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Evaluation and Contribution of Major Chromosomal Abnormalities in Couples with Recurrent Miscarriage

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Abstract:

Background: *Pregnancy loss is a common phenomenon. Most of the pregnancy losses which happen in the first and second trimesters are caused by chromosomal abnormalities. Repeated pregnancy loss is an extremely stressful condition for both the partners and physicians because it is difficult to find a reason behind it.*

Materials and Methods: *Cytogenetic analysis was performed according to standard methods on lymphocyte cultured cells obtained from the patient peripheral blood. In order to assess the frequency and nature of chromosomal aberrations that contribute to the occurrence of reproductive failure, we investigated 200 couples (400 individuals). Clinical diagnostic indications for chromosome analysis was recurrent abortion (at least three) were studied.*

The ANOVA test was used for statistical evaluation. The level of $p<0.05$ was considered as significance.

Results: *Most of the patients had 3 repeated abortions (66.6%).Cytogenetic analysis performed on 200 couples and karyotype of 5% of them were abnormal. These include translocation in 7 cases and sex chromosomal mosaicism in one case.*

Conclusion: *The present study demonstrates the importance of cytogenetic analysis in elucidating the recurrent miscarriage etiology, and enables healthcare professionals to properly conduct genetic counseling, allowing couples to make correct decisions about their reproductive life.*

Key words: *Chromosomal Abnormality, metaphase analysis, recurrent miscarriage*

I. Introduction

Reproductive failures include wide variety of problems such as infertility, pregnancy loss, abnormal pregnancy and birth defects. Recurrent miscarriage is also referred to as recurrent pregnancy loss or habitual abortion, is historically defined as three consecutive pregnancy losses prior to 20 weeks from the last menstrual period [1]. According to this definition the frequency of recurrent miscarriage is one in 300 women [2]. The American Society for Reproductive Medicine defines recurrent miscarriage as the two or more failed pregnancies [3]. As per this definition the prevalence of recurrent miscarriage is higher i.e. one in 100 women [2].

Recurrent miscarriage is an extremely stressful condition for both the partners and physicians because it is difficult to find a reason behind it. The frequency of first trimester pregnancy losses are more than that of second trimester.

Recurrent miscarriage has been directly associated with parental chromosomal anomalies, maternal thrombophilic disorders and structural uterine anomalies and indirectly with maternal immune dysfunction and endocrine abnormalities, advanced maternal age, decreasing semen quality in males [4,5]. Genetic imbalances are commonly reported under chromosomal anomalies and its unsteadiness in the affected couples as well as in the conceptus. The analysis of structural and numerical aberrations of the chromosomes has greatly helped to determine the etiology in majority of cases of reproductive failure.

In the present study we have tried to assess the frequency and nature of chromosomal aberrations that contribute to the occurrence of reproductive failure in India.

II. Materials And Methods

Study Population

This study was carried out in the department of cytogenetics and molecular biology at PreventiNe Life care Pvt. Ltd. in Navi Mumbai. 200 couples (400 individual) with three or more pregnancy losses were recruited from the outpatient clinic. We excluded the women with two miscarriages, also the possible etiological factors such as consanguineous marriage, diabetes mellitus, essential hypertension, thyroid dysfunction were excluded while recruitment. The control group consisted of 100 couples (200 individuals) with same age group having at least one normal child.

Chromosome preparations

Chromosomes from cultured peripheral blood lymphocytes were analyzed using Trypsin-Giemsa (GTG) banding: Karyotyping was conducted by analysis of G-banded chromosomes using 2 mL heparinized peripheral blood sample. Metaphase spreads were made from phytohemaglutinin stimulated peripheral lymphocytes using standard cytogenetic techniques. Cultures were harvested and Karyotyping was performed on G-bands produced with Trypsin and Giemsa (GTG)-banded chromosome preparations. At least 20 chromosome spreads were counted and analyzed for each patient. If there was any sign of mosaicism, 50 metaphases were analyzed. All chromosomal abnormalities were reported in accordance with the current international standard nomenclature ISCN [6].

The ANOVA were used for statistical evaluation. The level of $p<0.05$ was considered as significance.

III. Results

Chromosomal studies were performed in 200 couple (400 individuals) facing repeated pregnancy loss. The study population was classified in four groups according to the number of previous abortion. In group one couples had three abortions, in group two couples had four abortions, in group three couples had five abortions and in group four had six abortions. The highest numbers of patients were seen in group one (83.5%) (Table -1). The women ages were between 19 to 45 years old (mean 29.3 years old). The highest frequency of abortion was seen in women who belonged to age group 30-34 year old.

The results showed the number of abortion increased in older age, and the relation was significant (ANOVA test, p=0.044, Fig.1)

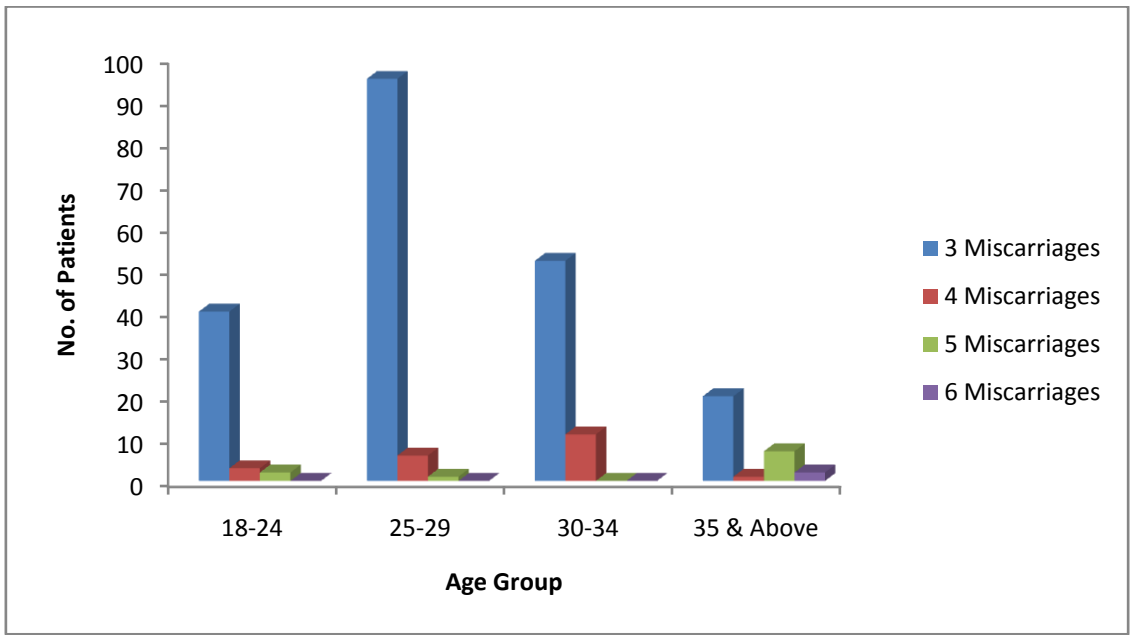

Figure 1 – Frequency of miscarriages according to the maternal age

Table 1 – The incidence of chromosomal abnormality in couples with specific number of miscarriages and percentage of couples studied.

No. of Miscarriages	**No. of Couples studied**	**Percentage of Couples studied**	**Incidence of chromosomal abnormality**
3	167	83.5%	7 (4.19%)
4	21	10.5%	0
5	10	5%	2 (20%)
6	2	1%	1 (50%)

Table 2 - Incidence of chromosomal abnormalities observed in 200 couples with recurrent miscarriages in India.

Karyotype	Incidence
Normal	
46,XX/46,XY	**390**
Robertsonian Translocation	**2**
45,XY,t(13;14)(q10;q10)	

45,XX,t(13;14)(q10;q10)	
Balanced Translocations	**7**
46,XX,t(2;12)(p23;q24.3)	
46,XX,t(5;15)(q23;q26)	
46,XX,t(7;14)(q23;p11)	
46,XX,t(7;22)(q11;p11)	
46,XY,t(5;14)(q34;q13)	
46,XY,t(7;18)(p11;q23)	
46,XY,t(11;22)(q23;q11)	
Mosaic	**1**
45,X[20];46,X;(X)(q10)[10]	
Total number of chromosomal abnormalities	10
Total	400

Table 3 - Incidence of chromosomal abnormalities observed in 200 couples (200 females and 200 males) with recurrent miscarriages in India.

Karyotype	Male	Female	Total
Normal			
46,XX	Nil	194	**194**
46,XY	196	Nil	**196**
Total Normal Karyotype	196	194	**390**
Robertsonian Translocation			**2**
45,XY,t(13;14)(q10;q10)	1	Nil	1
45,XX,t(13;14)(q10;q10)	Nil	1	1
Balanced Translocations			**7**
46,XX,t(2;12)(p23;q24.3)	Nil	1	1
46,XX,t(5;15)(q23;q26)	Nil	1	1
46,XX,t(7;14)(q23;p11)	Nil	1	1
46,XY,t(5;14)(q34;q13)	1	Nil	1
46,XX,t(7;22)(q11;p11)	Nil	1	1
46,XY,t(7;18)(p11;q23)	1	Nil	1
46,XY,t(11;22)(q23;q11)	1	Nil	1
Mosaic			**1**
45,X[20];46,X;(X)(q10)[10]	Nil	1	1
Total number of chromosomal abnormalities	4 (2%)	6(3%)	10(5%)
Total	200	200	400

Among 200 couples (400 individuals) studied, abnormal karyotype were found in 10 (5%) patients. (Table-2)

Of all the 10 patients studied, exhibit autosomal abnormalities in 9 individuals. Of those 9 individuals exhibiting autosomal abnormalities, 2 showed Robertsonian translocations which involved chromosome number 13, 14 in addition 7 had balanced translocations involving chromosome number 2,5,7,11,12,14,15,18,22 also 1 sex chromosomal abnormality was also noted.

The frequency of structural abnormalities was found to be (5/200) 2.5 % in female partners and (4/200) 2% in male partners. The frequency of sex chromosomal abnormality was found to be 0.5% (1/200). The incidence of chromosomal abnormalities among the participant was 5% individuals, which was similar to the incidence reported in other studies. In control group no major chromosomal abnormality observed.

IV. Discussion

Recurrent miscarriage is a crucial clinical and stressful situation that has been studied enormously but the causes and treatment for this condition is not yet fully determined. Even with wide-ranging research to explain the contributory things of recurrent miscarriage, about 50%-60% of the recurrent miscarriages are still idiopathic. This states that the recurrent miscarriages occur due to the multifactorial state that entails several gene-gene and gene-environment relations.

The prevention and control of reproductive failures begin with the study of etiology and necessitate multidisciplinary investigations. Among these, cytogenetic investigations are very useful.

Recurrent miscarriage may result from two types of chromosomal abnormalities such as the recurrence of a numerical abnormality such as aneuploidy in the embryo, which is usually not inherited or a structural abnormality derived from one of the parent. In approximately 3–5% of couples with recurrent miscarriage, one of the partners carries a balanced structural chromosomal anomaly. The most common types of parental chromosomal abnormality are balanced reciprocal or Robertsonian translocations [7]. When one member of a couple carries balanced reciprocal translocations; the risk of having a miscarriage is approximately doubled [8]. Although carriers of a balanced translocation are usually phenotypically normal, their pregnancies are at increased risk of miscarriage and may result in a live birth with multiple congenital malformation and/or mental disability secondary to an unbalanced chromosomal arrangement. The risk of miscarriage is influenced by the size and the genetic content of the rearranged chromosomal segments [9].

The risk of miscarriage resulting from chromosomal abnormalities of the embryo increases with advancing maternal age. However, it is important to note that as the number of miscarriages increases, the risk of euploid pregnancy loss increases [10]. It is also well known that women with previous spontaneous abortion have an increased risk of spontaneous abortion [11].

According to the literature review, the prevalence of chromosomal aberrations among the couples with recurrent miscarriages varied in different studies from none to as high as 15%. For example; in a study by Mustaqahamed et al in 2011using 30 couples, the ratio was 15%; in a Indian study by Dutta et al, 2011using 1,162 couples, the ratio was 3.35%; whereas in 193 Saudi couples study by Al Hussain et al in 2000, the ratio was 3.88% [12, 13, 14]. The overall chromosomal anomaly found in our study was 5.0% (Table-3). Variations in sample size, evaluation criteria for couples and techniques of cytogenetic analysis have all contributed to these differences among studies. It is also possible that the incidence of chromosomal aberrations may vary across different populations.

Translocation was the common abnormalities in our study too with 4.5 % (9/200). The incidence of translocation carriers in couples facing recurrent miscarriages were found to be 8.8% in a study conducted by Karaman and Ulug, 2013 and 2.88% in study by Farcas et al 2007[15,16].

It has been observed that the balanced chromosomal rearrangements have been detected to be present twice often in the female partners. This predominance of females appears to be due to the fact that chromosomal abnormalities that are compatible with fertility in females may be associated with sterility in males. As a result, a history of an abnormal offspring is about fourfold more likely if it is the mother who carries the chromosomal rearrangement [17]. The inheritance patterns of translocations are relatively unpredictable, and are determined by various modes of segregation at meiosis I. The pattern of segregation and the implications for progeny gametes depends on the particular chromosome involved and the size of the rearrangement [9].

In addition to clinical, environmental, and life-style risk factors, there is growing evidence that recurrent miscarriage has also genetic susceptibility. A review of initial observations indicated two to sevenfold increased prevalence of recurrent miscarriage among first-degree blood relatives compared to the background population. Population-based register studies done by Rull et al showed that overall frequency of miscarriage among the siblings of idiopathic recurrent miscarriage is approximately doubled compared to general population [18].

Failure of implantation and/or poor foeto-uterine interaction that caused by chromosomal and non-chromosomal factors, represent the main cause of miscarriage. Chromosomal analysis of abortus materials (between 8–15 weeks gestation) reveals an abnormal karyotype in 50 –60% of cases; i.e. most embryonic chromosome anomalies are incompatible with life [10]. In contrast, 5–7 % of couples with recurrent miscarriage show abnormal karyotype; so the vast majority of foetal chromosome anomalies are de-novo, while the parent's karyotype is normal [19]. Therefore, pre-fertilization factors (sperms and/or oocytes) or post-fertilization factors (mitotic and /or consequences of meiotic errors) might represent major problems that affect the foetal chromosome integrity.

Genetic counseling offers a prognosis for the risk of future pregnancies in these couples with an unbalanced chromosome complement and the opportunity for familial chromosome studies. Reproductive options in couples with chromosomal rearrangements include proceeding to a further natural pregnancy with or without a prenatal diagnosis test, gamete donation and adoption.

Pre-implantation genetic diagnosis has been proposed as a treatment option for translocation carriers [20]. Since pre-implantation genetic diagnosis necessitates that the couple undergo in vitro fertilization to produce embryos, couples with proven fertility need to be aware of the financial cost as well as implantation and live birth rates per cycle following in vitro fertilization/ pre-implantation genetic diagnosis. Furthermore, they should be informed that they have a higher (50–70%) chance of a healthy live birth in future untreated pregnancies following natural conception [7].

V. Conclusion

The causes of recurrent miscarriage are variable and chromosomal aberration is the commonest cause. Several sporadic cases of recurrent miscarriage have been noticed to carry chromosomal rearrangements, however large-scale studies by others revealed chromosome anomalies among the aetiology of recurrent miscarriage.

We conclude that chromosomal disorders are the underlying bases of reproductive failure in a high proportion of cases. The identification of chromosomal abnormality as the etiology has facilitated the counseling before conception and appropriate management.

The development of techniques allowing pre-implantation diagnosis of structural anomalies is of particular relevance to infertile couples with balanced autosomal translocations and may reduce the disappointment associated with chromosomally unbalanced embryos.

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