Obstetrics; Maternal-Fetal Medicine and Perinatology

Cytogenetic Investigation in Couples with Recurrent Abortion and Poor Obstetric History

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OBJECTIVES: The cause of recurrent early pregnancy wastage is often unknown. Cytogenetic studies have an important role in the evaluation of couples with repeated miscarriages and poor obstetric history.

STUDY DESIGN: To estimate the prevalence of chromosomal abnormalities and polymorphic variants, we performed G-banded chromosome analysis on 470 couples with 2 or more spontaneous abortions or bad obstetric history, the east of Turkey from 1998 to 2008.

RESULTS: Major chromosomal aberrations and polymorphic variants were found in 2.12% and 2.34%, respectively. Complex chromosomal rearrangements it was detected in one female patient.

CONCLUSION: Our study suggest that chromosomal abnormality incidence in patients with fetal deaths/abnormality is much higher than in the patients with first trimester or second trimester recurrent abortion. Prenatal diagnosis should be offered to couples with balanced chromosomal carrier and in vitro fertilization to couples with complex chromosomal rearrangements in the case of future pregnancies.

Key Words: Spontaneous abortion, Complexes chromosomal rearrangement, Cytogenetic

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Introduction

Chromosomal aberrations lead to reduced fertility in both men and women. Of all recognized pregnancies, about 10-15% result in miscarriage/spontaneous abortion. The majority of spontaneous abortions occur during the first trimester, and over 50% of these early miscarriages are chromosomally abnormal. Most women with a history of recurrent spontaneous abortion (RSA) will be under the care of a gynaecologist, who will have already searched for a gynaecological cause and will have excluded most serious maternal disorders. Recurrent miscarriages have a range of possible causes including genetic, anatomic, endocrine, immune, infective and thrombophilic in 60% cases. In the other 40% of cases no association with these factors could be found or unexplained causes. Maternal problems consist of uteral malformations, immunological factors, endocrine problems and so on. However, most spontaneous miscarriages are caused by chromosomal abnormalities in the embryo or fetus. Results of the numerous studies showed that approximately 50% to 80% of all pregnancy losses, depend on the maternal and gestational age at the time of loss, caused by chromosomal abnormalities. In 4-8% of couples with recurrent pregnancy loss, at least one of the partners has chromosomal abnormality that probably contains balanced chromosomal abnormalities. The most frequent is a reciprocal translocation in which a segment of a chromosome has exchanged places with a segment of another nonhomologous chromosome. In these cases, the chromosomes have difficulties pairing up and dividing during meiosis. As consequence, gametes will have unbalanced chromosomal aberrations (duplications and/or deletions). Usually, these imbalances are lethal to the developing embryo or fetus, causing spontaneous abortion. Sometimes, the pregnancy continues to term, leading to the birth of an infant with multiple congenital anomalies and mental retardation. When a parent carries a balanced chromosome rearrangement, the chance of having a live birth with an unbalanced chromosome complement is usually about 1% to 15%. The exact risk depends on the specific chromosomes involved, size of the segment involved in the rearrangement, genes contained in the segment, sex of the transmitting parent, family history, and mode of ascertainment. It is estimated that the medium risk is 12% if the translocation is present in the female and 5% if it is present in males. When one parent carries a chromosome rearrangement, the chance of spontaneous...
abortion is usually 25% to 50%. Empirical and/or hypotheti-
cal data are available for predicting the risk of adverse preg-
nancy outcome for various rearrangements.\textsuperscript{6,7,8}

Determining the causes of spontaneous abortion may re-
require extensive evaluation of both parents. The present study
tries to find the rate of chromosomal abnormalities in couples
with recurrent pregnancy loss. We present a retrospective
study of the cytogenetic data abnormalities in 470 couples
(940 patients) who were examined for RSA at the Firat
University Hospital in the 1998-2008 period.

Material and Method

This study was carried out at the Department of Medical
Biology and Genetics, Elazig, Turkey, and included 470 cou-
ples (940 patients) with recurrent spontaneous abortions, fetal
abnormality, fetal death, mentally retarded or malformed chil-
dren. Repeated abortion was defined as at least two first
trimester abortions or one spontaneous first trimester abortion
followed by a second or third trimester fetal death and/or mal-
formed child. Pedigree analysis (at least 3 generations) of all
couples having reproductive failure was performed in order to
determine the degree of consanguinity. These couples were
categorized as\textsuperscript{1} couples only with repeated spontaneous abor-
tions (RSA),\textsuperscript{2} couples with RSA preceded by stillbirth (SB),
malformed (MC) or mental retarded child (MRC), and\textsuperscript{3} cou-
ples with RSA and normal live issue/s (NC).

Peripheral blood (2 ml) was collected in heparin vacutain-
ers. For every subject whole blood (0.5 ml) cultures was set up
in 5 ml RPMI 1640 media (GIBCO BRL, USA) containing
15% fetal calf serum (Biological Industries, KBH, Israel), anti-
biotic mixture and phytohemagglutinin P (DIFCO Lab,
USA) for 72 h. Chromosome preparations were obtained from
lymphocyte cultures and analyzed after GTG-banding.\textsuperscript{9} In all
cases, at least 20 metaphases were analyzed. In cases of sus-
pected mosaicism, 50 cells were counted. The karyotypes
were interpreted using the recommendation of International
System for Human Cytogenetic Nomenclature.

Results

Of all couples, 278 (59.1\%) had two, 118 (25.10\%) had
three, 53 (11.27\%) had multiple (more than three) abortions,
and 20 (0.42\%) had fetal deaths/abnormality. The interval of
ages of patients had chromosomal abnormalities was from 18
to 42. The mean maternal age and paternal age of the entire
patient group was 33.52±4.50 (range 18-43) and 35.34±3.85
(range 21-43) years. Major chromosome abnormalities were
identified in 10 (2.12\%) and chromosomal variants in 11
(2.34\%). The gestational history in a male patient with t
(11;22) karyotype revealed a previous abortion and a normal
child. However, there was a history of multiple fetal losses in
the paternal grandmother and great-grandmother, suggesting
that the translocation may be segregating in the family for
some generations. Table 1 summarizes the number and re-
pective incidence of the different structural and numerical re-
arrangements in the 470 couples with RSA (1998-2008 pe-
riod). A female to male ratio of 1:1 for chromosome abnor-
ality ratio was observed. The chromosomal abnormalities
were robertsonian (20\%) and structural (80\%). Polymorphisms were detected in 55 (7.96\%) patients. These
polymorphisms were 1qh+, 8qh+, 9qh+, 16qh+, inv
(9)(p11;q13), 22pstk+, Yqh+ and Yqh-. The frequency of chro-
mosomal polymorphism are summarized in Table 2. Fetal
deaths/abnormality couples have high chromosomal abnor-
mality rate (20\%, 2/20).

Table 1: Specification of chromosome abnormalities found in
the study group.

<table>
<thead>
<tr>
<th>Carrier</th>
<th>Age</th>
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<tbody>
<tr>
<td>Female carrier</td>
<td></td>
</tr>
<tr>
<td>1. 46,XX, add(22)pter</td>
<td>29</td>
</tr>
<tr>
<td>2. 46,XX, del13(q21-qter)</td>
<td>22</td>
</tr>
<tr>
<td>3. 46,XX, t(13;15)</td>
<td>38</td>
</tr>
<tr>
<td>4. 46,XX,t(1;9;8;21)(q33;p22;q13;q21)</td>
<td>19</td>
</tr>
<tr>
<td>5. 46,XX,+mar</td>
<td>33</td>
</tr>
<tr>
<td>Male carrier</td>
<td></td>
</tr>
<tr>
<td>1. 46,XY, t(9,15)(q21;p?)</td>
<td>28</td>
</tr>
<tr>
<td>2.46,XY, t(14:21)</td>
<td>40</td>
</tr>
<tr>
<td>3.46,XY, t(5;4)(pter;q28), del4(q28→q28)</td>
<td>36</td>
</tr>
<tr>
<td>4.46,XY, t(11;22)(q23;q11)</td>
<td>23</td>
</tr>
<tr>
<td>5. 46,XY, inv(Y)(p11;q11)</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 2: Chromosomal polymorphisms.

<table>
<thead>
<tr>
<th>Chromosomal polymorphism</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>46,XX, 1qh(+)</td>
<td>0.1 (1)</td>
</tr>
<tr>
<td>46,XX, 8qh+</td>
<td>0.1 (1)</td>
</tr>
<tr>
<td>46,XY, inv(9)</td>
<td>1.17 (11)</td>
</tr>
<tr>
<td>46,XX, inv(9)</td>
<td>1.27 (12)</td>
</tr>
<tr>
<td>46,XY, 9qh+</td>
<td>0.1 (1)</td>
</tr>
<tr>
<td>46,XY, 16qh+</td>
<td>0.1 (1)</td>
</tr>
<tr>
<td>46,XX, 22pstk+</td>
<td>0.2 (2)</td>
</tr>
<tr>
<td>46,XY,Yqh(-)</td>
<td>1.0 (5)</td>
</tr>
<tr>
<td>46,XY, Yqh(+)</td>
<td>4.46 (21)</td>
</tr>
</tbody>
</table>

One (9\%) subject showed deletion in chromosome 13. The
deleted portion of this chromosome was present in all the
metaphases appearing as marker. Since this subject was clin-
cally normal, it was assumed that there was no loss of chro-
matin following deletions and these markers were actually the
deleted part of the chromosomes which otherwise was quite
evident from their banding pattern. One case of multiple
translocation involving chromosomes 1, 8, 9 and 21 was also
reported in a woman, have mentally retarded one children and eight abortion. The mother of the patient with complex chromosomal rearrangements (CCR) have no history of exposure to known mutagens, drugs, radiation or viral infections before or during pregnancy and parent’s karyotypes was normal.

Discussion

This study revealed that the incidence and distribution of chromosomal abnormalities among the investigated couples with repeated fetal loss is comparable to that reported worldwide. Cytogenetic examination of a large series of 470 couples with RSA led to the detection of structural chromosomal rearrangements in 2.34% of this selected population. In data from previously reported studies, the frequency of chromosome abnormalities remains low (5.7%) among stillbirths and only 0.5% of live births have chromosome abnormalities.7,8 This is much higher than the incidence of chromosomal abnormalities in the general population and may provide an explanation for the problem of RSA.

Physicians should bear in mind that in at least 5% of the couples they examine, chromosomal abnormality is the cause of abortions.10 The chromosomal aberration frequency reported in literature varies11,12 except few studies,13,14 in which higher frequencies have been reported but the number of subjects studied were less. Our results similar the three previous reports of chromosomal abnormalities in couples with recurrent pregnancy wastage which suggest that chromosomal translocations are found at a rate of from 3% to 31% (average 9.3%) in these couples.6,7,8 The incidence of chromosomal abnormality in couples with recurrent abortions reported in one cumulative study was 2.86% on an average,15 which is high than that of the present study. It has been estimated that the risk of miscarriage in couples with reciprocal translocations is approximately 25-50% whereas with Robertsonian translocation it is approximately 25%.16 We detected very high chromosomal abnormality frequencies in bad obstetric history couples (%20). Possible explanations for the divergent frequencies of chromosomal abnormality in couples with spontaneous abortion may be populational, geographical, environmental and genetic heterogeneities, methodological detection problems (for especially minor chromosomal abnormalities) and patients inclusion criteria.

It is usually a normal polymorphism; however, its clinical consequences remain unclear. The most frequent (0.95%) pericentric inversion in humans is inv9 in the general population. An association of pericentric inversions of the heterochromatic secondary constriction of chromosome 9 and reproductive failure has been suggested. The rate of pericentric inversions of chromosome 9 in our study (2.44%) is the similar that reported for the general population and for the rate observed in the control group (1.1%).17 Chromosomal inversions are not generally noted for their effects on spermatogenesis. A study on inversion of the Y chromosome in the Gujarati Muslim Indian population of South Africa failed to show any impairment in their reproductive fitness.18 Y chromosome varies in size in the normal male population, due to variability in the size of the heterochromatic portion of Yq (Yqh or Yq12). This is not associated with phenotypic abnormalities or infertility (19). Therefore, within our sample of patients, there is no apparent relationship between recurrent pregnancy wastage and inversion 9 or Yq polymorphism.

CCRs are rare chromosomal structural rearrangements characterized by three or more breakpoints located on two or more chromosomes.20 A de novo apparently balanced CCR or MCR probably has a high risk for abnormal phenotypes. Madan et al.21 reviewed 60 cases of balanced complex translocations and concluded that the chance that a de novo balanced complex translocation is associated with an abnormal phenotype increases with the number of breakpoints. More than 130 CCRs have been reported in the literature, most of them de novo and ascertained through a history of infertility or recurrent miscarriages.22 The Patsalis et al.23 study made an estimate of about 10% of hidden CCR in apparently balanced simple or complex structural rearrangements. It was observed as 10% CCR ratio in detected translocations in our studies. The the patients with CCR karyotype was very rare because of include four breakpoints. In our case the clinical examination revealed no phenotypic abnormalities or a mental retardation. CCRs in phenotypically normal persons are extremely rare. Therefore, the complex rearrangement was believed to be balanced. No further analyses (i.e. through array CGH) were performed in order to prove that the rearrangement is balanced.

There are several limitations in our study. Daniely et al. used chromosome-CGH in cases in which the karyotype found by cytogenetics was normal they detected additional numerical and structural anomalies in 8% of the abortion material from couples with recurrent abortions.24 Because of no performing comparative genomic hybridization (CGH) or spectral karyotyping (SKY) in abortion material or patients with abnormal karyotype and CCR, we no detected the cryptic imbalances of the chromosomes involved in the rearrangement, as well as alterations in the copy number of any other chromosome.

The evaluation of patients with a history of repeated spontaneous abortions requires careful consideration of potential genetic, anatomic, endocrine, infectious, and immunologic factors. Assigning proper etiological role to each of these contributing factors is often unclear. Evaluation of spontaneous abortion is important to exclude possible genetic causes. The specific information about the cytogenetic makeup of the couples and if possible of the abortus, still remains a primary focus during evaluation of such cases. Those cases have to be
detected as early as possible to arrange for adequate genetic counseling and to allow parents to make an informed reproductive decision regarding subsequent pregnancies. Therefore all the couples with balanced translocations should be strongly advised to monitor their future pregnancies by prenatal diagnosis to exclude the possibility of a chromosomally unbalanced zygote. In addition, genetic counselling in couples with CCR carrier should be different from balanced translocation carriers because of a 53.7% incidence of an abnormal pregnancy outcome to CCR carriers. This patients was advised in vitro fertilization (IVF) and genetic screening of embryos.

References


