Article

Cytogenetic studies in couples with recurrent miscarriage in the Sultanate of Oman

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Abstract

Miscarriage, defined as spontaneous pregnancy loss at <20–28 weeks' gestation, is a common clinical problem. Balanced chromosomal rearrangements in either parent are an important cause of repeated pregnancy loss, particularly in the first trimester. In this study, chromosomal abnormalities that cause recurrent miscarriage were evaluated in Omani parents and some of their dysmorphic children. A total of 380 couples (760 individuals) with two or more recurrent miscarriages were examined for chromosomal aberrations during the period 1999–2006. For each proband the chromosomal preparations were analysed and karyotyped after applying a Giemsa–trypsin banding method. The overall incidence of chromosomal anomaly was 26 out of 760 individuals (3.42%). These abnormalities included 21 (2.8%) structural aberrations and 5 (0.7%) numerical anomalies. In addition to these abnormalities, 39 (5.1%) chromosomal variants were also found. The nature of these abnormalities and their relation to obstetric history are discussed. In conclusion, chromosomal abnormality is one of the causes of recurrent miscarriage. This study illustrates the incidence and distribution of chromosomal abnormalities among Omani couples with recurrent miscarriage. Cytogenetic findings could provide valuable information for genetic counselling and allow monitoring of future pregnancies by prenatal diagnosis in couples with a history of recurrent miscarriage.

Keywords: *aneuploidy, chromosomal abnormalities, reciprocal translocation, recurrent miscarriage, Robertsonian translocation, spontaneous abortion*

Introduction

Miscarriage, defined as spontaneous pregnancy loss at <20–28 weeks' gestation, is a common clinical problem. Early pregnancy loss in the first trimester is the most common complication affecting at least 15–20% of clinically recognised pregnancies (Boue *et al*., 1985). Recurrent miscarriage, defined as three or more consecutive miscarriages, affects up to 3% of couples trying to establish a family (US Department of Health Services, 1982; Franssen *et al*., 2005). Some investigators feel that even two spontaneous abortions constitute recurrent miscarriage and deserve evaluation (Coulam, 1991). Historically, structural genetic, endocrine, anatomic and autoimmune factors were associated with recurrent miscarriage in about 60% of cases (Hill *et al*., 1992; Clifford *et al*., 1994; Stephenson, 1996). In the other 40% of cases, no association with these factors could be found. Repeated pregnancy losses during the first trimester are usually due to fetal genetic defects. Pregnancies lost in late gestations also have a high rate of chromosomal anomalies, about 30% in the second trimester and 5% in the third trimester (Lee and Silver, 2000). The mean incidence of cytogenetic anomalies in 6639 couples investigated for recurrent miscarriage was 6.65% (Fryns *et al*., 1984; Chandley, 1990; Kalpana *et al*., 2004).

Balanced structural chromosome abnormalities (abnormalities that involve the rearrangement of genetic material but no overall gain or loss, such as inversions and translocations) in parents can cause recurrent miscarriage. In couples with two or more miscarriages the incidence of these abnormalities varies between 3% and 6% (Brackeeleer and Dao, 1990; Clifford *et al*., 1994;

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Franssen *et al.*, 2005). When one parent carries a chromosome rearrangement the chance of miscarriage is usually 25–50% (Gardner and Sutherland, 1996). Empirical and/or hypothetical data are available for predicting the chance of adverse pregnancy outcome for various rearrangements (Daniel *et al.*, 1989). A history of any infertility, miscarriage, stillbirth or the birth of a child with multiple congenital abnormalities and/or mental retardation is significant because each is characteristic of chromosomal anomaly (Gardner and Sutherland, 1996).

In carrier couples, the products of conception can have a normal karyotype, the same balanced structural chromosome abnormality as the carrier, or an unbalanced structural chromosome abnormality. The last scenario can lead to the fetus being miscarried, a stillborn child, or a child born with major congenital defects and severe mental handicap. Current guidelines for the management of recurrent miscarriage recommend chromosome analysis in both parents (Royal College of Obstetricians and Gynaecologists, 2003). Once a structural chromosome abnormality has been detected, prenatal diagnosis in subsequent pregnancies and termination of pregnancy in the case of an unbalanced fetal karyotype is available.

Cytogenetic studies have been reported to determine the contribution of chromosomal abnormalities in parents with reproductive failure from various other countries. As far as is known, no such studies have been performed in the Sultanate of Oman. The aim of the present study was to evaluate the frequency and nature of chromosomal aberrations in Omani couples that contribute to miscarriages.

Materials and methods

In this descriptive case series study all 380 couples (760 individuals) with no fewer than two miscarriages were referred for cytogenetic investigation by various gynaecologists, obstetricians, and medical geneticists in the Sultanate of Oman between June 1999 and December, 2006. The obstetric history of the couples was recorded. The couples were grouped as: couples only with recurrent miscarriage; couples with recurrent miscarriage preceded by stillbirth or an abnormal child; and couples with recurrent miscarriage and a healthy child. All the couples were in the age range 17–45 years and the number of miscarriages ranged from two to 10.

For routine cytogenetic analysis, peripheral blood (2–3 ml) was collected in heparin vacutainers. For every case whole blood (0.5–0.6 ml) cultures were set up in 5 ml Roswell Park Memorial Institute 1640 media (GIBCO BRL, USA) containing 20% fetal bovine serum (GIBCO BRL), antibiotic mixture (10,000 units of Penicillin and 10 mg of stabilised streptomycin solution; SIGMA) and phytohaemagglutinin (PHA; GIBCO BRL) for 72 h (Moorhead *et al.*, 1960). Giemsa–trypsin banding of metaphase chromosomes was performed using standard methodology (Seabright, 1971). In each case, 25–30 metaphase plates were microscopically examined and scored and at least five metaphases were analysed per karyotype. In cases of suspected mosaicism, 50 cells were counted. After the detection of a chromosomal abnormality, other banding techniques (C-banding, NOR-banding) were used, if necessary. Microscopic photography and karyotype were carried out to document abnormal cases. A fluorescence in-situ hybridization technique was not available for the current study.

Results

In this study, the 380 couples (760 individuals) studied were classified into three groups according to the number of previous miscarriages. In group 1, couples had two or three miscarriages, in group 2, they had four or five and in group 3 they had six or more miscarriages. The highest number of patients was seen in group 1 (63.7%) (**Table 1**). The women were aged 17–45 years, with a mean of 27.66 years (SD = 5.01). The number of previous miscarriages varied from two to 10 (mean 3.1 miscarriages/couple; SD = 1.46). Among cases with abnormal karyotype, the maternal age was 27.31 years (SD = 4.55). The mean number of miscarriages was 3.9 per couple (SD = 1.63).

Among 380 couples (760 individuals), chromosome abnormalities were detected in 26 individuals (3.42%). Details of the abnormal karyotypes and the obstetric history of the couples are shown in **Tables 2** and **3**. Fourteen females (3.7%) and 12 males (3.2%) were found to have abnormal karyotypes. Among 26 cases, 21 (80.8%) showed structural aberrations (**Table 2**) and five (19.2%) carried numerical abnormalities (**Table 3**). Among 21 cases of structural abnormalities that formed the largest group of chromosomal anomalies, reciprocal translocations (**Figure 1**) were seen in 18 cases (85.7%), which frequently involved chromosomes 1, 5, 7, 10 and 18. Robertsonian translocations (**Figure 2**) were found only in three cases (14.3%) involving chromosome 13;14 and 13;22 (**Table 2**). Apart from these major chromosomal abnormalities, 39 (5.1%) individuals were found to have chromosomal variants, of which 32 cases included enlarged heterochromatin, extended satellites, variations in Y chromosome and additions of 14p, 15p and 21p and in seven patients the karyotypes revealed pericentric inversions (**Table 4**).

Five dysmorphic newborn baby samples were referred to the laboratory for karyotyping. One showed a non-immune hydrops baby (stillbirth); fetal karyotype of the amniotic fluid showed 46,XY,del10p, this derivative chromosome 10 derived from the mother having 46,XX,t(2;10). A baby with dysmorphic features showed 21q+, which resulted from his father who had a reciprocal translocation t(8;21)(p11.2;p11). Another baby had partial trisomy of 18q; his father was a carrier of balanced translocation t(10;18)(p13;q23). A dysmorphic child was reported as 10p+; the mother of this child revealed t(10;18)(p12;p11.2). Another dysmorphic child had an addition of chromosome 12(q24); the father of this child showed t(1;12)(q42;q24) (**Table 2**).

Table 5 shows the patients with chromosomal abnormalities (structural abnormalities such as reciprocal and Robertsonian

Table 1. Couples grouped according to the number of miscarriages.

Number of miscarriages	*No. of couples*	*Percentage*
2 or 3	242	63.7
4 or 5	109	28.7
6 or more	29	7.6
Total	380	100

Table 2. Cytogenetic findings, number of miscarriages and maternal/paternal age in recurrent miscarriage cases with structural aberrations.

Reciprocal translocation	*No. of miscarriages*	*Sex*	*Maternal/ Paternal age (years)*
46,XX,t(1;6)(q25;q16)/46,XX	9	F	33
46,XX,t(7;20)(p15;q13)	5	F	30
46,XY,t(1;5)(qter;p14)	4	M	29
46,XY,t(16;18)(p12;q23)	4	M	35
46,XY,t(1;12)(q32;q24)	4	M	29
46,X,der(X)t(X;6)(q22;q15)	2	F	20
46,XX,t(7;18)(q22;p11)	5	F	36
46,XXt(2;10) [fetal karyotype 46,XY,del(10p)]	3	F	25
46,XX,t(7;14)(q22;p10)	3	F	26
46,XY,t(10;18)(p13;q23) [Baby with partial trisomy of 18q]	4	M	34
46,XXt(10;18)(p12;p11.2)[dysmorphic child with 10p+]	3	F	25
46,XY,t(8;21)(p11.2;p11) [dysmorphic child with 21q+]	4	M	43
46,XY,t(5;10)(q35;q24)	4	M	35
46,XX,t(4;18)(q22;q23)	5	F	29
46,XX,t(10;11)(p13;q13)	3	F	28
46,XY,t(10;18)(p13;q21.1)	5	M	30
46,XY,t(1;12)(q42;q24) [dysmorphic child with (12)(q24)]	nr	M	35
46,XX,t(8;18)(q23;q22)	2	F	26
45,XX,t(13;14)(q10;q10)	3	F	23
45,XX,t(13;22)(q10;q10)	3	F	24
45,XX,t(13;14)(q10;q10)	4	F	25

nr = information not recorded.

Table 3. Cytogenetic findings, number of miscarriages and maternal/paternal age in recurrent miscarriage cases with numerical chromosomal abnormalities.

Numerical	*No. of miscarriages*	*Sex*	*Maternal/ paternal age (years)*
47,XXX/46,XX	3	F	34
47,XYY	3	M	39
47,XXY/XY	nr	M	35
47,XXY(90)/46,XY(10) with long Y	3	M	27
47,XXY(80)/46,XY(20)	2	M	27

nr = information not recorded.

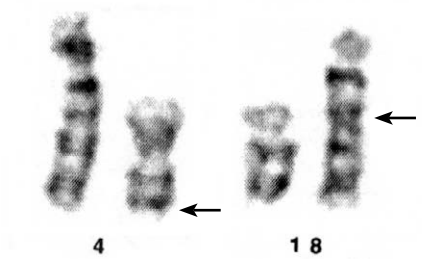

Figure 1. Female partial karyotype showing a reciprocal translocation between the long arms of chromosomes 4 and 18: t(4;18)(q22;q23).

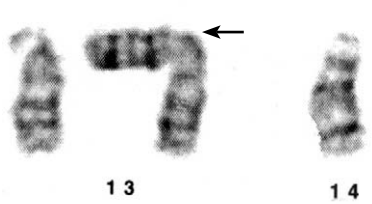

Figure 2. Female partial karyotype showing a Robertsonian translocation between the long arms of chromosomes 13 and 14: t(13;14)(q10;q10).

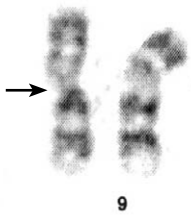

Figure 3. Male partial karyotype showing a pericentric inversion of chromosome 9: inv(9)(p13q12).

Table 4. Polymorphic chromosomal variants in recurrent miscarriage cases.

Variants	*No. of cases*	*%*
15p+	19	48.72
Short Y (yq–)	8	20.51
Pericentric inversion (9)(p13q12)	6	15.40
16qh+	2	5.13
Pericentric inversion (y)	1	2.56
22pstk+	1	2.56
14p+	1	2.56
21p+	1	2.56

Table 5. Major chromosomal anomalies in recurrent miscarriage cases according to obstetric history.

Indications	*No. of couples*	*Major chromosomal anomalies*	*%*
RM only	240	14	5.8
RM + abnormal child or stillbirth	104	8	7.7
RM + healthy child	36	4	11.1

RM = recurrent miscarriage

translocations and numerical abnormalities) according to their obstetric history. Chromosomal anomalies were found to be 5.8% in couples with only recurrent miscarriage. In couples with a history of stillbirth or an abnormal child along with recurrent miscarriage, the frequency of chromosomal aberrations was 7.7%, while in couples with recurrent miscarriage and a healthy child, this frequency was the highest at 11.1%. In this study, in couples who reported chromosomal abnormalities only one partner in each couple was affected and the affected individuals showed only one anomaly.

Discussion

Recurrent miscarriage is a difficult medical problem occurring in about 1–2% of fertile women (Wuu *et al*., 1991). Cytogenetic studies often form important parameters for the medical evaluation of subjects presenting with recurrent miscarriages. The incidence of chromosomal abnormalities among the couples with recurrent miscarriage in the present study was 6.84% (3.42% of individuals); this was higher than the incidences found in previous studies (2.9–6%) (Tharapel *et al*., 1985; Brackeeleer and Dao, 1990; Clifford *et al*., 1994; Franssen *et al*., 2005). Worldwide studies showed considerable differences in the frequency of chromosomal aberrations, which ranged from 2.76 to 18.75% (**Table 6**). The results of numerous cytogenetic studies on couples with recurrent miscarriage vary considerably. This is partly due to sample size, criteria used for selecting the cases and classification of cytogenetic diagnosis. Furthermore, the evaluation is affected by the timing of the cytogenetic study in the workup of the couple. Recurrent miscarriages have a range of possible causes including genetic, anatomical, endocrine, immune, infective, thrombophilic and unexplained causes (Kavalier, 2005). Maternal problems include uterine malformations, immunological factors and endocrine problems (Kavalier, 2005). Exclusion of these major causes prior to cytogenetic studies should increase the yield of parental chromosome abnormalities.

In general, the incidence of chromosomal abnormalities is higher in females than in males (Muneera *et al*., 2000; Dubey *et al*., 2005) since those that are compatible with fertility in females may be associated with sterility in males (Marmor *et al*., 1980; Brackeeleer and Dao, 1990; Dubey *et al*., 2005). However, in the present study, the incidence was almost equal in males (46%) and females (54%), suggesting that paternal chromosomal abnormality may have a role in the pathogenesis of miscarriages. In one case the male partner had a t(10;18) balanced translocation. His wife had five miscarriages in the first trimester and no live births, probably due to malformed concepti. In the present study, about 80% of the miscarriages were in the first trimester, 15.1% in the second and 4.9% in the third trimester showing a negative relationship between the number of miscarriages and the gestational age (i.e. the number of miscarriages decreased with increasing gestational age). The mean age of the women carrying chromosomal aberrations was 27.5 years. There was apparently no association of advanced maternal age with the number of miscarriages observed in these cases, indicating that the chromosomal anomalies could arise due to reasons other than advanced maternal age, although the number of cases in this study was too small to perform statistical analysis. One couple had 10 miscarriages and no living child, and the parental karyotypes were normal. There was apparently no increase in the rate of chromosomal abnormalities relative to the number of miscarriages in this study (although numbers were too small to perform statistical analysis), which is in agreement with earlier reports (Portnoi *et al*., 1988). Frequency of chromosomal anomalies according to the obstetric history was highest in couples with recurrent miscarriage and a healthy child (11.1%) compared with couples with recurrent miscarriage and an abnormal child or stillbirth (7.7%) and couples with only recurrent miscarriage (5.8%) (**Table 5**); these observations were in agreement with previous reports (Schwartz and Palmer 1983; Portnoi *et al*., 1988; Dubey *et al*. 2005). In fact, Coulam (1986) found a higher incidence of chromosome rearrangement in couples who had experienced both recurrent miscarriage and viable pregnancies than in couples with recurrent miscarriage and no viable pregnancies.

Among structural abnormalities that formed the largest group of chromosomal anomalies in the present study, reciprocal translocations were the most common (85.7%) and frequently involved chromosomes 1, 5, 7, 10 and 18. Robertsonian translocations were less frequently found (14.3%) and were only found in women. Two cases with t(13q;14q) and one with t(13q;22q) were observed. Some authors have indicated that when the Robertsonian translocation is maternal, there is greater risk of the fetus showing an unbalanced phenotype (Boue and Gallano, 1984; Muller and Young, 2001). In fact Lee and Silver (2000) estimated that the risk of miscarriage

Table 6. Worldwide studies of chromosomal rearrangements observed in couples with recurrent miscarriages.

	No. of couples studied	*Chromosomal rearrangement*				*Total (%)*
		Reciprocal	*Robertsonian*	*Inversion*	*Other*	
Belgium (Gent)	96	6	2	–	–	8 (8.33)
Japan	639	19	9	1	–	29 (4.54)
Netherlands (Leiden)	67	5	3	1	–	9 (13.43)
Italy (Padrea)	145	4	4	4	2	14 (9.66)
Netherlands (Rotterdam)	148	6	3	3	2	14 (9.46)
Switzerland (Zurich)	96	4	2	–	1	7 (7.29)
Sultanate of Oman	380	18	3	7	5	33 (8.68)
France (Strasbourg)	217	–	4	2	–	6 (2.76)
India (Hyderabad)	160	–	1	13	4	18 (11.25)
Saudi Arabia (Riyadh)	193	10	1	2	–	13 (6.74)
France (Paris)	315	7	5	4	–	16 (5.08)
India (New Delhi)	742	15	4	1	11	31 (4.18)
Spain (Barcelona)	32	3	1	1	1	6 (18.75)

Data from Boue *et al.*, 1985; Chandley, 1990; Butler and Hamil, 1995.

in couples with Robertsonian translocation is approximately 25% whereas with reciprocal translocations it is approximately 25–50%. A carrier of a balanced Robertsonian translocation has 45 chromosomes including the translocated chromosome. Robertsonian translocations arise either by mutation or by segregation in the offspring of a balanced carrier. Although a carrier of a Robertsonian translocation is phenotypically normal, there is a risk of unbalanced gametes and therefore of unbalanced offspring. In one interesting case (unpublished data) a mother had a Robertsonian translocation (13;14) which she transmitted to her two children.

Aneuploidy contributed to 19.2% (5/26) of the chromosomal aberrations analysed (**Table 3**), which included mosaics with two cell lines. Klinefelter mosaics were the most frequent (60%) followed by mosaicism of sex chromosome polysomy (20%) and 47, XYY (20%).

Pericentric inversion on chromosome 9 (**Figure 3**), which is common in humans, is considered to be a normal variant rather than an abnormal karyotype (Gardner and Sutherland, 1979). The risk of pregnancy loss with an inversion is not known. However, there are studies reporting an association of inversion 9 with subfertility, recurrent miscarriage and abnormal phenotypes (Uehara *et al.*, 1992). In the present study, there was a frequent occurrence of inversion 9 among the inversions analysed. In six cases, pericentric inversion of chromosome 9 occurred and in one case pericentric inversion of chromosome Y was detected (**Table 4**). All six cases with pericentric inversion 9 had a history of recurrent miscarriage (two to several miscarriages). In the one case with inversion (Y) the babies were affected with Smith–Lemli–Optiz syndrome. This indicates the possibility of inversion having a role in the aetiology of recurrent miscarriage, which may be confirmed by molecular studies. An increased tendency to early miscarriages in familial pericentric inversions has been well documented (Wenger and Steele, 1981; Rao *et al.*, 2005). Pericentric inversions were the most frequent chromosomal rearrangements with a frequency of 1–2% in the general population. In addition, subfertility and sterility of male inversion carriers has frequently been reported. Sutherland *et al.* (1976) estimated that the risk of a chromosomal imbalance (duplication/deficiency syndrome) in the offspring of carriers of pericentric inversions was 5–10% (Gardner and Sutherland, 1996). Conception by the recombinant gamete usually results in miscarriage or in the birth of a seriously affected child with partial trisomy/monosomy, which has important clinical consequences depending on the length of the inverted segment.

A number of minor polymorphic chromosomal variants were observed (**Table 4**) such as quantitative (16qh+) and qualitative (14p, 15p, 21p2 and 22p) heterochromatic polymorphisms, large satellites and fragments, which have been implicated in mitotic instability and a tendency towards an increased risk of aneuploidy (Ward, 2000). It is assumed that gain or loss (addition or deletion) of chromosomal fragments during gametogenesis could have led to the chromosomal imbalance in the fetus resulting in miscarriages.

Ideally, chromosomal studies should be performed on the abortus material to determine the contributory cause for that miscarriage. In such cases prenatal fetal karyotyping plays an important role in diagnosing the chromosomal abnormalities. Boue and Gallano (1984), in a collaborative study involving 71 European prenatal diagnosis centres, found a rate of 3.4% unbalanced fetal karyotypes in couples in which a parent had a balanced chromosomal structural rearrangement. A similar study was performed more recently by Caron *et al.* (1999). Therefore all couples with balanced translocations should be strongly advised to have their future pregnancies monitored by prenatal diagnosis to exclude the possibility of a chromosomally unbalanced zygote. The chance of a balanced chromosome rearrangement in the partner of a couple with two or more miscarriages is about 7% (Coulam, 1986; Kavalier, 2005). Determining the presence of such a rearrangement in a parent is useful because it provides: an explanation for the miscarriages; information about the risk for future miscarriages; availability of prenatal diagnosis in a future pregnancy; and information for members of the extended family who may be at risk and may wish to undergo chromosomal testing.

Chromosomal rearrangements in carrier parents are one of the causes of recurrent miscarriage. The present study illustrates the incidence and the distribution of chromosomal abnormalities among Omani couples with recurrent miscarriage is apparently higher than the global incidence. This information should assist physicians in Oman by increasing their awareness of cases of cytogenetically abnormal pregnancies with repeated pregnancy loss. There was apparently no increase in the rate of chromosomal abnormalities relative to the number of miscarriages and maternal age. Cytogenetic analysis should be part of the investigation of any couple who have experienced at least two pregnancy losses of unknown origin. Genetic counselling is essential in the management of couples who have had multiple pregnancy failures. The finding of translocation (reciprocal or Robertsonian) or an inversion in either parent is a strong indication for prenatal diagnosis (amniocentesis or chorionic villus biopsy) in making a precise reproductive decision regarding subsequent pregnancies.

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