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First-trimester maternal serum matrix metalloproteinase-9 (MMP-9) and adverse pregnancy outcome

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Objective To investigate the potential value of maternal serum matrix metalloproteinase-9 (MMP-9) in first-trimester screening for preeclampsia and spontaneous early preterm delivery.

Methods The concentrations of MMP-9, tumour necrosis factor soluble receptor-1 (TNF-R1), pregnancy-associated plasma protein-A (PAPP-A) and uterine artery pulsatility index (UA-PI) were measured at $11^{+0} - 13^{+6}$ weeks in cases of preeclampsia ($n = 128$), gestational hypertension ($n = 88$), small for gestational age ($n = 296$), spontaneous early preterm delivery ($n = 57$) and controls ($n = 569$). The distributions of measured metabolites and UA-PI in the control and adverse outcome groups were compared. Logistic regression analysis was used to determine the significant contributors in the prediction of adverse outcomes.

Results The median MMP-9 was higher than controls in the preeclampsia (1.190 MoM) and preterm delivery (1.187 MoM) groups. In the preeclampsia group there was a significant association between serum MMP-9 and TNF-R1 ($r = 0.523$, $P < 0.0001$). Significant prediction of preeclampsia was provided by history and UA-PI, and prediction of preterm delivery was provided by history and neither was improved by the addition of MMP-9.

Conclusion In pregnancies developing preeclampsia, the increased level of MMP-9 and the good correlation with TNF-R1 suggest the presence of an underlying inflammatory process. In the pregnancies resulting in spontaneous preterm delivery the small increase in MMP-9 is not useful in the prediction of preterm delivery.

KEY WORDS: first-trimester screening; matrix metalloproteinase-9; preeclampsia; preterm delivery; uterine artery doppler

INTRODUCTION

Matrix metalloproteinase-9 (MMP-9), is a zinc-dependent proteinase involved in inflammation, tissue remodelling, mobilization of matrix-bound growth factors and processing of cytokines (Stamenkovic, 2003). In pregnancy the maternal plasma concentration of MMP-9 is about 15 times higher than in non-pregnant women and the enzyme may be involved in trophoblastic invasion and placentation and in later pregnancy it is implicated in cervical ripening and rupture of the chorioamniotic membranes (Librach *et al*., 1991; Vettriano *et al*., 1996; Tu *et al*., 1998; Athayde *et al*., 1999; Stygar *et al*., 2002).

In normal pregnancy extravillous trophoblast invades the maternal spiral arteries destroying their muscular layer and converts these vessels into low-resistance and high-capacitance channels that supply increased maternal blood to the developing fetoplacental unit (Pijnenborg *et al*., 2006). There is evidence that extravillous trophoblast invasion may be impaired by increased secretion of MMP-9 by decidual cells after stimulation by proinflammatory cytokines such as tumour necrosis factor α (TNFα) (Lockwood *et al*., 2008).

Preeclampsia and the birth of small for gestational age (SGA) neonates are thought to be a consequence of impaired placentation (Pijnenborg *et al*., 2006). These complications can be predicted from the first trimester of pregnancy by the Doppler ultrasound evidence of increased pulsatility index (PI) in the uterine arteries and the altered maternal serum concentration of pregnancy-associated plasma protein-A (PAPP-A) and TNF soluble receptor-1 (TNF-R1), which are implicated in placental development (Lawrence *et al*., 1999; Laursen *et al*., 2001; Plasencia *et al*., 2007; Poon *et al*., 2009; Leal *et al*., 2009).

There is evidence that the timing of spontaneous onset of labour is dependent on programmed development of the uterus in early pregnancy (Garfield *et al*., 1998). A short cervix at mid-gestation is associated with increased risk of spontaneous preterm delivery before 34 weeks, whereas a long cervix is associated with increased risk of caesarean delivery for poor progress of labour at term (Celik *et al*., 2008; Smith *et al*., 2008).

The aim of this study is to first examine the possible interrelation of maternal serum concentration of MMP-9 with the other Doppler and biochemical markers of placentation, and then to investigate the extent to which the serum concentration of MMP-9 in the first-trimester of pregnancy can provide useful prediction for subsequent development of preeclampsia and SGA neonates and spontaneous early preterm delivery.

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MATERIALS AND METHODS

Study population

This was a case-control study. In our centre we performed screening for adverse pregnancy outcomes in women attending for routine assessment of risk for chromosomal abnormalities by measurement of fetal nuchal translucency thickness and maternal serum free ß-human chorionic gonadotropin (ß-hCG) and PAPP-A at $11^{+0} - 13^{+6}$ weeks' gestation (Snijders *et al*., 1998; Nicolaides *et al*., 2005). We recorded maternal characteristics and medical history and measured the uterine artery pulsatility index (UA-PI) by transabdominal colour Doppler (Plasencia *et al*., 2007). Approximately 10 mL of blood were drawn from patients to a plain Vacutainer glass tube, which was left to clot at room temperature for 10–15 min. The blood sample was then centrifuged at 3000 × g for 10 min and the supernatant (serum) was collected. The serum samples were stored at −20 °C for the initial 8–24 h and subsequently stored at −80 °C until being analysed.

Written informed consent was obtained from the women agreeing to participate in the study, which was approved by King's College Hospital Ethics Committee.

In this study we measured MMP-9 in samples from 128 cases with preeclampsia, including 29 requiring delivery before 34 weeks' gestation, 88 with gestational hypertension, 296 with SGA neonates and 57 with spontaneous preterm delivery before 34 weeks' gestation. Each case with complications was matched with one control case that had blood collected and stored on the same day, which did not develop any pregnancy complications and resulted in the live birth of phenotypically normal neonates.

Maternal history

Patients were asked to complete a questionnaire on maternal age, racial origin (White, Black, Indian or Pakistani, Chinese or Japanese and Mixed), cigarette smoking during pregnancy (yes or no), method of conception (spontaneous, use of ovulation drug and *in-vitro* fertilization), medical history (including chronic hypertension, diabetes mellitus, antiphospholipid syndrome, thrombophilia, human immunodeficiency virus infection, sickle cell disease), medication (including antihypertensive, antidepressant, antiepileptic, anti-inflammatory, aspirin, betamimetic, insulin, steroids, thyroxine), parity (parous or nulliparous if no delivery beyond 23 weeks), obstetric history (including previous pregnancy with preeclampsia or spontaneous preterm delivery before 34 weeks) and family history of preeclampsia (mother). The maternal weight and height were measured and the body mass index (BMI) was calculated in kg/m^2.

Outcome measures

The definitions of preeclampsia and gestational hypertension were those of the International Society for the Study of Hypertension in Pregnancy (Davey and MacGillivray, 1988). In gestational hypertension the diastolic blood pressure should be 90 mmHg or more on at least two occasions 4 h apart developing after 20 weeks' gestation in previously normotensive women in the absence of significant proteinuria and in preeclampsia there should be gestational hypertension 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 preeclampsia superimposed on chronic hypertension significant proteinuria (as defined earlier) should develop after 20 weeks' gestation in women with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks' gestation in the absence of trophoblastic disease).

The newborn was considered to be SGA if the birth weight was less than the fifth percentile after correction for gestation at delivery and sex, maternal racial origin, weight, height and parity (Gardosi and Francis, 2006).

Sample analysis

Samples and controls were diluted to 1 in 100 according to kit manufacturer's instruction; 100 µL of the prediluted sample/control was used to measure MMP-9 concentration by a quantitative sandwich enzyme immunoassay based on monoclonal antibody coated onto a microplate and an enzyme-linked polyclonal antibody added to the well after standards and samples (Quantikine human MMP-9 Immunoassay, R&D Systems Inc, Abingdon, England). Fresh aliquots of MMP-9 quality control samples of 117.9, 480.7 and 1191.1 pg/mL concentration were measured in duplicate at the beginning and the end of each run. The mean coefficients of variation were 9.7%, 11.5% and 11.9%, respectively.

In the same samples we measured TNF-R1 and PAPP-A and these data were presented in previous publications (Poon *et al*., 2009; Leal *et al*., 2009).

Statistical analysis

The measured concentration of MMP-9 was log transformed to make the distribution Gaussian. Multiple regression analysis was then used to determine which of the factors amongst the maternal characteristics were significant predictors of log MMP-9 in the control group and from the regression model the value in each case and control was expressed as a multiple of the expected median in the control group (MoM). Mann–Whitney test was used to determine the significance of differences in the median MoM in each pregnancy complication group to that in the controls.

In each case and control the measured UA-PI, serum TNF-R1 and serum PAPP-A were converted into MoMs after adjustment for gestation, maternal age, racial origin, weight or BMI, parity, method of conception and

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previous history of preeclampsia as previously described (Kagan *et al*., 2008; Poon *et al*., 2009; Leal *et al*., 2009). Linear regression analysis was then used to determine the significance of association between log MMP-9 MoM with log TNF-R1 MoM, log PAPP-A MoM or log UA-PI MoM in each outcome group.

Logistic regression analysis was used to determine which of the factors amongst the maternal characteristics, log MMP-9 MoM, log TNF-R1 MoM, log PAPP-A MoM and log UA-PI MoM had a significant contribution in predicting adverse pregnancy outcome. The performance of screening was determined by receiver operating characteristic (ROC) curves.

The statistical software package SPSS 15.0 (SPSS Inc., Chicago, IL) and Medcalc (Medcalc Software, Mariakerke, Belgium) were used for all data analyses.

RESULTS

The maternal characteristics of each of the outcome groups are summarized in Table 1.

Control group

Multiple regression analysis in the control group demonstrated that for log MMP-9 significant independent contributions were provided by maternal weight and racial origin but not by age ($P = 0.816$), fetal crown-rump length (CRL) ($P = 0.170$), parity ($P = 0.094$), method of conception ($P = 0.211$) and smoking ($P = 0.592$): log expected MMP-9 = $2.547 + 0.002 \times$ weight in kg + (-0.095 if Black, 0 if other racial origins); $R^2 = 0.027$, $P < 0.0001$. In each patient we used this formula to derive the expected log MMP-9 and then expressed the observed value as a MoM of the expected (Table 2).

There was a significant association between log MMP-9 MoM and log TNF-R1 MoM ($r = 0.443$, $P < 0.0001$; Figure 1) but not log PAPP-A MoM ($P = 0.563$) and log UA-PI MoM ($P = 0.075$).

Preeclampsia

In the preeclampsia group, compared to the controls, MMP-9, TNF-R1 and UA-PI were higher and PAPP-A was lower (Table 2). There was a significant association between log MMP-9 MoM and log TNF-R1 MoM ($r = 0.523$, $P < 0.0001$; Figure 1) but not log PAPP-A MoM ($P = 0.457$) and log UA-PI MoM ($P = 0.931$). There was a significant association between log UA-PI MoM and PAPP-A ($r = 0.273$, $P = 0.002$) but not TNF-R1 ($P = 0.814$).

In the preeclampsia group there was a significant association with gestational age at delivery for UA-PI ($r = 0.364$, $P < 0.0001$) and PAPP-A ($r = 0.345$, $P < 0.0001$), but not for MMP-9 ($P = 0.873$) and TNF-R1 ($P = 0.070$).

The median (interquartile range) values for UA-PI and maternal serum metabolites for the cases of preeclampsia requiring delivery before 34 weeks (early preeclampsia) and those delivery at or after 34 weeks (late preeclampsia) are shown in Table 2.

Prediction of early preeclampsia

Logistic regression analysis demonstrated significant contributions for the detection of early preeclampsia from maternal factors, PAPP-A and UA-PI but not MMP-9 ($P = 0.468$) and TNF-R1 ($P = 0.107$).

$Y = -5.155 - 3.107 \times \log$ PAPP-A MoM $+ 13.314 \times \log$ UA-PI MoM + (6.002 if history of chronic hypertension) + (1.305 if Black, 0 if other racial origins); $R^2 = 0.449$, $P < 0.0001$).

The areas under ROC curves (AUROC) and detection rates of early preeclampsia at a 5% false positive rate in screening by maternal factors only, PAPP-A only, UA-PI only and by their combinations are summarized in Table 3. The prediction provided by history with UA-PI was not improved by the addition of PAPP-A ($P = 0.854$).

Prediction of late preeclampsia

Logistic regression analysis demonstrated significant contributions for the detection of late preeclampsia from maternal factors, MMP-9 and UA-PI but not PAPP-A ($P = 0.093$) and TNF-R1 ($P = 0.829$).

$Y = -5.214 + 1.698 \times \log$ MMP-9 MoM $+ 3.019 \times \log$ UA-PI MoM $+ 0.114 \times$ BMI in kg/m^2+ (1.360 if family history of preeclampsia) + (1.408 if Black, 1.178 if Indian or Pakistani, 1.211 if Mixed, 0 if other racial origins) + (0 if nulliparous or parous with previous preeclampsia, -1.484 if parous without previous preeclampsia); $R^2 = 0.290$, $P < 0.0001$).

The AUROC and detection rates of late preeclampsia at a 5% false positive rate in screening by maternal factors only, MMP-9 only, TNF-R1 only, PAPP-A only, UA-PI only and by their combinations are summarized in Table 3. The prediction provided by history with UA-PI was not improved by the addition of MMP-9 ($P = 0.258$).

Gestational hypertension

In the gestational hypertension group, compared to the controls, there were no significant differences in MMP-9, TNF-R1, PAPP-A or UA-PI (Table 2).

SGA

In the SGA group, compared to the controls, there were no significant differences in MMP-9 but PAPP-A was lower and TNF-R1 and UA-PI were higher (Table 2). There was a significant association between log MMP-9 MoM and log TNF-R1 MoM ($r = 0.344$, $P < 0.0001$) but not log PAPP-A MoM ($P = 0.250$) and log UA-PI MoM ($P = 0.410$). There was a significant association between log UA-PI MoM and PAPP-A ($r = 0.277$, $P < 0.0001$) but not TNF-R1 ($P = 0.202$).

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Table 1—Maternal characteristics in the five outcome groups

Maternal characteristic	Control ($n = 569$)	Preeclampsia ($n = 128$)	Gestational hypertension ($n = 88$)	Small for gestational age ($n = 296$)	Preterm delivery ($n = 57$)
Maternal age in years, median (range)	32.6 (16–45)	31.6 (17–49)	33.3 (18–46)	32.2 (17–44)	33.2 (18–46)
Body mass index in kg/m^2, median (range)	24.6 (17.4–46.7)	27.1 (18.9–46.4)***	26.5 (19.6–53.9)***	24.5 (17.3–43.1)	25.1 (19.1–51.9)
Crown-rump length in mm, median (range)	64.0 (45–84)	62.3 (46–84)	62.5 (47–83)	61.6 (46–84)***	64.1 (47–84)
Racial origin					
White, *n* (%)	410 (72.1)	53 (41.4)***	67 (76.1)	206 (69.6)	42 (73.7)
Black, *n* (%)	95 (16.7)	55 (43.0)***	16 (18.2)	57 (19.3)	11 (19.3)
Indian or Pakistani, *n* (%)	31 (5.4)	9 (7.0)	0*	17 (5.7)	4 (7.0)
Chinese or Japanese, *n* (%)	12 (2.1)	2 (1.6)	1 (1.1)	1 (0.3)*	0
Mixed, *n* (%)	21 (3.7)	9 (7.0)	4 (4.5)	15 (5.1)	0
Parity					
Nulliparous, *n* (%)	266 (46.7)	79 (61.7)**	49 (55.7)	152 (51.4)	24 (42.1)
Parous—no previous preeclampsia, *n* (%)	287 (50.4)	30 (23.4)***	29 (33.0)**	134 (45.3)	29 (50.9)
Parous—previous preeclampsia, n (%)	16 (2.8)	19 (14.8)***	10 (11.4)*	10 (3.4)	4 (7.0)
Parous—previous spontaneous preterm delivery, *n* (%)	3 (0.5)	0	2 (2.3)	5 (1.7)	6 (10.5)***
Cigarette smoker, *n* (%)	25 (4.4)	6 (4.7)	7 (8.0)	53 (17.9)***	7 (12.3)*
Family history of preeclampsia—Mother, *n* (%)	22 (3.9)	15 (11.7)**	9 (10.2)*	10 (3.4)	1 (1.8)
Conception					
Spontaneous, *n* (%)	554 (97.4)	120 (93.8)	85 (96.6)	284 (95.9)	55 (96.5)
Ovulation drugs, *n* (%)	10 (1.8)	6 (4.7)	0	7 (2.4)	0
In-vitro fertilization, *n* (%)	5 (0.9)	2 (1.6)	3 (3.4)	5 (1.7)	2 (3.5)
Medical history					
None, *n* (%)	559 (98.2)	117 (91.4)***	85 (96.6)	281 (94.9)**	50 (87.7)***
Chronic hypertension, *n* (%)	1 (0.2)	8 (6.3)***	0	10 (3.4)***	1 (1.8)
Diabetes mellitus, *n* (%)	4 (0.7)	1 (0.8)	2 (2.3)	3 (1.0)	5 (8.8)***
Antiphospholipid syndrome, *n* (%)	3 (0.5)	1 (0.8)	1 (1.1)	0	1 (1.8)
Thrombophilia, *n* (%)	0	1 (0.8)	0	0	0
Sickle cell disease, *n* (%)	1 (0.2)	0	0	0	0
Human immunodeficiency viral infection, *n* (%)	1 (0.2)	0	0	0	0
Medication during pregnancy					
None, *n* (%)	533 (93.7)	115 (89.8)	76 (86.4)*	258 (87.2)***	50 (87.7)
Antihypertensives, *n* (%)	0	4 (3.1)*	0	7 (2.4)***	0
Insulin, *n* (%)	3 (0.5)	1 (0.8)	2 (2.3)	3 (1.0)	4 (7.0)**
Steroids, *n* (%)	1 (0.2)	0	0	1 (0.3)	0
Betamimetics, *n* (%)	10 (1.8)	4 (3.1)	4 (4.5)	17 (5.7)**	0
Thyroxine, *n* (%)	9 (1.6)	2 (1.6)	2 (2.3)	4 (1.4)	1 (1.8)
Aspirin, *n* (%)	3 (0.5)	0	2 (2.3)	0	0
Antiepileptic, *n* (%)	2 (0.4)	1 (0.8)	1 (1.1)	1 (0.3)	2 (3.5)
Antidepressants, *n* (%)	6 (1.1)	1 (0.8)	1 (1.1)	3 (1.0)	0
Anti-inflammatory, *n* (%)	2 (0.4)	0	0	2 (0.7)	0

Comparison with control group (Chi-square test or Fisher exact test for categorical variables and *t*-test for continuous variables):* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

Spontaneous preterm delivery

In the preterm delivery group, compared to the controls, MMP-9 was significantly higher but there were no significant differences in PAPP-A, TNF-R1 or UA-PI (Table 2).

Logistic regression analysis demonstrated significant contributions for the detection of spontaneous preterm

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Table 2—Comparison of median (interquartile range) MoM of MMP-9, TNF-R1, PAPP-A and UA-PI of each adverse outcome group with the controls

Outcome group	*n*	MMP-9 MoM	TNF-R1 MoM	PAPP-A MoM	UA-PI MoM
Control	569	1.019 (0.722–1.411)	0.996 (0.883–1.134)	1.070 (0.734–1.457)	1.037 (0.839–1.247)
Preeclampsia					
Total	128	1.190 (0.938–1.660)***	1.062 (0.924–1.218)**	0.783 (0.520–1.243)***	1.305 (0.984–1.542)***
Early	29	1.189 (0.816–1.739)	1.086 (0.907–1.303)	0.535 (0.391–0.961)***	1.512 (1.204–1.653)***
Late	99	1.195 (0.962–1.612)**	1.060 (0.923–1.185)*	0.929 (0.574–1.310)*	1.220 (0.927–1.448)***
Gestational hypertension	88	1.109 (0.833–1.345)	0.995 (0.932–1.138)	0.895 (0.622–1.442)	1.100 (0.885–1.287)
Small for gestational age	296	1.088 (0.784–1.476)	1.030 (0.901–1.180)*	0.778 (0.520–1.163)***	1.087 (0.889–1.332)*
Preterm delivery	57	1.187 (0.875–1.654)**	1.029 (0.921–1.119)	0.954 (0.591–1.389)	1.047 (0.800–1.282)

Mann–Whitney test to compare each group with controls: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

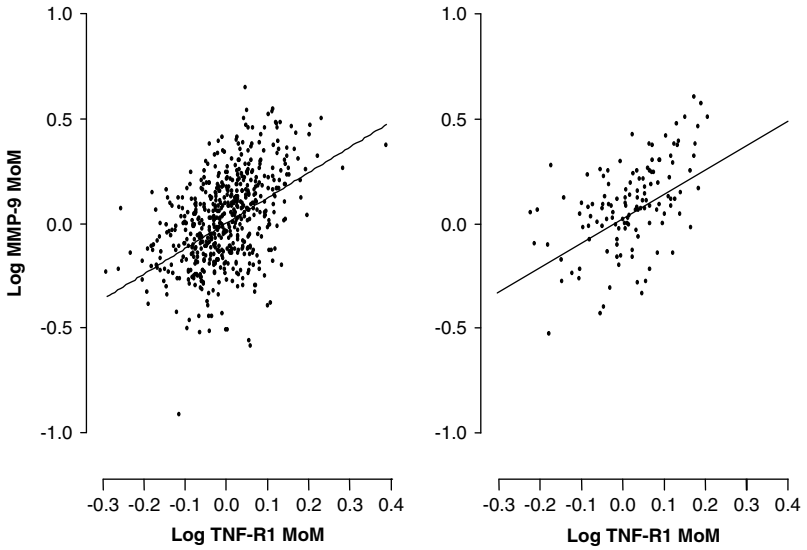

Figure 1—Relationship between maternal serum MMP-9 and TNF-R1 in the control (left) and preeclampsia (right) groups

Table 3—Comparison of the performance of screening for early and late preeclampsia by maternal factors, MMP-9, TNF-R1, PAPP-A, UA-PI and by their combinations

	Early preeclampsia		Late preeclampsia	
Method of screening	AUROC (95% CI)	DR for FPR 5%	AUROC (95% CI)	DR for FPR 5%
History	0.760 (0.652–0.868)	39.0	0.793 (0.747–0.838)	33.3
MMP-9	—	—	0.605 (0.546–0.663)	9.1
TNF-R1	—	—	0.579 (0.516–0.643)	12.1
PAPP-A	0.745 (0.641–0.849)	24.1	0.580 (0.516–0.643)	9.1
UA-PI	0.823 (0.735–0.911)	41.4	0.625 (0.559–0.690)	16.2
History plus				
MMP-9	—	—	0.801 (0.758–0.845)	35.4
TNF-R1	—	—	—	—
PAPP-A	0.842 (0.747–0.937)	51.7	0.793 (0.747–0.838)	31.3
UA-PI	0.902 (0.831–0.972)	69.0	0.802 (0.756–0.848)	29.3
MMP-9, UA-PI	—	—	0.814 (0.768–0.859)	34.3
PAPP-A, UA-PI	0.906 (0.840–0.971)	69.0	—	—
PAPP-A, MMP-9, UA-PI	—	—	—	—

delivery from previous history of spontaneous delivery before 34 weeks and MMP-9:

$Y = -2.470 + 1.628 \times \log$ MMP9 MoM + (2.999 if previous spontaneous preterm delivery); $R^2 = 0.083$, $P < 0.0001$.

The detection rate in predicting spontaneous delivery was 12.3% for a 5% false positive rate and the AUROC was 0.642 (95% CI 0.569–0.715). The prediction provided by history was not improved by the addition of MMP-9 ($P = 0.058$).

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DISCUSSION

The findings of this study demonstrate that the maternal serum concentration of MMP-9 at $11^{+0} - 13^{+6}$ weeks' gestation is increased in pregnancies that subsequently develop preeclampsia and in those resulting in spontaneous early delivery. In normal pregnancies the maternal serum concentration of MMP-9 increased with maternal weight and was lower in Black than in White women and therefore the measured concentration was adjusted for these variables before comparing results with pathological pregnancies. The finding that within the gestational range of $11^{+0} - 13^{+6}$ weeks there was no significant change with fetal CRL is compatible with the findings of a previous study that although the levels in pregnancy are about 15 times higher than in non-pregnant women there is no increase with gestation (Tu *et al*., 1998).

In both the normal pregnancies and in those that subsequently developed preeclampsia there was a significant association between serum MMP-9 and TNF-R1 but not UA-PI or serum PAPP-A. Doppler studies assessing placental perfusion showed that the prevalence of increased UA-PI in pregnancies developing preeclampsia is considerably higher in those developing early-onset disease requiring delivery before 34 weeks rather than late-onset disease (Plasencia *et al*., 2007; Poon *et al*., 2009). Similarly, pathological studies demonstrated that the prevalence of placental lesions in women with preeclampsia is inversely related to the gestation at delivery (Moldenhauer *et al*., 2003; Sebire *et al*., 2005). There was an association between UA-PI and serum PAPP-A concentration, which is thought to be involved in placentation through its proteolytic activity against insulin-like growth factor (IGF) binding proteins with low PAPP-A resulting in higher levels of bound and therefore biologically inactive IGFs (Lawrence *et al*., 1999; Laursen *et al*., 2001). The levels of PAPP-A were substantially lower in those developing early than late preeclampsia. In contrast, in patients with preeclampsia, although there was a significant increase in MMP-9 and TNF-R1, there was no significant association between the levels of these metabolites and the gestation at delivery. These results demonstrate that in women destined to develop preeclampsia there is evidence of an inflammatory process from as early as the first trimester of pregnancy and precedes the clinical onset of the disease by several months. The association between the levels of MMP-9 and TNF-R1 is compatible with the suggestion that the proinflammatory cytokine TNFα stimulates increased secretion of MMP-9 (Lockwood *et al*., 2008). However, the results do not provide support for the hypothesis linking impaired trophoblastic invasion of the maternal spiral arteries with placental hypoxia and the release of cytokines and MMP-9 (Kupferminc *et al*., 1994; Redman *et al*., 1999; Granger *et al*., 2001). Consequently, the underlying mechanism for the observed association between increased MMP-9 and development of preeclampsia is uncertain.

The finding that serum concentration of MMP-9 was not altered in pregnancies developing gestational hypertension is not surprising because in such pregnancies placentation is essentially normal. Similarly, in SGA pregnancies in the absence of preeclampsia MMP-9 was not altered despite some evidence of impaired placentation provided by the increased UA-PI and decreased serum PAPP-A.

In pregnancies resulting in spontaneous preterm delivery the maternal serum concentration of MMP-9 was significantly increased. This finding provides further support for the hypothesis that the timing of spontaneous onset of labour is dependent on programmed development of the uterus in early pregnancy (Garfield *et al*., 1998). However, the increase in serum MMP-9 in spontaneous preterm deliveries was small and the measurement of MMP-9 together with maternal history identified only 12% of such pregnancies at a false positive rate of 5%. A previous longitudinal study reported that although with the onset of labour there was a threefold increase in maternal levels of MMP-9, levels before labour remained unchanged throughout pregnancy (Tu *et al*., 1998).

In conclusion, at $11^{+0} - 13^{+6}$ weeks' gestation there is a good correlation between the maternal serum MMP-9 and TNF-R1. Although the serum concentration of both metabolites in pregnancies that subsequently develop preeclampsia is increased providing support for an underlying inflammatory process and their measurement is not useful in predicting preeclampsia. Similarly, although serum MMP-9 is increased in pregnancies complicated by spontaneous preterm delivery measurement of MMP-9 does not improve the prediction of preterm delivery provided by maternal history alone.

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