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A Cytogenetic Study of Couples with Miscarriages: An Experience from Manipal Referral Centre

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ABSTRACT Miscarriages are sporadic and are thought to result from genetic causes that are greatly influenced by parental chromosomal abnormalities. The researchers studied two hundred and ten couples to look for the prevalence of chromosomal abnormalities in couples with history of recurrent miscarriages. Karyotyping analysis was done by peripheral blood culture and GTG banding. Chromosomal aberrations were found in 8.57% patients: Numerical abnormalities - 0.95%, Structural abnormalities – 2.87% and polymorphic variants -4.76%. However, seven new balanced translocations detected in these patients have not been reported elsewhere in the literature. Couples whose carrier status was ascertained after two or more miscarriages have a low risk of viable offspring with unbalanced chromosomal abnormalities. Therefore, genetic counseling including karyotype is a prerequisite to identify risk factors in couples with recurrent miscarriages.

INTRODUCTION

Recurrent pregnancy loss is defined as three or more consecutive pregnancy loss before 20 weeks of gestation. It affects 1% of general population and approximately 15% of pregnancies. Although extensive studies over a decade has been carried out to identify the underlying causes, the definite reason of pregnancy loss is identified in only ~50% of cases (Andersen et al. 2000), which include chromosomal abnormalities, uterine anomalies, and immunologic factors. Chromosomal etiology is very common in miscarriages since 50% of first trimester miscarriages are due to a chromosome abnormality in the fetus (Ljunger et al. 2005; Alonso et al. 2011) and second trimester loss may be associated with anatomical and other genetic factors of the mother as well as fetus (Michel and Tiu 2007; Elghezal et al. 2007). The objective of the study was to determine the prevalence and type of chromosomal abnormalities in couples who experience first and/or second trimester recurrent pregnancy loss and history of miscarriages as well as congenital anomalous child.

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METHODOLOGY

Couples with recurrent pregnancy loss and willing for evaluation were included in the study for a period of 7 years (during 2005-2012). A total of 210 couples underwent karyotype testing. All patient samples were subjected to chromosome analysis using peripheral blood. For routine analysis, lymphocyte culture was set up using 5ml of RPMI 1640 medium supplemented with L-glutamine, appropriate antibiotic (streptomycin and penicillin), 20% of fetal bovine serum (FBS) and 0.1% of Phytohemagglutinin (PHA) in culture flask as per Moorhead et al. (1960). The T25 cultured flasks were incubated in CO_2 incubator at 37°C for 72 hours. Cultured cells were harvested by adding 50µl colchicine (0.1µg/ml) and incubated for 30 minutes to arrest dividing cell at metaphase, followed by 0.075M KCl (0.56%) for 20 minutes and fixation using standard fixative (3:1 methanol: acetic acid). After subsequent washing steps with fixative the metaphase slides were prepared by dropping method. Microscopic examination of 50 metaphases per case was done after standardized GTG banding techniques (Seabright 1971). Chromosomes were analyzed using image processing and Ikaros (version 5.4) software. Karyotypes were reported as per International Society

of Human Cytogenetic Nomenclature (ISCN 2009) guidelines.

RESULTS

A total of 420 cases (210 couples) with the history of reproductive loss were screened for karyotyping analysis. Thirty-six (8.57%) subjects were found have chromosomal abnormalities including polymorphic variants. Among them male partners (n=13, 6.19%) and females (n=23, 10.95%), (Table 1) possessed chromosomal aberrations, including numerical abnormalities such as TS and KS including mosaics (n=4, 0.97%) and structural abnormalities (n=12, 2.92%) such as translocations (n=10, 2.38%), inversions (n=2, 0.47%). Polymorphic/ heteromorphic chromosomal variants (n=20, 4.76%) including satellite associations of acrocentric chromosomes of 13, 14, 15 and 22) and chromosome inversions of 7, 8, 9 and Y as shown in Figures 1a-1h and Table 1. The frequency of chromosomal polymorphic variants in these subjects were almost equal in both male 10 (4.73%) and female 11(4.31%) partners. However, the present reports of chromosomal translocations in these subjects with recurrent pregnancy loss have not been found elsewhere in literature viz.,46,XX,t(4;6)(p12;p24), 46,XX, t(4;21)(q22;q21), 46,XX,t(1;3)(p32;q27), 46,XX,t(5;6)(p15;q25), 46,XXt(10;14) (q24;q32), 46,XX,t(10;22)(q24;q14), 46,XX, t(7;20)(p15;q13.3); Clinical details are summarized in Table 2.

DISCUSSION

Chromosome abnormalities are the most common cause of spontaneous miscarriage during the first and second trimesters amounting to about 70% within first 6 weeks, 50% before 10 weeks and 5% after 12 weeks (Hassold 1980; Munne 2002; Robberecht et al. 2012). Many cytogenetic studies have documented that majority of fetal chromosome abnormalities are *de novo* and parental karyotypes appeared normal (Shaffer et al. 1996). However, recent report by Niroumanesh et al. (2011) showed that 12% of the patients with the history of recurrent miscarriages were detected to have chromosomal abnormalities. Therefore, full term conception and live birth might depend on both parental and fetal chromosomal status along with environmental interactions that is, multifactorial. To determine etiology of miscarriage we need to consider genetic as well as non- genetic factors such as cytogenetic, hormonal, immunological, and infections. Since cytogenetic finding is considered as a "gold standard" it may give valuable clues (Franssen et al. 2006) for definitive diagnosis of patients with recurrent pregnancy loss. The frequency of chromosomal abnormalities depends on the selection criteria that may be directly proportional to number of miscarriages and history of bad obstetric, and also advanced maternal age associated with increased meiotic non-disjunction causing increased abortion rate (Jaslow et al. 2010). In the present study, out of 420 subjects, 36 (8.57%) have shown

Table 1: Cytogenetic reports in 210 couples with recurrent miscarriages

	In female partners (no. of cases)	*In male partners (no. of cases)*
Normal	46,XX (187 cases)	46,XY (197 case)
Numerical Abnormalities	45,X/46,XX (2 cases)	46,XY/47,XXY (1 case) 46,XY(12p+)/47,XY+ mar(?) (1 case)
Balanced Translocations	46,XX,t(4;6)(p12;p24) (1 case) 46,XX,t(4;12)(q24;q21) (1 case) 46,XX,(1;3)(p32;q27) (1 case) 46,XX,t(5;6)(p15.1;q25) (1 case) 46,XX,t(10;14)(q24;q32) (1 case) 46,XX,(10;22)(q24;q13) (1 case) 46,XX,t(7;20)(p15;q13.3) (1 case) 46,XX,rob(13;14)(q10;q10) (2 cases)	46,XY,t(13;14)(q10;q10) (1 case)
Inversions	46,XX, inv(7)(p12p22)	46,XY inv(8)(p12q21) (1 case)
Polymorphic/ Heteromorphic Variants	46,XX inv(9qh) (2 cases) 46,XX(14ps+) (2 cases) 46,XX(15ps+) (2 cases) 46,XX(22p+) (1 case) 46,XX, with D/D, D/G, G/G associations (4 cases)	46,XY inv(9qh) (1 case) 46,XY(13ps+) (2 case) 46,XY(15ps+) (2 case) 46,XY(9qh+) (p11q13) (1 case) 46,X,inv(Y) (1 case) 46,X, poly(Y) (2 cases)

Table 2: Clinical and karyotyping details of 9 couples with recurrent miscarriages

	Karyotype		*Age and clinical history*
	Female partners'	*Male partners'*	
1.	46,XX,t(4;6) (p12;p24) [Fig1a]	46,XY	25 yrs. History of three first trimester spontaneous abortions, and had a child 5 months possessed insertion of 46,XY,rec(6pter)… (p24), t(4;6) (p12;p24) and with developmental delay facial dysmorphism
2.	46,XX,t(4;21) (q23;q21) [Fig1b]	46,XY	30 yrs.History of two missed abortions and in third instance birth of a daughter with Down syndrome who died at the age of 4 ½ years due to severe congenital heart disease. The fourth instance ended up in an abortion at 12 weeks of gestation. The fetal cord blood was shown to have had 47,XY,t(4;21)(q23;q21)+21
3.	46,XX,t(1;3)(p32;q27) [Fig1c]	46,XY	32 yrs.History of two miscarriages carries chromosomal translocation of 46,XX,t(1;3)(p32;q27) and also had BOH with a malformed fetus.
4.	46,XX,t(5;6)(p15.1;q25) [Fig1d]	46,XY	23 yrs.History of three recurrent miscarriages and have no history of BOH
5.	46,XX,t(10;14)(q24;q32) [Fig1e]	46,XY	History of 4 recurrent miscarriages and have no history of BOH
6	46,XX,t(10;22) (q24;q13) [Fig1f]	46,XY	28 yrs.History of two first trimester miscarriages and also had history of one neonatal death (with multiple congenital anomalies).
7.	46,XX,t(7;20)(p15;q13.3) [Fig1g]	46,XY	22 yrs.History of missed abortion and lost her child with multiple congenital anomalies
8.	46,XX	46,XY, inv(8) (p12q21)[Fig1h]	28yrsHistory of two spontaneous abortions and her karyotype was normal. However, 33 year old male partner had paricentric inversion 8
9	46,XX,inv(7) (p12p22) [Fig1h]	46,XY	28yrsHistory of BOH (of two anomalous babies). The third was missed abortions and the fourth was a live baby with similar karyotype as mother, have that is 46,XX,inv(7) (p12p22) (paracentric inversion)

chromosomal variations in which 16 (3.8%) were chromosomal abnormalities and 20 (4.76%) were chromosomal variants.

Unbalanced chromosomal rearrangements are highly imperative for the failure to implant or abort the fetus at different stages of gestation. Fetal aneuploidy (trisomy in particular) is the most common cause of miscarriage before 10 weeks of gestation. In this study the researchers carried out cytogenetic analysis in about 142 products of conception, we found only 8.45% with abnormalities. In 1985, Boue and colleagues reported that one-fourth of fetal loss between 8-12 weeks had abnormal karyotypes. However, in an epidemiological survey Yamamoto and Wantanabe in 1979, reported approximately 6% loss in 8-10 weeks of gestation. The researchers hypothesized that early pregnancy loss might have other genetic causes such as copy number changes. It is important to keep in mind that even with history of a chromosomally abnormal pregnancy; most couples have good chance for a subsequent successful outcome unless they have a chromosomal abnormality. Parental chromosomal translocation that is, heterozygote (carrier) may have risk to lead to chromosomal imbalance of gametes and may produce spontaneous fetal death and malformed offspring (Gardner and Sutherland 2004). Typically, the imbalance is due to a segment of the other chromosome being deleted/inserted. It might be expected that the distribution of normal and abnormal conceptions would reflect the distribution of karyotypes in the gametes. Even though polymorphic variants including pericentric inversions of chromosome 9 and Y are said to be common in general population (Teo 1995), there is a need to consider the chromosomal inversions and polymorphic variants in recurrent pregnancy loss to determine future risk and better genetic counseling and management (Farcas 2007). In this study group, overall incidence was 2.4% for chromosomal translocations and 5.23% for chromosomal inversions and polymorphic variants as shown in Table 1.

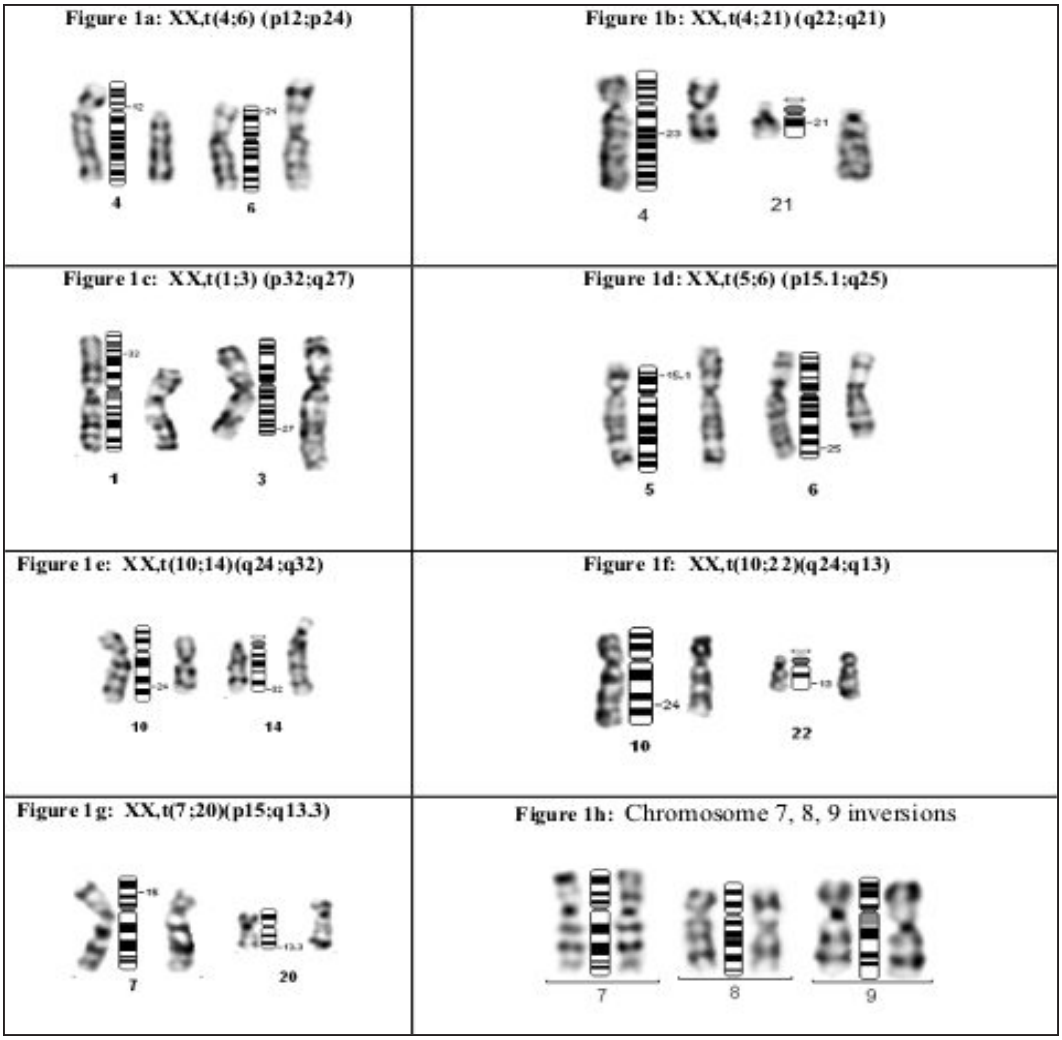

Fig. 1 (a-h): Chromosome translocations/inversion including polymorphic variants in couples with recurrent miscarriages

CONCLUSION

Karyotype analysis is a clinically useful test in cases of recurrent pregnancy loss. Multidisciplinary approaches comprising of obstetrician, clinical geneticist would help in good outcome in these patients.

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