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Original Research Article

Recurrent pregnancy loss − Chromosomal anomalies in couples

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Abstract

Background: Recurrent Pregnancy Loss (RPL) is an important reproductive health problem. The present study was carried out to find out the frequency and types of chromosomal anomalies in couples who consulted the Department of Obstetrics and Gynaecology, seeking treatment for RPL. They were examined and investigated at the Institute of Maternal and Child Health, Government Medical College, Kozhikode, which is a Tertiary care Centre of North Kerala.
Study Settings and Design: A retrospective study of karyotypes of 125 couples was done. The results of cytogenetic analysis over a period of 7 years were assessed.
Method: Peripheral blood lymphocytes were cultured according to standard protocol and karyotypes were analysed using Cytovision Software Version 7 and expertise of staff.
Results: Out of 125 couples, 114 (91.2%) couples had apparently normal karyotypes. Remaining 11 couples (8.8%) showed chromosomal anomalies.
Conclusion: This study shows the importance of karyotyping in couples with recurrent pregnancy loss. Early diagnosis and genetic counseling can be offered to affected couples.
Keywords: Recurrent Pregnancy Loss (RPL), Reciprocal Translocation, Robertsonian Translocation.

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1. Introduction

American Society of Reproductive Medicine (ASRM) has defined Recurrent Pregnancy Loss (RPL) as two or more pregnancy losses. A pregnancy loss is an involuntary end of pregnancy before 20 weeks. Pregnancy losses result from the following causes:

1. Abnormality of uterus
2. Hormone imbalance in the female partner including thyroid disorders and diabetes mellitus.
3. Abnormalities of immune system of the female partner.
4. Antiphospholipid antibodies, especially anticardiolipin antibody and lupus anticoagulant.
5. Chromosomal anomalies.

The treatment of a patient with RPL is based on the underlying cause. In our Institution, couples are referred to the Cytogenetic Lab for karyotyping. Simultaneously, investigations to rule out other causes are also performed.

Aim: The aim is to identify the anatomical, immunological, endocrine and cytogenetic causes. This will help the clinician to intervene, if required. To certain extent, uterine abnormalities and endocrine diseases are treatable. When one of the partners is diagnosed to have a balanced translocation, a Preimplantation Genetic Testing (PGT) can be advised.

2. Observation and results

A total of 125 coupleswho had 3 or more pregnancylosses were evaluated for chromosomal anomalies. 114 couples (91.2%) had normal karyotypes. 11 (8.8%) of the couples had chromosomal abnormalities. 9 of them were in the female partner and the remaining two in the male partner. Polymorphic variants of chromosomes are said to be normal: however, in our study, 30 individuals showed polymorphic variants, which is not included in the statistical analysis.

Details of chromosomal anomalies detected in our study are shown in **Table 1.**

Table 1: Chromosomal anomalies in couples with RPL

Sl No:	Types of Chromosomal anomaly	No: of Cases	Percentage (%)
	Total No: of couples (n)	125	
1	Total no: of anomalies	11	8.8
2	Reciprocal, balanced translocation	4	3.2
3	Robertsonian translocation	3	2.4
4	Inversion	3	2.4
5	Robertsonian + Reciprocal translocation	1	0.8

3. Discussion

Recurrent Pregnancy Loss (RPL) is defined as two or more pregnancy losses by American Society of Reproductive Medicine (ASRM). RPL is an important reproductive health problem. It is estimated that RPL occurs one in 100 pregnant women. The etiology of RPL remains unknown in 50% of the cases. [1] Many factors have been implicated namely chromosomal anomalies, congenital anomalies of uterus (e.g. Septate or bicornuate) endocrine disorders (thyroid diseases, diabetes mellitus) antiphospholipid antibodies and infections. Increased Maternal and Paternal age have also been associated with RPL.

Chromosomal anomalies are one of the leading causes for fetal loss. About 50-60% cases of RPL are the consequence of chromosomal abnormalities which can of prenatal origin or arise de novo in the embryo even if the parents have normal chromosomes. [2]

The most common parental chromosomal anomalies are balanced translocation, which occur in 2-5% cases of RPL. [3] Translocations can be reciprocal (approximately 60%) or Robertsonian (approximately 40%).

Paracentric or pericentric inversions, even though rare, are associated with RPL. [4] Parents carrying balanced translocations are usually asymptomatic. Pregnancies with unbalanced translocations can end in abortion, still birth or live birth with multiple congenital anomalies. If the fetus gets normal chromosomes from the parents, a normal healthy baby will be born.

Most common cause of early pregnancy loss (less than 10 weeks) is embryonic aneuploidy. Chromosomal anomalies such as autosomal trisomy, polyploidy, monosomy X are most commonly detected in the products of conception. The risk of aneuploidy increases with maternal age. [5]

The effects of paternal meiotic errors related to paternal age are less defined, as opposed to maternal age. This is because errors of non disjunction occur to a lesser extent in sperms than in oocytes. Paternal errors are mainly responsible for sex chromosome anomalies such as XXY and XYY.

Cytogenetic studies in couples with RPL are carried out in various Centres across the world. The results are compared in Table 2.

Table 2: Comparison of frequency of chromosomal anomalies in various studies

Sl No.	Authors	Year	No: of couples	Percentage of chromosomal anomalies	
				Affected couples	%
1	Present study	2020	125	11	8
2	Pal *et al* [6]	2017	172	17	9.88
3	Sudhir *et al* [7]	2016	440	15	3.41
4	Ghazaey *et al*[8]	2015	728	43	5.91
5	Goncalves *et al*[9]	2014	151	11	7.28
6	Flynn *et al* [10]	2014	795	28	3.52
7	Sheth *et al*[11]	2013	2428	170	7.00
8	Dutta *et al*[12]	2011	1162	78	6.71
9	Goud *et al*[13]	2009	380	26	6.84
10	Nazmy *et al*[14]	2008	376	34	9.04

The percentage of chromosomal anomalies ranges from 3.41-9.88. Our results are almost similar to those of Goncalves, Sheth, Nazmy *et al.* Most of the authors have reported the predominance of chromosomal anomalies in the female partner. In our study, out of 125 couples, only two males had chromosomal aberrations. Remaining nine were females. However the percentage of affected males was higher than in females in a study conducted by Neha Sudhir *et al*.

Structural abnormalities of chromosomes in RPL

Structural abnormalities of chromosomes are an important cause of RPL. Reciprocal translocations are the most common structural anomalies reported in several studies. In our study 40% of all chromosomal anomalies were reciprocal translocations; they were

1. 46, XX, t (10; 13) (q22; q12)
2. 46, XX, t (6; 8) (q25; q11.2)
3. 46, XX, t (2; 3; 12) (p24; q29; q21.3)
4. 46, XY, t (1; 10) (p36; q11)

Chromosomes involved were 2, 3, 6, 8, 10, 12 and 13. In one case, three chromosomes were involved. (**Figure 1**)

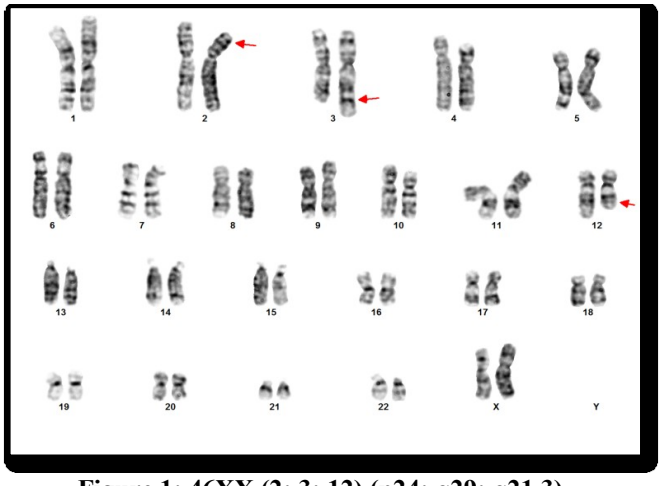

Figure 1: 46XX (2; 3; 12) (p24; q29; q21.3)

Pal *et al* reported 50% incidence of reciprocal translocation in their study, involving chromosomes 1, 4, 5, 6, 7, 10, 13, 15 and 16.

Erdal Tunc *et al* [15] report 30 cases of balanced translocation out of 62 chromosomal anomalies (48.38%). Malik Ejder *et al* [16] detected 33.3% cases of balanced reciprocal translocations. Wiem Ayed [17], Zouhair Elkarhat[18], also have almost similar reports.

Robertsonian translocation and RPL

When compared to reciprocal translocation, Robertsonian translocation is less frequent in RPL. In our study, there were 2 cases (2/125) of Robertsonian translocation; the karyotype of both were same - 45, XX, rob (13; 14) (q10; q10). It is estimated that the risk of abortion in couples with this chromosomal anomaly is approximately 25%; it is increased to 25-50% in reciprocal translocation. The phenotype of carrier of Robertsonian translocation is normal. But there is a high risk of production of unbalanced gametes which results in a zygote with a chromosomal anomaly; this leads to pregnancy loss or Down syndrome or a baby with congenital anomalies [19].

Pal *et al* have reported 2 cases of Robertsonian translocation in 172 couples, involving chromosomes 13, 14 and 21. Goncalves, Malik Ejder Yildirim, Wiem Ayed, Erdal Tuncand Neha Sudhir also report a lower frequency of Robertsonian translocations when compared to reciprocal balanced type.

Other anomalies

There was a male partner, with combined Robertsonian and reciprocal anomalies **(Figure 2)**. The karyotype was 45, XY, rob (13; 14) (q10; q10) t (4; 22) (q33; q11.2)

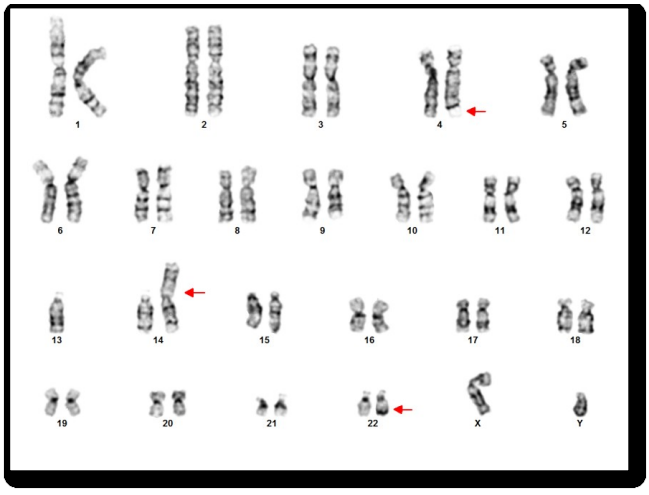

Fig 2.45, XY, rob (13; 14) (q10:q10), t (4:22) q (33:q11.2)

Inversion

The next common anomaly was inversion. There were 3 cases – 2 females and one male. In both female partners, chromosome 9 was involved whereas, chromosome 11 showed inversion in the male

Pal *et al* have reported 5 cases of inversion in 172 couples; 3 of which were in chromosome 9 and one each in chromosome 8 and Y. Pericentric inversion on chromosome 9 is considered as a normal variant. But there are studies reporting correlation between inversion 9 with subfertility, RPL and abnormal phenotype [20].

Even though structural anomalies (deletions, isochromosomes, insertions) and numerical anomalies are reported by various authors, in our study no such anomalies were detected.

Heteromorphism of chromosomes and satellite increments

Heteromorphism of long arm (q arm) of chromosomes 1,9,16 and Y is said to play a role in RPL. These are also associated with reproductive failure. It is assumed that the heterochromatin may contain genes that regulate cellular roles in reproduction [21].

In our study there were 30 karyotypes showing heteromorphic variants. The details are given in Table 3.

Table 3: Types of heteromorphism and satellite increments

Sl No.	Type	No. of cases
1	9 qh+	12
2	9qh-	4
3	1qh-	5
4	1qh+	1
5	15pstk+	2
6	14ps+	1
7	22ps+	2
8	21ps+	1
9	15ps+	2
	Total	**30 cases (24%)**

Malik Ejder Yildirim *et al* came across 19 cases (out of 300 couples) with heterochromatic changes and satellite increments.

There is a controversy in the literature as to screen for parental chromosomal anomalies after 2 or 3 previous pregnancy losses. In a meta analysis of 10 studies (n = 2498), there was no difference in the prevalence of chromosomal abnormalities in women with two versus three or more losses.[22] If an abnormal parental karyotype is found, referral to a clinical geneticist is indicated. Genetic counseling offers the couple a prognosis for future pregnancies with an unbalanced chromosome complement and the pedigree analysis. Reproductive options in couples with chromosomal rearrangements include proceeding to a further natural pregnancy with or without a prenatal diagnosis test, gametle donation and adoption. [23] For translocation carriers, counseling regarding Preimplantation Genetic Diagnosis (PGD) is warranted.

When both partners are confirmed to have normal karyotypes, we have to rule out other factors such as TORCH infections, uterine anomalies, endocrine disorders and immunological causes. In our study, one female had bicornuate uterus.

4. Conclusion

Recurrent Pregnancy Loss is a challenging problem for Obstetricians. Cytogenetic analysis is an essential investigation for couples, in whom genetic counseling and proper management can be planned accurately. Success of Assisted Reproductive Techniques (ART) can also be predicted. Some of the patients with apparently normal karyotypes may require molecular studies for the assessment of recurrent risk of miscarriages due to genetic anomalies. Despite the absence of any obvious reasons for RPL, the overall chance of pregnancy is good (more than 50%). No intervention is required in most couples.

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