Description of cytogenetic abnormalities and the pregnancy outcomes of couples with recurrent pregnancy loss in a tertiary-care center in Saudi Arabia

Khalid A. Awartani, FRCSC, FACOG, *Maryam S. Al Shabibi,* MD.

ABSTRACT

الأهداف: لتحديد انتشار اضطرابات الكروموسومات عند الأزواج الذين يعانون من الاجهاض المتكرر وتحديد عوامل أخرى التي قد ترتبط مع اضطرابات الكروموسومات و لتقيم نتائج الأزواج الذين خضعوا لإجرات متعددة الاختصاصات بناء على ارتباط العوامل المسببة .

الطريقة: أجريت هذه الدراسة بأثر رجعي على 1074 من الأزواج الذين حضروا في عيادة الاجهاض المتكرر خلال فترة 11 عاما من يناير 2006 إلى ديسمبر 2016 في مركز واحد وهو مستشفى الملك فيصل التخصصي ومركز الأبحاث، الرياض، المملكة العربية السعودية. جميع الأزواج خضعوا للتقييم والمتابعة عن كثب أثناء الحمل.

النتائج: من بين 1074 من الأزواج ،77 (7.2%) لديهم بعض من اضطرابات الكروموسومات، الأناث في الغالب تأثروا أكثر من الذكور (48, 62.3%) ومن بين 77 حالة من اضطرابات الكروموسومات . 46.8% انتقال متبادل و 10.3% إزْفاءٌ روبرتسونيّ و3.9% اضطرابات هيكلية معقدة. حدث انقلاب انعكاس كروموسومي في 14.3% و 2.6% كانت إضافات بهيكل الكروموسومات. تم الكشف عن اضطرابات الكروموسومات كسبب وحيد للاجهاض المتكرر في 25 من 77 حالة (32.5%) من الأزواج وتمت متابعة حالة الأزواج عن كثب حيث أن 67% من حالات الحمل انجبوا مواليد أحياء.

الخاتمة: توضح نتائج الدراسة نظرة ثاقبة عن انتشار اضطرابات الكروموسومات عند الأزواج الذين يعانون من الأجهاض المتكرر في منطقتنا و العوامل التي قد تكون متعلقة به. وتساعد هذه المعلومات على ضمان توفير الموارد اللازمة لرعاية هؤلاء المرضى.

Objectives: To determine the prevalence of chromosomal abnormalities in couples with recurrent pregnancy loss (RPL), to determine other factors that may be associated with the chromosomal abnormalities, and to assess the outcomes of couples who had undergone multidisciplinary interventions according to associated etiological factors.

Methods: This retrospective cohort study involved 1074 couples who attended RPL clinic during an 11-year period from January 2006 to December 2016 at a single center, King Faisal Specialist Hospital and Research Centre, Riyadh, Kingdom of Saudi Arabia. All of the couples had undergone complete RPL evaluations and were closely monitored and managed during pregnancy.

Results: Out of the 1074 couples, 77 (7.2%) carried some form of chromosomal abnormality, and the female (48, 62.3%) patients were affected more frequently than the male (29, 37.3%) patients. Out of the 77 cases with chromosomal abnormalities, 46.8% had reciprocal translocations, 10.3% had Robertsonian translocations, and 3.9% had complex structural abnormalities. Inversions had occurred in 14.3% and chromosomal additions had occurred in 2.6% of the patients. Isolated chromosomal abnormalities were detected in 25 out of 77 (32.5%) couples. The couples were closely followed, and 67% of the subsequent pregnancies resulted in live births.

Conclusion: This study's findings provide an insight into the prevalence of chromosomal abnormalities in couples with RPL in our region and the factors that may be associated with RPL. This information will help to ensure the required resources are provided to care for these patients.

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From the Department of Obstetrics and Gynecology, King Faisal Specialist Hospital and Research Center, Riyadh, Kingdom of Saudi Arabia.

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Address correspondence and reprint request to: Dr. Maryam S. Al Shabibi, Department of Obstetrics and Gynecology, King Faisal Specialist Hospital and Research Center, Riyadh, Kingdom of Saudi Arabia. E-mail: mrym.sh@hotmail.com
ORCID ID= orcid.org/0000-0002-8478-0539

Spontaneous pregnancy loss is a common occurrence. Approximately 15% of all clinically recognized pregnancies result in spontaneous losses, but many more pregnancies fail before they are recognized clinically.[1] Indeed, only 25–30% of all conceptions result in live births.[2] Recurrent pregnancy loss (RPL) is defined as ≥2 clinically recognized pregnancies that have failed before 20 weeks.[3] The findings from epidemiologic studies have shown that 1-4% of women experience RPL.[3,4] Recurrent pregnancy loss is a challenging area of reproductive medicine, because its etiology is unknown in 30–50% of the cases.[5,6] Recurrent pregnancy loss has been associated with factors that are related to genetics, age, antiphospholipid syndrome (APS), uterine anomalies, thrombophilia, hormonal or metabolic disorders, infections, autoimmunity, sperm parameters, and lifestyle issues.[2] Between 2% and 8% of recurrent pregnancy losses are associated with parental balanced structural chromosome rearrangements, and, most commonly, balanced reciprocal or Robertsonian translocations.[7-10] Additional structural chromosome abnormalities that are associated with RPL include chromosomal inversions, insertions, and mosaicism.[4,5] This study was based on a large database of patients who were referred to RPL clinics at a tertiary care referral center in Saudi Arabia, and it aimed to determine the prevalence of chromosomal abnormalities in couples with RPL, to determine other factors that may be associated with the chromosomal abnormalities, and to assess the outcomes of couples who had undergone multidisciplinary interventions.

Methods. This was a retrospective cohort study of the charts of all of the women who attended RPL clinics over an 11-year period from January 2006 to December 2016 at King Faisal Specialist Hospital and Research Centre (KFSH&RC-Riyadh). The study was based on a database created by one of the authors (KA). The study was approved by the Research Advisory Council. This article does not contain any studies with human participants or animals performed by any of the authors in accordance with the ethical standards of the institutional and/or national research committee and with 1964 Helsinki declaration and its later amendments or comparable ethical standards. Furthermore, there was no requirement to obtain informed consent from the study's participants.

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The data were collected from the patients' medical charts, and the computerized Integrated Clinical Information System. Telephone calls were used to contact the patients regarding their pregnancy outcomes. The patients' demographic and clinical data, including their age, weight, body mass index, and parities, the numbers of term deliveries and miscarriages, and the treatments used, and each couples' chromosomal analyses, diagnoses, and pregnancy outcomes, were obtained.

As part of the RPL workup undertaken for all of the couples, the complete blood count and the coagulation profiles, including the activated partial thromboplastin time, prothrombin time, and fibrinogen, were determined. The thyroid-stimulating hormone, prolactin, and glycated hemoglobin levels were evaluated to determine whether there were endocrine etiologies underlying the RPLs. Thrombophilia screening included assessments of the protein C and S, activated protein C resistance, antithrombin III, homocysteine, and lipoprotein (a) levels, and evaluations of the presence of methylenetetrahydrofolate reductase , prothrombin G20210A, and factor V Leiden gene mutations. The antiphospholipid antibody and antinuclear antibody levels were assessed to determine whether there were autoimmune factors underlying the RPLs. Three-dimensional pelvic ultrasound examinations, office-based hysteroscopies,and hysterosalpingographies were carried out to determine whether there were anatomical causes underlying the RPLs. All of the couples underwent karyotyping.

For the cytogenetic analyses, peripheral whole blood (0.4 mL) was incubated in complete Roswell Park Memorial Institute-1640 medium for 72h at 37°C, then 50 mL colcemid was added. After incubation for 20 min, a hypotonic solution of potassium chloride was added and the mixture was incubated for 10 min. A 3:1 methanol-acetic acid mixture was used to fix the samples before the slides were made. All of the data where entered into the database prospectively.

The statistical analyses were undertaken using the chi-square test for binomial data and S-plus 2000 software (MathSoft, Inc., Seattle, WA, USA). A value of $p< 0.05$ was considered statistically significant.

Results. A total of 1074 couples and 2148 individuals attended the RPL clinics at KFSH&RC from January 2006 to December 2016. The women's mean (standard deviation [±SD]) age was 30.6±5 years. The mean (±SD) number of previous miscarriages was 5±3.

Out of the 1074 couples, 77 (7.2%) had chromosomal abnormalities, and more female patients

had chromosomal abnormalities compared with the male patients (p=0.03). Reciprocal translocations was the most common chromosomal abnormalities (46%) followed by Robertsonian translocation (**Table 1**).

Most of the couples (67.5%) with chromosomal abnormalities had 1 or 2 additional etiological factors that were associated with RPL, and only 32.5% of the couples had isolated chromosomal abnormalities. Congenital thrombophilia was present in 36.4% of the females and 24.7% of the females had APS or other immunological etiologies that were associated with RPL. Anatomical factors (Uterine septum or Bicornuate uterus) that may have contributed to RPL were found in 6.5% of the females.

Before the investigations and management were undertaken, 34 out of 77 couples (44.2%) had at least one living child. These couples had undergone a total of 332 pregnancies, of which 85% had resulted in miscarriages and 15% had resulted in live births. Following their enrollment with the RPL clinic, 37 women became pregnant either spontaneously or through preimplantation genetic diagnosis (PGD). Out of the 66 pregnancies that were closely monitored and involved the treatment of all of the associated factors, 44 were successful and the live birth rate was 67% and the miscarriage rate was 34% (p=0.001). No chromosomal analyses were undertaken on the products of conception. The group of couples with reciprocal translocations had a live birth rate of 79% and a miscarriage rate of 21%. All of the subsequent pregnancies resulted in miscarriages in the group of couples with Robertsonian translocations. The couples with inversion chromosomal abnormalities had a live birth rate of 40% and a miscarriage rate of 60%, and those with numerical chromosomal abnormalities had a live birth rate of 83% and a miscarriage rate of 17%.

Table 1 - Types of chromosomal abnormality diagnosed.

Chromosomal abnormality	Maternal	Paternal	Total	(%)
Reciprocal translocation	19	17	36	(46.8)
Robertsonian translocation	7	1	8	(10.3)
Inversion	6	5	11	(14.3)
Addition	0	2	2	(2.6)
Complex structural rearrangement	2	1	3	(3.9)
Turner/ mosaic Turner syndrome	14	0	14	(18.2)
X or Y aneuploidy	0	3	3	(3.9)
Total	**48**	**29**	77	**(100)**

Discussion. Cytogenetic studies are important for evaluating couples with RPL. The prevalence of chromosomal abnormalities in the present cohort was 7.2%, which is similar to the rates reported from other studies conducted in the Arab states of the Persian Gulf, namely 7.8% in Saudi Arabia,[11] and 3.84%,[12] and 8%[13] in Oman. The findings from studies conducted in other countries have demonstrated RPL rates that range between 2.7% and 13% (**Table 2**).

Regarding the distribution of chromosomal abnormalities according to gender, there was a higher prevalence of chromosomal abnormalities among the female patients compared with that in the male patients, which concurs with the findings reported from other countries.[7] Among the structural chromosomal aberrations, reciprocal translocations occurred most frequently (46.8%), followed by inversions (14.3%) then Robertsonian translocations (10.3%).

One study reported pregnancy outcomes in couples with chromosomal abnormalities and RPL.[7] Live birth

Table 2 - Worldwide studies of chromosomal abnormalities diagnosed in couples with recurrent miscarriage.

Reference	Country	Number of couples studied	Reciprocal translocation	Robertsonian translocation	Inversion	Others	Total	(%)
Current study	Saudi Arabia	1074	36	8	11	22	77	(7.2)
Stephenson MD, Sierra S[7]	USA	1893	28	12	7	4	51	(2.7)
Makino T et al[8]	Japan	639	19	9	16	11	54	(8.5)
Al-Hussain M et al[11]	Saudi Arabia	193	10	1	2	2	15	(7.8)
Goud TM et[12]	Oman	380	18	3	-	5	26	(3.84)
Eltayeb SM et al[13]	Oman	290	-	-	3	3	23	(8.0)
Stephenson MD[14]	Canada	100	4	3	4	2	13	(13.0)
Gaboon NE[15]	Egypt	125	7	1	-	-	8	(6.4)
Fan HT et al[16]	China	1948	42	11	5	-	58	(2.98)
Kochhar PK, Ghosh P[17]	India	788	47	6	1	-	54	(6.8)
Flynn H[18]	UK	795	18	3	2	5	28	(3.5)
Tunç E[19]	Turkey	1510	30	12	9	11	62	(4.1)
Ghazaey S[20]	Iran	728	37	7	21	20	85	(11.7)

rate improvement after evaluations and interventions from 15% to 67% is in concordance with live birth rates reported.

Ideally, chromosomal studies should be performed on the products of conception to complete the cytogenetic evaluations, but these tests are not available at our center.

The management options available for the couples included complete RPL workups and interventions to manage all of the factors associated with RPL in accordance with our protocol. The patients were closely followed up during pregnancy, starting from 6 weeks of gestation by doing weekly ultrasound till 10 weeks. The patients then will be referred to obstetric high risk clinic at 12 weeks of gestation. Regardless of whether a pregnancy was achieved spontaneously or through in vitro fertilization or PGD, the patients were offered chorionic villus sampling or amniocentesis as perinatal diagnostic tests during pregnancy. The live birth rate was very high. This large data was in concordance to other prior findings in the area and world-wide with demonstration of reasonable outcome for this patient's population.[7]

Study limitations. Since this was a retrospective study, we had to obtain fundamental information about the patients from their medical records, and some of the patients' key data could not be evaluated (for example, the previous treatments used).

In conclusion, in this large set of data from Saudi Arabia, we have shown that it is not uncommon to see couples carrier for chromosomal abnormalities as part of their RPL work up, and it is common to be part of a multifactorial problem where intervention and close follow-up yield a very good live birth rate for these couples.

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