ORIGINAL RESEARCH PAPER

Genetics

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NEED OF GENETIC STUDIES IN COUPLES WITH RECURRENT MISCARRIAGE

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ABSTRACT

Human reproduction is considered as the most inefficient event as approximately 15–20% of human pregnancies end in miscarriage. There are numerous factors that may cause RM, but the exact cause of RM is difficult to ascertain. One of the major risk factor for miscarriage is genetic abnormalities. Thus cytogenetic study should be offered to all couples with more than two miscarriages especially in idiopathic cases. Hence the aim of the present study was to evaluate the need for genetic studies among couples with recurrent abortion. Cytogenetic analysis was carried out in 27 couples with two or more pregnancy loss as study subjects and 10 healthy couples without any chronic illness were also selected as control for this study. The results were correlated with various demographic, clinical and lifestyle aspects of couples with miscarriage. Changing lifestyle, timely marriage as well as planned and safe pregnancy can minimize the risk of miscarriage to some extent.

INTRODUCTION

Recurrent miscarriage (RM), defined as three or more consecutive pregnancy losses before 20-22 weeks of gestation (Gada Saxena et al., 2012). 10–15% of the recognized pregnancies end in miscarriage, mostly losses occurring in first trimester (Gardner and Sutherland, 2004; Harper, 2004). The risk for a miscarriage increases with advancing maternal age due to a higher incidence of concept uses with a chromosomal aneuploidy (Gardner and Sutherland, 2004; Harper, 2004). It is estimated that couples with idiopathic RM can have up to a 75% chance of having a successful pregnancy (Habayeb and Konje, 2004; Li et al., 2002).

The exact prevalence of RM is difficult to ascertain because RM is considered a multifactorial problem, with different causes involved in its etiology. These include parental chromosomal abnormalities, hypothyroidism, uterine abnormalities and antiphospholipid antibody syndrome (APS). Other probable or possible etiologies include additional endocrine disorders, heritable and/or acquired thrombophilias, immunologic abnormalities, infections, environmental and genetic factors.

Genetic factors contribute more than 50% of the cases of miscarriage. The miscarriages in women with RM may be due to de novo numerical chromosome abnormalities, in particular autosomal trisomies of chromosomes 13, 14, 15, 16, 21, and 22, and monosomy X (Pandey et al., 2005; Warren and Silver, 2008). Cytogenetic screening of couples with RM has revealed that parental chromosomal abnormalities occur in either partner in 5-7% of couples with RM, while the rate in the normal population is approximately 0.2% (Fryns and Buggenhout, 1998).

Genetic studies have important role in couples with the history of RPL. They can significantly improve the rate of successful pregnancies by prenatal genetic diagnosis. Routine cytogenetic analysis of miscarriages remains an uncommon practice till today. This unfortunate omission has impacted the management of couples with RM. The need of genetic studies is important to rule out a specific genetic or chromosomal conditions, prognosis and possible treatment. It also facilitates the provision for accurate information about the recurrence of future siblings.

MATERIALS AND METHODS

Twenty seven couples suffering with bad obstetric history were selected as study subjects and 10 healthy couples as control. Detailed demographic, clinical and lifestyle characteristics were recorded using proforma. These couples were referred from various maternity centers of Kerala to Genetika, Centre for Advanced Genetic Studies, Trivandrum, Kerala.

The fresh blood was collected by venipuncture and transferred into sodium heparin vacutainer. Added 5 to 6 drops of whole blood samples to a vial containing 10ml of RPMI 1640 medium supplemented with 15% foetal bovine serum. Then phytohaemagglutinin (PHA, 10µg/ml) was added to proliferate the lymphocyte cells and incubated at 37^{0}C for 72 hrs. At the 70th hour to the culture added a drop of colchicine (0.04µg/ml) to arrest the cell division at metaphase, then mixed gently and kept in incubator at 37^{0}C for 2 hours. After incubation they were treated with hypotonic KCl solution (0.075M) for 20 min and fixed in fresh fixative solution (methanol: acetic acid, 3:1). The cells were dropped onto slides and the slides were air dried and stained with 10% Giemsa. Washed the slides in distilled water and observed under a research microscope through 100x objective. For karyotyping and detecting the structural anomalies, GTG banding technique was performed. To detect numerical and structural abnormalities 20-25 metaphases were analyzed and 5-6 metaphases were karyotyped.

OBSERVATIONS AND RESULTS

Twenty seven couples suffering with recurrent pregnancy loss were selected as study subjects and 10 healthy couples as control. The age of subjects were ranged from 20 to 35 years with a mean age of 28.8 years. The age of the control subjects ranged from 20 to 35 years with a mean age of 31.98 years. The birth order of the study subjects ranged from 1 to 6 and majority of the study subjects had birth order between 2 to 4. Majority of the study subjects belonged to rural area followed by urban and coastal area. The duration of married life of these subjects were ranged from 1 to 26 years. Parental consanguinity was noticed in 4 couples. Majority of the study subjects had average socio economic status followed by good and low status.

COMPARISON OF KARYOTYPE ON STUDY AND CONTROL SUBJECTS

Table 1:

Subjects	Number of couples	Normal karyotype	Abnormal karyotype
Study subjects	27	21	6
Control subjects	10	10	0

21 out of 27 couples were showing normal chromosome pattern and 6 couples having abnormality in their chromosome structure. All the control subjects were showing normal karyotype.

DISTRIBUTION OF KARYOTYPE ACCORDING TO DEMOGRAPHIC CHARACTERISTICS

Table 2:

Variables	Category	Number of couples		Normal karyotype		Abormal karyotype	
		Husband	Wife	Husband	Wife	Husband	Wife
Age (Years)	<30	4	19	4	17	0	2
	30 to 35	12	6	12	5	0	1
	>35	11	2	9	1	2	1
Birth order	<2	10	10	9	7	1	3
	2 to 4	16	15	15	14	1	1
	>4	1	2	1	2	0	0
Age at marriage (Years)	<25	1	17	1	15	0	2
	25-30	14	8	14	7	0	1
	>30	12	2	10	1	2	1
BMI (Kg/m$_2$)	<25	9	14	9	14	0	0
	25-30	9	11	9	7	0	4
	>30	9	2	7	2	2	0

In table 2, age of the subjects were given and grouped into <30, 30 to 35 and >35 years. Abnormal karyotype was showed in couples having advanced age. Subjects having increased age at marriage were showed abnormality. Obese male subjects were showed abnormal karyotype. 4 couples have consanguineous marriage and 3 of them have abnormal pattern. Majority of the subjects were residing in rural area and highest abnormality were showed in subjects residing in urban area. Abnormal karyotype was showed in subjects having highest duration of married life. Subjects having low socio economic statuses were showed highest number of abnormal karyotype. Clinical characteristics of subjects with H/o infertility, H/o diabetes mellitus and H/o hypertension and H/o genetic disorder was showed highest number of abnormal karyotype.

DISTRIBUTION OF KARYOTYPE ACCORDING TO PHYSIOLOGICAL CHARACTERISTICS OF FEMALE SUBJECTS

Table 3:

Variables	Category	Number of wives	Karyotype	
			Normal	Abnormal
Menstrual periods	Regular	19	19	0
	Irregular	8	4	4
Age at menarche (Years)	≤14	20	20	0
	>14	7	3	4
Number of pregnancies	2 to 5	23	19	4
	>5	4	1	3
Number of abortion	2 to 5	24	20	4
	>5	3	0	3
Contraceptive drug used	Yes	3	2	1
	No	24	23	1
Uterine abnormality	Yes	1	0	1
	No	26	24	2
Endometriosis	Yes	2	0	2
	No	25	21	4
H/o mental stress	Yes	4	1	3
	No	23	22	1
H/o TORCH infection	Yes	5	2	3
	No	22	21	1

According to table 3, 50% of subjects with irregular menstruation were showed abnormal karyotype. Subjects having age above 14 years of menarche were showed abnormal karyotype. Highest number of pregnancy and abortion of subjects showed abnormality in their chromosome pattern. Subjects having endometriosis and uterine abnormality were showed abnormal karyotype. 3 subjects with history of TORCH infection were showed abnormal karyotype. Hormone analysis of subjects was studied. Subjects with abnormal level of hormone such as luteinizing hormone and follicle stimulating hormone were showed abnormal chromosome structure.

DISTRIBUTION OF KARYOTYPE ACCORDING TO LIFESTYLE AND PHYSIOLOGICAL CHARACTERISTICS OF MALE SUBJECTS

Table 4:

Variables	Category	Number of wives	Karyotype	
			Normal	Abnormal
Smoking	Yes	6	4	2
	No	21	21	0
Alcohol conception	Yes	7	5	2
	No	20	20	0
Semen analysis	Azoospermia	2	1	1
	Normal	23	23	0
	Oligospermia	2	1	1

Here 6 subjects having the habit of smoking and 2 of them were showed abnormal karyotype. 7 having habit of alcohol consumption and 2 of them were showing abnormal karyotype. According to semen analysis, 23 were normal, 2 subjects were azoospermic and remaining 2 were showed oligospermic. 50% of azoospermic and oligospermic subjects were showed abnormal karyotype.

DISCUSSION

In this study, more than 40% study subjects were above the age of 35 years. Advanced maternal age has been associated with increased number of miscarriages (Rocherbrochard and Thonneau, 2002). Maternal age and previous miscarriage rates increases the risk of subsequent miscarriages (de et al., 2002).

The present study also observed that increased paternal and maternal age is a risk factor for abnormal karyotype and recurrent abortion. Subjects with high number of spontaneous abortion showed abnormal karyotype.

According to Abetew et al., (2011) younger age of menarche i.e. less than 11 years is associated with pregnancy complications. In the present study indicates that those who had menarche at >14 years showed abnormal karyotype.

According to Glinoer et al., (2004) impaired maternal thyroid hormone availability may induce irreversible brain damage with consequent neurological abnormalities. But the present study observed that frequency of abnormal karyotype was increased in subjects with thyroid disorder. Poorly controlled diabetes mellitus results in increased risk for foetal loss (Melamed and Hod, 2009). Subjects with diabetes mellitus in the current study were observed with highest number of abnormal karyotype.

Parental chromosomal abnormalities are detected in about 14% of subjects with recurrent miscarriages (Elghezal et al., 2007). Several types of genetic problems like parental structural chromosomal abnormalities and recurrent aneuploidies may be associated with recurrent miscarriage. In the present study, it was also observed that increased paternal and maternal karyotype abnormality is a risk factor for fetal karyotype abnormality and recurrent abortion.

TORCH infections were reported in 18% of cases in the present study and 60 % of this affected subjects showed abnormal karyotypes which indicates that abnormality increases with infection. According to Charles and Larsen (1990), it is very unlikely that maternal infection causes recurrent abortion.

According to Balen, (2007) the endocrine abnormalities in RPL include raised concentrations of luteinising hormone (LH; seen in about 40% of women), testosterone and androstenedione in association with low or normal concentrations of follicle stimulating hormone. In the current study, the FSH level above 30 mUl/mg and LH above 30

mUl/mg showed increased number of abnormal karyotype than other subjects.

CONCLUSION

This study has shown that the incidence and distribution of chromosomal anomalies in couples with repeated foetal loss is comparable with control subjects. Subjects having major risk factors such as advancing age, age at marriage, BMI, parental consanguinity, infections and hormonal variations have showed positive correlation with their karyotype. Thus cytogenetic study should be offered to all couples with more than two miscarriages especially in idiopathic cases. Increasing awareness of the role of genetics in the etiology of RPL and its overall impact on the burden imposed on individuals, families and society has led to the emergence on modern clinical cytogenetics.

Up to 20 percent of pregnancies may end in miscarriage. A number of factors impact a woman's chance of miscarrying, from biological to lifestyle factors. Changing lifestyle can minimize the risk of miscarriage to some extent. Miscarriage often cause due to infection. These were caused due to unhygienic conditions of the couples. Vaccinations are the preventive measure for infection. Timely marriage as well as planned and safe pregnancy is also a right decision to escape from the chances of recurrent miscarriage.

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