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Original Research Article

Assessment of Chromosomal Abnormalities in Couples with Recurrent Pregnancy Loss

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ABSTRACT

Previous studies have demonstrated the type of chromosomal abnormalities found among couples with recurrent pregnancy loss. But a majority of the couples show a normal karyotype. The present study was undertaken to inquire the nature of cytogenetic abnormalities by routine karyotyping and c-banding whenever required, among couples with recurrent pregnancy loss and to compare it with fertile couples. Among couples, women with the history of two or more than two spontaneous abortions ≤ 24 weeks of gestation were included and couples with the history of Diabetes mellitus, thyroid disorders and hypertension, etc. were excluded. The lymphocytes were cultured as per the standard protocol and metaphase spreads stained by GTG banding technique. The presence of chromosomal aberrations was analyzed by semi-automated karyotyping software (Ikaros). In the present study, abnormal karyotypes were found in 3 cases. All the controls had a normal karyotype.

Keywords: Chromosomal abnormalities, Karyotyping, Recurrent pregnancy loss, C-banding, Heteromorphisms.

INTRODUCTION

Clinically recognized pregnancy loss is a common problem affecting 15-20 % of pregnancies. Recurrent pregnancy loss (RPL) as per the modern definition by American Society for Reproductive Medicine (ASRM) is "loss of two or more failed clinical pregnancies". Two losses are seen in 5% of couples and three losses or more seen in 1% of couples". [1]

As per the World Health Organization (WHO) "Reproductive health is a state of complete mental, physical, and social well-being which is related to all stages of reproductive processes". [2]

The etiology for RPL are enlisted as genetic, any pathology or hindering factors related to implantation, autoimmunity, endocrine abnormalities, anatomic uterine defects, paternal factors, alloimmunity, etc. But in half of the cases, etiology for RPL cannot be determined.[3] Few epidemiological studies have suggested that it could be multifactorial, involving the interaction of predisposing genetic and environmental factors in its pathogenesis. [4]

As per the previous studies, genetic abnormalities among aborted fetuses are high, compared to karyotype abnormalities among couples with RPL, which is about 3-5%. But this is five times greater than in the common population. [4]

The type of structural karyotype abnormalities that have been reported

include- reciprocal translocations (most common), followed by Robertsonian translocation, deletion, and inversion. Apart from significant chromosomal abnormalities, the other chromosomal variants detected include heterochromatin, Y chromosome variations, and fragile sites. [3]

As evidenced by previous literature, unexplained factors contribute to recurrent pregnancy loss in majority of the cases. Karyotyping is the only conventional test for couples with RPL to evaluate the genetic factor.

MATERIALS AND METHODS

The study was conducted in the Department of Anatomy in collaboration with Department of Obstetrics and Gynecology, JIPMER, Puducherry. The Institute ethics committee (IEC) & Postgraduate Research Monitoring Committee (PGRMC) approval were obtained prior to the study. It was a case-control study. Subjects were selected among couples attending outpatient department, OBGY by convenient sampling. Cases comprised of couples with RPL (gestational age ≤ 24 weeks). Controls were healthy fertile couples. Couples who have proved their fertility by birth of one or two children were selected. Sample size was calculated as 27 couples in each group. After informed written consent, demographic details and medical history of the couple were obtained in the predesigned data collection proforma. 3ml of heparinized peripheral blood was withdrawn by venipuncture under aseptic precautions. Peripheral blood lymphocyte cell culture was carried out as per the protocol. [5] GTG banding of the captured metaphases was done using Giemsa stain.

Analysis of slides

The slides were analyzed under the Trinocular microscope *Olympus BX51*. Twenty metaphase spreads were captured and analyzed for each of the samples. Images were obtained using *automated karyotyping software*, ***IKAROS***.

Whenever heterochromatin (chromosome 1, 9, 16, Y) was observed in the karyotype, such slides were further stained by C-banding technique for confirming heterochromatin.

Statistical Parameters & Analysis

Statistical analysis was done using SPSS version 19. Categorical data like presence or absence of chromosomal abnormalities (translocations, inversions) and heterochromatic regions was described as proportions. The difference in the proportion of chromosomal aberrations and heterochromatin in cases and controls was tested using Fisher's exact test.

RESULTS

The cases were 54 in number, i.e. 27 couples. Women with history of two or more than two spontaneous abortions ≤ 24 weeks of gestation were included in the study. The majority of the cases in both the genders were in the age group of 26-35 years. Both male and female controls were in the age group of 26-30 years. The present study included both first-trimester and second-trimester pregnancy losses up to 24 weeks of gestation. Most of the cases (approximately 70%) had more than two abortions. (Refer Figure-1).

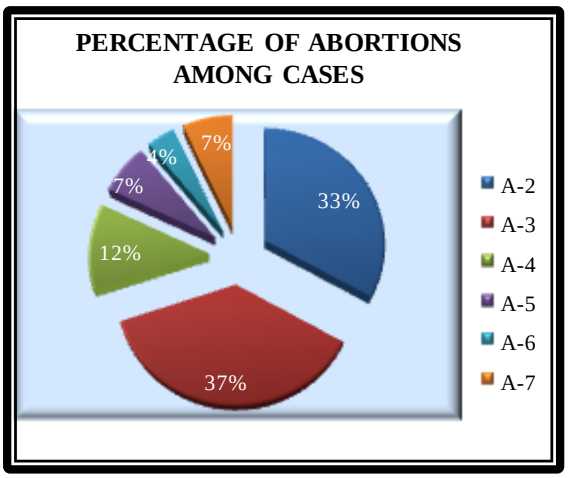

Figure-1: Percentage of abortions among Cases

Abortions

TABLE- I: Observations in Chromosomal abnormality

CASES	GENDER	KARYOTYPING	
n=27couples	**M/F**	**Structural Chromosomal Abnormalities**	**Numerical Chromosomal Abnormalities**
1.	M	46,XY,9qh+,16qh+[20]*	Nil
2.	M	46,XY,1qh+[33]*	Nil
3.	F	46,XX,del(22)(p11.1)[20]*	Nil

*-Numbers within square brackets are the number of metaphases captured and analyzed.

Chromosomal abnormalities (Table-I, Figure 2-7):

Structural chromosomal abnormalities (deletions and heterochromatic regions) were found in 3 out of 54 cases, and it was absent in all the controls. Numerical abnormalities were absent in both cases and controls. Heteromorphisms were detected among two male cases which included 9qh+, 16qh+ (Figure-2) and 1qh+ (Figure-3). Metaphase spread of the case with karyotype showing 1qh+ is depicted in Figure-4 and C-banding of same metaphase spread in Figure-5. 22 p deletion was noted in one female out of 54 cases (Figure-6). Thus three cases had chromosomal derangements. Two out of 27 male cases (7.4%), one out of 27 female cases (3.7%) on the whole 11% had chromosomal abnormalities (Figure-7). The presence of chromosomal abnormalities in cases and controls was not statistically significant (Fisher's exact test), p=0.24; OR=2.06, 95% CI of 1.69 – 2.51.

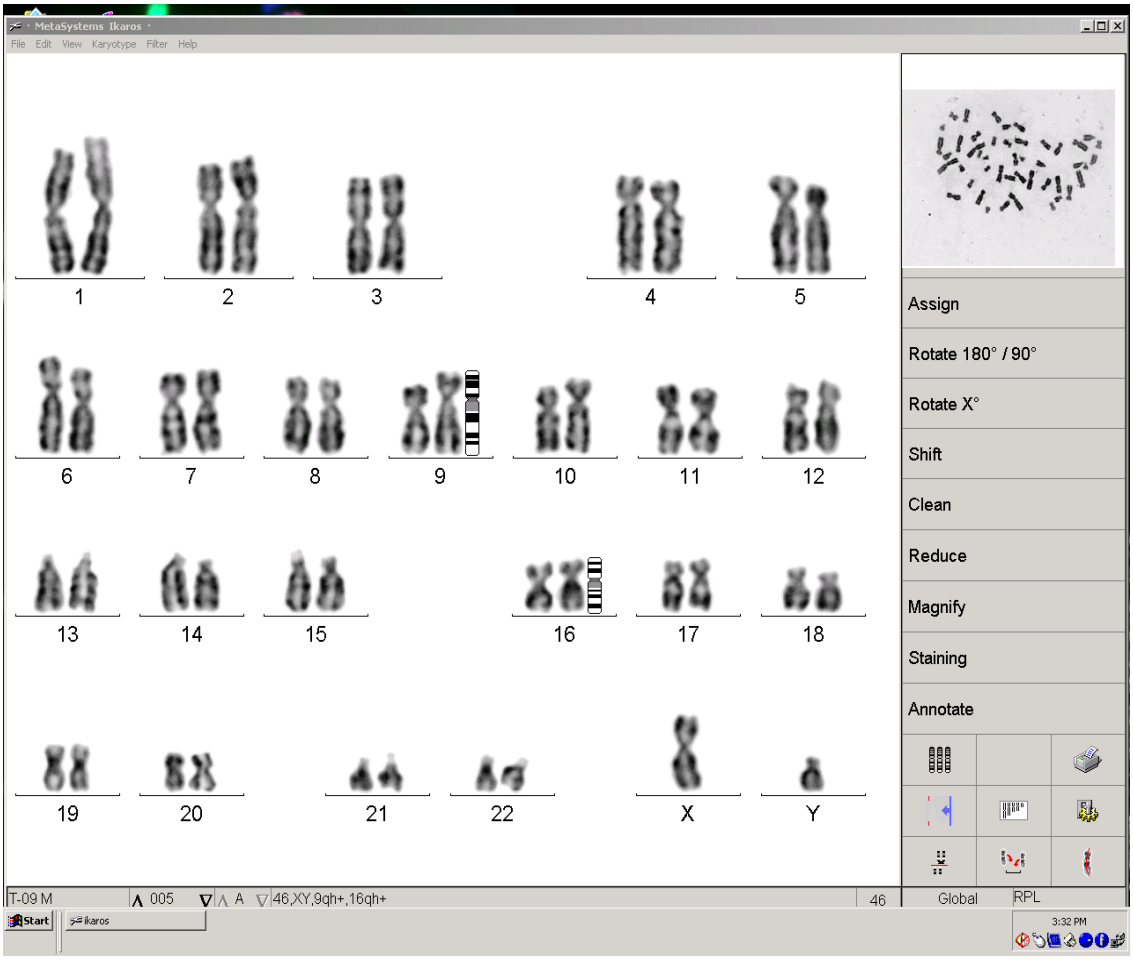

Figure-2: Male karyotype showing heterochromatin in chromosome 9 and 16 (46,XY,9qh+,16qh+)

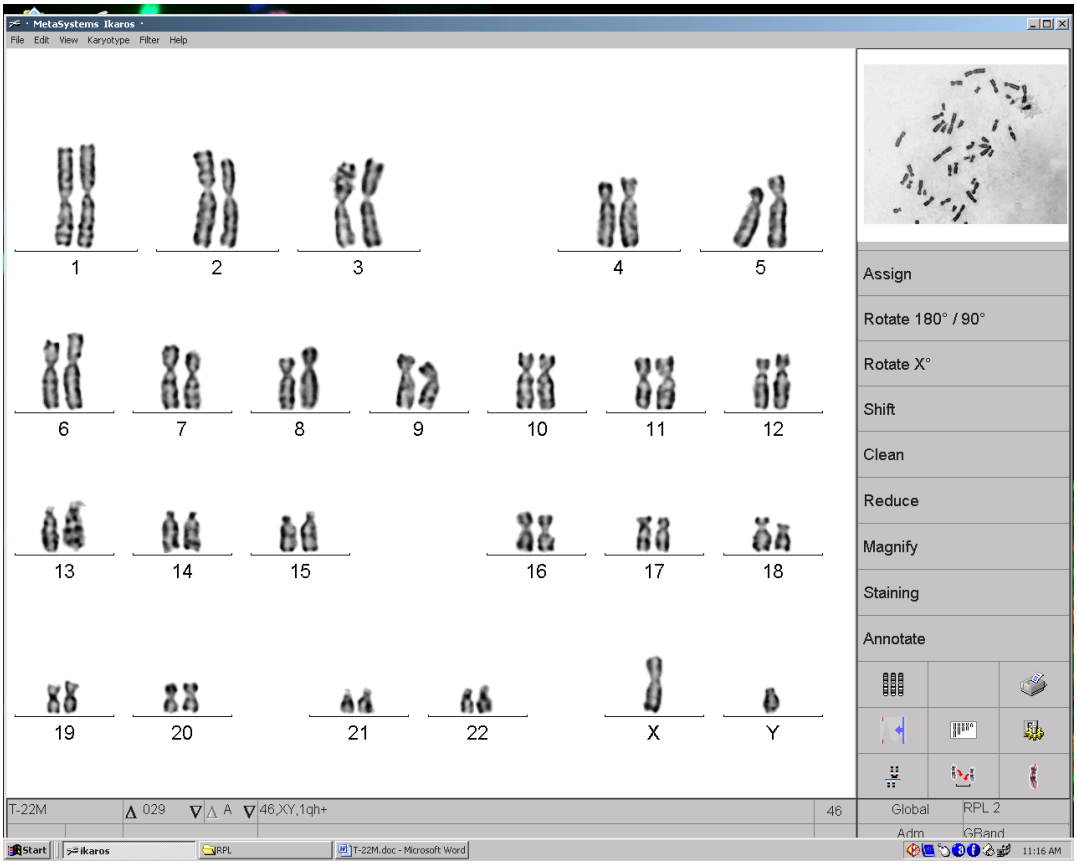

Figure-3: Male karyotype showing heterochromatin in chromosome 1(46,XY,1qh+)

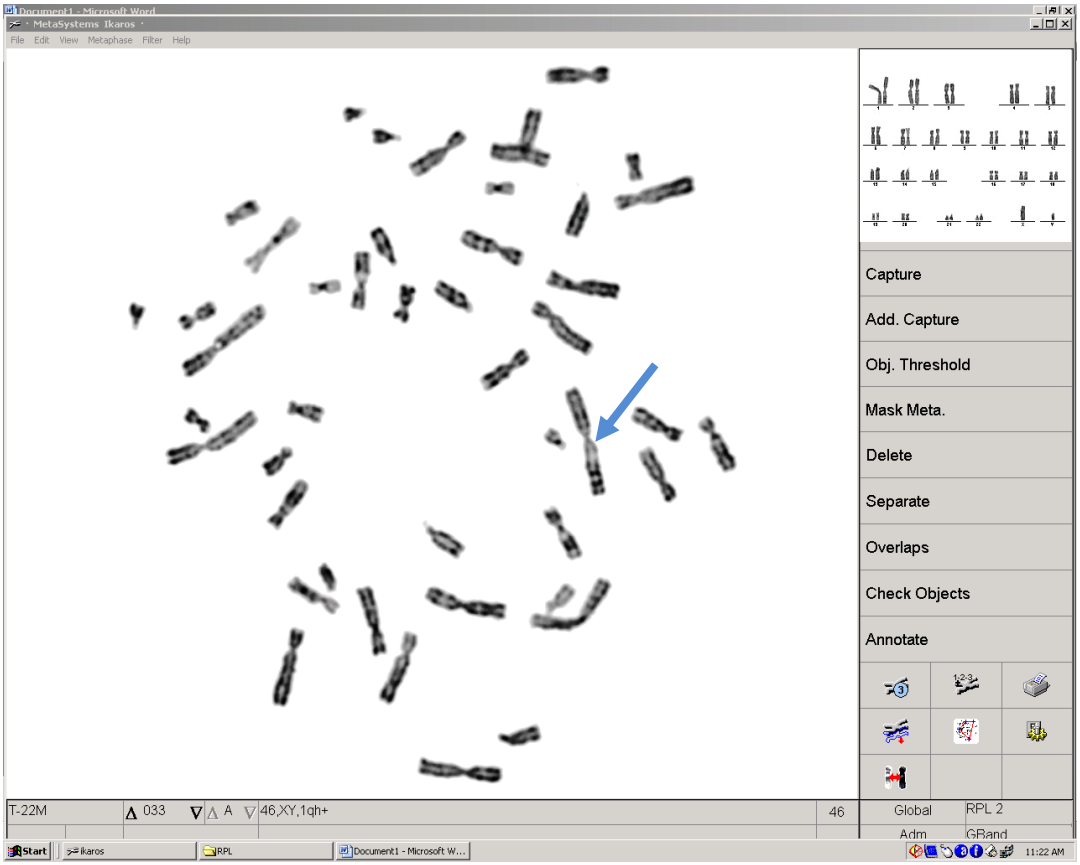

Figure 4- Male karyotype showing metaphase spread (46,XY,1qh+)

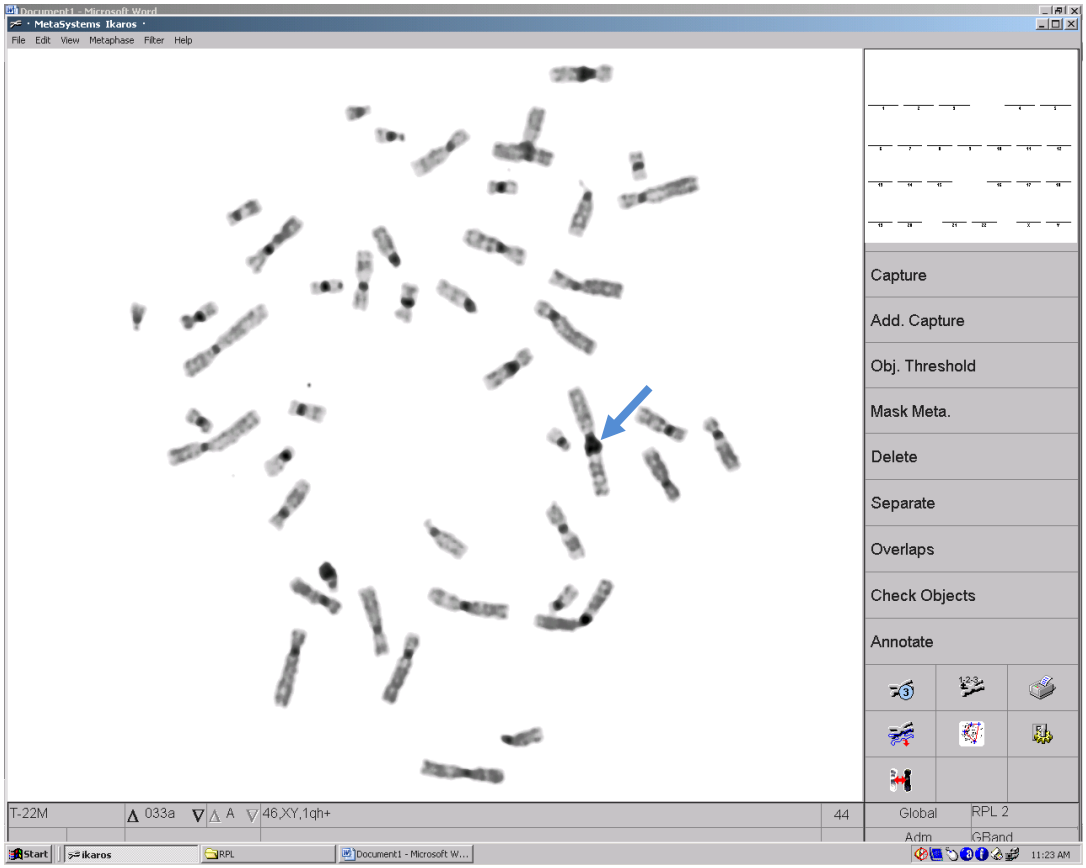

Figure-5: Male karyotype showing C-banding of same metaphase spread in Figure-4 (46,XY,1qh+)

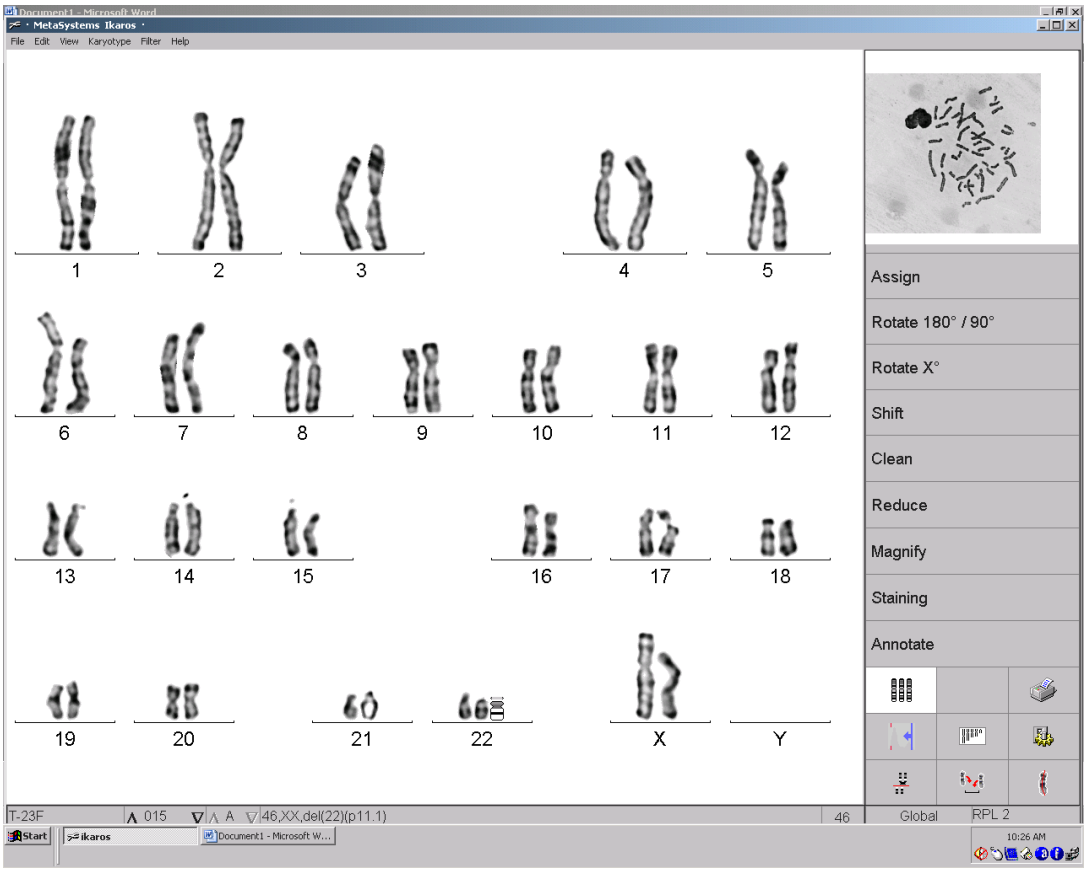

Figure-6: Female karyotype showing 22p deletion (46,XX,del(22)(p11.1)

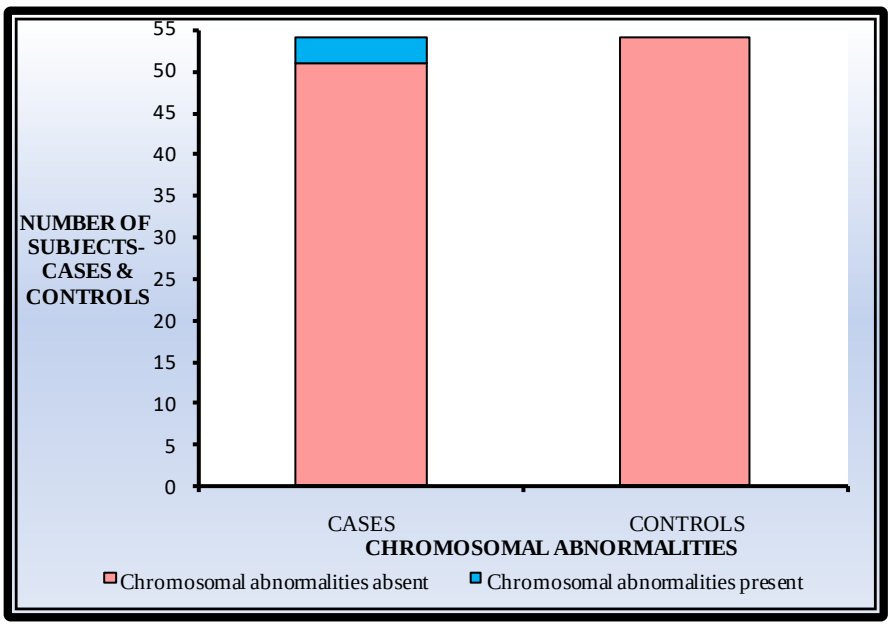

Figure – 7: Chromosomal abnormalities in cases and controls

DISCUSSION

In this case-control study, couples with RPL were evaluated for cytogenetic abnormalities. The frequency of chromosomal abnormalities to some extent was higher among couples with RPL compared to controls but was not statistically significant. These findings are in agreement with the previous studies which highlight the fact that genomic irregularities are one of the factors for causing RPL. [6-8]

Chromosomal aberrations were present in three cases out of 54 and it was absent in all the controls.

Studies vary with regards to inclusion criteria, in relation to the number of weeks at which pregnancy loss occurred. In the study on Iranian women, abortions less than 12 weeks were considered as cases. [8] The study in Turkish women and few other Indian studies less than 20 weeks of gestation were considered. [9,10]

Females with two or more than two miscarriages were considered in all the studies including the present study. The study involving Iranian population, couples were sorted into three groups; one with two abortions, second with three abortions, and compared with the controls. Karyotypic abnormalities were higher in the group with three abortions. [8]

Among the studies done in India, the prevalence of chromosomal aberrations varied between 7-18 %. [3, 6, 11] The present study showed 11% prevalence which is nearer to the prevalence of 8.4% as per similar study by Rajesh et al. [10] The prevalence among the North Indian population was 7%, and that from Mumbai had 18%. [3,11] This could be due to different sampling population with different ethnicity. Table-II shows the various studies done to evaluate the nature of chromosomal abnormalities among different study groups.

The association between RPL and aberrations could not be commented as the sample size was small. The chromosomal abnormalities that have been reported in previous studies include - structural abnormalities such as Robertsonian translocations, reciprocal translocations, inversions, deletions, the presence of satellites and numerical abnormalities such as mosaicism. But we could not report several structural and numerical chromosomal abnormalities as cited in previous studies. [3, 8, 9, 10]

A study among Iranian population reported a higher rate of chromosomal abnormalities in the group with three

abortions (5.3%) than the group with two abortions (3.07%). [8] Such correlation could not be commented on the present study.

Heterochromatic variations:

In the present study heterochromatic variations were noted to be 66.7% (of 11% chromosomal defects) whereas it was 40.4% (of 7%) as per Dubey et al. [3] It could be due to larger sample size in the latter study. In the study done among Iranian population, heterochromatic variations were higher (average 66%) compared to 7.5% in control individuals. [8] The highest prevalence of heterochromatic variations can be observed in the study among Turkish population (91.8% of 69.5%). [9] The common heterochromatic variations noted were those involving chromosome 1, 9, 16 & Y and also acrocentrics satellites and stalks. [3]

As per studies by Purandare et al. and Pokale et al., heterochromatic variations have been implicated in pregnancy losses. Inversion in Y-chromosome and 9qh+ may be associated with RPL. [6,11] Identification of marker chromosome in the form of heterochromatin could be utilized to figure out the future risk of RPL. [12]

The common heterochromatic variations described are - in q arm (qh+) followed by the occurrence of satellite and inversion. [11] In the study by Purandare et al, the qh+ and the satellite in p arm were higher than inversions and deletions. The frequency of occurrence of qh+ among chromosomes is highest on chromosome 1 followed by that in 9 and 16. [6]

From Table-II, it could be inferred that even though prevalence included abnormalities and variations, majority consisted of heterochromatic variations. As per the results of this study, heterochromatic variations could be one of the factors leading to RPL in absence of evident chromosomal abnormality.

TABLE-II: Comparison of studies done for Chromosomal aberrations and Heterochromatic variations

S. No.	Author details & Study population	Year of study	Sample size (Couples)	Abnormalities + Variations	Results	
					Chromosomal Abnormalities	Heterochromatic variations
1.	Pokale et al. [11] Mumbai, India	2015	Cases - 200	18%	27.78%	72.2%
			Controls-100	9%	Nil	9%
2.	Purandare et al. [6] India	2011	Cases - 440	17%	24%	76%
			Controls-200 (I)	3.5%	Nil	3.5%
3.	Asgari et al. [8] Iran	2013	Cases- 75 (with 3 abortions)	14.7%	36.4%	63.6%
			Cases- 65 (with 2 abortions)	9.2%	33.3%	66.7%
			Controls - 40 (I)	7.5%	Nil	7.5%
4.	Ocak et al. [9] Turkey	2013	495	69.5%	8.1%	91.8%
5.	Dubey et al. [3] AIIMS,India	2005	742	7%	59.6	40.4%
6.	Rajesh et al. [10] India	2008	72	8.4%	8.4%	Nil
7.	Present study India	2015-2017	Cases - 27	11%	33.3%	66.7%
			Controls - 27	Nil	Nil	Nil

[(I)-Individuals]

CONCLUSION

Though genetic cause was one among many of the factors leading to RPL, it could not be left without being assessed. Hence evaluation of karyotype and heteromorphisms is essential in cases with RPL. This will be helpful in genetic counseling and for reassuring the couples.

Limitations of the study were as follows- a) small sample size, b) In relation to the technique, trypsinization and banding vary depending on the climatic conditions. c) Though most of the cases diagnosed as RPL were pregnant at the time of the study, follow-up of the cases regarding the outcome of their present pregnancy could not be done.

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