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CYTOGENETIC STUDY OF LEUCOCYTE CULTURE IN MULTIPLE PREGNANCY LOSS IN MUMBAI

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Abstract- Multiple abortions constitute a significant percentage in married couples. The study was undertaken to explore the possibility of any cytogenetic abnormalities in karyotype of couples having a history of multiple abortions. A study consist of 50 married couples sufferings from recurrent abortions. Parental age was taken into consideration. Peripheral blood lymphocyte of each couple was used for cell culture. Metaphase spread was obtained by Giemsa Trypsin Banding technique.Minimum 15 metaphase were prepared. First slides were observed under microscope using applied spectrum imaging software. Prepared karyotypes were classified using Denver classification. Study showed incidence of chromosomal abnormalities is 6 %.Abnormalities were balanced translocation including 2 reciprocal translocation &1 Robertsonian translocation.
Key words- Cytogenetics, recurrent abortions, karyotyping, pregnancy loss.

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Introduction

Pregnancy loss can be considered as nature's method to select for genetically normal offspring. Reproduction in human being is genetically complex process and terribly incompetent so that under the best circumstance as approximately 70 % of fertilized ova are lost [4].The most common outcome of conception is embryonic or fetal death. Near 1/3 of conception do not result in the delivery of baby [4].

Life of baby begins in mother's womb & progresses till it become safe to expose the baby to surrounding world. Abortion or miscarriage is clinically detectable pregnancy that fails to progress i.e. premature stoppage of development & expulsion of product of conception from uterus before it is capable of living outside [4].Abortion is common occurrence and often remains unexplained. About 15-20 % of clinically recognized pregnancies end in abortions. Abortion is most common in 1st trimester. When abortion takes place 2 or 3 times before 20-22 weeks of gestational period it is termed as multiple,recurrent spontaneous abortion which affects 5 % couple trying to establish family [2,16].Such couple with history of recurrent abortions has increased risk of subsequent abortion.

American & Royal college of obstetricians and gynaecologists recommend to karyotype both parent in h/o recurrent abortion. Cytogenetics is specialized branch of medicine deals with chromosomal abnormalities and their role in various health hazards. Advances in cytogenetics such as banding technique have proved very useful in case of multiple abortions. It revealed that 50-60 % of cases recurrent abortion due to Genetic causes i.e. Chromosomal Aberration [5].

Other causes of multiple abortions can be anatomic, endocrine, immunological, infective, thrombophilic. Multiple abortions in 1st trimester which are due to chromosomal abnormality in one of the parent or in their gametes that occurs during gametogenesis, which is extremely difficult to assess. These arrangements are seen twice as often in female partner than in male. Increase in parental age also have role in causing recurrent abortion. Chromosomal translocation is the most common structural rearrangement involved in multiple abortions [3].Other causes are aneuplodies (trisomies, monosomies), polyploidy and other structural aberration.

Present study aims to assess cytogenetics of lymphocyte culture in 50 couples. Parental age is also taken into consideration.

Aim

To study cytogenetics of leucocyte culture in couples having history of multiple abortions.

Objectives

1. To study Incidence of chromosomal abnormalities in cases of multiple abortions.
2. To evaluate chromosomal abnormality responsible for early miscarriage.
3. To study distribution of balanced chromosomal abnormalities and its percentage in affected cases.
4. To study male: female distribution in affected individuals.
5. To study effect of parental age in case of multiple abortions.

Material

In this study 50 couples with recurrent spontaneous abortion were selected. Patients were thoroughly examined to rule out other non genetic causes of pregnancy losses. Detailed clinical and family histories were recorded in predesigned proforma. The predesigned proforma included details of time and number and number of abortions, parity, living children, family histories of recurrent abortions, consanguinity, caste, religion and ethnicity etc.

In present study, patients had a history of two or more abortions. All the couples were in age group ranging from 22-42 years and number of abortions ranges from 2-6 .The couples were subjected to clinical examination by Gynecologists and Urologist (to rule out anatomical abnormalities) and then by Genetic counsellor. After genetic counselling, family pedigree is drawn by genetic counsellor. Different age groups were selected to show the relation between advance parental age and chromosomal abnormality .After genetic counselling family pedigree were recorded for 50 couples.

Method

The blood samples of both male and female partners are subjected to a white cell chromosome analysis. Karyotyping is conducted by analysis of G-banded chromosomes using 3 ml heparinized peripheral blood sample. Metaphase spreads are made from peripheral lymphocytes using standard cytogenetic techniques. Cultures are harvested and Karyotyping is performed on G-bands produced with Giemsa Trypsin banding (GTG) preparations. The chromosomal status is analyzed.15 metaphases were analyzed for each patient. Initially slides were observed under Labomed Microscope under oil immersion. Then karyotypes were prepared on Olympus microscope using Applied Cytovision Software.All chromosomal abnormalities are reported in accordance with the current international standard nomenclature. Statistical study can be performed on results of group of such patients.

Results

Chromosomal analysis was performed in 50 couples (100 individuals) having history multiple (two or more) pregnancy loss. The ages of referred wives ranged from 22 yrs to 42 years old with mean of 29.1years. Number previous abortions vary from 2-6 abortions with mean of 2.7.It was found that out of 50 couples, only one male and two female were affected cytogenetically. Case no.3, case no.6, case no. 35 were having chromosomal abnormalities. Incidence of chromosomal abnormalities is 6%.Abnormalities included 2 reciprocal translocation and one Robertsonian translocation.

In Case: 3 male partner showing balanced reciprocal translocation 46XY; t (1; 2) (q32.3; qter) Fig(1).Here female partner is normal i.e. 46 XX.In Case No: 6 balanced reciprocal translocation is observed in female partner 46XX, t (1; 4) (q32-qter; qter)Fig(2) ,male partner is normal. In Case No: 35 female partner is showing balanced Robertsonian translocation 45XX, der, (14; 21) (q10; q10) Fig (3). Karyotype of male partner is normal. Out of 3 affected individuals two are females and one is male. Incidence of female partner is twice than that of male**.** Female / male Ratio is 2: 1.In this study mean maternal age is 29.1 yrs. Mean paternal age is 31.16 yrs. Maximum number of female partners were in 26-30 year age group (Table 1).Maximum number of male partners in couples is in 31-35 years age group (Table 1).Maximum number of couples are from group having history of 2 abortions. Here number of couples decreases as number of abortions increases (Table 2) (Figure 4).All the affected cases are of balanced structural chromosomal arrangement. It shows 2 cases of reciprocal translocation (66.6%) and 1 case of Robertsonian translocation (33.3 %) (Table 5).We found more number of cases of reciprocal Translocation. Maximum abnormalities are seen among the couples having history of 2 abortions. Out of 25 couples having history of only 2 abortions, we got 2 couples with chromosomal abnormalities (8 %) Fig (5) (Table: 3).Out of 3 abnormal cytogenetic findings 2 are seen in females of age group 25-30 years. Case no 48 shows presence of large Y, which is example of chromosomal variation Fig (8).

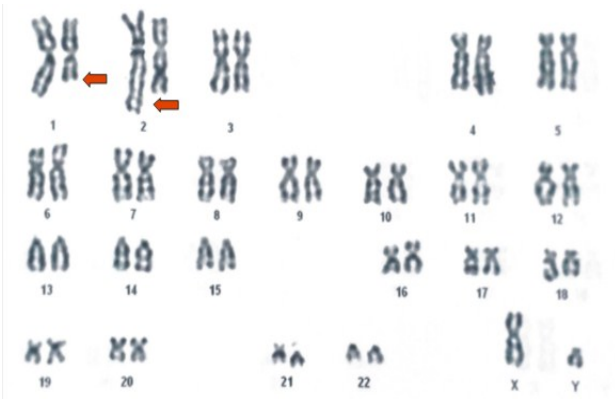

Fig. 1- Balanced reciprocal translocation: 46XY; t (1; 2) (q32.3; qter).

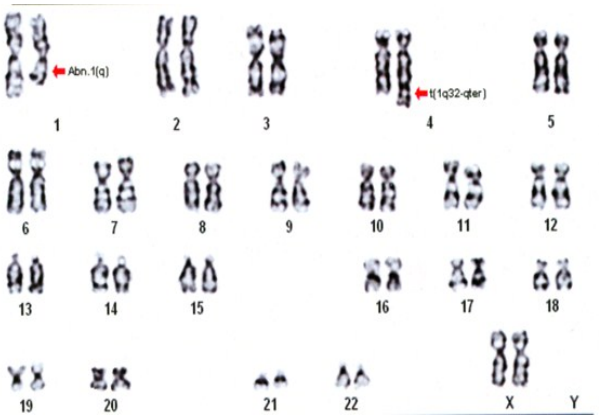

Fig. 2- Balanced reciprocal translocation: 46XX, t (1; 4) (q32-qter; qter).

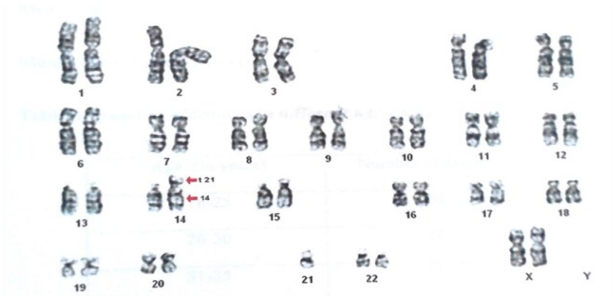

Fig. 3- Balanced Robertsonian translocation 45XX, der, (14; 21) (q10; q10)

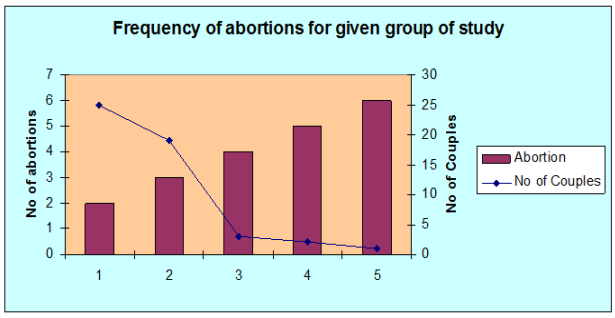

Fig .4-

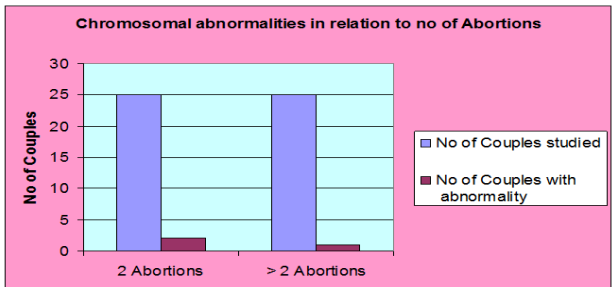

Fig. 5-

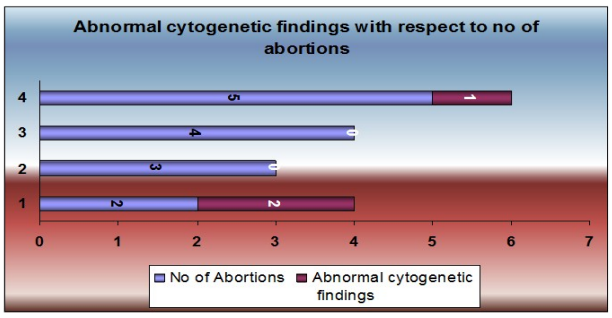

Fig .6-

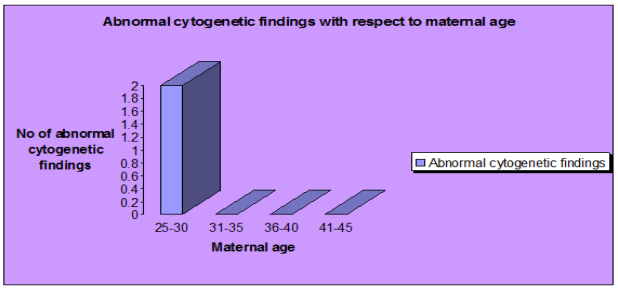

Fig. 7-

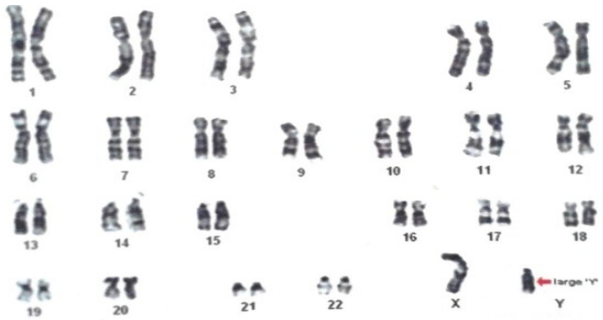

Fig. 8- 46 X Large Y

Table 1- Number of males & females in different age group

Age. (in years)	Number of Males	Number of females.
18-25	1	10
26-30	18	27
31-35	21	11
36-40	9	1
41 & above	1	1

Table 2- Frequency of abortions for given group of study

Number of abortions	Number of couples	%
2	25	50
3	19	38
4	3	6
5	2	4
6	1	2

Table 3- Chromosomal abnormalities in relation to no. of abortion

Case No.	Translocation type	Karyotype	No. of abortions
3	Reciprocal T.	46XY;t(1;2)(q32.3;qter)	5
6	Reciprocal T.	46XX;t(1;4)(q32-qter;qter)	2
35	Robertsonian T.	45XX;der,(14;21)(q10;q10)	2

Table 5- Frequency of chromosomal abnormalities

Translocation type	No. cases
Reciprocal T.	2
Robertsonian T.	1

Table 6- Abnormal Cytogenetic Finding w.r.t. No. of abortions

No. of Abortions	Abnormal cytogenetic findings
2	2
3	-
4	-
5	1

Table 7- Abnormal cytogenetics findings w.r.t. maternal Age

Maternal age.(in years)	Abnormal cytogenetic findings
25-30	2
31-35	-
36-40	-
41-45	-

Discussion

Multiple abortions are difficult medical problem happening in about 1-2% fertile women[11].The incidence of chromosomal abnormalities among different studies from none to as much as 30% [1].The variations in the size of the sample ,criteria used for ascertainment of cases and techniques of cytogenetic study have contributed to these wide differences between various studies [6]. It was seen that the balanced structural translocation represent the main cause of multiple abortions. In this study, we have studied 50 couples having history of multiple abortions which showed 3 cases (3 couples) with chromosomal abnormalities, all are having balanced chromosomal rearrangement. Findings are as follows.

Case No: 3 shows male partner is affected, 46XY, t (1; 2) (q32.3; qter) i.e.balanced reciprocal translocation.Case No: 6 shows Female partner is affected, 46XX, t (1; 4) (q32-qter; qter)which is balanced reciprocal translocation. Case No: 35 shows female is affected, 45XX, der (14; 21) (q10; q10) which is a balanced Robertsonian translocation. In our study the incidence is 6%, which is not significantly different from Global. Dubey et al. 2005, Osztovics MK et al. 1982, and Low PS et al. 1989 reported the low frequency of chromosomal abnormality i.e. 2 %, 4.78 %, 1.1% respectively[2,1,7]. Mean maternal age in our study is 29.1 years .Dubey et al. 2005 in their work got the 30.2 years as mean maternal age, which is more than that of our study [2] .Maximum numbers of females which are having abortion history are from age group 26-30 years**.** In further age group number decreases. Mean maternal age in chromosomally affected cases is 26.5 years .Therefore it is difficult to comment on these two parameters. Dubey S et al 2005 has also mentioned that there is no positive correlation of advance maternal age with number abortions observed [2].Mean Paternal Age in our study is 31.16 yrs**.** In our study only male who is affected is at age of 35 years**.** Maximum number of abortions we get in paternal age group 31-35 years. We found that 1 male and 2 female in study of 50 couples having chromosomal abnormality which was a ratio of 2:1. Stoll et al. 1981 reported the higher incidence of Female / Male ratio i.e. 7:1 [15].Ohno M et al. 1989 studied 19 females and 7 male which were chromosomally abnormal. (Female /male ratio =2.7/1)[11]. But Yuce H et al 2007 mentioned in his study more number of males than that of females which are cytogenetically affected (Female/Male Ratio =2.73/0.95) [18].We have reported 3 (6%) cases are of translocation. All the cases are carrier of balanced translocation. Mamelli et al 1984 reported 8% i.e. 4 cases of translocation in 50 couples [8]. In present study overall incidence of reciprocal translocation 4% and Robertsonian translocation is of 2 %**.**Fortuny et al 1988 reported the 0.64 % cases of reciprocal translocation and 3.59 % cases of Robertsonian translocation[4]. In our study we have reported all cases are of translocation. Similar result we observed in studies performed by Mamelli et al. 1984, and Low PS et al. 1989 i.e. all the cases were affected by translocation [8, 7].

Hussain MA et al.2000, Ohno M et al. 1989 showed 66.6%, 77% of cases were of translocations in affected individuals respectively.[6][11]Fortuny et al. 1988 reported 70% cases of translocations among affected couples[4]. Among translocation, we found that 75% affected cases are of reciprocal translocation and remaining cases are of Robertsonian translocation(Table:6).Mamelli et al 1984 also had more cases of Robertsonian translocation[8]. In present study translocation is predominantly seen of female partner than male partner (Table 5). Stoll et al 1981, Hussain MA et al 2000 reported the similar result[15,6] .No sex predominance was observed in case of study presented by Fortuny et al 1988[4].

In our study we presented one case of Robertsonian translocation (case no35) involving chromosome no 14 and 21. Yuce H et al 2007 had also recorded the Robertsonian translocation involving chromosome 14 and 21[16] (Table 5). Dubey S et al. 2005 in his study got the Robertsonian translocation of chromosome 14 and 21 in two different cases [2]. Low PS et al. 1989 in his work got the one case of Robertsonian Translocation involving chromosome 14[7]. Pantzar et al. 1984 also reported the balanced Robertsonian translocation involving 3 cases of chromosome 14[13].In case no. 35 the chromosomal abnormality is Robertsonian translocation in maternal .Chromosome in such case shall have greater risk, that the fetus will exhibit an unbalanced phenotype. Pantzar et al. 1984 also reported maternal Robertsonian translocation [13]. The case no.48 in present study show the presence of large Y**.** Blumberg BD et al 1982 reported the presence of large Y in his study [1]. Different studies have shown that the presence of large Y does not play any role for the reproduction outcome. The present study showed that maximum number of couples (50%) is from group having history of 2 abortions. (Table 3)

In present study 12.5 % of cases in couples having only 2 abortions history are affected. Blumberg BD et al 1982 detected 3 (10%) cases in 30 couples having only 2 abortions. We reported maximum number of chromosomal abnormalities in group having 2 abortions (Table: 7) [1].Ideally chromosomal studies should also be done on the abortus material to know the contributory factor for that abortion. This study has shown that the incidence and distribution of chromosomal abnormalities among Indian couples with repeated pregnancy loss is similar to that reported worldwide.

Summary and Conclusion

Cytogenetic study was performed in 50 couples having history of multiple pregnancy loss. Couples with different age groups were selected. Abortion number varies from 2-6.Anatomic and other abnormalities had been ruled out. Cytogenetic study was performed using Giemsa Banding technique .Karyotypes were analyzed. Parameters were parental age and number of abortions. Incidence of chromosomal abnormalities, types and their distribution with respect to sex had been observed. It was observed that the incidence of chromosomal abnormality is found to be 6% .All affected cases were of balanced translocations including two cases were reciprocal translocation and one case was of Robertsonian translocation. We did not find any significant relation in parental age and frequency of abortions. Also there was no relation between number of abortions and frequency of chromosomal abnormalities. It was seen that the female partners were affected more than that of male. Female /male ratio in chromosomally affected individuals in this study was 2:1.The finding co-relates with similar studies conducted by various other Geneticist.

List of abbreviation

List of abbreviation	
Dept-department	w.r.t-with respect to
no.-number	i.e.-that is

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