

Correlation between intestinal flora and serum inflammatory factors in patients with Crohn's disease

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Abstract. – **OBJECTIVE:** We investigated the correlation between intestinal flora and serum inflammatory factors in patients with Crohn's disease.

PATIENTS AND METHODS: From February 2014 to June 2016, 132 patients with Crohn's were enrolled in this study. There were 84 males and 48 females. The age range was from 28 to 72 years. We had 62 patients in active stage (the activity group) and 70 patients in remission stage (the remission group). We also enrolled 71 healthy cases in the control group. The expression levels of serum inflammatory factors including IL-6, IL-17, IL-22, and IL-33 were measured using ELISA. Fresh feces samples were diluted and, after cultivating the bacteria for 48 hours at 37°C, the number of colonies was counted. The number of flora per gram of feces (CFU/g) was determined.

RESULTS: The number of *Escherichia coli* and *Enterococcus sp.* in feces was significantly higher in the activity group compared to that of the control group and the remission group. The levels IL-1, IL-17, IL-22, and IL-33 in the activity group were significantly higher than those of other groups. The number of *Escherichia coli* and *Enterococcus sp.* was positively correlated with the levels of IL-6, IL-17, IL-22, and IL-33, while the number of *Bifidobacteria* and *Bacillus lactic acid* was negatively correlated with the levels of IL-6, IL-17, IL-22, and IL-33.

CONCLUSIONS: The number of conditional pathogenic bacteria in the activity group, was higher than other groups, while the number of probiotics bacteria decreased distinctly. We concluded that monitoring the changes in distribution and composition of intestinal flora as well as the levels of blood inflammatory factors could play a significant role in the treatment process of Crohn's disease.

Key Words:

Crohn's disease, Intestinal flora, Inflammatory factor.

Introduction

As a chronic inflammatory granuloma disease of the gastrointestinal tract, the etiology of Crohn's disease is still unclear¹. The location of the lesions is usually at terminal ileum and adjacent colon, but each section of the digestive tract from mouth to anus, all could be affected². The etiology of Crohn's disease is unidentified. Changes in intestinal flora and inflammatory factors play an important role in the pathogenesis of Crohn's disease³. In this work, intestinal flora and the levels of inflammatory factors in patients with Crohn's disease were studied, and the correlation between them was analyzed.

Patients and Methods

Patients

From February 2014 to June 2016, 132 patients with Crohn's, were enrolled in this study. There were 84 males and 48 females. The age range was from 28 to 72 years (average = 38.5±12.3). 62 patients were in active stage (the activity group), and 70 patients were in remission stage (the remission group). We also enrolled 71 healthy cases in the control group. This study was approved by the Ethics Committee of The First Affiliated Hospital of Guangdong Pharmaceutical University. Signed written informed consents were obtained from all participants before the study.

Inclusion Criteria

All the patients were consistent with the diagnosis guideline made by the inflammatory bowel disease cooperative group of a Digestive Branch of Chinese Medical Association², as well as the American and European guidelines^{4,5}.

Table I. General clinical condition.

Groups	Cases	Age	Gender ratio (Male/Female)	BMI
Activity group	62	38.8±23.4	45/17	21.3±2.2
Remission group	70	39.4±21.8	39/31	22.4±3.2
Health control	71	38.3±29.2	34/37	20.6±3.3
T/X2 value	-	0.43	0.58	0.33
p-value	-	0.68	0.57	0.69

Table II. Number of intestinal flora (CFU/g, $\bar{x}\pm s$).

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Exclusion Criteria

(1) Patients with cardiovascular and cerebrovascular disease; (2) Patients with tumors of each system, (3) Patients with autoimmune and inflammatory diseases, and (4) Patients who took probiotic preparations, antibiotics, and other drugs within four weeks before the examination date.

Serum Inflammatory Factors

The expression levels of serum inflammatory factors including IL-6, IL-17, IL-22, and IL-33 were measured using ELISA. All reagents used in this study were purchased from Wuhan Boshide Biological Engineering Co., Ltd. and we followed the instructions provided by the manufacturer.

Intestinal Flora

Fresh feces samples (0.5 g) were collected and diluted (10-8). 10 μ l of diluted sample was taken for inoculation. For *Escherichia coli* and *Enterococcus sp.* cultures we used aerobic medium, and for *Bacillus lactic acid* and *Bifidobacteria* we used the anaerobic medium. After cultivating the bacteria for 48 hours in 37°C, the number of colonies was counted. We then stained (gram stain) the bacteria and conducted biochemical identification. The number of bacteria per gram of feces (CFU/g) was determined.

Statistical Analysis

We used SPSS17.0 software (SPSS Inc., Chicago, IL, USA) for our statistical analysis. All measurement data were expressed as mean±standard

deviation. The variance analysis of repeated measurement data was used for statistical treatment. For comparison between groups, we used the t-test. The paired t-test was used for comparison in each group. The chi-square test was used for enumeration data. $p<0.05$ meant the difference was statistically significant.

Results

The age, gender ratio, BMI and other indexes of subjects were compared, with no statistically significant difference among the groups ($p>0.05$) (Table I). The number of *Escherichia coli* and *Enterococcus sp.* in feces was significantly higher in the activity group compared to that of the control group and the remission group. The difference was statistically significant ($p<0.05$). No significant difference was detected between the control group and the remission group (Table II).

The levels IL-1, IL-17, IL-22, and IL-33 in the activity group were significantly higher than those of the control group and the remission group. The difference was statistically significant ($p<0.05$). No significant difference was detected between the control group and the remission group ($p>0.05$) (Table III).

The number of *Escherichia coli* and *Enterococcus sp.* was positively correlated with the levels of IL-6, IL-17, IL-22, and IL-33, while the number of *Bifidobacteria* and *Bacillus lactic acid*

Table IV. Correlation between intestinal flora and inflammatory factors.

Groups	IL-1	IL-17	IL-22	IL-33
<i>Escherichia coli</i>	0.33	0.48	0.69	0.17
<i>Enterococcus</i>	0.24	0.35	0.77	0.55
<i>Bifidobacteria</i>	-0.23	-0.34	-1.06	-1.27
<i>Bacillus lactic acid</i>	-0.33	-0.48	-0.49	-0.33

was negatively correlated with the levels of IL-6, IL-17, IL-22, and IL-33 ($p < 0.05$) (Table IV).

Discussion

The bacterial flora in human digestive tract can reach more than 500 species. They are mostly concentrated in the colon and small intestine. Under physiological conditions, these bacteria are divided into three categories, including physiological bacteria, conditional pathogenic bacteria and pathogenic bacteria⁶⁻⁸. However, due to the effect of maintaining normal function of host gastrointestinal tract, the physiological bacteria and conditional pathogenic bacteria belong to the intestinal autochthonous flora⁹. In some pathological conditions, such as Crohn's disease, the abnormal distribution of intestinal autochthonous flora could trigger the release of inflammatory factors¹⁰.

Prior studies showed that in patients with inflammatory bowel disease in the active stage, the balance of *Bifidobacteria* and *Bacillus lactic acid* was broken. Other reports suggested that in patients with inflammatory bowel disease, the number of *Clostridium sp.* declined significantly in the intestinal flora while the number of *Escherichia coli* increased distinctly¹¹. Patients with Crohn's disease often suffer from mucosal fissuring ulcers, which affects all layers of intestinal wall, stiffen the intestinal tube, and eventually cause intestinal obstruction¹². Damage to the intestinal tract, triggers the release of a large number of various inflammatory factors, which changes local micro-environment and affects intestinal pH. These changes alter the intestinal micro-environment and put the pathogenic bacteria in an advantageous position¹³⁻¹⁶. Among the inflammatory factors, IL-1, IL-17, IL-22, and IL-33 are the most common factors¹⁷. IL-1 plays an important role in the inflammatory process of Crohn's disease by inducing the expression of cyclooxygenase-2, phospholipase A2, nitric oxide synthase, interferon and adhesion molecule¹⁸.

IL-17 is largely secreted by CD4⁺ T cells, and promotes the generation of epithelial cells, endothelial cells, and fibroblasts. The release IL-17 may accelerate the formation of the fibrotic scar at the damaged part of intestinal tissues and destroy the structure of intestinal canal¹⁹. IL-33 can activate NF- κ B and MAPK, promote the production of Th2 cytokines, participate in the allergic reaction, and also can interact with IL-6 to promote the micro-thrombus in intestinal microcirculation²⁰. The joint action of the above-mentioned inflammatory factors can disturb the intestinal environment and further affects the distribution of intestinal flora²¹. In this study, we discovered that the levels of inflammatory factors including IL-1, IL-17, IL-22, and IL-33 in the activity group were significantly higher than those in the remission group and the health control group. Prior studies showed^{22,23} that the level of IL-22 in patients with Crohn's disease in active stage increased significantly. These results suggested that there was a mutual adjustment amongst various inflammatory factors in the pathogenesis of Crohn's disease.

Our results showed that the average number of *Escherichia coli* in feces samples taken from patients with Crohn's disease in the active stage was (8.2 ± 2.1) CFU/g, and the average number of *Enterococcus sp.* was (7.9 ± 1.7) CFU/g, which were significantly higher than those in other groups. We discovered that the Crohn's disease in active stage had clinical manifestation such as systemic aggravating, and was accompanied by some changes of intestinal flora distribution and composition. Our findings were consistent with results reported in the previous studies. Results obtained from previous studies²⁴, revealed that an imbalance in intestinal flora was frequently occurred in patients with inflammatory bowel disease. Additionally, the number of conditional pathogenic bacteria such as *Escherichia coli* and *Enterococcus sp.* in the activity group, was higher than other groups, while the number of probiotics bacteria represented by *Bifidobacteria* and *Bacillus lactic acid* decreased distinctly. This may lead to intestinal mucosal bar-

rier dysfunction, decline in immunity, and deterioration of patient's condition.

The number of *Escherichia coli* and *Enterococcus sp.* was positively correlated with the levels of IL-6, IL-17, IL-22, and IL-33, while the number of *Bifidobacteria* and *Bacillus lactic acid* was negatively correlated with the levels of IL-6, IL-17, IL-22, and IL-33. These results suggested that the evaluation of inflammatory factor levels could primarily prompt the distribution of intestinal flora and also could comprehensively assess the progression of Crohn's disease.

Conclusions

Monitoring the changes in distribution and composition of intestinal flora as well as the levels of blood inflammatory factors could play a significant role in the treatment process of Crohn's disease.

Conflict of interest

The authors declare no conflicts of interest.

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