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# Excess Syncytiotrophoblast Microparticle Shedding is a Feature of Early-onset Pre-eclampsia, but not Normotensive Intrauterine Growth Restriction

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*Rationale*: Syncytiotrophoblast microparticles (STBM) are shed into the maternal circulation in higher amounts in pre-eclampsia compared to normal pregnancy and are believed to be the stimulus for the systemic inflammatory response and endothelial cell damage which characterises the maternal syndrome. The excess shedding of STBM may be caused by hypoxia as a result of poor placentation, which is often a feature of pre-eclampsia. Similar placental pathology occurs in some cases of normotensive intrauterine growth restriction (nIUGR), but in the absence of maternal disease.

*Objective*: To examine whether the shedding of STBM in nIUGR occurs to the same extent as in pre-eclampsia.

*Methods*: A prospective case–control study in a tertiary referral centre of: 1) women with early-onset pre-eclampsia (EOPET $<34$ week), 2) women with late-onset pre-eclampsia (LOPET $\geq 34$ week), 3) women with nIUGR), 4) matched normal pregnant women (NPC), and 5) non-pregnant women. An ELISA using the antitrophoblast antibody NDOG2 was used to measure STBM levels in peripheral venous plasma. Non-parametric analyses were conducted with statistical significance set at $p < 0.05$.

*Results*: STBM levels rise during normal pregnancy. EOPET was associated with increased STBM levels (EOPET (median): 41 ng/ml, $n = 15$) compared with matched normal pregnancy (16 ng/ml, $n = 15$; Wilcoxon $p = 0.005$). LOPET (50 ng/ml, $n = 10$) and nIUGR (18 ng/ml, $n = 8$) STBM levels did not differ from matched normal pregnancy (36 ng/ml, $n = 15$, and 36 ng/ml, $n = 8$, respectively). Background levels in non-pregnant plasma were 0.49 ng/ml, $n = 10$.

*Conclusions*: Increased STBM levels were found in EOPET but not in nIUGR providing further evidence for their role in the pathogenesis of the maternal syndrome.

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## INTRODUCTION

Pre-eclampsia remains one of the most common causes of maternal mortality in the developed world [1,2]. The maternal syndrome of pre-eclampsia, characterised by hypertension and proteinuria, defines the disease. When pre-eclampsia presents remote from term, the fetus is at increased risk of intrauterine growth restriction (IUGR). IUGR can also occur in the absence of maternal hypertension, a state that we have termed normotensive IUGR. Given that incomplete placentation is shared by pre-eclampsia and normotensive IUGR [3], the latter may represent the fetal consequences of a shared placental pathology occurring in isolation.

The cogent model for the pathogenesis of the maternal syndrome of pre-eclampsia describes a process by which a placental factor is released into the maternal circulation, which damages the maternal endothelium, causing a syndrome of systemic endothelial dysfunction [8]. It is now apparent that this endothelial dysfunction is part of a wider maternal systemic inflammatory response which occurs in normal pregnancy but is far more intense in pre-eclampsia [4–6]. The placental factor responsible is not known but candidates

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include sFlt-1, peroxides, eicosanoids, cytokines, and syncytiotrophoblast microparticles (STBM).

We have previously shown that STBM prepared from normal placentae cause endothelial cell dysfunction in vitro [7] and in isolated vessels [8], and that pre-eclampsia plasma inhibits endothelial cell proliferation [9]. STBM are detectable in the plasma of pregnant women by both flow cytometry and enzyme-linked immunosorbent assay (ELISA) [10] and significantly higher levels were found in women with pre-eclampsia [10]. A significant correlation was found between the plasma concentration of STBM and endothelial inhibition, suggesting that STBM may contribute to the maternal endothelial dysfunction [10]. There is also an excess of circulating cellular syncytial debris in pre-eclampsia [11]. The release of syncytiotrophoblast debris into the maternal circulation is thought to be the result of syncytial apoptosis, which is part of a normal process of turnover and repair [12] and/or necrosis [13]. Syncytiotrophoblast apoptosis is increased in pre-eclampsia [14] and this could explain the increased debris in the maternal circulation. It has been proposed that this increase in apoptosis may result from oxidative stress in the placenta caused by a failure of spiral artery adaptation leading to a poorly developed blood supply [12]. The other consequence of this placental pathology is intrauterine growth restriction (IUGR) of the fetus.

The poor placentation and fetal growth restriction seen in some cases of pre-eclampsia, however, is not unique to this disorder. Similar pathology is also seen in some, but not all, cases of normotensive IUGR. Interestingly, increased syncytial apoptosis has also been reported in these pregnancies [15] which would be expected to result in the increased shedding of syncytiotrophoblast debris. According to this hypothesis, this should precipitate the maternal syndrome which it clearly does not. This could be due either to a lack of increased shedding in normotensive IUGR or a difference in the way that the mother's innate immune system and endothelial cells respond in this condition. Therefore, the purpose of this study was to measure STBM levels in the maternal circulation in normal pregnancy and to compare them with those seen in pre-eclampsia, normotensive IUGR, and non-pregnancy.

## METHODS

This was a prospective case–control study using clinical plasma samples obtained from the maternity services at a tertiary referral centre (Children's and Women's Health Centre of British Columbia). These samples were frozen at −80 °C and transported to Oxford, UK, for analysis.

Pre-eclampsia was defined by the criteria of the National High Blood Pressure Education Program [16]. Only singletons were investigated. IUGR was defined as either an ultrasound estimate of fetal weight or an ultrasound measurement of the fetal abdomen $<$5th centile for gestational age, confirmed at delivery (birthweight $<$5th centile for age and gender) and associated with neither aneuploidy, structural anomalies, nor congenital infection. The histopathology diagnoses of all women were reviewed, when available, to confirm the presence or absence of abnormal placental findings in cases and controls, respectively.

Following informed consent, peripheral venous blood was drawn from the following:

1. 15 women with early-onset pre-eclampsia ($<$34 weeks' gestation),
2. 10 women with late-onset pre-eclampsia ($\geq$ 34 weeks' gestation),
3. 10 women with normotensive IUGR (abdominal circumference $<$5th centile for gestational age with birthweight $<$5th centile confirmed postnatally, excluding both aneuploidy and congenital infections),
4. 35 normal pregnant women matched for age ($\pm$5 years), gestation ($\pm$14 days) and parity (0, 1, $\geq$ 2) (one control per case in groups 1–3), and
5. 10 non-pregnant women aged 20–40 years, not using hormonal contraception.

The sample collection was co-ordinated by a dedicated full-time research co-ordinator and was approved by both the University of British Columbia Clinical Research Ethics Board and the Children's and Women's Health Centre of British Columbia Ethics Board. For women with both pre-eclampsia and normotensive IUGR blood sampling was performed at the time of diagnosis of the respective pregnancy complication.

Following informed consent, 5 ml of antecubital vein blood was taken antenatally. The plasma was prepared from this lithium heparin anticoagulated peripheral venous blood by high speed centrifugation, and stored at −80 °C for transport from Vancouver to Oxford. The tube containing the plasma was thawed to room temperature and 2 ml of plasma was used per sample assay. The sample was topped up with endotoxin free phosphate buffered saline (PBS-E, Sigma, St Louis, MO), ensuring the sample was diluted at least 1:2. The plasma/PBS-E mixture was then transferred to an ultracentrifuge tube (14 × 89 mm Ultra-Clear tube, Beckman Coulter, High Wycombe, Bucks, UK). To pellet any STBM, the samples were spun on a Beckman L8-80M ultracentrifuge at 150,000*g* for 45 min at 4 °C. This was based on a protocol known to pellet ribosomes. The supernatant was discarded and the final pellet was resuspended in 350 µl 0.1% bovine serum albumin (BSA, Research Diagnostics Inc, Flanders, NJ) in PBS-E. The samples were then transferred to 0.7 ml screw-top tubes and kept at −80 °C until use.

### Standards for the STBM enzyme-linked immunosorbent assay (ELISA)

Syncytiotrophoblast microparticles (STBM) were prepared from normal placentae by a modification of the method of Smith et al. [7] and used as standards for the STBM ELISA. The protein content of the STBM suspension was 9.9 mg/ml. Fifty microlitres of this was added to 1 ml of diluting buffer