Structural Chromosomal Abnormalities in Couples with Recurrent Pregnancy Loss

Tekrarlayan Gebelik Kaybı Olan Çiftlerde Yapısal Kromozom Bozuklukları

Ayşe ÇIRAKOĞLU, MD,[a]
Şükriye YILMAZ, MD,[a]
Dilhan KURU, MD,[a]
Yelda TARKAN-ARGÜDEN, MD,[a]
Gülgün S. GÜVEN, MD,[a]
Ayhan DEVİREN, MD,[a,b]
Seyfettin ULUDAĞ, MD,[c]
Seniha HACIHANEFİOĞLU, MD,[a,b]

Departments of [a]Medical Biology,
[b]Medical Genetics,
[c]Gynecology and Obstetrics,
İstanbul University
Cerrahpaşa Medical Faculty, İstanbul

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Yazışma Adresi/*Correspondence:*
Ayse ÇIRAKOĞLU, MD
İstanbul University
Cerrahpaşa Medical Faculty,
Department of Medical Biology, İstanbul,
TÜRKİYE/TURKEY
cirakay@istanbul.edu.tr

ABSTRACT Objective: Recurrent pregnancy loss is an important problem affecting couples trying to conceive. Genetic factors, particularly chromosomal abnormalities appear to be highly associated with reproductive loss. The frequency of presence of at least one partner, who is a carrier of a structural chromosome rearrangement, varies from 3% to 11% among couples with a history of recurrent pregnancy loss. The aim of this study was to introduce the cytogenetic data of couples that referred with recurrent pregnancy loss to our center. **Material and Methods:** Chromosome analyses were performed in 449 couples with more than one pregnancy loss using GTL banding. **Results:** Chromosome abnormalities were detected in one partner in 19 of 449 couples. All chromosome abnormalities were structural, and 18 of them were balanced. Autosomal reciprocal translocations were the most frequent type (2.9%) of abnormalities. The unique Robertsonian translocation found in our study was t(13;14), which was observed in two patients. Chromosomal heteromorphisms were determined in 19.59% of patients. **Conclusion:** The frequency of chromosomal abnormalities were found as 4.23% in our series. Cytogenetic investigation of couples with recurrent pregnancy loss is necessary as chromosomal abnormalities constitute a very important part of factors that cause pregnancy loss.

Key Words: Cytogenetics; abortion, habitual; chromosomes

ÖZET Amaç: Tekrarlayan gebelik kayıpları, çocuk sahibi olmayı amaçlayan çiftleri etkileyen önemli bir sorundur. Genetik etkenler, özellikle de kromozom anomalilerinin üreme kayıplarıyla yakın ilişkisinin olduğu bilinmektedir. Tekrarlayan gebelik kaybı öyküsü olan çiftlerde, eşlerin en az birinin kromozom yapı anomalisi taşıyıcısı olma sıklığının %3-11 arasında değiştiği bildirilmektedir. Bu çalışmada, merkezimize tekrarlayan gebelik kayıpları nedeniyle başvuran çiftlerden elde edilen sitogenetik verilerin değerlendirilmesi amaçlanmıştır. **Gereç ve Yöntemler:** Birden fazla gebelik kaybı olan 449 çiftte G bantlama kullanılarak kromozom analizi gerçekleştirildi. **Bulgular:** 449 çiftin 19'unda eşlerden birinde kromozom anomalisi saptandı. Tüm kromozom anomalileri yapı anomalisi, 18'i ise dengeli olarak bulundu. En sık gözlenen kromozom anomalisi türü otozomal resiprokal translokasyonlar (%2.9) olarak bulundu. İki olguda saptanan t(13;14) bu çalışmada gözlenen tek Robertson tipi translokasyon oldu. %19.59 oranında kromozomal heteromorfizm gözlendi. **Sonuç:** Bu çalışmada, tekrarlayan gebelik kaybı öyküsü olan çiftlerde kromozom anomalilerinin sıklığı %4.23 olarak bulunmuştur. Kromozom anomalileri tekrarlayan gebelik kayıp sebeplerinin önemli bir bölümünü oluşturduğundan, tekrarlayan gebelik kayıpları olan çiftlerde sitogenetik inceleme yapılması gereklidir.

Anahtar Kelimeler: Sitogenetik; düşük, tekrarlayan; kromozomlar

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Recurrent pregnancy loss (RPL) defined as three or more spontaneous abortions before 20th week of gestation.[1] According to this definition, RPL occurs in approximately 1% of all pregnancies. This frequency increases up to 5% when RPL is defined as two or more losses of

pregnancy. There are many causes of RPL, but genetic factors, particularly chromosomal abnormalities are the most common ones. It has been reported that the frequency of chromosomal abnormalities in first trimester miscarriages is 50%-80%.[2,3] These chromosomal abnormalities may be either numerical or structural. The frequency of presence of at least one partner, who is a carrier of a structural chromosome rearrangement, varies from 3% to 11% among couples with a history of RPL.[4-16] The majority of chromosome rearrangements are balanced reciprocal and Robertsonian translocations. It is known that such abnormalities cause no phenotypic effect on the carrier but lead to increased risk to produce unbalanced gametes. Therefore, they have not only high risk to give abnormal offspring with unbalanced karyotypes, but also have increased prevalence of miscarriages.[3-17]

Here, we present a retrospective study including cytogenetic data of 449 couples (898 patients) with recurrent miscarriages.

MATERIAL AND METHODS

After investigations to exclude immunologic effects, uterine malformations and other causes of RPL, 449 couples with at least two pregnancy losses were referred to Cytogenetic Laboratory of Medical Biology Department of Cerrahpasa Medical Faculty from Department of Gynecology and Obstetrics of Cerrahpasa Medical Faculty between 1998 and 2008. Ethical Approval of The No.1 Ethical Board of Clinical Research of Istanbul was obtained for reporting the results. The mean age of the females was 29 years, while it was 33 years for the males. Chromosomes were obtained from peripheral blood cultures and analyzed by G-banding with Trypsin-Leishman using standard techniques. At least 20 metaphases were analyzed for each patient. If any mosaisizm was suspected, at least 50 metaphases were counted. Additional banding techniques like C-banding and nucleolus organizer region (NOR), and fluorescence in situ hybridization (FISH) were used for characterizing chromosomal rearrangements when a chromosomal rearrangement was detected. Chromosomal abnormalities were designated and described according to the International System for Human Cytogenetic Nomenclature (ISCN) 2005.[18]

RESULTS

As summarized in Table 1, chromosomal abnormalities were detected in 19 of a total of 449 couples. All chromosomal abnormalities were structural, and 18 of them were balanced. Reciprocal translocations were shown in 13 subjects (six females and seven males). Robertsonian translocations were another group of balanced rearrangements which were detected in our study. The same der(13;14) Robertsoinan translocation was observed in two cases (one female and one male).

Inversions, which were found in three males, were paracentric in two patients and pericentric in one patient. Paracentric inversions were inv(13)(q12q34) and inv(14)(q12q32). Pericentric inversion was inv(7)(p15q22). All inversions were in mosaic conditions. Supernumerary marker chromosome was observed in one case (male), the only subject who had an unbalanced karyotype. Multi-

TABLE 1: Chromosomal abnormalities in our cases.

Balanced rearrangements			
Reciprocal translocations			
Female carriers	**Age**	**Male carriers**	**Age**
46,XX,t(1;6)(q13;q22)	24	46,XY,t(2;4)(p21;q35)	27
46,XX,t(2;10)(p13;q22)	27	46,XY,t(2;6)(q21;q27)	25
46,XX,t(2;18)(p25;q21)	30	46,XY,t(8;11)(p11;q25)	29
46,XX,t(4;10)(p16;q11)	28	46,XY,t(8;14)(q22;q13)	29
46,XX,t(4;15)(q31;q15)	35	46,XY,t(10;16)(q26;q21)	29
46,XX,t(10;11)(q21;q14)	35	46,XY,t(4;6)(q21;q13)	38
		46,XY,t(14;18)(q11.2;q21)	31
Robertsonian translocations			
Female carriers		**Male carriers**	
45,XX,der(13;14)(q10;q10)	28	45,XY,der(13;14)(q10;q10)	25
Inversions			
No female carrier		**Male carriers**	
		mos46,XY,inv(7)(p15q22)/46,XY	33
		mos46,XY,inv(13)(q12q34)/46,XY	27
		mos46,XY,inv(14)(q12q32)/46,XY	29
Unbalanced rearrangements			
No female carrier		**Male carriers**	
		47,X,der(X)(:p11→q13:),Y	28

probe centromeric FISH was used to identify the origin of supernumerary marker chromosome. It was identified that the marker chromosome contained X centromere, and final karyotype was formulated as 47,X,der(X)(:p11→q13:),Y.

The couples who had chromosomal abnormalities had no live births in our series. Of 19 carriers of chromosomal abnormalities, seven were females and 12 were males.

At least one chromosomal polymorphism was shown in 77 of 398 couples. In 11 of these 77 couples (88 patients in total) both spouses had chromosomal polymorphisms. Pericentric inversion 9, which has been considered as a population variant, was shown in 10 cases. 9qh+ was the most common chromosomal variant that was found in 31 cases. Yqh+ and 16qh+ were observed in 16 cases, while 1qh+ was found in 15 cases (Table 2).

DISCUSSION

It was reported that the incidence of couples, who have RPL, varied between 2.7 and 11% in previous studies.[6-16] The frequency of chromosomal abnormalities in our series was 4.23% (19 of 449 couples) which was in concordance with literature. Autosomal reciprocal translocations have been proposed as the most common chromosomal changes in couples who have RPL.[11,13,17] In the same way, reciprocal translocations were the most common abnormalities (2.9%) in our series as reported in literature, and all the chromosomes that involved in these reciprocal translocations were found in autosomes.

Although reciprocal translocations are balanced rearrangements, they are important for the offspring of carriers that have increased risk of chromosomal imbalance during gametogenesis due to unequal meiotic segregation. When one of the parents is a carrier of a balanced reciprocal translocation, a pregnancy can result in three types of offspring: a child with a normal karyotype, a child with a balanced reciprocal translocation, or a conceptus with an unbalanced karyotype that may lead spontaneous miscarriage or live-born child with malformations and mental retardation.[17] Since cytogenetic findings do not only lead to RPL, but also increase frequency of bearing malformed child, genetic counseling for subsequent pregnancies of couples who have balanced translocation is important.

The distribution of breakpoints in translocations in our series revealed that one of the breakpoints (18q21) involved twice in different translocations [t(2;18)(p25;q21) and t(14;18)(q11.2;q21)], while the other breakpoints occurred only once. The present data on translocation breakpoints in carriers of autosomal reciprocal translocations and RPL do not indicate that specific breakpoints are preferentially involved in these translocations.[11]

Robertsonian translocations are another type of abnormalities that have been reported to be frequently seen in couples with RPL. D/D translocations are very common in general population with a frequency of about 1/1000 newborns.[11] We found the same Robertsonian translocation, t(13;14), in two cases.

Inversions are another group of chromosomal abnormalities which have been frequently reported in couples with RPL. It is known that paracentric inversion carriers have risk to produce unbalanced gametes with a dicentric chromosome and an acentric fragment due to homolog pairing in meiosis occuring through the formation of an inversion loop between the normally structured and inverted loop. Because of these chromosomally unbalanced gametes, the incidence of fetal loss increases in paracentric inversion carriers. Pericentric inversion carriers also have increased risk for pregnancy loss. The consequences of crossing over in a pericentric inversion loop may be deletion or dup-

TABLE 2: Chromosome variants in our series.

Chromosome variant	inv(9)(p11q13)	1qh+	9qh+	16qh+	Yqh+	Total
No. of cases (%)	10 (2.23 %)	15 (3.34%)	31 (6.9%)	16 (3.56%)	16 (3.56%)	88 (19.59%)

lication of a chromosome segment. The size of loss or gain of genetic material depends on the length of the inversion segment.[19,20] Inversions were found in three cases in our study, one of them was pericentric and two of them were paracentric.

The most frequent (1-2.8%) pericentric inversion in humans which has been considered as a population variant is inv(9)(p11q13).[21] However, there are several studies reporting an association of inv(9) with sub-fertility, recurrent abortions and abnormal phenotypes.[21] Ten subjects in our study had inv(9)(p11q13) (2.25%).

In conclusion, we found that cytogenetic investigation of couples with RPL revealed an incidence of structural chromosomal rearrangements of 4.23% in our series. As cytogenetic abnormalities constitute a very important portion of the causes of fetal loss, we suggest that it is necessary to perform cytogenetic investigation for couples who have recurrent miscarriages and fetal losses.

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