The HELLP syndrome. Clinical course, underlying disorders and long-term follow-up
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High prevalence of hemostatic abnormalities in women with a history of severe preeclampsia

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Abstract

In patients with a history of severe preeclampsia an increased frequency of hemostatic abnormalities has recently been suggested in small studies without control groups. Our purpose was to investigate the prevalence of such abnormalities in a large patient group with a history of severe hypertensive disorder in pregnancy in comparison with an appropriate control group.

A total of 345 patients with a history of severe preeclampsia were investigated at a minimum of 10 weeks post partum for the presence of activated protein C resistance, the associated factor V mutation, hyperhomocysteinemia and anticardiolipin antibodies. The control group consisted of 67 healthy women with a history of uncomplicated pregnancies only. Blood was obtained during the second half of a normal menstrual cycle and none of the patients or controls used oral contraceptives.

Of all patients 11.3% had activated protein C resistance (controls subjects 1.5%, p= 0.025). Only half of these patients had the factor V mutation. Hyperhomocysteinemia was present in 12.1% of all patients in comparison with 4.5% in the control group (p=0.115). Anticardiolipin antibodies were observed in 20.9% of the patients, whereas these antibodies were found in 7.5% of the control subjects (p=0.016). In general the prevalence of these abnormalities was 1.5 to 2 times higher in patients who were delivered before 28 weeks, in comparison with patients who were delivered after 28 weeks.

Hemostatic abnormalities, associated with an increased risk of thrombosis are present in approximately 40% of patients with a history of severe preeclampsia, which is almost four times higher than in control subjects. These findings might suggest a cause of preeclampsia and could have implications in subsequent pregnancies and general health.
Introduction

Preeclampsia, with its reported incidence of about 5% of all deliveries, is a major cause of maternal and perinatal morbidity and death. The acronym HELLP syndrome (Hemolysis, Elevated Liver enzymes and Low Platelet count) was introduced by Weinstein in 1982 as a way of describing a particular group of women with preeclampsia. The causes of both preeclampsia and HELLP syndrome are still unknown, but in recent years many studies have demonstrated that endothelial cell dysfunction is intimately involved in their pathogenesis. Severe early-onset preeclampsia has been associated with disorders which increase the risk of thrombosis. It has recently been suggested that the relationship between inherited coagulation abnormalities leads to a prethrombotic state and an increased risk of fetal loss, but this association with early fetal loss remains controversial. Resistance to activated protein C may contribute to the pathogenesis of preeclampsia. The factor V Leiden mutation may result in a predisposition to severe preeclampsia.

Hyperhomocysteinemia, which is associated with an enhanced risk of arterial and venous thrombosis, has also been described as a risk factor in women with preeclampsia, as well as in those with unexplained recurrent early pregnancy loss or abruptio placentae. Since the 1980’s the presence of anticardiolipin antibodies has been associated with adverse perinatal outcome. It is currently unclear whether these hemostatic abnormalities are indeed found more frequently in patients with a history of preeclampsia. Therefore we initiated this study (1) to assess the prevalence of hemostatic abnormalities, which are associated with an increased risk of thrombosis, in a large group of consecutive patients with a history of severe preeclampsia and (2) to compare prevalence in this group with the prevalence of these factors in a control group of women with a history of uncomplicated pregnancies only. If the results of this study confirm a higher prevalence of hemostatic abnormalities, treatment aimed at correcting the underlying abnormalities could have implications for future pregnancies and for public health.

Material and Methods

The patient group, recruited between January 1995 and October 1996, consisted of women, consecutively examined at the departments of obstetrics and gynecology in the Academical Medical Center and the Free University Hospital in Amsterdam, all with a history of severe preeclampsia (defined as a diastolic blood pressure of ≥ 110 mm Hg and the presence of proteinuria (urinary protein concentration ≥ 0.5 g/L) diagnosed before 34 weeks’ gestation, with delivery before 36 weeks’ gestation) or with the HELLP syndrome (as determined by
the following simultaneous measurements: lactate dehydrogenase > 600 U/L, serum aspartate aminotransferase or alanine aminotransferase > 50 U/L, and platelet count < 100x10⁹/L), or with eclampsia irrespective of gestational age of the foetus. Those patients with preexisting hypertension, vascular or renal disease or diabetes were excluded. The control group, recruited by advertisement between January 1995 and October 1996, consisted of healthy, fertile women with normal menstrual cycles who were between the ages of 20 and 40 years. Their medical history was uncomplicated, they had no history of miscarriage or ectopic pregnancy, and all their pregnancies were uneventfully carried and resulted in the delivery of an infant with a normal birth weight according to the Amsterdam birth weight chart. Patients and control subjects were investigated from 10 weeks post partum in the second half of a normal menstrual cycle, for the presence of activated protein C resistance, factor V mutation, hyperhomocysteinemia and anticardiolipin antibodies. Most were tested at more than 6 months post partum. The interval between delivery and investigation was similar for both patients and control subjects. Patients and control subjects did not use oral contraceptives or were asked to stop doing so at least 8 weeks before testing. None took vitamins known to influence homocysteine metabolism within 6 months before investigation. We divided the patient population into 2 groups: those who were delivered before 28 weeks (early patient group) and those who were delivered after 28 weeks of gestation.

Informed consent was obtained from all participants and the study was approved by the institutional review boards.

Laboratory tests
Resistance to activated protein C was assessed by the activated protein C-dependent prolongation of the activated partial thromboplastin time, performed in both the absence and the presence of activated protein C (Coated APC Resistance, Chromogenix, Mölndal, Sweden). Results were expressed as the ratio of the 2 values. An activated protein C sensitivity ratio ≤ 2.0 was considered to represent true resistance to activated protein C. If the ratio was > 2.7, the presence of factor V mutation was searched for by standard polymerase chain reaction assay. Women with an activated protein C ratio > 2.7 were considered not to have factor V mutation.

Hyperhomocysteinemia was assessed both during fasting and after methionine loading. At 8 AM, after an overnight fast, we obtained a blood sample (with ethylenediaminetetraacetic acid used for anticoagulation) for determination of fasting homocysteine concentration. After being centrifuged for 10 minutes at 1800 G, plasma was stored at -30 C until analysis. Subsequently, an oral dose of L-methionine (0.1 g/kg body weight) was
administered in orange juice. During the test a standardized methionine-poor breakfast and lunch (containing 14 mg methionine per gram protein) were given. Six hours after the methionine load, a second blood sample was drawn for determination of homocysteine concentration. Total (free plus protein bound) homocysteine concentration (disulfide homocysteine plus mixed disulfides) was measured by established methods.\(^{16,17}\) Patients were considered to have hyperhomocysteinemia if homocysteine concentration at baseline or 6 hours after methionine loading exceeded the 97.5 percentile of that observed in healthy premenopausal women.\(^4\) Anticardiolipin antibodies were determined by enzyme-linked immunosorbent assay according to the Harris directives.\(^{18}\) Results were measured spectrophotometrically (492 nm), and expressed as \(\geq 10\) (low positivity or higher) or \(\geq 20\) (moderately or highly positive) immunoglobulin G (IgG) or M (IgM) anticardiolipin antibodies.

**Statistical analysis**

Data were analyzed using BMDP (Los Angeles, Calif) statistical software. Differences between groups were tested with a 2-sided method by use of the chi-square test or Student t test as appropriate. Statistical significance was considered at \(p < 0.05\). Trend analysis between categories was performed by chi-square.

**Results**

A total of 158 patients met the criteria for severe preeclampsia, 164 had HELLP syndrome and 23 had a history of eclampsia during the index pregnancy. Of the 345 patients 81% were primiparous. Because no differences in the studied indices were observed among the different patient groups (preeclampsia, HELLP or eclampsia) results for the entire patient group were combined. Sixty-seven healthy women served as control subjects, and 44% of them were primiparous.

The prevalence of activated protein C resistance in the control group was 1.5%. In all patients, activated protein C resistance was present in 11.3%, with the prevalence almost twice as high in those who were delivered before 28 weeks of gestation in comparison with those who were delivered later (Table 1). One patient had a homozygote mutation of factor V and 16 patients were heterozygous (6%). In the control group a factor V mutation (heterozygous) was confirmed in a single patient with activated protein C resistance (prevalence 1.5%). In contrast, this mutation was only found in half of the patients with activated protein C resistance. Of the control subjects, 4.5% were found to have hyperhomocysteinemia, though this was present in 12.1% of all patients (\(p=0.115\)). In patients who had delivered before 28 weeks, hyperhomocysteinemia was found in 19%.
Anticardiolipin antibodies were present in 7.5% of the control subjects, with a 3- to 4-fold higher prevalence in the patient subgroups (Table 1). A statistically significant difference between patients and control subjects was observed at an anticardiolipin-antibody cutoff point of $\geq 10$ IgG or IgM, whereas at $\geq 20$ IgG or IgM the difference was not statistically significant. Table 2 summarizes odds ratios with 95% confidence intervals for the studied defects, in comparison with control subjects, in those patients who were delivered before week 28 of gestation.

None of the control subjects had a combination of the investigated defects, whereas 2 or more abnormalities were seen in 17.6% of the early patient group and in 6.7% of the patients who were delivered after week 28 of gestation (Table 3).

**Table 1.** Prevalence of activated protein C resistance (APCR), Factor V Mutation (FVM), Hyperhomocysteinemia (HHC) and Anticardiolipin Antibodies (ACA) $\geq 10$ and $\geq 20$ GPL or MPL in controls and patients with a history of severe preeclampsia.

<table>
<thead>
<tr>
<th>Defect</th>
<th>Controls</th>
<th>Patients $&lt; 28$ weeks</th>
<th>Patients $\geq 28$ weeks</th>
<th>All Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>APCR **</td>
<td>1/67</td>
<td>9/50</td>
<td>23/234</td>
<td>32/284</td>
</tr>
<tr>
<td>(1.5%)</td>
<td>(18.0%)*</td>
<td>(9.8%)*</td>
<td>(11.3%)*</td>
<td></td>
</tr>
<tr>
<td>FVM</td>
<td>1/67</td>
<td>4/50</td>
<td>13/234</td>
<td>17/284</td>
</tr>
<tr>
<td>(1.5%)</td>
<td>(8.0%)</td>
<td>(5.6%)</td>
<td>(6.0%)</td>
<td></td>
</tr>
<tr>
<td>HHC **</td>
<td>3/67</td>
<td>11/58</td>
<td>24/231</td>
<td>35/289</td>
</tr>
<tr>
<td>(4.5%)</td>
<td>(19.0%)*</td>
<td>(10.4%)</td>
<td>(12.1%)</td>
<td></td>
</tr>
<tr>
<td>ACA $\geq 10$ GPL/MPL **</td>
<td>5/67</td>
<td>17/62</td>
<td>50/259</td>
<td>67/321</td>
</tr>
<tr>
<td>(7.5%)</td>
<td>(27.4%)*</td>
<td>(19.3%)*</td>
<td>(20.9%)*</td>
<td></td>
</tr>
<tr>
<td>ACA $\geq 20$ GPL/MPL</td>
<td>1/67</td>
<td>1/62</td>
<td>9/259</td>
<td>10/321</td>
</tr>
<tr>
<td>(1.5%)</td>
<td>(1.6%)</td>
<td>(3.5%)</td>
<td>(3.1%)</td>
<td></td>
</tr>
</tbody>
</table>

* chi square analysis $p < 0.05$ versus controls
** significance for trend analysis comparing controls with the 2 subgroups of patients

**Table 2.** Odds ratios for the presence of a hemostatic abnormality in women with a history of severe preeclampsia who delivered before 28 weeks of gestation as compared to controls

<table>
<thead>
<tr>
<th>Defect</th>
<th>Odds Ratio</th>
<th>95% limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>APCR</td>
<td>14.5</td>
<td>1.7 - 322</td>
</tr>
<tr>
<td>FVM</td>
<td>5.7</td>
<td>0.6 - 140</td>
</tr>
<tr>
<td>HHC</td>
<td>4.9</td>
<td>1.2 - 24</td>
</tr>
<tr>
<td>ACA 10</td>
<td>4.7</td>
<td>1.5 - 16</td>
</tr>
</tbody>
</table>
Table 3. Prevalence of one or more abnormalities of APCR, FVM, HHC, ACA in patients with a history of severe preeclampsia and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Single abnormality</th>
<th>Two or more abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 28 weeks</td>
<td>≥ 28 weeks</td>
</tr>
<tr>
<td></td>
<td>15/51 (29.4%) *</td>
<td>54/210 (25.7%) *</td>
</tr>
<tr>
<td>Controls</td>
<td>9/67 (13.4%)</td>
<td>0/67 (---)</td>
</tr>
<tr>
<td></td>
<td>9/51 (17.6%) *</td>
<td>14/210 (6.7%) *</td>
</tr>
</tbody>
</table>

* chi square analysis p < 0.05 versus controls

No differences of birth weight were observed between the infants born of patients with or without underlying disorders. Perinatal death was more frequent in patients with activated protein C resistance than in patients without activated protein C resistance (odds ratio 2.6, 95% CI 1.0-6.6). Moreover, in patients with anticardiolipin antibodies, perinatal death was more frequent than in patients without anticardiolipin antibodies (odds ratio 2.0, 95% CI 0.95-4.2). Patients with or without the other abnormalities had similar perinatal mortality rates.

Discussion

This study demonstrates that in consecutive patients with a history of severe preeclampsia the hemostatic abnormalities known to be associated with increased thrombotic risk are present in approximately 40% of cases (Table I). In those who were delivered before 28 weeks of gestation, this figure was found to be as high as 60%. These figures are 4 to 6 times higher than those observed in a control group. Half of the women with hemostatic abnormalities had anticardiolipin antibodies, one fourth had activated protein C resistance, and one fourth had hyperhomocysteinemia. It is to some extent surprising that there were no differences in the studied coagulation indexes among the different patient groups (preeclampsia, HELLP or eclampsia). It should be noted, however, that we did not study platelet function. The prevalence of these abnormalities, as well as for the factor V mutation, observed in the control group is consistent with earlier reports on healthy subjects. However, it should be realized that the control group differed from the general population because we included only women with a normal menstrual cycle, not taking oral contraceptives, who had completed their pregnancies uneventfully. All women with a history of any perinatal complications such as preterm labor, fetal death, or miscarriage, were excluded for optimal to the patient group. The strict criteria for inclusion in the control group limited the number to 67 women. As a result, some of the individual comparisons of the prevalence of the studied abnormalities did not reach statistical significance. However, the odds ratios observed in those patients
delivered before 28 weeks of gestation are similar for the factor V mutation, hyperhomocysteinemia and anticardiolipin antibodies, indicating a comparable importance (Table 2). In those patients with activated protein C resistance, only half had a factor V mutation as determined by the polymerase chain reaction assay. Thus activated protein C resistance not associated with a mutation in factor V appears to be more prevalent in women with a history of severe preeclampsia, which is in concordance with observations of patients with cerebral ischemic disorders. Activated protein C resistance without factor V mutation could not be attributed to the use of oral contraceptives or to a recent pregnancy. Furthermore, the results of this study indicate that a combination of ≥ 2 of the hemostatic abnormalities that we studied were present in about a fourth of the patients, whereas this did not occur in the control group. Although some of the abnormalities investigated, in particular anticardiolipin antibodies and activated protein C resistance, have been implicated in the pathogenesis of early fetal loss, little is known about the relevance of these abnormalities to patients with hypertensive disorders in pregnancy. Our findings are in agreement with our earlier observations in a small set of patients with severe early-onset preeclampsia, a study without a control group. The mechanism by which the presence of prethrombotic abnormalities contribute to the development of preeclampsia is unknown. The promotion of local microthrombosis in the developing uteroplacental arteries may induce endothelial cell dysfunction. This possibility, however, remains speculative. It is noteworthy, however, that recent randomized clinical trials in patients with anticardiolipin antibodies and who had recurrent miscarriages indicated that prophylaxis with unfractionated heparin, in combination with low doses of aspirin, improved perinatal outcome. It remains to be demonstrated in a comparative trial whether treatment of women with a history of preeclampsia and with hemostatic abnormalities will improve perinatal outcome in subsequent pregnancies.

This study demonstrates that preeclampsia could be a first manifestation of a thrombotic disorder. These patients should be evaluated for thrombotic disorders because knowledge of such disorders could have consequences for their general health, including the risk of postpartum venous thromboembolism and oral contraceptive-associated thromboembolism.

Acknowledgments: we are grateful to the many patients and controls who made this study possible. We highly valued the help of Dr.M.H. Prins for the statistical analysis and O.P. Bleker and H.P. van Geyn for support.
References
