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Endostatin levels and the risk of subsequent preeclampsia

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ABSTRACT

Objective: To evaluate endostatin, an anti-angiogenic factor, in relation to the risk of preeclampsia (PE).
Study design: In this case control study, serum samples were collected at 11–17 weeks and 18–26 weeks' gestation. Endostatin levels were expressed as adjusted multiples of the median (MoM). Logistic regression was used to calculate adjusted odds ratios (aORs) for the prediction of PE.
Results: A total of 77 women with PE and 150 controls were studied. Endostatin levels were significantly higher in women with PE compared to controls in both the first and the second trimester. At a cut-off level of 75th percentile of endostatin MoMs, the aORs for PE were 1.33 (95% confidence interval [CI], 0.68–2.58) at 11–17 weeks and 1.77 (95% CI, 0.94–3.34) at 18–26 weeks, after adjustment for ethnicity and chronic hypertension. The aORs for early-onset PE were 3.51 (95% CI, 1.18–10.43) at 11–17 weeks and 2.17 (95% CI, 0.67–7.06) at 18–26 weeks.
Conclusions: Higher endostatin levels are associated with an increased risk of early onset PE. Endostatin alone, however, has a poor predictive value for clinical usefulness.

1. Introduction

Preeclampsia is a major cause of maternal and perinatal mortality and morbidity [1]. This disease affects 3–7% of pregnant women [2]. Underlying causes are heterogeneous and remain unclear. It has been hypothesized that preeclampsia is a disease of the maternal endothelium due to defective placentation. Placental factors enter the maternal circulation and cause endothelial dysfunction resulting in hypertension and proteinuria [1–4]. Although underlying mechanisms are likely set up during the first trimester, clinical signs do not appear until the second or third trimesters. The ability to predict patients at risk would be of great value and would enable a closer monitoring of high risk women and possibly the use of preventive strategies. In recent years there has been a growing interest in early screening tests for preeclampsia [5].

Placentation is under the influence of angiogenic and anti-angiogenic factors and their imbalance generates abnormal placental function [6]. In patients who develop preeclampsia, trophoblastic cells have lost their ability to colonize the maternal spiral arteries [7]. This dysfunction could be related to an imbalance between angiogenic and antiangiogenic factors, including endostatin, an inhibitor of tumor growth and a potent antiangiogenic factor [8,9]. A few small studies have highlighted the role of endostatin in preeclampsia [10–13]. Endostatin is a 20 kDa molecule derived from the noncollagenous domain at the C-terminal of collagene type XVIII [9,14]. In vitro, endostatin specifically inhibits endothelial cell proliferation and migration [9]. Dhanabal et al. have demonstrated that endostatin causes apoptosis specifically of endothelial cells in vitro [8]. In vivo, Wen et al. showed that trophoblastic cells attenuated their own invasion by producing proteases that locally release endostatin from the decidua [15].

The aim of this study was to analyze endostatin levels in the first and second trimesters of pregnancy in order to determine the association of endostatin levels with the risk of subsequent preeclampsia.

2. Methods

2.1. Study design

This was a case–control study nested in two separate prospective cohorts – Maternal Infant Research on Oxidative Stress (MIROS), and Preeclampsia Assessment of Risk by Integrated Screening (PARIS). The MIROS study was based on a randomized

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placebo-controlled trial of antioxidant supplementation (vitamins C and D). The trial was conducted in Canada (17 centers) and Mexico (10 centers) from January 2004 to March 2006. The design and methods of the trial have been described elsewhere [16]. The MIROS study included only patients from all centers in Canada, who consented to participate in a biobank. The PARIS study was conducted from November 2006 to June 2008 and was specifically designed to study early markers of preeclampsia in the first trimester, combining maternal serum markers and uterine artery Doppler. Other biomarkers were tested in parts of the two cohorts described in this study, and were published previously [17–19]. Written informed consent was obtained from all women in both cohorts, and the project was approved by the institutional review board.

Maternal blood samples were collected during two visits. Samples for the MIROS study were collected between 12 and 18 weeks (visit 1) then between 24 and 26 weeks of gestation (visit 2). Samples for the PARIS study were collected between 11 and 13 weeks (visit 1) then between 18 and 22 weeks (visit 2). In pooled analyses, biomarker data were grouped into two periods by timing of blood sampling: early (11–17 weeks) versus mid-gestation (18–26 weeks).

The definitions of preeclampsia and gestational hypertension were those stated by the International Society for the Study of Hypertension in Pregnancy [20]. Gestational hypertension was defined as blood pressure higher or equal to 140/90 mmHg on two readings at least 4 h apart after 20 weeks of gestation. Preeclampsia was defined as gestational hypertension with proteinuria higher or equal to 0.3 g in 24-h urine collection or $\geq$2+ in urine protein dip test. Early-onset preeclampsia was defined as preeclampsia diagnosed before 34 weeks. Severe preeclampsia was defined by a blood pressure higher or equal to 160/110 mmHg, proteinuria higher or equal to 5 g/d, or the presence of an adverse condition, including maternal symptoms, maternal signs of end-organ dysfunction, abnormal maternal laboratory testing, or fetal compromise [20]. All cases of preeclampsia were reviewed by two independent investigators. In both studies, gestational age was calculated from the date of last menstrual and confirmed by first trimester ultrasound.

Controls were selected from patients without preeclampsia, gestational hypertension, intrauterine growth retardation, or placental abruption. In the MIROS study cases and controls were matched by their allocation strata (low-risk vs. high-risk). Women were considered as being "high risk" if they had pre-pregnancy chronic hypertension, pre-pregnancy diabetes, or a history of preeclampsia [16]. In the PARIS study cases and controls were matched by date and gestational age at recruitment.

In both studies, maternal non-fasting blood samples were collected, immediately centrifuged, and frozen at −80 °C until biochemical analyses. Endostatin was measured using enzyme-linked immunosorbent assays (ELISA) by the Human Endostatin kit (QUANTIKINE, R&D systems, Minneapolis). Although the batches were different between the two studies, the same technique was used with similar reagents, the same laboratory and the same operator. The lower limit of detection of the assay was 0.023 ng/ml. The intra- and inter-assay coefficients of variation were 5.5% and 6.6%, respectively. The laboratory professionals who performed the biochemical assays were blinded to the clinical status of the subjects.

2.2. Statistical analysis

For statistical analysis, the following steps were taken:

The normality of endostatin levels was confirmed by Kolmogorov test. We used multiple linear regressions in the unaffected group to obtain expected endostatin levels depending on gestational age, maternal age, ethnicity and cohort of origin. Endostatin levels were then expressed in multiples of the median (MoM) for each subject.

Maternal characteristics were compared between cases and controls by chi-square test and Fisher's exact test for categorical variables and by the Student *t*-test for continuous variables.

Endostatin levels, expressed in MoM, were compared by the Student *t* test between cases and controls. We categorized endostatin MoMs into percentiles, with a cut-off of the 75th percentile.

We conducted univariate and multivariate logistic regression analyses by adjusting for potential confounding factors to determine the risk of preeclampsia in subjects who had endostatin levels higher than the 75th percentile, as compared to those below the 75th percentile. Maternal characteristics that were associated with preeclampsia in univariate analysis with $p < 0.2$ were tested as potential confounders. The performance of screening was determined by ROC curves and area under the curve (AUC).

Table 1
Maternal and pregnancy characteristics by clinical outcome.

	All preeclampsia $n = 77$	Early-onset preeclampsia $n = 21$	Severe preeclampsia $n = 41$	Controls $n = 150$
Maternal age, median (IQR)	30 (27–33.5)	32 (27.5–35.5)	29 (26.5–34.5)	30 (27–33)
Smoking, *n* (%)	8 (10.4)	4 (19.0)	8 (19.5)	15 (10.0)
Nulliparous, *n* (%)	60 (77.9)	15 (71.4)	33 (80.5)	127 (84.7)
Pre-pregnancy BMI, kg/m^2, median (IQR)	26.75 (22.5–30.5)**	28.9 (22.4–32.6)*	25.2 (22.5–29.9)**	23.1 (20.8–25.7)
Ethnicity, *n* (%)				
White	61 (79.2)	14 (66.7)	32 (78.0)	124 (82.7)
African	10 (13.0)**	5 (23.8)	6 (14.6)**	4 (2.7)
Hispanic	2 (2.6)	0	1 (2.4)	6 (4.0)
Asian	1 (1.3)	1 (4.8)	1 (2.4)	3 (2.0)
Other/mixed	3 (3.9)	1 (4.8)	1 (2.4)	13 (8.7)
Gestational age at onset of disease, weeks, median (IQR)	37.0 (33.0–38.0)	31.5 (26.5–33.0)	34.0 (32.0–37.0)	
Gestational age at delivery, week, median (IQR)	37.3(35.8–39.0)**	34 (31.1–36.1)**	36 (33.7–38.0)**	39.7 (38–40.4)
Preterm delivery	26 (33.8)**	18 (85.7)**	22 (53.7)**	13 (8.7)
Birthweight, g, median (IQR)	2905 (2185–3356.5)**	1825 (1410–2512.5)**	2394 (1733–3277)**	3407.0 (3085.8–3675.3)
Chronic hypertension, *n* (%)	16 (20.8)**	8 (38.1)**	12 (29.3)**	6 (4.0)

X^2 test for categorical variables and Mann–Whitney test for continuous variables were used to compare adverse outcome groups with control group. All comparisons are versus control group. BMI, body mass index.

* $p < 0.05$.

** $p < 0.001$.