

CYTOGENETIC STUDY IN CASES WITH RECURRENT ABORTION IN SAUDI ARABIA

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Background: A proportion of cases with repeated abortion are caused by chromosomal abnormality in one of the parents. Several studies have been done to determine the role of chromosomal abnormalities in couples with repeated fetal loss in various countries. None of these studies was done in the Arab Peninsula.

Material and Methods: Cytogenetic study was done for 193 consecutive Saudi couples who presented with repeated abortion at the King Khalid University Hospital in Riyadh, Saudi Arabia.

Results: We found that the frequency of chromosomal abnormalities was not significantly different from that reported worldwide. The nature of those abnormalities and their relation to the obstetric history of cases were discussed.

Conclusion: This study should help physicians working in the region to realize the contribution of chromosomal abnormalities to cases of repeated fetal loss. It should also help in setting priorities of cytogenetic screening in individual cases.

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Approximately 15%-20% of clinically recognizable pregnancies end in spontaneous abortion.^{1,2} The incidence of chromosomal abnormalities in those abortions is as high as 50%.³ A modest but clinically important proportion of spontaneous abortions is caused by a balanced chromosomal aberration in one of the parents.⁴⁻⁸ This results from the production of gametes and embryos with unbalanced chromosome sets.^{9,10} The clinical consequences of such abnormal gametes include sterility, repeated abortions, and giving birth to malformed children.^{11,12}

Several studies have been done in various countries to determine the contribution of chromosome abnormalities in parents with fetal wastage.¹³ To our knowledge, no such studies have been done in the Arabian Peninsula. The aim of this study was to assess the frequency and nature of chromosomal aberrations that contribute to the occurrence of repeated abortions in Saudi Arabia. This should assist physicians in Saudi Arabia and other neighboring countries by increasing their awareness of the frequency of cytogenetic abnormalities in cases with repeated abortions. It also provides figures for comparison with other countries and research centers.

This study included all Saudi couples with repeated abortions who were referred for cytogenetic studies between December 1994 and December 1998 at King Khalid University Hospital in Riyadh, Saudi Arabia. All cases were ascertained to have had two or more spontaneous abortions. Couples who were referred because of having previous children with congenital anomalies and abortions were not included in the study. The obstetric history of couples was either recorded on the request form or retrieved from the files of patients.

For routine cytogenetic analysis, 0.3 mL of peripheral blood was incubated in complete lymphocyte culture medium (10% fetal bovine serum in RPMI 1640, with 0.15% phytohemagglutinin and 1% Penstrep in 5% CO₂ incubator at 37°C for three days). Metaphases were harvested by adding colcemid for 20 minutes, followed by hypotonic KCl treatment for 5 minutes and fixation, using standard 3:1 methanol-acetic fixative (all the reagents were from GIBCO Life Technologies Ltd., Paisley, Scotland). The high-resolution study was done by synchronization, using methotrexate (10⁻⁷M) for 17 hours, and thymidine (10⁻⁵M) for 5.5 hours before harvesting, as mentioned elsewhere.¹⁴

Microscopic examination of 15-20 cells and photography of two cells were done after standard trypsin-Wright G-banding (GTW) and/or Quinacrine Q-banding (QFQ). Chromosomes were visually analyzed and

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Materials and Methods

FIGURE 1. Female karyotype showing a small paracentric inversion of chromosome 8:46, XX, inv (8) (p12 p23).

abnormalities were detected during microscopy in all cases. Microscopic photography and karyotype were done for documentation in abnormal cases.

The correlation between the obstetric history of cases with and without abnormal karyotype was computed using the Spearman test for correlation using the SPSS program.¹⁵ The frequencies of chromosomal abnormalities were compared to similar studies using Z-test for comparison of two frequencies with unequal variance.

Results

A total of 193 Saudi couples with history of repeated abortions were examined. The age of the referred wives ranged from 16 to 50 years, with a mean of 30.31 years (SD=6.78). The number of previous deliveries ranged from 0 to 13 (mean 2.1; SD=2.4511). The number of previous abortions varied from 2 to 16 abortions (mean 4.2 abortions/couple; SD=2.1801).

Eleven females (5.7%) and four males (2.07%) were found to have abnormal karyotypes. These abnormalities included 10 balanced reciprocal translocations, one Robertsonian translocation, two inversions and two cases of mosaic X-chromosome monosomy (Table 1). Examples of the encountered chromosomal abnormalities are shown in Figures 1 and 2.

Among cases with abnormal karyotype, the mean maternal age was 28.8 (SD=5.78). The mean number of previous deliveries was 1.4667 deliveries/couple (SD=2.0307), and the mean number of abortions was 5.73 per couple (SD=2.4631).

The Spearman test for correlation showed that older maternal age, the average gestational age at time of abortion, and the number of previous deliveries were not statistically associated with the occurrence of chromosomal aberrations. However, the number of previous abortions showed a significant influence on the possibility of having a chromosomal abnormality ($P=0.005$).

FIGURE 2. Male karyotype showing a reciprocal translocation between the short arms of chromosomes 2 and 6:46, XY, t (2; 6) (p13; p21.3).

TABLE 1. Cytogenetic findings, parity, number of abortions and maternal age in cases with abnormal karyotype.

Karyotype	Parity	No. of abortions	Maternal age
46, XX, t(7;8) (p15;q23)	1	4	28
46, XY, t(5;12) (q11;p13)	1	10	30
46, XX, t(4;17) (p16;q24)	0	5	28
46, XY, t(2;11) (q24;q32)	0	3	27
46, XY, t(5;7) (p11;q11)	3	8	32
46, XX, t(7;11) (p13;q24)	1	3	27
46, XX, t(10;14) (p13;q14)	1	7	32
46, XX, t(1;3) (q22;q23)	2	3	23
45, XX, t(13;14) (p11;q11)	0	9	26
46, XY, t(2;6) (p13;p21.3)	2	9	39
46, XX, inv (8) (p12;p23)	0	3	19
46, XX, [113]/45, X[4]	0	6	26
46, XX, t(6;11) (q23;q22)	2	4	25
46, XX [210]/45, X [7]	8	5	42
46, XY, inv (4) (p14 q31.3)	1	7	28

Discussion

Schmidt (1962) was the first to report the results of cytogenetics analysis in patients with a history of two or more spontaneous abortions. This was followed by a series of cytogenetic studies of couples with a history of repeated pregnancy loss.¹⁶ The incidence of chromosomal abnormalities among those cases varied in different studies, from none¹⁷ to as high as 21.4%.¹⁸ The variations in the size of the sample, the criteria used for ascertainment of cases, and the technique of cytogenetic study have contributed to these wide differences between various studies.¹⁹ It is also possible that different populations vary in the incidence of carriers of chromosomal aberrations.

Several studies have been carried out to determine the prevalence of chromosomal aberrations among couples with repeated fetal loss. This was found to be 5.8%, 5% and 4.7% in three of the largest reviews that were reported by Tharapel et al.,¹⁹ Campana et al.²⁰ and Braekeleer and

TABLE 2. Structural chromosome rearrangements found in couples with recurrent abortions compared to similar studies.^{5,22,23}

	No. of couples studied	Robertsonian	Reciprocal	Inversion	Others	Number	%
Spain (Barcelona)	32	1	3	1	1	6	17.8
Netherlands (Leiden)	67	3	5	1	—	9	13.4
Italy (Padua)	145	4	4	4	2	14	9.6
Netherlands (Rotterdam)	148	3	6	3	2	14	9.6
Belgium (Gent)	96	2	6	—	—	8	8.3
Switzerland (Zurich)	96	2	4	—	1	7	7.3
Saudi Arabia (Riyadh)	193	1	10	2	—	13	6.7
France (Paris)	315	5	7	4	—	16	5.1
Japan	639	9	19	1	—	29	4.5
France (Strasbourg)	217	4	—	2	—	6	2.8

Dao,¹⁶ respectively. In this study, we found that the incidence of chromosomal abnormalities among couples with repeated abortions was 7.7% (SD=1.92), which is not significantly different from the global incidence.

We found that 11 women and four men had chromosomal abnormalities, which was a ratio of 2.75:1. A similar male to female ratio has been found in most of the reported studies. This predominance of females appears to be due to the fact that chromosomal abnormalities that are compatible with fertility in females may be associated with sterility in males.^{11,16}

The structural chromosomal abnormalities that we encountered were divided into balanced reciprocal chromosomal translocations (10/15), Robertsonian translocation (1/15), and inversions (2/15). Table 2 shows that the distribution of structural chromosomal rearrangements in our study is similar to that reported worldwide.

Numerical chromosomal aberrations are less frequently encountered among couples with repeated abortions. Those aberrations are usually in the form of sex chromosomal aneuploidy, and they occur in a low frequency (<0.15% of cases).²⁰ We encountered two cases with X-chromosome mosaicism. Reviewing the clinical features in both cases showed that none of them had the features of Turner syndrome. The two cases showed a low level of monosomic cells (Table 1). However, the X monosomy could not be ascribed to a normal background level of monosomic cells, as the level of X-chromosome monosomy in our laboratory never exceeded 0.5% in other groups of referrals. The low level of mosaicism and/or confinement of the aneuploid cell line to genital organs may explain the normal phenotype of both cases.

Regarding the relation of the obstetric history to the frequency of chromosomal aberrations, no statistically significant correlation was found between the number of deliveries or gestational age and the occurrence of chromosomal abnormalities. However, a statistically significant correlation was found between the number of previous abortions and the occurrence of chromosomal abnormalities ($P=0.005$). Thus, in cases of paucity of resources and the need to limit cytogenetics study to a fraction of cases, those with a larger number of abortions

should be given priority for screening, even if they had previously normal deliveries.

This study has shown that the incidence and distribution of chromosomal abnormalities among Saudi couples with repeated fetal loss is comparable to that reported worldwide. Physicians in charge of clinics for repeated fetal loss should bear in mind that in at least 5% of the couples they examine, chromosomal abnormality is the cause of abortions. Those cases have to be detected as early as possible to arrange for adequate genetic counseling and to allow parents to make an informed reproductive decision regarding subsequent pregnancies.

Cytogenetics studies should be offered to all couples with more than two spontaneous abortions. However, in cases where cytogenetic studies are not freely available, priority should be given to cases with larger numbers of abortions.

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