Correlation of late-pregnancy serum 25-OH-VitD3 content with maternal endothelial injury and placental apoptosis

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ARTICLE INFO
Objective: To study the correlation of late-pregnancy serum 25-OH-VitD3 content with maternal endothelial injury and placental apoptosis in pregnant women with preeclampsia (PE).

Methods: PE pregnant women and healthy pregnant women who gave birth in the First People’s Hospital of Yichang between June 2014 and February 2017 were selected as PE group and control group respectively. At 32 weeks of gestation and before delivery, the serum was collected respectively to determine the contents of 25-OH-VitD3 and endothelial lesion markers; after delivery, the placenta was collected to determine the expression of apoptosis molecules.

Results: 25-OH-VitD3 contents in serum as well as XIAP and Survivin mRNA expression in placental tissue of PE group were significantly lower than those of control group while sEng, sFlt-1, IFI16 and tTG contents in serum as well as AP-2, Smac and PTEN mRNA expression in placental tissue were significantly higher than those of control group; sEng, sFlt-1, IFI16 and tTG contents in serum as well as AP-2, Smac and PTEN mRNA expression in placental tissue of PE pregnant women with low 25-OH-VitD3 content were significantly higher than those of PE pregnant women with high 25-OH-VitD3 content while XIAP and Survivin mRNA expression were significantly lower than those of PE pregnant women with high 25-OH-VitD3 content.

Conclusions: The decline of late-pregnancy serum 25-OH-VitD3 content can aggravate the maternal endothelial injury and placental apoptosis in pregnant women with PE.

1. Introduction

Preeclampsia (PE) is a common complication during pregnancy, which is with increasing incidence rate year by year and will increases the risk of maternal and newborn mortality[1]. At present, the pathogenesis of preeclampsia is still not fully clear, and the excessive apoptosis of placental cells and the excessive injury of maternal endothelial function caused by the placental superficial implantation are the important characteristics of the disease[2,3]. In recent years, the relationship between vitamin D and pregnancy complications has received more and more attention, vitamin D is a kind of fat-soluble vitamin, with a broad range of biological activities and it includes five forms of compounds from vitamin

D1 - D5. Vitamin D3 is the main form of vitamin D in the human body, the vitamin D3 from food absorption or skin synthesis is metabolized in the body and transformed to 25-hydroxyvitamin D3 (25-OD-VitD3), and 25-OD-VitD3 is the main form of vitamin D3 in blood circulation and also the main index to evaluate vitamin D metabolism[4,5]. In the following study, in order to define the correlation between 25-OD-VitD3 and the onset of preeclampsia, we specifically analyzed the correlation of late-pregnancy serum 25-OH-VitD3 content with maternal endothelial injury and placental apoptosis in patients with preeclampsia.

2. Materials and methods

2.1. General information

Preeclampsia pregnant women and healthy pregnant women who gave birth in the First People’s Hospital of Yichang between June 2014 and February 2017 were selected and enrolled in PE group.
and control group respectively. PE group of pregnant women were in accordance with the diagnostic criteria for preeclampsia, the pregnant women combined with gestational diabetes mellitus and other pregnancy complications were ruled out. A total of 42 cases from 24 to 35 years old were enrolled, 27 cases of which were primiparous and 15 cases of which were multiparous, and the gestational age at delivery was 35-38 weeks; control group of pregnant women were excluded from pregnancy complications by regular antenatal care, a total of 60 cases from 23 to 35 years old were enrolled, 37 cases of which were primiparous and 23 cases of which were multiparous, and the gestational age at delivery was 37-39 weeks. There was no significant difference in general information between the two groups of pregnant women ($P>0.05$).

2.2. Research methods

2.2.1. Serum sample collection and detection
At 32 weeks of gestation and before delivery, 3-5 mL of cubital venous blood was collected from two groups of pregnant women, let stand and centrifuged to separate the upper serum, and enzyme-linked immunosorbent assay kit was used to determine serum 25-OH-VitD, sEng, sFlt-1, IFI16 and tTG contents.

2.2.2. Placenta sample collection and detection
After delivery, right amount of placental tissue was collected and washed with saline to remove the blood, the kits were used to separate the total RNA in the tissue and synthesize it into cDNA by reverse transcription, then fluorescence quantitative PCR kit was used to amplify cDNA, and the AP-2α, Smac, PTEN, XIAP and Survivin mRNA expression were determined.

2.3. Statistical methods

SPSS 20.0 software was used to input data, the median of serum 25-OH-VitD3 content in PE pregnant women before delivery was calculated and used to divide groups into those with low 25-OH-VitD3 content and with high 25-OH-VitD3 content, and the differences in measurement data between two groups was analyzed by t test.

<table>
<thead>
<tr>
<th>Groups</th>
<th>$n$</th>
<th>sEng</th>
<th>sFlt-1</th>
<th>IFI16</th>
<th>tTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE group</td>
<td>42</td>
<td>5.68±0.72</td>
<td>6.41±0.87</td>
<td>32.41±5.84</td>
<td>13.15±1.89</td>
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<tr>
<td>Control group</td>
<td>60</td>
<td>1.98±0.25</td>
<td>1.36±0.20</td>
<td>12.56±2.03</td>
<td>6.58±0.71</td>
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<tr>
<td>$t$</td>
<td>18.398</td>
<td>27.584</td>
<td>16.408</td>
<td>11.285</td>
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</tr>
<tr>
<td>$P$</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>25-OH-VitD3</th>
<th>$n$</th>
<th>sEng</th>
<th>sFlt-1</th>
<th>IFI16</th>
<th>tTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low content</td>
<td>21</td>
<td>8.03±1.06</td>
<td>9.21±1.16</td>
<td>43.89±7.41</td>
<td>18.72±2.95</td>
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<tr>
<td>High content</td>
<td>21</td>
<td>3.31±0.46</td>
<td>3.76±0.65</td>
<td>21.36±3.48</td>
<td>8.85±1.15</td>
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<tr>
<td>$t$</td>
<td>12.948</td>
<td>13.586</td>
<td>10.938</td>
<td>14.128</td>
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<tr>
<td>$P$</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

3. Results

3.1. Serum 25-OH-VitD3 contents in two groups of pregnant women
At 32 weeks of gestation and before delivery, serum 25-OH-VitD3 contents of PE group were (19.28±2.83) nmol/L and (17.58±2.41) nmol/L respectively; serum 25-OH-VitD3 contents of control group were (31.25±5.82) nmol/L and (33.12±4.91) nmol/L respectively. The t test analysis showed that serum 25-OH-VitD3 contents of PE group were significantly lower than those of control group.

3.2. Serum endothelial injury marker contents in two groups of pregnant women

Before delivery, analysis of serum endothelial injury markers sEng, sFlt-1, IFI16 and tTG contents between two groups of pregnant women was as follows: serum sEng, sFlt-1, IFI16 and tTG contents of PE group were significantly higher than those of control group. Analysis of serum endothelial injury markers sEng, sFlt-1, IFI16 and tTG contents between PE pregnant women with different 25-OH-VitD3 contents was as follows: serum sEng, sFlt-1, IFI16 and tTG contents of PE pregnant women with low 25-OH-VitD3 content were significantly higher than those of PE pregnant women with high 25-OH-VitD3 content.

3.3. Apoptosis molecule expression in placenta of two groups

After delivery, analysis of apoptosis molecules AP-2α, Smac, PTEN, XIAP and Survivin expressions in placenta between two groups was as follows: AP-2α, Smac and PTEN mRNA expressions in placental tissue of PE group were significantly higher than those of control group while XIAP and Survivin mRNA expressions were significantly lower than those of control group. Analysis of apoptosis molecules AP-2α, Smac, PTEN, XIAP and Survivin expressions in placenta between PE pregnant women with different 25-OH-VitD3 contents was as follows: AP-2α, Smac and PTEN mRNA expressions were significantly lower than those of control group.
Comparison of apoptosis molecule mRNA expression in placenta between PE pregnant women with different 25-OH-VitD3 contents.

Table 4.

Comparison of apoptosis molecule mRNA expression in placenta between PE pregnant women with different 25-OH-VitD3 contents.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>AP-2</th>
<th>Smac</th>
<th>PTEN</th>
<th>XIAP</th>
<th>Survivin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE group</td>
<td>42</td>
<td>3.28±0.52</td>
<td>2.74±0.41</td>
<td>2.04±0.36</td>
<td>0.31±0.07</td>
<td>0.27±0.06</td>
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<tr>
<td>Control group</td>
<td>60</td>
<td>1.02±0.15</td>
<td>1.04±0.17</td>
<td>0.98±0.11</td>
<td>1.05±0.18</td>
<td>0.96±0.14</td>
</tr>
<tr>
<td>( t )</td>
<td></td>
<td>23.109</td>
<td>16.384</td>
<td>12.348</td>
<td>19.209</td>
<td>25.866</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>

in placental tissue of PE pregnant women with low 25-OH-VitD3 content were significantly higher than those of PE pregnant women with high 25-OH-VitD3 content while XIAP and Survivin mRNA expressions were significantly lower than those of PE pregnant women with high 25-OH-VitD3 content.

4. Discussion

Preeclampsia is a common complication during pregnancy. During the progression of preeclampsia, superficial placental implantation as well as insufficient remodeling and increased resistance of spiral artery will affect the placental blood perfusion, which will cause excessive trophoblast apoptosis in placenta and a variety of "toxic factors" to be released into the blood circulation, leading to maternal endothelial injury. Although the pathologic features of preeclampsia have been confirmed by more and more studies, the mechanism causing the above pathological features remains unclear. Vitamin D is a fat-soluble vitamin with multiple biological activities in the body. 25-OH-VitD3 is the main form of vitamin D in the body. Research in recent years has confirmed that 25-OH-VitD not only regulates calcium phosphorus metabolism, but also has the following biological activities: (1) has negative regulatory effect on the renin - angiotensin - aldosterone system, and can inhibit the vasoconstriction mediated by angiotensin so as to increase the placental blood perfusion[6]; (2) regulates the differentiation and maturation of Treg cells, induces the establishment of immune tolerance in the female fetal interface, and helps the implantation of placenta and the infiltration of trophoblast cells[7]; (3) increases the secretion of anti-inflammatory factors and inhibits the damage of inflammatory response to the placental trophoblast cells and maternal endothelial function[8,9]. In order to define the role of 25-OH-VitD in the development and change of preeclampsia, the changes of late-pregnancy serum 25-OH-VitD contents in pregnant women with preeclampsia were analyzed in the study, and the results showed that serum 25-OH-VitD3 contents of PE group were significantly lower than those of control group at 32 weeks of gestation and before delivery. This indicates that the decrease of serum 25-OH-VitD3 is closely related to the occurrence of preeclampsia.

Maternal endothelial injury is a prominent feature of preeclampsia, and the increased release of many placenta-derived "toxic factors" is the key to the maternal endothelial injury. sEng and sFlt-1 are the extracellular structures of Endoglin and Flt-1 respectively, which do not have transmembrane and intracellular structure domain, and can enter into the maternal blood circulation in the soluble forms and compete with Endoglin and PLT-1 to be combined with VEGF, PLGF, TGF- \( \beta \) 1 and other cytokines, antagonize the endothelial protective effect mediated by the above cytokines and cause endothelial damage[10-12]. IFI16 is derived from the placental trophoblasts and endothelial cells, it can directly cause the damage of endothelial cells which further release IFI16[13]. tTG is the TG family member that catalyzes the glutamine residue and lysine residues crosslinking, and hypoxia-related transcription factors HIF-1 and pro-inflammatory transcription factor NF-kB can promote the formation and release of tTG[14]. Analysis of the changes in serum contents of the above endothelial injury markers in pregnant women with pre-eclampsia in the study showed that serum sEng, sFlt-1, IFI16 and tTG contents of PE group were significantly higher than those of control group. This indicates that there is significant maternal endothelial injury in pregnant women with preeclampsia, and the release of multiple endothelial injury markers increases. Further analysis of the effect of 25-OH-VitD3 on maternal endothelial injury in pre-eclampsia showed that serum sEng, sFlt-1, IFI16 and tTG contents of PE pregnant women with low 25-OH-VitD3 content were significantly higher than those of PE pregnant women with high 25-OH-VitD3 content. This indicates that the decrease of 25-OH-VitD3 content in the serum of pregnant women with preeclampsia can increase maternal endothelial injury.

The placenta is the main source of the "toxic factors" in pre-eclampsia, and the insufficient placental blood perfusion is an important factor causing the massive release of "toxic factors". The low perfusion of placenta will not only increase the release
of "toxic factors" but also cause the apoptosis of trophoblast cells and further aggravate the placental superficial implantation and placental hypoxia[15]. AP-2 is a kind of specific DNA-binding transcription activator that can induce apoptosis by activating Bax/cytochrome C/Caspase pathway[16]; Smac is a molecule that interacts with a variety of anti-apoptotic proteins, and it antagonizes the anti-apoptotic effect of anti-apoptosis proteins by means of the related role of amino terminal so as to promote apoptosis; PTEN is a widespread tumor suppressor gene in cells, which can resist the proliferation mediated by PI3K/AKT pathway so as to inhibit proliferation and promote apoptosis. XIAP and Survivin are the main anti-apoptosis proteins in the placenta, on the one hand, their anti-apoptotic activity will reduce under the inhibition of Smac, and on the other hand, their expression will reduce under the influence of related transcription factors[17,18]. The analysis of the changes in above apoptosis molecule expression in placenta of pregnant women with preeclampsia in the study showed that AP-2, Smac and PTEN mRNA expressions in placental tissue of PE group were significantly higher than those of control group while XIAP and Survivin mRNA expressions were significantly lower than those of control group. This indicates that there is significant placental trophoblast apoptosis in pregnant women with preeclampsia. Further analysis of the influence of 25-OH-VitD on placental trophoblast apoptosis in the course of preeclampsia showed that AP-2, Smac and PTEN mRNA expressions in placental tissue of PE pregnant women with low 25-OH-VitD3 content significantly increased while XIAP and Survivin mRNA expressions significantly decreased. This indicates that the decrease of 25-OH-VitD3 in the serum of pregnant women with preeclampsia can aggravate the apoptosis of the placental trophoblast cells.

It can be concluded that serum 25-OH-VitD3 content significantly reduces in pregnant women with PE; abnormally reduced 25-OH-VitD3 can aggravate maternal endothelial injury and placental apoptosis.

References


