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Association of first-trimester angiogenic factors with placental histological findings in late-onset preeclampsia

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ABSTRACT

Objective: To explore in women with late-onset preeclampsia (PE) the association between maternal levels of angiogenic/antiangiogenic factors in the first trimester of pregnancy and histological findings attributable to placental underperfusion (PUP).
Methods: A nested case-control cohort study was conducted in 73 women with pregnancies complicated by late-onset PE (>34 weeks at delivery) matched with controls. First trimester uterine artery Doppler (UtA); maternal levels of placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) were retrieved. Placentas were histologically evaluated using a hierarchical and standardized classification system. One-way ANOVA with linear polynomial contrast or linear-by-linear association test was performed to test the hypothesis of a linear association across study groups (controls, PE without PUP and PE with PUP).
Results: In 54 (74%) placentas, 89 placental histological findings qualifying for PUP were found. Across study groups, significant values were observed in maternal levels of decreased PlGF (MoM values: 1.53, 1.41 and 1.37; $p < 0.001$), increased sFlt-1 (MoM values: 3.11, 3.11 and 3.22; $p = 0.002$), increased sFlt-1/PlGF ratio (MoM values: 2.3, 2.3 and 2.44; $p < 0.001$), abnormal UtA Doppler (MoM values: 1, 1.26 and 1.32; $p < 0.001$), and worse perinatal outcomes in terms of gestational age at delivery, cesarean section for not reassuring fetal status, birth weight and neonatal acidosis.
Discussion: In late-onset PE an imbalance of circulating angiogenic and anti-angiogenic factors already present at 8–10 weeks of pregnancy was associated with histological findings reflecting placental insufficiency. An early first trimester screening by angiogenic factors might help to identify patients with placental involvement among late-onset PE cases.
Conclusion: In late-onset preeclampsia, first-trimester uterine Doppler and circulating levels of angiogenic/antiangiogenic factors are associated with placental underperfusion.

1. Introduction

Preeclampsia (PE) is a major contributor to maternal and perinatal morbidity and mortality [1,2]. Accumulating evidence indicates that a central feature in the pathophysiology of PE is a failure of the physiologic transformation of spiral arteries [3–6]. Although the primary insults for these abnormalities remain elusive [7,8], it is postulated that the resulting poor placentation

ABBREVIATIONS: PE, preeclampsia; FGR, fetal growth restriction; GA, gestational age; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; MoM, multiples of normal median; EFW, estimated fetal weight; UtA, uterine artery; UA, umbilical artery; MCA, middle cerebral artery; CPR, cerebroplacental ratio; PI, Doppler pulsatility index; BW, birth weight; PUP, placental underperfusion.

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and reduced blood supply to the placenta in early pregnancy lead to the release of factors into the maternal circulation causing the systemic manifestations of the syndrome.

While this pathophysiology is behind the vast majority of cases of early-onset PE, in late-onset PE there is a broader spectrum of involved mechanisms [9]. This has a major clinical impact because whilst a combination of maternal characteristics and some biomarkers (such as blood pressure (BP) and uterine Doppler evaluation) at first-early second trimesters predict most cases that develop early-onset PE, this same predictive strategy fails to detect the majority of instances of late-onset PE [10]. This poor predictive performance could be overcome by a better phenotyping of the late PE syndrome, similarly what has been proposed in other obstetric syndromes (i.e. prematurity) [11].

A proportion of women with late-onset PE had signs of placental insufficiency at histological evaluation [12], and they exhibited an imbalance in their angiogenic profile [13]. Increased risks of adverse perinatal outcome have been described in presence of simultaneous late-onset PE and placental insufficiency [14]. It follows from this evidence that for an efficient prevention of late-onset PE, targeting those instances that exhibit placental insufficiency is key. However, it remains unclear whether late-onset PE cases with true placental under perfusion already show an imbalance in their angiogenic profile in the first trimester. This could help to define predictive and preventive strategies.

The aim of this study was to explore the association between maternal plasma concentrations of angiogenic factors in the first trimester of pregnancy and histological findings attributable to PUP in late-onset PE.

2. Patients and methods

2.1. Participants

A nested case-control study nested in a cohort of unselected singleton pregnancies referred for routine first-trimester screening for aneuploidies (11+0–13 + 6 weeks of gestation) was conducted. Eligible cases were women without chronic hypertension subsequently developing late-onset PE (delivering above 34 weeks of gestation). Cases were matched with controls, defined as pregnancies not complicated by PE delivering immediately after each case. Exclusion criteria were congenital malformations and suspected chorioamnionitis.

Plasmatic levels of angiogenic factors were determined in stored samples. Gestational age (GA) in all pregnancies was calculated based on the crown-rump length (CRL) at first-trimester ultrasound [15].

The local Ethics Committee approved the study protocol and each patient provided written informed consent.

2.2. Data collection

Baseline maternal characteristics, including age, ethnicity, body mass index (BMI), parity, maternal and paternal smoking, known chronic disease (i.e., diabetes mellitus, renal disease, and autoimmune disorders), and obstetric history were recorded in the hospital database upon study admittance. Data on postpartum follow-up or subsequent complications of pregnancy, ultrasound evaluations, and perinatal outcomes were also collected prospectively.

2.3. Outcome measures

PE was defined according to the guidelines of the International Society for the Study of Hypertension in Pregnancy. This requires two recordings of systolic BP $\geq$ 140 mmHg or diastolic BP $\geq$ 90 mmHg at least 4 h apart in previously normotensive women after 20 weeks of gestation, and proteinuria of 300 mg or more in 24 h [16].

Small for gestational age (SGA) newborns were defined with birth weight below the 10th centile according to local standards [17].

Intrauterine growth restriction (IUGR) fetus was defined with an estimated fetal weight (EFW) below the 10th centile according to local standards [17], with abnormalities in Doppler evaluation (cerebroplacental ratio (CPR) < 5th centile or uterine artery (UtA) Doppler pulsatility index (PI) > 95th centile) [18,19].

2.4. Biophysical parameters

In each patient transvaginal UtA Doppler assessment was performed at the time of first-trimester scan (11+0–13 + 6 weeks of gestation) in order to measure the PI in the left and right uterine arteries, as previously described [18]. Maternal BP was recorded at time of ultrasound with automated devices OMRON M6 Comfort (OMRON Corporation, Kyoto, Japan). Mean arterial pressure (MAP) was measured in one arm (right or left) while women were seated and after a 5-min rest. MAP was calculated as: diastolic BP + (systolic BP − diastolic BP)/3.

2.5. Blood sampling and testing procedures

Venous blood samples were drawn from each woman between 8 + 0 and 11 + 6 weeks' gestation (mean 10.6, SD 3.85). They were collected in an EDTA-containing tube and processed within one hour. Plasma was separated by centrifugation at 3000 rpm for 10 min at 4 °C, and samples were immediately stored at −80 °C until assayed.

Levels of PlGF and sFlt-1 in maternal plasma were measured by the corresponding ELISA kits according to the manufacturer's instructions (R&D Systems Europe Ltd, Abingdon, UK). The assay kits used detect only free analytics, not the bound fractions. All samples were collected, handled, and stored under the same conditions and tested in duplicate. Plate-to-plate variability was controlled by running an internal control along the samples. Test sera and matched controls were simultaneously and blindly run on the same plates. Intra-assay (<5%) and inter-assay (10%) precision of all kits were consistent. Linear regression coefficients of standard curves were never <0.99.

2.6. Clinical management

Deliveries were attended by a staff obstetrician blinded to angiogenic factor determinations. Continuous fetal heart monitoring was performed during labor, graded (normal, suspicious, or abnormal) in terms of presence, type, and length of decelerations; bradycardia; tachycardia; and assessment of variability [20,21]. In instances of two or more suspicious variables or with at least one abnormal parameter unresponsive to digital fetal scalp stimulation, fetal scalp blood pH was tested [22]. Any pH value < 7.15 or <7.20 on two attempts 30 min apart were regarded as abnormal [22]. Cesarean section was done for non-reassuring fetal status, based on intrapartum fetal abnormalities in heart rate and scalp blood pH [22]. Cesarean delivery for non-reassuring fetal status was indicated for a persistently abnormal heart tracing after pessary withdrawal, oxytocin suspension, and a 10-min intravenous infusion of ritrodine (200 μg/min). All cases with adverse outcome were formally assessed to ensure that the management protocol had been followed correctly.