There is No Relationship Between the Number of Subsequent Pregnancy Losses and Thrombophilic Factors

Tekrarlayan Gebelik Kaybı Sayısı ile Trombofilik Faktörler Arasında İlişki Yoktur

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ABSTRACT Objective: If there are two previous miscarriages, the risk of miscarriage in the next pregnancy is 26% . Presence of three previous miscarriages increases this risk upto 32%. We have aimed to find whether or not there is a difference between the women with habitual abortion (three or more fetal losses) and women with two fetal losses in relation to thrombophilic factors. **Material and Methods:** A total of 108 women aged between 17-40 years who admitted to the infertility department were included in the study. None of the patients were pregnant. Patients were divided into two groups as women with three or more subsequent pregnancy losses, which was defined as habitual abortus, and women with two abortuses, and then both groups underwent thrombophilia workup. There were 66 patients in the first group and 42 patients in the second group. **Results:** We did not find any significant difference between the groups in relation to folic acid, vitamin B12, homocystein, antithrombin III, protein S levels or presence of activated protein C resistance, prothrombin G mutation, and factor V mutation. There were no pathological findings on hysterosalpingography and pelvic ultrasonography of the patients. **Conclusion:** As a result, we believe that investigation for thrombophilia should be performed in women with two recurrent miscarriages (without regarding the early or late abortions), before the occurence of three fetal losses. One may expect that this approach will protect patients from losing their next pregnancies by preventing late diagnosis and the treatment of thrombophilia. However, our opinion should be supported by prospective cohort studies with a greater sample size.

Key Words: Pregnancy; thrombophilia; abortion, habitual

ÖZET Amaç: Daha önce iki düşüğü olanlarda sonraki gebelikte düşük riski %26'dır. Ancak, eğer hastanın daha önce üç düşüğü varsa, bu risk %32'ye çıkar. Biz bu çalışmada habituel düşük (üç veya daha fazla fötal kayıp) yapan kadınlarla iki fötal kaybı olan kadınlar arasında trombofilik faktörler açısından fark olup olmadığını bulmak istedik. **Gereç ve Yöntemler:** İnfertilite bölümüne başvuran 17-40 yaş arasında 108 kadın çalışmaya alındı. Hastaların hiçbiri gebe değildi. Hastalar habituel düşük olarak tanımlanan üç veya daha fazla ardışık düşüğü olan kadınlar ve iki düşüğü olan kadınlar olarak iki gruba ayrıldı ve daha sonra her iki grup trombofilik faktörler açısından karşılaştırıldı. İlk grupta 66 hasta ve ikinci grupta 42 hasta vardı. **Bulgular:** Gruplar arasında folik asit, vitamin B12, homosistein, antitrombin III, protein S düzeyleri ve aktive protein C direnci, protrombin G mutasyonu ve faktör V mutasyonu varlığı açısından önemli fark bulmadık. Hastaların histerosalfingografi ve pelvik ultrasonografilerinde patolojik bulgu yoktu. **Sonuç:** Sonuç olarak, hastalar üç fötal kayıp yaşamadan önce, iki rekürren düşükte (erken ve geç düşük olmasına bakılmaksızın) trombofili için araştırma yapılması gerektiğine inanıyoruz. Bu yaklaşımın, trombofilinin geç tanı ve tedavisini önleyerek, hastaların sonraki gebelik kayıplarını önlemesi beklenir. Fakat bizim fikrimiz daha büyük örneklemli prospektif kohort çalışmalarla desteklenmelidir.

Anahtar Kelimeler: Gebelik; trombofili; düşük, tekrarlayan

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Three or more early pregnancy losses affect 1-2% of women in the reproductive age, whereas two subsequent pregnancy losses affect 5% of the women in the same age group. Etiologic factors of recurrent pregnancy losses (RPL) include chromosomal translocations or inversions, Mullerian anomalies, endocrinological or autoimmune diseases, and recently thrombophilia, on which more investigations have been focused.[1] It is debated that whether the investigation for thrombophilia should be done im the women with habitual abortus (three or more fetal losses) only, or it could be done for women with a smaller number of subsequent pregnancy losses.

There is no specific number of miscarriages or firmly established criterion that justifies evaluation for recurrent pregnancy loss or defines the scope of investigation. The risk of miscarriage in next pregnancy increases with the number of prior miscarriages. If there is one or two previous miscarriages, the risk of miscarriage in the next pregnancy is 24% and 26%, respectively. However, if there are three previous miscarriages, this risk increases up to 32%.[2]

In this study, we aimed to find if there is a difference between the women with habitual abortus and women with two fetal losses in relation to the presence of thrombophilic factors.

MATERIAL AND METHODS

This study includes 108 women aged between 17-40 years with a history of recurrent pregnancy losses, who admitted to the infertility department with desire of having a baby. None of the patients were pregnant during the study. This study has been performed in accordance with Helsinki Declaration. Written informed consents were obtained from all patients before the study.

Detailed medical history was taken from the patients, and physical examination was done. We performed pelvic ultrasonography and hysterosalpingography (HSG) in all patients. Blood samples were obtain to study antithrombin III, protein S, protein C, activated protein C resistance, folic acid, vitamin B 12, homocystein, factor V Leiden and prothrombin G mutations. All blood samples were collected in plastic tubes containing sodium citrate as the anticoagulant, they were centrifuged, the plasma was separated and the sample was rapidly cooled to -20 ºC. Then the tests were done within 3-5 days.

Anticardiolipin (ACA) antibody and Lupus anticoagulant were also investigated. We investigated the antibody titers for toxoplasma, rubella, and cytomegalovirus (CMV). Table 1 presents the names of the tests used, their methods of evaluation, and the normal ranges of the results.

Statistical analyses were done using Chi-square test in SPSS (Statistical Package for Social Sciences) software version 10.0 for Windows.

RESULTS

The mean age of 108 women was 27.6 ± 4.3 years, their mean body mass index (BMI) was 23.8 ± 3.6, mean gravida was 3.8 ± 1.9, and mean number of abortions was 3.1 ± 1.7. There were 19 patients (17.6%) with the history of a previous surgery, one patient (0.9%) with the history of previous pelvic inflammatory disease, one patient (0.9%) with diabetes mellitus and one patient (0.9%) with cardiac disease. ACA and Lupus anticoagulant were negative in the woman with diabetes mellitus.

In their family histories, there were 10 (9.3%) patients with diabetes mellitus, 10 (9.3%) with cardiac disease, 17 (15.7%) with hypertension and 3 (2.8%) with tuberculosis. No abnormalities were found on HSG or pelvic ultrasonographies of the patients.

Toxoplasma antibody Ig G was positive in 11 (10.2%) patients, rubella antibody Ig G was positive in 24 (22.2%) patients, and CMV antibody Ig G was positive in 11 (10.2%) patients. Ig M antibodies for toxoplasma, rubella and CMV were all negative.

Anticardiolipin antibody (ACA) was present in one (0.9%) patient, but no positivity was detected for Lupus anticoagulant.

Factor V Leiden mutation was present in nine patients (8.4%) whereas prothrombin G mutation was present in one (0.9%). Activated protein C resistance was low in 19 (17.9%) patients. Protein S and antithrombin III levels were low in 15 (13.9%)

TABLE 1: The names, methods and normal ranges of the tests used for investigation of the patients.

Name of the test	Test method	Normal ranges
Anti-Toxoplasma Ig G	Elisa	4-6 IU/ml negative
Anti-Toxoplasma IgM	Elisa	<100 AU/ml negative
Anti-Rubella Ig G	Elisa	7.5-12.5 IU/ml negative
Anti-Rubella Ig M	Elisa	0.3-0.6 AU/ml negative
Anti-Cytomegalovirus Ig G	Elisa	0.5-0.8 IU/ml negative
Anti-Cytomegalovirus Ig M	Elisa	1-2 AU/ml negative
Anti Cardio Lipin Ig M	Elisa	Negative MPL/ml
Lupus anticoagulant (screening)	Coagulative assay	31-44 seconds
Factor V Leiden mutation	Light cycler (Roche)	G/G
Prothrombin mutation	Light cycler (Roche)	G/G
Folic acid		3-17 ng/ml
Vitamin B 12		193-982 pg/ml
Fasting homocystein	Elisa	5-15 µmol/L
Anti thrombin 3	Chromogenic assay	75-125%
Protein C	Chromogenic assay	70-130%
Protein S	Coagulative assay	55-160%
Activated protein C resistance	Coagulative assay	2.6-7.8 negative <2.6 abnormal

and in six (5.6%) patients, respectively. We found high homocystein levels in nine (8.3%) patients.

There were 66 patients (61.1%) in the first group (habitual miscarriages) and 42 patients (38.9%) in the second group (women with two miscarriages). The mean age of the women in the first and second groups were 28.2 ± 4.4 years and 26.7 ± 4.1 years, mean body mass index (BMI) was 23.7 ± 4.0 and 24.0 ± 3.1, mean gravida was 4.6 ± 2.0 and 2.5 ± 0.8, and mean number of abortions was 4.0 ± 1.6 and 1.8 ± 0.4, respectively.

The ratio of first trimester losses to total losses in the first group was 29/66 (43.9%), and this ratio was 25/42 (59.5%) in the second group.

There were no differences between the groups in regard to personal and family histories of diseases and in regard to toxoplasma, rubella, and CMV antibodies (Table 2).

TABLE 2: Comparison of the groups acoording to history of diseases and toxoplasma, rubella and CMV antibodies.

	Groups		
	Two abortions	>=3 abortions	
Variables	n=42 (%)	n=66 (%)	p
History of previous surgery	6 (14.3%)	13 (19.7%)	0.35
History of pelvic inflammatory disease	-	1 (1.5%)	0.72
History of diabetes	-	1 (1.5%)	0.72
History of cardiac disease	-	1 (1.5%)	0.72
Family history of diabetes	2 (4.8%)	7 (10.6%)	0.27
Family history of cardiac disease	3 (7.1%)	7 (10.6%)	0.55
Family history of hypertension	5 (11.9%)	12 (18.2%)	0.51
Toxoplasma IgG	4 (9.5%)	7 (10.6%)	0.55
Rubella IgG	8 (19%)	16 (24.2%)	0.80
CMV IgG	2 (4.8%)	9 (13.6%)	0.33

We did not find any significant differences between the groups for the thrombophilic factors (Table 3).

We also compared these variables between the first and second trimester pregnancy losses, however we could not do statistical comparison because of the small sample size of the groups.

DISCUSSION

Pregnancy itself is a hypercoagulative state. During pregnancy, factor VII, VIII, X, and fibrinogen levels rise and antithrombin III, protein C levels decrease, and protein S level decreases by 40-50%. Therefore, the investigation for thrombophilic factors should be done during nonpregnant state.

Factor V Leiden and prothrombin G mutations are the most frequently seen thrombophilic factors. Hyperhomocysteinemia is the third in order. The other hypercoagulative states are deficiencies of antithrombin III, protein C, and protein S.

There are many reports comparing the results of women with habitual abortus with normal population. Ridker et al.[3] compared 113 patients with factor V Leiden mutation with 437 postmenopausal parous women. Grandone et al. compared the patients with first trimester loss with patients with second and third trimester losses, and found a strong association of factor V mutation with second and third trimester losses.[4] Clark et al. showed that activated protein C resistance enhanced thrombus formation during pregnancy, and led to intrauterine fetal loss.[5] Preston et al. reported that deficiencies of antithrombin III, protein C, and protein S are possibly responsible from a fetal loss beyond 28 weeks of gestation.[6] Wouters et al. reported that hyperhomocysteinemia was present in 21% of women with habitual abortus and increased homocystein levels prevented implantation by causing decidual and chorionic vessel damage.[7]

Screening for the general population is not justified. The prevalence of factor V Leiden, the most common defect in European countries, ranges between 2 and 15% in Caucasian populations, and is greater in Northern European than in Southern European populations, with an ethnic distribution. In the Caucasian population, the G20210A prothrombin gene mutation is detected in about 2% of subjects, with wide geographic differences. Antithrombin heterozygous deficiency occurs in about 1 in 2500 (0.04%) members of the general population. The prevalence of protein C deficiency in healthy individuals is 0.2 to 0.3%. The prevalence of protein S deficiency was found as 2.1% in a Dutch population, 1.3% in an Italian population, and 5.7% in a Spanish population. In a large Scottish study, the prevalence of protein S deficiency was estimated to range from 0.03% to 0.13%. The prevalence of moderate hyperhomocysteinemia is estimated as 5 to 25% in European population studies.[8]

In our study population, prevalence of factor V mutation was between 7.5 to 9.5%; activated protein C resistance was between 16.6 and 18.2%; prothrombin mutation was 1.5%; antithrombin deficiency was between 4.5 and 7.1%; protein S deficiency was between 12.2 and 16.6%; and hyperhomocysteinemia was between 7.5 and 9.5%. We

TABLE 3: Comparison of the two groups by thrombophilic factors.

	Groups		
	Two abortions	>=3 abortions	
Variables	n= 42 (%)	n= 66 (%)	p
High homocystein level	4 (9.5)	5 (7.5)	0.20
Low vitamin B12 level	4 (9.5)	1 (1.5)	0.09
Low antithrombin III level	3 (7.1)	3 (4.5)	0.14
Low protein S level	7 (16.6)	8 (12.2)	0.20
Prothrombin G mutation	0 (0)	1 (1.5)	0.88
Factor V mutation	4 (9.5)	5 (7.5)	0.84
Activated protein C resistance	7 (16.6)	12 (18.2)	0.054

do not know the prevalence of thrombophilic factors in our country. However, in our study population, prevalence of activated protein C resistance, antithrombin deficiency, and protein S deficiency seems higher than some European countries.

In our study, we compared women with two previous abortions with women with three or more abortions, and we could not find any significant differences in the levels of thrombophilic factors between the two groups. Finan et al. reported that when they compared habitual aborters with women with two abortions, they did not find any statistical significant difference between the groups for factor V Leiden and prothrombin G mutation prevalence.[9] Abraitis et al. similarly reported that there was no statistically significant difference when they compared the groups with two abortions and with three or more abortions in relation to activated protein C resistance.[10]

It was previously suggested that the first or second trimester pregnancy miscarriages had different etiologic factors. It was reported that the presence of Lupus anticoagulant with other antiphospholipid antibodies were strongly associated with first trimester abortions.[11] also In addition, antithrombin III deficiency was reported to be associated with first trimester abortions and preeclampsia as well.[12] Although, a higher prevalance of late pregnancy losses was reported in women with thrombophilia, activated protein C resistance and factor V Leiden mutation were also found to be related to recurrent first trimester pregnancy losses.[13,14] Increased factor VIII level was also reported to be responsible from recurrent early pregnancy losses.[15] With all these contradictory reports, it becomes more complicated to decide the best options upon laboratory testing for women with early or late miscarriages.

A meta-analysisperformed in 2004 examined the subgroups of recurrent pregnancy losses in relation to factor V Leiden and prothrombin G mutations.[16] According to this analysis, prothrombin G mutation showed a higher Odds ratio in women with two abortions whyen compared to habitual aborters. Again, if there was prothrombin G mutation, Odds ratio was higher for first trimester losses only when compared to fetal losses both in the first and second trimesters.[17-19]

We tried to compare thrombophilic factors between first or second trimester pregnancy losses within the groups, however we could not do a statistical comparison because of the small sample size of the groups.

Screening for congenital thrombophilia is suggested for women with recurrent pregnancy loss (three or more miscarriages), and for women with fetal loss, including three or more first trimester losses, two or more second trimester losses, or any stillbirth.[20] In our study population, there first trimester losses were more in women with two miscarriages, when compared to women with three or more fetal losses. However, we did not find any difference between the groups in relation to thrombophilic factors. Lavigne et al. recommended consideration of earlier screening, at least in (childless) women with a primary first unexplained fetal loss from the 10th week of gestation.[20]

As a result, we believe that investigation for thrombophilia should be done for women with two recurrent miscarriages (without regarding the early or late abortions) before the occurence of a third fetal loss. This approach will prevent late diagnosis and the treatment of thrombophilia, and will prevent patients from losing their next pregnancies. However, our opinion should be supported by prospective cohort studies with a greater sample size.

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