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Maternal serum angiopoietin-2 at 11 to 13 weeks of gestation in hypertensive disorders of pregnancy

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Objective In women with preeclampsia (PE), the serum concentration of the growth factor angiopoietin-2 (Ang-2) is significantly lower than in unaffected controls. The objective of this study is to determine if the decrease in serum Ang-2 is evident from the first trimester of pregnancy before the clinical onset of PE.

Methods Serum Ang-2 and uterine artery pulsatility index (PI) were measured at 11 to 13 weeks in 126 pregnancies that subsequently developed PE, 88 cases that developed gestational hypertension (GH) and 214 unaffected controls.

Results Maternal serum Ang-2 in the PE group [0.96 multiple of the median (MoM)] and in GH (1.12 MoM) was not significantly different from the unaffected group (1.07 MoM). Uterine artery PI was significantly higher in the PE group (1.32 MoM) but not in GH (1.11 MoM) compared to the unaffected group (1.05 MoM).

Conclusion In pregnancies that develop PE there is Doppler evidence of impaired placentation from the first trimester of pregnancy. However, the impaired placentation is not reflected in altered maternal serum levels of Ang-2. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS: angiopoietin-2; first-trimester screening; preeclampsia; uterine artery Doppler

INTRODUCTION

Angiopoietins are angiogenic growth factors produced by the villous trophoblast and they are thought to play an important role in placental vascular development (Charnock-Jones *et al*., 2004; Seval *et al*., 2008). There are three stages in the vascular development of the placenta: first, vasculogenesis which refers to the formation of a primitive vascular network from endothelial progenitor cells, second, branching angiogenesis and third, non-branching angiogenesis both of which involve the formation of new blood vessels from already existing ones (Carmeliet *et al*., 2000; Yancopoulos *et al*., 2000; Geva *et al*., 2002). The angiopoietins act on the second and third stages: angiopoietin-1 (Ang-1) causes endothelial maturation and vascular stabilization, whereas angiopoietin-2 (Ang-2) acts as an antagonist of Ang-1 thereby leading to further angiogenesis (Suri *et al*., 1996; Maisonpierre *et al*., 1997; Zhang *et al*., 2001).

In preeclampsia (PE), there is histological and Doppler evidence of impaired placentation (Khong *et al*., 1986; Pijnenborg *et al*., 1991; Yu *et al*., 2005; Plasencia *et al*., 2007). Two studies have reported that in women with PE the serum concentration of Ang-2 is significantly lower than in unaffected controls (Hirokoshi *et al*., 2005; Nadar *et al*., 2005). Hirokoshi *et al*(2005) reported that the mean level of Ang-2 in 26 women with PE at 25 to 40 weeks of gestation was 4.5 ng/mL, compared to 18.9 ng/mL in unaffected controls of the same gestation and 2.1 ng/mL in non-pregnant controls. Similarly, Nadar *et al*. (2005) reported that the median level of Ang-2 in 35 women with PE at 26 to 40 weeks was 9 ng/mL, compared to 20 ng/mL in unaffected controls and 6 ng/mL in non-pregnant women. It is uncertain whether the low levels of Ang-2 in PE are the consequence of the disease or whether the decrease precedes the clinical onset of PE and is a reflection of the involvement of this growth factor in the pathogenesis of the disease.

The aim of this study was to investigate whether in pregnancies that develop hypertensive disorders the maternal serum concentration of Ang-2 is altered in the first trimester of pregnancy.

METHODS

Study population

This was a prospective screening study for hypertensive complications of pregnancy in women attending for their routine first hospital visit in pregnancy. In this visit, which is held at $11+0$ to $13+6$ 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 (Snijders *et al*., 1998; Kagan *et al*., 2008). We record maternal characteristics and medical history, measure the uterine artery pulsatility index (PI) by transabdominal colour Doppler (Plasencia *et al*., 2007), and store serum and plasma at $-80\,^{\circ}C$ for subsequent biochemical analysis. This study is part of a

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research programme on the early prediction of pregnancy complications, and the data from these patients on uterine artery PI were included in previous publications (Akolekar *et al*., 2008; Poon *et al*., 2009) The data on Ang-2 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.

We prospectively examined 8234 singleton pregnancies between March 2006 and March 2007. 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 this study, we measured maternal serum Ang-2 in 126 cases that developed PE, 88 cases that developed GH and 214 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 Kg/m^2.

Outcome measures

The definitions of PE and GH were those of the International Society for the Study of Hypertension in Pregnancy (Davey *et al*., 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 serum samples of 50 μL were analysed using a Dissociation-Enhanced Lanthanide Fluorescent Immunoassay (DELFIA) Ang-2 sandwich immunoassay (PerkinElmer Life and Analytical Sciences, Turku, Finland) using a two step protocol. The analysis was done using two monoclonal anti-human angiopoietin antibodies (R & D Systems, Minnesota, USA; catalogue number MAB098 and MAB0983). Firstly, standards and samples (50 μL/well) in duplicates were added to the wells followed by assay buffer (150 μL/well) and then incubated at room temperature for 2 h. The plate was then washed four times and then DELFIA Enhancement solution (200 μL/well) was dispensed. Fluorescence readings were measured on a VICTOR2D plate reader (PerkinElmer Life and Analytical Sciences, Turku, Finland). The concentrations of Ang-2 were determined using MultiCalc software (PerkinElmer Life and Analytical Sciences, Turku, Finland). The between-batch coefficient of variation was less than 5% in all the samples analysed.

Statistical analysis

The distributions of serum Ang-2 and uterine artery PI were made Gaussian after logarithmic transformation. Normality of distributions was checked by Kolmogorov-Smirnov test and probability plots. Regression analysis was used to determine which of the factors among the maternal characteristics and gestation were significant predictors of log Ang-2 in the unaffected group. Then the distribution of log Ang-2 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). Kruskall-Wallis test with Dunn's procedure and Bonferroni correction was used to compare median MoM of Ang-2 and uterine artery PI between the outcome groups.

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL) and XLSTAT-Pro 2008 (Addinsoft, USA) were used for data analyses.

RESULTS

The maternal characteristics of each of the outcome groups are compared in Table 1. In the PE group compared to controls, the BMI was significantly higher, there were more Black women and more women had PE in their previous pregnancies were chronic hypertensives on antihypertensive medication and their mother had PE. In the GH group compared to controls, there were fewer Indian or Pakistani women and more women had PE in their previous pregnancies and their mother had PE.

The serum Ang-2 values were log-transformed to make the distribution Gaussian. The normality of the distribution was tested with Kolmogorov-Smirnov test ($p = 0.196$). Multiple regression analysis in the unaffected group demonstrated that for log Ang-2 significant independent contributions were provided by maternal weight but not fetal CRL ($p = 0.877$), smoking ($p = 0.863$), parity ($p = 0.797$) or Black racial origin ($p = 0.093$).

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Table 1—Maternal characteristics in the three outcome groups

Maternal characteristics	Control ($n = 214$)	Preeclampsia ($n = 126$)	Gestational hypertension ($n = 88$)
Maternal age in years, median (IQR)	31.9 (28.7–35.2)	31.7 (26.7–36.4)	33.4 (30.1–36.2)
BMI in kg/m^2, median (IQR)	25.1 (23.0–29.0)	27.1 (23.8–32.2)*	26.5 (24.2–31.5)
CRL in mm, median (IQR)	64.2 (59.7–71.0)	62.9 (58.1–71.0)	62.5 (57.7–68.8)
Gestational age at sampling, median (IQR)	12.6 (12.3–13.0)	12.4 (12.1–13.0)	12.4 (12.3–13.0)
Racial origin			
White, *n* (%)	150 (70.1)	51 (40.5)	67 (76.1)
Black, *n* (%)	43 (20.1)	55 (43.7)***	16 (18.2)
Indian or Pakistani, *n* (%)	14 (6.5)	9 (7.1)	0*
Chinese or Japanese, *n* (%)	2 (0.9)	2 (1.6)	1 (1.1)
Mixed, *n* (%)	5 (2.3)	9 (7.1)	4 (4.6)
Parity			
Nulliparous, *n* (%)	87 (40.7)	79 (62.7)	50 (56.8)
Parous—no previous preeclampsia, *n* (%)	120 (56.1)	29 (23.0)***	28 (31.8)***
Parous—previous preeclampsia, *n* (%)	7 (3.3)	18 (14.3)***	10 (11.4)*
Family history of preeclampsia—mother (*n*, %)	6 (2.8)	15 (11.9)**	9 (10.2)*
Cigarette smoker, *n* (%)	16 (7.5)	6 (4.8)	7 (8.0)
Conception			
Spontaneous, *n* (%)	205 (95.8)	118 (93.7)	84 (95.5)
Assisted, *n* (%)	9 (4.2)	8 (6.3)	4 (4.5)
Medical history			
None, n (%)	208 (97.2)	116 (92.1)	85 (96.6)
Chronic hypertension, *n* (%)	1 (0.5)	8 (6.3)**	0
Diabetes mellitus, *n* (%)	2 (0.9)	0	2 (2.3)
Thrombophilia, *n* (%)	3 (1.4)	2 (1.6)	1 (1.1)
Medication during pregnancy			
None, *n* (%)	194 (90.7)	114 (90.5)	76 (86.4)
Antihypertensives, *n* (%)	0	4 (3.2)*	0
Insulin, *n* (%)	2 (0.9)	0	2 (2.3)
Anti-asthmatics, *n* (%)	5 (2.3)	4 (3.2)	4 (4.5)
Thyroxin, *n* (%)	2 (0.9)	2 (1.6)	2 (2.3)
Aspirin, *n* (%)	4 (1.8)	1 (0.8)	2 (2.3)
Antidepressant, *n* (%)	2 (1.0)	1 (0.8)	1 (1.1)
Antiepileptic, *n* (%)	4 (1.9)	0	1 (1.1)
Others, *n* (%)	1 (0.5)	0	0

Comparisons between outcome groups (Chi-square test and Fisher exact test for categorical variables and Kruskall-Wallis test and Dunn's procedure with Bonferroni correction for continuous variables): $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.
IQR, interquartile range; CRL, crown-rump length; BMI, body mass index.

$$\log \text{expected Ang-2} = 0.995 + (-0.003 \times \text{maternal weight in kg}); R^2 = 0.016, p = 0.036.$$

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

There was no significant difference in maternal serum Ang-2 levels in PE or GH compared to controls (Table 2). In contrast, uterine artery PI was significantly increased in PE.

DISCUSSION

The results of this study demonstrate that at 11 to 13 weeks of gestation, the maternal serum concentration of Ang-2 in women who subsequently develop PE is not significantly different from unaffected controls. Consequently, the previously reported association between established PE and decreased levels of Ang-2 (Hirokoshi *et al*., 2005; Nadar *et al*., 2005) is not evident before the clinical onset of the disease and certainly not from the first trimester of pregnancy.

In unaffected controls, the measured concentration of maternal serum Ang-2 decreased with maternal weight. Consequently, as in the case of uterine artery PI the measured concentration of Ang-2 must be adjusted for this variable before comparing with pathological pregnancies (Plasencia *et al*., 2007). A previous cross-sectional study of 20 normotensive pregnancies at 8 to 41 weeks reported that there is a highly significant association between placental protein concentration and mRNA expression of Ang-2 and that they both decrease with gestation (Geva *et al*., 2002). In our study, there was no change in serum Ang-2 with fetal

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Table 2—Median (interquartile range) for maternal serum angiopoietin-2 and uterine artery pulsatility index (PI) in the three outcome groups

	Serum angiopoietin-2 (median, IQR)		Uterine artery PI (median, IQR)	
Outcome group	MoM	ng/mL	MoM	Unit
Unaffected	1.07 (0.70–1.45)	6.84 (4.52–9.48)	1.05 (0.84–1.30)	1.68 (1.33–2.07)
Preeclampsia	0.96 (0.62–1.48)	6.27 (3.70–9.19)	1.32 (0.99–1.56)*	2.10 (1.57–2.50)
Gestational hypertension	1.12 (0.68–1.60)	6.92 (4.42–10.06)	1.11 (0.89–1.31)	1.75 (1.42–2.06)

Comparisons between outcome groups by Kruskall-Wallis test and Dunn's procedure with Bonferroni correction; Bonferroni corrected significance level, $^{*}p < 0.0167$.
MoM, multiple of the median.

CRL within the narrow gestational window of 11 to 13 weeks.

Wang *et al*. (2007) analysed the maternal serum levels of Ang-2 at 10 to 13 weeks in 13 women who subsequently developed intrauterine growth restriction (IUGR) and 23 normal controls and found that the levels in women destined to develop IUGR were significantly lower compared to controls. The authors do not distinguish between IUGR with or without PE and therefore the results of their study cannot be extrapolated to hypertensive disorders in pregnancy as the mechanism causing altered levels may be different. All the studies examining maternal serum Ang-2 levels in either PE or IUGR are cross-sectional studies in the first (Wang *et al*., 2007) or the third-trimester (Hirokoshi *et al*., 2005; Nadar *et al*., 2005), and these findings need to be confirmed in larger cohort studies and longitudinal studies.

In contrast to serum Ang-2, the uterine artery PI at 11 to 13 weeks is increased in women who subsequently develop PE supporting the hypothesis that PE is a consequence of impaired placentation evident from the first trimester of pregnancy. Previous studies have also demonstrated that in pregnancies destined to develop PE there is an altered maternal serum biochemical profile of placental products, including pregnancy associated plasma protein-A (PAPP-A) and placental growth factor, from the first trimester (Akolekar *et al*., 2008; Poon *et al*., 2009). Consequently, our findings that the levels of Ang-2 are not altered question the suggested central role for this growth factor in placental development.

The hypothesis that Ang-2 plays a key role in placental vascular development has been raised by the finding that the mRNA expression of Ang-2 in placental tissue declines with gestation but in the first trimester is 400 times higher than the expression of the vascular endothelial growth factor-A (VEGF-A) (Geva *et al*., 2002) which has been widely acknowledged as a major factor in placentation (Ferrara 2000; Levine *et al*., 2004; Maynard *et al*., 2008; Steinberg *et al*., 2009). However, the findings of high placental mRNA expression in normal pregnancy (Geva *et al*., 2002) and the low serum levels in association with established PE (Hirokoshi *et al*., 2005; Nadar *et al*., 2005) do not prove a role for Ang-2 in either normal placentation or in the pathophysiology of PE. Indeed, two studies examining placental expression of Ang-2 in PE reported contradictory results (Zhang *et al*., 2001; Geva *et al*., 2002). Zhang *et al*(2001) examined nine women with PE at 31 to 40 weeks and reported that Ang-2 mRNA expression in PE was nearly half than in nine normotensive controls at the same gestation. In contrast, Geva *et al*. (2002) reported that placental Ang-2 mRNA expression in five cases of PE in the third trimester was not significantly different from ten normotensive controls.

Irrespective of the potential role of Ang-2 in normal and impaired placentation, the findings of our study demonstrate that the maternal serum level at 11 to 13 weeks is not altered in pregnancies that subsequently develop hypertensive disorders in pregnancy.

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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