EVALUATION OF ETIOLOGIC CAUSES OF RECURRENT PREGNANCY LOSS

Mehmet OBUT[1], Mehmet Siddik EVSEN[1], Hatice Ender SOYDINC[1], Muhammet Erdal SAK[1], Ali OZLER[1], Mehmet FIDANBOY[2], Mahmut BALKAN[2], Aysegul TURKYILMAZ[2], Talip GUL[1],

[1] Department of Obstetrics and Gynecology, Dicle University, Faculty of Medicine, Diyarbakır
[2] Department of Medical Biology and Genetic, Dicle University, Faculty of Medicine, Diyarbakır

SUMMARY

Objective: *The aim of the present study is to evaluate etiologic factors in patients with recurrent pregnancy loss (RPL).*

Material and methods: *The records of patients admitted to Obstetrics& Gynecology Clinic of Dicle University Medical Faculty Hospital, from 2008 to 2011 were evaluated. Of the 114 patients who all tests were applied for diagnosis in our hospital were enrolled to study. For etiologic evaluation; Parental chromosome analysis, maternal antiphospholipid antibodies, hysterosalpingography for mullerian anomalies, thrombophilic disorders (factor V leiden mutation, prothrombin gene mutation) and endocrine (diabetes mellitus, thyroid hormone disorder) tests applied to patients.*

Results: *The mean age and mean number of abortus were 29.7±6.6, 3.2±1.3 respectively. Fifty (43.9%) patients had at least one etiologic factor where as 64 (56.1%) were idiopathic. Major chromosomal aberrations were detected in four (%3.5) couples as inversion of the ninth chromosome. The frequency for other pathologies; mullerian anomaly:14 (%12.3), factor V leiden mutation:7 (%6.1), prothrombin gene mutation:6 (%5.3) and antiphospholipid antibodies in 10 (%8.8) patients.*

Conclusion: *The prevalance for major chromosomal aberrations, mullerian anomalies, thrombopylic disorders and other pathologic conditions which evaluated in the study were found similar to reported previously. Polymorphism of 9qh+ (heterochromatic centromeric regions) was seen in 39 (%34.2) parents. Further studies are needed to understand that, if this result related to the genetic polymorpisym of the study population? or the pathology associated with RPL?.*

Key words: *diagnosis, etiology, recurrent pregnancy loss*

Journal of Turkish Society of Obstetrics and Gynecology, (J Turk Soc Obstet Gynecol), *2013; Vol: 10, Issue: 2, Pages: 67- 71*

TEKRARLAYAN GEBELİK KAYIPLARINDA ETİYOLOJİK NEDENLERİN DEĞERLENDİRİLMESİ

ÖZET

Amaç: *Bu çalışmanın amacı tekrarlayan gebelik kaybı (TGK) olan hastalarda etiyolojik nedenlerin değerlendirilmesidir.*

Gereç ve yöntemler: *Dicle Üniversitesi Tıp Fakültesi Hastanesi kadın hastalıkları ve doğum kliniğine, 2008'den 2011 tarihine kadar başvuran hastaların verileri incelendi. Tanıya yönelik tüm testleri hastanemizde yapılmış olan 114 hasta çalışmaya alındı. Etiyolojiye yönelik ebeveyn kromozom analizi, annede antifosfolipid antikor pozitifliği, kongenital uterin anomaliler için histerosalfingografi, trombofilik bozukluklar (faktör V leiden mutasyonu, protrombin gen mutasyonu) ve endokrinolojik (diabetes mellitus, tiroit endokrinopatisi) yönden tetkikleri değerlendirildi.*

Address for Correspondence: Dr. Mehmet Sıddık Evsen. Yenişehir mah. Mimar Sinan cad. Aslan Apt B Blok Kat: 7 No: 20 Yenişehir, Diyarbakır
Phone: + 90 (505) 357 43 63
e-mail: mevs26@yahoo.com
Received: 12 October 2012, revised: 13 January 2013, accepted: 20 January 2013, online publication: 25 January 2013

DOI ID:10.5505/tjod.2013.61587

Bulgular: *Hastaların ortalama yaşı ve abortus sayıları sırasıyla 29.7±6.6, 3.2±1.3 idi. Elli (%43.9) hastada en az bir etiyolojik neden saptanırken, 64(%56.1) hasta idiopatik olarak değerlendirildi. Majör kromozom anomalisi olarak; dört ebeveynde dokuzuncu kromozomda inversiyon saptandı. Diğer patolojilerin oranı; kongenital uterus anomalisi:14 (%12.3), faktör V Leiden mutasyonu:7 (%6.1), protrombin gen mutasyonu: 6 (%5.3) ve 10 (%8.8) hastada antifosfolipid antikor pozitifliği idi. Ebeveynlerin %34,2'sinde karyotip analizinde 9qh+ izlendi.*

Sonuç: *Major kromozomal anomali, kongenital uterus anomalileri, trombofilik bozukluklar ve çalışmada değerlendirilen diğer patolojik durumlarının oranı daha önce yayınlanmış veriler ile benzer olduğu saptandı. Ebeveynlerin 39'unda (%34,2) 9. kromozomda qh+ (sentromer bölgesinde heterokromazi) polimorfizmi izlendi. Bu sonucun çalışılan toplumun genetik polimorfizmimi? yoksa TGK ile ilişkilimi? olduğunu anlamak için daha ileri çalışmalara ihtiyaç vardır.*

Anahtar kelimeler: *etiyoloji, tanı, tekrarlayan gebelik kaybı*

Türk Jinekoloji ve Obstetrik Derneği Dergisi, (J Turk Soc Obstet Gynecol), *2013; Cilt: 10, Sayı: 2, Sayfa: 67- 71*

INTRODUCTION

Recurrent pregnancy loss (RPL) was traditionally described as 3 or more clinically diagnosed consecutive pregnancy losses prior to the 20th gestational week (molar and ectopic pregnancies were not included in the definition)[1]. Since similar etiologic factors have been identified between 2 or ≥ 3 pregnancy losses has been detected in recent years, RPL has been defined as ≥ 2 consecutive pregnancy losses and etiological investigation has been recommended[2-4].

Two or 3 consecutive pregnancy losses among the female population of child-bearing age have been observed at an incidence of 5% and 0.3-1%, respectively [1,5]. A consensus has not been reached on a uniform screening algorithm related to the etiological evaluation of RPL. Although many etiologic factors (genetic, anatomic, hormonal, environmental and immunological) have been implicated for the development of this condition with complex pathophysiological characteristics, any distinct pathology cannot be detected in 50% of the cases[6]. Successful pregnancy outcomes have been reported in 71-76% of the cases in the later period[7,8].

From etiological perspective, detection of any cause of repeated miscarriages and to find a treatable abnormality in couples with a history of RPL is important considerations. Etiological factors can demonstrate significant variations among populations because of genetic polymorphism. Aim of the present study is to evaluate patients with RPL in our region regarding etiological factors.

MATERIAL and METHODS

The records of patients which investigated for RPL in Obstetrics& Gynecology Clinic of Dicle University Medical Faculty Hospital, from 2008 to 2011 were evaluated. Institutional ethic approval was obtained from the Local Ethical Committee. The study conducted in a tertiary referral center in the southeast Anatolian region and patients with RPL generally referred to this clinic for etiological evaluation. Abortus defined as clinically fetal losses which weighing less than 500 g or miscarriages before the 20th gestational age[6]. Five hundred and seventy-two patients consulted to our clinic for the etiological investigation, because of RPL for ≥ 2 times. A total of 114 patients who had undergone all etiological analyses in our hospital such as parental chromosome analyses, hysterosalpyngography, tests for the detection of diabetes mellitus (DM), thyroidal endocrinopathies, factor V Leiden (FVL) mutation, prothrombin gene mutation and antiphospholipid antibodies were included in the study.

Age, number of gravidy, parity, miscarriages and live births of the patients were recorded. Body mass indices (BMIs) of the patients were also calculated [BMI =bodyweight (kg)/height (m)2].

For parental chromosome analyses, firstly peripheral blood cultures were obtained in the genetic laboratory. Then the prepared slides were stained using Giemsa banding technique. In this technique, chromosomes in 30- 50 metaphase plates were evaluated as for numerical and structural abnormalities and karyotyping was performed based on a metaphasis with at least 10 chromosome bands. Detection of any abnormal karyotype necessitated determination of a karyotype

from a metaphase with at least 50 chromosome bands. In cases with detected chromosomal inversion, the presence of an abnormal region was investigated. C banding or NOR banding techniques were applied for cases in the presence of chromosomal qh+ and increased ps+ respectively.

In the hematology laboratory, venous blood samples were drawn into tubes with EDTA and analyzed as for Factor V Leiden and prothrombin gene mutation in a Light cycler device using Real Time PCR method. For the analysis of lupus anticoagulant, Hemosil test kit was used for samples drawn into Na citrate tubes, while anticardiolipin IgG and IgM was studied in Alegra analyzer (Orgentec Diagnostika) test kit using ELISA method. TSH, fT3, fT4 tests were performed using Cobas analyzer kits in the hormone laboratory.

Fifty one patients were primary (with no live birth) whereas 63 patients were secondary habitual (at least one live birth) miscarriages. Two, three and ≥ 4 miscarriages were detected in 34 (29.9%), 54 (47.4%) and 26 (22.8%) patients, respectively. Miscarriages occurred in the first (n=51; 51.8%), second (n=15; 13.2%) and the first or second (n=40; 35.1%) trimesters of pregnancies.

Statistical analysis: Data were evaluated using SPSS 15.0 (Statistical Package for Social Science) statistical package program for Windows. Frequency, mean and standard deviation of data were calculated.

RESULTS

Mean age (29.7 ± 6.6 years), number of miscarriages (3.2 ± 1.3) and demographic data of the patients are shown in Table I. At least one etiologic factor was detected in 50 (43.9%) patients, while any etiologic factor was not found in 64 (56.1%) patients.

Table I: *Demographic characteristics of the patients.*

Characteristics	Mean±SD	Min-Max
Age, years	29,7±6,6	19-42
BMI	23,5±3,3	17,8-31.2
Gravidity	4,1±1,9	2-12
Parity	0,95±1,1	0-5
Abortus	3,2±1,1	2-10
Alive	0,81±0,95	0-5

BMI= *Body mass index,* ***SD=*** *Standard deviation,* ***Min-Max=*** *Minimum-Maximum.*

As a major parental chromosomal abnormality, an abnormal inversion of the 9th chromosome was detected in 4 (3.5%) parents (3 females and 1 male). All cases with major chromosomal abnormalities had experienced primary habitual abortus. In the chromosomal analysis, at least one of 48 (42.1%) couples had karyotypes different from 46XX or 46XY cell lines. Polymorphisms of qh positivity were detected on the 9th chromosome in 27 female and 20 male patients. Major parental chromosome abnormalities and abnormal karyotype/ chromosomal variants are seen in Table II.

Table II: *Results of Abnormal Parental Chromosomes.*

Caryotype	46 XX, n (%)	46XY,n (%)
9qh+	27 (23,7)	20 (17,5)
inv 9	3 (2,6)	1 (0,9)
14ps+	1 (0,9)	1 (0,9)
1qh+	1 (0,9)	-
22ps+	-	2 (1,8)

n= *Number of patients,* ***%=*** *Percent.*

Endocrinological disorders detected in a total of 17 patients consisted of type 2 DM (n=6), thyroid endocrinopathies (total n=11; clinical hypothyroidism, n=5; subclinical hypothyroidism, n=4; hyperthyroidism, n= 2). Anatomical uterine assessments revealed the presence of congenital uterine anomalies in 14 (12.3 %) patients. As congenital anomalies, bicornuate uterus (n= 6), septate uterus (n=4), T-shaped uterus (n=3) and uterus didelfis (n=1) were observed. Heterozygous FVL (n=7; 6.1%) and prothrombin gene (n=6: 5.3%) mutations were also observed. Any homozygous FVL or prothrombin gene mutation was not noted in any of our study participants. Antiphospholipid antibody positivity was detected in 10 (8.8%) patients.

DISCUSSION

In cases with RPL, karyotype analyses of parents have been recommended. In addition to numerical or structural abnormalities in chromosomes, single gene defect, X-linked or polygenic/multifactorial anomalies have been revealed as an etiologic factor for RPL. In the literature, the most frequently reported major chromosomal anomalies have been chromosomal translocation and inversion. Major chromosomal aberrations have been reported at a rate of 0.7% in general population, while in cases with RPL it rises to

3-5%[1,9]. Alp et al.[10] analyzed karyotypes of 434 couples with a history of RPL in our region and reported chromosomal aberrations in 6.9% of one of the parents. Minor chromosomal aberrations were not included in their study and other etiologic factors were not analyzed. In this study, major parental chromosomal abnormalities were detected in 3.5% of the cases and qh positivity was observed in heterochromatic centromeres of the 9th chromosome in 23.7% of the female and 17.5% of the male partners. Polymorphisms have been described on heterochromatic centromeric regions of the chromosomes[11]. It has been suggested that this type of variations have no phenotypic impact on carriers but they occurs more frequently in infertile couples or patients with history of RPL[12]. However, Blumberg et al[11] suggested heterochromatic polymorphic variants of chromosomes have not any significance in cases with RPL. Minor aberrations such as qh positivity are not usually reported. However in this study because of its high incidence, we thought that it should be evaluated in future studies in consideration of genetic differences among the study population or its role as an etiologic factor.

Congenital uterine abnormalities were detected in 3-7% of normal female population whereas in 10-15% in cases with RPL. Most frequently reported mullerian anomalies have been septate uterus and bicornuate uterus. Especially the relationship between septate uterus and RPL was demonstrated[5,13,14]. In the present study the frequency of congenital uterine anomalies were detected as 12.3%.

Predisposition to coagulopathy secondary to physiological alterations in coagulation factors in pregnancy is aggravated in the presence of hereditary thrombophilic mutations. Hereditary thrombophilia has been reported to be correlated with increased incidence of complicated outcomes as venous thrombosis, pulmonary embolism, miscarriages, intrauterine fetal loss, intrauterine growth restriction (IUGR) and preeclampsia[6,15]. Controversial outcomes have been published in the literature related to the association between thrombophilic disorders and RPL. However two metaanalyses recommended to investigate patients for FVL mutation, prothrombin gene mutation and protein S deficiency[16,17]. Prevalence of protein S deficiency has been reported to range between 0.03%-0.13%. Since any consensus has not been reached about the low-normal reference value of protein S levels and because of necessity of confirmatory measurement of the values obtained and variations in reference values caused by factors as age and gender, it is difficult to diagnose protein S deficiency[18,19]. Because of rarity of protein S deficiency and above-mentioned difficulties, protein S deficiency is not evaluated in the present study.

Since a statistically significant correlation could not be demonstrated between fetal loss and MTHFR gene mutation, protein C and antithrombin 3 deficiencies, the indicated thrombophilic disorders were not included in the study[16,17]. In our country, the incidence of FVL mutation positivity has been reported as 7.9-18.2% and prothrombin gene mutation as 1.7-5.37%[20,22]. In cases with demonstrated prothrombin gene or FVL mutations, thrombophylaxis has been recommended because of improvements in pregnancy outcomes. In this study, we detected rates of FVL and prothrombin gene mutations as 6.1% and 5.3%, respectively.

Some autoimmune diseases have been blamed as an etiologic factor for RPL. However only in the diagnosis of antiphospholipid antibodies syndrome history of RPL is a criteria[23]. In the literature, incidence of the presence of antiphospholipid antibodies has been reported to be significantly higher in cases with RPL when compared with the general population and its inclusion in etiological surveys has been recommended. In women with antiphospholipid antibody positivity, increased risks of IUGR and prematurity have been reported[1,23]. In this study, antiphospholipid antibody positivity was observed in 8.8% of the patients in compliance with the literature.

Although controversial, associations among DM and thyroid endocrinopathy with infertility, congenital anomalies and RPL have been reported in the literature[1,6,24-26]. In this study, type 2 DM or thyroid endocrinopathy was observed in 14.9% of the patients. Apart from this, endocrinopathies as luteal phase defect, hyperprolactinemia, polycystic ovarian syndrome, insulin resistance have been implicated among etiologic factors[27,28]. Endocrinologic factors excepting DM and thyroid endocrinopathy were not investigated in this study.

Our study had some limitations including its retrospective design, scarce number of cases and absence of any control group. However, we think that this survey will be especially helpful in the evaluation of cases with RPL in our region.

In conclusion, in the assessment of cases with RPL in our region, we have detected genetic, anatomic and immunologic factors in cases with RPL comparable to those found in the literature. Detection of 9qh positivity in 39 (34.2%) parents reminded us the need for further studies in order to understand whether 9 qh positivity is related to the genetic polymorphism in the studied population? or is it associated with RPL?.

REFERENCES

1. Fox-Lee Laura, Schust DJ. Recurrent pregnancy loss. In Berek JS ed, Berek & Novak Gynecology, 14th ed. Philadelphia: Lippincott Williams &Wilkins, 2007.p. 1277- 322.
2. Jaslow CR, Carney JL, Kutteh WH. Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. Fertil Steril 2010; 93(4): 1234- 43.
3. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. Fertil Steril 2008; 89: 1603.
4. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertil Steril 2013; 99(1): 63.
5. Rai R, Regan L. Recurrent miscarriage. Lancet 2006; 12; 368: 601- 11.
6. Ford HB, Schust DJ. Recurrent pregnancy loss: etiology, diagnosis, and therapy. Rev Obstet Gynecol 2009; 2(2): 76- 83.
7. Shapira E, Ratzon R, Shoham-Vardi I, Serjienko R, Mazor M, Bashiri A. Primary vs. secondary recurrent pregnancy loss-epidemiological characteristics, etiolgy and next pregnancy pregnancy outcome. J Perinat Med 2012; 29; 40(4): 389- 96.
8. Harger JH, Archer DF, Marchese SG, Muracca-Clemens M, Garver KL. Etiology of recurrent pregnancy losses and outcome of subsequent pregnancies. Obstet Gynecol 1983; 62(5): 574- 81.
9. Franssen MT, Korevaar JC, Leschot NJ, Bossuyt PM, Knegt AC, Gerssen-Schoorl KB, et al. Selective chromosome analysis in couples with two or more miscarriages: case-control study. BMJ 2005; 16(33): 137- 41.
10. Alp MN, Oral D. Tekrarlayan spontan abortusları olan çiftlerde genetik araştırmalar. Dicle Tıp Dergisi 2006; 33(2): 71- 80.
11. Blumberg BD, Shulkin JD, Rotter JI, Mohandas T, Kaback MM. Minor chromosomal variants and major chromosomal anomalies in couples with recurrent abortion. Am J Hum Genet 1982; 34(6): 948- 60.
12. Dundar M, Caglayan AO, Saatci C, Batukan C, Basbug M, Ozkul Y. Can the classical euchromatic variants of 9q12/qh+ cause recurrent abortions? Genet Couns 2008; 19(3): 281- 6.
13. Devi-Wold AS, Pham N, Arici A. Anatomic factors in recurrent pregnancy loss. Semin Reprod Med 2006; 24(1): 25- 32.
14. Proctor JA, Haney AF. Recurrent first trimester pregnancy loss is associated with uterine septum but not with bicornuate uterus. Fertil Steril 2003; 80(5): 1212- 5.
15 Lockwood CJ. Heritable coagulopathies in pregnancy. Obstet Gynecol Surv 1999; 54(12): 754- 65.
16 Kovalevsky G, Gracia CR, Berlin JA, Sammel MD, Barnhart KT. Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss: a meta-analysis. Arch Intern Med 2004; 164: 558- 63.
17 Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: A meta-analysis. Lancet 2003; 361: 901- 8.
18. Dykes AC, Walker ID, McMahon AD, Islam SI, Tait RC. A study of Protein S antigen levels in 3788 healthy volunteers: influence of age, sex and hormone use, and estimate for prevalence of deficiency state. Br J Haematol 2001; 113: 636- 41.
19. Ten Kate MK, Van Der Meer J. Protein S deficiency: a clinical perspective. Haemophilia 2008; 14(6): 1222- 8.
20. Özdemir S, Balcı O, Görkemli H, Taşçı E, Acar H. Tekrarlayan gebelik kaybı olan hastalarda trombofili mutasyon sıklığının değerlendirilmesi. Genel Tıp Derg 2010; 20(3): 93- 7.
21. Şahin Fİ, Ataç B, Yılmaz Z, Zeyneloğlu HB. Tekrarlayan gebelik kayıplarında trombofili mutasyon sıklıkları. Erciyes Tıp Dergisi 2009; 31(2): 104- 9.
22. Altintas A, Pasa S, Akdeniz N, Cil T, Yurt M, Ayyildiz O, et al. Factor V Leiden and G20210A prothrombin mutations in patients with recurrent pregnancy loss: data from the southeast of Turkey. Ann Hematol 2007; 86: 727- 31.
23 Di Prima FA, Valenti O, Hyseni E, Giorgio E, Faraci M, Renda E, et al. Antiphospholipid Syndrome during pregnancy: the state of the art. J Prenat Med 2011; 5(2): 41- 53.
24. Greene MF. Spontaneous abortions and major malformations in women with diabetes mellitus. Semin Reprod Endocrinol 1999; 17(2): 127- 36.
25. Dönmez M, Şişli T, Atış A, Aydın Y. Spontan abortus ve tiroit fonksiyonları. Perinataloji Dergisi 2005; 13(2): 110- 3.
26. Stagnaro-Green A, Glinoer D. Thyroid autoimmunity and the risk of miscarriage. Best Pract Res Clin Endocrinol Metab 2004; 18(2): 167- 81.
27. Cocksedge KA, Li TC, Saravelos SH, Metwally M. A reappraisal of the role of polycystic ovary syndrome in recurrent miscarriage. Reprod Biomed Online 2008; 17(1): 151- 60.
28. Celik N, Evsen MS, Sak ME, Soydinc E, Gul T. Evaluation of the relationship between insulin resistance and recurrent pregnancy loss. Ginekol Pol 2011; 82(4): 272- 5.