Cytogenetic Analysis of Couples with Recurrent Pregnancy Loss

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Abstract - The goal of this cytogenetic study was to evaluate the various risk factors and chromosome abnormalities in couples experiencing recurrent pregnancy loss (RPL). This study was conducted among 50 couples with RPL. These study subjects were in the age group of 20 to 50 years. The cytogenetic analysis results were correlated with various demographic, lifestyle and clinical aspects of couples with RPL. Increase in number of gestations, spontaneous abortions and MTPs showed increased incidence of abnormal karyotypes among the study subjects with RPL. The study demonstrated that there is a positive correlation between duration of married life, history of infection, chronic illness and drug intake with chances for recurrent pregnancy loss. A significant abnormal karyotype among the study subjects along with the above parameters is suggestive of increased risk of recurrent pregnancy loss. Modification of life style along with proper medication for teratogenic infection and awareness of the role of Genetics in the etiology of RPL will help in reducing the risk for recurrent pregnancy loss.

Index Terms- Carrier, Cytogenetics, Genetic testing, Karyotype, Miscarriage, Recurrent Pregnancy Loss (RPL), Spontaneous abortion

1 INTRODUCTION

Recurrent pregnancy loss (RPL), defined as two or more consecutive pregnancy losses before 20th week of pregnancy is a frequent obstetric complication [1]. Couples with recurrent miscarriage (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 [2].

Factors associated with RPL include parental chromosome translocations, uterine malformations, endocrine, hematological and autoimmune factors. In spite of exhaustive investigation, approximately 40% of RPL cases remain unexplained and are classified as idiopathic RPL (iRPL) [3]. Other risk factor includes loss of euploid pregnancy, loss after the first trimester, difficulty in conceiving, and delivery of a very low birth weight baby. The most prevalent abnormality of spontaneous abortion is chromosomal aneuploidy. Other etiologic factors of spontaneous abortions are anatomic anomalies, endocrine or hormonal problems, coagulation protein defects, and nutritional and environmental factors [4].

Several studies have implicated the role of female factor in recurrent miscarriages, but the role of male factor has only recently been realized in couples experiencing sporadic assisted reproductive technique failure. Till date few studies have evaluated the role of sperm integrity in iRPL cases following spontaneous conception. Analysis of DNA integrity in understanding the role of sperm factor in iRPL may reduce the number of cases diagnosed as idiopathic and aid in providing most adapted therapeutics to the couple [3].

The clinical association of RPL in polycystic ovarian syndrome (PCOS) is more than common. However, the incidence rate between PCOS and recurrent miscarriage remains uncertain due to its wide variation in different studies. The high prevalence of hyper-secretion of LH and obesity in the syndrome contributes to the conclusion which has been reported as a risk factor for spontaneous abortion. Hyperinsulinemia has been proposed as the pathway for the effect of obesity on some reproductive abnormalities, probably through its effect on androgen production. There are many other reasons for recurrent miscarriages including chromosomal abnormalities, maternal age, medical illness, intrauterine illness, infections and immunological, endocrine and psychological factors [4].

Cytogenetic Analysis of recurrent miscarriages provides valuable insights into the cause of miscarriage which can eliminate further costly testing. In addition, recurrent risk estimates for subsequent pregnancies can also be determined. Genetic testing of prospective parents can identify those who carry a disease that can be passed

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onto their children [5]. Genetic testing of fetuses provides information about potential birth defects and gives couples high level of probability information upon which to base decisions. These new technologies have given sophisticated information and advanced technical solutions to this problems. Hence the present study was undertaken to evaluate various risk factors and chromosome abnormalities, if any, in couples with recurrent pregnancy loss and to evaluate the role of various demographic and clinical aspects of couples with RPL.

2 Materials and Methods

Fifty couples suffering with varying degrees of pregnancy loss were selected for this study. All these couples have at least one year duration of married life. These couples were referred from various infertility clinics and maternity centers of Kerala for genetic testing to Genetika, Centre for Advanced Genetic Studies, Trivandrum, Kerala. Detailed demographic and lifestyle characteristics were recorded using proforma. Chromosome preparation was done by peripheral blood lymphocyte culture method [6] and banded with GTG banding technique [7].

The fresh blood collected by venepuncture was transferred in to 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 20% foetal bovine serum. Then phytohaemagglutinin (PHA, 10µg/ml) was added to proliferate the lymphocyte cells and incubated at 37ºC 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 37ºC for 2 hours. Then transferred the whole content into a sterile centrifuge tube and centrifuged at 1000 rpm for 10 minutes. Discarded the supernatant, mixed the contents using cyclomixer, and re-suspend the pellet in pre-warmed hypotonic KCl (0.075M) and incubated for 20 minutes at 37 ºC. 2-3 drops of Methanol: Acetic acid fixative (3:1 ratio) which was freshly prepared was added to the culture and centrifuged at 1000 rpm. Supernatant was discarded and the cell contents were mixed in a cyclomixer. 10ml of fresh fixative was added to the pellets present at the bottom of the centrifuge tube and kept for at least 30 minutes in refrigerator. Again the preparation was centrifuged at 1000 rpm for 10 minutes and the supernatant was discarded. Added fresh fixative centrifuged and repeated the process 3 or 4 times until the supernatant appeared clears. Clear cell suspension was prepared and dropped the cell suspension, drop by drop on to pre cleaned labelled and chilled slides from a particular height so as to get good quality metaphases. The slides were air dried and the slides were stained with 10% Giemsa staining solution for 10 minutes. 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 [8].

3 Results

The demographic and clinical characteristic findings are given in the table1. The age of husbands and wives ranged from 20 to 50 years. The clinical characteristic findings are given in the table 2. The number of gestation of the couples was observed and among them the abnormal karyotype was found in 33.33% on those with number of gestation >6. Moreover subjects with more than six times spontaneous abortions showed increased (66.66%) incidence of abnormal karyotypes. Simultaneously couples with more medical termination of pregnancies (MTP) showed increased (40%) incidence of abnormal karyotypes. In short, increase in number of gestations, spontaneous abortions and MTPs showed increased incidence of abnormal karyotypes among the study subjects with RPL.

The life style characteristic findings are given in the table 3. The duration of married life of the couples were observed and among them those which had a duration >10 years showed 66.66% of abnormal karyotypes. Subjects with history of infections viz. fever, urinary tract infection, allergy due to dust, etc reported increased incidence of abortions. 27.2% of the couples with history of chronic illness had abnormal karyotype. The illnesses observed were PCOD, APLA Syndrome, asthma and diabetes. These findings demonstrated that there is a positive correlation between duration of married life, history of infection, chronic illness and drug intake with chances for recurrent pregnancy loss.

TABLE: 1

Distribution of Karyotype according to Demographic characteristics

	Variable	Number (Percentage)	Abnormal Karyotype (Percentage)	Normal Karyotype (Percentage)
Age of Husband	21 – 30	10 (20%)	1(10%)	9 (90%)
	31– 40	32 (62%)	1(3.12%)	31(96.87%)
	41 – 50	8 (16%)	2 (25%)	6 (75%)
Age of Wife	21-30	30(60%)	1(3.3%)	29 (96.6%)
	31-40	15(30%)	1(6.66%)	14(93.33%)
	41-50	5(6%)	3(60%)	2(40%)

TABLE: 2

Distribution of Karyotype according to Clinical characteristics

	Variable	Number (Percentage)	Abnormal Karyotype (Percentage)	Normal Karyotype (Percentage)
Number of Gestations	<6	47(94%)	2(4.25%)	45(95.74%)
	>6	3(6%)	1 (33.33%)	2(66.66%)
Number of Spontaneous Abortions	<6	47(94%)	1(2.12%)	46(97.8%)
	>6	3(6%)	2(66.66%)	1(33.33%)
Number of MTPs	0-1	45(90%)	1(2.22%)	43(95.5%)
	2-3	5(10%)	2 (40%)	3(60%)

TABLE: 3

Distribution of Karyotype according to Lifestyle characteristics

	Variable	Number (Percentage)	Number of Abnormal Karyotype (Percentage)	Number of Normal Karyotype (Percentage)
Duration of Married Life	<10	44 (88%)	5 (11.36%)	39 (88.6%)
	>10	6 (12%)	1 (16.6%)	5 (83.3%)
H/o Infection	YES	3 (6%)	2 (66.66%)	1 (33.33%)
	NO	47 (94%)	1 (2.12%)	46 (97.8%)
H/o Illness	YES	11 (22%)	3 (27.2%)	8 (72.7%)
	NO	39 (78%)	2 (5.12%)	37 (94.87%)
H/o Drug Intake	YES	9 (18%)	3 (33.33%)	6 (66.66%)
	NO	41 (82%)	2 (4.87%)	39 (95.12%)

4 Discussions

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 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 [9].

In most cases more than 99% of chromosomally abnormal pregnancies result in miscarriage which occurs prior to 10 weeks of gestation. Such miscarriages are thought to occur on a random basis, with increasing frequency of trisomy with advancing maternal age [10]. In this study the cytogenetic analysis of 50 couples with recurrent pregnancy loss was conducted. Regarding the parental chromosome analysis 10% out of 100 subjects were found abnormal.

Some couples with habitual abortion, have an increased proportion of aneuploid cells in their peripheral blood lymphocytes. Some studies have described increased sex chromosome aneuploidy in couples with recurrent abortions [11]. In the present study, the karyotype analysis was found 10% abnormal for the male subjects ranging from 21-30, 3.12% abnormal for the male subjects ranging from 31-40 years of age group and 25% abnormal karyotype for the male subjects of age group 41-50 years. The karyotype analysis was observed as 3.33% and 6.66% abnormal for the women ranging from 21 - 30 and 31 - 40 years respectively and 60% abnormal for the female subjects from age group of 41-50 years. Epidemiological evidence shows a steep increase in sporadic miscarriage rate in women aged more than 36 years [12]. This age related risk is due to a higher number of aneuploides, mainly trisomies [10]. The age dependent increase of trisomies, with repeated miscarriages as its manifestation, may be due to a recurrence of sporadic chromosome abnormalities [13].

Numerous studies have shown that abortion is closely associated with other confounding factors, such as smoking, alcohol, drug use, promiscuity and venereal disease. These behaviors may help women to cope with unresolved anxiety and depression after abortion. Women with a history of abortion are especially likely to persist in using tobacco, alcohol, and illegal drugs during subsequent pregnancies [14]. This maybe because the subsequent wanted pregnancy is a connector to unresolved issues after abortion. In the present study, the karyotype analysis on duration of married life among couples showed 11.36% abnormal karyotype from the group of less than 10 years and 16.6% abnormal karyotype from the group of more than 10 years.

The major cause of Spontaneous abortion is fetal chromosomal abnormalities, contributing to about 50~60% of the cases. The most prevalent abnormality of Spontaneous abortion is chromosomal aneuploidy. Other etiologic factors of Spontaneous abortion are anatomic anomalies, endocrine or hormonal problems, coagulation protein defects, and nutritional and environmental factors [4]. In the present study 94% of the subjects had spontaneous abortions less than 6 times with 2.12% abnormal karyotype and 6% subjects had spontaneous abortions more than 6 times with the abnormal karyotype of 66.66%.

Recurrent pregnancy loss (RPL) in polycystic ovary syndrome (PCOS), which occurs in ~50% of total pregnancies, is a frequent obstetric complication. Polycystic ovary syndrome (PCOS) is a common condition estimated to affect 4 – 18% of women of reproductive age [15]. Several studies have implicated the role of female factor in recurrent miscarriages. In the present study 22% of subjects were found with history of illness having 27.2% abnormal karyotype and 18% of subjects were found with history of drug intake and the abnormal karyotype analysis was found to be 33.33%. Thus the present study suggests the evaluation of genetic instabilities in couples with recurrent pregnancy loss especially in idiopathic cases.

5 Conclusions

The couples who had reported for the recurrent pregnancy loss showed a higher percentage of abnormal karyotype. The abnormal 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 recurrent risk is more in couples with structural anomalies and so such couples are suggested to undergo genetic counseling before they are planning to get conceived again to avoid further miscarriages. This study has shown that the incidence and distribution of chromosomal abnormalities among couples with repeated fetal loss is comparable to that reported worldwide. Thus cytogenetic studies should be offered to all couples with more than 2 spontaneous abortions 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, through this individuals can be better informed about extent of DNA damages, Genetic risks and reproductive options.

6 References

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