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CLINICAL ARTICLE

Association between the maternal serum levels of 19 eicosanoids and pre-eclampsia

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ABSTRACT

Objective: To investigate whether serum levels of 19 eicosanoids are associated with pre-eclampsia. *Methods:* A case–control study was performed using data for pregnant women with pre-eclampsia, normotensive pregnant women, and nonpregnant women, for all of whom serum samples had been collected at a hospital in Shanghai, China, between December 2012 and December 2013. Ultra-performance liquid chromatography–tandem mass spectrometry was used to measure the serum levels of 19 eicosanoids. *Results:* Overall, 49 pregnant women with pre-eclampsia, 26 normotensive pregnant women, and 14 nonpregnant women were included. Women with pre-eclampsia had significantly higher serum levels of 11,12-epoxyeicosatrienoic acid (11,12-EET), the hydroxyeicosatetraenoic acids 5-HETE, 8-HETE, 12-HETE, and 15-HETE, and leukotriene B4 than did women with a normal pregnancy and nonpregnant women, both before and after the onset of pre-eclampsia ($P < 0.01$ for all comparisons). Women with severe pre-eclampsia had significantly higher serum levels of 5-HETE, 15-HETE, and leukotriene B4 than did women with mild pre-eclampsia, women with a normal pregnancy, and nonpregnant women ($P < 0.01$ for all comparisons). *Conclusion:* The eicosanoids 11,12-EET, 5-HETE, 8-HETE, 12-HETE, 15-HETE, and leukotriene B4 might play important parts in the occurrence and development of pre-eclampsia.

1. Introduction

Pre-eclampsia is a severe complication that occurs during human pregnancy, with hypertension and proteinuria as the main features. Pre-eclampsia is one of the primary causes of morbidity and mortality of mothers and neonates in the perinatal period, with a prevalence of approximately 5%–8% [1].

Placental ischemia and hypoxia in the early stage of pregnancy could cause excessive oxidative stress reactions at the maternal–fetal interface, making the placenta release large amounts of inflammatory mediators that enter the maternal circulation and cause vascular endothelial injury to the mother. The presence of vascular inflammation could be the main cause of the occurrence and development of pre-eclampsia [2]. One study [3] has shown that eicosanoids, which are generated from arachidonic acid in three metabolic pathways, have important roles in the regulation of vascular inflammation, vasoconstriction, and angiogenesis, and could be involved in the occurrence of pre-eclampsia.

Arachidonic acid is a polyunsaturated fatty acid that is most commonly present in phospholipids. The main products of arachidonic acid processing by the cyclooxygenase pathway include prostaglandins (PGs) and thromboxane A2 (TXA2); the main products of the lipoxygenase pathway include leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs); and the main products of the cytochrome P450 (CYP) pathway include epoxyeicosatrienoic acids (EETs) and 16-, 17-, 18-, 19-, and 20-HETE [4]. The EETs can be further catalyzed to dihydroxyeicosatrienoic acids (DHETs) by soluble epoxide hydrolase.

PG I2 is a vasodilator and platelet aggregation inhibitor, whereas TXA2 is a vasoconstrictor and can promote platelet aggregation. LTs mainly regulate vasoconstriction and vascular permeability, and leukotriene B4 (LTB4) is an important pro-inflammatory cytokine. The HETEs mainly regulate vascular contraction and promote inflammation [5,6], whereas the EETs are a class of vascular protective factors that have anti-inflammatory effects, induce blood vessel relaxation, and promote angiogenesis. During pregnancy, EET synthesis has been detected in the placenta, trophoblast cells, the amnion, the chorion, the decidua, and the myometrium [7].

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The aim of the present study was to determine the association between pre-eclampsia and serum levels of 19 eicosanoids with important roles in vasoconstriction and vascular inflammation.

2. Materials and methods

A case–control study was undertaken using data for pregnant women with pre-eclampsia, normotensive pregnant women, and nonpregnant women whose serum samples had been collected in the Clinical Laboratory Department of Baoshan Branch of Shanghai First People's Hospital, Shanghai, China, between December 1, 2012, and December 31, 2013. The study was approved by the Ethics Committee of the Baoshan Branch of Shanghai First People's Hospital. Patients had provided informed consent for any future use of their anonymized data at the time their serum samples had been collected.

Women with pre-eclampsia were identified by searching the hospital database. The diagnostic criteria of pre-eclampsia were a pregnancy duration of at least 20 completed weeks, a systolic blood pressure of at least 140 mmHg and/or a diastolic blood pressure of at least 90 mmHg, and proteinuria (≥300 mg protein per 24-h urine collection, protein/creatinine ratio ≥ 0.3, or a urine dipstick reading of 1 + or more [protein concentration ≥ 0.3 g/L]). Severe pre-eclampsia was defined as a systolic blood pressure of at least 160 mmHg and/or a diastolic blood pressure of at least 110 mmHg, a platelet count of less than 100 000 per μL, aspartate aminotransferase or alanine transaminase levels more than 70 U/L or twice the normal concentration, serum creatinine levels more than 97.2 μmol/L or twice the baseline value, or presence of pulmonary edema/headache or visual changes. Participants in the other two groups were matched for age and body mass index.

The serum samples for the present study were obtained from the biobank of the hospital. Each sample had been centrifuged within 2 h of collection at 3000 rpm for 10 min, and then separated and stored at −80 °C until use. For women with pre-eclampsia, two serum samples obtained before the onset of pre-eclampsia and two serum samples obtained after the onset of pre-eclampsia were analyzed. The mean of all four measurements was used to evaluate the eicosanoid levels in the pre-eclampsia group overall. For the participants with a normal pregnancy, two serum samples were analyzed. For women in the nonpregnant control group, one serum sample was analyzed. Means were then calculated.

Nineteen eicosanoid standards and five internal standards were purchased from Cayman Chemical (Ann Arbor, MI, USA). The standards comprised 5,6-DHET, 8,9-DHET, 11,12-DHET, 14,15-DHET, 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET, 5-HETE, 8-HETE, 12-HETE, 15-HETE, 20-HETE, 6-keto-PGF1α, TXB2, LTB4, LTC4, LTD4, and LTE4. The deuterated internal standards comprised PGE-d_4, LTB4-d_4, 11,12-EET-d_{11}, 15-HETE-d_8, and 14,15-DHET-d_{11}.

A 400-μL blood sample from each woman was added to 400 μL 4% phosphoric acid solution and 10 μL internal standard solution (the concentration was 100 ng/mL for PGE-d_4, LTB4-d_4, and 11,12-EET-d_{11}, and 2 μg/mL for 14,15-DHET-d_{11} and 15-HETE-d_8), vortex-mixed, and subjected to solid-phase extraction using Oasis HLB μElution 96-well plates (Waters, Milford, MA, USA). The elution process was as follows: (1) activation: 200 μL methanol was added into each well twice; (2) balance: 200 μL ultra-pure water was added into each well three times; (3) sample loading: 700 μL acidified sample was added into each well; (4) first washing: 200 μL 5% ammonia solution was added to each well; (5) second washing: 200 μL methanol/water (70%/30%) was added to each well; (6) elution: 25 μL acetonitrile/isopropanol (65%/35%) containing 2% formic acid was added to each well twice. After the elution, 50 μL water was used for dilution and a 100-μL sample was loaded for evaluation.

An Acquity Ultra-Performance Liquid Chromatography/Xevo TQ Mass Spectrometer system with a CortersC18 column (1.6 μm, 2.1 × 100 mm; all from Waters, Milford, MA, USA) was used to examine the prepared samples. The mobile phase consisted of 0.1% formic acid (phase A) and acetonitrile (phase B); both reagents were from Sigma-Aldrich(St. Louis, MO, USA).The elution gradient was as follows: 0–2 min phase A 70% to 30%; 2–5 min phase A 70% to 0%; 5–6 min phase A maintained at 0%; 6–8 min phase A 0% to 80%; the flow rate was 0.35 mL/min; and the injection volume was 5 μL. The operating conditions of the Xevo TQ-MS spectrometer were as follows: ion mode, negative electrospray ionization; capillary voltage, 2.5 kV; source temperature, 150 °C; desolvation temperature, 550 °C; desolvation gas flow, 800 L/h; and cone gas flow, 150 L/h. A MassLynx 4.1 (Waters, Milford, MA, USA) software was used to analyze the data.

The statistical analysis was performed using SPSS version 20.0 (IBM, Amonk, NY, USA) and graphs were prepared using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). Data that met the criteria for normal distribution and homogeneity of variance were subjected to analysis of variance; multiple independent data that did not meet the criteria for homogeneity of variance were analyzed using the Kruskal–Wallis *H* test, and independent samples were analyzed using the Mann–Whitney *U* test. Pair-wise comparison of multiple sample averages was performed using the Student–Newman–Keuls *q* test, and pair-wise comparison of multiple independent samples was performed using the Nemenyi test. $P < 0.05$ was considered statistically significant.

3. Results

The study included 49 pregnant women with pre-eclampsia, 26 normotensive pregnant women, and 14 nonpregnant women. Of the women with pre-eclampsia, 31 had severe disease, and 18 had mild disease. The systolic pressure and diastolic pressure in the pre-eclampsia group were significantly higher than the corresponding values in the normal pregnancy group and the nonpregnant control group (Table 1), but there were no significant differences between the latter two groups.

The levels of DHETs in the pre-eclampsia group and the normotensive group were significantly higher than those in the nonpregnant group ($P < 0.01$ for all comparisons), whereas there was no significant difference between the former two groups. The serum levels of EETs, HETEs, LTs, and TXB2 in the pre-eclampsia group were significantly higher than those in the other two groups ($P < 0.01$ for all comparisons), whereas there was no significant difference between women with a normal pregnancy and nonpregnant women (Fig. 1).

Further analysis of the relationship between each individual serum eicosanoid and pre-eclampsia revealed that the serum levels of 8,9-HETE, 11,12-HETE, 5-HETE, 8-HETE, 12-HETE, 15-HETE, and LTB4 in the pre-eclampsia group were significantly higher than those in the other two groups ($P < 0.01$ for all comparisons), whereas no significant difference was found between the normotensive pregnancy group and the nonpregnant group (Fig. 2).

A comparison of the 19 serum eicosanoids between women with severe pre-eclampsia and women with mild pre-eclampsia showed that the levels of HETs, EETs, HETEs, and LTs in general were not significantly different between these two groups. However, when individual eicosanoids were compared, the serum levels of 5-HETE, 15-HETE, and LTB4 in women with severe pre-eclampsia were significantly higher than those in women with mild pre-eclampsia, women with a normotensive pregnancy, and nonpregnant women ($P < 0.01$ for all comparisons) (Fig. 3). No significant differences were found between the latter three groups, and the remaining eicosanoids were not significantly different between women with severe versus mild pre-eclampsia.

A comparison of the serum levels in the pre-eclampsia group before the onset of pre-eclampsia (pregnancy duration 14–36 weeks) with the levels among women with a normal pregnancy (pregnancy duration 24–36 weeks) and among nonpregnant controls showed that the serum levels of EETs, HETEs, and LTs in women who subsequently developed pre-eclampsia were significantly higher than those in the latter two groups ($P < 0.01$ for all comparisons), whereas there was no

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