AUTHOR'S QUERY SHEET

Author(s): I. Cetin et al.
Article title: SOBS
Article no: 397316

Dear Author

The following queries have arisen during the editing of your manuscript and are identified on the proofs. Unless advised otherwise, please submit all corrections using the CATS online correction form.

AQ1 Please check the corresponding author's info.

AQ2 Please check the number agreement.

AQ3 Please spell out [LDL] in full at first mention.

AQ4 A declaration of interest statement reporting no conflict of interest has been inserted. Please confirm the statement is accurate.

Acta Obstetricia et Gynecologica. 2009; 00: 1–4

informa
healthcare

SHORT REPORT

First trimester PTX3 levels in women who subsequently develop preeclampsia and fetal growth restriction

IRENE CETIN[1], VERONICA COZZI[1], ARIS T. PAPAGEORGHIOU[2], VIRGINIA MAINA[3], ALESSANDRO MONTANELLI[4], CECILIA GARLANDA[4] & BASKY THILAGANATHAN[2]

[1]*Unit of Obstetrics and Gynecology I, Department of Clinical Sciences Luigi Sacco, University of Milan, Italy,* [2]*Fetal Medicine Unit, Division of Clinical and Developmental Sciences, St George's, University of London, Cranmer Terrace, London SW17 0RE, UK,* [3]*Centro IDET, Institute of General Pathology, University of Milan, Italy, and* [4]*Istituto Clinico Humanitas, via Manzoni 56, 20089 Rozzano, Milan, Italy*

Abstract

Pentraxin 3 (PTX3) and C-reactive protein (CRP) levels were measured in the first trimester of pregnancy in women who subsequently developed preeclampsia (PE, $n=16$) and fetal growth restriction (FGR, $n=12$) requiring iatrogenic delivery before 37 weeks, and those who had uncomplicated pregnancies delivering at term ($n=60$). Mean PTX3 levels were significantly higher in women who subsequently developed PE (7.31 ng/ml, SD = 4.12) when compared to those with normal pregnancy outcome (4.92 ng/ml, SD = 1.94, $p=0.0046$). There were no significant differences between PTX3 levels in women with FGR (4.82 ng/ml, SD = 2.35) compared to normal pregnancy outcome ($p=0.88$). The median CRP levels did not vary significantly between the three groups ($p=0.26$). PTX3 levels in women who subsequently develop PE are already elevated in the first trimester, but not in those that develop FGR. This supports the hypothesis of an excessive maternal inflammatory response to pregnancy in the etiology of PE.

Key words: *Preeclampsia, inflammation, screening, pentraxin*

Introduction

The etiology of preeclampsia (PE) and fetal growth restriction (FGR) remains unclear despite their obvious importance from the public health perspective as leading causes of maternal and perinatal mortality worldwide. What is known is that disorders of trophoblast development, endothelial activation, angiogenesis, and abnormal oxidative stress may contribute to the etiology or pathophysiology of PE (1). The maternal serum markers related to these mechanisms have been evaluated for the early identification of women at high risk of developing PE (2).

There is increasing evidence that an abnormal immunological or inflammatory response between the developing trophoblast and maternal decidua may have an important role in the etiology of PE (1). Pentraxin 3 (PTX3) is a recently described inflammatory molecule which belongs to the same family as C-reactive protein (CRP). It is expressed in response to inflammatory stimuli by a variety of cells, including endothelial cells, monocytes, macrophages, and fibroblasts (3). PTX3 is known to be present in receptive endometrium, and abnormal expression is implicated in pregnancy failure (4). Previous studies have shown that maternal PTX3 levels are significantly higher in women with established PE in the third trimester when compared to those with normal pregnancies (5,6). The aim of this study is to compare first trimester serum levels of PTX3 in women who subsequently developed preterm PE to those requiring preterm delivery for FGR or with a normal pregnancy outcome.

Material and methods

This is a prospective nested case–control study from a larger ongoing study examining first trimester

AQ1

Correspondence: Basky Thilaganathan, Fetal Medicine Unit, St George's Hospital, Blackshaw Road, London SW17 0QT, UK. E-mail: basky@pobox.com

(Received 3 November 2008; accepted 9 April 2009)

ISSN 0001-6349 print/ISSN 1600-0412 online
DOI: 10.1080/00016340902971441

ultrasound and serum markers for PE. Unselected women with singleton pregnancies attending St George's Hospital for their first trimester ultrasound scan between 11 and 14 weeks of gestation were invited to participate. The study protocol was approved by the medical ethics committee of the hospital and written informed consent was obtained from each participant. Mothers with pre-existing medical disorders or receiving drug therapy were excluded from the study. Maternal blood was collected and stored at the time of the ultrasound examination. Maternal demographic characteristics and ultrasound findings were entered onto a computerized database at the time of the ultrasound examination. Data on pregnancy outcome were entered onto the same database when these became available from the delivery records. A database search was performed to identify all cases meeting the inclusion criteria, and their stored blood samples were then retrieved.

Maternal blood samples were analyzed from three groups of patients: those with pregnancies complicated by preterm PE with normal fetal growth; and those with preterm FGR in the absence of PE. For the purpose of this study, only cases of PE and FGR requiring delivery before 37 weeks of gestation were included. For every case, the next two cases with full term uncomplicated pregnancies with normal fetal growth were selected as controls.

PE was defined according to the guidelines of the International Society for the Study of Hypertension in Pregnancy. This requires two recordings of systolic blood pressure $\geq$140/90 mMHg at least 4 h apart in a previously normotensive woman, and proteinuria of 300 mg or more in 24 h, or two readings of at least 2+ on dipstick analysis of midstream or catheter urine specimens if no 24 h collection is available. FGR was defined as a birth weight below the fifth percentile for gestational age necessitating delivery because of abnormal tests of fetal well-being (abnormal umbilical artery pulsatility index [>95th centile], abnormal biophysical profile, or abnormal cardiotocography). Uncomplicated pregnancies were defined as pregnancies which remained normotensive throughout gestation and resulted in full-term delivery of a healthy infant with appropriate weight for gestational age.

Maternal blood samples were centrifuged and serum was stored at –80°C for up to two years until analysis. Analysis was performed at the Istituto Clinico Humanitas, where the researchers were blinded to the pregnancy outcome. The Sandwich ELISA for PTX3 was performed as previously described (7). The PTX3 ELISA system has a detection limit of 0.1 ng/mL with an intra-assay and inter-assay coefficient of variation (CV) of <5%. Serum levels of CRP were measured using the ultrasensitive latex immunoassay CRP Vario (Abbott Diagnostics Europe) with the intra- and inter-assay CV both <10%. All assays were performed without knowledge of pregnancy outcome.

Statistical analysis

Sample size was calculated on the basis that the difference in serum PTX3 levels between women with PE and controls would be at least two-fold (5). Setting type I and II errors at 0.05 and 0.20, respectively, we calculated the required sample size should be at least 10 cases of PE and FGR. Data in the three groups were analyzed for evidence of non-normality using the Shapiro–Wilk W test. Data were presented as median and interquartile range (IQR) if non-parametric or mean and standard deviation (SD) if parametric. The three groups were compared using the Fisher–Freeman–Halton exact test for dichotomous variables, and ANOVA or Kruskal–Wallis test for continuous variables. If there was evidence of difference between the three groups, subgroup analysis between pairs of outcomes were performed using the t-test or Mann–Whitney U test. For multiple comparisons, statistically significant results were confirmed using Scheffé test. Two-sided p-values are reported throughout.

Results

Sixteen cases of PE, 14 of FGR and 60 controls were identified. Assay results were only available from 12 women with FGR. There were no significant differences in maternal age, parity, and BMI between the three groups of patients (Table I). There were significantly more Afro-Caribbean women in the group that later developed preterm PE. The gestational age at delivery and birth weight in pregnancies complicated by either PE or FGR was lower than in normal pregnancies, as preterm delivery was included in the definition of these outcomes (Table I).

There were significant differences in mean PTX3 levels between the three groups (Figure 1, ANOVA $F=6.03$, $p=0.0036$). Mean PTX3 levels were significantly higher in women who subsequently developed preterm PE (7.31 ng/ml, SD = 4.12) compared to those with normal pregnancy outcome (4.92 ng/ml, SD = 1.94, $p=0.0046$). There were no significant differences between PTX3 levels in women with preterm FGR (4.82 ng/ml, SD = 2.35) compared to those with normal pregnancy outcome ($p=0.88$). Scheffé's test was used to confirm these differences were not due to multiple comparisons (normal versus PE, $p=0.0046$, FGR versus normal,

Table I. Maternal demographic characteristics and pregnancy outcomes in the study groups.

	Normal ($n=60$)		FGR ($n=12$)		PE ($n=16$)		
	n or median	Percentage (%) or IQR	n or median	Percentage (%) or IQR	n or median	Percentage (%) or IQR	$p^{\star}=$
Parity							0.30
Nulliparous	32	53%	6	50%	5	31%	
Parous	28	47%	6	50%	11	69%	
Race							0.017
Caucasian	49	82%	7	58%	9	56%	
African	2	3%	2	17%	5	31%	
Asian	3	5%	2	17%	1	6%	
Other	6	10%	1	8%	1	6%	
Age (years)	32.6	29.1–5.8	33.3	28.5–5.9	34.7	28.5–6.9	0.72
BMI	22.9	21.1–5.1	24.2	22.8–8.6	25.2	21.9–8.5	0.17
Birth weight (grams)	3,390	3,106–3,700	1,514	1,349–3,819	2,495	2,315–2,640	–**
Birth weight (centile)	43.6	24.8–6.0	1.3	0.3–0.8	21.9	8.6–32.0	–**
Gestational age at delivery (weeks)	40^{+3}	39^{+3}–41^{+1}	34^{+4}	33^{+3}–35^{+3}	36^{+3}	36^{+1}–36^{+4}	–**

Note: BMI = body mass index; IQR = interquartile range; FGR = fetal growth restriction; PE = preeclampsia.
*p-values comparing the three groups were calculated using the Fisher–Freeman–Halton exact test for dichotomous variables and Kruskal–Wallis test for continuous variables.
**p-values for birth weight and gestational age are not reported as these variables were used to define the three groups.

$p=0.993$, and FGR versus PE, $p=0.039$). Levels above the 95th percentile for PTX3 in normal pregnancy (8.55 ng/ml) were found in 5/16 (31%) of those that subsequently developed PE. There was no significant correlation between PTX3 and BMI overall, or in any of the three groups ($p=0.174$).

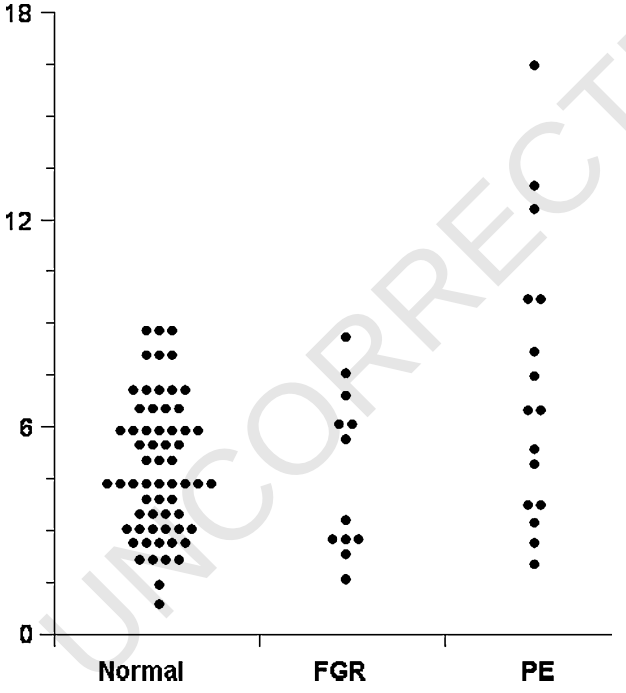

Figure 1. First trimester maternal serum PTX3 levels (ng/ml) in women with normal outcome, fetal growth restriction (FGR), and those that developed preeclampsia (PE).

There were no significant differences in the median CRP levels (Kruskal–Wallis $T=2.73$, $p=0.26$) between the women with normal pregnancy outcome (0.30 mg/dL, IQR 0.17–1.37), preterm PE (0.37 mg/dL, IQR 0.20–1.21), and preterm FGR (0.24 mg/dL, IQR 0.18–0.65). No correlation between CRP and PTX3 was seen in women with normal outcome ($r=-0.02$, $p=0.88$).

Discussion

The data of this study suggest that abnormally high maternal serum long PTX3 levels in early pregnancy are associated with the subsequent development of preterm PE, but not FGR. PTX3 is a member of the pentraxin family, which includes CRP and serum amyloid P component (SAP). The cross-species evolutionary conservation of PTX3, in contrast to CRP and SAP, suggests an important role for this molecule. PTX3 appears to have a major role in resistance against selected pathogens by acting as a predecessor of antibodies, recognizing microbes, activating complement, and facilitating pathogen recognition by phagocytes. However, recent animal studies have also shown coordinated temporal PTX3 expression at uterine sites during implantation (4). Furthermore, deletion of this gene results in early pregnancy failure suggesting that PTX3 plays a crucial role in implantation and decidualization (4).

In the human, PTX3 is seen in receptive endometrium, and in-vitro co-culture studies of trophoblast and uterine stromal cells have demonstrated significant upregulation of inflammatory genes, especially

IL8 and PTX3 (8,9). This suggests that successful implantation may rely on the modulation of the immune environment of the decidua by the trophoblast to ensure an enriched cytokine/chemokine environment. The abnormal pro-inflammatory maternal status (IL1 and TNF-α), pre-existing endothelial damage, and excess of oxidized LDL seen in the first trimester in women who subsequently develop PE may all induce PTX3 elevation (3,10). Further studies are needed to address the potential involvement of PTX3 in the pathogenesis of subsequent poor trophoblast invasion. Differences in placental histology seen between PE and FGR may explain the lack of PTX3 elevation in FGR cases, suggesting that abnormal maternal immune responses may not be responsible for the placental abnormalities seen in FGR (11). The additional study finding that neither preterm PE nor FGR have elevated CRP levels is consistent with most but not all previous studies (12). The lack of correlation between CRP and PTX3 suggest that pregnancy may exert independent mechanisms for modulating these inflammatory markers.

One of the aims of antenatal care has been early identification of women at risk of adverse pregnancy outcomes such as PE. There is evidence from a number of trials that aspirin or calcium therapy may be particularly effective when started in the early part of pregnancy, and this has highlighted the need for identifying high-risk women in the first trimester. The finding of this study that PTX3 is significantly higher in women with subsequent PE as early as the first trimester could make this a useful and biologically plausible marker. PTX3 unlike uterine artery Doppler, PlGF, VEGF, sFLt-1, or PP13, is a marker of maternal inflammatory response rather than placental function, and therefore may be an independent, synergistic biomarker for PE (5). Further evaluation of PTX3 in the prediction of PE should be carried out, and its relationship to uterine artery Doppler and other maternal serum markers examined.

Acknowledgements

This work has been financially supported by the 6th Framework Programme of the European Union (Project EMBIC, Contract no. LSHM-CT-2004-512040), by Istituto Superiore di Sanitá, MIUR, Ministero della Salute and Fondazione CARIPLO (Project 'Genetics and postgenomic of major defects in human reproduction' Contract no. 2005.1055/104878). Professor Thilaganathan holds a patent on multiple first trimester serum biomarker screening for preeclampsia.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005;308:1592–4.
2. Papageorghiou AT, Campbell S. First trimester screening for preeclampsia. Curr Opin Obstet Gynecol. 2006;18:594–600.
3. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. Annu Rev Immunol. 2005;23:337–66.
4. Tranguch S, Chakrabarty A, Guo Y, Wang H, Dey SK. Maternal pentraxin 3 deficiency compromises implantation in mice. Biol Reprod. 2007;77:425–32.
5. Cetin I, Cozzi V, Pasquini F, Nebuloni M, Garlanda C, Vago L, et al. Elevated maternal levels of the long pentraxin 3 (PTX3) in preeclampsia and intrauterine growth restriction. Am J Obstet Gynecol. 2006;194:1347–53.
6. Rovere-Querini P, Antonacci S, Dell'Antonio G, Angeli A, Almirante G, Dal Cin E, et al. Plasma and tissue expression of the long pentraxin 3 during normal pregnancy and preeclampsia. Obstet Gynecol. 2006;108:148–55.
7. Peri G, Introna M, Corradi D, Iacuitti G, Signorini S, Avanzini F, et al. PTX3, a prototypic long pentraxin, is an early indicator of acute myocardial infarction in man. Circulation. 2000;102:636–41.
8. Hess AP, Hamilton AE, Talbi S, Dosiou C, Nyegaard M, Nayak N, et al. Decidual stromal cell response to paracrine signals from the trophoblast: amplification of immune and angiogenic modulators. Biol Reprod. 2007;76:102–17.
9. Popovici RM, Betzler NK, Krause MS, Luo M, Jauckus J, Germeyer A, et al. Gene expression profiling of human endometrial–trophoblast interaction in a co-culture model. Endocrinology. 2006;147:5662–75.
10. Williams MA, Farrand A, Mittendorf R, Sorensen TK, Zingheim RW, O'Reilly GC, et al. Maternal second trimester serum tumor necrosis factor-alpha-soluble receptor p55(sTNFp55) and subsequent risk of preeclampsia. Am J Epidemiol. 1999;149:323–9.
11. Newhouse SM, Davidge ST, Winkler-Lowen B, Demianczuk N, Guilbert LJ. In vitro differentiation of villous trophoblasts from pregnancies complicated by intrauterine growth restriction with and without pre-eclampsia. Placenta. 2007;28:999–1003.
12. Djurovic S, Clausen T, Wergeland R, Brosstad F, Berg K, Henriksen T. Absence of enhanced systemic maternal inflammatory response at 18 weeks of gestation in women with subsequent preeclamsia. Br J Obstet Gynaecol. 2002;109:759–64.

ACTA OBSTETRICIA ET GYNECOLOGICA

Colour figure order form

Article details

Manuscript ID: _____ First author: _____

Title: _____

1. COLOUR FIGURES

Figures will appear in colour in the online version of the article free of charge. Authors or their institutions must bear the cost of any colour in the printed journal. This is charged at a fixed rate of USD 1000 for the first page and 500 for the second and subsequent pages.

I require _____ number of pages to be printed in colour. Figure numbers: _____

2. PAYMENT

Page Charges Cost	Colour Figures Cost	Total Cost

Terms of payment: Payment to be made 30 days from receipt of invoice from Informa UK Ltd, which will be sent out using the details you provide below immediately following publication of the print issue containing the article. (Payment/Account details for Informa UK Ltd is available on request). Invoice to:

Name: _____

Position: _____ Institution: _____

Invoice Mailing Address: _____

_____

_____

Email address: _____

PO or other Billing Reference: _____ VAT/IVA No. _____

Other invoicing instructions: _____

Alternatively, provide credit card details below (your card will not be debited until publication):

Credit Card Payment:

Credit Card ☐ Please charge my AMEX/VISA/MasterCard (delete as appropriate)

Card No ☐☐☐☐/☐☐☐☐/☐☐☐☐/☐☐☐☐ Exp.date ☐☐/☐☐

CVV number (last 3 or 4 digits on reverse of card):

Name on credit card:

Address where registered:

Signature: **Date:**

All orders are accepted subject to Informa Healthcare's standard Terms and Conditions.

Please fax a copy of this form to the editorial office at +46 8 440 80 50 and send the original copy (by non-priority/ regular (air)mail, retaining a copy for your files) to: Informa Healthcare, PO Box 3255, SE-103 65 Stockholm, Sweden

NOTE: This form must be signed and returned before publication can proceed.