Ultrasonic evaluation of central retinal artery hemodynamics in patients with hypertensive disorder complicating pregnancy and the correlation with disease

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Objective: To study the correlation between central retinal artery hemodynamic characteristics in patients with hypertensive disorder complicating pregnancy and endothelial injury molecules as well as trophoblast cell apoptosis molecules. Methods: 45 healthy pregnant women, 37 patients with gestational hypertension and 24 patients with preeclampsia who gave birth in Obstetrics Department of our hospital between May 2013 and December 2015 were selected and included in the control group, GH group and PE group respectively. Central retinal artery ultrasonography was done to determine peak systolic velocity (PSV), end-diastolic velocity (EDV) and resistance index (RI), serum was collected to determine interleukin-6 (IL-6), IL-17, IL-24, chemokine ligand 10 (CXCL10) and cartilage glycoprotein 40 (YKL40) content, and placenta tissue was collected to determine Fas, FasL, Bax, Caspase-3, Caspase-9, XIAP, Survivin and Livin expression. Results: Central retinal artery PSV and EDV as well as XIAP, Survivin and Livin expression in placenta of GH group and PE group were significantly lower than those of control group \( (P<0.05) \) while central retinal artery RI, serum IL-6, IL-17, IL-24, CXCL10 and YKL40 content as well as Fas, FasL, Bax, Caspase-3 and Caspase-9 expression in placenta were significantly higher than those of control group \( (P<0.05) \). Central retinal artery PSV and EDV as well as XIAP, Survivin and Livin expression in placenta of PE group were significantly lower than those of GH group \( (P<0.05) \) while central retinal artery RI, serum IL-6, IL-17, IL-24, CXCL10 and YKL40 content as well as Fas, FasL, Bax, Caspase-3 and Caspase-9 expression in placenta were significantly higher than those of GH group \( (P<0.05) \). Serum IL-6, IL-17, IL-24, CXCL10 and YKL40 content as well as Fas, FasL, Bax, Caspase-3 and Caspase-9 expression in placenta were negatively correlated with PSV and EDV, and positively correlated with RI; XIAP, Survivin and Livin expression in placenta were positively correlated with PSV and EDV, and negatively correlated with RI. Conclusions: Central retinal artery blood flow characteristics in patients with hypertensive disorder complicating pregnancy are the significantly increased blood flow resistance and the significantly decreased blood flow volume, and the above blood flow characteristics are associated with maternal endothelial injury and trophoblast cell apoptosis.

1. Introduction

Hypertensive disorder complicating pregnancy (HDCP) is the pregnancy complication that causes serious harm to maternal and child health, it is mainly shown as hypertension, albuminuria and edema, and meantime, and it is accompanied by early fundus change. Current studies on the pathogenesis of HDCP believe that maternal vasoactive substance changes, endothelial dysfunction as well as the arteriolar spasm caused by increased susceptibility to vasoactive substances are the key links in the process of disease, and the arteriolar spasm of multiple organs in the body occurs and leads to the corresponding clinical symptoms[1-2]. Central retinal artery diameter is about 170 μm, it belongs to the small arteries,
and it is the only small artery in living body whose morphology can be directly observed and whose function can be directly determined. Study has shown that the central retinal artery blood flow is significantly abnormal in patients with HDCP[3], but there is no clear report on the relationship between the arterial blood flow state changes and maternal endothelial cell as well as the trophoblast cell damage in the placenta. In the following study, central retinal artery hemodynamics in patients with HDCP was evaluated and the correlation with the endothelial injury molecules and trophoblast cell apoptosis molecules was analyzed.

2. Materials and methods

2.1. Research subjects

45 healthy pregnant women, 37 patients with gestational hypertension and 24 patients with preeclampsia who gave birth in Obstetrics Department of our hospital between May 2013 and December 2015 were selected and included in control group, gestational hypertension (GH) and preeclampsia (PE) group respectively. Gestational hypertension and preeclampsia were in accordance with the related diagnostic criteria for HDCP in the 8th edition of Obstetrics and Gynecology, and healthy pregnant women received regular prenatal examination and were without complications during pregnancy. Control group were \((29.4\pm3.4)\) years old, with gravidity \((1.58\pm0.22)\) times and with gestational age at delivery \((38.59\pm4.32)\) weeks; GH group were \((30.1\pm3.3)\) years old, with gravidity \((1.63\pm0.21)\) times and with gestational age at delivery \((38.22\pm4.19)\) weeks; PE group were \((30.8\pm3.9)\) years old, with gravidity \((1.39\pm0.18)\) times and with gestational age at delivery \((37.41\pm4.19)\) weeks. The three groups of subjects were not statistically different in general information \((P>0.05)\).

2.2. Central retinal artery ultrasound evaluation methods

Color Doppler diasonograph was used for inspection, linear array probe was used, the frequency was 7.5–10.0 MHz, and the inspection method is as follows: the subjects took supine position and gently closed their eyes, the probe was placed on the eyelids, the horizontal section of the eyeball was taken, the central retinal artery blood flow chart was obtained from the optic nerve “V” shape dark space 1.0 cm from posterior eyeball wall, the sampling volume was 1.5 mm, and the peak systolic velocity (PSV), end-diastolic velocity (EDV) and resistance index (RI) were detected.

2.3. Serum sample collection and detection methods

At 35 weeks of pregnancy, 5 mL of fasting venous blood was collected from the three groups in the morning and centrifuged to get serum, and then enzyme-linked immunosorbent assay kits were used to determine interleukin-6 (IL-6), IL-17, IL-24, chemokine ligand 10 (CXCL10) and cartilage glycoprotein 40 (YKL40) content.

2.4. Placenta sample collection and detection methods

The placenta tissue was collected from three groups of pregnant women within 30 min after delivery of placenta, sampling site was the central maternal placenta, and the placenta tissue blocks were washed with normal saline for 5–10 times and then stored in liquid nitrogen. For detection, the placenta tissue samples were taken out, about 30 mg specimens were weighed, and the RNA extraction kits and first cDNA strand synthesis kits were used to extract the RNA in the tissue and reversely transcribe it to cDNA. Finally, cDNA samples were used for fluorescence quantitative PCR reaction, Fas, FasL, Bax, Caspase-3, Caspase-9, XIAP, Survivin and Livin were amplified respectively, and the above gene mRNA levels were calculated.

2.5. Statistical analysis

SPSS20.0 software was used to input and analyze data, measurement data analysis among three groups was by variance analysis and \(P<0.05\) indicated statistical significance in differences.

3. Results

3.1. Central retinal artery hemodynamic parameters

Central retinal artery ultrasound atlas of typical cases in normal pregnant women and patients with HDCP, analysis of the specific parameters PSV, EDV and RI is shown in Table 1. Central retinal artery PSV and EDV of GH group and PE group were significantly lower than those of control group \((P<0.05)\) while central retinal artery RI were significantly higher than that of control group \((P<0.05)\).

Central retinal artery PSV and EDV of PE group were significantly lower than those of GH group \((P<0.05)\) while central retinal artery RI was significantly higher than that of GH group \((P<0.05)\).

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>PSV (cm/s)</th>
<th>EDV (cm/s)</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>45</td>
<td>14.86±1.88</td>
<td>5.56±0.72</td>
<td>0.61±0.07</td>
</tr>
<tr>
<td>GH group</td>
<td>37</td>
<td>13.04±1.56</td>
<td>4.20±0.64</td>
<td>0.73±0.09</td>
</tr>
<tr>
<td>PE group</td>
<td>24</td>
<td>10.42±1.34</td>
<td>3.16±0.39</td>
<td>0.79±0.08</td>
</tr>
</tbody>
</table>

*: compared with control group, \(P<0.05\); &: compared with GH group, \(P<0.05\).

3.2. Central retinal artery gene expression parameters

Central retinal artery ultrasound atlas of typical cases in normal pregnant women and patients with HDCP, analysis of the gene expression parameters (Fas, FasL, Bcl-2, Bax, Caspase-3, Caspase-9, XIAP, Survivin and Livin) is shown in Table 2. Central retinal artery Fas, FasL, Bcl-2, Bax, Caspase-3, Caspase-9, XIAP, Survivin and Livin were significantly lower than those of control group \((P<0.05)\) while central retinal artery Caspase-9 was significantly higher than that of control group \((P<0.05)\).
3.2. Serum cytokine content

Analysis of serum cytokines IL-6, IL-17, IL-24, CXCL10 and YKL40 among the three groups is shown in Table 2. Serum IL-6, IL-17, IL-24, CXCL10 and YKL40 content of GH group and PE group were significantly higher than those of control group (P<0.05); serum IL-6, IL-17, IL-24, CXCL10 and YKL40 content of PE group were significantly higher than those of GH group (P<0.05).

3.3. Apoptotic molecule expression in placenta

Analysis of pro-apoptotic molecules Fas, Fasl, Bax, Caspase-3 and Caspase-9 expression in placenta tissue among the three groups is shown in Table 3. Fas, Fasl, Bax, Caspase-3 and Caspase-9 mRNA content in placenta tissue of GH group and PE group were significantly higher than those of control group (P<0.05), and Fas, Fasl, Bax, Caspase-3 and Caspase-9 mRNA content in placenta tissue of PE group were significantly higher than those of GH group (P<0.05).

Analysis of anti-apoptotic molecules XIAP, Survivin and Livin expression in placenta is shown in Table 4. XIAP, Survivin and Livin mRNA content in placenta tissue of GH group and PE group were significantly lower than those of control group (P<0.05), and XIAP, Survivin and Livin mRNA content in placenta tissue of PE group were significantly lower than those of GH group (P<0.05).

### Table 4

Anti-apoptotic molecule expression in placenta tissue of three groups (\( \times 10^3 \)).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>XIAP</th>
<th>Survivin</th>
<th>Livin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45</td>
<td>1.08±0.15</td>
<td>0.94±0.12</td>
<td>0.99±0.11</td>
</tr>
<tr>
<td>GH group</td>
<td>37</td>
<td>0.74±0.09*</td>
<td>0.79±0.10*</td>
<td>0.71±0.08*</td>
</tr>
<tr>
<td>PE group</td>
<td>24</td>
<td>0.43±0.05*</td>
<td>0.38±0.04*</td>
<td>0.48±0.07*</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; *: compared with GH group, P<0.05.

### Table 2

Comparison of serum cytokine content among the three groups (\( \times 10^3 \)).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>IL-6 (pg/mL)</th>
<th>IL-17 (pg/mL)</th>
<th>IL-24 (μg/mL)</th>
<th>CXCL10 (pg/mL)</th>
<th>YKL40 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45</td>
<td>22.51±3.52</td>
<td>63.65±7.87</td>
<td>0.52±0.08</td>
<td>82.35±9.35</td>
<td>1.39±0.14</td>
</tr>
<tr>
<td>GH group</td>
<td>37</td>
<td>37.69±5.23*</td>
<td>89.34±9.35*</td>
<td>0.81±0.10*</td>
<td>121.67±16.79*</td>
<td>3.24±0.46*</td>
</tr>
<tr>
<td>PE group</td>
<td>24</td>
<td>69.54±7.83*</td>
<td>132.54±17.58*</td>
<td>1.21±0.15*</td>
<td>164.51±20.38*</td>
<td>8.59±1.04*</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; *: compared with GH group, P<0.05.

### Table 3

Pro-apoptotic molecule expression in placenta tissue of three groups (\( \times 10^3 \)).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Fas</th>
<th>Fasl</th>
<th>Bax</th>
<th>Caspase-3</th>
<th>Caspase-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45</td>
<td>1.05±0.14</td>
<td>0.96±0.11</td>
<td>1.12±0.15</td>
<td>1.02±0.10</td>
<td>0.98±0.13</td>
</tr>
<tr>
<td>GH group</td>
<td>37</td>
<td>1.89±0.24*</td>
<td>1.74±0.19*</td>
<td>1.64±0.20*</td>
<td>2.05±0.28</td>
<td>1.73±0.22*</td>
</tr>
<tr>
<td>PE group</td>
<td>24</td>
<td>3.29±0.44*</td>
<td>2.95±0.38*</td>
<td>3.58±0.31*</td>
<td>3.45±0.49*</td>
<td>2.42±0.31*</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; *: compared with GH group, P<0.05.
and mononuclear macrophage recruitment in vascular endothelium, which activate inflammation and cause endothelial damage[11]. IL-24 is the new member of the interleukins family, placenta trophocyte can synthesize and secrete IL-24, and the IL-24 that enters into the blood circulation from the placenta can cause endothelial injury; YKL40 and CXCL10 are the cytokines with chemotactic function, and they mainly mediate inflammatory cell recruitment to the inflammatory site[12]. In the study, the analysis of the content of these cytokines in serum showed that serum IL-6, IL-17, IL-24, CXCL10 and YKL40 content in patients with HDCP significantly increased ($P<0.05$), and serum IL-6, IL-17, IL-24, CXCL10 and YKL40 content of PE group were significantly higher than those of GH group ($P<0.05$), negatively correlated with PSV and EDV, and positively correlated with RI. This means that the changes of central retinal artery blood flow characteristics in patients with HDCP are associated with the endothelial injury caused by abnormally increased serum cytokines.

Systemic arteriolar spasm in patients with HDCP can cause multiple organ perfusion reduction, and the uterine spiral artery spasm can directly cause the placenta blood perfusion reduction and local tissue ischemia hypoxia. There will be abnormal trophoblast cell apoptosis in ischemic-hypoxic placenta, which affects the placental material transport function and the placental nutrition supply; meantime, ischemia hypoxia can also cause trophoblast cells to synthesize a variety of traumatic cytokines and secrete them into the maternal, thus aggravating the maternal endothelial injury and increasing the degree of arteriolar spasm[13,14]. Fas/FasL and Bax mediate death receptor apoptosis and mitochondrial apoptosis respectively, and both apoptotic pathways can activate Caspase-3 and Caspase-9 and cause cells apoptosis[15,16]; XIAP can antagonize mitochondrial pathway of apoptosis, and Survivin and Livin can antagonize death receptor pathway of apoptosis[17,18]. In the study, analysis of the expression of these apoptotic molecules showed that Fas, Fasl, Bax, Caspase-3 and Caspase-9 expression in placenta tissue of patients with HDCP significantly increased, were negatively correlated with PSV and EDV, and positively correlated with RI; XIAP, Survivin and Livin expression significantly decreased ($P<0.05$), were positively correlated with PSV and EDV, and negatively correlated with RI. This means that the changes of central retinal artery blood flow characteristics in patients with HDCP are associated with excessive trophoblast cell apoptosis in the placenta tissue.

In conclusion, central retinal artery blood flow characteristics in patients with HDCP are the significantly increased blood flow resistance and the significantly decreased blood flow volume, and the above blood flow characteristics are associated with maternal endothelial injury and trophoblast cell apoptosis.

References