Exploration of serum TNF− and IL−6 change in fetuses with fetal stress and its correlation with brain damage

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ABSTRACT

Objective: To study serum TNF− and IL−6 change in fetuses with fetal stress and its correlation with brain damage. Methods: A total of 30 cases of fetuses with fetal stress were selected as pathology group, 30 cases of healthy fetuses were selected as healthy group, and serum was collected to detect the contents of inflammatory factors TNF− and IL−6 as well as nerve injury molecules S100B, NSE, CK-BB and Tau; ambulatory electroencephalography (AEEG) and brainstem auditory evoked potential (BEAP) were conducted to judge the abnormality. Results: Serum TNF−, IL−6, S100B, NSE, CK-BB and Tau contents of pathology group were significantly higher than those of healthy group; serum TNF− and IL−6 contents of pathology group fetuses with abnormal AEEG and BEAP were higher than those of fetuses with normal AEEG and BEAP, and the more severe the abnormality of AEEG and BEAP, the higher the serum TNF− and IL−6 contents; serum TNF− and IL−6 contents were positively correlated with S100B, NSE, CK-BB and Tau contents. Conclusion: Serum TNF− and IL−6 contents abnormally increase in fetuses with fetal stress, and serum TNF− and IL−6 contents have good correlation with the abnormal degree of ambulatory electroencephalography and brainstem auditory evoked potential as well as the contents of nerve injury molecules.

1. Introduction

Fetal stress is a common complication in perinatal period, fetuses are in intrauterine hypoxia environment and it causes acidosis, and about 2/3 of fetal stress will last and become neonatal asphyxia and systemic hypoxia and ischemia. Nervous system has poor tolerance to hypoxia, fetal stress-induced hypoxia will cause nerve dysfunction, and severe cases may affect the development of maturation of nervous system. At present, sensitive indicators for clinical early judgment of nerve dysfunction are still needed, and in cases of corresponding clinical symptoms or electrophysiological changes, nerve function has mostly had different degree of irreversible damage. Therefore, exploring sensitive indicators of nerve dysfunction is research emphasis of fetal stress and neonatal hypoxic-ischemic encephalopathy. Studies in recent years have shown that excessive secretion of inflammatory factors under hypoxia conditions is an important cause of nerve dysfunction. TNF− and IL−6 are two inflammatory factors closely related to nerve injury. In the following research, serum TNF− and IL−6 change in fetuses with fetal stress and its correlation with brain damage were analyzed.

2. Subjects and methods

2.1. Subjects

Puerperas treated in Obstetrics Department of our hospital from May 2012 to October 2014 were selected as research subjects, 30 cases of puerperas with fetal stress and 30 cases of puerperas with normal pregnancy were screened and enrolled in pathology group and control group of the research respectively, and inclusion criteria of subjects were as follows: (1) puerperas who received regular antenatal examination in our hospital; (2) singleton, head delivery, primipara; (3) pathology group met the criteria for fetal stress and healthy group were excluded of pregnancy complications; (4) obtaining informed consent and approval of the hospital ethic committee. Excluded subjects included twin or multiple pregnancy and malpresentation puerperas as well as those without signing

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informed consent. Pathology group were (28±2) years old with BMI (25±3) kg/m²; healthy group were (28±3) years old with BMI (25±4) kg/m².

2.2 Specimen collection methods

After fetal delivery of two groups, peripheral blood specimens were collected from the fetuses and centrifuged to get upper serum, transfer it to 1.5 mL EP tube and preserve it at -80°C.

2.3 Serum index detection methods

Serum specimens were taken, and ELISA was used to detect TNF-α, IL-6, S100B, NSE, CK-BB and Tau contents.

2.4 Ambulatory electroencephalography

AEEG from US Nicolet Company was used for examination, low-frequency filter was 0.5 Hz, high-frequency filter was 70 Hz, scalp electrodes were placed, the middle of the forehead and calvarium was as reference electrodes, 24 h EEG was recorded, and abnormal degree was judged as follows: (1) mildly abnormal: background activity slight delay of maturation, less than 20 s, and occurrence of abnormal discharge in local lesion; (2) moderately abnormal: background activity maturation delay for 20-60 s, and occurrence of continuous voltage below 25 μV; (3) severely abnormal: background activity maturation delay for more than 60 s, and occurrence of continuous voltage below 5 μV.

2.5 Brainstem auditory evoked potential

EMG-evoked potential apparatus from US Nicolet Company was used for examination, recording electrodes were placed on the forehead, reference electrodes were placed on bilateral mastoids, earth electrodes were placed on lower limbs, click with frequency 11.1 Hz and superposition 1000 times was used for stimulus, stimulus intensity started from 65 dB and changed by 10 dB, minimum sound intensity that could detect repeatable V-wave was used as V-wave reaction threshold, and abnormal degree was judged as follows: (1) mildly abnormal: V-wave reaction threshold 40-65 dB; (2) moderately abnormal: V-wave reaction threshold 65-85 dB; (3) severely abnormal: V-wave reaction threshold more than 85 dB.

2.6 Statistical methods

SPSS 21.0 was used for data input and analysis, comparison between two groups was by t test, comparison among groups was by variance analysis and differences were considered to be statistically significant at a level of P<0.05.

3. Results

3.1. Assessment of serum inflammatory factor and nerve injury molecule contents in fetuses with fetal stress and normal fetuses

Analysis results of serum inflammatory factor contents was shown in Figure 1, and details were as follows: serum TNF-α and IL-6 contents of pathology group were significantly higher than those of healthy group; analysis of serum nerve injury molecule contents was shown in Table 1, and details were as follows: serum S100B, NSE, CK-BB and Tau contents of pathology group were significantly higher than those of healthy group.

![Figure 1](image.png)

Figure 1. A was the comparison of serum TNF-α contents between fetuses with fetal stress and normal fetuses; B was the comparison of serum IL-6 contents between fetuses with fetal stress and normal fetuses.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
</table>

Comparison of serum nerve injury molecule contents between fetuses with fetal stress and normal fetuses

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>S100B (ng/mL)</th>
<th>NSE (ng/mL)</th>
<th>CK-BB (ng/mL)</th>
<th>Tau (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathology</td>
<td>30</td>
<td>1.16±0.11</td>
<td>78.4±9.1</td>
<td>108.9±12.5</td>
<td>1.59±0.16</td>
</tr>
<tr>
<td>Healthy</td>
<td>30</td>
<td>0.30±0.04</td>
<td>20.4±2.6</td>
<td>36.7±4.1</td>
<td>0.42±0.06</td>
</tr>
<tr>
<td>t</td>
<td>28.385</td>
<td>31.342</td>
<td>23.185</td>
<td>30.933</td>
<td></td>
</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>

3.2. Relationship between AEEG and serum inflammatory factor and nerve injury molecule contents

Serum TNF-α, IL-6, S100B, NSE, CK-BB and Tau contents of fetuses with different abnormal degree of AEEG were different; serum TNF-α, IL-6, S100B, NSE, CK-BB and Tau contents of fetuses with mildly abnormal, moderately abnormal and severely abnormal AEEG were higher than those of fetuses with normal AEEG; the more severe the abnormal degree of AEEG, the higher...
the serum TNF-α, IL-6, S100B, NSE, CK-BB and Tau contents.

3.3. Relationship between BEAP and serum inflammatory factor and nerve injury molecule contents

Serum TNF-α, IL-6, S100B, NSE, CK-BB and Tau contents of fetuses with different abnormal degree of BEAP were different; serum TNF-α, IL-6, S100B, NSE, CK-BB and Tau contents of fetuses with mildly abnormal, moderately abnormal and severely abnormal BEAP were higher than those of fetuses with normal BEAP; the more severe the abnormal degree of BEAP, the higher the serum TNF-α, IL-6, S100B, NSE, CK-BB and Tau contents.

Table 3
Relationship between BEAP and serum inflammatory factor and nerve injury molecule contents

<table>
<thead>
<tr>
<th>BAEP abnormality</th>
<th>TNF-α (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>S100B (ng/mL)</th>
<th>NSE (ng/mL)</th>
<th>CK-BB (ng/mL)</th>
<th>Tau (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>128.3±14.9</td>
<td>131.8±14.3</td>
<td>0.52±0.05</td>
<td>33.7±4.1</td>
<td>51.2±6.3</td>
<td>0.65±0.06</td>
</tr>
<tr>
<td>Mildly abnormal</td>
<td>218.5±25.6</td>
<td>201.3±22.5</td>
<td>0.89±0.09</td>
<td>66.2±7.3</td>
<td>85.1±9.3</td>
<td>1.08±0.12</td>
</tr>
<tr>
<td>Moderately abnormal</td>
<td>303.2±35.9</td>
<td>341.7±38.9</td>
<td>1.35±0.14</td>
<td>91.9±9.7</td>
<td>114.4±12.8</td>
<td>1.61±0.18</td>
</tr>
<tr>
<td>Severely abnormal</td>
<td>371.4±39.5</td>
<td>421.4±42.9</td>
<td>1.80±0.20</td>
<td>130.4±14.5</td>
<td>172.3±18.6</td>
<td>2.19±0.22</td>
</tr>
</tbody>
</table>

Table 2
Relationship between AECCG and serum inflammatory factor and nerve injury molecule contents

<table>
<thead>
<tr>
<th>AEEG abnormality</th>
<th>TNF-α (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>S100B (ng/mL)</th>
<th>NSE (ng/mL)</th>
<th>CK-BB (ng/mL)</th>
<th>Tau (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>133.2±15.8</td>
<td>124.5±14.1</td>
<td>0.55±0.06</td>
<td>32.5±3.8</td>
<td>50.3±6.4</td>
<td>0.62±0.07</td>
</tr>
<tr>
<td>Mildly abnormal</td>
<td>204.5±20.9</td>
<td>195.5±20.7</td>
<td>0.93±0.10</td>
<td>64.4±7.6</td>
<td>83.5±9.6</td>
<td>1.14±0.18</td>
</tr>
<tr>
<td>Moderately abnormal</td>
<td>293.4±31.6</td>
<td>336.1±41.3</td>
<td>1.25±0.15</td>
<td>93.5±10.1</td>
<td>110.4±12.8</td>
<td>1.52±0.17</td>
</tr>
<tr>
<td>Severely abnormal</td>
<td>367.5±35.9</td>
<td>435.5±52.3</td>
<td>1.77±0.18</td>
<td>127.6±13.8</td>
<td>170.9±20.5</td>
<td>2.24±0.25</td>
</tr>
</tbody>
</table>

4. Discussion

Fetal stress is the most common reason that causes neonatal asphyxia and hypoxic-ischemic encephalopathy. Brain tissue is with fast metabolism and intensive physiological activities, and although the mass of brain tissue accounts for only 2% of total body mass, its oxygen consumption accounts for more than 20% of that of body tissue. When fetal stress causes hypoxia, brain tissue is the first damaged visceral organ. The main way of current clinical judgment on brain tissue injury and nerve dysfunction is judging clinical symptoms and signs, detecting serum nerve injury molecule contents as well as carry out ambulatory electroencephalography and brainstem auditory evoked potential. Above methods can effectively assess the degree of nerve dysfunction, but when corresponding indicators are abnormal, brain tissue has mostly had different degree of injury, and intervention and treatment at this time will cause legacy of nerve dysfunction.

In recent years, more and more studies have confirmed that activation of inflammatory response and excessive secretion of inflammatory factors under hypoxic-ischemic state is one of the important causes of brain tissue injury, and the change of inflammatory factor contents is earlier than the change of nerve injury molecules and the change of electrophysiological indicators. After fetuses with fetal stress have blood-cerebrospinal fluid barrier injury, locally excessively generated inflammatory factors will be released into bloodstream. Thus it was speculated that detection of the contents of related inflammatory factors in serum could provide basis for the judgment of the degree of brain damage in fetuses with fetal stress. TNF-α and IL-6 are the most important two inflammatory factors causing nerve injury, and they are mainly synthesized and secreted by monocytes, glialocytes and neurons in brain tissue. In the research, analysis of serum TNF-α and IL-6 contents between fetuses with fetal stress and normal fetuses showed that serum TNF-α and IL-6 contents of pathology group were significantly higher than those of control group. It indicated that increased contents of TNF-α and IL-6 were associated with the occurrence of fetal stress.

TNF-α is a cytokine with multiple biological effects, it can activate NF-kB in astrocytes, on the one hand, it induces neuron apoptosis and causes nerve dysfunction through Caspase pathway, and on the other hand, it exerts synergistic effect with other inflammatory factors and pro-inflammatory factors and causes inflammatory injury to neurons. IL-6 has endogenous chemotaxis, and it can recruit a variety of inflammatory factors in brain tissue and mediate cascade amplification of inflammatory response. According to the analysis results of serum TNF-α and IL-6 contents in fetuses with fetal stress and normal fetuses, above two cytokines were involved in the occurrence of fetal stress. In order to further clarify the relationship
between increased TNF-α and IL-6 contents and nerve dysfunction, subgroup analysis was carried out to test results of ambulatory electroencephalography and brainstem auditory evoked potential, and differences in serum TNF-α and IL-6 contents among different subgroups were compared.

Ambulatory electroencephalography (AEEG) and brainstem auditory evoked potential (BAEP) are common auxiliary examination ways of assessing neonatal nerve function. AEEG can record electrical activity of brain, and assess the basic function and development status of brain tissue; BEAP can assess the function of brainstem auditory pathway and peripheral nerves. Fetal stress-induced hypoxia and ischemia will cause extensive damage of brain, and the more severe the degree of damage, the more significant the abnormal degree of AEEG and BEAP. After AEEG and BEAP examination, serum TNF-α and IL-6 contents of fetuses with normal, mildly abnormal, moderately abnormal and severely abnormal AEEG and BEAP in pathology group were compared, and results showed that serum TNF-α and IL-6 contents of fetuses with abnormal AEEG and BEAP in pathology group were higher than those of fetuses with normal AEEG and BEAP, and the more severe the abnormality of AEEG and BEAP, the higher the serum TNF-α and IL-6 contents. It indicated that serum TNF-α and IL-6 contents in fetuses with fetal stress had good correlation with the abnormal degree of AEEG and BEAP, and could accurately reflect the degree of nerve injury.

In cases of hypoxic and ischemic injury of brain tissue, neurons and glial cells rupture, and a variety of components in cells will be released into cerebrospinal fluid, and thus enter into blood circulation through blood-brain barrier. In clinical practice, detecting the contents of serum nerve cell-related molecules can judge the degree of nerve injury. S100B protein is a marker molecule located in glial cells,NSE is the catalyzing enzyme in neurons promoting glycolysis, CK-BB is the catalyzing enzyme involved in energy metabolism of nerve cells, and Tau protein is microtubule-associated protein widely expressed in nerve cells. The four above are marker molecules commonly used for reflecting nerve dysfunction, and detection of the contents of serum nerve dysfunction molecules in fetuses with fetal stress showed that serum S100B, NSE, CK-BB and Tau contents of pathology group were significantly higher than those of healthy group; the more severe the abnormality of AEEG and BEAP, the more significant the increase of serum S100B, NSE, CK-BB and Tau contents. Further analysis of the correlation between inflammatory factor contents and nerve injury molecule contents showed that serum TNF-α and IL-6 contents were positively correlated with S100B, NSE, CK-BB and Tau contents. Thus it was further confirmed that serum TNF-α and IL-6 contents in fetuses with fetal stress could accurately reflect the degree of nerve dysfunction.

Based on above discussion, it is believed that serum TNF-α and IL-6 contents abnormally increase in fetuses with fetal stress, and serum TNF-α and IL-6 contents have good correlation with abnormal degree of ambulatory electroencephalography and brainstem auditory evoked potential as well as the contents of nerve injury molecules.

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