Correlation of intestinal flora disorder with systemic inflammatory response and stress response in children with severe pneumonia

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ABSTRACT

Objective: To study the correlation of intestinal flora disorder with systemic inflammatory response and stress response in children with severe pneumonia. Methods: The children who were diagnosed with severe pneumonia in Xiangyang No. 1 People’s Hospital between April 2014 and December 2017 were selected as the pneumonia group of the study, and the healthy children who received physical examination in Xiangyang No. 1 People’s Hospital during the same period were selected as the control group. The feces was collected to determine the number of intestinal flora bifidobacteria and Escherichia coli (E. coli). Besides, the serum was collected to determine the contents of inflammatory cytokines and oxidative stress indexes, and the peripheral blood was collected to determine the expression intensity of inflammatory molecules and oxidative stress molecules. Results: The number of bifidobacteria and the level of Bifidobacterium and E. coli ratio B/E in feces as well as SOD content in serum of pneumonia group were significantly lower than those of control group whereas the number of E. coli in feces, TLR2, TLR4, NOX2, iNOS and FOXP3 expression intensity in peripheral blood as well as G-CSF, sTREM1, TNF-α, LPO and NO contents in serum were significantly higher than those of control group; Pearson correlation analysis showed that B/E level in feces of pneumonia group was negatively correlated with TLR2, TLR4, NOX2, iNOS and FOXP3 expression intensity in peripheral blood as well as G-CSF, sTREM1, TNF-α, LPO and NO contents in serum, and positively correlated with SOD content in serum. Conclusion: The intestinal flora disorder in children with severe pneumonia can aggravate the degree of systemic inflammatory response and stress response in the course of disease.

1. Introduction

Pneumonia is a common disease in pediatric respiratory system. As the children’s respiratory system and immune system development are not mature, and their resistance and removal to pathogens are weak, the pathogen infection can easily progress to severe pneumonia, which increases the mortality rate of children(1). Activation of systemic inflammation is the most prominent pathological feature of severe pneumonia, and the cascade release of inflammatory cytokines can cause multiple visera function damage and increase the occurrence risk of multiple organ dysfunction. Pulmonary pathogen infection is the initial factor of inflammation activation, but the excessive activation of systemic inflammatory response will only occur in part of the children, and lead to the occurrence of severe pneumonia and the secondary activation of stress reaction. It is still not clear at present about the mechanism of the excessive activation of systemic inflammation and the secondary activation of stress response in the process of severe pneumonia. Intestinal tract is where microorganisms gather in the body, and the activation of the inflammatory response in the course of pneumonia will destroy the balance of intestinal flora and cause the bacterial translocation into blood circulation so as to mediate the activation of systemic inflammation(2). In the following study, in order to define the role of intestinal flora disorder in the occurrence and development of severe pneumonia, we analyzed the correlation of intestinal flora disorder with systemic inflammatory response and stress response in children with severe pneumonia.
2. Clinical information and research methods

2.1 Clinical information of research subjects

The children who were diagnosed with severe pneumonia in Xiangyang No. 1 People’s Hospital between April 2014 and December 2017 were chosen as the pneumonia group of the research, and they were diagnosed with severe pneumonia by pathogen detection and imageological examination and met with one of the following conditions: (1) requiring mechanical ventilation; (2) with the increase of lung lesion scope by more than 50% within 48 hours; (3) with renal failure, daily urine volume < 400 mL or blood creatinine >177 μmol/L. Moreover, the healthy children who received physical examination in Xiangyang No. 1 People’s Hospital during the same period were chosen as the control group. There were 85 cases in the pneumonia group, including 48 males and 37 females who were 4-11 years old, and with body mass of (29.6±6.2) kg; there were 60 cases in the control group, including 33 males and 27 females who were 4-10 years old, and with body mass of (28.8±5.4) kg. There was no significant difference in general information between the two groups of children (P>0.05).

2.2 Research methods

2.2.1 Intestinal flora detection

The feces specimens were collected from pneumonia group on the same day of admission, and also collected from control group during physical examination. The bacterial DNA extraction kit was used to separate the bacterial genomic DNA from the feces, then Bifidobacterium and Escherichia coli (E. coli) 16S rDNA primer sequences were designed, PCR reaction system was configured for fluorescence quantitative PCR reaction, and the reaction curves and the wet feces weight were referred to calculate the number of bifidobacteria and E. coli.

2.2.2 Serum index detection

Serum was collected from pneumonia group before treatment, and obtained from control group during physical examination. Elisa kit was used to detect the contents of G-CSF, sTREM1, TNF-α and NO. Thiobarbituric acid chromatometry kit was used to determine the contents of LPO, and nitroblue tetrazolium kit used to determine the content of SOD.

2.2.3 Peripheral blood index detection

Peripheral anticoagulant blood was obtained from pneumonia group before treatment, and obtained from control group during physical examination. The fluorescent antibody of TLR2, TLR4, NOX2, iNOS and FOXP3 were incubated, and flow cytometer was used to measure their expression intensity.

2.3 Statistical analysis

Software SPSS 22.0 was used to input data. The differences in measurement data between groups were analyzed by t test and P<0.05 indicated statistical significance in the differences.

3. Results

3.1 The number of intestinal flora in feces

Analysis of the number of intestinal flora bifidobacteria and E. coli as well as the Bifidobacterium and E. coli ratio B/E in feces between the two groups of patients was as follows: the number of bifidobacteria and the level of Bifidobacterium and E. coli ratio B/E in feces of pneumonia group were significantly lower than those of control group whereas the number of E. coli was significantly higher than that of control group. Differences in the number of bifidobacteria and E. coli as well as the level of B/E were statistically significant between the two groups (P<0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Bifidobacteria (copy number/g wet feces)</th>
<th>E. coli (copy number/g wet feces)</th>
<th>B/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia group</td>
<td>85</td>
<td>3.27±0.52</td>
<td>5.76±0.77</td>
<td>0.411±0.064</td>
</tr>
<tr>
<td>Control group</td>
<td>60</td>
<td>7.95±0.93</td>
<td>2.09±0.42</td>
<td>2.756±0.351</td>
</tr>
</tbody>
</table>

3.2 Inflammatory molecules in peripheral blood and serum

Analysis of inflammatory molecules TLR2, TLR4, G-CSF (mg/L), sTREM1 (ng/L) and TNF-α (ng/L) in peripheral blood and serum between the two groups of patients was as follows: TLR2 and TLR4 expression intensity in peripheral blood as well as G-CSF, sTREM1 and TNF-α contents in serum of pneumonia group were significantly higher than those of control group. Analysis of the correlation between inflammatory molecules and intestinal flora B/E in pneumonia group was as follows: B/E was negatively correlated with TLR2 and TLR4 expression intensity in peripheral blood as well as G-CSF, sTREM1 and TNF-α contents in serum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>TLR2 (ng/mL)</th>
<th>TLR4 (ng/mL)</th>
<th>G-CSF (ng/mL)</th>
<th>sTREM1 (ng/mL)</th>
<th>TNF-α (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia group</td>
<td>85</td>
<td>5.48±0.73</td>
<td>9.38±1.15</td>
<td>35.2±5.5</td>
<td>67.6±8.4</td>
<td>164.8±22.3</td>
</tr>
<tr>
<td>Control group</td>
<td>60</td>
<td>2.13±0.34</td>
<td>4.37±0.55</td>
<td>10.3±1.5</td>
<td>24.1±3.6</td>
<td>42.6±6.2</td>
</tr>
</tbody>
</table>

P<0.05
3.3 Stress molecules in peripheral blood and serum

Analysis of stress molecules NOX2, iNOS, FOXP3, LPO (μmol/L), SOD (U/L) and NO (μmol/L) in peripheral blood and serum between the two groups of patients was as follows: NOX2, iNOS and FOXP3 expression intensity in peripheral blood as well as LPO and NO contents in serum of pneumonia group were significantly higher than those of control group whereas SOD content in serum was significantly lower than that of control group. Analysis of the correlation between inflammatory molecules and intestinal flora B/E in pneumonia group was as follows: B/E was negatively correlated with NOX2, iNOS and FOXP3 expression intensity in peripheral blood as well as LPO and NO contents in serum, and positively correlated with SOD content in serum.

4. Discussion

Severe pneumonia is a critical pediatric respiratory disease, whose basic pathological characteristic is the excessive activation of systemic inflammatory response. It will cause multiple viscera function damage, and serious cases may develop into multiple organ failure and increase the mortality rate. At present, the mechanism of systemic inflammatory response activation in severe pneumonia has not been fully explained. Pulmonary pathogen infection is the cause of inflammation activation in the local tissue in the course of pneumonia, but only part of the children will develop severe pneumonia and systemic inflammatory reaction activation on the basis of local inflammation, so pulmonary pathogen infection alone cannot explain the excessive activation of systemic inflammatory response in the course of severe pneumonia. The intestinal tract is where the microorganisms accumulate in the body. There are more than 1 000 species of bacteria, viruses and fungi known to be colonized in the intestinal tract, and different intestinal microorganisms are in equilibrium under physiological conditions. Bifidobacterium is an important probiotic in the intestines, which is microorganisms are in equilibrium state and has an inhibiting effect on transient bacteria and pathogen involved in the formation of intestinal mucosal biological barriers, Bifidobacterium is an important probiotic in the intestines, which is to be colonized in the intestinal tract, and different intestinal more than 1 000 species of bacteria, viruses and fungi known to be colonized in the body. There are 1 000 species of bacteria, viruses and fungi known to be colonized in the intestinal tract, and different intestinal microorganisms are in equilibrium under physiological conditions. Bifidobacterium is an important probiotic in the intestines, which is involved in the formation of intestinal mucosal biological barriers, and has an inhibiting effect on transient bacteria and pathogen reproduction, making the intestinal flora in equilibrium state.[3,4]. When the body suffers from severe infection, severe trauma and other attacks, the balance of intestinal flora will be damaged, the reproduction of probiotic Bifidobacterium is restrained, and pathogen E. coli massively reproduces and transfers into blood circulation to cause the occurrence of bacteremia and promote the excessive activation of systemic inflammation.[5,6]. In order to define the role of intestinal flora disorder in the course of severe pneumonia, the number and ratio of intestinal flora in feces of children with severe pneumonia were analyzed in the study, and the results showed that the number of bifidobacteria and the level of Bifidobacterium and E. coli ratio B/E in feces of pneumonia group were significantly lower than those of control group whereas the number of E. coli was significantly higher than that of control group. This shows that there is intestinal flora disorder in children with severe pneumonia, and the probiotic Bifidobacterium decreases while the pathogen E. coli increases.

The excessive activation of systemic inflammatory response is the basic pathological feature of severe pneumonia, and also an important pathological factor in the occurrence of multiple organ dysfunction.[7,8]. After intestinal flora is disturbed and pathogens transfer into the blood circulation, the abnormally reproduced pathogens, as the pathogen pattern molecules, can be combined with the pattern recognition receptors to mediate the inflammatory reaction activation. TLR2 and TLR4 are important pattern recognition receptors in the body, which can identify the pathogens transferring into blood circulation and initiate the downstream signal transduction pathway to activate the transcription factor NF-κB and start the expression of a variety of inflammatory genes.[9,10]. G-CSF is a pro-inflammatory glycoprotein that can promote the neutrophils and monocytes in peripheral blood to be activated and infiltrate in multiple organs so as to mediate the cascade activation of inflammation in local viscera[11]; sTREM1 is a cytokine that activates myeloid cells and participates in the triggering process of inflammatory response[12]; TNF-α is a pro-inflammatory factor that is massively secreted in early stage of mononuclear macrophage activation, and it can, on the one hand, mediate the cascade activation of inflammatory response, and on the other hand, directly damage organ tissues[13]. In the study, analysis of the change of these inflammatory molecules in children with severe pneumonia indicated that TLR2 and TLR4 expression intensity in peripheral blood as well as G-CSF, sTREM1 and TNF-α contents in serum of pneumonia group were significantly higher than those of control group. It shows that the over-activation of TLR2 and TLR4 inflammatory response pathways is related to the occurrence of severe pneumonia. Further analysis of the relationship between intestinal flora disorder and excessive inflammation activation in children with severe pneumonia showed that B/E level in feces of pneumonia group was negatively correlated with TLR2 and TLR4 expression intensity in peripheral blood as well as G-CSF, sTREM1 and TNF-α contents in serum. This indicates that the disorder of intestinal flora in children with severe pneumonia can cause the excessive activation of TLR2 and TLR4 inflammatory response pathways to mediate the activation of systemic inflammatory response in the course of disease.

The systemic inflammatory response that continues to be activated in children with severe pneumonia can further induce the activation of stress response[14,15]. Increased formation of reactive oxygen...
species is an important characteristic of stress reaction activation. NOX2-mediated electron that transfers from NADPH to oxygen molecules is the main source of reactive oxygen species in the process of severe infection, and the massively generated reactive oxygen species can on the one hand, oxidize the lipid, generate LPO and cause tissue damage, and on the other hand, consume antioxidant enzyme SOD and weaken the antioxidant capacity[16,17]. NO is the gas signal molecule that plays an important role in the process of oxidative stress, and the increased iNOS expression induced by severe infection can promote the formation of NO, then enhance oxidative stress reaction through the signal transduction of NO[18]. In addition, the excessive activation of stress response can also inhibit immune response. FOXP3 is a molecule with negative immunoregulatory effect, and the high expression of FOXP3 in the process of stress can inhibit the immune response[19,20]. In the study, analysis of the change of above stress molecules in children with severe pneumonia showed that NOX2, iNOS and FOXP3 expression intensity in peripheral blood as well as LPO and NO contents in serum of pneumonia group were significantly higher than those of control group whereas SOD content in serum was significantly lower than that of control group. This means that there is excessive activation of stress response in children with severe pneumonia; both the generation of reactive oxygen species and NO and the consumption of antioxidant enzymes significantly increase; and the expression of immunosuppression signal molecules caused by stress is also up-regulated. Further analysis of the relationship between intestinal flora disorder and excessive stress reaction activation in children with severe pneumonia showed that the B/E level in feces of children with pneumonia group was negatively correlated with NOX2, iNOS and FOXP3 expression intensity in peripheral blood as well as LPO and NO contents in serum, and positively correlated with SOD content in serum. This indicates that the disorder of intestinal flora in children with severe pneumonia can cause excessive activation of stress response.

The results of the above study show that there is intestinal flora disorder in children with severe pneumonia; the probiotic Bifidobacterium decreases and the pathogen E. coli increases; the disordered intestinal flora can aggravate the degree of systemic inflammatory response and stress response in the course of disease.

References


