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 rearragements 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 wiht balanced chromosomal carrier and in vitro fertilization to couples with complex chromosomal rearragements in the case of future pregnancies.

Key Words: Spontaneous abortion, Compleks chromosomal rearragement, Cytogenetic

Gynecol Obstet Reprod Med;16:1 (4-8)

Introductıon

Chromosomal aberrations leads 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.[1] 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.[2] 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.[3] However, most spontaneous miscarriages are caused by chromosomal abnormalities in the embryo or fetus.[4] 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.[5] In 4-8% of couples with recurrent pregnancy loss, at least one of the partners has chromosomal abnormality that probably contains balance chromosomal abnormalities.[3]

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

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Submitted for Publication: *14.08.2009*
Accepted for Publication: *08.12.2009*

abortion is usually 25% to 50%. Empirical and/or hypothetical data are available for predicting the risk of adverse pregnancy outcome for various rearrangements.[6,7,8]

Determining the causes of spontaneous abortion may 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 couples (940 patients) with recurrent spontaneous abortions, fetal abnormality, fetal death, mentally retarded or malformed children. 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 malformed 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[1] couples only with repeated spontaneous abortions (RSA),[2] couples with RSA preceded by stillbirth (SB), malformed(MC) or mental retarded child (MRC), and[3] couples with RSA and normal live issue/s (NC).

Peripheral blood (2 ml) was collected in heparin vacutainers. 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), antibiotic mixture and phytohemagglutinin P (DIFCO Lab, USA) for 72 h. Chromosome preparations were obtained from lymphocyte cultures and analyzed after GTG-banding.[9] In all cases, at least 20 metaphases were analyzed. In cases of suspected 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 respective incidence of the different structural and numerical rearrangements in the 470 couples with RSA (1998-2008 period). A female to male ratio of 1:1 for chromosome abnormality 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 freguency of chromosomal polymorhism are summarized in Table 2. Fetal deaths/abnormality couples have high chromosomal obnormality rate (20%, 2/20).

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

Carrier	Age
Female carrier	29
1. 46,XX, add(22)pter	32
2. 46,XX, del13(q21-qter)	22
3. 46,XX, t(13;15)	38
4. 46,XX,t(1;9;8;21)(q33;p22;q13;q21)	19
5. 46,XX,+mar	33
Male carrier	
1. 46,XY, t(9,15)(q21:p?)	28
2.46,XY, t(14;21)	40
3.46,XY, t(5;4)(pter;q28), del4(q28→q28)	36
4.46,XY, t(11;22)(q23;q11)	23
5. 46,XY, inv(Y)(p11;q11)	27

Table 2: Chromosomal polymorphisms.

Chromosomal polymorphism	Frequency (%)
46,XX, 1qh(+)	0.1 (1)
46,XX, 8qh+	0.1 (1)
46,XY, inv(9)	1.17 (11)
46,XX, inv(9)	1.27 (12)
46,XY, 9qh+	0.1 (1)
46,XY,16qh+	0.1 (1)
46,XX, 22pstk+	0.2 (2)
46,XY,Yqh(-)	1.0 (5)
46,XY, Yqh(+)	4.46 (21)
	7.96 (55)

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 clinically normal, it was assumed that there was no loss of chromatin 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 retarted 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 varies[11,12] 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 expecially 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 inv[9] 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 phenotipic 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 criptic 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.[25] This patients was advised in vitro fertilization (IVF) and genetic screening of embriyos.

Kötü Obstetrik Öykü ve Tekrarlayan Düşüklere Sahip Çiftlerde Sitogenetik İncelemeler

AMAÇ: Tekrarlayan rekürren abortusun nedeni sıklıkla bilinmemektedir. Sitogenetik çalışmalar tekrarlayan düşük ve kötü obstetrik hikayeli çiftlerin değerlendirilmesinde önemli bir role sahiptir.

GEREÇ VE YÖNTEM: Kromozomal anomalilerin ve polimorfik varyantların sıklığının belirlenmesi için, Türkiye'nin doğusunda 1998-2008 yılları arasında 2 veya daha fazla spontan abortus veya kötü obstetrik hikayeye sahip 470 çiftin G bantlamayla kromozom analizini yaptık.

BULGULAR: Major kromozomal anomaliler ve polimorfik varyantlar sırasıyla %2.12 ve %2.34 oranında bulundu. Bir dişi hastada kompleks kromozomal yeniden düzenlenme tespit edildi.

SONUÇLAR: Çalışmamız fetal anomali ve ölümün görüldüğü ailelerdeki kromozomal anomali oranının ilk veya ikinci trimester düşük hikayesi olan ailelere göre daha yüksek olduğunu göstermektedir. Gebeliğin istendiği durumlarda dengeli kromozomal taşıyıcı çiftlere prenatal tanı ve kompleks yeniden düzenlenmelere sahip ailelere in vitro fertilizasyon önerilmelidir.

Anahtar Kelimeler: Spontan abortus, Sitogenetik, Kompleks yeniden düzenlenme, Genetik danışmanlık

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