Factor V Leiden and recurrent miscarriage—prospective outcome of untreated pregnancies

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BACKGROUND: Some cases of recurrent miscarriage and later pregnancy complications have a thrombotic basis. Factor V Leiden is a common thrombophilic mutation. METHODS: The prospective outcome of untreated pregnancies amongst 25 women heterozygous for the Factor V Leiden allele who had a history of either recurrent early miscarriages only (three or more miscarriages at <12 weeks gestation; n = 19) or of late miscarriage (>12 weeks gestation; n = 9) was studied. Control groups of women with a similar pregnancy history but who had a normal Factor V genotype were also studied. RESULTS: The live birth rate was significantly lower amongst women with a history of recurrent early miscarriage who carried the Factor V Leiden allele (6/16; 37.5%) compared with that amongst those with a normal Factor V genotype (106/153; 69.3%; odds ratio 3.75, 95% confidence intervals 1.3–10.9). The live birth rate was 11.1% (1/9) amongst those with a history of late miscarriage carrying the Factor V Leiden allele and 48.9% (22/45) amongst those with a normal Factor V genotype. CONCLUSIONS: Attention should be directed at screening women with recurrent miscarriage associated with placental thrombosis for Factor V Leiden and a policy of targeted thromboprophylaxis during future pregnancies should be assessed in the form of a randomized controlled trial.

Key words: Factor V Leiden/pregnancy outcome/prospective study/recurrent miscarriage

Introduction

Pregnancy is a hypercoagulable state (Stirling et al., 1984). Since antiphospholipid antibodies (aPL), an acquired thrombophilic defect, have been established as an important and treatable cause for pregnancy loss at all gestational ages (Rai et al., 1997), the potential role that other thrombophilic defects may play in adverse pregnancy outcome is under investigation. One such thrombophilic defect is Factor V Leiden, a common mutation (G→A at nucleotide position 1691 in the Factor V gene) which is associated with a significant increased risk for systemic venous thrombosis (Svensson and Dahlback, 1994; Dahlback, 1995). Factor V Leiden has also been reported in association with placental thrombosis (Rai et al., 1996; Dizon et al., 1997).

Existing data on the association between Factor V Leiden and pregnancy outcome is weak in three important aspects. Firstly, the prevalence of Factor V Leiden amongst women with recurrent miscarriage has been variously reported to be either increased or similar to that amongst parous controls (Balasch et al., 1997; Brenner et al., 1997; Dizon-Townson et al., 1997; Grandone et al., 1997; Metz et al., 1997; Kutteh et al., 1998; Ridker et al., 1998; Souza et al., 1999; Tal et al., 1999; Rai et al., 2001). This discrepancy may partly be explained by selection bias and the small numbers of women that have been included in some studies. Secondly, whilst placent al thrombosis in association with Factor V Leiden and other thrombophilic defects has been reported, this is neither a universal nor a specific finding (Mousa and Alfirevic, 2000) and finally, there are no data on the prospective outcome of untreated pregnancies amongst women with Factor V Leiden and a history of pregnancy loss. Despite these limitations, some pregnant women with Factor V Leiden and a history of recurrent miscarriage have been subjected to heparin thromboprophylaxis, together with its attendant risks, in an attempt to improve their chance of a successful pregnancy (Brenner et al., 2000; Younis et al., 2000).

The aim of this prospective observational study was to determine the pregnancy outcome of women with recurrent miscarriage who carry the Factor V Leiden allele and who receive no pharmacological treatment, except for folic acid, during pregnancy.

Materials and methods

This study was conducted between April 1998 and March 2001. The study population comprised 25 consecutive Caucasian women with a history of either recurrent early miscarriage only (three or more consecutive miscarriages at ≤12 weeks gestation; n = 16)
age 32 years (range 24–42); median number of miscarriages 3 (3–5) or a history of at least one late miscarriage (>12 weeks gestation; \(n = 9\)) [median age 28 years (range 20–35); median number of late miscarriages 1 (1–3)] who were heterozygous for the Factor V Leiden allele. The control populations consisted of 198 consecutive Caucasian women with a history of either recurrent early miscarriage only (\(n = 153\)) [median age 33 years (range 20–46); median number of miscarriages 3 (3–8)] or a history of at least one late miscarriage (\(n = 45\)) [median age 32 years (range 20–42); median number of late miscarriages 1 (1–3)] who had a normal Factor V genotype. All women had persistently negative tests for aPL, normal uterine anatomy and a normal peripheral blood karyotype, as did their partner. No woman in this study had a personal history of systemic venous thrombosis. There was no significant difference in either the age or number of previous miscarriages between women with Factor V Leiden and their appropriate control group.

Antiphospholipid antibody assays

All women were screened for aPL on at least two occasions >6 weeks apart prior to pregnancy. Lupus anticoagulant (LA) was detected using the dilute Russell’s viper venom time (dRVVT) together with a platelet neutralization procedure. Patient samples with a dRVVT ratio (test/control) of ≥1.1 were retested with a platelet neutralization procedure. A decrease of ≥10% in the ratio was considered to be positive for LA (Lupus Anticoagulant Working Party on behalf of the BCSH Haemostasis and Thrombosis Taskforce, 1991). Anticardiolipin antibodies (aCL) were identified using a standardized enzyme-linked immunosorbent assay. An IgG aCL level ≥5 GPL units and an IgM aCL ≥3 MPL units was considered to be positive (Khamashta and Hughes, 1993). Women with a positive test for LA or a positive aCL titre had a confirmatory test performed on a second sample taken at least 6 weeks after the initial sample. Women with persistently positive tests for either LA or aCL were considered to have the antiphospholipid syndrome and were treated with aspirin and heparin during pregnancy (Rai et al., 1997).

Factor V Leiden

Genomic DNA was extracted from EDTA whole blood using standard techniques. PCR using known primers was used to amplify exon 10 of the Factor V gene, which contains the G→A mutation at nucleotide position 1691 (Bertina et al., 1994). Following amplification, a 20 ml aliquot of the product was digested overnight with 5 IU of the enzyme MnlI (New England Biolabs, Hitchin, Herts, UK) at 37°C. Samples of the digested and undigested PCR product were separated electrophoretically in a 3% agarose gel and the bands visualized using ethidium bromide. The undigested PCR product measures 223 base-pairs (bp) in size. Following cleavage with MnlI, a normal allele produces bands of 37, 82 and 104 bp. A mutant allele produces bands of 82 and 141 bp due to loss of one MnlI cleavage site. Controls on each gel included a known heterozygote, a normal control known not to possess the Factor V Leiden allele and a water blank containing no input DNA.

Management during pregnancy

No woman received pharmacological treatment during pregnancy except for folic acid (400 mg/day) as prophylaxis against neural tube defects. All women attended a dedicated early pregnancy clinic on a weekly basis from 5 weeks of amenorrhoea until 14 weeks gestation. At these visits, ultrasound scans were performed to confirm fetal viability and to assess fetal growth.

Statistical analysis

Discrete variables were analysed using the \(\chi^2\)-test and continuous variables analysed using the Mann–Whitney \(U\)-test. Yates’s correction was used for cell values of ≤5. Survival data was analysed using the logrank test. \(P\)-values < 0.05 were taken as statistically significant.

Results

Recurrent early miscarriages

The live birth rate was significantly lower amongst women with a history of recurrent early miscarriages who carried the Factor V Leiden allele (6/16; 37.5%) compared with that amongst those with a normal Factor V genotype (106/153; 69.3%; odds ratio 3.75, 95% confidence intervals 1.3–10.9). Three woman in this cohort who carried the Factor V Leiden allele had a pregnancy loss after 12 weeks gestation, including one who had a severely growth-restricted stillborn infant delivered at 36 weeks gestation. In contrast, all miscarriages amongst those with a normal Factor V genotype occurred...
before 12 weeks gestation. The survival plot of the outcome of each pregnancy in this cohort of women who presented with recurrent early miscarriage is shown in Figure 1.

**Previous late pregnancy loss**

The live birth rate was 11.1% (1/9) amongst those with a history of late miscarriage carrying the Factor V Leiden allele and 48.9% (22/45; odds ratio 7.6, 95% confidence intervals 0.9–66.0) amongst those with a normal Factor V genotype. One woman in this cohort who carried the Factor V Leiden allele had a placental abruption at 28 weeks gestation and delivered a stillborn infant.

No woman in this study had a symptomatic venous thrombosis during the antenatal, intra-partum or post-partum periods.

Discussion

This is the first report of the prospective outcome of untreated pregnancies amongst a cohort of women with recurrent miscarriage who carry the Factor V Leiden allele. The finding that these women are at significantly increased risk for miscarriage compared with those with a normal Factor V genotype highlights the importance of thrombophilic defects in the aetiology of adverse pregnancy outcome. However, it is equally important to note that some women who carry the Factor V Leiden allele have uncomplicated pregnancies and that maternal carriage of this thrombophilic mutation does not therefore preclude a successful, uncomplicated live birth at term. The dual challenges raised by this study are (i) how to discriminate between those women with Factor V Leiden who are destined to miscarry or suffer a late pregnancy complication from those who will have a successful pregnancy and (ii) how should those women with Factor V Leiden identified as being at increased risk for future pregnancy loss be treated.

Pregnancy complications associated with Factor V Leiden and other genetic thrombophilic mutations (e.g. prothrombin G20210A and methylenetetrahydrofolate reductase C677T) are thought to be due to thrombosis of the uteroplacental vasculature. However, the relative risk for the development of systemic thrombosis associated with these mutations is much lower (heterozygous Factor V Leiden: 5-fold; heterozygous Factor II G20210A: 2-fold; homozygous MTHFR C677T: 2-fold) than that of previously identified haemostatic defects, such as heterozygous antithrombin deficiency (20–50-fold) (Cattaneo et al., 1997; Alhenc-Gelas et al., 1999; Lane and Grant, 2000). There has consequently been a shift in emphasis from the restrictive concept of single, dominant causes of thrombosis to emphasizing the potential role of multiple, inherited risk factors. This concept of the aetiology of systemic thrombosis is also likely to be applicable to placental thrombosis. Kupferminc et al. reported that whilst 52% (57/110) of women with an obstetric complication (pre-eclampsia, placental abruption or intrauterine growth restriction) carried one or more of three thrombophilic mutations (Factor V Leiden, prothrombin G20210A or methylenetetrahydrofolate reductase C677T) these mutations were also present in 17% of women with normal pregnancies (Kupferminc et al., 1999). Clearly not all women who carry a thrombophilic mutation suffer a pregnancy loss and perhaps it is those who carry multiple thrombophilic defects who are at greatest risk. This is supported by the results of a retrospective study which reported that the odds ratio for stillbirth was significantly higher amongst women with combined thrombophilic defects (14.3-fold) compared with those with single defects (between 2.0- and 5.2-fold) (Preston et al., 1996).

The contribution of the fetal genotype in determining pregnancy outcome demands further investigation. The placenta receives two arterial supplies (one maternal and one fetal) and placental infarction should only occur if both vascular supplies are compromised. The importance of this concept was illustrated in an earlier study which reported that placental infarction was significantly more often seen when the fetus carried the Factor V Leiden allele compared with when it had a normal Factor V genotype (Dizon et al., 1997).

Two small studies, from the same group of investigators, have reported that heparin thromboprophylaxis during pregnancy leads to a high live birth rate amongst women with a history of adverse pregnancy outcome and a thrombophilic defect (Brenner et al., 2000; Younis et al., 2000). However, both studies were uncontrolled and the results must therefore be interpreted with caution.

The results of our study suggest that attention should be directed at screening women with recurrent miscarriage associated with placental thrombosis for Factor V Leiden and a policy of targeted thromboprophylaxis during future pregnancies should be assessed in the form of a randomized controlled trial.

**References**


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