Ultrasonic evaluation of fetal ventricular systolic function in hypertensive disorder complicating pregnancy and its correlation with the expression of placental hypoxia-related genes

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ABSTRACT

Objective: To discuss the ultrasonic evaluation of fetal ventricular systolic function in hypertensive disorder complicating pregnancy and its correlation with the expression of placental hypoxia-related genes. Methods: A total of 98 late pregnant women with hypertensive disorder complicating pregnancy who were treated in the hospital between December 2014 and February 2017 were selected as hypertensive disorder complicating pregnancy group and 100 normal late pregnant women who received physical examination in the hospital during the same period were selected as normal control group. The ultrasonic parameters of fetal ventricular systolic function in two groups of pregnant women were detected before delivery, and the expression of hypoxia-related genes in placental tissue was detected by fluorescence quantitative PCR. Pearson test was used to assess the correlation between the parameters of fetal ventricular systolic function and placental hypoxia in hypertensive disorder complicating pregnancy. Results: The fetal left ventricular fractional shortening and right ventricular fractional shortening levels of hypertensive disorder complicating pregnancy group were lower than those of normal control group. Angiogenesis-related genes VEGF, netrin-1 and XIAP mRNA expression in placental tissue of hypertensive disorder complicating pregnancy group were lower than those in placental tissue of normal control group while HIF-1α mRNA expression was higher than that in placental tissue of normal control group; oxidative stress-related genes Nrf2, TAC3 and PrxⅡ mRNA expression in placental tissue were lower than those in placental tissue of normal control group; apoptosis genes Fas, p53 and caspase-9 mRNA expression in placental tissue were higher than those in placental tissue of normal control group while Bcl-2 and SFRP2 mRNA expression were lower than those in placental tissue of normal control group. Pearson test showed that the ultrasonic parameter levels of fetal ventricular systolic function in hypertensive disorder complicating pregnancy were directly correlated with the degree of placental hypoxia. Conclusion: Ultrasonic testing of fetal ventricular systolic function in hypertensive disorder complicating pregnancy can be used as a reliable method to measure the degree of placental hypoxia.

1. Introduction

Hypertensive disorder complicating pregnancy belongs to idiopathic disease in pregnancy, the incidence is 5%-10% of all pregnant women, it is the second leading cause of death of pregnant and lying-in woman, and early diagnosing the disease and confirming the fetal intrauterine damage are required to smoothly end the pregnancy and ensure maternal and fetal safety to the greatest extent[1,2]. The latest studies have shown that there may be fetal cardiac function hypoplasia in hypertensive disorder complicating pregnancy, which is mainly manifested as the decrease of ventricular systolic function, causes that the blood supply from the right ventricle cannot meet the need for fetal intrauterine growth, and eventually results in fetal hypoplasia, intrauterine asphyxia and
other serious events[3,4]. Ultrasonography is the major noninvasive means to determine fetal heart development status and can indirectly reflect the fetal intrauterine blood supply, so some scholars have pointed out that the fetal ultrasound heart parameters can be used to measure the existence and severity of fetal intrauterine hypoxia[5,6]. In this study, the differences in ultrasound cardiac function parameter levels were compared between fetuses with hypertensive disorder complicating pregnancy and normal fetuses, and the correlation between cardiac function parameters and placental hypoxia was further analyzed to lay the foundation for subsequent auxiliary application of ultrasound in the diagnosis and treatment of the disease.

2. Information and methods

2.1 General information

A total of 98 late pregnant women with hypertensive disorder complicating pregnancy who were treated in the hospital between December 2014 and February 2017 were selected as hypertensive disorder complicating pregnancy group, 100 normal late pregnant women who received physical examination in the hospital during the same period were selected as normal control group, and the families of pregnant women signed the informed consent. Hypertensive disorder complicating pregnancy group were 23-38 years old, and the gravidity was 1-3 and (1.73±0.59) in average; normal control group were 22-39 years old, and the gravidity was 1-3 and (1.69±0.54) in average. The differences in age and gravidity distribution were not significant between the two groups, and the hospital ethics committee approved the study.

2.2 Inclusion and exclusion criteria

Inclusion criteria: (1) meeting the diagnostic criteria for hypertensive disorder complicating pregnancy; (2) diagnosed with hypertensive disorder complicating pregnancy for the first time and never receiving independent treatment outside the hospital; (3) cooperating with related inspection and giving birth smoothly. Exclusion criteria: (1) with cardiac dysfunction before pregnancy; (2) dead fetus in uterus; (3) combined with systemic infectious diseases.

2.3 Fetal ventricular systolic function ultrasound

1 week before delivery, color Doppler diasonograph was used to determine the fetal left ventricular systolic function parameter levels, including left ventricular fractional shortening and right ventricular fractional shortening.

2.4 Hypoxia–related gene expression in placental tissue

After the delivery of fetus and placenta, the placenta tissue samples were collected, fluorescence quantitative PCR was used to determine the expression of hypoxia-related genes in it, and the specific steps were as follows: the cells were broken with Trizol reagent and mixed with chloroform to precipitate total RNA, and 75% ethanol was used to clean the RNA precipitation. Reverse transcription kits were used to synthesize sample cDNA, and fluorescence quantitative PCR kit was used to amplify the target genes, including angiogenesis-related genes VEGF, HIF-1 α, netrin-1 and XIAP, oxidative stress-related genes Nrf2, TAC3 and Prx II as well as apoptosis genes Fas, Bcl-2, p53, caspase-9 and SFRP2. The corresponding PCR amplification curves were obtained in the software and the mRNA expression of the above target genes were calculated.

2.5 Statistical processing

Statistical software was SPSS 25.0 and \( P<0.05 \) indicated statistical significance in differences. Fractional shortening, angiogenesis-related genes, oxidative stress-related genes and apoptosis genes were in terms of mean ± standard deviation, the comparison was by t test and correlation analysis was by Pearson test.

3. Results

3.1 Fractional shortening

Comparison of ultrasonic parameter levels of fetal ventricular systolic function between two groups of pregnant women was as follows: the fetal left ventricular fractional shortening level of hypertensive disorder complicating pregnancy group was (0.30±0.04), the right ventricular fractional shortening level was (0.26±0.03), and they were greatly lower than the fetal left ventricular fractional shortening level of normal control group (0.35±0.06) and the right ventricular fractional shortening level (0.36±0.05). The differences in ultrasonic parameter levels of fetal ventricular systolic function were significant between the two groups (\( P<0.05 \)).

3.2 Angiogenesis–related genes

Comparison of angiogenesis-related genes VEGF, HIF-1 α, netrin-1 and XIAP mRNA expression in placental tissue between the two groups was as follows: VEGF, netrin-1 and XIAP mRNA expression in placental tissue of hypertensive disorder complicating pregnancy group were lower than those in placental tissue of normal
control group whereas HIF-1α mRNA expression was higher than that in placental tissue of normal control group. The differences in angiogenesis-related genes VEGF, HIF-1α, netrin-1 and XIAP mRNA expression in placental tissue were significant between the two groups \( (P<0.05) \), shown in Table 1.

### Table 1.
Comparison of angiogenesis-related gene expression in placental tissue between the two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>VEGF</th>
<th>HIF-1α</th>
<th>netrin-1</th>
<th>XIAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100.74±12.85</td>
<td>99.92±10.63</td>
<td>101.64±13.78</td>
<td></td>
</tr>
<tr>
<td>HDCP group</td>
<td>98</td>
<td>79.84±9.15</td>
<td>85.71±9.63</td>
<td>65.88±7.19</td>
<td></td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>13.284</td>
<td>11.639</td>
<td>15.982</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

### 3.3 Oxidative stress-related genes

Comparison of oxidative stress-related genes Nrf2, TAC3 and Prx mRNA expression in placental tissue between the two groups was as follows: Nrf2, TAC3 and Prx mRNA expression in placental tissue of hypertensive disorder complicating pregnancy group were greatly lower than those in placental tissue of normal control group. The differences in oxidative stress-related genes Nrf2, TAC3 and Prx mRNA expression in placental tissue were significant between the two groups \( (P<0.05) \), shown in Table 2.

### Table 2.
Comparison of oxidative stress-related gene expression in placental tissue between the two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Nrf2</th>
<th>TAC3</th>
<th>Prx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100.74±12.85</td>
<td>99.92±10.63</td>
<td>101.64±13.78</td>
</tr>
<tr>
<td>HDCP group</td>
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<td></td>
<td>13.284</td>
<td>11.639</td>
<td>15.982</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

### 3.4 Apoptosis genes

Comparison of apoptosis genes Fas, Bcl-2, p53, caspase-9 and SFRP2 mRNA expression in placental tissue between the two groups was as follows: Fas, p53 and caspase-9 mRNA expression in placental tissue of hypertensive disorder complicating pregnancy group were higher than those in placental tissue of normal control group whereas Bcl-2 and SFRP2 mRNA expression were lower than those in placental tissue of normal control group. The differences in apoptosis genes Fas, Bcl-2, p53, caspase-9 and SFRP2 mRNA expression in placental tissue were significant between the two groups \( (P<0.05) \), shown in Table 3.

### Table 3.
Comparison of apoptosis gene expression in placental tissue between the two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Fas</th>
<th>Bcl-2</th>
<th>p53</th>
<th>caspase-9</th>
<th>SFRP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>98.63±10.74</td>
<td>100.93±12.64</td>
<td>101.64±13.29</td>
<td>98.46±10.58</td>
<td>102.53±13.28</td>
</tr>
<tr>
<td>HDCP group</td>
<td>98</td>
<td>128.49±15.36</td>
<td>73.26±8.19</td>
<td>118.35±14.28</td>
<td>132.16±14.88</td>
<td>75.31±8.64</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

### 3.5 Correlation analysis

Pearson test showed that the ultrasonic parameters left ventricular fractional shortening and right ventricular fractional shortening levels of fetal ventricular systolic function in pregnant women with hypertensive disorder complicating pregnancy were positively correlated with angiogenesis-related genes VEGF, netrin-1 and XIAP mRNA expression, and negatively correlated with HIF-1α mRNA expression; they were positively correlated with oxidative stress-related genes Nrf2, TAC3 and Prx mRNA expression; they were negatively correlated with apoptosis genes Fas, p53 and caspase-9 mRNA expression, and positively correlated with Bcl-2 and SFRP2 mRNA expression \( (P<0.05) \).

### 4. Discussion

The main pathological changes of hypertensive disorder complicating pregnancy are the maternal systemic vasospasm, the increase of placental vascular resistance and the decrease of placental blood perfusion, which can lead to fetal hypoxia and even distress with disease progress. In the above process, the fetal myocardium develops compensatory hyperplasia, and the ventricular systolic dysfunction and fetal blood supply insufficiency will occur when the compensatory mechanism reaches the limit. Ultrasonic fetal cardiac function testing is the most common means for objective judgment of fetal cardiac function, and the value of ventricular fractional shortening for evaluating ventricular systolic function in adults and children has been confirmed[6-8]. It was found in the study that compared with those of normal control group, the fetal left and right ventricular fractional shortening levels of hypertensive disorder complicating pregnancy group were lower, explaining that there is a certain degree of fetal ventricular systolic dysfunction in women with hypertensive disorder complicating pregnancy. The direct relationship between fetal ventricular systolic function and placental hypoxia will be further discussed in this paper.

The placental hypoxia is one of the most important pathological changes in hypertensive disorder complicating pregnancy, which is directly related to the placental vascular recasting and insufficient pro-angiogenesis factor expression. VEGF is the strongest factor that promotes angiogenesis, and it has been found that its expression...
decreases in preeclampsia placenta tissue, which directly affects the trophoblast cell differentiation and implant ability, and causes the shallow placenta implantation[9,10]. HIF-1α is a hypoxia response regulator in the body that is activated under hypoxia condition, and the HIF-1α expression increases when the placental vascular recasting is impaired and in relatively hypoxia state. netrin-1 is an axon guidance factor that leads the neural orientated growth and induces vascular growth, and there is its expression decrease and even deletion in the placenta of patients with preeclampsia eclampsia[11]. XIAP inhibits vascular endothelial cell apoptosis in normal anischemic hypoxic placenta villi, and it can maintain placental vascular physiological function[12]. It was found in the study that compared with those in placenta tissue of normal control group, VEGF, netrin-1 and XIAP mRNA expression in placental tissue of hypertensive disorder complicating pregnancy group were lower whereas HIF-1α mRNA expression was higher, showing that hypertensive disorder complicating pregnancy can lead to the decrease of pro-angiogenesis gene expression and the increase of hypoxia-related gene expression in placenta. Further Pearson test showed that the fetal left and right ventricular fractional shortening levels of hypertensive disorder complicating pregnancy group were positively correlated with VEGF, netrin-1 and XIAP mRNA expression, and negatively correlated with HIF-1α mRNA expression, confirming that ultrasonic parameter levels of fetal ventricular systolic function of hypertensive disorder complicating pregnancy can objectively measure the exuberant extent of placental angiogenesis, and indirectly reflect the extent of placental hypoxia.

Hypoxia can directly result in the increase of oxidative stress in placental tissue, and excessive production of hypoxic metabolites further damage placental cells and impede fetal growth and development[13,14]. Nrf2 is a key target regulating oxidative stress in the body and plays an important antioxidant effect, and it has been found that its expression decreases in preeclampsia placenta tissue, which is speculated to be because that the oxidative stress caused by hypoxia inhibits its expression[15]. The expression of TAC3 significantly reduces in the hypoxic preeclampsia placenta tissue, and the reduction of its specific expression is highly consistent with the degree of placental hypoxia. Prx II is an important enzyme of oxidative metabolism in the body, which can remove the H2O2 from metabolism and exert antioxidant effect. Reduced Prx II expression can increase the cell sensitivity to oxidative stress and the generation of oxygen free radicals in cells. It was found in the study that compared with those in the placenta tissue of normal control group, Nrf2, TAC3 and Prx II mRNA expression in placental tissue of hypertensive disorder complicating pregnancy group were lower, indicating that the anti-oxidative stress gene expression decreases in placenta tissue of hypertensive disorder complicating pregnancy. Further Pearson test showed that the fetal left and right ventricular fractional shortening levels of hypertensive disorder complicating pregnancy were positively correlated with Nrf2, TAC3 and Prx II mRNA expression, showing that ultrasonic ventricular systolic function parameter levels can objectively reflect the oxidative stress degree in placenta tissue of hypertensive disorder complicating pregnancy, and indirectly reflect the degree of hypoxia.

The final outcome caused by hypoxia is placental apoptosis as well as fetal growth and developmental disorders and even intrauterine death, and many apoptosis genes are involved in the process and can also be used as the objective indicator to indirectly reflect the degree of placental hypoxia. Fas is the cell surface receptor mediating apoptosis. Bcl-2 is the first discovered anti-apoptotic gene, and it is negatively correlated with the apoptosis rate of placental choriocarcinoma cells[16-18]. p53 is the upstream regulatory gene of Bcl-2, which can reduce Bcl-2 expression and induce apoptosis[19]. caspase-9 is the initiation and execution gene of apoptosis, and trophoblast cells can stimulate the increase of caspase-9 expression under the regulation of hypoxia[20]. SFRP2 is a gene with anti-apoptotic effect, which can inhibit apoptosis by inhibiting the activity of caspase-3. It was found in the study that compared with those of normal control group, Fas, p53 and caspase-9 mRNA expression in placental tissue of hypertensive disorder complicating pregnancy group were higher whereas Bcl-2 and SFRP2 mRNA expression were lower, which indicates that the pro-apoptotic gene expression increases and the anti-apoptotic gene expression decrease in the placenta of hypertensive disorder complicating pregnancy. Further Pearson test showed that the fetal left and right ventricular fractional shortening levels of hypertensive disorder complicating pregnancy were negatively correlated with Fas, p53 and caspase-9 mRNA expression, and positively correlated with Bcl-2 and SFRP2 mRNA expression, indicating that ultrasonic ventricular systolic function parameter levels can objectively reflect the placental apoptosis activity of hypertensive disorder complicating pregnancy.

The fetal ultrasonic ventricular systolic function parameter levels of hypertensive disorder complicating pregnancy decrease, and the specific levels are directly correlated with placenta angiogenesis, oxidative stress and apoptosis-related gene expression, can be used as the noninvasive and objective indicators to measure the degree of placental hypoxia, and are worthy of popularization and application in clinical practice in the future.

References


