

Cytogenetic Screening in Couples with Recurrent Pregnancy Loss: A Single-Center Study and Review of Literature

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ABSTRACT

Context: Recurrent pregnancy loss (RPL) is a devastating reproductive problem that affects more than 2% of couples who are trying to conceive. Chromosomal rearrangements in either carrier are a major cause of clinically recognized abortion. **Aims:** The purpose of this study is to report the prevalence of chromosome abnormalities in RPL and provide clinical characteristics of couples with two and more miscarriages. **Settings and Design:** Genetic counseling in laboratory of histology housed in a Faculty of Medicine of Sfax. **Materials and Methods:** Karyotype was generated from the peripheral blood lymphocyte cultures and the cytogenetic analysis was performed using R-bands after heat denaturation and Giemsa (RHG) banding. A multiplex polymerase chain reaction wherever necessary was done. **Statistical Analysis Used:** SPSS version 17. **Results:** A total of 104 couples with RPL were carried out in this study. The frequency of chromosomal rearrangements was 11.5%, three times more prevalent in men than women ($P = 0.08$). In addition, the prevalence of chromosomal anomalies increases according to the number of miscarriages (from 4.8% to 7.6%, with 2 or ≥ 3 miscarriages, respectively; $P = 0.9$). Finally, a particular familial adverse reproductive background was found in these carriers ($P = 0.03$). **Conclusions:** These data highlight that an RPL evaluation is appropriate after the second miscarriage and that cytogenetic evaluation is necessary for an accurate approach to elucidate the causes of RPL. Moreover, familial adverse reproductive backgrounds have an impact of being carrier of chromosome abnormalities and a larger study is mandatory to define reproductive characteristics of carriers.

KEYWORDS: Chromosome X, genetic counseling, pericentric inversion, reciprocal translocation, recurrent pregnancy loss

INTRODUCTION

Recurrent pregnancy loss (RPL) is classically defined as the occurrence of three or more consecutive abortions; yet, the American Society of Reproductive Medicine (ASRM) has recently redefined RPL by two or more failed pregnancies. It is a devastating reproductive problem that affects more than 2% of couples who are trying to conceive and a variety of possible etiologies have been described such as genetic, endocrinologic, immunologic, and anatomic. Often, chromosomal rearrangements in either carrier are a

major cause of clinically recognized miscarriage and studies published elsewhere have shown a prevalence of chromosomal anomalies that varies from 4% to 8% of couples who are affected by at least two pregnancy losses.^[1-3]

The presence of chromosomal rearrangements leads to unequal crossing over during meiosis which can result in gametes with unbalanced chromosomes. The

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clinical consequences of such imbalances usually are spontaneous miscarriages or early neonatal deaths.^[4]

This study was carried out to determine the frequency of chromosomal abnormalities in couples with RPL and provide clinical characteristics of carriers.

MATERIALS AND METHODS

A 2-year retrospective study (January 2007–December 2008) was carried out on couples who presented for genetic counseling because of at least two or more miscarriages. It is the first study in our laboratory, which enrolled all patients with RPL during 2 years, and hence calculation for study sample size was not done.

Couples enrolled in this study had two or more consecutive spontaneous abortions, with or without previous gestation(s). In previous reproductive history in these couples with RPL (i.e. number of miscarriages, age at the first conception, and familial infertility, repetitive miscarriages, or malformative syndrome) were recorded. Informed consent was obtained from couples.

Parental karyotyping is a part of the recommended systematic investigation of RPL; we, therefore, did not require the approval of the local institutional review board. The karyotype was performed elsewhere. It was generated from the peripheral blood lymphocyte cultures during 72 h and the cytogenetic analysis was performed according to the standard cytogenetic protocols by RHG banding. Fifteen metaphases were systematically studied, and if any mosaicism was suspected, the number of analyzed metaphases was enlarged to 50. Chromosomal abnormalities were reported in accordance with the current international standard nomenclature international system for human cytogenomic nomenclature (ISCN).^[5]

In the case of cytogenetic deletion on the long arm of chromosome Y, a molecular screening of AZFa, AZFb, and

AZFc was performed using multiplex polymerase chain reaction (PCR) method according to European guidelines.^[6]

All statistical analysis was done using Statistical Package for the Social Sciences software (Version 17) (SPSS/IBM Inc., Chicago, IL, USA). Quantitative variables were expressed as mean, median, and range, while qualitative variables were presented as a total number and proportion. Statistical significance was defined as $P \leq 0.05$.

RESULTS

This study included a total of 104 couples with a history of 2 or more pregnancy losses. The age ranged from 18 to 42 (mean = 28 years) and from 24 to 68 (mean = 33 years), respectively, for women and men. The mean number of miscarriages was 3 (range from 2 to 6).

The clinical characteristics of RPL couples with and without chromosome abnormalities are shown in Table 1.

Abnormal karyotype was detected only in 12 patients (11.5%) while 92 couples (88.5%) have a normal chromosome formula ($P = 0.00$). In the both groups of couples with RPL, the median number of miscarriages was 3 (range [2–4] and [2–6], $P = 0.25$). In addition, the number of carriers increased according to the number of miscarriage (for 2 vs. 3 or more miscarriages: 5 (4.8%) to 7 (6.7%) versus 40 (38.5%) to 52 (50%), respectively, in couples with and without chromosome abnormalities; $P = 0.9$).

For the gender, the median age at first conception was 23.5 years (20–37)–27 years (15–40) ($P = 0.69$) and 31 years (26–43)–31.5 years (22–66) ($P = 0.84$), respectively, for female and male carriers or not of chromosome abnormalities. Nine (75%) couples with abnormal karyotype have familial adverse reproductive

Table 1: Clinical characteristics of couples with recurrent pregnancy loss

	Carriers of chromosome abnormalities	Carriers without chromosome abnormalities	<i>P</i>
Number of Couples: N (%)	12 (11.5%)	92 (88.5%)	0.00*
Number of miscarriages			
Median (range)	3 (2-4)	3 [2-6]	0.25
=2 miscarriages: N (%)	5 (4.8%)	40 (38.5%)	0.9
>=3 miscarriages: N (%)	7 (6.7%)	52 (50%)	
Age at first conception			
Female: Median (range)	23.5 years (20-37)	27 years (15-40)	0.69
Male: Median (range)	31 years (26-43]	31.5 years (22-66)	0.84
Familial adverse reproductive backgrounds <i>n</i> (%)	9 (75%)	3 (25%)	0.03*

N: number of couples with RPL, * $P < 0.05$

backgrounds. However, the last was observed in only 3 (25%) couples without a chromosome abnormalities ($P = 0.03$).

Chromosome abnormalities were found in 12 cases; among those, 9 cases (75%) showed structural aberrations and 3 cases (25%) of numerical anomaly were found. Majority of the abnormalities were (balanced reciprocal translocations [BRTs]) (5 cases, i.e. 4.8%) in four men and one women ($P = 0.63$). Three pericentric inversions of chromosome 9 were determined (2.8%). One case of microdeletion on the long arm of chromosome Y, suspected on karyotype, was confirmed by multiplex PCR in a 43-year-old patient with more than 3 RPLs. Three aneuploidies of chromosome X (2.8%) were also identified: a Turner syndrome with 45,X/46, XX mosaicism, a Triple X syndrome, and a Klinefelter syndrome with 47,XXY[1]/46, XY[49]. Chromosome abnormalities were three times more prevalent in men than women ($P = 0.08$).

DISCUSSION

The aim of this study is to report the prevalence of chromosome abnormalities in RPL and provide clinical characteristics of carriers. There are very few studies in the Tunisian population focusing on karyotypic abnormalities among couples with RPL.^[7]

The overall frequency of chromosomal anomalies found in this study was 11.5%. This prevalence is as higher as reported previously in several populations.^[1,8] Studies conducted around the world indicate considerable differences in the reported chromosome aberration frequency which ranges from 4% to 12% [Figure 1]. These differences may be related to sample size or to different criteria for sample selection such as the number of miscarriages.^[2,3,9] Nevertheless, the pattern

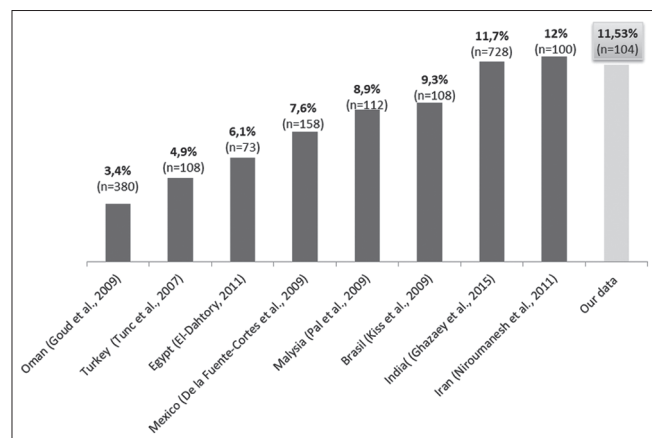


Figure 1: Review of literature among prevalence of chromosomal abnormalities in couples with recurrent pregnancy loss (n: Number of couples with recurrent pregnancy loss)

of chromosomal abnormalities is similar to that seen in previous studies.^[1,3] As has been reported in other studies, reciprocal translocations were the most frequent balanced chromosomal anomalies detected in this study.^[1,3,8] The prevalence of BRT found here is 4.8% which is near to the mode (about 3%–6%) observed in the previous studies.^[3,4,10]

In addition, we have determined more male than female carriers for reciprocal translocations ($P = 0.63$). A likely explanation is that autosomal reciprocal translocations in male carriers may have a severe effect leading to the improper spermatogenetic function and resulting in reproductive failure (ranging from pregnancy loss to infertility).^[11,12] In literature, there have been reports of reciprocal translocation carriers with varying combination of the involved chromosomes, resulting in RPL and reproductive failure.^[4,13] The size of the chromosomal segment involved and the breakpoint positions have a vital role in meiosis. Thus, molecular characterization of these chromosomal breakpoint regions could pave way for identification of genes involved in RPL, in order to elucidate the molecular mechanism underlying the aberrations. This molecular characterization could be helpful in assisted reproduction where the zygote would be checked or sperm chromosomes would be considered for further evaluation. One specific characteristic of BRTs is the absence of phenotypic manifestations in carriers, and another is the high risk to give birth to children with unbalanced chromosomal rearrangements. The estimated risk of an unbalanced fetus for a carrier couple has been reported to be between 3% and 5%.^[14]

The inversion^[9] (p12; q13) is a commonly reported inversion which was detected in three patients with RPL. Despite the fact that it is classified as a minor chromosomal rearrangement which does not correlate with abnormal phenotypes, many reports in the literature raised conflicting views regarding the chromosomal association of inversion^[9] with abnormal clinical conditions such as infertility and RPL.^[15-17]

Couples with RPL show a high incidence of X chromosome aneuploidies (3 cases; 2.8%). It has been postulated that there is an association between X chromosome aneuploidies and RPL. A loss of X chromosome copy particularly may involve a diminished ovarian reserve and many anatomical uterine anomalies.^[18] Besides, the supernumerary X chromosome, both in Klinefelter syndrome and 47, XXX, may disturb the meiotic segregation which explains the occurrence of RPL.^[19-21]

In addition, a microdeletion of chromosome Y was detected in this study and the association between such

chromosome abnormalities and RPL is under debate.^[22-25] It has been demonstrated that the microdeletion of chromosome Y may be an important hidden cause of RPL.^[23] Yet, screening for this abnormality is not recommended for the routine evaluation of RPL couples.^[24,25]

Second, with this study, we aimed at finding a subgroup with apparently high frequency of carrier status of chromosome abnormalities but until now did fail to detect such a subgroup. However, in this study, chromosome abnormalities seem to be prevalent in younger couples (the mean age was 31 years). This finding is probably caused by a demographic characteristic of RPL couples (an early marriage and younger age of carriers). In fact, advanced parental age (men > age 40 or women >35 years of age) is widely recognized as increasing the risk of spontaneous pregnancy loss and chromosomal abnormalities.^[26-28] Besides, the prevalence of chromosomal anomalies increases according to the number of miscarriages (from 4.8% to 7.6%, $P = 0.9$). In addition, focusing on reproductive backgrounds, when a history of infertility, repetitive miscarriages, or malformative syndrome is found in the family of one of the two parents, the risk of finding a structural chromosomal anomaly is significantly higher ($P = 0.03$). Such result highlights the impact of personal reproductive backgrounds on the risk of being carrier of structural abnormalities.^[29]

Finally, regardless of the small sample size of this study, these data highlight that a cytogenetic assessment of couples is appropriate after the second miscarriage.

Yet, the Practice Committee of the ASRM recommended a careful review of each pregnancy loss after the third miscarriage. Evidence based for karyotyping of couple with RPL from the second miscarriage would dramatically increase health-care costs,^[30] however, deferring testing until after the third recurrent loss does not improve the odds of discovering an abnormal factor. It would cause additional emotional distress for the patient who incurs another loss, along with increasing the age at which the couple attempts a next pregnancy. For women in their 30s, increased maternal age increases the risk of subsequent fetal loss.^[31] Thus, couples with chromosomal abnormalities should be referred to a clinical geneticist for an informed prognosis for a subsequent pregnancy. *In vitro* fertilization plus preimplantation genetic diagnosis (PGD) is an important step in the management of these couples; however, it cannot be generalized as a standard treatment for couples with RPL and major abnormalities but may be considered as an alternative treatment for a certain subset

of the couples with RPL and reciprocal translocation. Although it is difficult to differentiate cases that require PGD, reports elsewhere suggested that cytogenetic findings such as the karyotype of the past miscarriage specimens^[32] and the distribution of balanced and unbalanced karyotype in the ejaculated sperm of male carriers^[33] may help to determine whether PGD will be beneficial. It is also important to evaluate which type of translocation has a high probability for repeated miscarriage. For that purpose, further studies will be needed that include a long follow-up of the carrier couples, taking into account the type of translocation as classified by the cytogenetic characteristics, such as the number and breakpoint of the chromosome involved and the potential imbalance that will be produced through malsegregation.^[34]

CONCLUSIONS

These data highlight that cytogenetic evaluation is both appropriate after the second and necessary for an accurate approach to elucidate the causes of RPL. About clinical characteristics of carriers with history of fetal losses, a larger study can give the opportunity to define such criteria as parental age, number of miscarriage, and reproductive backgrounds.

Data available on request from the authors

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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