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Maternal plasma pentraxin 3 at 11 to 13 weeks of gestation in hypertensive disorders of pregnancy

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Objective To investigate whether in pregnancies that subsequently develop preeclampsia (PE), the maternal plasma concentration of the inflammatory factor pentraxin 3 (PTX3) at 11–13 weeks of gestation is increased and whether such a possible increase is associated with uterine artery pulsatility index (PI).

Methods The concentration of plasma PTX3 at 11–13 weeks was measured in a case-control study from 120 pregnancies that developed PE, including 27 who required delivery before 34 weeks (early PE), 87 cases of gestational hypertension (GH) and 207 normal controls. The median PTX3 multiple of the median (MoM) in the control and hypertensive groups were compared. Regression analysis was used to determine the significance of the association between plasma PTX3 and uterine artery PI.

Results Plasma PTX3 was significantly higher in the early PE group (1.44 MoM; $p < 0.0083$) but not in late PE (1.11 MoM) or GH (1.10 MoM) compared to the controls (0.97 MoM). There was no significant association between plasma PTX3 levels and uterine artery PI in either the PE group ($p = 0.693$) or in the controls ($p = 0.209$).

Conclusion Increase in maternal plasma PTX3 in pregnancies that subsequently develop early PE is evident from 11–13 weeks but the underlying mechanism for such an increase remains uncertain. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS: pentraxin 3; first trimester screening; preeclampsia; uterine artery Doppler

INTRODUCTION

Innate defense mechanisms against pathogens and damaged tissues consist of a cellular and a humoral arm. Pentraxins (PTX), a superfamily of proteins highly conserved during evolution, are an essential component of the humoral immune system (Garlanda *et al*., 2005). There are two types of PTXs. The short ones, such as C-reactive proteins, which are produced by the liver and the long ones, such as PTX3, which is expressed by many cells including vascular endothelial cells, monocytes, macrophages and fibroblasts (Garlanda *et al*., 2005). Plasma levels of PTX3 are increased in shock, sepsis, and vascular disorders such as myocardial infarction and small vessel vasculitis (Peri *et al*., 2000; Fazzini *et al*., 2001; Muller *et al*., 2001). A suggested role for PTX3 is binding to dying or apoptotic cells and their constituents thereby limiting their immunogenicity and reducing the risk of autoimmunity (Rovere *et al*., 2000; Baruah *et al*., 2006; Manfredi *et al*., 2008). Apoptosis is a normal event during pregnancy (Smith *et al*., 1997; Huppertz & Kingdom, 2004) and PTX3 may play a role in preventing maternal alloimmunization against the fetus (Rovere-Querini *et al*., 2006).

Preeclampsia (PE), which affects about 2% of pregnancies, is a major cause of maternal and perinatal morbidity and mortality (ACOG Committee on Practice Bulletin–Obstetrics, 2002; WHO, 2005). The underlying mechanism for PE is thought to be impaired placentation due to inadequate trophoblastic invasion of the maternal spiral arteries, documented by the findings of histological studies in affected pregnancies (Brosens *et al*., 1967; Khong *et al*., 1986; Pijnenborg *et al*., 1991). Additionally, in pregnancies destined to develop PE, Doppler ultrasound studies reported increased pulsatility index (PI) in the uterine arteries from as early as 11–13 weeks of gestation (Yu *et al*., 2005; Plasencia *et al*., 2007). The resulting placental hypoxia leads to trophoblastic cell death and the release of inflammatory factors that cause endothelial cell activation and damage (Redman, 1991; Roberts and Redman, 1993; Granger *et al*., 2001). Two studies have reported that in patients with PE there is a 6 to 9-fold increase in maternal plasma levels of PTX3 (Cetin *et al*., 2006; Rovere-Querini *et al*., 2006).

The aim of this study is to investigate whether the increase in maternal plasma PTX3 concentration precedes the clinical onset of PE and whether there is a relation between plasma PTX3 levels and uterine artery PI.

METHODS

Study population

This was a case–control study drawn from a large prospective observational study for hypertensive complications of pregnancy in women attending for their

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routine first hospital visit in pregnancy at Kings College Hospital, London, UK. In this visit, which is held at 11^{+0}–13^{+6} weeks of gestation, all women have an ultrasound scan to firstly, confirm gestational age from the measurement of the fetal crown–rump length (CRL), secondly, diagnose any major fetal abnormalities and thirdly, measure fetal nuchal translucency thickness as part of screening for chromosomal abnormalities (Kagan *et al*., 2008). We record maternal characteristics and medical history, measure the uterine artery PI by transabdominal colour Doppler (Plasencia *et al*., 2007), and store serum and plasma at −80 °C for subsequent biochemical analysis. This study is part of a research programme on the early prediction of pregnancy complications. The data on PTX3 were not published previously. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by King's College Hospital ethics committee.

The base cohort study population, wherein the present case–control study was nested, was examined between March 2006 and March 2007 and constituted 8 234 singleton pregnancies. In 147 (1.8%) cases, there was subsequent development of PE, 135 (1.6%) cases developed gestational hypertension (GH) and 7922 cases were unaffected by PE or GH. In addition, 30 (0.4%) pregnancies, in which there was at least one episode of hypertension but on the basis of the available data it was not possible to determine whether the diagnosis was PE, were also excluded from further analysis. In this study, we measured maternal plasma PTX3 in 120 cases who developed PE, including 27 who required delivery before 34 weeks (early PE) and 93 cases of late PE, 87 cases that developed GH and 207 unaffected controls. The selection of the specific samples from each group of hypertensive disorders was simply based on availability. The cases and controls were matched for length of storage of their blood samples and none of the samples were previously thawed and refrozen.

Maternal history

Patients were asked to complete a questionnaire on maternal age, racial origin, cigarette smoking during pregnancy, method of conception, medical history, medication, parity, obstetric history and family history of PE in the mother. The questionnaire was then reviewed by a doctor together with the patient. The maternal weight and height were measured and the body mass index (BMI) was calculated in kilograms per metre squared.

Outcome measures

The definitions of PE and GH were those of the International Society for the Study of Hypertension in Pregnancy (Davey and MacGillivray, 1988). In GH the diastolic blood pressure should be 90 mmHg or more on at least two occasions 4 h apart developing after 20 weeks of gestation in previously normotensive women in the absence of significant proteinuria. In PE there should be GH with proteinuria of 300 mg or more in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-h collection is available. In PE superimposed on chronic hypertension significant proteinuria (as defined above), should develop after 20 weeks of gestation in women with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks of gestation in the absence of trophoblastic disease).

Sample analysis

Duplicate plasma samples were analyzed using a PTX3 enzyme-linked immunosorbent assay (ELISA) kit (R & D Systems Europe Ltd, Abingdon, United Kingdom). The assay was performed according to the manufacturer's instructions. Sample pre-treatment was performed manually. The ELISA assay was performed on an automated ELISA processor (Dade-Behring BEP 2000, Liederbach, Germany). Substrate and stop reagents were added manually. Absorbance readings were taken on a VICTOR3 plate reader (PerkinElmer Life and Analytical Sciences, Turku, Finland). The concentrations of PTX3 were determined using MultiCalc software (PerkinElmer Life and Analytical Sciences, Turku, Finland). The lower limit of detection of the assay was 0.025 ng/mL and the between-batch imprecision was 8.4% at PTX3 concentration of 0.9 ng/mL, 8.3% at 3.4 ng/mL and 5.6% at 9.0 ng/mL. All samples were analyzed in duplicate and those with a large coefficient of variation (more than 20% for values of less than 1 ng/mL and more than 15% for values of 1 ng/mL or more) were reanalyzed.

Statistical analysis

The distributions of plasma PTX3 and uterine artery PI were made Gaussian after logarithmic transformation. The normality of the distribution was confirmed with Kolmogorov–Smirnov test and probability plots. Regression analysis was used to determine which of the factors amongst the maternal characteristics and gestation were significant predictors of log PTX3 in the unaffected group. Then the distribution of log PTX3 expressed as multiples of the median (MoM) of the unaffected group was determined in the PE and GH groups. Similarly, the measured uterine artery PI was converted into MoM after adjustment for gestation, maternal age, BMI and racial origin, as previously described (Plasencia *et al*., 2007). Kruskal–Wallis test with Dunn's procedure for *post hoc* evaluation was used to compare median MoM of PTX3 and uterine artery PI between the outcome groups. Chi square test and Fisher exact test were used to compare categorical variables between the outcome groups. Regression analysis was used to determine the significance of association between log PTX3 MoM and log uterine artery PI MoM in the different outcome groups.

The statistical software package SPSS 15.0 (SPSS Inc., Chicago, IL) and XLSTAT-Pro 2008 (Addinsoft, USA) were used for data analyses.

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RESULTS

The maternal characteristics of each of the outcome groups are compared in Table 1. In the early PE group, compared to controls there were more black women, more women had PE in their previous pregnancy, were chronic hypertensives on antihypertensive medication and delivered fetuses with a lower birth weight centile at an earlier gestation. In the late PE group compared to controls, women had significantly higher BMI, there were more black women, more women had PE in their previous pregnancies, were chronic hypertensives, their mother had PE and they delivered fetuses with a lower birth weight centile at an earlier gestation. In the GH group, compared to controls there were fewer Indian or Pakistani women and more women had PE in their previous pregnancies, their mother had PE and they delivered fetuses with a lower birth weight centile.

Unaffected group

Multiple regression analysis in the unaffected group demonstrated that for log PTX3 significant independent contributions were provided by racial origin and maternal weight but not fetal CRL ($p = 0.658$), smoking ($p = 0.089$), parity ($p = 0.923$) or method of conception ($p = 0.170$):

$$\log \text{expected PTX3} = -0.078 + (-0.003 \times \text{maternal weight in kilograms}) + (-0.122 \text{ if Asian, 0 if other racial origins});$$

$$R^2 = 0.042,\ p = 0.004.$$

In each patient, we used this formula to derive the expected log PTX3 and then expressed the observed value as a MoM of the expected (Table 2). Similarly, we used previously derived formula for uterine artery PI to calculate the MoM values (Plasencia *et al*., 2007).

Table 1—Maternal and fetal characteristics in the four outcome groups

Maternal and fetal characteristics	Control ($n = 207$)	Early preeclampsia ($n = 27$)	Late preeclampsia ($n = 93$)	Gestational hypertension ($n = 87$)
Maternal age in years, median (IQR)	31.9 (28.7–35.5)	32.7 (27.1–38.6)	31.6 (26.5–36.5)	33.4 (30.1–35.9)
Body mass index in Kg/m^2, median (IQR)	25.1 (22.9–28.7)	27.7 (23.9–32.0)	27.0 (23.7–33.4)*	26.6 (24.1–31.1)
Crown–rump length in mm, median (IQR)	64.1 (59.7–71.0)	67.0 (58.1–74.0)	62.0 (57.8–69.4)	62.2 (57.6–68.8)
Racial origin				
White, *n* (%)	144 (69.6)	11 (40.7)	41 (44.1)	65 (74.7)
Black, *n* (%)	41 (19.8)	12 (44.4)*	38 (40.9)*	17 (19.6)
Indian or Pakistani, *n* (%)	15 (7.2)	2 (7.4)	7 (7.5)	0*
Chinese or Japanese, *n* (%)	2 (1.0)	0	2 (2.2)	1 (1.1)
Mixed, *n* (%)	5 (2.4)	2 (7.4)	5 (5.4)	4 (4.6)
Parity				
Nulliparous, *n* (%)	83 (40.1)	14 (51.9)	60 (64.5)	48 (55.2)
Parous—no previous preeclampsia, *n* (%)	117 (56.5)	6 (22.2)*	22 (23.7)*	29 (33.3)*
Parous—previous preeclampsia, *n* (%)	7 (3.4)	7 (25.9)*	11 (11.8)*	10 (11.5)*
Family history of PE—Mother (*n*, %)	7 (3.4)	3 (11.1)	11 (11.8)*	9 (10.3)*
Cigarette smoker, *n* (%)	16 (7.7)	0	6 (6.5)	7 (8.0)
Conception				
Spontaneous, *n* (%)	199 (96.1)	24 (88.9)	89 (95.7)	84 (96.6)
Assisted, *n* (%)	8 (3.9)	3 (11.1)	4 (4.3)	3 (3.4)
Medical history				
None, *n* (%)	200 (96.6)	22 (81.5)	87 (93.5)	84 (96.6)
Chronic hypertension, *n* (%)	1 (0.5)	4 (14.8)*	4 (4.3)*	0
Diabetes mellitus, *n* (%)	2 (1.0)	0	1 (1.1)	2 (2.3)
Others, n (%)	3 (1.9)	1 (3.7)	1 (1.1)	1 (1.1)
Medication during pregnancy				
None, *n* (%)	187 (90.3)	23 (85.2)	85 (91.4)	75 (86.2)
Antihypertensives, *n* (%)	0	2 (7.4)*	2 (2.2)	0
Aspirin, *n* (%)	3 (1.5)	1 (3.7)	0	2 (2.3)
Others, *n* (%)	17 (8.2)	1 (3.7)	6 (6.5)	10 (11.5)
Fetal birth weight centile, median (IQR)[a]	51.6 (28.1–79.9)	0.49 (0.07–7.96)*	19.5 (6.6–63.1)*	29.2 (15.0–69.8)*
Gestation at delivery (weeks), median (IQR)	39.5 (38.5–40.3)	31.4 (28.5–32.3)*	38.3 (37.2–39.6)*	40.0 (38.6–40.5)

IQR, interquartile range.

* $p < 0.05$. for comparisons between outcome groups (Chi square test and Fisher exact test for categorical variables and Kruskal–Wallis test and Dunn's procedure for continuous variables).

[a] In all patients, the birth weight was converted into a percentile after correction for gestation at delivery and sex of the newborn, maternal ethnic origin, weight, height and parity (Gardosi and Francis, 2007).

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Table 2—Median (interquartile range) for plasma pentraxin 3 and uterine artery pulsatility index (PI) in the outcome groups

		Plasma pentraxin 3 (median, IQR)		Uterine artery PI (median, IQR)	
Outcome group	n	MoM	ng/mL	MoM	Unit
Unaffected	207	0.97 (0.74–1.22)	0.48 (0.37–0.61)	1.05 (0.84–1.31)	1.68 (1.37–2.10)
Total preeclampsia	120	1.17 (0.80–1.71)[a]	0.51 (0.37–0.80)	1.31 (0.99–1.56)[a]	2.10 (1.58–2.50)
Early preeclampsia	27	1.44 (0.83–2.00)[b]	0.59 (0.43–.92)	1.54 (1.20–1.68)[b]	2.49 (2.00–2.66)
Late preeclampsia	93	1.11 (0.78–1.63)	0.48 (0.36–0.78)	1.26 (0.94–1.45)[b]	2.00 (1.55–2.37)
Gestational hypertension	87	1.10 (0.83–1.52)	0.50 (0.39–0.69)	1.11 (0.89–1.31)	1.76 (1.42–2.07)

MoM, multiple of the median; IQR, interquartile range; comparisons between outcome groups by Kruskal–Wallis test and Dunn's procedure.
[a] Adjusted significance level $p < 0.0167$.
[b] Adjusted significance level $p < 0.0083$.

Hypertensive disorders

Plasma PTX3 MoM was significantly higher in early PE compared to controls but not in late PE or GH (Table 2). Uterine artery PI MoM was significantly increased in both early PE and late PE than in controls but not in GH (Table 2).

Relationships between plasma PTX3 and uterine artery PI

There were no significant associations between plasma PTX3 and uterine artery PI in either the PE group ($p = 0.693$, Figure 1), the GH group ($p = 0.411$) or the controls ($p = 0.209$).

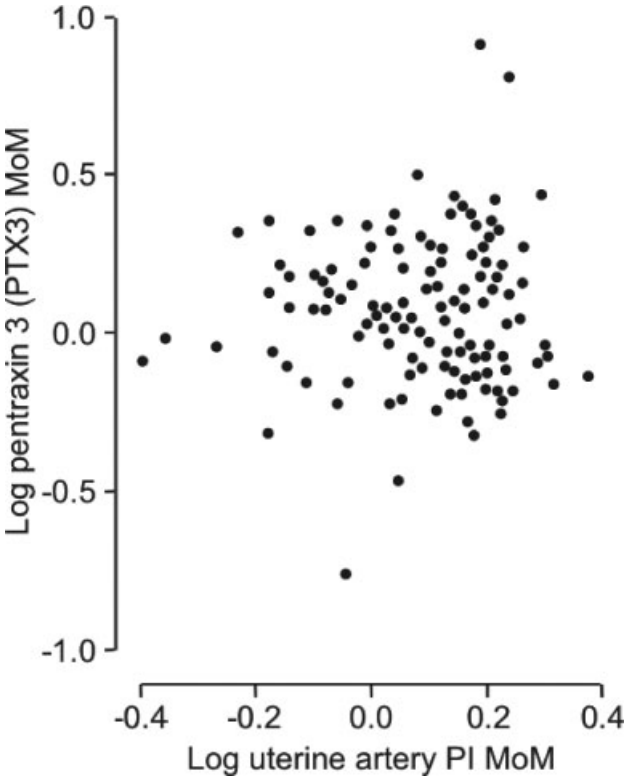

Figure 1—Association between log pentraxin 3 (PTX3) multiple of the median (MoM) and log uterine artery pulsatility index (PI) MoM in the preeclampsia group

DISCUSSION

The finding of this study confirm the association between PE and increased maternal plasma concentration of PTX3 (Cetin *et al*., 2006; Rovere-Querini *et al*., 2006) and demonstrate that the increase in this acute phase protein is evident from as early as the first trimester of pregnancy and precedes the clinical onset of the disease by several months.

In the unaffected controls, the measured concentration of maternal plasma PTX3 decreased with maternal weight and was lower in Asians than in white women. Consequently, as in the case of uterine artery PI the measured concentration of plasma PTX3 must be adjusted for these variables before comparing with pathological pregnancies (Plasencia *et al*., 2007).

In pregnancies destined to develop PE, the uterine artery PI at 11–13 weeks was increased providing further evidence that PE is the consequence of reduced placental perfusion due to impaired trophoblastic invasion of the maternal spiral arteries and their conversion from narrow muscular vessels to wide non-muscular channels (Brosens *et al*., 1967; Khong *et al*., 1986; Pijnenborg *et al*., 1991). The differences between pregnancies developing PE from controls in both plasma PTX3 and uterine artery PI were particularly marked in severe early onset disease requiring delivery before 34 weeks. This is compatible with the findings of firstly, histological studies that early PE is a more severe disease with greater impairment in placentation and secondly, the results of clinical studies that it is early PE rather than late disease, which is associated with increased risk of perinatal mortality and morbidity and both short-term and long-term maternal complications (Witlin *et al*., 2000; von Dadelszen *et al*., 2003; Irgens *et al*., 2001; Egbor *et al*., 2006).

Impaired perfusion of the placenta is thought to cause hypoxia-related trophoblastic cell death and the release of inflammatory factors, which in turn cause endothelial dysfunction and the development of the clinical symptoms of the disease (Redman, 1991; Roberts & Redman, 1993; Granger *et al*., 2001). The extent to which PTX3, which is expressed in vascular endothelial cells (Garlanda *et al*., 2005), is involved in the pathogenesis of PE

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or is a mere marker of the altered endothelial function observed in this disease remains to be determined. In PE there is increased apoptosis, evidenced by both histological studies of the placenta and raised maternal plasma concentration of cell-free fetal DNA (Lo *et al*., 1999; Leung *et al*., 2001; Zhong *et al*., 2005). PTX3 binds to dying or apoptotic cells, and the increased plasma levels of PTX3 in PE may reflect an immunological response to the damaged placenta (Rovere *et al*., 2000; Baruah *et al*., 2006; Manfredi *et al*., 2008).

Plasma PTX3 and uterine artery PI at 11–13 weeks were increased in women who subsequently developed early PE, rather than in late PE or GH. However, there was no significant association between the degree of impairment in placental perfusion, as measured by uterine artery PI, and the subsequent inflammatory and immunological response, manifested by increased plasma PTX3 level, which suggests that there may not be a causal association between the degree of impairment in placental perfusion and the subsequent inflammatory and immunological response. Further studies are required to establish whether the cause of the increased plasma PTX3 levels is endothelial, immunological or placental.

Original publication

All authors have read and approved the submission of the manuscript; the manuscript has not been published and is not being considered for publication elsewhere, in whole or in part, in any language.

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