MEDICAL SCIENCE MONITOR

Correlations of Inflammatory Factors with Intestinal Flora and Gastrointestinal Incommensurate Symptoms in Children with Asthma

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Background: Bronchial asthma is a common pediatric disease, the pathogenesis of which is complicated. The correlations of the levels of inflammatory factors in peripheral serum with intestinal flora and gastrointestinal incommensurate symptoms in children with asthma remain to be further elucidated.

Material/Methods: A total of 70 children diagnosed with asthma in the Pediatric Department of our hospital from February 2016 to March 2017 were enrolled as an observation group, and another 25 healthy children in the same age range were selected as a control group. The levels of inflammatory factors [C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6)], the total load of intestinal flora, and the main strains were detected among all included patients. Moreover, incommensurate symptoms of patients in the observation group were recorded and gastrointestinal symptom rating scale (GSRS) scores were calculated. The differences in indexes between the observation group and the control group were compared.

Results: The levels of CRP, TNF-α, and IL-6 in peripheral serum in the observation group were significantly higher than those in the control group (p<0.05). The analysis of the correlations of inflammatory factors in peripheral serum with intestinal flora and GSRS scores showed that C-reactive protein (CRP) was positively correlated with GSRS scores (r=0.696, p<0.001) and the total load of intestinal bacteria (r=0.813, p<0.001).

Conclusions: The inflammatory factors in peripheral serum of children with asthma are closely correlated with intestinal flora and gastrointestinal function. With the increasingly high levels of inflammatory factors in peripheral serum, the probability of intestinal flora disturbance and gastrointestinal incommensurate symptoms will be increased.

MeSH Keywords: Asthma • Inflammatory Bowel Diseases • Only Child

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Background

Bronchial asthma is a common pediatric disease and factors such as interferon-gamma (IFN-γ) and interleukin (IL) play vital roles in the pathogenetic process [1]. The pathogenesis of pediatric bronchial asthma is very complicated. The main clinical manifestations include repeated coughing and wheezing, which are dangerous symptoms that often mimic dyspnea. Pediatric bronchial asthma is primarily characterized by airway overreaction and obvious airway obstruction [2]. A study has shown that overreaction of the inflammatory system plays an important role in the occurrence and development of asthma [3]. Asthma is an immunoreactivity disease mainly caused by imbalance of the proportion of T helper type 1 (Th1)/T helper type 2 (Th2) cytokines and the dysfunction [4]. Common manifestations are decreased levels of Th1 cells (such as IFN-γ and IL-2) and increased levels of Th2 cells (such as IL-3 and IL-4), as well as the hyperfunction of Th2 cells [5]. Chronic airway inflammatory reaction is more likely to occur when Th2 cytokines are at a high level and become overactive, which eventually results in airway overreaction and thus causes asthma [6]. Patients with bronchial asthma usually also have long-term repeated bacterial or viral infections, often manifested as allergy [7]. In recent years, research has found that asthma is correlated with the decrease of intestinal probiotics, in which *Bifidobacterium* and *Lactobacillus* are the most representative. There is a low risk that children with normal intestinal flora will develop high levels of T cell factors and hyperfunction [7]. When intestinal flora disturbance occurs, patients are more likely to suffer from a series of incommensurate symptoms such as abdominal pain and distention, constipation, and diarrhea [8]. Therefore, the correlations of inflammatory factors with intestinal flora in children with asthma were analyzed in this study in order to provide new ideas for the prevention and treatment of asthma in children and to improve the prognosis.

Material and Methods

General data

A total of 70 children diagnosed with asthma in the Pediatric Department of our hospital from February 2016 to March 2017 were selected as the observation group, while another 25 healthy children in the same age range were enrolled as the control group. There were 33 males and 60 females, aged 6–14 years old, with an average age of 8.79±2.83 years old. All included patients in the observation group conformed to the Conventional Diagnostic Criteria for the Prevention and Treatment of Bronchial Asthma in Children revised in 2014 by the Prevention and Treatment Group of Pediatric Departments in China. Exclusion criteria were: patients with multiple system organ failure, patients with other severe infections or autoimmune diseases, patients having taken antibiotics and intestinal microecologics before diagnosis, patients with family history of allergic diseases, patients with mental diseases, and patients with incomplete clinical data or who refused to sign the informed consent. The study was reviewed and approved by the Ethics Committee of our hospital and all patients who participated in the study signed the informed consent.

Methods

The clinical data of all patients, including age, sex, height, and weight, were analyzed retrospectively. After 10 h of solid and liquid fasting, fasting peripheral blood was extracted from all the included patients. The upper-layer serum was taken to detect the relevant biochemical indexes. The levels of total cholesterol (TC) and triglyceride (TG) were detected with an automatic biochemical analyzer provided by Hitachi, and the levels of inflammatory factors [C-reactive protein (CRP), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)] were detected via immunoturbidimetry.

Detection of the total load of intestinal bacteria and main strains was conducted. The stools in observation group and control group were collected, and 350 g of middle stools were selected as the final sample. The sample was detected via SYBR GREEN I fluorescence quantitative polymerase chain reaction (qPCR). We assessed the total load of all bacteria and the load of the main bacterial species in stools, including the 2 probiotics (*Bifidobacterium* and *Lactobacillus*) and the harmful bacteria (such as *Escherichia coli*, *Helicobacter pylori*, *Streptococcus*, *Staphylococcus,* and *Pseudomonas aeruginosa*).

Data collection of gastrointestinal incommensurate symptoms and gastrointestinal symptom rating scale (GSRS) scores was performed, including: symptoms of abdominal pain (abdominal pain, nausea, vomiting), symptoms of regurgitation (heartburn, belching, sour regurgitation), symptoms of diarrhea (loose stools, fecal incontinence, fecal urgency), symptoms of dyspepsia (borborygmus, abdominal distension, an increase in flatus), and symptoms of constipation (infrequent bowel movement, fecaloma, unfinished feeling of defecation). The score for each symptom ranged from 0 to 3 points. The scores of no symptoms, mild symptoms, moderate symptoms, and severe symptoms were 0 points, 1 point, 2 points, and 3 points, respectively. The total score of the symptoms ranged from 0 to 15 points.

Statistical methods

Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA) was used for data processing. The collected data are presented as ($\bar{x}\pm s$). The chi-square test was used for the comparison of enumeration data. Correlation analysis

Table 1. Comparisons of general data between observation group and control group.

General data	Observation group (n=70)	Control group (n=25)	p Value
Age (years old)	9.03±2.01	8.12±2.13	0.068
Gender (Male/Female)	25/45	8/15	0.710
BMI (kg/m²)	17.85±2.18	18.04±1.95	0.065
TC (mmol/L)	5.69±1.10	4.99±1.03	0.063
TG (mmol/L)	1.85±1.03	1.86±0.59	0.793

Table 2. Comparisons of the levels of inflammatory factors between observation group and control group.

Relevant index	Observation group (n=70)	Control group (n=25)	p Value
CPR (mg/L)	5.59±3.21	1.32±1.10	0.001
TNF-α (ng/L)	5.19±2.38	3.18±1.93	0.001
IL-6 (ng/L)	4.45±2.19	1.58±1.03	0.001

Table 3. Comparisons of the total load of bacteria and the load of main strains between observation group and control group.

Load of bacteria	Observation group (n=70)	Control group (n=25)	p Value
Total load of bacteria	12.12	7.81	0.001
Bifidobacterium	2.99	6.57	0.000
Lactobacillus	1.27	5.39	0.001
Escherichia coli	8.94	5.38	0.019
Helicobacter pylori	9.39	6.56	0.001
Streptococcus	8.11	3.87	0.001
Staphylococcus	6.51	3.12	0.039
Pseudomonas aeruginosa	5.70	6.19	0.067

was used for the difference between 2 factors. $p<0.05$ suggested that the difference was statistically significant.

Results

Comparisons of general data between observation group and control group

There were no statistically significant differences in age, sex, BMI, TG level, or TC levels between the observation group and control group ($p>0.05$), and the data were comparable (Table 1).

Comparisons of the levels of inflammatory factors between observation group and control group

The levels of CRP, TNF-α, and IL-6 in peripheral serum in the observation group were significantly higher than those in the control group ($p<0.05$) (Table 2).

Comparisons of the total load of bacteria and the load of main strains between observation group and control group

The total load of bacteria in the observation group was significantly increased compared to that in the control group. The levels of the 2 probiotics (*Bifidobacterium* and *Lactobacillus*) in the observation group were significantly reduced compared to those in the control group, while the levels of harmful bacteria (*Escherichia coli, Helicobacter pylori, Streptococcus,* and *Staphylococcus*) in the observation group were significantly up-regulated compared with those in the control group ($p<0.05$) (Table 3).

Proportions of gastrointestinal symptoms of patients in observation group

The proportion of patients in the observation group suffering from gastrointestinal incommensurate symptoms was as high as 91.43%. Dyspepsia accounted for the largest proportion (57.14%), followed by the symptoms of abdominal pain

Table 4. Proportions of gastrointestinal symptoms of patients in observation group.

Relevant symptom	n (70)	%
Gastrointestinal incommensurate symptoms	64	91.43
Symptoms of abdominal pain	29	45.31
Symptoms of regurgitation	18	25.71
Symptoms of diarrhea	25	35.71
Symptoms of dyspepsia	40	57.14
Symptoms of dyspepsia	27	38.57

Table 5. Proportions of GSRS scores in observation group.

GSRS score (point)	n	%
0–5	8	11.43
6–10	47	67.14
11–15	15	21.43
Total	70	100.00

(45.31%). Other incommensurate symptoms included regurgitation, diarrhea, and constipation (Table 4).

Proportions of GSRS scores in observation group

In the observation group, patients with GSRS scores of 0–5 points, 6–10 points, and 11–15 points accounted for 11.43%, 67.14%, and 21.43%, respectively. The majority of patients had scores of 6–10 points (Table 5).

Correlation of inflammatory factors in peripheral serum with intestinal flora and GSRS scores

The results showed that CRP was positively correlated with the GSRS scores ($r=0.696$, $p<0.001$) and the total load of intestinal bacteria ($r=0.813$, $p<0.001$) (Figures 1, 2).

Discussion

At present, the pathogenesis of asthma in children is unclear, and it is generally considered that asthma in children is correlated with genetic factors, acute respiratory infections, mental factors, and unstable environment [9]. Bronchial asthma is a chronic respiratory inflammatory disease, mainly characterized by obvious airway overreaction, also referred to as hyperreactivity [10]. During the occurrence and development of bronchial asthma, the most important pathogenic factors are the
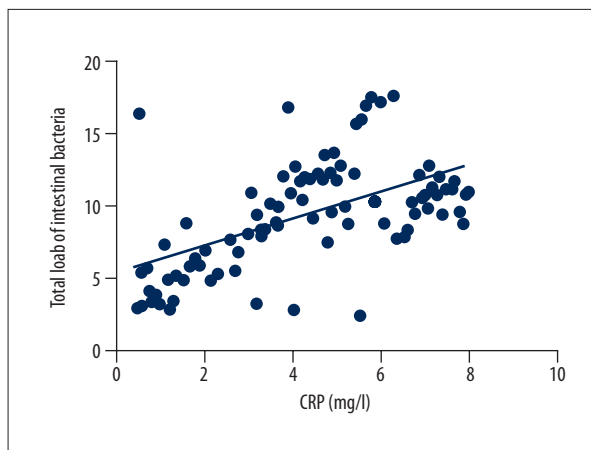

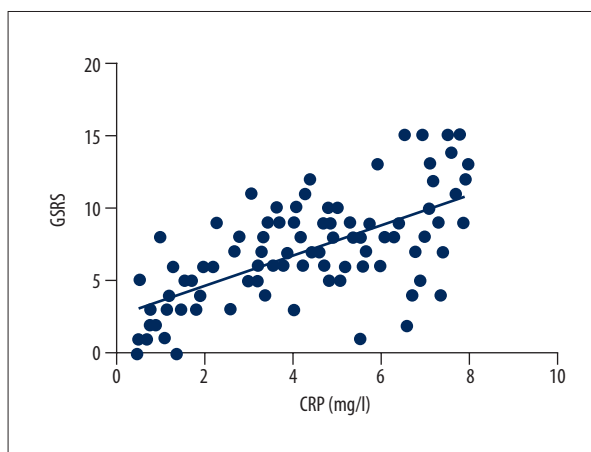**Figure 1.** Correlation of CRP with the total load of intestinal bacteria.

Figure 2. Correlation of CRP with GSRS scores.

imbalance in the levels of Th1/Th2 cytokines and dysfunction. Th1 cytokines exert an anti-infection function and Th2 cytokines promote the synthesis of B cytokines and the release of immunoglobulin [11]. During the occurrence and development of bronchial asthma, with the decrease in the level of Th1 cells and the increase of Th2 cells, the synthesis and secretion of immunoglobulin E (IgE) are enhanced and the levels of IL-5 and IL-8 are also elevated. The rise of interleukin levels results in the accumulation of eosinophils in the respiratory tract. Thus, the occurrence and development of bronchial asthma are caused or aggravated by the above factors to different degrees [12,13]. As bronchial asthma is a chronic respiratory inflammatory disease, inflammatory reaction plays a major role in pediatric asthma [14]. CRP is an acute inflammatory reactive protein, and its synthesis and release result from the stimulation of cells and epithelins via lymphokines. CRP levels in patients with asthma are ubiquitously increased slightly or moderately and are significantly increased when bronchial asthma is combined with acute infection [15]. As the immune function of normal children is weaker than that

of adults, the reaction of children with asthma to infection is thus relatively inconspicuous [16]. During asthma attacks in children, Th16 cytokines launch the synthesis of several pro-inflammatory cytokines, which cause epithelial cells from the respiratory tract to induce the expression of cytokines such as TNF-α and IL-6. These cytokines result in the increase in the number of smooth muscle cells, thus aggravating the airway inflammatory reaction [17]. We found in this study that the levels of inflammatory factors in peripheral serum were significantly higher than those in healthy children through physical examination, which is consistent with the above conclusion.

Exposure to microorganisms at an early age can affect immune function in children and alter various immune cytokines. The self-receptor repertoire and non-self-receptor repertoire are further changed correspondingly [15]. During the entire growth process from a newborn to a child, with the changes of the living environment, the intestinal flora has been changing all the time but remains in a state of homeostasis [18]. At a very early stage, some facultative anaerobes such as *Escherichia coli* are found in the intestinal tract firstly, and then some obligate anaerobes like *Bifidobacterium* are gradually observed at the late stage [19]. Research has shown that the increase of probiotics in the intestinal tracts can alleviate the inflammatory reaction to different extents, decrease the levels of inflammatory factors in peripheral serum, reduce hypersensitivity of the intestinal tract, and enhance the protective function of the gastrointestinal tract, which finally

lowers the incidence rate of gastrointestinal incommensurate symptoms [1]. In children with asthma, the airway inflammatory reaction can result in intestinal flora disturbance, and then a series of gastrointestinal incommensurate symptoms occur [18]. The present study revealed that the total load of intestinal bacteria in children with asthma was higher than that in healthy children and the level of probiotics was lower than in healthy children. Moreover, the total load of intestinal bacteria was positively correlated with GSRS scores and inflammatory factors.

Conclusions

These findings suggest that with the increasingly high levels of inflammatory factors in the peripheral serum of children with asthma, the risk of intestinal flora disturbance and gastrointestinal incommensurate symptoms is increased. The inflammatory factors in peripheral serum of children with asthma are closely correlated with intestinal flora and gastrointestinal incommensurate symptoms. This study indicates that the treatment of intestinal flora disturbance and the decrease in the level of inflammatory factors can play roles in preventing, improving, and treating pediatric asthma.

Conflict of interest

None.

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