Activated protein C resistance shows an association with pregnancy-induced hypertension

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A common mutation in the factor V gene, the Leiden mutation, is the most frequent genetic cause of resistance to activated protein C (APC). Recent studies have shown that the prevalence of APC resistance is associated with severe pregnancy-induced hypertension (PIH). Our objective was to determine whether the factor V Leiden mutation is more prevalent in patients who developed severe PIH than in normotensive pregnant women. In 70 women with a history of severe PIH, of whom 15 had pre-eclampsia, we investigated common coagulation factors as well as APC resistance (factor V related). We found that seven of these 70 women showed low values for APC. Out of these, five were heterozygous and none was homozygous for factor V Leiden mutation. In a control group of normotensive pregnant women we found a 3.0% rate of APC resistance and a 3.0% rate of carriers of the Leiden mutation. These results indicate a significantly higher prevalence of both APC resistance and factor V Leiden mutation in women with severe PIH. Placental infarctions and micro-embolisms are considered to be one of the principle pathophysiological changes in severe PIH. Our results suggest that APC resistance is a risk factor for severe PIH, in addition to its well-known role in macrothrombo-embolism.

Key words: APC resistance/factor V Leiden mutation/placental infarction/pre-eclampsia/pregnancy-induced hypertension

Recent studies have shown that the heterozygous carrier rate of the Leiden mutation depends on ethnic origin. The carrier rate ranges from about 15–20% in Scandinavian countries (Hallam et al., 1991) to an overall rate of about 4.4% in other European countries, to 0.6% in Asia Minor, while it is thought to be absent in Africa and Australia (Rees et al., 1995). The incidence of this mutation in an obstetric population in Utah, USA, was 3% (Dizon-Townson et al., 1997), thus corresponding with the overall European carrier rate. Similar investigations have suggested a carrier rate for the Leiden mutation of 4.2% in a group of normotensive pregnant women (Dizon-Townson et al., 1996).

Heterozygous carriers of the Leiden mutation have an up to 10-fold higher risk for thrombo-embolic events. Carrier rates in individuals with a personal history of thrombosis range up to 40%; in those affected, events during pregnancy occur in up to 60% (Koster et al., 1993; Svensson and Dahlbäck, 1994; Hellgren et al., 1995). The risk of thrombosis among women who are carriers of this mutation and who are users of oral contraceptives is increased more than 30-fold compared to non-carriers who do not take oral contraceptives (Vandenbroucke et al., 1994).

We have investigated the prevalence of the Leiden mutation in a population of women in Berlin, Germany, with pregnancy-induced hypertension (PIH) in order to compare this prevalence with carrier rates in a group of normotensive women and with rates quoted in other studies (Dekker et al., 1995; Dizon-Townson et al., 1996; Lindoff et al., 1997). Additionally, we looked for common clinical characteristics of women with PIH.

Materials and methods

Study population

The study was approved by the local Ethics Committee of the Faculty of Medicine, Charité, Humboldt University, Berlin.

We investigated 70 women who had been treated for PIH in the Department of Obstetrics, Berlin, between January 1991 and December 1997.

All women had PIH according to the diagnostic criteria described by the German Section of the International Society for the Study of Hypertension in Pregnancy (ISSHP); PIH was defined as hypertension without proteinuria which occurs after the 20th gestational week and lasts no longer than 6 weeks post-partum. Of the 70 patients, 15 had proteinuria according to the ISSHP definition (one 24 h urine collection with a total protein excretion of ~300 mg, or two ‘catch-catch midstream’ or catheter specimens of urine collected 4 h apart with 1 mg albumin per litre or 2+ or more on reagent strip). Those patients with proteinuria were defined as having pre-eclampsia. Four women developed HELLP (haemolysis, elevated liver function tests and low platelet count) syndrome. All women classified as having

Introduction

The factor V Leiden mutation is the most common genetic cause of resistance to activated protein C (APC) and has recently been associated with severe pre-eclampsia (Dekker et al., 1995; Dizon-Townson et al., 1996; Lindoff et al., 1997) and other obstetric complications (Kupferminc et al., 1999). The factor V Leiden mutation is related to a single point mutation of G to A at nucleotide position 1691 in the gene encoding for coagulation factor V. This mutation results in the substitution of arginine by glutamine at position 506 and thereby causes a poor anticoagulant response to activated protein C (i.e. APC resistance) (Bertina et al., 1994; Dahlbäck, 1995).
PIH including those with pre-eclampsia and HELLP syndrome had a diastolic blood pressure ~110 mm Hg on two or more occasions. None of the women had had hypertension before the 20th gestational week or before pregnancy.

All women who delivered between January 1991 and December 1997 were asked to return for a single post-partum visit. All study participants were interviewed about their blood pressure, their current medication, and their personal and family history of thrombo-embolic events. Additionally, medical charts were reviewed and patient demographics including age, weight, height, gravidity, parity, mode of delivery and personal and family history of hypertension, were recorded. Fetal outcome data including sex, weight, length, head circumference, Apgar scores, umbilical artery and vein pH were tabulated.

Control group

A control group was established to find out the prevalence of APC resistance in a group of normotensive pregnant women. Between January and May 1998, blood from 100 pregnant women was drawn during ante-natal visits to the Department of Delivery for routine tests. These women did not have elevated blood pressure, i.e. not above 140/90 mm Hg. Laboratory tests for APC resistance (including the accelerin inactivation test, see below) and polymerase chain reaction (PCR) for the factor V Leiden mutation were performed.

Coagulation tests

Platelet-poor citrated plasma was obtained from the 70 women entering the study. The following parameters of coagulation were investigated: PT (prothrombin time) activated partial thromboplastin time (aPTT), thrombin time, fibrinogen, antithrombin III (AT III), von Willebrand antigen, ristocetin cofactor, protein C and S, APC resistance and accelerin inactivation.

Polymerase chain reaction

The factor V Leiden mutation was verified by using appropriate primers and red blood cell DNA as previously described (Bertina et al., 1994).

APC resistance and accelerin inactivation test

APC resistance was determined using the Coatest APC Resistance kit® according to the original instructions provided by the manufacturer (Chromogenix, Mölndal, Sweden). In addition, the accelerin inactivation test was used to detect factor V Leiden-related APC resistance even in patients undergoing anticoagulation therapy (Hintz et al., 1995). In contrast to the APC procedure, the patients’ standard and control plasmas were diluted 1:20 with barbitol buffer to a pH of 7.6 (Behringwerke, Marburg/Lahn, Germany) and genuine human factor V-deficient plasma (Immuno, Heidelberg, Germany) was added. From each patient’s blood sample, 50 µl of plasma (1:20 diluted) was mixed with 50 µl of factor V-deficient plasma and 50 µl of APTT reagent which was then incubated for 7 min at 37°C. Thereafter, 50 µl of undiluted APC-CaCl2 solution was added. The times of analysis were measured with the Coa Screener coagulometer (Labor, Ahrensburg, Germany).

To establish a reference curve, the APC-CaCl2 solution was diluted with CaCl2 in a concentration of 0.025 mol/l. The degree of dilution of the APC solution was defined as accelerin inactivation (AK-INAKT) and was described as a percentage. Accordingly, APC solutions of 1:1 (undiluted), 1:2, etc. corresponded to 100%, 50%, etc. AK-INAKT. As a reference standard, pooled plasma from 15 healthy blood donors without a history of thrombophilia was used. The current experimental set-up was similar to that described for previous investigations (Hintz et al., 1995) except that, in this study, pooled plasma was used. The clotting times (in seconds) were plotted in the log/log divided co-ordinate scheme. The percentage of AK-INAKT for the clotting times of the patients’ plasma in seconds was read off from the curve.

Results

APC resistance frequencies of 10.0% (7/70) in the PIH group versus 3.0% (3/100) in the control group were found. Five of the 70 women in the PIH group carried the factor V Leiden mutation (all heterozygotes, no homozygous mutants). Two of the seven women in this group with APC resistance showed low values of APC resistance but no reduced accelerin inactivation tests; both of these women were not carriers of the Leiden mutation. In the control group we found a 3.0% carrier rate for the factor V Leiden mutation (three heterozygotes, no homozygotes). All women were of German origin.

The two study groups were well matched and there were no significant differences regarding gravidity (Table I). The median maternal age in the PIH group was 28 years (range 16–40) and 30 years (range 16–43) in the control group.

In the PIH group 63 (90%) women were Europeans; 53 were from Germany (75.7% of the whole group), nine from Turkey (12.9%), and one from Yugoslavia (1.4%); seven women (10.0%) were born in other countries. In comparison, the control group showed that 77 women originated from Germany (77.0%), 14 women from Turkey (14.0%), three women from Yugoslavia (3.0%) and six from other countries (6.0%).

In the PIH group the non-carriers of the factor V Leiden mutation showed neither antithrombin III nor protein C deficiency. Low levels of protein S (<65%) were measured in 19 pregnant women; this finding was consistently present 1–2 days post-partum. Thus, this should be regarded as a pregnancy-induced deficiency of protein S. In the group without APC resistance, 11 had elevated fibrinogen concentrations (>450 mg/dl, maximum: 690 mg/dl) which could be linked to gravidity. Among women without APC resistance, the median APC ratio was 2.7 (±0.4; normal >2.0) versus a ratio of 2.6 (±0.35) in the control group.

In the group of women who had PIH and showed APC resistance (Table II), the median gestational age was 37 ± 3.7 weeks (range 30–39), the mean maternal age 29.6 (range 25–35), the median gravidity 2.4 ± 2.2 (range 1–7) and the median parity 1.6 ± 0.8 (range 1–3). We found a median diastolic blood pressure of 115 mm Hg. The Caesarean section rate was 42.9%.

| Table I. Gravidity of the study and control groups expressed as percentages |
|----------------|----------------|----------------|
| Gravidity | Study group | Control group |
|   1    | 52.9 (n = 37) | 55.0 (n = 55) |
|   2    | 15.7 (n = 11) | 15.0 (n = 15) |
|   3    | 14.3 (n = 10) | 14.0 (n = 14) |
| >4    | 17.1 (n = 12) | 16.0 (n = 16) |
In the same investigation, a heterozygous carrier rate of 8.9% for factor V Leiden was found in 158 women with severe pre-eclampsia. The patient population and results were similar to ours, although only 15 women in our study population had pre-eclampsia according to the ISSHP classification. We focused on patients with PIH and found a greater prevalence of decreased APC ratios in this patient group with a history of PIH compared to a control group. Seven out of 70 women in the current study with a history of PIH showed a reduced response to APC. Five of these were heterozygous carriers of the factor V Leiden mutation. No carrier of the Leiden mutation was missed by our accelerin inactivation test.

Recently, Kupferminc et al. (1999) showed an overall higher frequency of genetic thrombophilia in women with pregnancy complications. The incidence of factor V Leiden carriers among women with severe pre-eclampsia was 20.0% compared to a rate of 6.0% in a group of women with uneventful pregnancy.

We did not find unexpected laboratory tests within the group of women with PIH. All values which differed from normal values could be explained by physiological changes during pregnancy (Öhian, 1997). Therefore, we did not perform laboratory tests other than tests for APC resistance, accelerin inactivation and PCR for factor V Leiden mutation in our control group.

Other risks for women with the factor V Leiden mutation include an up to 10-fold higher overall risk for thrombosis (Koster et al., 1993), as well as a 30% higher risk for thrombosis during oral contraception use (Vandenbroucke et al., 1994), and a higher risk for thrombosis during in-vitro fertilization (IVF) treatment (Horstkamp et al., 1996a,b).

Our investigation indicates a higher prevalence of APC resistance in women with pregnancy-induced hypertension. As APC resistance is linked to thrombosis and other serious complications leading to maternal morbidity, our study supports the idea of screening tests for APC resistance in women with such problems during pregnancy. We suggest that knowledge of patients with the factor V Leiden mutation might and should alter the treatment, e.g. by adding low-dose aspirin and following these women even more closely throughout their pregnancy. However, the high variation of prevalence of the factor V Leiden mutation in different ethnic groups requires its definition before screening.

### Acknowledgements

The authors wish to thank the clinical staff of the Department of Obstetrics and the laboratory staff of the Department of Internal Medicine.

### References


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Received on March 2, 1998; accepted on September 7, 1999