Effects of Huaiqihuang Granule on immunoglobulin, T lymphocyte subsets and cytokines in children with cough variant asthma

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Objective: To observe the effect of Huaiqihuang granule on immunoglobulin, T lymphocyte subsets and cytokines in children with cough variant asthma (CVA).

Methods: 80 cases of children with CVA were enrolled in our hospital from June 2015 to June 2016, and were randomly divided into study group and control group. Two groups were both given salbutamol aerosol powder. On this basis, the control group was given Montelukast Sodium Chewable Tablets, while the study group was treated with Huaiqihuang granules. The interleukin 4 (IL-4), interferon-γ (IFN-γ), IgA, IgG and IgE levels and CD4⁺/CD8⁺ T lymphocyte ratio analyze in children after 3 months of treatment.

Results: There were no significant differences between the two groups before treatment ($P>0.05$). Both IgA and IgG were significantly higher in the two groups after treatment, while IgE was significantly lower ($P<0.05$). Compared with the control group, the IgA and IgG in the study group were significantly higher than those in the control group ($P<0.05$), while IgE was significantly lower ($P<0.05$). After treatment, compared with the control group, the proportion of CD4⁺ and CD4⁺/CD8⁺ in the study group were significantly lower than that of the control group, and the CD8⁺ ratio increased more significantly ($P<0.05$). After treatment, IL-4 decreased significantly, while IFN-γ and IFN-γ/IL-4 were significantly increased ($P<0.05$), IL-4 level of the study group decreased significantly compared with the control group, and IFN-γ increased significantly compared with the control group, and the level of IFN-γ/IL-4 after treatment was significantly higher than that of the control group ($P<0.05$).

Conclusions: Huaiqihuang granule can improve humoral immunity and cellular immunity of children with CVA, and can adjust the immune cells balance of Th1/Th2, and improve the immune function and prognosis of children with CVA.

1. Introduction

Cough variant asthma (CVA) is a common clinical pediatric chronic cough, atypical asthma, and diagnosis and treatment not in time can be developed for the typical asthma. At present, CVA treatment mainly by leukotriene receptor antagonists, and its role in reducing the high rate of CVA recurrence is weak[1]. Huaiqihuang granule is a kind of fungus medicine composed of trametes versicolor matter, medlar and polygonatum, which contains Huaiier polysaccharide protein has a strong biological regulation function, lycium barbarum polysaccharides can enhance the body immunity, and studies have shown that Huaiqihuang particles can reduce respiratory infections[2]. In order to further understand the mechanism of Huaiqihuang Granule in treating CVA, we analyzed the effect of Huaiqihuang granule on the immune function of children with CVA, and the results are reported as follows.

2. Materials and methods

2.1. General Information

80 cases of children with CVA were selected from June 2015 to June 2016 in our hospital, All children were diagnosed according
to CVA diagnostic criteria[3], and were treated for the first time or stop treatment for more than 3 months of recurrence treatment, randomly divided into two groups of 40 cases. The study group with 26 males and 14 females, aged 2–8 years, duration of 2–12 months, the control group with 25 males and 15 females, aged 2–9 years, duration of 1–13 months. Excluding acute respiratory infection, other causes of chronic cough, combined with cardiovascular and cerebrovascular diseases, liver and kidney dysfunction in children, excluding drug allergy, the recent use of immune function drugs and those who failed to comply with the instructions, all the guardians of the children signed informed consent. There was no significant difference in age, sex ratio and course of disease between the two groups. 

2.2. Methods

All patients were given salbutamol sulfate aerosol, 0.2 mg/time, 4 times/d. On the basis, children in the control group were given montelukast sodium chewable tablets according to age, 2–5 years old children 1 times/d, 4 mg/times, 6–9 years old children 1 times/d, 5 mg/times, bedtime meal. The children in the study group on the basis of age given salbutamol sulfate Huaqihuang granules, 2–3 years 2 times/d, 5 g/times, 3–9 years 2 times/d, 10 g/times. All children were treated for 3 months, before treatment, the guardians of children should be given the right usage and dosage of the model drug, and told them not to switch to other drugs, to ensure that all children are prescribed medication to take drugs.

2.3. Detection indicators and methods

Before and after treatment, respectively, 10 mL fasting peripheral venous blood of children was collected in the morning and placed in heparin anticoagulant tube (1:20 U). Using double antibody sandwich enzyme linked immunosorbent assay (ELISA) to detect the level of interleukin 4 (IL-4) and interferon-γ (IFN-γ), and immunoglobulin IgA, IgG and IgE were detected by immuno-turbidimetry. CD4+ and CD8+ T cells were detected by flow cytometry, and the proportion of CD4+/CD8+ T cells was calculated.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>IgA (g/L)</th>
<th>IgG (g/L)</th>
<th>IgE (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The study group</td>
<td>Before treatment</td>
<td>0.91±0.24</td>
<td>7.98±1.24</td>
<td>305.14±47.05</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>1.35±0.48</td>
<td>10.81±2.57</td>
<td>143.67±31.48</td>
</tr>
<tr>
<td>The control group</td>
<td>Before treatment</td>
<td>0.92±0.27</td>
<td>8.05±1.35</td>
<td>309.07±49.23</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>1.12±0.36</td>
<td>9.13±2.13</td>
<td>179.98±43.54</td>
</tr>
</tbody>
</table>

* compared with before treatment, P<0.05; † compared with the control group after treatment, P<0.05.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>CD4+</th>
<th>CD8+</th>
<th>CD4+/CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>The study group</td>
<td>Before treatment</td>
<td>48.07±5.02</td>
<td>20.89±3.12</td>
<td>2.17±0.42</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>41.43±4.56</td>
<td>25.59±3.99</td>
<td>1.58±0.47</td>
</tr>
<tr>
<td>The control group</td>
<td>Before treatment</td>
<td>47.75±4.29</td>
<td>21.17±2.82</td>
<td>2.25±0.51</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>45.76±3.69</td>
<td>21.12±3.04</td>
<td>2.48±0.64</td>
</tr>
</tbody>
</table>

* compared with before treatment, P<0.05; † compared with the control group after treatment, P<0.05.

2.4. Statistical analysis

All the data were analyzed by SPSS21.0 software, t test was used to compare the measurement data, the difference was statistically significant when P<0.05.

3. Results

3.1. Changes of immunoglobulin IgA, IgG and IgE levels in two groups before and after treatment

Before treatment, there was no significant difference in the levels of immunoglobulin IgA, IgG and IgE between the two groups (P>0.05). Both IgA and IgG were significantly higher in the two groups after treatment, while IgE was significantly lower (P<0.05). IgA and IgG of the study group were significantly higher than the control group, while IgE was significantly decreased compared with the control group (P<0.05) (Table 1).

3.2. Changes of the proportion of T cell subsets in two groups before and after treatment

There was no significant difference in the CD4+, CD8+ and CD4+/CD8+ ratio between the two groups before and after treatment (P>0.05). After treatment, CD4+ of the two groups decreased significantly (P<0.05), CD8+ of the study group increased significantly, and CD4+/CD8+ decreased significantly (P<0.05). In the control group CD8+ decreased slightly, and CD4+/CD8+ increased slightly, and there was no statistically significant difference compared with before treatment (P>0.05); Compared with the control group, the CD4+ and CD4+/CD8+ of study group were lower than those in the control group, however, CD8+ was significantly increased compared with the control group (P<0.05) (Table 2).
3.3. Changes of cytokines IL-4 and IFN-γ levels of two groups children before and after treatment

The levels of IL-4, IFN-γ and IFN-γ/IL-4 in the two groups before treatment were not significantly different (P>0.05). The IL-4 levels of the two groups decreased significantly after treatment, while IFN-γ and IFN-γ/IL-4 levels increased significantly (P<0.05). The IL-4 level of study group was significantly decreased compared with the control group, while IFN-γ and IFN-γ/IL-4 levels were significantly higher than the control group (Table 3).

4. Discussion

CVA, also known as allergic asthma, would be induced and aggravated by climate mutations, exercise or upper respiratory tract infection, and clinical manifestations mainly are severe dry cough and shortness of breath[4,5]. The pathogenesis of CVA is complex, and modern medical research suggest that its pathogenesis is mainly composed of a variety of immune cells involved in chronic airway inflammation and cause airway hyperresponsiveness, which can cause severe airway obstruction. Studies show that about 30% of children with CVA can be changed from atypical asthma to typical asthma[6], so timely diagnosis and treatment of CVA are effective in preventing airway injury in children. The current clinical treatment of CVA drugs are glucocorticoids, bronchodilators, theophylline drugs and leukotriene receptor antagonist[7], while long-term glucocorticoid is not conducive to the physical and mental health of young children[8]. Experimental study shows that theophylline sustained-release tablets CVA treatment adverse reaction rate is as high as 18.42%, while the combination of proprietary Chinese medicine preparation of cold Chuba particles can significantly reduce the adverse reaction to 5.26% (P<0.05).

Huaqihuang granule is a kind of fungus medicine composed of trametes versicolor matter, medlar and polygonatum, huai Q bacteria as monarch drug, the remaining two taste as adjuvant drug, Huai Qi, medlar Ziyan, Huang Qi and Yin tonifying, three drugs are combined, invigorating qi and nourishing Yin without greasy, suitable for repeated the respiratory tract infection of Qi Yin deficiency in children with cough and asthma[8,10]. Huai Qi bacteria contained in the huaiyl polysaccharides protein has strong biological regulation, Lycium barbarum polysaccharides can enhance immunity, and studies have shown that Huaqihuang particles can reduce respiratory infections, and Sun et al.[11] by comparison found that Huai Qi Yellow granules in children with recurrent respiratory tract infection, and the recurrence rate of children with respiratory tract infection can be reduced from 96.42% to 31.03%. In this paper, the preliminary analysis of Huaqihuang particles on CVA in children with immune function, reduce the role of CVA recurrence remains to be further follow-up.

Recent studies have shown that mycoplasma pneumoniae is one of main the cause closely related to CVA, Which is also one of the main reasons caused long-term move CVA , Mycoplasma pneumoniae-dependent PI protein adhesion to the surface of the body cells, but also absorb nutrients and excrete metabolism, and induce immune cells to participate in the formation of airway hyperresponsiveness[12,13]. In addition, mycoplasma pneumoniae can also induce the secretion of immunoglobulin IgE, which combined with basophil receptors to cause hypersensitivity and further strengthen airway hyperresponsiveness[14].

T cell subsets have a role in regulation of immunoglobulin level, CD4+ and CD8+ are auxiliary effector T cells (Th) and cytotoxic T cells (Tc), and the former combined with peptide-MHC-II complex to secrete cytokines to promote the differentiation of B cells into plasma cells then secrete antibody, the latter combined with antigen peptide-MHC-I through the release of pore forming, particle enzyme or activated FasL pathway to kill the infected cells; CD4+CD8+ as an immune regulation index can effectively reflect the immune state of the body[15]. T cells differentiate into CD4 and CD8. CD8 is cytotoxic cells, kill the damaged cells, CD4 secreting cytokines to activate macrophages and NK cells killing the damaged cells, which is the two pathway of T cell differentiation of immune function. In general, the CD4+/CD8+ ratio was maintained between 1.4~2, the CD4+/CD8+ of CVA in this study was greater than 2, which showed that the body's immune function disorder. A large number of clinical studies show that the IgE level in CVA patients was significantly higher than that in healthy children, and positively correlated with CD4+, CD4+/CD8+, negatively correlated with CD8+[16], and the incidence of asthma is closely related to the imbalance of Th1/Th2, and the decreased expression of CD4+CD25Foxp3+Treg can induce Th1 cytokine decreased secretion, Th2 type cytokine increased secretion, leading to the imbalance of Th1/Th2[17,18]. IFN-γ and IL-4 are the characteristic factors of Th1 cells and Th2 cells, which are involved in the process of airway inflammation in children with CVA. The increase of IL-4 secretion can induce asthma, and the
ratio can effectively respond to Th1/Th2 cell immune status, thus effectively regulate IgE, T cell subsets and cytokine levels, which is helpful to correct the imbalance of Th1/Th2 and contributes to the treatment and prognosis of CVA.

This study shows that taking with Huaiqihuang granules after 3 months after treatment, the level of IgE of the study group was reduced, showing that Huaqiuhuang granules significantly reduce children with airway hyperresponsiveness, and found that the IgE, CD4+ and CD4+/CD8+ after treatment all decreased, while CD8+ increased, consistent with the reported[16]. The results of the study showed that compared with the control group, the proportion of CD4+ and CD4+/CD8+ in the study group after treatment decreased more significantly, the ratio of CD8+ were significantly higher (P<0.05), the IL-4 level in study group was significantly decreased, IFN-γ and IFN-γ/IL-4 significantly increased (P<0.05), and the IFN-γ/IL-4 level was significantly higher than the control group (P<0.05). This may be due to fungal substance containing PS-T Huaqiuhuang granules in promoting phagocytosis, enhancing the activity of lysozyme, promoting the proliferation and differentiation of T cells, and increasing the Tregs cell expression, to correct the imbalance of Th1/Th2. On the other hand, the polysaccharide contained in the polysaccharide further stimulated T cells, promoting the expression of CD25 and inducing the gene transcription of IFN-γ cytokine gene[19-21].

In summary, Huaqiuhuang granule can effectively reduce the levels of IgE and IL-4 in children with CVA, adjust the balance of Th1/Th2 cellular immunity, and contribute to the prognosis of CVA. CVA recurrence in children in this study remains to be followed up.

References


