General Section

Inherited thrombophilia screening in Greek women with recurrent fetal loss

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Summary

Objective: The present study was designed to determine the prevalence of factor V Leiden (FVL), prothrombin gene G20210A (PTG) and methylenetetrahydrofolate reductase (MTHFR C677T) mutations in women from South-Western Greece with recurrent fetal loss (RFL) and negative personal thromboembolic history. *Materials and Methods:* 212 women with RFL and 181 women with at least two pregnancies with normal outcome and no history of pregnancy loss were investigated for the commonest thrombophilic mutations (FVL, PTG, MTHFR C677T). Comparisons between groups were performed by Pearson's chi-square test and odd ratios were calculated. *Results:* An abnormal genotype was detected in 49 women of the study group (23.1%) and in 41 women of the control group (22.6%). *Conclusion:* Inherited thrombophilia screening is not indicated as an initial approach in Greek women with RFL and negative personal thromboembolic history.

Key words: First trimester recurrent fetal loss; Factor V Leiden; Prothrombin G20210A; MTHFR C677T; Molecular thrombophilic testing.

Introduction

Early fetal loss is separated into recurrent fetal loss in the 1st trimester and single fetal loss in the 2nd trimester [1]. First trimester recurrent fetal loss (RFL) has been defined as three or more consecutive pregnancy losses before the 12th week of gestation, affecting 1% of the women in reproductive age [2].

Several factors have been reported as causes of RFL. These include chromosomal abnormalities, uterine structural abnormalities, infections, metabolic disorders, autoimmune factors, drugs and enviromental factors [3].

The present study was designed to determine the prevalence of factor V Leiden (FVL), prothrombin gene G20210A (PTG) and methylenetetrahydrofolate reductase (MTHFR C677T) mutations in women from South-Western Greece with RFL and negative personal thromboembolic history.

Materials and Methods

From October 1999 to December 2006, 212 women with a history of RFL attending the Outpatient Clinic of the Obstetrics and Gynecology Department of the University of Patras, were included in the study. A control group consisted of 181 age- and race-matched women, with at least two pregnancies with normal outcome and no history of pregnancy loss. Demographic data were collected during the first appointment at the Clinic and included information on thromboembolic and obstetric history, current medications, and family history.

Exclusion criteria for entry in the study were known risk factors for RFL. These included inherited thrombophilic factors (FVL, PTG, homozygous MTHFR C677T mutations as well as protein C, protein S and antithrombin III deficiencies), personal thromboembolic history, preexisting antiphospholipid syndrome (APS), chromosomal abnormalities, uterine structural abnormalities, hormonal imbalances, infections, metabolic disorders, autoimmune disorders and drugs. The study was approved by the Ethical Committee of the Hospital. Informed consent was obtained from each woman.

Blood samples in EDTA were collected from all women. Molecular diagnosis of the FVL, PTG, homozygous MTHFR C677T mutations was performed after DNA isolation, polymerase chain reaction (PCR) amplification, and hybridization of amplification products using allele specific oligonucleotide probes for the detection of factor V G506A, prothrombin G20210A and MTHFR C677T normal and mutated alleles (Thrombophilia Gene Mutation Assay Kit, Vienna Lab, Austria).

Comparisons between groups were performed by Pearson's chi-square test. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. The significance level was set at 0.05. Statistical analyses were performed by the Statistical Package for Social Sciences for Windows (SPSS-12).

Results

An abnormal genotype was detected in a total of 49 of the 212 women in the study group (23.1%). Among them, eight women (3.8%) were heterozygous for the FVL mutation (FVL (±) genotype), eight (3.8%) were heterozygous for the PTG mutation (PTG (±) genotype) and 33 women (15.6%) were homozygous for the MTHFR C677T mutation (MTHFR T677T genotype). Combined thrombophilic genotypes or homozygosity for the FVL mutation and PTG mutation were not detected.

An abnormal genotype was detected in a total of 41 from the 181 women in the control group (22.6%). Among them, seven women (3.9%) were heterozygous for the FVL mutation (FVL (±) genotype), seven (3.9%)

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were heterozygous for the PTG mutation (PTG (±) genotype) and 27 women (14.9%) were homozygous for the MTHFR C677T mutation (MTHFR T677T genotype). Combined thrombophilic genotypes or homozygosity for the FVL mutation and PTG mutation were not detected.

The ORs for the incidence of thrombophilic mutations in the women with RFL as compared with the control group are shown in Table 1.

Table 1. — *Prevalence of thrombophilic genotypes among women with RFL and control women.*

Thrombophilic genotype	Women with (n = 212)	Control women (n = 181)	OR (± 95% CI)
FVL (±)	8 (3.8%)	7 (3.9%)	0.97 (0.35-2.74)
PTG (±)	8 (3.8%)	7 (3.9%)	0.97 (0.35-2.74)
MTHFR T677T	33 (15.6%)	27 (14.9%)	1.05 (0.61-1.87)
Total	49 (23.1%)	41 (22.6%)	1.03 (0.64-1.65)

(±) = heterozygocity; OR = Odds Ratio; CI = Confidence Interval.

Discussion

Successful pregnancy outcome depended on the development and maintenance of adequate placental circulation. Abnormalities of placental vasculature may result in a number of gestational pathologies, including 1st and 2nd trimester fetal loss, intrauterine growth restriction (IUGR), intrauterine fetal death (IUFD), placental abruption (PA) and preeclampsia (PE) [4].

The pathogenetic mechanisms responsible for placental vascular pathologies in women with thrombophilia have not been fully elucidated. It is yet unknown why only some women with thrombophilia express vascular gestational pathologies while others do not. It is possible that this may relate to local factors affecting coagulation, fibrinolysis, and vascular tone at the level of placental vessels [5].

Many studies confirmed that women with inherited thrombophilia are at increased risk of developing pregnancy complications [1, 6]. In our study, we investigated the association between inherited thrombophilia and RFL in women from South-Western Greece with a negative personal thromboembolic history.

The presence of FVL (±) genotype in our study showed a negative association with RFL, but this finding was not significant (OR 0.97; 95% CI 0.35-2.74). According to the international literature the presence of FVL (±) genotype increase the risk for RFL (OR 1.91; 95% CI 1.01-3.61), compared to a normal genotype [6-13].

The presence of PTG (±) genotype in our study, showed a negative association with RFL, but this finding was not significant (OR 0.97; 95% CI 0.35-2.74). According to the international literature the presence of PTG (±) genotype increase the risk for RFL (OR 2.70; 95% CI 1.37-5.35), compared to a normal genotype [6-8, 11, 12, 14-16].

The presence of MTHFR T677T genotype in our study showed a positive association with RFL, but this finding was not significant (OR 1.05; 95% CI 0.61-1.87). According to the international literature the presence of MTHFR T677T genotype showed a negative association with RFL, but this finding was not significant (OR 0.86; 95% CI 0.44 to 1.69) [6, 11, 17, 18].

The findings from many studies have shown that selective inherited thrombophilia screening in women with personal thromboembolic history and/or adverse pregnancy outcome is more cost-effective than universal screening [6, 19, 20]. As these complications tend to recur in subsequent pregnancies, the identification of inherited thrombophilia markers in these patients is necessary. It offers an opportunity to reduce the risk of recurrence with prophylactic anticoagulant therapy [21-23].

Although many studies confirmed that women with inherited thrombophilia are at increased risk of developing pregnancy complications [1, 6], the absolute risk of venous thromboembolic disease and adverse pregnancy outcome remains low. Thus, at present, universal inherited thrombophilia screening in women during pregnancy cannot be justified clinically [1, 6].

The main limitation of our study was the small number of cases. Our results suggest that, inherited thrombophilia screening is not indicated as an initial approach in Greek women with RFL and negative personal thromboembolic history. These data require confirmation in larger clinical trials.

Conclusion

Our study suggests that inherited thrombophilia screening is not indicated as an initial approach in Greek women with RFL and negative personal thromboembolic history.

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