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• CLINICAL RESEARCH •

Intestinal microecology and quality of life in irritable bowel syndrome patients

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Abstract

AIM: It has been noticed that gastroenteritis or dysentery plays a role in pathogenesis of irritable bowel syndrome (IBS), and antibiotics can increase functional abdominal symptoms, both of which may be partly due to intestinal flora disorders. This study was to determine the change of gut flora of IBS, a cluster of abdominal symptoms. Because of the chronic course and frequent occurrence of the disease, IBS patients suffered much from it. So the quality of life (Qol) of IBS patients was also evaluated in this study.

METHODS: Twenty-five Rome II criteria-positive IBS patients were recruited, and 25 age and gender-matched healthy volunteers were accepted as control. The fecal flora, including *Lactobacillus, Bifidobacterium, Bacteroides , C. perfringens Enterobacteriacea* and *Enterococus*, were analyzed quantitatively and qualitatively. We also calculated the ratio of *Bifidobacterium* to *Enterobacteriaceae* (B/E ratio) in both IBS patients and controls. In both groups, the data were further analyzed based on age difference, and comparisons were made between the younger and elder subgroups. We also evaluated the quality of life (QoL) of IBS patients and the control group using the Chinese version of SF-36 health questionnaire.

RESULTS: In IBS patients, the number of fecal *Bifidobacterium* was significantly decreased and that of *Enterobacteriaceae* was significantly increased compared with that in healthy controls (both $P<0.05$).The mean microbial colonization resistance (CR) of the bowel in IBS patients was smaller than 1, making a significant difference compared with that in control which was more than 1 ($P<0.01$). There was no significant difference in gut flora between two subgroups. While in control, the elder subgroup presented more *Enterobacteriacea* than the younger one ($P<0.05$). Compared with the control group, IBS patients had significantly lower scores on all SF-36 scales, with the exception of physical functioning. However, there was no significant correlation between quality of life and enteric symptoms in IBS patients.

CONCLUSION: There are intestinal flora disorders in IBS patients, which may be involved in triggering the IBS-like symptoms. IBS patients experience significant impairment in QoL, however, the impairment is not caused directly by enteric symptoms.

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INTRODUCTION

Many patients with typical irritable bowel syndrome (IBS) blamed all their bowel symptoms for acute gastroenteritis or dysentery, and it has been shown that intestinal infection does play an important role in the pathogenesis of IBS[1,2]. Although the mechanisms underlying postinfectious IBS are not clear, microbiological environmental alterations may be partly responsible for the pathogenesis. There is even evidence showing that antibiotics can increase functional abdominal symptoms[3]. In some cases, this is due to the colonization of pathogenic bacteria such as *Clostridium difficile*. In others it may be due to changes in bowel flora, which have been shown to persist for many months after a single antibiotic course.

In this study, the changes of intestinal microecology were investigated. Since age or sex may influence the composition of gut flora[4,5], age and sex-matched controls were recruited and the gut flora was also studied. Furthermore, the impact of IBS on quality of life (QoL) was evaluated using SF-36 health questionnaire (Chinese Version). Finally, the correlation between enteric symptoms scores and QoL in IBS patients was analyzed.

MATERIALS AND METHODS

Subjects

From September 2002 to March 2003, 25 Rome II criteria-positive[6] IBS patients diagnosed at the Department of Gastroenterology, Sir Run Run Shaw Affiliated Hospital of Zhejiang University were recruited (8 males, 17 females, mean age 45.40±10.56 years,ranging from 26 to 64 years). Subjects were excluded if they had any organic disease and those who had taken antibiotics or microecological modulators within 2 weeks before study were also ruled out. Twenty-five healthy volunteers (age and gender-matched) were accepted as control. All procedures were approved by the Ethical Committee of Medical College of Zhejiang University.

Bacteriogical analysis of intestinal flora

Selective culture medium for *Lactobacillus* (LBS), *Bifidobacterium* (Bs), *Bacteroides* (NBGT), *Enterococus* (EC) and *Enterobacteriacea* (EMB) was prepared by the Institute of Infectious Diseases, the First Affiliated Hospital of Zhejiang University. The culture medium for *C. perfringen* (TSN) was bought from Biomerieux (French). Glove box (Forma Scientific, Lnc., USA) was also needed.

Bacteriological analysis of intestinal flora was performed using the method that was essentially the same as that established by Okusa *et al*[7]. In brief, 10 g fresh stool was collected and sent to laboratory within 30 min in an anaerobic

jar. After 1 g fresh stool was aseptically quantified and homogenized, then diluted with an anaerobic solution in an anaerobic chamber (decimal dilutions up to 10^{-7} were prepared). Fifty μL of serial dilution volume (10^{-1}, 10^{-3}, 10^{-5}, 10^{-7}) of the specimens was spread over the above agar media with a L-shape stick. Aerobic bacteria were cultivated at 37 ℃ in an incubator for 48 h and anaerobic bacteria were cultivated in glove box (800 mL/L nitrogen, 100 mL/L hydrogen, and 100 mL/L carbon dioxide) at 37 ℃ for 48-72 h. After incubation, morphologically distinct colonies were described, calculated, isolated, and identified by morphology, Gram reaction and API fermentation tests, *etc*. The results were expressed as $\log_{10}$ of the number of bacteria per gram wet weight of feces (colony forming units/g , CFU/g). In our study, 2×10^2 (CFU) were set as the lowest detection limit. The incidence of each bacterial group recorded as the percentage of each bacterial group relative to the total bacteria was calculated. Furthermore, the ratio of *Bifidobacterium* to *Enterobacteriaceae* (B/E ratio) was calculated as the microbial colonization resistance (CR) of the bowel according to literature[8].

Evaluation of enteric symptoms

IBS patients were evaluated by enteric symptom questionnaire[9] which included 6 scales concerning abdominal pain, mucous stool and distention, *etc* (Table 1). Each scale was scored as "0" to "3" according to the severity and frequency of symptoms, whereas a higher score meant a more severe and frequent occurrence. The sum of each score was regarded as the general assessment of enteric symptoms.

Assessment of quality of life

The quality of life (QoL) of IBS patients and controls was evaluated with the Chinese version of short form 36 (SF-36) established by Wang *et al*.[10], which included 8 multiple dimensions as physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE) and mental health (MH). Scores were calculated according to corresponding formula and rules. Each dimension was scored from 0 to 100, with higher scores indicating better QoL.

Statistical analysis

Quantitative data were compared using different *t* test according to different situations, with the results expressed as mean±SD. The detection frequency was analyzed by Chi-squares test. The correlation between enteric symptom scores and QoL in IBS patients was detected by Pearson's correlation analysis. A *P* value less than 0.05 was considered statistically significant. All analysis was conducted using SPSS10.0 statistical passage.

RESULTS

Gut flora and microbial colonization resistance and detection frequency

Compared with the control group, IBS patients showed a significant decrease in *Bifidobacterium* but an increase in *Enterobacteriacea* (both $P<0.05$). The mean microbial colonization resistance (CR) of the bowel in IBS patients was smaller than 1, which was significantly different from that in control ($P<0.01$). There were no significant differences in *Lactobacillus*, *Bacteroides*, *Enterococus* between two groups. The detection frequency of *C. perfrigens* in IBS group was much lower than that in the control group ($P<0.01$, Table 2).

Table 2 Number of bacteria and B/E value in two groups (mean±SD, LgN/g stool)

Bacteria (%)	IBS patients (n=25)	Controls(n=25)
Lactobacillus	6.79±1.73 (92)	6.79±1.94 (84)
Bifidobacteria[a]	8.14±2.14 (96)	9.32±1.22 (100)
Bacteroides	10.49±0.56 (100)	10.62±0.79 (100)
C.perfringens[d]	7.32±2.12 (40)	6.66±1.68 (80)
Enterococus	7.96±1.53 (100)	7.49±1.29 (100)
Enterobacteriaceae[a]	9.02±1.04 (100)	8.44±0.95 (100)
B/E value[b]	0.91±0.27 (96)	1.12±0.23 (100)

The data in the bracket mean positive rate (This is applicable for the following tables including flora.). [a]$P<0.05$ flora *vs* control, [b]$P<0.01$ B/E value *vs* control, [d]$P<0.01$ detection frequency *vs* control.

The data were further analyzed based on age difference. Both groups were sorted on ascending order, the first 12 persons were chosen as younger subgroup (mean age 36.25±5.17) and the rest 13 (mean age 53.85±6.14) as elder one. We found that in IBS group, there was no significant difference in gut flora between two subgroups, but CR was smaller than 1 in both subgroups. While in control, the elder subgroup presented more *Enterobacteriacea* than the younger subgroup ($P<0.05$), but smaller CR (Tables 3, 4).

Table 3 Number of bacteria and B/E value in younger subgroup and elder subgroup in IBS group (mean±SD, LgN/g stool)

Bacteria (%)	Younger subgroup (n=12)	Elder subgroup (n=13)
Lactobacillus	6.63±1.83 (100)	6.98±1.70 (84)
Bifidobacteria	7.60±2.65 (100)	8.68±1.38 (92)
Bacteroides	10.52±0.42 (100)	10.47±0.72 (100)
C.perfringens	8.84±1.07 (25)	6.89±2.20 (54)
Enterococus	8.26±1.58 (100)	7.69±1.50 (100)
Enterobacteriaceae	9.16±1.22 (100)	8.90±0.88 (100)
B/E value	0.85±0.33 (100)	0.98±0.19 (92)

QoL of IBS patients

IBS patients experienced significant impairment in QoL (Table 5). Compared with control group, IBS patients had significantly lower scores on all SF-36 scales, with the exception of physical functioning. Decrements in QoL were most pronounced in general heath (GH, mean score 41.40), role physical (RP, mean score 52.00) and vitality (VT, mean score 53.40).Others like mental health (MH), role emotional (RE), bodily pain (BP) and social functioning (SF) were also impaired.

Table 1 Enteric symptom scales

Symptoms	0	1	2	3
Duration of abdominal pain (h/d)	None	<1	2-8	>8
Frequency of abdominal pain (d/wk)	None	1-2	3-5	6-7
Ratio of abnormal shape of stool	None	<1/4	1/4-3/4	>3/4
Ratio of abnormal passage of defecation	None	<1/4	1/4-3/4	>3/4
Ratio of mucous stool	None	<1/4	1/4-3/4	>3/4
Distention or gastrectasia when defecation	None	<1/4	1/4-3/4	>3/4

Table 4 Number of bacteria and B/E value in younger subgroup and elder subgroup of control group. (mean±SD, LgN/g stool)

Bacteria (%)	Younger subgroup (*n*=12)	Elder subgroup (*n*=13)
Lactobacillus	6.18±2.07 (75)	7.20±1.83 (92)
Bifidobacteria	9.32±1.08 (100)	9.32±1.38 (100)
Bacteroides	10.49±0.71 (100)	10.75±0.89 (100)
C.perfringens	6.45±1.82 (83)	6.89±1.59 (77)
Enterococus	7.09±1.49 (100)	7.86±1.00 (100)
Enterobacteriaceae[a]	8.05±1.14 (100)	8.81±0.57 (100)
B/E value	1.19±0.28 (100)	1.06±0.14(100)

[a]*P*<0.05 flora *vs* control.

Table 5 QoL of IBS patients and normal control (mean±SD)

QoL scale	IBS patients (*n* =25)	Controls (*n* =25)
PF	74.40±26.78	85.40±15.53
RP[a]	52.00±44.44	77.00±37.44
BP[b]	73.33±17.57	84.89±16.00
GH[b]	41.40±15.04	73.40±13.52
VT[b]	53.40±16.94	69.60±17.38
SF[b]	75.00±12.50	84.50±12.12
RE[b]	61.33±41.59	96.00±11.06
MH[b]	58.24±16.21	80.00±13.52

[a]*P*<0.05 ,[b]*P*<0.01 *vs* healthy control .

Correlation between QoL and enteric symptoms in IBS patients

Although IBS patients presented a negative correlation between bodily pain (BP) and enteric symptoms (correlation coefficient: 0.347), the difference was not significant (*P*>0.05). While vitality (VT), role emotional (RE), general health (GH) and physical functioning (PF), showed no significant difference with QoL in IBS patients (Table 6).

Table 6 Correlation between enteric symptoms and QoL in IBS (mean±SD)

	Score of QoL	Score of enteric symptoms	Correlation coefficient (*r*)
PF	74.40 (26.78)	7.56 (2.31)	-0.062
RP	52.00 (44.44)	7.56 (2.31)	0.060
BP	73.33 (17.57)	7.56 (2.31)	-0.347
GH	41.40 (15.04)	7.56 (2.31)	-0.137
VT	53.40 (16.94)	7.56 (2.31)	-0.280
SF	75.00 (12.50)	7.56 (2.31)	0.018
RE	61.33 (41.59)	7.56 (2.31)	-0.257
MH	58.24 (16.21)	7.56 (2.31)	0.001

DISCUSSION

The etiology of IBS is still unclear and the pathogenetic mechanisms are only partly understood. Intestinal motility alteration, visceral hypersensitivity, disturbed intestinal reflexes, psychological disorders, food intolerance and gastrointestinal infection and imbalance of gut flora were involved in the pathogenesis of IBS[11]. IBS patients presented various intestinal motility alterations, which were recognized as the basic pathophysiological factor. Evidences have shown that there were gastroparesis and small bowel dysmotility in IBS[12]. The motility index (MI),mean number and peak amplitude of high amplitude propagating contractions (HAPCs) in IBS patients were significantly greater than those in controls. These abnormalities might be related to shortened colonic transit time[13]. The migrating motor complex (MMC) was found to be an important mechanism controlling bacterial growth in the upper small bowel. Its disruption could promote duodenal bacterial overgrowth and bacterial translocation[14].

Food-related microbial alteration of the bowel might be partly responsible for the occurrence of IBS, since many IBS symptoms might be triggered or aggravated by food intake[15]. King and his colleagues found that colonic-gas production, particularly hydrogen, was greater in IBS patients, and both symptoms and gas production were reduced by an exclusion diet. This reduction might be associated with alterations in the activity of hydrogen-consuming bacteria[16].It has been shown that IBS patients have abnormal lactulose breath test (LBT), suggesting the presence of small intestinal bacterial overgrowth (SIBO) or an increased number of enteric organisms. Normalization of LBT could lead to a significant reduction in IBS symptoms[17]. On the other hand, some studies reported that the incidence of IBS was increased after acute gastroenteritis or dysentery. Under the circumstance of infection, an alterant microbiological environment might influence the number of lymphocytes, mast cells and enteroendocrine cells in the mucosa, rapid transit and a tendency to a secretory state was often found[1].

In this study, we found that IBS patients had a decrease of beneficial *Bifidobacteria* and overgrowth of potentially pathogenic *Enterobacteriaceae*. The ratio of B/E was smaller than 1, suggesting impairment of microbial colonization resistance (CR) of the bowel in IBS. Once CR was destroyed by antibiotics or other reasons, bacterial infection or dysbacteriosis might emerge[18].

Intestinal dysbacteriosis may be related to the occurrence of symptoms of IBS, since *Bifidobacteria* are considered to be beneficial to health. It involves in the production of essential mucous nutrients, such as short-chain fatty acids(SCFAs) and lactic acid, by lactose fermentation. They have been shown to eliminate toxins and unnecessary substances, such as hydroxybenzene, ammonia and steroids. They may also participate in the regulation of intestinal functions, such as nutrient synthesis and absorption. *Bifidobacteria* could prevent the overgrowth of potentially pathogenic organisms by bacterial barrier or by producing antibiotics[5,19]. *Enterobacteriaceae*, the main cause of endotoxin, may produce toxins such as ammonia , sulfureted hydrogen[5]. Endotoxin could temporarily impair canine gut absorptive function both in colon and in jejunum, with decreased absorption of water, glucose and eletrolytes, including sodium, chloride, and potassium[20]. When endotoxin of Gram-negative bacteria was administered intravenously in rats, the migrating myoelectric complex was replaced by spike bursts accompanied by rapid transit[21].These effects may contribute to the occurrence of diarrhea. Microecological modulators could prominently relieve IBS-like enteric symptoms, suggesting the close relationship between gut flora and IBS[22,23].

Ageing may affect the flora residing in the gut and outside of it[5]. In our study, we found that the intestinal microecology showed an overgrowth of *Enterobacteriaceae* with age in normal control, implying that age may interact with the gut flora. The overgrowth of *Enterobacteriaceae* may also account for the susceptibility to intestinal infection and endotoxemia in elderly people. *Bifidobacteria* have been shown to have a close relationship with human longevity[5]. While in IBS group, age had no influence on gut flora, since there was intestinal dysbacteriosis in both younger and elder subgroups.

There was a high detection frequency of *C. perfringens* in normal control, without any apparent pathogenic effect. It was supposed that changes of gut flora in IBS might result in a relatively high detection frequency of *C. perfringens* .Therefore, further studies are needed to elucidate this phenomenon.

Anyway, microbial metabolic processes have been going on like in a black box[24]. Although almost all organic compounds and nutrients, fiber, digestive secretions and desquamated

epithelial cells of the host can enter into microorganismic metabolic chains and processes, little is known about this metabolic chain and process. Further studies are still needed. However, there was dysbacteriosis in IBS patients. Whether it is the effect or just cause of IBS remains unclear.

The purpose of focusing on health-related quality of life (HRQL) is to go beyond the presence and severity of symptoms of disease and to examine how patients perceive and experience these manifestations in their daily lives[25].Briefly, HRQL concerns more about the physical, psychological and social functioning.

IBS is not a life-threaten disease. Its impacts include costs associated with diagnosis and treatment, production losses due to morbidity and pain and diarrhea, *etc*.[26]. With a questionnaire, Silk[27] found that IBS impacted significantly on personal relationships and working practices. Some even complained that IBS prevented them from applying for promotion or a new job. IBS patients had impaired quality of life in general health, vitality, social functioning, *etc*[9,28]. According to the research of Gralnek *et al*.[29], IBS patients experienced significantly worse HRQL in some aspects than those with gastroesophageal reflux disease (GERD) or diabetes mellitus (DM). IBS patients had even worse bodily pain, fatigue, and social functioning when compared with dialysis-dependent end-stage renal disease patients,

In our study, we also found that IBS patients had significantly lower scores on all SF-36 scales with the exception of physical functioning, when compared with the age and sex-matched control group. Decrements in QoL were most pronounced in general health, role physical and vitality. However, there was no significant correlation between QoL and enteric symptoms, which might be due to the frequent presence of anxiety, depression, fatigue and anorexia in IBS patients[11,30].Compared with the control, neuroticism, hypochondriasis and depression were significantly more prevalent in IBS patients attending a clinic, which would have a prominently negative influence on QoL. Hypnotherapy was effective in treating IBS, suggesting that psychological factors may play an important role in IBS. Thus the general impacts of IBS may go far beyond those of enteric symptoms, implicating that IBS is a psychosomatic disease.

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