 4% of the cases (n=16). Of 16 patients with chromosomal abnormalities, 10 had translocations (10 of 16 (63%); overall 2.5%; 9 reciprocal and 1 Robertsonian; Fig. 1), 4 had mosaicism, and 2 had inversions (1 paracentric and 1 pericentric; 2/16 (12.5%); overall 0.5%) (Fig. 2 and Table 1). No significant association was found between the presence of chromosomal abnormalities and gender (7/193 females vs. 9/191 males; p=0.61). Although the frequency of chromosomal abnormality tended to increase with age, there was no significant association between the presence of chromosomal abnormality and age (p>0.05 for both). Six of 16 patients with chromosomal abnormalities had healthy children, and pregnancy was achieved by preimplantation genetic diagnosis (PGD) in 2 patients.

Table 1: Chromosomal abnormalities detected in the study group

Case no.	Age	Karyotype
Structural abnormalities		
1	26	45,XY,der(13;13)(q10;q10)
2	60	46,XY,t(7;19)(p13;q13.1)
3	33	46,XX,t(6;18)(p23;q11.2)
4	36	46,XX,t(1;6)(q25;p21.3)
5	30	46,XX,t(4;18)(p15.2;p11.2)
6	26	46,XY,inv(12)(q21.2;q24.1)
7	39	46,XY,inv(3)(p14q24)
8	36	46,XY,t(15;17)(q11.2;p11.2)
9	34	46,XY,t(5;21)(p11;q11.2)
10	33	46,XY,t(6;7)(q21;p15)
11	35	46,XY,t(7;10)(p11.2;q22.1)
12	29	46,XY,t(7;11)(p15;q23)
Numerical abnormalities		
13	35	mos 45,X[3]/47,XXX[3]/46,XX[43]
14	31	mos 45,X(6)/46XX(94)
15	40	mos 47,XXX[3]/46,XX[47]
16	36	mos 47,XXX[5]/46,XX[43]

DISCUSSION

In the present study, we used karyotyping technique and found the frequency of chromosomal abnormalities as 4%, a 20-fold higher incidence compared with the general population (0.2%–0.6%).[7–10] While this frequency is consistent with some of the previously published data, some researchers have reported a higher incidence (up to 11%) of chromosomal abnormalities in individuals with RPL.[4,11–13] Moreover, it has been claimed that, compared with karyotyping, low-pass genomic sequencing is significantly more effective in the detection of chromosomal abnormalities in individuals experiencing RPL.[12] Popescu et al.[4] have demonstrated that chromosome microarray analysis (CMA) provides a probable/definitive cause of RPL in more than 90% of the patients. Similarly, SNP-based CMA of the product of conception has been suggested to be more successful than karyotyping and that the chromosomal abnormality may be missed by karyotyping in 20%–40% of the cases.[14] Quantitative fluorescent-polymerase chain reaction (QF-PCR) has been reported as a superior method to array comparative genomic hybridization (a-CGH) and karyotyping.[15] Therefore, it is possible that the frequency of chromosomal abnormalities in RPL cases is dependent on the technique that was used, and the frequency may increase in the future following the adaptation of more sensitive techniques in routine practice. However, karyotyping still remains to be the most cost-effective method. It may not be possible to differentiate between *de novo* and inherited chromosomal abnormalities by analysis of the product of conception via techniques such as QF-PCR and SNP-CMA, and further testing would be required prior to genetic counseling.

The most common type of chromosomal abnormality in our study group was translocation similar to previous studies.[12,13] One partner carries a balanced reciprocal or a Robertsonian translocation in ~4% of couples with a history of RPL.[16] Individuals carrying a reciprocal translocation, which is one of the most frequent chromosomal rearrangements in humans, are asymptomatic other than showing an increased risk of having children with unbalanced translocation.[17] Robertsonian translocation carriers may also present with RPL. Robertsonian translocations are significantly more common in infertile men compared with the general population (3% vs. 0.1%),[9] and abnormalities involving chromosomes 13 and 14 represent the majority of all Robertsonian translocations.[18] One of our patients, who was also a male, had a Robertsonian translocation. As this patient had 45 chromosomes due to derivation of chromosome 13, the chance of having healthy offspring was virtually impossible for this couple. Of note, other factors may contribute to RPL in individuals with balanced translocations. While we did not observe any translocations related to the sex chromosomes, a complete spermatogenic arrest is more likely in men with translocations involving the X chromosome due to incomplete inactivation.[17,19,20]

Another type of structural chromosomal abnormality detected in this study was inversion albeit less frequent than previously reported among RPL cases.[12,13] To the best of our knowledge, this is the first study documenting inv(12)(q21.2;q24.1) although other inversions involving chromosome 12 have previously been reported to be associated with spontaneous miscarriages.[21,22] The second case with an inversion had inv(3)(p14q24), which was first described in 1974 and later reported in individuals with RPL, particularly in familial cases.[23–25] On the other hand, Lindberg et al.[25] have claimed that inv(3)(p14q24) is simply a polymorphism and not a cause for RPL although they discovered the familial inversion while investigating a patient with RPL. Regardless, it should be kept in mind that unbalanced paracentric inversions are important chromosomal abnormalities, as they are characterized by unviable pregnancies.[17]

In the present study, X chromosome mosaicism was the second most common type of chromosomal abnormality. Two of these patients had mosaic monosomy X while the remaining two had mosaic trisomy X. The most common sex chromosome abnormality in abortion samples is monosomy X, which is claimed to be responsible for 10% of chromosomal abnormalities that cause fetal loss.[26,27] Women with X-chromosome mosaicism have been reported to have a diminished ovarian reserve and that their oocytes are prone to embryonic lethality.[28] As the percentage of mosaicism X increases, the rate of fetal losses is expected to increase in parallel with nondisjunction in meiosis. While the diminished ovarian reserve is significantly associated with a higher likelihood of RPL and abnormal karyotypes in individuals with X chromosome mosaicism, the risk of full aneuploidy infants is also increased in these families.[28,29] Thus, PGD should be considered in cases with mosaicism, to reveal the gonadal chromosomal status.

There is no association between chromosomal abnormalities and gender, as we also observed in the present study. However, in many cultures, women have to take the blame for RPL due to cultural taboos, and RPL has major effects on the quality of life of individuals. Not only women with a history of RPL are more likely to develop depression and anxiety, but anxiety and depression have also been

shown to be risk factors for RPL.[30,31] As it is clear that couples with a history of RPL would benefit from psychological/psychiatric support, such issues may be briefly addressed during genetic counseling as well. Several factors may increase the probability of chromosomal abnormality carrier status: low maternal age at second miscarriage, history of ≥3 pregnancy losses, history of ≥2 miscarriages in a sibling, and history of ≥2 miscarriages in the parents of either partner.[32] As the risk of carrier status significantly increases after the second miscarriage and the efficiency of parental chromosome analysis has been suggested to be increased by withholding the test from couples with a low probability, parental karyotyping is recommended after the second miscarriage.[32] Some chromosomal abnormalities such as inversion may be difficult to be detected by routine karyotyping due to limitations of resolution and banding.[12] Therefore, genomic sequencing may be a good alternative to traditional karyotyping in selected patients; however, further study is required on how to determine the candidate patients for sequencing.

CONCLUSION

In conclusion, the incidence of structural and/or numerical chromosomal abnormalities is significantly higher than the general population in patients with RPL. The chance of achieving a healthy live birth is affected by several contributing factors as well as the type of the abnormality, that is, whether it is possible to obtain healthy germ cells or not. Prenatal diagnosis and PGD options with the detection of chromosomal abnormality should be evaluated in genetic counseling to prevent both possible pregnancy losses and live births with a chromosomal abnormality. Although routine karyotyping remains to be the most preferred method to screen for the presence of chromosomal abnormalities as the cause of RPL, alternative methods such as CGH or genomic sequencing may be applied in selected patients. However, further evidence is needed to show the superiority of these methods to traditional karyotyping.

Statement

Ethics Committee Approval: The Cemil Taşçıoğlu City Hospital Clinical Research Ethics Committee granted approval for this study (date: 30.06.2020, number: 281).

Informed Consent: Informed consent had been obtained from all patients during genetic counseling. The data was retrospectively retrieved and anonymously analyzed during the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – BE, ÖFK, OŞ; Design – BE, ÖFK; Supervision – OŞ; Resource – BE, ÖFK, OŞ; Materials – BE; Data Collection and/or Processing – BE, ÖFK, OŞ; Analysis and/or Interpretation – BE; Literature Search – BE; Writing – BE; Critical Reviews – BE, ÖFK, OŞ.

Conflict of Interest: The authors have no conflict of interest to declare.

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