Is Recurrent Abortion an Indication for Subtelomeric Region Analysis?

Tekrarlayan Gebelik Kayıpları Subtelomerik Bölge Analizi İçin Bir Endikasyon mudur?

Beyhan DURAK,[a]
Mesut YEŞİL,[a]
Muhsin ÖZDEMİR,[a]
Oğuz ÇİLİNGİR, MD,[a]
Turgay ŞENER, MD,[b]
Güney BADEMCİ, MD,[a]
M. Hamza MÜSLÜMANOĞLU, MD,[a]
Sevilhan ARTAN[a]

Departments of
[a]Medical Genetics,
[b]Obstetrics and Gynecology,
Eskişehir Osmangazi University
Faculty of Medicine, Eskişehir

Geliş Tarihi/*Received:* 06.05.2009
Kabul Tarihi/*Accepted:* 18.10.2009

Yazışma Adresi/*Correspondence:*
Beyhan DURAK
Eskişehir Osmangazi University
Faculty of Medicine,
Department of Medical Genetics,
Eskişehir
TÜRKİYE/TURKEY
bdurak@ogu.edu.tr

ABSTRACT Objective: Invertion and balanced chromosome translocations are most frequently seen chromosomal rearrangements in couples with recurrent reproductive failure. About 5% of the couples with recurrent losses have chromosomal structural rearrangements. Balanced cryptic chromosomal rearrangements that can be missed by conventional cytogenetic karyotyping could be responsible for recurrent abortions in couples with apparently normal karyotypes. The aim of this prospective study was to determine the frequency of balanced subtelomeric chromosomal rearrangements by chromosomal subtelomeric region screening in karyotypically normal couples with idiopathic recurrent spontaneous abortions. **Material and Methods:** The study was performed on 70 couples proven to be clinically normal who had three or more spontaneous abortions and whose karyotypes were found to be normal. Hybridization and analysis of the telomere regions of the all autosomal and gonosomal chromosomes were performed by fluorescence in situ hybridization technique with the usage of the specific probes of the subtelomeric regions. **Results:** No chromosomal rearrangements including subtelomeric translocations could be identified in 140 cases. Besides, no polymorphic increased and/or decreased signals were seen in the analysis of 1400 metaphases. **Conclusion:** Balanced cryptic chromosomal rearrangements can cause balanced reciprocal translocations in the couples with recurrent spontaneous abortions. Subtelomeric cryptic rearrangements of these couples were screened in this study and no relations were found between the subtelomeric rearrangements and the multiple miscarriages, concordant with the reports in the literature. However, having not only multiple miscarriages but also infants with mental and physical abnormalities may an important indication for subtelomeric region analysis.

Key Words: In situ hybridization, fluorescence; abortion, spontaneous; telomere

ÖZET Amaç: Kötü obstetrik öyküye sahip olan çiftlerde inversiyon ve dengeli translokasyonlar en sık rastlanan kromozom yeniden düzenlenmeleridir. Tekrarlayan kendiliğinden düşüğü olan çiftlerin % 5 kadarının bir yapısal kromozom düzensizliği taşıyıcısı olduğu bilinmektedir. Normal kromozom yapısına sahip olan çiftlerde, konvansiyonel karyotipleme ile saptanamayan dengeli kriptik kromozomal yeniden düzenlenmeler, tekrarlayan abortuslara neden olabilir. Bu çalışmada, tekrarlayan ve sebebi açıklanamayan düşükleri nedeniyle kliniğe başvuran, normal karyotipe sahip çiftlerde kriptik subtelomerik bölgelerin taraması yapılarak bu bölgelere ilişkin anomali sıklığının belirlenmesi amaçlanmıştır. **Gereç ve Yöntemler:** Çalışma klinik olarak normal olduğu doğrulanmış, üç veya daha fazla spontan abortus yaşamış ve karyotipleri normal 70 çift üzerinde yapıldı. Tüm otozomal ve gonozomal kromozomların telomer bölgeleri, floresan in situ hibridizasyon tekniği kullanılarak subtelomerik bölgelere özgü problar ile hibridize edilerek analiz edildi. **Bulgular:** Çalışma kapsamındaki 140 hastada, translokasyonlar dahil olmak üzere, telomer bölgelerinde herhangi bir yeniden düzenlenim tesbit edilemedi. Bunun yanı sıra, analiz edilen 1400 metafazda, herhangi bir kromozomun telomer bölgesine ait artmış ve/veya azalmış polimorfik sinyal görülmedi. **Sonuç:** Tekrarlayan gebelik kayıpları olan çiftlerde görülen dengeli translokasyonlardan, dengeli kriptik kromozomal yeniden düzenlenmeler sorumlu olabilir. Bu nedenle, bu çalışmada telomer bölgelerindeki kriptik kromozomal değişimler araştırılmış ve literatür ile uyumlu olarak tekrarlayan kendiliğinden düşükler ile subtelomerik değişimler arasında ilişki bulunamamıştır. Bununla birlikte, çalışma grubundaki hastaların, sadece tekrarlayan kendiliğinden düşüklere değil aynı zamanda mental ve fiziksel anomalileri olan bebeklere sahip olması, subtelomerik bölge analizi için önemli bir edikasyon olabilecektir.

Anahtar Kelimeler: İn situ hibridizasyon, flöresans; düşük, kendiliğinden; telomer

Turkiye Klinikleri J Med Sci 2010;30(5):1465-8

Spontaneous abortion (SAB) is the most common complication of human gestation. Recurrent SAB, defined as three or more consecutive abortions, occurs in approximately 1% to 2% of couples and about 50% of cases remain idiopatic. Although the etiology of early pregnancy loss varies, chromosome abnormalities are known to be the most common cause of SABs. At least 50% of all first-trimester and 10-15% of all second-trimester SABs have chromosome aberrations.[1-7] The most frequently seen abnormalities in conceptuses are monosomy X, autosomal trisomies, polyploidy, and structural rearrangements.[1] The majority of these abortions occur in chromosomally normal parents but balanced structural chromosomal abnormalities are found in about 2-7% of parents with recurrent reproductive failures. Individuals with balanced chromosome rearrangements are at an icreased risk of producing an unbalanced genetic material during gametogenesis. Balanced reciprocal and Robertsonian translocations are the most commonly seen parental karyotypes in these cases. Because of abnormal segregation, the gametes have imbalanced amount of chromosomal materials. These imbalances are fatal to the embriyo/fetus and cause spontaneous abortions. Therefore if there is as history of recurrent SAB, parental karyotypes must be investigated for the presence of a balanced chromosome abnormality.[8-10]

The subtelomeric regions are gene-rich, transcriptionally active regions. The frequency of genetic recombination increases towards the telomeres. Owing to the repeat motifs found in these areas, they may be prone to rearrangements. Unbalanced chromosomal rearrangements involving telomeres have been reported as an important cause of idiopathic mental retardation and congenital malformations.[8-17]

Conventional cytogenetic analysis at a 400-550 band resolution is used for the diagnosis of chromosomal rearrangements larger than 4-5 megabases. High resolution karyotyping has not been used even though it may be more efficient for improving the diagnosis of rearrangements 2-4 megabases in size, since it is time consuming and does not guarantee the diagnosis of undetectable chromosomal rearrangements. Moreover, the telomeric regions of the chromosomes are usually G-band negative and therefore small translocations with other chromosomal telomeric regions may be almost impossible to discriminate by using the conventional banding techniques.[8-15] With the advent of chromosome-specific telomeric fluorescence in situ hybridization (FISH) probes, it is now possible to identify submicroscopic rearrangements of distal ends of the chromosomes that cannot be detected by conventional cytogenetic methods.[1]

The aim of this prospective study was to screen cryptic chromosomal abnormalities in 140 patients (70 couples) referred to our department for recurrent unexplained miscarriages.

MATERIAL AND METHODS

PATIENTS

This prospective study was performed on 70 clinically normal couples who had had three or more spontaneous abortions before 20 weeks of gestation. The frequent causes of abortions including maternal diseases and hormonal and infectious factors were previously excluded in these patients. Only the couples who had an apparent normal karyotype were included into the study. The couples included into the study had a history of three or more abortions and did not have any children. Consanguinity was not seen in couples and there were no special issues in Their pedigrees. The patient consent forms including details of the study were signed by the patients. The study protocol was approved by the Ethics Committee of the University depending on the provisions of the Helsinki Declaration.

FISH ANALYSIS

Specific probes for chromosomal telomeric regions (ToTel Vysion Multicolor FISH Probe Panel Vysis 33-270000) were used for FISH experiments following the recommended manufacturer's protocol with minor modifications. The slides were treated with 100 %, 70 %, 50% and 30% alcohol series and then washed in 0.1xSSC (standard saline citrate) solution for 1 min. Then the slides were denaturated in 2xSSC solution at 70°C for 30min and 0.07 M Na-

OH at room temperature for 1 min. Following denaturation, the slides were washed in 1xSSC and 2xSSC solutions at +4ºC for 1 min and dehydrated in alcohol series (30%-50%-70%-100%). After overnight hybridization with the probe panels, each mix of probes was evaluated for a minimum of 10 metaphases by using Zeiss Axiophot Fluoresence microscope and PSI Applied Imaging software.

RESULTS

Out of 70 couples, 12 had three, 25 had four, 21 had five, four had six, two had seven, three had eight and three had nine SABs. The couples had only recurrent SABs. There was no mentally and/or physically retarded child in their reproductive history. The couples did not have living children. The maternal age was between 20 and 35 years whereas paternal age interval was between 22 and 42 years.

Subtelomeric FISH analysis was performed in a total of 140 patients. Hybridization success for all subtelomeric "probe panels" was 100%. No abnormalities could be found among couples with recurrent SABs. All fluoresence spots of subtelomeric regions were normal and no polymorphic increased and/or decreased signals were seen in 1400 metaphases analysed.

DISCUSSION

In the present study, no subtelomeric region rearrangement was found in a systematic screening of 70 couples with recurrent first-trimester miscarriages. The results of the previous studies and the results of the present study are given in Table 1. As seen in the table, the present study shows the results of a larger population and only four cases with subtelomeric region rearrangements have been reported in a total of 524 cases. Therefore, the relative incidence of cryptic rearrangements seen in couples with recurrent miscarriages is in the ranges from 0% to 3%.[5,15,18-21]

In the study by Cockwell et al., the frequency of subtelomeric region rearrangements was reported as 1% whereas no subtelomeric region abnormality was detected in the studies of Fan et al., Benzacken et al. and Jalal et al.[18-21] However, a significantly high incidence rate (20%) has been reported by Yakut et al. It was a great chance to diagnose subtelomeric region rearrangements in two of the five couples analysed.[15] Moreover, subtelomeric FISH-detected abnormality incidence might have been higher if the aberrations that can be diagnosed by the conventional chromosome analysis had been included into the study. In that study, it is not clear whether the aberrations were visible through microscopic analysis. The selection criteria of the patients are also important for determination of the incidence of subtelomeric region imbalances in couples with SABs.

TABLE 1: FISH studies on subtelomeric rearrangements in different series of patients with recurrent miscarriages.

	Number of Patients	Normal	Detected Cryptic Subtelomeric Abnormalities
Present Study	140	140	0
Monfort (2006)5	36	35	1
Cockwell (2003)21	100	99	1
Yakut (2002)15	10	8	2
Fan (2002)18	80	80	0
Benzacken(2002)20	114	114	0
Jalal(2001)19	44	44	0
Total	524	520	4

The results of the present study supports the conclusion of Monfort et al. who reported that subtelomeric translocations were infrequently related with multiple miscarriages. The subtelomeric region analysis by the FISH technique might not be an indication in the couples with multiple pregnancy losses. The technique is expensive, time consuming and needs huge labor.[5]

Although subtelomeric translocations are not frequently seen in couples with multiple fetal losses, these balanced rearrangements are seen in high percentages among couples with fetal losses and infants with mental retardation (MR) and/or congenital anomalies (CA)[12]. The couples with recurrent SABs and infants with MR/CA are not included into the study although maternal or paternal balanced cryptic subtelomeric region translocations have been di-

agnosed in couples with not only recurrent SABs but also infants with MR and/or CA (unpublished data).

In conclusion, subtelomeric region translocations are rarely seen in couples with multiple miscarriages. However, the subtelomeric region analysis is becoming very valuable if a couple has had not only abortions, but also an offspring with mental and physical abnormalities.

Acknowledgments

This work was supported by Eskisehir Osmangazi University Reserach Foundation (Project No. 2004110006 Date: 20.02.2004 B.30.2.OGÜ.0.05.02.00/119). All authors contributed significantly into the study, in examination of the patients, laboratory experiments, analysis of subtelomeric regions, discussion of the results and writing the manuscript.

REFERENCES

1. Gardner RJM, Sutherland GR. Reproductive failure. Chromosome Abnormalities and Genetic Counselling. 3rd ed. New York: Oxford University Press; 2004. p.339-49.
2. Dejmek J, Vojtassák J, Malová J. Cytogenetic analysis of 1508 spontaneous abortions originating from south Slovakia. Eur J Obstet Gynecol Reprod Biol 1992;46(2-3):129-36.
3. Gardó S, Bajnóczky K. Cytogenetic analysis of spontaneous abortions with direct analysis of chorionic villi. Eur J Obstet Gynecol Reprod Biol 1992;47(2):117-20.
4. Goddijn M, Leschot NJ. Genetic aspects of miscarriage. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14(5):855-65.
5. Monfort S, Martínez F, Roselló M, Badia L, Prieto F, Orellana C. A subtelomeric translocation apparently implied in multiple abortions. J Assist Reprod Genet 2006;23(2):97-101.
6. Rodriguez-Revenga L, Badenas C, Sánchez A, Mallolas J, Carrió A, Pedrinaci S, et al. Cryptic chromosomal rearrangement screening in 30 patients with mental retardation and dysmorphic features. Clin Genet 2004;65(1): 17-23.
7. Stirrat GM . Recurrent miscarriage. Lancet 1990;336(8716):673-5.
8. Kilby MD, Brackley KJ, Walters JJ, Morton J, Roberts E, Davison EV. First-trimester prenatal diagnosis of a familial subtelomeric translocation. Ultrasound Obstet Gynecol 2001;17 (6):531-3.
9. Knight SJ, Flint J. Perfect ending: a review of subtelomeric probes and their clinical diagnosis. J Med Genet 2000;37(6):401-9.
10. Knight SJ, Horsley SW, Regan R, Lawrie NM, Maher EJ, Cardy DLN, et al. Development and clinical application of an innovative fluorescence in situ hybridization technique which detects submicroscopic rearrangements involving telomeres. Eur J Hum Genet 1997;5(1):1-8.
11. Davies AF, Kirby TL, Docherty Z, Ogilvie CM. Characterization of terminal chromosome anomalies using multisubtelomere FISH. Am J Med Genet A 2003;120A(4):483-9.
12. de Vries BB, White SM, Knight SJ, Regan R, Homfray T, Young ID, et al. Clinical studies on submicroscopic subtelomeric rearrangements: a checklist. J Med Genet 2001;38(3):145-50.
13. Knight SJ, Lese CM, Precht KS, Kuc J, Ning Y, Lucas S, et al. An optimized set of human telomere clones for studying telomere integrity and architecture. Am J Hum Genet 2000;67(2):320-32.
14. Patsalis PC, Evangelidou P, Charalambous S, Sismani C. Fluorescence in situ hybridization characterization of apparently balanced translocation reveals cryptic complex chromosomal rearrangements with unexpected level of complexity. Eur J Hum Genet 2004;12(8): 647-53.
15. Yakut S, Berker-Karaüzüm S, Simşek M, Zorlu G, Trak B, Lüleci G. Telomere-specific fluorescence in situ hybridization analysis of couples with five or more recurrent miscarriages. Clin Genet 2002;61(1):26-31.
16. De Vries BB, Winter R, Schinzel A, van Ravenswaaij-Arts C. Telomeres: a diagnosis at the end of the chromosomes. J Med Genet 2003;40(6):385-98.
17. Knight SJ, Regan R, Nicod A, Horsley SW, Kearney L, Homfray T, et al. Subtle chromosomal rearrangements in children with unexplained mental retardation. Lancet 1999;354 (9191):1676-81.
18. Fan YS, Zhang Y. Subtelomeric translocations are not a frequent cause of recurrent miscarriages. Am J Med Genet 2002;109(2):154.
19. Jalal SM, Harwood AR, Sekhon GS, Pham Lorentz C, Ketterling RP, Babovic-Vuksanovic D, et al. Utility of subtelomeric fluorescent DNA probes for detection of chromosome anomalies in 425 patients. Genet Med 2003;5(1):28-34.
20. Benzacken B, Carbillon L, Dupont C, Siffroi JP, Monier-Gavelle F, Bucourt M, et al. Lack of submicroscopic rearrangements involving telomeres in reproductive failures. Hum Reprod 2002;17(5):1154-7.
21. Cockwell AE, Jacobs PA, Beal SJ, Crolla JA. A study of cryptic terminal chromosome rearrangements in recurrent miscarriage couples detects unsuspected acrocentric pericentromeric abnormalities. Hum Genet 2003;112(3): 298-302.