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Cytogenetic Analysis of Patients with Recurrent Miscarriages

Muhammad Umar, Hamid Saeed Malik, Hira Nadeem, Babar Zaman, Noor ul Huda Alhadi, Fauzia Khan

ABSTRACT

Objective: To evaluate the cytogenetic analysis of patients with recurrent miscarriages.

Study design and setting: Cross-sectional study, Department of Hematology, Armed Forces Institute of Pathology, Rawalpindi from February 2022 to August 2022.

Methodology: 196 patients (98 couples) of recurrent miscarriages within the reproductive age group were included. Cases with known anatomical or endocrinal causes of recurrent miscarriages were excluded. Couples with abnormal reproductive tract anatomy or abnormal endocrine functions were excluded. A standardized system for human cytogenetic nomenclature was used for identifying all chromosomal aberrations. Axioscope microscopes (MetaSystems, Germany) were used for visualizing the metaphases, and MetaSystems software (MetaSystems, Germany) was used to determine the karyotype of each metaphase. Data were analyzed using the student t-test and Chi-square test. A p-value =0.05 was considered significant.

Results: Of 98 couples, most of the couples experienced 3 miscarriages. The difference in ages between males and females was significant (p-value <0.001). Chromosomal abnormalities were found in 7 (7.2%) of females and 5 (5.2%) of males. Positive family history of RPL was noted in 27 (13.8%) of the participants. A total of 12/196 (6.1%) males and females experiencing RPL had chromosomal anomalies. Out of these 1 individual (0.5%) had structural aberration, 1(0.5%) numerical abnormality, and 10 (5.1%) were found to have Chromosome Polymorphism.

Conclusions: Translocations, numerical aberrations, and chromosomal polymorphism are common cytogenetic abnormalities noted in cases with RPL. Clinicians should refer such couples for karyotyping to rule out the possible genetic causes of recurrent miscarriages.

Keywords: Chromosomes, Cytogenetics, Recurrent Pregnancy Loss

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INTRODUCTION:

One of the most common clinical complications during pregnancy is miscarriage. In nature, miscarriages usually occur at 20–28 weeks of pregnancy as a way to select genetically normal offspring. As nature's quality control, miscarriages occur spontaneously.[1] It is estimated that 15–20% of clinically recognized pregnancies end in early pregnancy loss in the first trimester.[2]

The term recurrent pregnancy loss (RPL) refers to three or more consecutive losses of pregnancies before 20 to 22 weeks of pregnancy, but in recent years, even two consecutive miscarriages have been considered RPL. In clinically recognized pregnancies, approximately 15% will result in pregnancy failure, with occult abortion being an unrecognized subset of these cases.[3]

Only 50% of RPL cases have an identifiable etiology, while the rest remain undetermined.[4] A chromosomal aberration in the embryo is thought to be responsible for 60% of RMs.[5] Several factors have been proposed as contributing factors to RPL, making it difficult to determine its exact cause. Among these factors are genetics, advanced maternal and paternal ages, luteal phase defects during pregnancy, endocrine dysfunction, autoimmunity, infectious diseases,

environmental toxins, congenital anomalies, and uterine anomalies.[6]

Statistically, 25–50% more chances for miscarriage are present when one parent carries chromosome abnormalities than when the other does.[7] In fact, 50% of spontaneously aborted infants have abnormal chromosomes.[8] The abnormalities include structural chromosomal aberrations as well as numerical chromosomal anomalies.[9] Fetal genetic defects are usually responsible for repeated pregnancy losses during the first trimester.

Chromosome anomalies are also common in late pregnancies. Approximately 30% of pregnancies lost in the second trimester of pregnancy and 5% in the third trimester are affected by chromosomal anomalies.[10]

As a result of balanced chromosomal rearrangements, unbalanced gametes can cause RPL, stillbirths, and neonates with multiple congenital anomalies. Recurrent miscarriages can be caused by balanced structural chromosome abnormalities (such as inversions and translocations) in parents.[10] Couples, who are unable to create a viable pregnancy, may feel guilty and physically exhausted because of RPL. Chromosome analysis of both parents is recommended for the management of recurrent miscarriages.[10] Researchers have reported varying frequencies of balanced chromosomal rearrangements between 2% to 8% in couples that experience RPL. The rate is much higher than the 0.2% to 0.5% observed in the general population. Chromosome rearrangements were not accurately estimated in our population of couples with RPL.[3]

Currently, cytogenetic analysis of miscarriages is an uncommon practice, and couples with RM have been negatively affected by this unfortunate omission. Unfortunately, this omission has negatively impacted the management of couples with RM. The study aims to improve the knowledge of clinicians in the region regarding cytogenetic analysis of repeated miscarriages as well as generate baseline data regarding chromosomal aberrations among RM patients.

METHODOLOGY:

This cross-sectional study was conducted at the Department of Hematology, Armed Forces Institute of Pathology Rawalpindi for a duration of six months (February 2022 to August 2022). Ethical approval was obtained from the institutional review board (IRB) vide reference number (FC-HEM21-13/READ.IRB/22/1293). After a thorough literature search, we calculated the sample size using a WHO calculator, keeping a 5% margin of error,95% confidence level, and prevalence of recurrent pregnancy loss (RPL) of 15%.[3] Sample size of 196 was calculated. Sampling was done using the nonprobability convenient sampling technique.

Inclusion criteria: Affected couple (Husband & wife) within the reproductive age group with two or more consecutive pregnancy losses.

Exclusion Criteria: Abnormal reproductive tract anatomy, abnormal endocrine functions, positive TORCH screening, and positive antiphospholipid antibody screen were excluded from the study.

Before enrolling all patients, we obtained their written consent, and the confidentiality of the patients was ensured at all levels. Detailed history and complete physical examination were done. Following standard protocols for preparing metaphase chromosomes, they were G-banded using Trypsin-Giemsa banding preparations. During this process, at least 20 metaphases were studied; however, if abnormal findings were observed, the metaphases were studied by 50. A standardized system for human cytogenetic nomenclature was used for identifying all chromosomal aberrations. Axioscope microscopes (MetaSystems, Germany) were used for visualizing the metaphases, and MetaSystems software (MetaSystems, Germany) was used to determine the karyotype of each metaphase.

Data were entered in Microsoft excel and later analyzed using Statistical Package for Social Sciences (SPSS) 21.0. A Chi-square test and a student t-test was used. A p-value =0.05 was considered significant. Calculation of mean and SD was done for quantitative variables while frequency and percentage calculation was done for qualitative variables.

RESULTS:

A total of 98 couples (196 individuals) with a history of recurrent pregnancy loss (RPL) were included in this study. Of these, the number of miscarriages ranged from 2 to 5. Most of the couples experienced 3 miscarriages. The mean age of patients was 30.16±3.23 years. The mean age of females was 27.87±1.85 years with a mean of miscarriage 3.27±0.53 while the mean age of males was 32.45±2.64 years with a mean of miscarriage 3.27±0.53. The difference in ages between males and females was found by using a student t-test which is significant (<0.001). (Table 1) Among all participants, chromosomal abnormalities were found in 7 (7.2%) of females and 5 (5.2%) of males whereas 91 females (92.8%) and 93 males (94.8%) had a normal karyotype. This study also revealed a positive family history of RPL in 27 (13.8%) of all participants. In our study, only 12/196 (6.1%) males and females experiencing RPL had chromosomal anomalies. Out of these 1 individual (0.5%) had structural aberration, 1(0.5%) numerical abnormality, and 10 (5.1%) were found to have Chromosome Polymorphism. Detailed information on the frequency of chromosomal abnormalities is provided in Table 2. The structural Chromosomal Abnormality included Robertsonian translocation detected in 1 female at Chromosome 45XX t(14;21). The only numerical anomaly was 47, XYY. Chromosome Polymorphism comprises about 10/12 (83.3%) of total chromosomal abnormality observed. The most frequent polymorphisms observed was $1qh^+$ (3 male and 4 females), followed by $9qh^+$ (1 female and 1 male) then $16qh^+$ (1 female).

DISCUSSION:

The consequence of RM is both physical and psychological. Physical issues include bleeding and infections whereas psychological consequences increase the risk of anxiety, depression, post-traumatic stress disorders, and suicidal thoughts. A study done in the year 2021 by Prof Sibohan *et al*,[11] stated that the risk of miscarriages is 15.3% (95% CI; 12.5%-18.7%) in all pregnancies. They noted a prevalence of 1.9% for two miscarriages (1.8%-2.1%) , 0.7%(0.5-0.8%) for three or more miscarriages. We also noted 1% of our cases with two miscarriages but in our study, the highest number of cases were with three miscarriages n=73, 74.5% followed by four miscarriages, n= 21 (21.6%). In a local study done in Pakistan, the mean abortions in cases with RPL was 3.40± 1.23 which is similar to our mean miscarriage of 3.27±0.53.[12] A study done by Santjohanser *et al*,[13] documented the mean age of participants as 37.3 ± 4.4 years. The mean age of patients in our study was 30.16 ± 3.23 years. In another study, the mean maternal age at the time of pregnancy loss was 34.3 years.[14]

Our study revealed a positive family history of RPL in 13.8% of n=27 cases. A positive family history was also noted by FABIO *et al*,[15] in a case-control study (13 cases versus 8 controls, RR 3.2%, CI (1.3- 8.1%). In another study done by Silvana *et al*,[16] the family reproductive data showed that there is a two to three times high risk of RPL in couples with positive family history than that in the general population. Similar results were observed by Andrea *et al*,[17] in a meta-analysis, and a systematic review was done in the year 2020. They documented that women who reported recurrent miscarriages were more likely to report a family history of miscarriage (OR 1.90,95% CI 1.37-2.63). They also documented that all the recurrent miscarriages occurred in the first trimester as noted in our study(100%, 98 couples).

In our study 92.8% females n= 91 and 94.8% males n=93 had normal karyotype. In a study done by Silvana *et al*,[16] the majority of spouses had normal karyotypes (88.5% females and 91% males). Among the remaining cases, 65% of females and 76% of males expressed constitutional chromosomal variation most frequently pericentric inversion of chromosome 9 was noted. However, in our study, the only structural abnormality noted was Robertsonian translocation in a female 45 XX t (14;21). A study done by SUDHIR *et al*,[18] noted a chromosomal aberration frequency of 3.4%.In our study chromosomal abnormality was noted in 6.1% cases (n=12/196, 5.2% males , 7.2% females). Balanced translocations formed the largest group in the study of SUDHIR et al 19 with 60% anomalies. In our study, only Robertsonian translocation was noted. 45 XX t(14;21) noted in our study are carriers and at risk of having a child with translocation Down Syndrome.[19] In another study by Kochhar *et al*,[20] chromosomal rearrangements were noted in 6.8%(54/788) cases. These chromosomal rearrangements included 5.9% reciprocal translocations, 0.7% Robertsonian translocations and 0.1% inversions.

In our study numerical abnormality in chromosomes found was 47, XYY (1, 0.5%). Chromosomal polymorphism comprised the highest chromosomal abnormality noted in our study (10/12 . 83.3%). Feng, X. *et al*,[21] in a study on the Chinese population noted that chromosomal polymorphism occurred most frequently in the RPL group as compared to the control group. This finding is similar to that ours**.** The most statistically significant chromosomal polymorphism they observed was in the acrocentric chromosome (p<0.001). We observed the most frequent polymorphism of 1qh+, followed by 9qh+ and 16qh+. They also observed statistically significant Polymorphism of 9qh+, inv (9) and Yqh+ among both groups(p=0.01).[21]

Table 1: Mean age of couples with recurrent miscarriages

Parameter	Females (%)	Males (%)	p-value
Mean age	27.87±1.848	32.45±2.640	<0.001

Table 2: Frequency of chromosomal Abnormality

Parameter		Females (%)	Males (%)	Total (%)	p-value
Numerical Chromosomal Abnormality	Yes	0 (0.00)	1 (1.00)	1 (0.5)	0.316
	No	98 (100)	97 (99.0)	195 (99.5)	
Structural Chromosomal Abnormality	Yes	1 (1.00)	0 (0.00)	1 (0.5)	0.316
	No	97 (99.0)	98 (100)	195 (99.5)	
Chromosome Polymorphism	Yes	6 (6.1)	4 (4.1)	10 (5.1)	0.516
	No	92 (93.9)	94 (95.9)	186 (94.9)	
Consanguineous Marriage	Yes	19 (19.4)	18 (18.4)	37 (18.9)	0.855
	No	79 (80.6)	80 (81.6)	159 (81.1)	
Family History of Congenital Abnormality	Yes	0 (0.00)	0 (0.00)	0 (0.00)	
	No	98 (100.0)	98 (100.0)	196 (100.0)	
Family History of Miscarriages	Yes	16 (16.3)	11 (11.2)	27 (13.8)	0.300
	No	82 (83.7)	87 (88.8)	169 (86.2)	
Time of Pregnancy Loss (trimester Wise)	First	98 (100.0)	98 (100.0)	196 (100.0)	

CONCLUSIONS

RPL is a concerning reproductive health issue that needs further research for correct treatment. Translocations, numerical aberrations, and chromosomal polymorphism are the common cytogenetic abnormalities noted in cases with RPL. Clinicians should understand the significance of cytogenetic analysis in couples with RPL and should refer them for karyotyping at least after two miscarriages to rule out the possible genetic causes of recurrent miscarriages.

Authors Contribution:
Muhammad Umar: Principal Investigator
Hamid Saeed Malik: Supervisor, Study Objective
Hira Nadeem: Proof reading, limitations
Babar Zaman: Biostatistics
Noor ul Huda Alhadi: Discussion
Fauzia Khan: Compilation of Data

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