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Risk Evaluation of Future Pregnancy among Patients with Recurrent Abortion

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ABSTRACT *Recurrent pregnancy loss (RPL) is a common occurrence, with approximately 15% of all clinically recognized pregnancies resulting in pregnancy failure. RPL also referred as recurrent miscarriage or habitual abortion, is historically defined as 3 consecutive pregnancy losses prior to 20 weeks from the last menstrual period. Based on the incidence of sporadic pregnancy loss, the incidence of recurrent pregnancy loss should be approximately 1 in 300 pregnancies. However, epidemiologic studies have revealed that 1% to 2% of women experience recurrent pregnancy loss. The etiology of recurrent miscarriage includes genetic abnormalities, autoimmune related conditions, structural abnormalities, infections, endocrine disorders, and unknown factors. The present study was carried out in 48 couples suffering with varying degrees of pregnancy loss were selected as study subject. The present study highlights the risk evaluation of future pregnancy among patients with recurrent abortion by cytogenetics. The cytogenetic analysis results were correlated with various demographic and clinical aspects of couples with RPL. Increase in number of gestations, spontaneous abortions and MTPs showed increased incidence of abnormal fetal karyotypes among the study subjects. The results were correlated with various demographic and clinical aspects of infertile couples. Risk factors such as increasing maternal age, increasing paternal age, duration of married life, history of parental infection, etc can lead to fetal loss. Modification of lifestyle, along with preventive measures against teratogenic infection, and awareness of the role of genetics in the etiology of RPL will help in reducing the risk for recurrent pregnancy loss.*

Introduction

Pregnancy is a unique and well choreographed physiological process that involves dynamic maternal and foetal dialogue. Local immune tolerance, angiogenesis, cytokine and hormonal balance, cellular and molecular mimicry, genetic and epigenetic as well as environmental factors can influence pregnancy outcomes (Dosiou and Giudice, 2005; Thaxton and Sharma, 2010; Zhang et al., 2011; Hemberger, 2012; Van and Oudejans, 2013; Hughes, 2014; Rai and Cross, 2014).

Recurrent miscarriage (RM) has recently been defined in the guidelines of the American Society of Reproductive Medicine as the loss of two or more consecutive pregnancies (Practice Committee of the American Society, 2008). Couples with RM are facing an increased risk of being carriers of a structural balanced chromosome abnormality. The incidence of carrier status is ~0.7% in the general population worldwide and increases to 2.2% after one miscarriage, 4.8% after two miscarriages and 5.2% after three miscarriages (Van, 2011).

The etiology of RM includes genetic abnormalities, autoimmune related conditions, structural abnormalities, infections and endocrine disorders, is unknown in a significant number of miscarriages. Although chromosomal anomalies are identified in about 50%–60% of all miscarriages, they seem to occur less frequently in the products of conception (POC) from recurrent abortions than in those from spontaneous abortions (Kano et al., 2009).

Following the loss of wanted pregnancy, most women are likely to keep trying until a live birth is achieved. Thus, the chance of conception following a miscarriage should be higher than those following a live birth. In a cohort of 261 women followed up for 6 year after a miscarriage, natural conception occurred in 97.7% of these without known fertility problems (Tam et al., 2005).

A significant proportion of cases of recurrent miscarriage remain unexplained despite detailed investigation. It affects about one in every hundred couples. Sometimes a treatable cause can be found, and sometimes not. But in either case, most couples are more likely to have a successful pregnancy next time than to miscarry again. The investigations and management of recurrent miscarriages is one of the most debated topics. This study is aimed to evaluate risk for future pregnancy among patients with recurrent abortion.

Materials and Methods

Forty eight couples suffering with varying degrees of pregnancy loss were selected as study subjects for this study. All these couples have at least one year duration of married life. Detailed demographic and socio-economic characteristics were recorded using proforma. These couples were referred from various infertility clinics and maternity centers of Kerala to Genetika, Centre for Advanced Genetic Studies, Trivandrum, Kerala.

The fresh blood was collected by venepuncture and trans-

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ferred into vacuutainer containing sodium heparin as anticoagulant. 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, 100µg/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 a hypotonic solution (0.075M KCl) for 1 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.

Results

Table 1: Distribution of fetal karyotype according to demographic characteristics

Category	Variables	Number (%)	Normal karyotype	Abnormal karyotype
Maternal age (Years)	21-32	38 (79.16%)	24(63.15%)	14 (36.84%)
	33-44	10 (20.83%)	3(30%)	7 (70%)
Paternal age (Years)	25-36	29 (60.41%)	20(68.96%)	9 (31.03%)
	37-47	19 (39.58%)	7(36.84%)	12 (63.15%)
Duration of married life (Years)	1-10	44 (91.66%)	26 (59.09%)	18 (40.90%)
	11-20	4 (8.33%)	1 (25%)	3(75%)

The distribution of fetal karyotype according to demographic characteristics of study subjects was studied (Table 1). The maternal age of the study subjects was observed and 33-44 years of age were showed 30% of normal fetal karyotype and 70% were showed increased incidence of abnormal fetal karyotype. 37-47 years of paternal age of the study subjects were showed 36.84% of normal fetal karyotype and 63.15% were showed abnormal fetal karyotype. The duration of married life of couples was analyzed and among them those had duration of 11-20 years of married life showed 25% normal fetal karyotype and 75% abnormal fetal karyotype.

Table 2: Distribution of fetal karyotype according to clinical characteristics

Category	Variables	Number (%)	Normal karyotype	Abnormal karyotype
Number of gestation	1-4	36 (75%)	24 (66.66%)	12 (33.33%)
	5-7	12 (25%)	3(25%)	9 (75%)
Number of spontaneous abortion	0-3	38 (79.16%)	23 (60.52%)	15 (39.47%)
	4-7	10 (20.83%)	4 (40%)	6 (60%)
MTP	0-1	42 (87.50%)	23 (54.76%)	19 (45.23%)
	2-3	6 (12.50%)	2 (33.33%)	4 (66.66%)
Paternal Karyotype	Normal karyotype	46 (95.83%)	27 (58.69%)	19 (41.30%)
	Abnormal karyotype	2 (4.16%)	0	2 (100%)
Maternal Karyotype	Normal karyotype	46 (95.83%)	27 (58.69%)	19 (41.30%)
	Abnormal karyotype	2 (4.16%)	0	2 (100%)
H/o Parental Infection	Yes	6 (12.50%)	1 (16.66%)	5 (83.33%)
	No	42 (87.50%)	26 (61.90%)	16 (38.09%)
H/o Parental Illness	Yes	11 (22.91%)	5 (45.45%)	6 (59.45%)
	No	37 (77.08%)	15 (40.54%)	22 (54.54%)
H/o Drug Intake	Yes	9 (18.75%)	3 (46.15%)	6 (66.66%)
	No	39 (81.25%)	21 (53.84%)	18 (33.33%)
USS Finding (ultra sound scan)	Normal	26 (54.16%)	14 (53.84%)	12 (46.15%)
	Abnormal	22 (45.83%)	13 (59.09%)	9 (40.90%)

The distribution of fetal karyotype according to clinical characteristics was studied (table 2). The number of gestations of the couple was observed and those with number of gestation 5-7 times showed 25% normal fetal kayotype and 75% showed abnormal fetal karyotype. Moreover the study subjects suffering with 4-7 times spontaneous abortions were showed normal fetal karyotype of 40% and increased incidence (60%) of abnormal fetal karyotype. In short, the study subject with 2-3 numbers of medical terminations of pregnancies (MTP) was showed high abnormal fetal karyotype (66.66%). The distribution of fetal karyotype according to paternal and maternal karyotype was studied. In these 95.83% subjects showed normal fetal karyotype and 4.16% subjects showed abnormal fetal karyotype. In the case of 46 normal subjects, 58.69% had normal fetal karyotype and 41.30% had abnormal fetal karyotype. About 100% abnormal fetal karyotype is observed in 2 abnormal karyotype of paternal and maternal subject. The study subjects having H/o parental infection and H/o parental illnesses were showed an increased incidence of abnormal fetal karyotype. Subjects with H/o parental infections were showed normal fetal karyotype of 16.66% and 83.33% of abnormal fetal karyotype. Subjects with H/o parental illnesses were showed normal fetal karyotype of 45.45% and 59.45% of abnormal fetal karyotype. The study subjects, 18.75% of them have the history of drug intake and 81.25% do not have any history of drug intake. Subject with history of drug intake, 46.15% had normal fetal karyotype and 66.66% had abnormal fetal karyotype. Among the study subject there was no significant effect of USS (ultra sound scan) finding on fetal karyotype.

Discussion

Recurrent Pregnancy Loss (RPL) also referred to as Recurrent Miscarriage or Habitual Abortion is a distinct disorder defined by two or more failed clinical pregnancies, and up to 50% of cases of RPL will not have a clearly defined etiology. Approximately 15-20% of clinically recognizable pregnancies end in spontaneous abortion. The incidence

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of chromosomal abnormalities in those abortions is as high as 50%. A modest but clinically important proportion of spontaneous abortions are caused by a balanced chromosomal aberration in one of the parents. This results from the production of gametes and embryos with unbalanced chromosome sets. The clinical consequences of such abnormal gametes include sterility, repeated abortions, and giving birth to malformed children (Philipp, 2003).

Maternal age and previous miscarriage rates increases the risk of subsequent miscarriages. Women experience an age dependent increase in various adverse reproductive events such as infertility, pregnancy complications (de et al., 2002). Approximately 2% to 4% of RPL is associated with a parental balanced structural chromosome rearrangement, most commonly balanced reciprocal or Robertsonian translocations. Additional structural abnormalities associated with RPL include chromosomal inversions, insertions, and mosaicism. Single gene defects, such as those associated with cystic fibrosis and sickle cell anemia, are seldom associated with RPL (Holly and Danny, 2009).

In the present study also observed that increased paternal and maternal age is a risk factor for fetal karyotype abnormality and recurrent abortion. From the data it is clearly demonstrated that maternal as well as paternal age increases the fetal karyotypic abnormalities. In the case of duration of married life, the fetal karyotype abnormality is increased with increased duration. Fetal karyotype abnormality was higher in those which had duration of married life ranging from 11-20 and lower for those ranging from 1-10 years.

A number of studies in women with RM showed that the higher the number of previous losses, the lower the live birth rate in a subsequent pregnancy (Brigham et al., 1999). In the current study, along with the characteristics such as number of gestation and number of spontaneous abortion, it also influences the parental karyotype. As the number of gestation increases fetal karyotype abnormalities will also increased and leads to recurrent abortion. Fetal karyotype abnormalities were increased with increasing number of spontaneous abortion. According to the numbers of MTP increased fetal karyotype abnormalities were identified. The present study clearly demonstrates a positive correlation between fetal karyotype and number of MTPs.

Parental chromosomal abnormalities are detected in about 2-8% of couples 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. Balanced chromosomal rearrangements are found in 2-5% of these couples, and among these balanced translocations are the most frequent abnormalities. In the present study, it was also observed that increased paternal and maternal karyotypic abnormality is a risk factor for fetal karyotype abnormality and recurrent abortion.

There are several studies that implicate the role of female factor in recurrent miscarriages. Certain illnesses, especially those that restrict blood flow to the uterus, may increase a woman's chances of miscarrying (because the growing fetus can't get enough oxygen to survive). These include diabetes, thyroid disease, lupus, and heart disease, as well as others like uterine infections. Managing the condition before and during pregnancy can reduce miscarriage risk. In the present study 22.91% of subjects were found with history of illness having 54.54% abnormal karyotype and 18.75% of subjects were found with history of drug intake and the abnormal karyotype analysis was found to be 33.33%.

Conclusion

The present study was carried out in 48 study subjects with recurrent abortion. The main objective of this study was to detect the various factors that associated with recurrent abortion and to detect the chromosome abnormalities in patients with recurrent abortion, to avoid risk for future pregnancies.

The couples who had reported for the recurrent pregnancy loss showed a higher percentage of abnormal fetal karyotype. The abnormal fetal karyotype was found higher in those couples with an increased age, increased duration of married life, increased number of gestations, increased number of spontaneous abortions and increased number of MTPs. The incidence of RPL was found also prevalent in those who had history of infection, illness and drug intake.

The fetal karyotype analysis with respect to demographic and clinical risk factors such as maternal age, paternal age, duration of married life, paternal karyotype, maternal karyotype, parental illness, H/o drug intake, number of spontaneous abortion, number of MTPs, H/o infection, abnormal USS finding, etc. showed increase level of abnormal fetal karyotype. The study demonstrated a positive correlation with karyotypic abnormality and future pregnancy loss. So, the risk of two consecutive losses can be thoroughly assessed through cytogenetic and biochemical analysis.

REFERENCE

1. Brigham SA, Conlon C, Farquharson RG. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. Hum Reprod 1999;14:2868–71.
2. De Bruin J P, Dorland M, Spek E R, Posthuma G, van Haaften M, Looman C W. Ultrastructure of the resting ovarian follicle pool in healthy young women. Biol Reprod. 2002; 66:1151–1160.
3. Dosiou C, Giudice L C. Natural killer cells in pregnancy and recurrent pregnancy loss: endocrine and immunologic perspectives. Endocr Rev. 2005; 26:44–62.
4. Elghezal H, Hidar S, Mougou S, Khairi H, Saad A. Prevalence of chromosomal abnormalities in couples with recurrent miscarriage. Fertil Steril. 2007; 88:721–723.
5. Hemberger M. Immune balance at the foeto-maternal interface as the fulcrum of reproductive success. J Reprod Immunol. 2012; 97:36–42.
6. Hughes V. Epigentics: The sins of the father. Nature. 2014; 507:22–24.
7. Kano T, Mori T, Kimura A. Gender ratio distortion in abortuses and live births from patients with recurrent spontaneous abortion. Am J Reprod Immunol. 2009; 62(3):125–127.
8. Philipp T. Embryoscopic and cytogenetic analysis of 233 missed abortions: factors involved in the pathogenesis of developmental defects of early failed pregnancies, Human reproduction. 2003; 18 (8); 1724 - 1732.
9. Practice Committee of the American Society for Reproductive Medicine Definitions of infertility and recurrent pregnancy loss. Fertil Steril. 2008; 89(6):1603.
10. Rai A, Cross JC. Development of the hemochorial maternal vascular spaces in the placenta through endothelial and vasculogenic mimicry. Dev Biol. 2014; 387:131–141.
11. Tam WH, Tsuni MHY, Lok IH et al., Long Term Reproductive Outcome Subsequent To Medical versus surgical treatment For Miscarrige Hum. Reproduction. 2005; (12), 3355-3359.
12. Thaxton JE, Sharma S. Interleukin-10: a multi-faceted agent of pregnancy. Am J Reprod Immunol. 2010; 63:482–491.
13. Van Dijk M, Oudejans C. (Epi) genetics of pregnancy-associated diseases. Front Genet.2013;4:180.

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14. Zhang J, Chen Z, Smith GN, Croy BA. Natural killer-triggered vascular transformation: maternal care before birth? Cell Mol Immunol. 2011; 8:1–11.
15. E.Van den Boogaard. Selective karyotyping in recurrent miscarriage: are recommended guidelines adopted in daily clinical practice? ; Human Reproduction. 2011;26 (8); 1965-1970.
16. Holly B. Ford and Danny J Schust, Recurrent Pregnancy Loss: Etiology, Diagnosis, and Therapy, Rev Obstet Gynecol. 2009 Spring; 2(2): 76–83.