Do placental lesions reflect thrombophilia state in women with adverse pregnancy outcome?

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We examined the relationship between placental histology and thrombophilia status in women who were admitted with severe pre-eclampsia/eclampsia, placental abruption, intrauterine growth restriction or unexplained stillbirth. All women had thrombophilia screen at least 10 weeks after delivery (antithrombin III, protein C, protein S, activated protein C resistance, anticardiolipin antibodies, lupus anticoagulant, fasting plasma homocysteine and specific mutations to methylenetetrahydrofolate reductase C677T, G20210A prothrombin gene and factor V Leiden).

Placental histology reports were examined to identify the frequency of thrombotic lesions in the placenta including fetal stem vessel thrombosis, fetal thrombotic vasculopathy, placental infarction, perivillous fibrin deposition, intervillous thrombosis and placental floor infarction. During a 17 month period, a cohort of 79 women met the study criteria. Thirty (70%) out of 43 women with abnormal thrombophilia screen had abnormal placental histology. Twenty-eight (78%) out of 36 women with negative thrombophilia screen had abnormal placentae. No specific histological pattern could be identified when thrombophilia positive and thrombophilia negative groups were compared. We propose that there is a poor correlation between thrombophilia status and pathological changes of the placenta in women with severe pregnancy complications.

Key words: adverse pregnancy outcome/placental thrombosis/thrombophilia

Introduction

Thrombotic lesions of the placenta are a common finding in women with major complications of pregnancy such as pre-eclampsia (Kitzmiller and Benirschke, 1973), stillbirth (Fujikura and Benson, 1964; Naeye, 1977), fetal growth restriction (Fox, 1976; Salafia et al., 1992; Salafia et al., 1995), and placental abruption (Goddijn-Wessel et al., 1996). Histological evidence of placental thrombosis may also indicate the presence of subclinical congenital or acquired haemostatic defects (Kraus, 1993). However, clinical significance of morphological changes in the placenta is still a matter of controversy (Driscoll and Langston, 1991). We were particularly interested in frequency and significance of placental lesions in women with adverse pregnancy outcome and abnormal thrombophilia status. Arias et al. (1998) found that women with a history of adverse pregnancy outcome and thrombotic lesions of the placenta exhibit laboratory abnormality consistent with a thrombophilic state. Khong and Hague (1999) examined pathological changes of the placenta in women with maternal hyperhomocystaemia and found that placental changes are non-specific and could not be identified in every placenta.

The aim of the present study was to provide additional data on the relationship between placental changes and thrombophilia status in women with serious pregnancy complications.

Materials and methods

The placental histology reports and results were studied of complete thrombophilia screen for women who were admitted to Liverpool Women’s Hospital with pregnancies complicated by: (i) severe pre-eclampsia (PET)/eclampsia requiring obstetric intensive care; (ii) placental abruption requiring immediate delivery; (iii) antenatally diagnosed intrauterine growth restriction (IUGR) requiring delivery before 36 weeks; or (iv) unexplained stillbirth after 23 completed weeks of gestation.

Cases with fetal congenital malformation, abnormal karyotype or known maternal history of deep venous thrombosis or pulmonary embolism were excluded.

Thrombophilia screen

All blood tests were done at least 10 weeks after delivery and included assays of antithrombin III, protein C activity, antigen protein S free and total, activated protein C resistance (APCR), anticardiolipin antibodies (IgG and IgM), lupus anticoagulant by activated partial thromboplastin time (APTT) and dilute Russell’s viper venom test (DRVVT) and fasting plasma homocysteine. Results were considered abnormal only if two consecutive tests were outside the reference range for the local population. Because these tests are subject to widespread inter-laboratory variation, specific cut-off points for abnormal dynamic thrombophilia have deliberately not been given: local reference ranges were derived from the local normal population and cannot and should not be extrapolated to other settings.

Genetic studies were performed to look for specific mutations to methylenetetrahydrofolate reductase (MTHFR C677T), G20210A prothrombin gene and factor V Leiden.

Coagulation investigations

Antithrombin III, protein C activity, APCR without predilution with factor V deficient plasma, lupus clotting screens [prothrombin time (PT), APTT, DRVVT] with phospholipid neutralisation procedure (PNP) were all measured on the multidiscree analyser (MDA-180 Organon-Teknika) with a variety of reagents according to the manufacturer’s instructions. Protein S assay was performed using...
Diagnostica Stago free and total Protein S kits (Asnieres, France) by an enzyme-linked immunosorbent assay (ELISA) method.

**Homocysteine and anticardiolipin antibodies**

Homocysteine was quantified by ELISA using Axis Homocysteine EIA® (Bio-Rad, Hemel Hempstead, UK). Anticardiolipin antibodies were measured using the Cambridge Life Science Melisa kit (Cambridge Life Science, Huntingdon, UK). Results were read on multispec reader (Titertek Multiscan II MCC/340®; from Laboratories Ltd, Finland) at 450 nm and calculated using the manufacturer’s software.

**DNA extraction and genetic analysis**

Genomic DNA was extracted from 400 µl of whole blood using the Puregene kit® (Gentra, MN, USA) (floogen) according to the manufacturer’s instructions. DNA sequences of factor V gene, prothrombin gene and MTHFR gene were amplified by the polymerase chain reaction (PCR) using primers (Bertino et al., 1994; Frooss et al., 1995; Poort et al., 1996).

**Definition of placental lesions**

The histological examination of the placenta was performed as a routine clinical practice in pregnancies with severe adverse outcome.

Fetal stem vessel thrombosis was diagnosed when a major placental vessel was completely or partially occluded by a thrombus. The vessel could be located either in the chorionic plate or in one of the stem vessel branches (arterial) or tributaries (vein).

Fetal thrombotic vasculopathy was identified by foci of more than five terminal villi showing lack of villous capillaries and with a hyalinized fibrinous stroma. The adjacent villi were normally vascularized and the distribution of avascular villi conformed to a single villous tree (Redline and Pappini, 1995).

Placental (villous) infarctions were diagnosed in the presence of an area of ischaemic necrosis of the placental villi with collapse of the intervillous space and tight clustering of the villous tree.

Perivillous fibrin deposition was diagnosed in the presence of increased and excessive accumulations of fibrin-like material in the intervillous space surrounding multiple villi that remained separated. Trophoblastic cells may have migrated into the fibrin deposits and the villous capillaries may be normal.

Intervillous thrombosis was diagnosed when a villous-free nodular thrombus was found in the intervillous space.

Placental floor infarction was diagnosed when the maternal surface of the placenta was extensively thickened and stiffened due to accumulation of excessive fibrinoid material which expands the basal plate, enrols anchoring villi and surrounds large numbers of basal and perisepial villi (Kraus, 1996).

Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated for comparison of frequencies. Chi-square (χ²) test, Fisher’s exact test, Student’s t-test were used where appropriate and \( P < 0.05 \) was taken as significant. Statistical analyses were performed using the statistical package of Arcus Quickstat 1.0 (Iain Buchan, 1997).

**Results**

In the period from July 1997 to November 1998, there were 102 women who delivered at the Liverpool Women’s Hospital with pre-eclampsia/eclampsia, intrauterine growth restriction, unexplained stillbirth or placental abruption as defined by our protocol. Placental histology and full thrombophilia screen were available for 79 of them who were studied in detail. There were no demographic differences between the 79 women included in the study and the 23 women in whom placental histology or thrombophilia screen was either incomplete or not performed. The general characteristics and pregnancy outcome of the study group (\( n = 79 \)) are described in Table I. Forty-three (54%) women had abnormal thrombophilia screen. There were no significant differences between the two groups regarding maternal age, gestational age at delivery, onset of labour, mode of delivery, and pregnancy outcome. The incidence of placental abruption was lower in women with abnormal thrombophilia (14 versus 36%, \( P = 0.04 \)).

Frequency of placental lesions and their relationship with abnormal thrombophilia screen is shown in Table II. Pathological changes in the placentae were identified in 30/43 (70%) women with abnormal thrombophilia compared to 28/36 (78%) women with negative thrombophilia screen (not significant). Placental infarction was the most common lesion and was

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**Table I. General characteristics of the study population**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Abnormal thrombophilia ( n = 43 )</th>
<th>Normal thrombophilia ( n = 36 )</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>27.7 (5.6)</td>
<td>28.5 (6.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age (weeks):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;28</td>
<td>5 (12)</td>
<td>3 (8)</td>
<td>NS</td>
</tr>
<tr>
<td>28–32</td>
<td>8 (18)</td>
<td>10 (28)</td>
<td></td>
</tr>
<tr>
<td>32–36</td>
<td>19 (44)</td>
<td>18 (50)</td>
<td></td>
</tr>
<tr>
<td>37–40</td>
<td>6 (14)</td>
<td>3 (8)</td>
<td></td>
</tr>
<tr>
<td>40–42</td>
<td>5 (12)</td>
<td>2 (6)</td>
<td></td>
</tr>
<tr>
<td>Indication for delivery:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-eclampsia/eclampsia</td>
<td>26 (61)</td>
<td>16 (44)</td>
<td>NS</td>
</tr>
<tr>
<td>placental abruption</td>
<td>6 (14)</td>
<td>13 (36)</td>
<td>0.04</td>
</tr>
<tr>
<td>unexplained stillbirth</td>
<td>8 (19)</td>
<td>5 (14)</td>
<td></td>
</tr>
<tr>
<td>IUGR</td>
<td>10 (23)</td>
<td>7 (19)</td>
<td></td>
</tr>
<tr>
<td>≥2 indications for delivery</td>
<td>11 (26)</td>
<td>8 (22)</td>
<td></td>
</tr>
</tbody>
</table>

**Table II. Relationship between placental lesions and thrombophilia screening tests**

<table>
<thead>
<tr>
<th>Placental pathology</th>
<th>Abnormal thrombophilia ( n = 43 )</th>
<th>Normal thrombophilia ( n = 36 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal placenta</td>
<td>13 (30)</td>
<td>8 (22)</td>
</tr>
<tr>
<td>Fetal stem thrombosis</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Thrombotic vasculopathy</td>
<td>0 (0)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Placental infarction</td>
<td>20 (47)</td>
<td>18 (50)</td>
</tr>
<tr>
<td>Perivillous fibrin deposition</td>
<td>11 (26)</td>
<td>6 (17)</td>
</tr>
<tr>
<td>Maternal floor infarction</td>
<td>3 (7)</td>
<td>9 (25)</td>
</tr>
<tr>
<td>Inter-villous thrombosis</td>
<td>11 (26)</td>
<td>9 (25)</td>
</tr>
<tr>
<td>Multiple changes</td>
<td>19 (44)</td>
<td>19 (53)</td>
</tr>
</tbody>
</table>

Values are number of cases (%).
identified in 48% of placenta of the study population, but there was no significant increase in the number of specific placental lesions in women with abnormal thrombophilia.

We compared the incidence of six types of pathological lesions of the placent in women with single (n = 23) and multiple (n = 20) abnormal laboratory tests and found no difference between the two groups (Table III).

The distribution of placental lesions according to state of thrombophilia screen and obstetric complications is shown in Tables IV and V. Odds ratios and 95% CI were calculated for all comparisons (Table II–V) but the differences were not statistically significant and therefore not shown in the tables.

### Discussion

The results of our study show that there is a poor correlation between thrombophilia state and placental pathological changes in women with adverse pregnancy outcome. The six types of placental lesions were selected based on previous reports suggesting that they are common in pregnant women with thrombophilia (Arias et al., 1998; Magid et al., 1998). It is important to emphasize that our study does not address the incidence of these patterns in all births, but rather shows their relative frequency and possible clinical implications in a high-risk obstetric population.

### Table III. Placental changes in women with abnormal thrombophilia screen

<table>
<thead>
<tr>
<th>Placental pathology</th>
<th>Abnormal thrombophilia screen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multiple (n = 23)</td>
</tr>
<tr>
<td>Normal placenta</td>
<td>8 (35)</td>
</tr>
<tr>
<td>Fetal stem thrombosis</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Thrombotic vasculopathy</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Placental infarction</td>
<td>10 (44)</td>
</tr>
<tr>
<td>Perivillous fibrin deposition</td>
<td>6 (26)</td>
</tr>
<tr>
<td>Maternal floor infarction</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Inter-villous thrombosis</td>
<td>7 (30)</td>
</tr>
<tr>
<td>Multiple changes</td>
<td>10 (44)</td>
</tr>
</tbody>
</table>

Values are number of cases (%).

Placental infarction was the most common abnormal histological finding in our cohort and this finding is consistent with previous reports (Laurini et al., 1994). Small placental

### Table IV. Relationship between placental lesions and type of abnormal thrombophilia screen

<table>
<thead>
<tr>
<th>Placental pathology</th>
<th>Low APCR (n = 20)</th>
<th>Low free protein S (n = 8)</th>
<th>Low total protein S (n = 4)</th>
<th>Prolonged DRVVT (n = 9)</th>
<th>ACA IgG (n = 1)</th>
<th>ACA IgM (n = 10)</th>
<th>Factor V Leiden mutation +,+/+,+,- (n = 3)</th>
<th>G20210A +,+/+,- (n = 2)</th>
<th>Hyperhomocysteinaemia MTHFR +,+ MTHFR +,+/-,- (n = 6)</th>
<th>Hyperhomocysteinaemia MTHFR +,+ MTHFR +,+/-,- (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal placenta</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Fetal stem thrombosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Thrombotic vasculopathy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Placental infarction</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Perivillous fibrin deposition</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Maternal surface infarction</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Inter-villous thrombosis</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table V. Relationship between obstetrical problems, thrombophilia screen, and placental lesions

<table>
<thead>
<tr>
<th>Placental pathology</th>
<th>Abnormal thrombophilia screen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n = 26)</td>
</tr>
<tr>
<td>Normal placenta</td>
<td>Normal (n = 23)</td>
</tr>
<tr>
<td>Fetal stem thrombosis</td>
<td>Normal (n = 3)</td>
</tr>
<tr>
<td>Maternal surface infarction</td>
<td>1</td>
</tr>
<tr>
<td>Inter-villous thrombosis</td>
<td>1</td>
</tr>
</tbody>
</table>

Values are number of cases (%).

IUGR = intrauterine growth restriction.

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infarctions are common in uncomplicated pregnancies at term
and of no importance, but extensive infarction, i.e. necrosis of
>10% of the placenta, is associated with fetal hypoxia, growth
retardation and fetal death, particularly in the second and early
third trimester (Fox, 1997; Rodriguez et al., 1997).

Although it is claimed that fetal thrombotic vasculopathy
and fetal stem vessel thrombosis are common findings in
women with adverse pregnancy outcome (Arias et al., 1998),
we were unable to confirm this observation.

The incidence of maternal floor infarction in unselected
pregnancies is <1% (Naeye, 1985; Andres et al., 1990). The
incidence in our cohort was much higher (15%) confirming
the previously observed association between maternal floor
infarction of the placenta and adverse pregnancy outcome
(Andres et al., 1990). As expected, perivillous fibrin deposition
and intervillous thrombosis were quite common, but their
clinical significance remains questionable (Fox, 1998).

Low activated protein C resistance (APCR) was the most
common abnormal finding in this cohort. It is important to
stress that only two women in our cohort had factor V Leiden
mutation. Despite that, placental infarction was demonstrated
in 50% of cases of low APCR ratio. The significance of this
finding remains uncertain.

Placental changes in women with abnormal anticardiolipin
antibodies and lupus anticoagulants are consistent with previ-
ous reports (Out et al., 1991; Magid et al., 1998). Non-specific
placental changes in women with hyperhomocysteinaemia
were recently reported (Khong and Hague, 1999) and were
confirmed in our study. It is noteworthy that four women with
hyperhomocysteinaemia had normal placentae. This suggests
that placental changes may not be the only factor responsible
for adverse pregnancy outcome in these women.

In summary, we found a poor correlation between throm-
bophilia status and pathological changes of the placenta in
women with severe pregnancy complications. If there is a
causal link between currently known thrombophilias, placental
pathological changes and severe pregnancy complications,
more sophisticated methods are required to prove it.

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73, 529–534.
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restriction in infants of less than thirty-two weeks’ gestation: associated

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