Thrombophilic Polymorphisms Are Common in Women with Fetal Loss without Apparent Cause

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Summary

An association between fetal loss and thrombophilia has recently been described but has not been yet fully elucidated. We have evaluated prospectively the prevalence of the three common thrombophilic polymorphisms (TP) factor V G1691A (Leiden), thermolabile-methylene tetrahydrofolate reductase (TL-MTHFR) C677T and factor II G20210A mutations, in 76 women with fetal loss (≥3 in first, ≥2 in second, ≥1 in third trimester) without apparent cause and 106 controls without fetal loss. Thirty seven out of 76 (49%) of the women in the fetal loss group had at least one TP compared to only 23/106 (22%) in the control group (p = 0.0001). Factor V-Leiden was more common in the fetal loss group 24/76 (32%) compared to the control group 11/106 (10%) (OR = 4.0, 95% CI: 1.8-8.8, p < 0.001). Five of the 76 patients (7%) were homozygous for factor V-Leiden compared to none of the controls (p = 0.012). A trend, albeit no statistically significant difference was found between women with fetal loss and control groups regarding factor II G20210A (8% vs. 4% respectively, OR = 2.2, 95% CI: 0.6-8.0, p = 0.23) and MTHFR C677T (18% vs. 10% respectively, OR = 1.95, 95% CI: 0.83-4.6, p = 0.12). Combined TP were documented in 6/76 (8%) patients compared to 1/106 (1%) in controls (OR = 9.0, 95% CI: 1.1-76, p = 0.02). Second or third trimester fetal loss were more common cause of pregnancy termination in 37 patients with TP compared to 39 patients without TP (57/158 (36%) vs. 23/135 (17%) respectively, (p = 0.0004). Thrombophilic polymorphisms are common in women with fetal loss without apparent cause and are associated with late pregnancy wastage. Combinations of TP increase the risk for fetal loss.

Introduction

Recurrent fetal loss is a common health problem with three or more successive losses affecting 1-2% and two or more affecting up to 5% of women at the reproductive age (1). Data accumulated over the past two years suggest a possible association between thrombophilia and fetal loss (2). A clear association has been established between fetal loss and certain thrombophilic states such as antithrombin III deficiency and combined thrombophilia (3, 4). While several reports have suggested an increase in the prevalence of activated protein C resistance and factor V G1691A (Leiden) mutation in women with fetal loss (5-7), not all reports concerning fetal loss and factor V-Leiden mutation include data supporting this association (4). These uncertainties concerning the exact role of factor V-Leiden in fetal loss may have resulted from studies of the prevalence of these polymorphisms in groups of women who had no prior evaluation for other known causes of fetal loss (4, 8). In addition, the role of the most common thrombophilic polymorphism (TP), thermolabile methylene tetrahydrofolate reductase (TL-MTHFR) (9) and that of the recently described G20210A mutation in the prothrombin gene (Factor II G20210A) (10) in fetal loss have yet not been evaluated. We therefore aimed to investigate the prevalence of the three most common hereditary TP factor V-Leiden, TL-MTHFR C677T and factor II G20210A in women with fetal loss without apparent mechanical, anatomical, endocrinological or immunological cause.

Methods

Patients

Seventy-six consecutive women with recurrent fetal loss without known cause who were referred for evaluation to the Thrombosis and Haemostasis Unit, of the Rambam Medical Center during an 18 months period from 1/1/1997 to 30/6/1998, were included. Inclusion criteria were three or more first trimester (7-12 weeks of gestation) fetal loss, two or more second trimester (12-24 weeks of gestation) fetal loss or at least one intrauterine fetal death (IUFD) (above 24th week of gestation).

Only cases with post-embryonic loss after an ultrasonic disappearance of fetal pulse from the intrauterine fetal pole were included in the study. Documented first trimester preclinical and blighted ovum abortions were excluded. Pregnancy losses that were the result of documented fetal malformation or the result of an infectious complication were also excluded.

All women were in good general health without previous history of venous or arterial thromboembolic disease, diabetes mellitus or thyroid dysfunction. They all had a thorough investigation which was negative for potential causes of fetal demise including fasting glucose, basal FSH, LH and estradiol levels on day 3 of a natural cycle, TSH and prolactin levels and antinuclear factor. Screening tests for antiphospholipid antibodies included lupus anticoagulant sensitive aPTT, thromboplastin titration index, diluted Russel Viper Venom test, and IgG anticardiolipin. If one of these assays were positive, the patient was not included in the study. In addition, transvaginal scanning was performed to verify ovarian morphology. In addition, women with 3 or more first trimester or 2 or more second trimester pregnancy losses underwent a hysterosalpingography and/or hysteroscopy to confirm uterine cavity normalcy and both partners were also investigated for chromosomal aberrations. Women were not pregnant at the time of investigation and none of them were taking oral contraceptives.

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Table 1  Thrombophilic polymorphisms in women with fetal loss and controls

<table>
<thead>
<tr>
<th>Polymorphism*</th>
<th>Patients (76)</th>
<th>Controls (106)</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV G1691A</td>
<td>24 (32)</td>
<td>11 (10)</td>
<td>4.0</td>
<td>1.8-8.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FII G20210A</td>
<td>6 (8)</td>
<td>4 (4)</td>
<td>2.2</td>
<td>0.6-8.0</td>
<td>0.23</td>
</tr>
<tr>
<td>TL-MTHFR C677T</td>
<td>14 (18)</td>
<td>11 (10)</td>
<td>1.95</td>
<td>0.83-4.6</td>
<td>0.12</td>
</tr>
<tr>
<td>FV G1691A only</td>
<td>18 (24)</td>
<td>10 (9)</td>
<td>3.0</td>
<td>1.3-6.9</td>
<td>0.009</td>
</tr>
<tr>
<td>FII G20210A only</td>
<td>5 (7)</td>
<td>3 (3)</td>
<td>2.4</td>
<td>0.56-10.4</td>
<td>0.28</td>
</tr>
<tr>
<td>TL-MTHFR C677T only</td>
<td>8 (11)</td>
<td>9 (9)</td>
<td>1.3</td>
<td>0.47-3.5</td>
<td>0.64</td>
</tr>
<tr>
<td>Combined**</td>
<td>6 (8)</td>
<td>1 (1)</td>
<td>9.0</td>
<td>1.1-76.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Any</td>
<td>37 (49)</td>
<td>23 (22)</td>
<td>3.4</td>
<td>1.7-6.1</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*  FV G1691A - heterozygotes and homozygotes, FII G20210A - heterozygotes and homozygotes, TL-MTHFR C677T - homozygotes
** Combined: 5 patients heterozygotes for FV G1691A and homozygotes for TL-MTHFR C677T, 1 patient heterozygote for FV G1691A and FII G20210A and homozygote for TL-MTHFR C677T.

Results

The group of women with fetal loss, and control group were not different concerning age and ethnicity. Mean age of the women with fetal loss was 31 ± 5 years (range 19-44 years) and of the controls 31 ± 6 years (range 19-44 years).

The distribution of TP is presented in Table 1. Twenty-four of the 76 women with fetal loss (32%) had factor V Leiden mutation compared to 11/106 (10%) of controls (OR = 4.0, 95% CI: 1.8-8.8, p < 0.001) (Table 1). Five of the 76 patients (7%) were homozygotes for factor V-Leiden compared to none of the 106 controls (p = 0.012).

Factor II G20210A was found in 6/76 (8%) patients, 5 heterozygotes and 1 homozygote compared to 4/106 (3.8%), all heterozygotes, in controls (OR = 2.2 95% CI: 0.6-8.0, p = 0.23) (Table 1). Homozygosity for TL-MTHFR was documented in 14/76 (18%) of patients compared to 11/106 (10%) of controls (OR = 1.95, 95% CI: 0.83-4.6, p = 0.12) (Table 1). To analyze their specific contribution for fetal loss we have evaluated the risk involved for each TP alone (Table 1). The OR for factor V G1691A alone was 3.0 (95% CI: 1.3-6.9, p = 0.009). For factor II G20210A alone the OR was 2.4 (95% CI: 0.56-10.4, p = 0.28) and for TL-MTHFR the OR was 1.3 (95% CI: 0.47-3.5, p = 0.64).

Six of 76 (8%) women with fetal loss had more than one TP compared to 1/106 (1%) of controls (OR = 9.0, 95% CI: 1.1-76.4, p = 0.02). In 5 of the patients the combination was homozygosity for TL-MTHFR and heterozygosity for factor V-Leiden mutation and one patient had all 3 polymorphisms, heterozygosity for factor V-Leiden and factor II G20210A and homozygosity for TL-MTHFR. Thirty-seven of the 76 women with fetal loss (49%) had at least one TP compared to 23/106 (22%) in controls (OR = 3.4, 95% CI: 1.7-6.1, p = 0.0001) (Table 1).

Pregnancy outcome in the 76 women with fetal loss is presented in Table 2. The group of 76 women with fetal loss had a total of 359 pregnancies. Median number of pregnancies was 5 (range 1-21). Only 66 gestations (18.4%) ended by live birth. Mean number of fetal loss was 4 (range 1-20). Two hundred and thirteen (59.3%) resulted in first trimester abortion, 63 (17.5%) in second trimester abortion and 17 (4.8%) in intrauterine fetal death. Live birth occurred in 17.3% of gestations in patients with TP compared to 19.6% in gestations of patients without TP (p = 0.56). First trimester abortions were more common in patients without TP 112/135 (83%) compared to 101/158 (64%) in patients with TP (p = 0.0004). Second trimester abortions were more common in patients with TP 44/158 (28%) compared to 19/135 (14%) in patients without TP (p = 0.007). Likewise, intrauterine fetal death (IUFD) occurred in 13/158 (8%) of fetal losses in patients with TP compared to 4/135 (3%) of fetal losses in patients without TP (p = 0.09). Of the 158 fetal losses in women with TP, 57 (36%) ended by either second trimester miscarriage or intrauterine fetal death compared to only 23 of the 135 fetal losses (17%) in women without TP (p = 0.0004).

Discussion

The present report documents a clear association between factor V-Leiden mutation and fetal loss. Factor V-Leiden was significantly more common in patients with fetal loss compared to controls with a 4 fold increase in odd ratio and 95% CI of 1.8-8.8. Evaluation of the prevalence of factor V-Leiden as the sole TP revealed that the association with fetal loss hold true with a OR of 3.0 and 95% CI of 1.3-6.9. Factor V-Leiden mutation (13) is the most common thrombophilic marker in Caucasians and a founder effect has recently been suggested to explain its particular high prevalence in this population (14). The prevalence of 10% in the control group is representative for the population of North-
ern Israel (15, Brenner et al., unpublished). Of interest, 7% of the women with fetal loss were homozygote for factor V-Leiden compared to none of the controls (p = 0.012) and significantly higher than the prevalence of homozygosity for factor V-Leiden in Caucasians which is reported to be between 0.1-1.0% (6, 14, 15). Thus our results demonstrate for the first time that homozygosity for factor V-Leiden is strongly associated with fetal loss. Two recent case-control studies have documented an association between factor V-Leiden and fetal loss. The study by Grandone et al. (7) demonstrated presence of factor V-Leiden in 16% of women with 2 or more pregnancy loss compared to 4% in controls. Rüdker et al. (8) documented a 2.2 fold increase of factor V-Leiden in North American non-selected Caucasian women with fetal loss compared to controls (8% vs. 3.7%). The higher prevalence of factor V-Leiden in women with fetal loss in our study, 32% vs. 10% in controls, may result from different ethnic background as demonstrated by the higher prevalence of factor V-Leiden in our controls as well as from different selection criteria of the study group. Women with fetal loss in our study were selected following an extensive work-up, which had ruled out apparent mechanical, anatomical, endocrinological or immunological causes, while patients in other studies (8) were selected by being Caucasians with recurrent fetal loss.

The prevalence of homozygosity for TL-MTHFR was somewhat higher in women with fetal loss compared to controls (OR = 1.95) but this association did not reach statistical significance. Furthermore, analysis of the TL-MTHFR mutation alone resulted in reduction of OR to 1.3 (Table 1). Homozygosity for TL-MTHFR in the absence of hyperhomocysteinemia is, if at all, a mild risk factor for venous thromboembolism (16, 17). However, homozygosity for TL-MTHFR may predispose to increased homocysteine levels, particularly in the setting of low folate status, which is a common gestational finding (18).

Factor II G20210A was reported to be more common in Southern Europe (21) and in Israel (22) with a prevalence of 3% and 5.5% respectively, in agreement with the prevalence of 4% in our controls. Recently, a founder effect was suggested to explain the relatively high prevalence of factor II 20210A in Caucasians (22). Factor II G20210A was more common in women with fetal loss compared to controls, OR = 2.4, albeit this association did not reach significance (p = 0.28). This may be due to size of the study groups in view of the relatively low prevalence of the factor II G20210A polymorphism in women with fetal loss and controls. Studies involving larger number of patients are indicated to verify the potential association of fetal loss and factor II G20210A.

Forty-nine percent of women with fetal loss had at least one TP, compared to 23% of controls (Table I). The 3.2 fold increase prevalence of at least one TP in women with fetal loss reflects largely the differences in prevalence of factor V-Leiden mutation although TL-MTHFR and factor II G20210A mutations could also contribute to the observed difference. This can be seen by the 9-fold increase in odd ratio for fetal loss in patients with multiple TP (Table 1). The combination of TL-MTHFR and factor V-Leiden was more common than expected in the women with fetal loss group suggesting that this combination may potentially contribute to fetal loss. Multiple thrombophilic defects have been reported to be associated with an increased risk of venous thrombosis (11, 23), as well as with an increased risk for fetal loss in patients with factor V-Leiden, protein C, protein S and antithrombin III deficiency (4). Recently, both factor V-Leiden and TL-MTHFR were found to be individually associated with preeclampsia without effect of TL-MTHFR on the association of factor V-Leiden with preeclampsia (24). The present study is the first demonstration that multiple TP increases the risk for fetal loss.

Women with fetal loss in the present study had a median of 4 fetal loss with less than 20% of gestations resulting in live birth. While live birth occurred at a similar rate in the fetal loss group in women with or without TP (17.3% vs 19.6% respectively) (Table 2), timing of fetal loss differed between women with and without TP. In particular, while first trimester fetal loss was more common in patients without TP, second trimester fetal loss and intrauterine fetal death were significantly more prevalent in women with TP (Table 2). These results strengthen previous observations that second trimester miscarriages are more common in women with APC-resistance (5) and that intrauterine fetal death is more common in women with factor V-Leiden mutation (4). However, factor V-Leiden can be associated with first trimester fetal loss as well (8).

Taken together the data of the present report suggest that one of the 3 common TP can be found in approximately half of the women with a history of fetal loss of unknown cause following an extensive anatomic, endocrinologic, immunologic and infectious work-up.

References


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