Decreased endovascular trophoblast invasion in first trimester pregnancies with high-resistance uterine artery Doppler indices

F.Prefumo1, N.J.Sebire2 and B.Thilaganathan1,3

1Fetal Medicine Unit, Department of Obstetrics and Gynaecology, St George’s Hospital Medical School, London SW17 0RE, UK and 2Department of Pathology, Great Ormond Street Hospital for Children, London WC1N 3JH, UK

3To whom correspondence should be addressed. E-mail: basky@pobox.com

BACKGROUND: Defective trophoblastic invasion in early pregnancy is involved in the pathogenesis of pre-eclampsia. This study investigates the relationship between Doppler assessment of uterine artery resistance and endovascular trophoblastic invasion in the first trimester of pregnancy. METHODS: Patients undergoing termination of pregnancy for non-medical reasons were categorized as having a low- or high-resistance uterine artery blood flow pattern by transabdominal Doppler ultrasound. Products of conception were examined histologically with regard to the extent of decidual endovascular trophoblast invasion. RESULTS: There were 14 low-resistance and 17 high-resistance uterine artery blood flow pregnancies identified at 10–14 weeks of gestation. Normal intra-decidual endovascular trophoblast invasion was identified with a similar frequency in both groups (P = 0.79). However, the proportion of decidual vessels with endovascular trophoblast invasion was significantly higher in the low-resistance pregnancies (49%) compared with the high-resistance ones (34%, P = 0.02). CONCLUSIONS: The findings of this study support the use of uterine artery Doppler investigation for the non-invasive assessment of trophoblast invasion in early pregnancy. Further studies are necessary to clarify the biological significance of these observations and their potential clinical applications.

Key words: Doppler/early pregnancy/placentation/trophoblast invasion/uterine arteries

Introduction

Successful placentation relies on normal trophoblastic invasion of the maternal decidua, myometrium and blood vessels. In particular, extravillous trophoblastic cells invade the uterine spiral arteries, modifying their endothelial lining and media, and leading to a progressive dilatation of these vessels (Pijnenborg et al., 1980; Kam et al., 1999).

Pre-eclampsia is characterized by a failure of normal trophoblastic invasion and lack of uterine spiral artery remodelling leading to a high-resistance uteroplacental circulation (Brosens, 1977). The degree of the uteroplacental resistance can be assessed by uterine artery Doppler, which is an effective clinical screening tool for the subsequent development of severe pre-eclampsia (Bower et al., 1993; Papageorghiou et al., 2001). A few previous studies have investigated the relationship between uterine artery Doppler indices and histological features of placentation in normal and pre-eclamptic pregnancies. However, all of them were carried out in third trimester on placental bed biopsies obtained after delivery, long after active trophoblastic invasion has ceased (Lin et al., 1995; Aardema et al., 2001). If the current model of pre-eclampsia is correct, high-resistance uterine artery blood flow indices should be related to failure of trophoblastic invasion in early, rather than late, pregnancy.

Extravillous trophoblast invasion of the decidual maternal vessels can be observed as endovascular trophoblast on histological examination of the products of conception obtained from early pregnancy losses or terminations (Sebire et al., 2001; 2002). It is hypothesized that defective trophoblastic invasion of decidual vessels in the first trimester may be associated with decreased trophoblastic conversion of the spiral artery branches to uteroplacental vessels in later pregnancy. The aim of this study was to examine the possible relationship between Doppler assessment of uterine artery resistance and endovascular trophoblastic invasion in the first trimester of pregnancy.

Materials and methods

Doppler ultrasound examination of the maternal uterine arteries was performed in women attending a clinic for termination of pregnancy in the late first trimester. Only singleton pregnancies were included. Women with a known medical condition (e.g. diabetes mellitus, connective tissue disease) or a history of recurrent miscarriage were excluded. Gestational age was calculated from the last menstrual period and confirmed by crown-rump length measurement. In all
cases, a careful search for fetal abnormalities was performed. Local ethical committee approval was obtained for this study, and all women gave their written informed consent.

Examinations were performed using an Acuson XP-10 system (Mountain View, CA) equipped with a 5 MHz curvilinear transabdominal probe, following a technique previously described (Hollis et al., 2001). The high-pass filter was set to the minimum and the pulse repetitive frequency was 2.5 kHz. The maximum achievable thermal and mechanical indices were 1.2 and 1.0 respectively. The size of the sampling gate was set to 2 mm. A mid-sagittal section of the uterus was obtained and the cervical canal was identified. The probe was then moved laterally until the paracervical vascular plexus was seen. Colour Doppler was turned on and the uterine artery was identified as it turned cranially to make its ascent to the uterine body. Measurements were taken at this point, before the uterine artery branched into the arcuate arteries. Once it was ensured that the angle was <60°, the pulsed Doppler gate was placed over the vessel. Angle correction was then applied and the signal updated until at least four consecutive flow velocity waveforms of good quality were obtained. The resistance index (RI) was measured and recorded, as well as the presence or absence of an early diastolic notch (Bower et al., 1993).

High-resistance cases were defined as those presenting with bilateral uterine artery notches and a mean RI above the 95th centile. Low-resistance cases were defined as those presenting with no uterine artery notches and a mean RI below the 5th centile. Previously calculated centiles were employed (Hollis et al., 2003).

Products of conception were obtained from all cases at the time of surgical termination of pregnancy and immediately fixed in formalin. Decidual fragments were identified, blocked, paraffin embedded and 4 µm sections cut and stained with haematoxylin and eosin. Histological sections were reviewed by a pathologist blinded to the clinical details. In each case, the presence or absence of implantation site fragments was determined. The implantation site was identified by the presence of interstitial extravillus trophoblast surrounding decidual vessels. All implantation site decidual vessels were examined and the presence or absence of endovascular trophoblast invasion determined (Figure 1) (Sebire et al., 2001; 2002).

Comparisons of the demographic characteristics and histological findings between groups were carried out using Mann-Whitney and comparison of proportions tests.

**Results**

Thirty-one pregnancies were examined, 17 with high-resistance uterine artery flow and 14 with low-resistance flow. The median maternal age was 26 years (range 18–40), gravidity 2 (range 1–6), parity 0 (range 0–3), gestational age at ultrasound scan 11.4 weeks (range 9.4–13.6), gestational age at sampling 12.0 weeks (range 10.0–14.1). The median interval between the ultrasound scan and sampling was 3 days (range 1–10). No significant differences in clinical characteristics were found between the two groups (Table I). Histological findings are provided in Table II. Pregnancies with a low-resistance uterine artery flow were associated with a significantly higher proportion of implantation site vessels with endovascular trophoblast invasion present compared with those with high resistance.

**Discussion**

The findings of this study demonstrate that pregnancies with a low-resistance uterine artery flow pattern in early pregnancy are associated with a more extensive trophoblastic invasion of the decidual vessels than pregnancies with a high resistance.

Various studies have reported on the correlation between uterine artery blood flow measurements obtained in vivo by Doppler ultrasound in early pregnancy and the subsequent development of pre-eclampsia and intrauterine growth
Table I. Demographic and clinical characteristics in pregnancies with high- and low-resistance uterine artery blood flow

<table>
<thead>
<tr>
<th></th>
<th>High-resistance (n = 17)</th>
<th>Low-resistance (n = 14)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>25.5 (18–36)</td>
<td>29.5 (19–40)</td>
<td>0.06</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2 (1–5)</td>
<td>3 (1–6)</td>
<td>0.34</td>
</tr>
<tr>
<td>Parity</td>
<td>0 (0–3)</td>
<td>0 (0–3)</td>
<td>0.38</td>
</tr>
<tr>
<td>Gestational age at scan (weeks)</td>
<td>11.1 (10.0–13.6)</td>
<td>12.3 (9.4–13.4)</td>
<td>0.19</td>
</tr>
<tr>
<td>Gestational age at sampling (weeks)</td>
<td>11.7 (10.4–14.1)</td>
<td>12.8 (10.0–14.0)</td>
<td>0.18</td>
</tr>
<tr>
<td>Scan to sampling interval (days)</td>
<td>4 (1–10)</td>
<td>3 (1–8)</td>
<td>0.95</td>
</tr>
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Table II. Histological findings in products of conception from pregnancies with high- and low-resistance uterine artery blood flow examined in the late first trimester

<table>
<thead>
<tr>
<th></th>
<th>High-resistance (n = 17)</th>
<th>Low-resistance (n = 14)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation site identified</td>
<td>13/17 (76%)</td>
<td>12/14 (86%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Endovascular trophoblast invasion present</td>
<td>8/13 (62%)</td>
<td>8/12 (67%)</td>
<td>0.79</td>
</tr>
<tr>
<td>No. of implantation site vessels per case</td>
<td>9 (1–17)</td>
<td>7.5 (3–32)</td>
<td>0.44</td>
</tr>
<tr>
<td>No. of implantation site vessels with endovascular trophoblast invasion</td>
<td>39/114 (34%)</td>
<td>70/143 (49%)</td>
<td>0.02</td>
</tr>
</tbody>
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restriction (van den Elzen et al., 1995; Harrington et al., 1997; Martin et al., 2001; Vainio et al., 2002). Moreover, it has previously been demonstrated that the uterine artery RIs obtained transabdominally at 10–14 weeks of gestation are repeatable and reproducible measurements (Hollis et al., 2001), and that a correlation exists between the RI and birth weight (Hollis et al., 2003). These clinical data have been interpreted as suggesting a correlation between uterine artery Doppler findings and the extent of trophoblastic invasion in early pregnancy, based on (i) the fact that defective placentation is considered to be the major aetiological factor in the development of pre-eclampsia and intrauterine growth restriction (Khong et al., 1986); and (ii) the analogy with the fact that uterine artery Doppler findings in the third trimester of pregnancy were shown to correlate with trophoblast invasion verified at histology (Lin et al., 1995). However, the latter finding was not confirmed in a more recent study (Aardema et al., 2001).

In the present study, trophoblastic invasion has been assessed by examining decidual endovascular trophoblast invasion in specimens obtained from suction termination of pregnancy. The implantation site was identified in ~80% of cases, a figure similar to that reported in other studies employing the same method (Sebire et al., 2001; 2002). The inability to identify the implantation site in all cases is due to the sporadic sampling methodology inherent in histopathological examination.

In those pregnancies where an implantation site was identified, endovascular trophoblast invasion was observed in ~65% of cases. Previous studies have reported a similar figure in normal pregnancies undergoing social termination, in some subgroups of spontaneous pregnancy loss and in partial moles, while significantly lower percentages of cases with endovascular trophoblast invasion are present in complete moles and in pregnancy losses related to primary antiphospholipid antibody syndrome (APLS) (Sebire et al., 2001; 2002).

The two groups compared in the present study, i.e. pregnancies with a high- and low-resistance uterine artery flow pattern, were unlikely to present with the frankly pathological features associated with complete moles and APLS-related pregnancy losses, since all pregnancies were viable and ongoing at the time of evacuation. Moreover, we know that only a minor proportion of the cases with high-resistance uterine artery flow will present with severe pre-eclampsia or fetal growth restriction in later pregnancy (van den Elzen et al., 1995; Harrington et al., 1997; Martin et al., 2001). Therefore, the lack of difference in the proportion of cases with endovascular trophoblast invasion in the two groups (62% versus 67%; P = 0.79) suggests that they represent the two extremes of biological variability in otherwise normal early pregnancies. However, the quantitative analysis of the extent of invasion, expressed as the percentage of implantation site vessels showing signs of endovascular trophoblast invasion, demonstrated a significant difference between the two groups. The higher percentage of invaded vessels (49%) in the low-resistance cases, as opposed to the high-resistance ones (34%; P = 0.02) provides unique evidence that in early pregnancy uterine artery Doppler findings correlate with the extent of trophoblastic invasion.

The haemodynamic interpretation of the observed decrease in uterine artery resistance with higher degrees of endovascular invasion is not completely clear. The data available on the process of endovascular and interstitial trophoblast invasion in normal pregnancies suggest that endovascular trophoblast ‘plugging’ does not allow intervillous blood flow in the early stages of gestation. Direct channels through the trophoblast plugs develop gradually between 8 and 12 weeks of gestation, and are unlikely to allow any substantial blood flow before this later date (Burton et al., 1999; Jauniaux et al., 2003). This delayed onset of intervillous blood flow protects from oxidative damage to the trophoblast. Oxidative damage, when induced by premature and widespread onset of the
maternal placental circulation secondary to shallow trophoblast invasion, is an important factor in early pregnancy loss (Jauniaux et al., 2003).

The presence of endovascular trophoblast ‘plugging’ might be expected to result in an increased resistance in the uteroplacental vascular bed prior to the establishment of an effective intervillous blood flow. As a result, we would expect to observe an increase in uterine artery vascular impedance in those pregnancies where trophoblast invasion is more successful. However, the data from the present study showed exactly opposite findings. There are two possible explanations for this phenomenon: (i) intervillous blood flow increases enough to affect uterine artery resistance earlier in gestation than expected; (ii) more likely, uterine artery resistance and endovascular trophoblast invasion of the decidual arteries are not directly related events, i.e. the efficiency of trophoblastic invasion observed at the decidual level is not the direct cause of variations in uterine artery resistance at this stage of pregnancy, but is rather one of the aspects of a process that influences flow resistance through a different but related pathway. Simultaneous events such as interstitial invasion, deeper decidual–myometrial junction vascular invasion or the release of vasoactive mediators may therefore explain changes in arterial resistance and be related to decidual endovascular trophoblastic invasion.

According to a departmental protocol, 800 µg of misoprostol was administered vaginally for cervical priming 2 h before the surgical termination of the pregnancy. While it is known that misoprostol may acutely increase uterine artery resistance to flow (Yip et al., 2000), there is no evidence that the use of any kind of cervical priming should in any way affect the findings of endovascular trophoblast invasion. In the unlikely case that the latter might happen, the two groups of pregnancies examined in this study would nevertheless have been exposed to the same effects.

The findings of this study support the use of uterine artery Doppler investigation for the non-invasive assessment of trophoblast invasion in early pregnancy. Further studies are necessary to clarify the biological significance of these observations and their potential clinical application.

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References


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