Expression of Soluble Endoglin and sFlt-1 in Peripheral Blood of Preeclampsia

Minghua Fan¹,², Yongping Xu¹, Jianqing Qiu¹

Abstract

Objective  To investigate the expression and significance of serum soluble endoglin (sEng) and soluble fms-like tyrosine kinase 1 (sFlt-1) in preeclampsia. Methods  Serum levels of sEng and sFlt-1 were determined by enzyme linked immunosorbent assay. Results  The serum level of sEng of the preeclampsia group (6.39±2.15) ng/ml was significantly higher than that of the control (2.16±0.31) ng/ml, P<0.01, especially of early-onset severe preeclampsia. The serum level of sFlt-1 of the preeclampsia group (411.11±80.19) pg/ml was significantly higher than that of the third trimester group (27.57±3.60) pg/ml, but sFlt-1 did not correlate with the most severe forms of the disease. Conclusion  Serum levels of sEng and sFlt-1 are significantly increased in preeclampsia, and the serum level of sEng is even higher in severe preeclampsia patients. Keywords: Preeclampsia; Soluble endoglin; Soluble fms-like tyrosine kinase receptor 1; Angiogenic factor

Introduction

Preeclampsia (PE) is a pregnancy-specific syndrome which is common but can badly affects maternal and fetal health. Studies have suggested that the onset of PE stems from pathophysiological changes of placental tissues and deficient spiral artery remodeling is believed to be the key link of PE. Placental release of angiogenetic factors plays a key role in spiral artery remodeling. sEng is a soluble transforming growth factor (TGF)-β receptor, and sFlt-1 is an effective vascular endothelial growth factor (VEGF) as well as an inhibitor of placental growth factor (PLGF), all of which could reduce the angiogenesis [1]. To investigate the expression and significance of sEng and sFlt-1 in peripheral blood in pathophysiologic process of preeclampsia, this study examined the expression of sEng and sFlt-1 in peripheral blood of patients with preeclampsia.

Materials and methods

Subjects and sample collection

Study Subjects  A total of 47 subjects in different trimesters of pregnancy were recruited from the Department of Obstetrics and Gynecology, the Second Hospital of Shandong University, Jinan, China

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University, Jinan, from April 2013 to September 2015. There were 12 patients with mild preeclampsia, 19 patients with early-onset severe preeclampsia (average gestational age: 32.53±1.98 weeks) and 16 patients with late-onset severe preeclampsia (average gestational age: 37.57±1.49 weeks). At the same time, 43 women in full-term pregnancy from the same hospital were investigated as a control (average gestational age: 38.3±0.7 weeks). All blood samples were collected from women delivered±24h. Pregnant women in two groups (average age: 31±6 years) have no history of either hypertension, heart disease, kidney disease, diabetes and hyperthyroidism, or blood transfusion and immunological therapy.

Inclusion Criteria Preeclampsia group: Pregnant women with childbearing age of 25-37 years old. Singleton pregnancy. No medical and surgery complications. Preeclampsia diagnosis is in accordance with the diagnostic criteria of Gynecology and Obstetrics. Developed at ≤34 weeks of gestation is defined as early-onset severe preeclampsia and late onset severe preeclampsia afterwards. Have regular check and decide to deliver in the Second Hospital of Shandong University.

Normal pregnancy group: Healthy pregnant women with childbearing age of 25-37 years old. Singleton pregnancy. No medical and surgery complications. No abnormal fetal development. Have regular check and decide to deliver in the Second Hospital of Shandong University.

Sample collection

Fasting venous blood was drawn with 3mL from the subjects in the morning, the separated serum was stored at -70°C until assay.

Measurement of the expression of serum sEng and sFlt-1 with ELISA

ELISA was used to determine the expression of serum sEng and sFlt-1 in preeclampsia group, subgroups of preeclampsia and normal pregnancy group, respectively. Kits were obtained from ADL company in America and operations were conducted according to the kit instructions. Replicate wells in all the samples determined in the same batch were measured and the mean was the final concentration. The variation for intra-assay was <5%.

Statistical analysis

SPSS 13.0 software package was used to carry out statistical analysis. Pairwise comparison was analysed by applying independent sample t-test and multiple samples comparison was analysed by variance analysis. Correlation was identified by correlation analysis.

Results

Expressions of serum sEng and sFlt-1 in different groups Table 1.

Our study suggested that expressions of serum sEng and sFlt-1 in preeclampsia group was significantly elevated compared with the normal pregnancy (P<0.01). Statistical significance was assessed in the comparison between either severe preeclampsia and mild preeclampsia (P<0.05) or early-onset severe preeclampsia and late-onset severe preeclampsia (P<0.05), and expression of serum sEng in early-onset severe preeclampsia was three times higher than the critical value.
Table 1. Comparison of sEng and sFlt-1 concentrations between the preeclampsia group and the control (X±s).

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>sEng (ng/ml)</th>
<th>sFlt-1 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>preeclampsia</td>
<td>47</td>
<td>6.39±2.15</td>
<td>411.11±80.19</td>
</tr>
<tr>
<td>mild preeclampsia</td>
<td>12</td>
<td>4.10±0.58</td>
<td>272.31±27.04</td>
</tr>
<tr>
<td>early-onset severe</td>
<td>19</td>
<td>6.12±1.17</td>
<td>451.15±107.39</td>
</tr>
<tr>
<td>preeclampsia</td>
<td>16</td>
<td>4.71±0.39</td>
<td>352.41±96.18</td>
</tr>
<tr>
<td>late-onset severe</td>
<td>12</td>
<td>2.16±0.31</td>
<td>27.57±4.36</td>
</tr>
<tr>
<td>control</td>
<td>43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correlation analysis of sEng and sFlt-1

The correlation coefficient between sEng and sFlt-1 is 0.168 and the difference has no statistical significance (P>0.05), indicating that sEng and sFlt-1 are independent factors in the pathological of preeclampsia; therefore, the mechanisms of action may be different.

Discussion

Uterine spiral artery remodelling is a complicated process, which shares similar biological behaviours with tumor cells. Normal placental development is characterized by cytotrophoblast cells in placental villous invasion into the maternal uterine spiral arteries. Then, trophoblast cells would gradually replace vascular endothelial cells, and further invade into muscular layer, destroy the myofiber and lamina elastica in uterine spiral arteries with low resistance and high capacity, thus increasing placental blood perfusion and contribute to the fetal growth and development [2].

Studies have indicated that clinical manifestations of preeclampsia such as hypertension and proteinuria are associated with anti-angiogenetic factors in the circulation. Scholars have proposed that the imbalance between pro-angiogenic factors and anti-angiogenetic factors (sEng or sFlt-1) is the leading cause of the onset of preeclampsia [3].

Endoglin, also called CD105, is in the localization of human chromosome 9q34. It is a homodimeric transmembrane glycoprotein and one of the components of TGF-β1 and TGF-β3 receptor complexes. Endoglin is mainly expressed on the surface of human endothelial cells and syncytiotrophoblast cells in term placenta, which has been qualified as one of the markers of proliferation of endothelial cells. Endoglin function is thought to be closely related to TGF-β and its receptors. On the one hand, TGF-β can regulate cell proliferation and differentiation, angiogenesis and the integrity of vascular wall structure and have impact on metabolism of extracellular matrix (ECM) [4]. On the other hand, Endoglin could participate in angiogenesis and remodeling via inhibiting the reaction of cells to TGF-β. The main function of Endoglin during pregnancy is to be involved in the formation of TGF-β1 and TGF-β3 receptor complexes and suppress the outward growth and migration of trophoblast cells. Endoglin produced by cells would be released to the circulation in a form of short and soluble sEng and it is shown to be up-regulated in placental tissues of patients with
Preeclampsia, increasing sEng levels released to maternal circulation, therefore, we considered that the expression of Eng could be precisely reflected by measuring sEng levels in the circulation.

Our data presented demonstrate that sEng levels in patients with preeclampsia was high in peripheral blood. It was significantly expressed in patients with early-onset preeclampsia, which was more than three times that of normal pregnancy, indicating that levels of increased sEng was positively correlated with the severity of hypertension and proteinuria appeared in preeclampsia and this was consistent with the observations of Venkatesha et al [5]. Uterine spiral artery remodelling starts from 6 to 8th gestational week and vascular remodelling inhibited by Endonlin are apparent in 4 to 6th gestational month, suggesting that sEng levels in patients with preeclampsia was significantly elevated during uterine spiral artery remodelling, which shows that sEng is likely to be a forecast index of preeclampsia and the degree could be a reflection of the severity of the disease.

Soluble FMS-like tyrosine kinase-1 (sFlt-1), also called soluble vascular endothelial growth factor receptor 1 (VEGFR-1), is a splice variant of the VEGF receptor Flt-1. It acts as a potent antagonist of VEGF and PLGF with which it combines in maternal circulation to impair its function and inhibit angiogenesis. Abnormal placental perfusion of patients with preeclampsia may lead to the overexpression of sFlt-1 in placenta. However, the abnormal expression of sFlt-1 is not specific for preeclampsia. Studies have found that overexpression of sFlt-1 was also examined in fetal growth restriction (FGR), abnormal placental perfusion in second trimester of pregnancy and women with pregnancy complication [6]. The data presented suggest that maternal serum level of sEng-1 in normal pregnancy and preeclampsia show significant difference, whereas the level is not directly related to the severity of preeclampsia and the types of preeclampsia. Therefore, we propose that sFlt-1 is more likely to be the main cause of clinical symptoms of preeclampsia but not the initial factor of the onset of preeclampsia.

**Conclusion**

In summary, sFlt-1 and sEng can block the angiogenesis promoting effect of TGF-β and VEGF in extracellular matrix, respectively. Although the mechanism of action is different, they can both cause the damage of vascular endothelial cells and induce the preeclampsia, thus, the exact mechanisms of sFlt-1 and sEng in the pathogenesis of preeclampsia need to be further investigated.

**References**

