Case report

Factor V Leiden and fetal loss in a 33-year-old Tunisian woman

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Introduction

Fetal loss may sometimes be a consequence of congenital thrombophilia. A variety of disorders such as the presence of antiphospholipid antibodies, hyperhomocysteinaemia, antithrombin deficiency, protein S deficiency and activated protein C resistance, or factor V Leiden, may constitute risk factors for fetal loss [*1,2*].

Hereditary resistance to activated protein C is regarded as the most frequent cause of familial thrombosis [*3,4*]. This resistance to activated protein C stems from a mutation (the replacement of arginine by glutamine at amino acid position 506) in the factor V molecule-designated factor V Leiden-resulting from a single point mutation in the factor V gene leading to a G→A substitution at nucleotide position 1691 which cannot be inactivated by the protease [*5*].

The role of factor V Leiden as a risk factor in fetal loss is still controversial. Factor V Leiden has been considered as a minor risk factor in comparison with deficiencies in antithrombin, protein C or protein S [*1–3*]. The incidence of factor V Leiden among women who have experienced recurrent fetal losses, however, has been shown by some researchers to be higher than among women with no history of fetal loss [*6 9*]. Other researchers, however, have found no differences [*10*].

This is a case report of a woman with factor V Leiden as a unique abnormality. She experienced 2 fetal losses, had a successful pregnancy after treatment and then experienced yet another fetal loss.

Methods

The patient was a 33-year-old woman who experienced 2 fetal losses. The second abortus was autopsied together with placental examination. Maternal blood was collected and serological evaluation was carried out. Activated partial thromboplastin time (APTT) and kaolin clotting time tests were performed and antithrombin activity was determined by chromogenic method (Stachrom AT III, normal range: 80%–140%). Protein C and protein S antigen levels were measured with enzyme immunoassays (Asserachrom Protéine C and Asserachrom Protéine S, normal range: 60%–140%). Factor V level was determined by the manual chronometric method using deficient plasma in factor V (normal range: 60%–140%). Reagents, enzyme immunoassays and plasma were purchased from Diagnostica Stago SA, Asnières, France.

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The anticardiolipin antibodies (IgG and IgM isotypes) were determined by standardized enzyme immunoassay with normal values less than 5 GPL units or MPL units respectively (Sigma, St Louis, Missouri, United States of America). Lupus anticoagulants were screened by clotting techniques as per Exner et al. [*11*].

Resistance to protein C was measured by determining the activated partial thromboplastin times in the absence and presence of activated protein C (purified activated protein C, Diagnostica Stago, Asnières, France) [*12*]. The activated protein C sensitivity ratio was expressed as the ratio of the 2 values and then normalized to the ratio obtained with a reference plasma (minimal normal ratio values: 0.8–0.85). Genetic analysis of the factor V Leiden mutation (1691 G→A) was performed as previously described [*13*]. Maternal DNA extracted from whole blood was subjected to polymerase chain reaction (PCR) amplification. The PCR-product was then digested by restriction enzyme *Mnl* I. Gel analysis was performed on 2% agarose gel. Genetic analysis of the mutation in the prothrombin gene (20210 G→A) was also carried out with multiplex allele specific PCR.

Results

The 33-year-old woman had 2 fetal losses at 37 and 32 weeks of amenorrhoea. Neither tests nor evaluations were carried out for the first fetal loss at 37 weeks. The second fetal loss occurred at 32 weeks and the fetus, dead *in utero*, was autopsied and the placenta was examined. This revealed no anatomic abnormalities. No evidence for chromosomal abnormalities was found for further karyotype analysis. There was evidence, however, for ischaemic lesions of maternal origin as the placental villosities on the maternal interface had restricted lumina, some of them thrombosed, with large areas of fibrin thrombi.

Maternal serological evaluation was carried out and clotting tests were performed. APTT and kaolin clotting time were 14 and 39 seconds respectively, i.e. normal. Antithrombin, protein C, protein S and factor V levels were also normal. Screening studies for antiphospholipid antibody syndrome included assays for anticardiolipin antibodies and lupus anticoagulant. The anticardiolipin antibodies were determined by standardized enzyme-linked immunosorbent assay (IgG 4 units GPL and IgM 4 units MPL; normal < 5 units for both). Plasma was also evaluated for lupus anticoagulant activity with clotting techniques; no evidence of such lupus anticoagulant activity was found. The functional assay for activated protein C resistance gave evidence for an activated protein C sensitivity ratio of 49.6/34.3, or 1.44. The normalized activated protein C sensitivity ratio—obtained by normalizing the activated protein C sensitivity ratio to the ratio obtained with a reference plasma—was 1.44/2, or 0.72 (minimum normal ratio values: 0.8–0.85). Maternal DNA extracted from whole blood was subjected to PCR amplification. The PCR product was then digested by *Mnl* I. Gel analysis revealed a heterozygote genotype for the 1691 G→A mutation with the loss of a cleavage site for *Mnl* I in one of the alleles of the factor V gene as a consequence. We found this mutation to be associated with activated protein C sensitivity ratio resistance, i.e. occurrence of a factor Va molecule that is not properly inactivated by activated protein C. Genetic analysis of the prothrombin gene revealed a normal heterozygote genotype.

After the second fetal loss, the patient received acetylsalicylic acid at 100 mg per day. She became pregnant 3 months later; in addition to acetylsalicylic acid, she then received enoxaparin sodium at 20 mg per day during the pregnancy. At 35 weeks, she underwent caesarean section and gave birth to a baby girl. After this successful experience, she became pregnant again but had a spontaneous abortion during the first trimester. She is presently pregnant at 33 weeks of amenorrhoea and is again undergoing treatment with acetylsalicylic acid and enoxaparin sodium.

Discussion

A placental infarct is an area of placental parenchyma that has undergone ischaemic necrosis. We had evidence that our patient had placental thrombi resulting in placental infarction on the maternal side of the placental unit. Thrombotic events in the placenta have been associated with maternal hypercoagulable states including deficiencies of antithrombin III, protein C, protein S and antiphospholipid syndrome [*14,15*]. Increased risk for fetal loss was also reported for women with heritable thrombophilia including combined deficiencies of antithrombin, protein C, protein S and factor V Leiden. Indeed, the factor V Leiden mutation was associated with a 2-fold increase in risk for third-trimester pregnancy loss associated with intrauterine death [*2*]. This agreed with our observations in the present case.

Bertina et al. reported that 80% of individuals with normalized activated protein C sensitivity ratio < 0.84 and 100% of those with it < 0.70 were heterozygotes or homozygotes for the mutation [*5*]. In our patient, the normalized activated protein C sensitivity ratio was 0.72 and the genotype was a heterozygote for the 1691 G→A mutation.

After the second fetal loss, our patient was given a treatment analogous to patients with antiphospholipid syndrome, i.e. acetylsalicylic acid and enoxaparin sodium. This treatment may account for the success of the third pregnancy and may confirm the importance of thromboprophylaxis on pregnancy outcome in patients with recurrent pregnancy loss associated with factor V Leiden mutation [*9,16,17*]. The treatment was ineffective insofar as the patient subsequently experienced a further miscarriage. This third miscarriage, however, might not have been related to factor V Leiden inasmuch as it occurred during the first trimester [*1*]. It has been reported recently that no significant difference was found in the occurrence of activated protein C resistance and factor V Leiden mutation in women with first or second trimester recurrent pregnancy loss [*8*]. The efficacy of thromboprophylaxis must be further confirmed.

For this woman, the factor V Leiden mutation associated with activated protein C resistance was a unique abnormality, likely to generate a hypercoagulable state and causing thrombosis to occur. Although the cause of recurrent pregnancy loss in most cases is unknown, our observations suggest that hypercoagulability and placental infarction due to the factor V Leiden phenotype could be etiologic factors.

Conclusion

Fetal losses at 37 and 32 weeks of amenorrhoea and the presence of placental infarcts in a mother with factor V Leiden but with no other known abnormalities suggests an association between factor V Leiden and fetal loss.

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