Original Article

Research on correlation between MMP-9 and early-onset preeclampsia

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Abstract: Objective: To investigate the expression changes of MMP-9 in serum and placenta tissues of patients with early-onset preeclampsia (EOPE), and to explore the relation between MMP-9 and EOPE. Methods: 40 pregnant women examined for pregnancy or delivered in our hospital were selected for this study; they were divided into EOPE group (n = 20, pregnant women with EOPE) and control group (n = 20, normal healthy maternal women). ELISA, immunohistochemical staining and Western blot were used to examine the expression levels of MMP-9 in serum and placenta tissues of both EOPE group and control group; and Spearman’s correlation analysis was used to analyze the correlation between the expression of MMP-9 and EOPE. Results: Compared with control group, the serum level of MMP-9 in EOPE group significantly increased (P<0.05); while the MMP-9 expression in placenta tissues was obviously decreased (P<0.05). Correlation analysis indicated that serum MMP-9 in EOPE group was positively related to mean arterial pressure (r = 0.685, P<0.05). Conclusion: The elevation of serum MMP-9 level and decline of placenta MMP-9 level may be related to the occurrence of EOPE, indicating MMP-9 may be involved in pathological and physiological process of preeclampsia.

Keywords: Early-Onset preeclampsia (EOPE), placenta shallow implantation, MMP-9

Introduction

Early-onset preeclampsia (EOPE) is a severe obstetrics disease that threatens mother and fetus [1], and its incidence keeps rising. EOPE usually develops within 34 weeks of pregnancy. Due to its early onset and rapid development, EOPE has been the main cause of death of maternal women and perinatal infants. So far, the pathogenesis of EOPE hasn’t been explained completely. In recent years, researches have shown that the failure of invasive ability of trophoblast cells and placenta shallow implantation are two crucial factors for EOPE; meanwhile, vascular endothelial cell damage that related with inflammation and oxidative stress is an essential part of EOPE [2, 3]. Placenta implantation can be influenced by several factors, such as integrin, matrix metalloproteinases, and insulin-like growth factors etc. Matrix metalloproteiilases-9 (MMP-9) is a rate-limiting enzyme during the implantation. Several researches have found that the plasma MMP-9 level of EOPE patients is lower than that of normal maternal women [4, 5].

MMP-9 is a proteolytic enzyme, involving in the degradation of extracellular matrix (ECM), embryo development, angiogenesis, inflammatory reaction, tissue repair and so on. Research has shown that MMP-9 plays an important part in embryo implantation of mice, and the abnormal expression of MMP-9 in trophoblastic cells can result in abnormal embryonic development [6]. Besides, it is reported that MMP-9 is not only the downstream product of inflammatory reaction, but also an important regulatory factor that has positive feedback effect on several pro-inflammatory cytokines [7, 8]. At present, the role of vascular endothelial injury in EOPE is concerned, but its regulation mechanism is not clear, and detection of MMP-9 expression in placenta and serum of EOPE patients has not been reported. The aim of this study was to detect the expression change of MMP-9 in EOPE patients and normal pregnant women
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and to analyze their correlations; the results of this study will provide experimental and theoretical basis for clinical prediction, prevention and treatment of early-onset preeclampsia.

Material and methods

Study subjects

40 cases of pregnant women examined for pregnancy or delivered at department of obstetrics and gynecology in our hospital from January 2013 to April 2014 were selected in this study. The subjects were divided into two groups: EOPE group (n = 20) and control group (n = 20). The gestational week of EOPE group was less than 34 weeks, and the patients in EOPE group aged from 21 to 38 years old with an average of (31.5±1.62) years old; the gestational week of control group was close to EOPE group (31.32±1.19) weeks, the patients in control group aged from 20 to 35 years old with an average of (30.7±1.72) years old; there was no significant difference between the two groups in terms of age and general information (P>0.05), the subjects in two groups were comparable. All selected patients had no diabetes, high blood pressure, nephropathy, acute or chronic infectious diseases or other chronic illness, besides, all the pregnant women were not parturient and without premature rupture of membranes. The subjects of this study all signed informed consent, and the study was approved by the Hospital Ethics Committee.

Main reagents and instruments

Rabbit-anti-human MMP-9 monoclonal antibody (Santa, USA); ELISA reagent for serum MMP-9 detection (R&D, USA); automatic Elisa reader (Rayto, USA); second antibody (Goat-anti-rabbit) and DAB kit (Beijing Zhong Shan Biological Technology CO., LTD.); Vertical electrophoresis chamber and transfer electrophoresis chamber (BIO-RAD company, USA).

Methods

Specimen collection: Placental tissue was collected during cesarean section; after delivering, a villus tissue (2.5 cm * 2.5 cm * 2.5 cm) in the center of placenta, avoiding the area of infarction, bleeding or calcification, was cut off immediately. After being washed with normal saline, the tissue was preserved in liquid nitrogen at once for subsequent experiments. Blood samples were extracted from antecubital vein after 8 hours of fasting and kept in room temperature for 20 min; then the samplers were centrifuged at 3000 r/min for 20 min, and the serum was separated and stored at -70°C.

The detection of serum MMP-9 level: Enzyme-linked immunosorbent assay (ELISA) was used to detect the serum MMP-9 level; the specific operation steps were carried out in accordance with the instructions, finally, the absorbance value of each hole was detected by ELISA reader with a 450 nm wave length.

Immunohistochemical staining: The placenta tissues were de-waxed by gradient ethanol to water, and incubated with 3% H₂O₂ at room temperature for 10 minutes to eliminate the activity of endogenous peroxidase. After being washed with PBS, the tissue samples were sealed with 10% normal goat serum and incubated at room temperature for 10 minutes. Gently remove serum and add Rabbit-anti-human MMP-9 monoclonal antibody (concentration at 1:100) to incubate overnight in a wet box at 4°C; then washed with PBS, 5 minutes * 3 times; add HRP labeled Sheep-anti-rabbit IgG antibody and incubated at 37°C for 30 minutes; PBS wash for 5 minutes * 3 times, and then use DAB for coloration, the coloration was terminated under optical microscope, hematoxylin was used to double staining, and at last the samples were mounted. The expression level of MMP-9 was assessed by using histochernistry score (H-SCORE). H-SCORE = I×PC, I indicates the intensity of staining, and PC indicates the proportion of cells being stained in each intensity.

Western blot detection: The placenta tissues were grinded and lysed, and the protein concentration in placenta tissues was detected by ELISA reader. According to the detected protein concentration, the total protein mass was 80 μg; after being separated by SDS-PAGE gel electrophoresis, the protein was transferred to PVDF membrane using electro-blotting method. The membrane was sheared according to the required target strip, and then sealed with TBS-T solution containing 5% skimmed milk under room temperature for 1 h; add Rabbit-anti-human MMP-9 monoclonal antibody and Rabbit-anti-human GAPDH antibody and incu-
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Table 1. Comparison of clinical data between two groups of patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Age (year)</th>
<th>Gestational age (week)</th>
<th>Total weight of placenta (g)</th>
<th>Neonatal weight (Kg)</th>
<th>Mean blood pressure (mmHg)</th>
<th>24 h urinary protein (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOPE group</td>
<td>20</td>
<td>31.5±1.62</td>
<td>31.32±1.19</td>
<td>368.67±42.17*</td>
<td>1.67±0.54*</td>
<td>122.52±8.16*</td>
<td>5.78±2.04*</td>
</tr>
<tr>
<td>Control group</td>
<td>20</td>
<td>30.7±1.72</td>
<td>31.62±2.21</td>
<td>535.71±72.87</td>
<td>2.75±0.22</td>
<td>82.79±7.66</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: *compared with the control group, P<0.05.

Results

Comparison of clinical data between two groups

There were no statistically significant differences in age and gestational weeks between EOPE group and control group (all P>0.05); placental weight of EOPE group (368.67±42.17) g was significantly less than that of normal group (535.71±72.87) g (P<0.05). And there were also statistically significant differences in the neonatal weight, mean blood pressure and 24 h urinary protein between two groups (all P<0.05), See Table 1.

Comparisons of MMP-9 expression level between two groups

The HE staining of placenta tissues showed that there were chorionic trophoblast and decidua basalis layer of the placenta in both groups, see Figure 1. Immunohistochemical staining results showed that cytokeratin-7 was positively expressed in the decidua basalis, and MMP-9 was mainly expressed in the cyto-

bated overnight at 4°C; after being washed with TBS-T solution at room temperature, the membrane was incubated with Sheep-anti-rabbit secondary antibody for 30 minutes; later, the membrane was prepared to react with coloration regent after washing with TBS-T solution, and exposed and scanned. The Image J software was used to calculate the optical density value of the target band, and the expression level of MMP-9 protein was evaluated by OD ratio of MMP-9 and GAPDH bands.

Statistical treatment

SPSS 17.0 software was used for statistical analysis; the measurement data were expressed as mean ± standard deviation, and count data were expressed as percentage; t test was used for the comparison of the measurement data between groups, while chi-square test was used for the comparison of count data between groups; Spearman correlation analysis was used to analyze the correlation between MMP-9 and EOPE. P<0.05, the difference was statistically significant.
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The staining results showed that MMP-9 was weakly expressed in the placental chori-onic trophoblast cells of EOPE patients, but highly expressed in the normal pregnant women, as shown in Figure 2. The H-SCORE of placenta MMP-9 expression in EOPE group and control group were 1.68±0.08 and 2.12±1.02, respectively, with statistical significance between the two groups, see Table 2 and Figure 3.

ELISA showed that the expression level of serum MMP-9 in EOPE group was higher than that of control group, and the difference was statistically significant, see Figure 4.

Comparisons of placenta MMP-9 levels between the two groups of patients by western blot

The expression of MMP-9 in placental chorionic trophoblasts of EOPE patients (0.47±0.07) was significantly lower than that of normal pregnant women (1.00±0.08), the difference was statistically significant. See Figures 5, 6; Table 3.

Results of spearman correlation analysis

There was no correlation between the serum MMP-9 level and placenta MMP-9 level in both EOPE group and control group (P>0.05). The expression level of MMP-9 in placenta tissues of EOPE group and control group were not correlated with mean arterial pressure and 24-hour urine protein (all P>0.05). In EOPE group, there was a positive correlation between serum MMP-9 and mean arterial pressure (r = 0.685, P<0.05), but there was no such correlation between serum MMP-9 and mean arterial pressure in the control group (P>0.05).

Discussion

Early-onset preeclampsia (EOPE) is a severe pregnancy hypertension that seriously affects

<table>
<thead>
<tr>
<th>Group</th>
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<th>H-SCORE</th>
<th>T value</th>
<th>P</th>
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<tbody>
<tr>
<td>EOPE group</td>
<td>20</td>
<td>1.68±0.08*</td>
<td>35.57</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Control group</td>
<td>20</td>
<td>2.12±1.02</td>
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</tr>
</tbody>
</table>

*P<0.05.

Figure 2. Immunohistochemical staining of MMP-9. A: EOPE group; B: Control group.

Figure 3. H-SCORE of placenta MMP-9 expression; *P<0.05, compare with EOPE group.
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the health of maternal women and fetus [9-11].

Due to its early onset, it can directly cause immature fetal development. Therefore, peri-
natal infants of EOPE patients usually have poor prognosis [12, 13]. There are several dif-
fferent theories on the pathogenesis of EOPE; among them, the placenta shallow implantation is now widely accepted [14, 15]. Patho-
logical study found that the chorionic epithelium cells of EOPE patients incompletely invaded the endometrium, causing abortion, limited fetal growth and development and preeclampsia etc. [16, 17].

MMP-9 is one of the important members of matrix metalloproteinases family [18]. MMP-9 can degrade the main ingredient in extracellu-
lar matrix-type IV collagen, and directly involve in the process of chorionic epithelium cells invading endometrium and embryo implantation [19, 20]. Clinical experiment results show that compared with normal pregnant women, patients with EOPE had a significantly elevated serum MMP-9 level, indicating that the elevated serum MMP-9 level may be one of the impor-
tant factors of EOPE [21, 22].

The experiment results of this study showed that MMP-9 was weakly expressed in the pla-
cental chorionic trophoblast cells of EOPE group but positively expressed in that of control group. The H-SCORE of placenta MMP-9 of EOPE group was apparently lower than that of control group. Western blot showed that the expression of MMP-9 in the placenta tissues of EOPE group (0.47±0.07) was apparently lower than that of control group (1.00±0.08), the dif-
fifference was statistically significant. The result of this study is consistent with the studies at home and abroad on the levels of serum
MMP-9.

In addition, we also compared the gestational weeks, the placenta weight, and the weight of newborns between two groups; there was no significant difference in gestational weeks between two groups, however, the placenta weight of EOPE group (368.67±42.17) g was obviously less than that of control group (535.71±72.87) g, the difference was statisti-

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>MMP-9 relative value</th>
<th>T value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOPE group</td>
<td>20</td>
<td>0.47±0.07</td>
<td>34.17</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Control group</td>
<td>20</td>
<td>1.00±0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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cally significant. Moreover, the weight of new-borns in EOPE group (1.67±0.54) kg was lower than that of control group (2.75±0.22) kg. These results suggest that EOPE patients may have development disorder with placenta, which directly causes the miscarriage, premature birth, limited growth and development of fetus. And the correlation analysis result showed that the serum level of MMP-9 in EOPE group was positively correlated with mean arterial pressure (r = 0.685, P<0.05), suggesting MMP-9 may play an important role in the process of EOPE.

In conclusion, the down-regulation of MMP-9 expressions in placenta of EOPE patients may inhibit the implantation ability of placenta chorionic trophocytes invading the endometrium, which leads to the development of EOPE. However, this research also has certain limitation, such as small sample size. Further in-depth researches with large samples are still needed to better provide experimental and theoretical basis for clinical prediction, prevention and treatment of EOPE.

Disclosure of conflict of interest

None.

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[17] Correlation between MMP-9 and EOPE.


