Expression of S100 calcium-binding protein A8 in peripheral blood of patients with preeclampsia during pregnancy

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Abstract
A total of 30 late onset severe preeclampsia (LS-PE) patients and 30 early onset severe preeclampsia (ES-PE) patients were selected as Experimental group, and 30 normal pregnant were selected as Control group. Expression of S100 calcium-binding protein A8 (S100A8) mRNA was detected by reverse transcription polymerase chain reaction (RT-PCR). Enzyme-linked immunosorbent assay (ELISA) was used to detect expression of S100A8 protein and inflammatory factors. Levels of uric acid (UA) and creatinine (CRE) were measured using an automatic biochemical analyzer. Urinary protein (UPRO) content was measured using biuret colorimetry. S100A8 levels were significantly higher in experimental groups than in control groups (P < 0.05). Significantly increased contents of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-12, UA, CRE, and UPRO, and decreased level of IL-10 were found in experimental groups than in control groups (P < 0.05). Compared with patients with ES-PE, significantly higher levels of TNF-α, IL-12, IL-6, UA, CRE, and UPRO, and lower level of IL-10 were found in patients with LS-PE. S100A8 plays pivotal roles in the development of preeclampsia through the interactions with other inflammatory factors.

Keywords
creatinine, inflammatory, preeclampsia, S100A8 protein, uric acid, urinary protein

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Introduction
Preeclampsia refers to the clinical syndrome of the development of proteinuria and hypertension after the first half of pregnancy.1 As a syndrome of systemic vascular disorder, preeclampsia can affect the functions of brain and liver in mothers.2 Preeclampsia is also proved to be an additional risk factor for the development of cognitive impairment during school age for children who experienced very preterm birth and intrauterine growth restriction.3 Studies on proteinuria have shown that preeclampsia is closely related to endothelium.4 Decrease in arterial compliance causes preeclampsia hypertension, and proteinuria of preeclampsia is caused by glomerular endotheliosis.5

As a mammalian calcium-binding protein, S100 calcium-binding protein A8 (S100A8) in peripheral blood of patients with preeclampsia during pregnancy has been proved to be involved in the development and progression of various human diseases. It has been reported that S100A8 content in plasma is closely correlated with the traditional risk factors, blood neutrophil counts, and cardiovascular disease in adults.6 In addition, S100A8 was found to be able to promote inflammatory responses in patients with age-dependent diseases or traumatic

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brain injury.\textsuperscript{7} In the studies of pregnancy loss, significantly increased expression levels of S100A8 mRNA and protein were observed in endometrial decidua tissue of patients with recurrent early pregnancy loss.\textsuperscript{8} Based on those previous studies, it will be reasonable to hypothesize that S100A8 may also play pivotal roles in the progression of preeclampsia.

**Patients and methods**

**Objects**

Sixty patients with severe preeclampsia, including 30 patients with early onset severe preeclampsia (ES-PE), 20–39 (29.5 ± 4.6) years old, 1–3 (1.9 ± 0.8) times of pregnancy, and 30 patients with late onset severe preeclampsia (LS-PE), 22–44 (32.2 ± 5.9) years old, 1–3 (2.0 ± 0.8) times of pregnancy, were selected from June 2016 to December 2016 in Department of Gynecology and Obstetrics, Women and Children’s Hospital to serve as Experimental group. At the same time, 30 normal pregnant women (uterine-incision delivery) with the same date of delivery were selected to serve as Control group, 23–36 (30.9 ± 3.6) years old, 1–3 (1.9 ± 0.8) times of pregnancy. All the pregnant women were cesarean delivery. The body mass index (BMI), gestational week at delivery, proteinuria level, systolic pressure, diastolic pressure, neonatal weight, and neonatal Apgar score in LS-PE group were 4.79 ± 0.21, 34.9 ± 3.3, 6.5 ± 0.73 g/24h, 175.46 ± 4.38 mmHg, 114.43 ± 3.76 mmHg, 21,952 ± 753 g, and 7.87 ± 0.06, respectively. The BMI, gestational week at delivery, proteinuria level, systolic pressure, diastolic pressure, neonatal weight, and neonatal Apgar score in ES-PE group were 4.52 ± 0.16, 34.4 ± 2.9, 5.3 ± 1.6 g/24h, 171.32 ± 5.42 mm Hg, 116.29 ± 3.94 mm Hg, 2011 ± 642 g, and 9.42 ± 0.08, respectively. The BMI, gestational week at delivery, proteinuria level, systolic pressure, diastolic pressure, neonatal weight, and neonatal Apgar score in LS-PE group were 4.79 ± 0.21, 34.9 ± 3.3, 6.5 ± 0.73 g/24h, 175.46 ± 4.38 mmHg, 114.43 ± 3.76 mmHg, 21,952 ± 753 g, and 7.87 ± 0.06, respectively. Research has been approved by the Ethics Committee of the hospital and that it conforms to the provisions of the Declaration of Helsinki. All the participants signed informed consent.

All participants were singleton pregnancy. Except preeclampsia, no other complications, such as cardiovascular and cerebrovascular diseases, blood diseases, hyperthyroidism kidney disease, diabetes, and endocrine diseases were found in patients of LS-PE and ES-PE groups. No significant differences in gestational age, age, and number of pregnancies were found between those three groups. The Ethics Committee of our institute has approved this study. All participants signed informed consent.

**Diagnostic criteria**

Diagnostic criteria of severe preeclampsia: first, continuous high blood pressure: diastolic blood pressure \(\geq 160\) mm Hg and/or systolic blood pressure \(\geq 160\) mm Hg; second, urinary protein (URO) \(\geq 5\) g/24h or random URO \(\geq 5\) g/24h; third, persistent headache or visual impairment or other neurological symptoms; fourth, persistent upper abdominal pain, subcapsular hematoma, or hepatic rupture; fifth, liver dysfunction: increased levels of alanine aminotransferase (ALT) or aspartate aminotransferase (AST); sixth, abnormal renal function: oliguria (24h urine output < 400 mL or hourly urine output < 17 mL) or serum creatinine > 106 \(\mu\)mol/L; seventh, hypoproteinemia combined with pleural effusion or peritoneal effusion; eighth, abnormalities in circulatory system: density of platelet declines and is lower than 100 \(\times\) 10\(^9\)/L, intravascular hemolysis, anemia, jaundice, or elevated lactate dehydrogenase (LDH) level; ninth, heart failure, pulmonary edema; tenth, fetal growth is limited or oligohydramnios. Preeclampsia occurred before the 34th week of pregnancy is defined as ES-PE, and occurred after the 34th week of pregnancy is defined as LS-PE.

**Specimen collection and processing**

Fasting blood (5 mL) was extracted from elbow vein between 7:00 and 8:00 a.m. 1 day before uterine-incision delivery. Blood samples were transferred to ethylenediaminetetraacetic acid (EDTA)-K2 anticoagulant tube and kept at room temperature for 30 min, followed by centrifugation at 3000t/min for 15 min to collect the supernatant. Supernatant was kept at −80°C before use; 24-h urine was collected 1 day before uterine-incision delivery to record the total volume of urine. Urine was mixed and at least 30 mL urine was subjected to examination. After uterine-incision delivery, placental tissue (about 1 cm \(\times\) 1 cm \(\times\) 1 cm) was collected from the central region of placenta. After washing with sterile saline, excess water was absorbed using
sterile gauze. Placental tissue was stored at −80°C before total RNA extraction.

**Real-time polymerase chain reaction to detect the expression of S100A8 mRNA in peripheral blood and placenta**

Placental tissue was grinded and Trizol reagent (TOYOBO, Japan) was used to extract total RNA from both peripheral blood and placental tissue. RNA concentration was measured using an ultraviolet spectrophotometer (KAIAO, Beijing, China). Reverse transcription was performed using a kit (Takara, Japan) to synthesize cDNA. With cDNA as template, SYBR Green PCR kit (TakaRa, Japan) was used to prepare the polymerase chain reaction (PCR) system. PCR reaction was performed on a real-time PCR detection system (Bio-Rad, USA) with GAPDH as endogenous control. Primers used in PCR reaction was as follows: S100A8 forward: 5′-CGGGATCCATGGCAACTGAACTGGAG-3′; S100A8 reverse: 5′-GCTCTAGATTACTCTGTCGTC-3′; GAPDH forward: 5′-GAGTCAGCGGATTTGGTCTG-3′; GAPDH reverse: 5′-GACAAAGCTCCGTCTTCT-CAG-3′. PCR reaction condition: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 60 s. Data were processed using 2-ΔΔCT with GAPDH as endogenous control to calculate the relative expression level of S100A8 mRNA.

**Enzyme-linked immunosorbent assay to detect the level of S100A8 protein in plasma and levels of inflammatory factors in peripheral blood**

Level of S100A8 protein in plasma was determined using enzyme-linked immunosorbent assay (ELISA) kit (Abnova, Taiwan). Absorbance at 450 nm was measured to determined concentration of S100A8 protein. Contents of inflammatory factors tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-10, and IL-12 in serum were determined using an ELISA kit provided by BOSTER (Wuhan, Hubei, China) according to the instruction.

**Determination of content of uric acid creatinine and UPRO in serum**

Levels of uric acid (UA) and creatinine (CRE) were measured using an automatic biochemical analyzer (Beckman, USA). UPRO content was measured by biuret colorimetry (Sangon, Shanghai, China).

**Statistical analysis**

SPSS19.0 statistical software (SPSS inc, Chicago, USA) was used. All data were expressed in the form of mean ± standard deviation. One-way analysis of variance (ANOVA) was used for the comparisons among multiple groups. Correlation between variables was analyzed using Pearson correlation analysis. Receiver operating characteristic (ROC) analysis was applied to determine optimal sensitivity and specificity to discriminate preeclampsia. P < 0.05 was considered to be statistically significant.

**Results**

**Comparison of expression levels of S100A8 mRNA and protein in peripheral blood among groups**

As shown in Figure 1(a), expression level of S100A8 mRNA was significantly higher in ES-PE and LS-PE groups than in Control group (P < 0.05). In addition, expression level of S100A8 mRNA in LS-PE group was significantly higher than that in ES-PE group (P < 0.05). Those results suggest that preeclampsia can upregulate the transcription level of S100A8, and the transcription level of S100A8 mRNA is related to the duration of preeclampsia.

As shown in Figure 1(b), consistent with the expression pattern of S100A8 mRNA, contents of S100A8 protein in ES-PE group (0.679 ± 0.073 ng/mL) and LS-PE group (0.943 ± 0.112 ng/mL) were significantly higher than the content of S100A8 protein in Control group (0.539 ± 0.062 ng/mL) (P < 0.05). In addition, content of S100A8 protein in LS-PE group was significantly higher than that in ES-PE group (P < 0.05). Those results suggest that preeclampsia can upregulate the transcription level of S100A8, and the transcription level of S100A8 mRNA is related to the duration of preeclampsia.

The ROC curve analysis showed that the area under the curve of S100A8 was 0.883 (95% confidence interval (CI) = 0.816–0.951, P < 0.034), and the sensitivity, specificity, positive predictive value, and negative predictive value were 96.7%, 76.7%, 85.6%, and 79.2%, respectively (Figure 1(c)). The result suggested that S100A8 can be used as an important predictor of preeclampsia.
Comparison of expression levels of inflammatory factors in peripheral blood among groups

As shown in Figure 2, levels of IL-6, TNF-α, and IL-12 in peripheral blood of ES-PE group were 16.24 ± 5.98, 21.64 ± 10.59, and 1.09 ± 0.18 pg/mL, respectively, which were significantly higher than those in Control group (8.93 ± 1.42, 12.26 ± 4.03, and 0.26 ± 0.06 pg/mL, respectively) (P < 0.05). Content of IL-10 was 1.63 ± 0.14 pg/mL, which is significantly lower than that of Control group (1.96 ± 0.12 pg/mL) (P < 0.05). Compared with ES-PE group, levels of IL-6, TNF-α, and IL-12 were significantly increased and level of IL-10 was significantly decreased in LS-PE group (P < 0.05). Those data suggest that preeclampsia can increase the levels of inflammatory factors and decreased the level of anti-inflammatory factor IL-10. And the changes in those factors were related with the duration of preeclampsia.

Comparison of UA, CRE, and UPRO among groups

As shown in Figure 3, highest levels of UA, CRE, and UPRO were found in LS-PE group, followed by SE-PE group and Control group, significant differences were found among those groups (P < 0.05). For example, the content of UPRO in LS-PE group was 1.4 ± 0.5 g/L, which was significantly higher than that in ES-PE group (0.9 ± 0.3 g/L; P < 0.05). In addition, content of UPRO in ES-PE group was significantly higher than that in Control group (0.3 ± 0.1 g/L; P < 0.05).

Correlation between S100A8, inflammatory factors, UA, CRE, and UPRO

Levels of S100A8 protein was positively correlated with the levels of TNF-α and IL-6, and was negatively correlated with level of IL-10, but no significant correlation with IL-12 was found. Further analysis showed that level of S100A8 protein was significantly positively correlated with the levels of UA, CRE, and UPRO (Table 1).

Discussion

In spite of the unclear pathogenesis, the development of preeclampsia is closely correlated with the increased inflammatory responses. In this study, significantly increased level of pro-inflammatory factors and decreased level of anti-inflammatory factors were found in patients with LS-PE than patients with ES-PE. A recent study reported that the causes of ES-PE and LS-PE might be different at molecular level.9 Therefore, the difference in levels of inflammatory factors may be explained by the differences in pathogenesis or difference duration of diseases.

Our study suggest that S100A8 may play important roles in the development of preeclampsia. The expression level of S100A8 is positively correlated with the contents of TNF-α, IL-6, and IL-12, but negatively correlated with IL-10, indicting the interactions among those inflammatory factors. Preeclampsia is also characterized by the increased levels of UA, CRE, and UPRO.10 Consistent results were found in our study. Level of UA, CRE, and UPRO were significantly higher in preeclampsia
Figure 2. Expression levels of inflammatory factors in peripheral blood: (a) TNF-α, (b) IL-6, (c) IL-10, and (d) IL-12. *Compared with Control group \( P < 0.05; \) #Compared with Control group ES-PE group \( P < 0.05.\)

Figure 3. Contents of UA, CRE, and UPRO in different groups: (a) CRE, (b) UA, and (c) UPRO. *Compared with Control group \( P < 0.05; \) #Compared with Control group ES-PE group \( P < 0.05.\)

Table 1. Correlation between S100A8, inflammatory factors, UA, CRE, and UPRO.

| S100A8 | TNF-α | IL-6 | IL-10 | IL-12 | CRE | UA | UPRO |
|--------|-------|------|-------|-------|-----|----|------|
| \( r \) | 0.829 | 0.886 | -0.829 | 0.771 | 0.943 | 0.922 | 0.918 |
| \( P \)  | 0.042 | 0.019 | 0.042 | 0.072 | 0.005 | 0.009 | 0.010 |

S100A8: S100 calcium-binding protein A8; UA: uric acid; CRE: creatinine; UPRO: urinary protein; TNF: tumor necrosis factor; IL: interleukin.
patients than in control. Moreover, expression level of S100A8 was also found to be positively correlated with the level of UA, CRE, and UPRO, indicating the complex regulatory network of the function of S100A8 in the pathogenesis of preeclampsia.

In conclusion, our study suggest that S100A8 protein play important roles in the development of preeclampsia by interacting with inflammatory factors, and the measurement of the contents of inflammatory factors, UA, CRE, and UPRO may provide valuable information for the prediction and prevention of preeclampsia.

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