Parental Karyotype and Genetic Markers for Thrombophilia in Recurrent Miscarriage

Çetin **SAATÇİ**[1], Gökalp **ÖNER**[2], Sener **TAŞDEMİR**[1], Aslıhan **KİRAZ**[1], Yusuf **ÖZKUL**[1], Munis **DÜNDAR**[1], İptisam İpek **MÜDERRİS**[2]

[1]*Department of Genetics, Faculty of Medicine, Erciyes University, Kayseri*
[2]*Department of Obstetrics and Gynecology, Faculty of Medicine, Erciyes University, Kayseri*

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Abstract

Objective: In the present study, our aim has been to examine the frequency of parental chromosomal aberrations and factor V Leiden (FVL), methylenetetrahydrofolate reductase (MTHFR), and prothrombin gene (FII) mutations as markers for thrombophilia in cases of recurrent miscarriage (RM).

Materials and Methods: A total of 120 patients presented to our service with RM who were diagnosed as cases of three or more consecutive spontaneous miscarriages before 24 weeks of gestation between 2005-2007. During the study period the women were not pregnant. Both partners were karyotyped as part of the primary investigation and parental chromosomal aberrations were investigated. Furthermore common inherited thrombophilia markers were studied. Genotype analysis for FVL, MTHFR, and FII mutations were assessed by polymerase chain reaction analysis.

Results: Chromosomal aberrations were found in 16 couples (13.3%). Maternal and paternal inversions were more commonly seen chromosomal abnormalities (56.25%). In 65 (54.2%) patients at least one thrombophilia marker was detected, whereas 55 (45.8%) patients had no thrombophilia. There were no significant differences in the prevalence of RM between patients with parental karyotypic aberrations and those with no aberrations (p=0.75). Similarly there were no significant differences between patients with mutation of genetic markers of thrombophilia and those with no mutations (p=0.67). Mean age of the patients increased with the number of RM (p<0.001), and chromosomal aberrations significantly increased with mutation of the genetic markers for thrombophilia (p=0.015).

Discussion: In this study, the frequency, relations, and type of chromosomal abnormalities and mutations of genetic markers of thrombophilia in parents with RM were presented.

Keywords: pregnancy, recurrent miscarriages, genetic markers

Özet

Tekrarlayan Gebelik Kayıplarında Trombofili İçin Genetik Belirteçler ve Parental Karyotipleme

Amaç: Bu çalışmada amacımız, tekrarlayan gebelik kayıplarında (TGK) parental kromozomal aberasyonları ve trombofili nedenleri arasında olan faktör V Leiden (FVL), metilentetrahidrofolat redüktaz (MTHFR) ve protrombin gen (FII) mutasyonu sıklığını değerlendirmektir.

Materyal ve Metot: 2005-2007 yılları arasında servisimize başvuran 24. gebelik haftasından önce arka arkaya gerçekleşen üç ve üzerinde spontan düşük olarak tarif edilen TGK olan toplam 120 hastayı inceledik. Çalışma sırasında kadınlar gebe değildi. İlk araştırmada, tüm partnerlere karyotipleme yapıldı ve parental kromozomal aberasyonlar araştırıldı. Ayrıca, sıklıkla görülen kalıtsal trombofili belirteçleri araştırıldı. FVL, MTHFR ve FII mutasyonların genotipik analizleri polimeraz zincir reaksiyonu ile değerlendirildi.

Sonuçlar: On altı çiftte (%13.3) kromozomal aberasyon bulundu. Maternal ve paternal inversiyon en sık rastlanan kromozom anomalisi idi (%56.25). Altmış beş (%54.2) hastada en az bir trombofili belirteci izlenirken, 55 (%45.8) kişide trombofili belirteçleri negatifti. Karyotip aberasyon olan ve olmayan hastalar arasında tekrarlayan gebelik kaybı prevalansında istatistiksel olarak anlamlı fark bulunamadı (p=0.75). Aynı şekilde trombofili genetik belirteç mutasyonu olan ve olmayan

Corresponding Author: Dr. Gökalp Öner
Erciyes Üniversitesi Tıp Fakültesi, 38039 Talas, Kayseri, Türkiye
Phone : +90 352 437 49 37
GSM : +90 506 234 68 22
E-mail : dr.gokalpw85@mynet.com

hastalar arasında istatistiksel olarak anlamlı fark bulunamadı (p=0.67). Ortalama hasta yaşı TGK sayısıyla artmaktadır (p<0.001) ve kromozomal aberasyonlar trombofili için genetik belirteç mutasyonuyla anlamlı olarak artmaktadır (p=0.015). **Tartışma:** Bu çalışmada, tekrarlayan gebelik kaybı olan hastalardaki kromozomal anomalilerin ve trombofilinin genetik belirteçlerinin sıklığı, aralarındaki ilişkileri ve çeşitleri gösterildi.

Anahtar sözcükler: gebelik, tekrarlayan gebelik kaybı, genetik belirteçler

Introduction

Recurrent miscarriage (RM) is usually defined as the loss of three or more consecutive pregnancies before 24 weeks of gestation (1,2). Within this definition is a large and heterogeneous group of patients with many different causes of miscarriage. Recurrent spontaneous abortion is a condition that occurs with 2-5% of couples. Approximately 10-15% of all clinical pregnancies end in miscarriage and most of these (80%) before 13 weeks' gestation (3). Chromosomal abnormalities, uterine malformations, and antiphospholipid syndrome are the three generally accepted etiologies of RM. Currently evidence is insufficient to support routine evaluation of other causes, including hormonal abnormalities, infection, systemic disease, environmental agents, and alloimmune factors (4). Up to 60% of sporadic miscarriages (5-7) and between 29% and 60% of RMs might be caused by chromosomal aberrations in the embryo (8-10). It is generally assumed that recurrent miscarriage might be due to recurrent chromosomal anomalies in the fetus due to a balanced aberration in one of the parents being inherited by the offspring in an unbalanced form. The parental chromosomal aberration might be either a structural anomaly, such as reciprocal or Robertsonian translocations, or mosaicism for numeric aberrations. Additionally pregnancy complications including idiopathic fetal loss are thought to result from placental under-perfusion due to occlusive events, including thrombosis of placental vessels. Thrombosis of placental vessels is multicausal in nature and may involve both acquired and inherited risk factors (11-12), leading to recurrent fetal loss. It has been reported that genetic tendencies to thrombosis may also be associated with recurrent pregnancy loss (13). The three most common genetic markers for thrombophilia (GMT) which are known to predispose to venous thrombosis are; factor V Leiden (FVL), methylenetetrahydrofolate reductase mutation (MTHFR, C677T) (14,15) and prothrombin gene mutation (FII, G20210) (16).

In this study, we investigated the frequency and type of chromosomal abnormalities and MTHFR, FVL, and FII gene mutations in the parents in our cohort of patients with recurrent spontaneous abortion.

Materials and Methods

A total of 120 patients presented to our service with RM between 2005-2007. Recurrent pregnancy loss was diagnosed as three or more consecutive spontaneous miscarriages before 24 weeks of gestation. Losses after 24 weeks are considered as stillbirths or intrauterine fetal death. Both partners were karyotyped as part of the primary investigation. All participating women were healthy, and exclusion criteria included induced abortions, a systemic disease (diabetes, thyroid disease, and lupus), uterine structural abnormalities, pregnancy at the time of investigation, and previous history of thrombosis. Before inclusion in the study, objective evidence of past pregnancies was required, such as a positive hCG test, histology of fetal or placental tissue, or ultrasound-based reports of a pregnancy sac. Chromosome studies were performed on routinely cultured peripheral blood lymphocytes. Colchicine was added 2 hours before the cytological preparation. Slides were processed for G banding with Trypsin-Giemsa by standard techniques. A minimum of 20 cells were microscopically analyzed at metaphase from two independent cultures prepared for each individual.

DNA was extracted from EDTA-anticoagulant blood samples using standard methods (17). FVL was detected by PCR amplification of a 223 bp fragment and MnLI digestion, as previously described (18). The C677T substitution in the MTHFR gene was identified using HinfI cleavage of a 198 bp PCR-amplified product (19). For identification of the G20210A substitution in the factor II gene, a slight modification of a published method was used (16). A 253 bp fragment of the 3' untranslated region of the gene was amplified by PCR using the same primers as described and digested simultaneously with HindIII and MspI. The A20210 and G20210 alleles were discernible by this procedure since the A20210 allele bears a restriction site for both enzymes, whereas the G20210 allele bears restriction site only for MspI.

All patients with parental aberrations were offered genetic counselling. The clinical features of each patient and her miscarriages were recorded, with particular attention being paid to whether the previous miscarriages occurred in the first, second and third trimester. The details of each individual were entered into a computerised database with statistical software (Statistical Package for Social Science; SPSS, Chicago, IL) for Windows. Analysis was performed by Pearson's χ^2 test and by Fisher's exact test. Data were analyzed by Spearman's correlation coefficient and were compared by Mann-Whitney Test and t-test. Also univariate odds ratios (OR) and 95% CI were estimated separately for each polymorphism. A value of p<0.05 was taken to represent statistical significance in all tests.

Results

A total of 120 couples were karyotyped. Chromosomal aberrations were found in 16 couples (13.3%). In our study the most frequent chromosomal rearrangement was paternal inversion (p11q12) (7.5%), which was found in 9 of 16 parents (9). The second most prevalent anomaly was maternal and paternal balanced translocations (4.2%), which were found in 5 of the patients. Maternal mosaicism for numerical aberration was found only in one patient. The other chromosomal aberration was 46XY (16qh+). Sixty-five (54.2%) were diagnosed as having at least one thrombophilia marker, whereas 55 (45.8%) had no thrombophilia. The prevalence of MTHFR carriers was 45.8% (55/120), forty-eight (40%) of whom were heterozygous and seven (5.8%) were homozygous. In addition, the prevalence of FVL mutation was 15% (18/120) and the prevalence of FII mutation was 4.1% (5/120). Overall, eight patients (6.6%) had both FVL and MTHFR mutations, three patients (2.5%) had both FII and MTHFR mutations, and one patient (0.8%) had either FVL or FII mutations. None of the patients was homozygous for FVL or FII mutations. Table 1 shows mean ±SD, median, minimum and maximum values for age, first or second trimester miscarriages, third trimester losses and live births in our patients. There were no significant difference between the age of the women (p=0.89) and their husbands (p=0.92) with or without chromosomal aberrations. There was no significant difference in the prevalence of recurrent miscarriages between patients with parental karyotypic aberrations and those with no aberrations (p=0.75). Similarly there was no significant difference between patients with mutation of genetic markers of thrombophilia and those with no mutations (p=0.67). So prevalence of recurrent miscarriages was not statistically different in any of the genetic mutations studied in each group. Inherited thrombophilias showed more correlation with the first trimester than the second trimester abortions (p<0.001). Table 2 shows the prevalence of parental chromosomal aberration and the prevalence of GMT mutations according the number of previous miscarriages. There was no increased risk for the presence of a chromosomal aberration (OR=1.09; 95% CI, 0.84-1.42) or GMT mutations (OR=1.81; 95% CI, 0.58-5.68) for an increased risk of a subsequent pregnancy loss. Table 3 shows the details of the number of previous spontaneous abortions, number of patients and age of the parents. Mean age of the patients and husbands increased with the number of recurrent abortions (p<0.001, r=0.340) (p<0.001, r=0.418). Table 4 shows the relation between chromosomal aberrations and mutations of GMT. Chromosomal aberrations significantly increased with mutation of GMT (p=0.015).

Discussion

It has been reported that abnormal chromosomes in either partner, antiphospholipid antibodies, uterine anomalies, luteal phase defect, endocrine disorders and thrombophilia cause recurrent miscarriages (20). We studied cytogenetic abnormalities that can be divided into numerical abnormalities (96%), structural chromosome abnormalities (%3) and other mechanisms, such as mosaicism (1%) and single gene mutations involving FVL, MTHFR and FII mutations. The prevalence of parental chromosomal aberrations was greater in this series (13.3%) than in most series in the literature, which have quoted 3-5% prevalence (21-23). The higher prevalence might have been due to the relatively higher number of previous miscarriages in this cohort of patients (3.55±1.32). Table 1 show that the hereditary thrombophilias studied were associated with the first trimester than the second trimester losses, concurring with reports in the literature (24-26). As thrombophilias are caused by genetic mutations, they should be absolute causes of pregnancy loss. In the present study the patients with live births and recurrent miscarriage did not have show a statistically significant difference in the prevalence of thrombophilia. Although, 65 (54.1%) were diagnosed as having at least one thrombophilia marker, 55 (45.8%) had no thrombophilia; the mean live birth rates and recurrent miscarriage rates in all trimesters of GMT mutations were similar to that in patients presenting without GMT mutations. In our study, the frequency of chromosomal aberrations and GMT mutations were not dependent on the number of previous abortions. In a study, maternal age and previous number of miscarriages were argued to be two independent risk factors for a further miscarriage (27). In our study, maternal age, paternal age and previous number of miscarriages may be associated risk factors for further miscarriages. Advanced maternal age adversely affects ovarian function, giving rise to a decline in the number of good quality oocytes, resulting in chromosomally abnormal conceptions (28). Also our study shows that recurrent miscarriages may be associated with paternal age.

The most common types of parental chromosomal abnormality are balanced reciprocal or Robertsonian translocations (29). In our study, maternal and paternal inversion (9) (p11q12) was more common than the others (56.25%). FVL, FII and MTHFR were found to be prominent inherited risk factors for venous thrombosis (16); and, as placental underperfusion due to thromboembolic events contributes to fetal loss, our results document the association between both mutations and primary RM. Although the prevalences of FVL, FII and MTHFR mutations were more prominent among women with 3 or more consecutive miscarriages, the correlation was not statistically significant in our study. Some studies have shown that GMT mutations are more common in early RM (30) as in our study, but some have not (31,32). Also, in line with our results, chromosomal abnormalities are more common in early RM (3). It has been suggested that the presence of both parental karyotypic aberrations and mutations of GMT define a high-risk group for RM (33). Likewise, we have observed a strong relation between karyotypic aberrations and mutations of GMT (p=0.015).

Table 1. Details of patients in the study

	Number of patients	Patients age (years)	Husbands age (years)	First trimester abortions median (min-max)	Second trimester abortions median (min-max)	Third trimester losses median (min-max)	Live birth median (min-max)
Chromosomal aberrations	16 (13.3)	29.44±5.45	32.47±5.67	3 (2-7)	0 (0-5)	0 (0-2)	0 (0-2)
Chromosomal normal	104 (86.6)	27.68±5.36	30.63±5.51	3 (1-9)	0 (0-3)	0 (0-2)	0 (0-2)
MTHFR heterozigot	48 (40)	28.33±5.12	31.49±5.27	3 (1-6)	0 (0-5)	0 (0-1)	0 (0-2)
MTHFR homozigot	7 (5.8)	29.17±5.7	30.25±5.56	3 (3-4)	0 (0-3)	0 (0-0)	0 (0-1)
FVL heterozigot	18 (15)	27.59±6.73	30.20±5.69	3 (2-5)	0 (0-3)	0 (0-1)	0 (0-1)
FII heterozigot	5 (4.1)	29±6.20	29.33±0.57	3 (1-4)	0 (0-2)	0 (0-0)	0 (0-1)
MTHFR/FVL/FII mutations	65 (54.2)	27.98±5.39	30.72±5.10	3 (1-6)*	0 (0-5)*	0 (0-1)	0 (0-2)
MTHFR/FVL/FII normal	55 (45.8)	27.85±5.43	31.08±5.98	3 (1-9)	0 (0-2)	0 (0-2)	0 (0-2)
Total	120	27.92±5.54**	30.90±5.55**	3 (1-9)	0 (0-5)	0 (0-2)	0 (0-2)

Note: Data are presented as mean ±SD (range).
Data are presented as n (%).
MTHFR: methylenetetrahydrofolate reductase.
FVL: factor V Leiden.
FII: prothrombin.
*p<0.001.
**p>0.05, p>0.05.

Table 2. Prevalence of chromosomal aberrations according to number of previous miscarriage and prevalence of genetic markers of thrombophilia mutations according to number of previous miscarriage

Number of previous miscarriages	Chromosomal aberrations	Chromosomal normal	Total	*p*	Odds ratio	95% CI
3-4	13 (12.4)	92 (87.6)	105	NS	0.6	0.14-2.27
≥5	3 (20)	12 (80)	15	NS	0.6	0.19-1.92
Total	16 (13.3)	104 (86.7)	120	NS	1.09	0.84-1.42
Number of previous miscarriages	**Genetic markers of thrombophilia mutations**	**Genetic markers of thrombophilia normal**	**Total**	***p***	**OR**	**95% CI**
3-4	50 (47.6)	55 (52.4)	105	NS	1.07	0.94-1.22
≥5	5 (33.3)	10 (66.7)	15	NS	0.6	0.21-1.62
Total	65 (54.2)	55 (45.8)	120	NS	1.81	0.58-5.68

Data are presented as n (%).
CI: confidence interval; NS: not significant.

Table 3. Miscarriage rates, age of parents and number of patients

Number of previous spontaneous abortions	Number of patients	Patients age (years)	Husbands age (years)
3	88	27.01±5.24	29.90±5.28
4	13	28.85±4.45	33.00±5.51
5	6	34.67±3.72	35.80±4.66
6	3	32.00±2.65	36.67±6.11
7	2	32.00±1.41	35.00±0.00
8	2	25.00±8.48	34.00±0.00
9	1	32.00±0.00	35.00±0.00
11	1	37.00±0.00	39.00±0.00

Note: Data are presented as mean ±SD (range).

Table 4. Chromosomal aberrations significantly increased with mutation of genetic markers of thrombophilia (p=0.015)

	Genetic markers of thrombophilia normal	Genetic markers of thrombophilia mutation	Total
Chromosomal aberrations	12	4	16
Chromosomal normal	43	61	104
Total	55	65	120

Recurrent fetal loss is multi-factorial and involves the participation of a number of acquired risk factors, including advanced parental age, systemic disease, inherited risk factors including FVL, FII, MTHFR mutations and parental karyotypic aberrations.

In our study, with a limited number of patients who had a history of recurrent pregnancy loss, we have found that chromosomal anomalies in both parents and MTHFR, FVL, and FII gene mutations in women may be associated with recurrent abortions. Further studies are needed to clarify whether parental chromosomal aberrations and mutations of GMT are directly related with RM. It seems more likely that the prognosis for a pregnancy with history of previous RM is more dependent on factors such as the number of previous miscarriages, maternal age, and paternal age.

References

1. Dawood F, Farquharson R, Quenby S. Recurrent miscarriage. Curr Obstet and Gynecol 2004;14:247-53.
2. Crosignani PC, Rubin BL. Recurrent spontaneous miscarriage. The recommendations of the ESHRE workshop on recurrent spontaneous miscarriage held in Anacapri. Hum Reprod 1991;6:609-10.
3. American College of Obstetricians and Gynaecologists. Management of recurrent early pregnancy loss. ACOG Practice Bulletin 2001;24:1-12.
4. Uzelac PS. Recurrent abortion: etiology and treatment. Current Obstet and Gynecol 2007;14:264.
5. Boue J, Boue A, Lazar P. The epidemiology of human spontaneous abortions with chromosomal anomalies. Basel; Karger 1975:330.
6. Stein Z. Early fetal loss. Birth Defects 1981;17:95-9.
7. Sanchez JM, Franzi L, Collia F et al. Cytogenetic study of spontaneous abortions by transabdominal villus sampling and direct analysis of villi. Prenat Diagn 1999;19:601-3.
8. Stern JJ, Dorfman AD, Gutierez-Najar MD. Frequency of abnormal karyotype among abortuses from women with and without a history of recurrent spontaneous abortion. Fertil Steril 1996;65:250-3.
9. Ogasawara M, Aoki K, Okada S et al. Embryonic karyotype of bortuses in relation to the number of previous miscarriages. Fertil Steril 2000; 73:300-4.
10. Carp HJA, Toder V, Orgad S et al. Karyotype of the abortus in recurrent miscarriage. Fertil Steril 2001;5:678-82.
11. Sheiner E, Shoham-Vardi I, Hallak M et al. Placenta previa: obstetric risk factors and pregnancy outcome. J Matern Fetal Med 2001;10:414-9.
12. Rai R, Shlebak A, Cohen H et al. Factor V Leiden and acquired activated protein C resistance among 1000 women with recurrent miscarriage. Hum Reprod 2001;16:961-96.
13. Reznikoff-Etievant MF, Cayol V, Carbonne B et al. Factor V Leiden and G20210A prothrombin mutations are risk factors for early recurrent miscarriage. Br J Obstet Gyneacol 2001;108:1251-4.
14. Arruda VR, Von Zuben PM, Chiapurini LC et al. The mutation Ala677-Val in the methylene tetrahydrafolate reductase gene: a risk factor for arterial disease and venous thrombosis. Thromb Haem 1997;77:818-21.
15. Nelen WL, Bloom HJ, Thomas CM et al. Methylene tetrahydrafolate reductase polymorphism affects the change in homocysteine and folate concentrations resulting from low dose folic acid supplementation in women with unexplained recurrent miscarriages. J Nutr 1998;128: 1336-41.
16. Poort SR, Rosendahl FR, Reissma PH et al. A common genetic variation in the 3-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996;88:3698-03.
17. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure of extracting DNA from human nucleated cells. Nucl Acid Res 1998;16: 1215-7.
18. Salomon O, Steinberg DM, Zivelin A et al. Single and combined prothrombothic factors in patients with idiopathic venous thromboembolism. Arterioscler Thromb Vasc Biol 1999;19:511-8.
19. Frost P, Blom HJ, Milos R et al. A candidate genetic risk factor for vascular disease: a common mutation MTHFR. Nat Genet 1995;10: 111-3.
20. Hatasaka HH. Recurrent miscarriage: epidemiological factors, definitions, and incidence. Clin Obstet Gynecol 1994;37:625-34.
21. De Braekeleer M, Dao TN. Cytogenetic studies in couples experiencing repeated pregnancy losses. Hum Reprod 1990;5:519-28.
22. Portnoi MF, Joye N, Van Den Akker J et al. Karyotypes of 1142 couples with recurrent abortion. Obstet Gynecol 1988;72:31-4.
23. Fortuny A, Carrio A, Soler A et al. Detection of balanced chromosome rearrangements in 445 couples with repeated abortion and cytogenetic prenatal testing in carriers. Fertil Steril 1988;49:774-9.
24. Brenner B, Mandel H, Lanir N et al. Activated protein C resistance can be associated with recurrent fetal loss. Br J Haematol 1997;97:551-4.
25. Tal J, Scliamer LM, Leibovitz Z et al. A possible role for activated protein C resistance in patients with first and second trimester pregnancy failure. Hum Reprod 1999;14:1624-7.
26. Younis JS, Ohel G, Brenner B et al. Familial thrombophilia the scientific rationale for thrombophylaxis in recurrent pregnancy loss. Am J Reprod Immunol 2000;43:31-5.
27. Regan L, Braude PR, Trembath PL. Influence of past reproductive performance on risk of spontaneous abortion. BMJ 1989;299:541-5.
28. Nybo Anderson AM, Wohlfahrt J et al. Maternal age and fetal loss: population based register linkage study. BMJ 2000;320:1708-12.
29. Carp H, Feldman B, Oelsner G et al. Parental karyotype and subsequent live births in recurrent miscarriage. Fertil and Steril 2004;81:5.
30. Reznikoff-Etievant MF, Cayol V, Carbonne B et al. Factor V Leiden and G20210A prothrombin mutations are risk factors for very early recurrent miscarriages. Br J Obst and Gynaec 2001;108:1251-4.
31. Kutteh WH, Park VM, Deitcher SR. Hypercoagulable state mutation analysis in white patients with early first trimester recurrent pregnancy loss. Fertil Steril 1999;71:1048-54.
32. Altintaş A, Pasa S, Akdeniz N et al. Factor V Leiden and G20210A prothrombin mutations in patients with recurrent pregnancy loss: data from the southest of Turkey. Ann Hematol 2007;86:727-31.
33. Goddjin M, Leschot NJ. Genetic aspects of miscarriage. Bailliere's Clin Obstet and Gynecol 2000;14:855-65.