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Effects of mesalazine combined with bifid triple viable on intestinal flora, immunoglobulin and levels of cal, MMP-9, and MPO in feces of patients with ulcerative colitis

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Abstract. – OBJECTIVE: Ulcerative colitis (UC) commonly occurs in young and middle-aged people and is characterized by frequent attacks and difficult cure. Mesalazine is often applied in the initial treatment of UC, but it can lead to serious adverse effects. Its combination with bifid triple viable could mitigate adverse effects. This study aims to explore the effects of mesalazine combined with bifid triple viable on the intestinal flora, immunoglobulin (Ig), and levels of calprotectin (Cal), matrix metalloproteinase-9 (MMP-9), myeloperoxidase (MPO) in feces of UC patients.

PATIENTS AND METHODS: A total of 180 UC patients in our hospital were divided into two groups with 90 cases in each one. Patients in control group were treated with oral mesalazine (1.5 g/d), and those in the treatment group were treated with oral mesalazine (1.5 g/d) combined with bifid triple viable (1.26 g/d) for 2 months. Treatment effects in both groups following the therapeutic period were observed accordingly. Feces of patients in the two groups were collected for detecting intestinal microorganism and the levels of Cal, MMP-9, and MPO. Serum levels of IgG, IgA, and IgM in each patient were detected by enzyme-linked immunosorbent assay (ELISA).

RESULTS: The overall response rate of the treatment group (91.11%) was significantly higher than that of control group (68.89%; *p*=0.000). The abundances of intestinal microflora, including Bifidobacterium, *Actinomycetes, Rikenellaceae, Genus VI* of *Acidaminococcaceae*, and *Metascardovia* in treatment group were remarkably higher than those in control group. The abundances of intestinal microorganisms such as *Phocaeicola, Lawsonia intracelluaris*, *Streptococcus*, *Ruminococcaceae*, and *Clostridium* in control group were significantly higher than those in treatment group. After treatment, serum levels of IgG and IgM of patients in treatment group were lower than those in control group, with IgG of (15.14±0.98) (*p*=0.031) and IgM of (1.50±0.18) (*p*=0.000). No statistical difference in IgG level was observed between treatment group and control group after treatment (*p*=0.871). After treatment, the levels of Cal and MMP-9 in feces of patients in treatment group were significantly lower than those in control group with Cal of (79.81±5.42) (*p*=0.000) and MMP-9 of (4.89±0.98) (*p*=0.000). There was no statistical difference in the MPO level between treatment group and control group after treatment (*p*=0.871).

CONCLUSIONS: Mesalazine combined with bifid triple viable is able to enhance the curative effect for UC, improve the composition of intestinal flora, weaken the immune response, and reduce levels of Cal and MMP-9 in the intestinal tract.

Key Words:
Ulcerative colitis (UC), Bifid triple viable, Intestinal flora.

Introduction

Ulcerative colitis (UC) is a chronic idiopathic disease, which commonly affects people in 30-50 years. UC may result in disability. Its incidence rate in Asia exceeds that in Europe[1,2]. However, due to the unclear pathogenesis of the disease, effective cure strategies for UC are lacking. UC patients gradually deteriorate in the long course of the disease. Up to 15% of UC patients require colectomy, and the annual direct and indirect costs related to UC are as high as 12.5 billion euros in Europe and 8.1 billion dollars in the United States[3]. Ulcerative proctitis is one subtype of UC and considered as its initial manifestation[4]. Prevention of the progression of ulcerative proctitis is vital to improve the prognosis of UC patients, and mesalazine serves as a great helper[5].

Multiple Guidelines and Consensus have stated that mesalazine is recommended as the initial

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treatment regimen for UC[6,7]. Moreover, topical use of mesalazine in colorectum can effectively deliver the drug to the lesion location, thereby achieving superior efficacy to that of steroid or oral administration of mesalazine. The combination of methalazine and aminosalicylate (oral administration and topical use) may be more effective than using either of them alone.

Currently, corticosteroids and anti-inflammatory agents (such as mesalazine) are preferred for UC treatment, as well as symptomatic treatments, including antidiarrheal agents and fluid infusion[8]. However, these treatments are not always reliable in the process of disease control. These drugs may lead to adverse events, such as allergy, occurring in patients with existing treatments[9]. The addition of bifid triple viable can greatly reduce the occurrence of adverse effects of drugs and improve the therapeutic effect, contributing to regulate the composition of intestinal flora and systemic immune response. However, the specific regulatory mechanism remains unclear.

As a result, this study explored the effects of mesalazine combined with bifid triple viable on intestinal flora composition, systemic immune response, and levels of related indicators in feces of UC patients. It is pointed out that the overall response rate of the combination of the two drugs was higher than that of taking one drug alone in UC patients. Combination of the two drugs can better regulate intestinal microorganisms and reduce the immune response of the body, which can also strongly regulate calprotectin (Cal) and matrix metalloproteinase-9 (MMP-9).

Patients and Methods

Basic Information

A total of 180 UC patients in our hospital were divided into two groups with 90 cases in each one. Patients in control group were treated with oral mesalazine (1.5 g/d), and those in treatment group were treated with oral mesalazine (1.5 g/d) combined with bifid triple viable (1.26 g/d). The age range of patients in control group was 24-64 years, with an average age of (45.12±3.81) years. The age range of the patients in treatment group was 22-65 years-, with an average age of (43.18±2.95) years. There were no statistical differences in age, gender, and other basic information between control group and treatment group ($p>0.05$).

Inclusion criteria for UC patients were applied: 1) UC diagnosis was in accordance to the Guidelines proposed by the Chinese Society of Gastroenterology, Chinese Medical Association, 2) patients underwent enteroscopy, and 3) patients agreed to participate in this study. Exclusion criteria were applied: 1) patients complicated with other serious physical diseases, 2) pregnant or lactating patients, 3) malignant tumor patients, or 4) patients with serious mental disorders.

Diagnostic criteria: 1) patients with clinical manifestations including recurrent abdominal pain and diarrhea accompanied by tenesmus and systemic symptoms, 2) colonoscopy examination showed the diffuse distribution of lesions, accompanied by hemorrhage, edema, suppuration, erosion, and ulcer in some parts with narrowing of colon pouch and polyp formation.

All the subjects signed relevant informed consent forms. This investigation was approved by the Ethics Committee of our hospital and conformed to relevant provisions.

Methods and Treatment Effects

All subjects were given symptomatic treatments, such as general treatment and anti-inflammatory treatment. Patients in control group were treated with mesalazine (Sunflower Pharmaceutical Group Jiamusiluling Pharmaceutical Co., LTD., H19980148, 0.25 mg × 24 tablets), 0.5 g each time, 3 times a day, after meals. In treatment group, mesalazine combined with Bifidobacterium triplex live bacteria (Shanghai Shangyao Xinyi Pharmaceutical Factory Co., LTD., Chinese drug approval number S10950032, 210 mg × 24 tablets) 0.42 g each time, 3 times a day, were given to each patient. All subjects were treated for 2 months.

Efficacy observation: according to the improvement of clinical symptoms and the changes of disease activity index (DAI) score before and after treatment, the efficacy was classified into three grades: complete remission, effective, and ineffective. Total score included three parts: weight loss percentage (weight into 0, 1-5 for 1 point, 5-10 to 2 points, 10 to 15 for the three points, greater than 15 to 4 points), defecate viscosity (normal to 0, loose stool to 2 points, diarrhea to 4 points), and defecate haemorrhage (normal to 0, occult blood positive to 2 points, overt bleeding to 4 points). Then, DAI value was calculated by total score/3. DAI value of patients with complete remission was significantly reduced, and their clinical symptoms such as abdominal pain, abdominal distension, purulent blood, and stool disappeared. The DAI value of patients with effective treatment decreased slightly but not significantly, and

the change of clinical symptoms was not significant. The DAI value of patients with ineffective treatment was unchanged or increased, and the clinical symptoms such as abdominal pain, pus, and blood stools were aggravated, and the number of stools per day increased.

Detection of Intestinal Flora in UC Patients

Feces of UC patients in control group and treatment group (15 cases in each group) were collected and sent to CapitalBio Co., Ltd. (Beijing, China) for analysis of enteric microorganisms. The team of this study sent middle and rear segment of feces collected from 30 UC patients to the company. After extraction, amplification, database building, and labeling of microbial genomic DNA, Illumina MiSeq was chosen to conduct high-throughput sequencing to detect the species and relative abundance of microorganisms in the samples. Relevant bioinformatics analysis was carried out [Linear discriminant analysis Effect Size (LEfSe) analysis].

Detection of Serum Level of Immunoglobulin (Ig)

Serum levels of Igs in patients of control group and treatment group were detected by enzyme-linked immunosorbent assay (ELISA) using commercial kits, including IgG, IgM, and IgA. Peripheral blood was respectively collected from 90 patients in control group and 90 patients in treatment group before and after treatment with 5 mL from each patient. After centrifugation at 3500 rpm/ min for 5 min, the upper phase of serum was transferred to a new centrifuge tube and stored in liquid nitrogen. The detection was strictly performed according to the instruction steps of the ELISA kit. After the operation, the absorbance at 560 nm was read on the microplate reader and converted into the actual concentrations of IgG, IgM, and IgA *via* standard curves.

Detection of Cal, MMP-9, and Myeloperoxidase (MPO) Levels in Feces

The feces of 90 patients in control group and 90 patients in treatment group before and after treatment were respectively collected with 5 g from each case and stored in a refrigerator at –20°C. The specimens were taken out for detection. After specimens warmed up, 2 g of feces were taken into a small test tube, added with 1:50 physiological saline, fully mixed for 15 min, and then centrifuged at 1500 rpm/min for 10 min. The super-

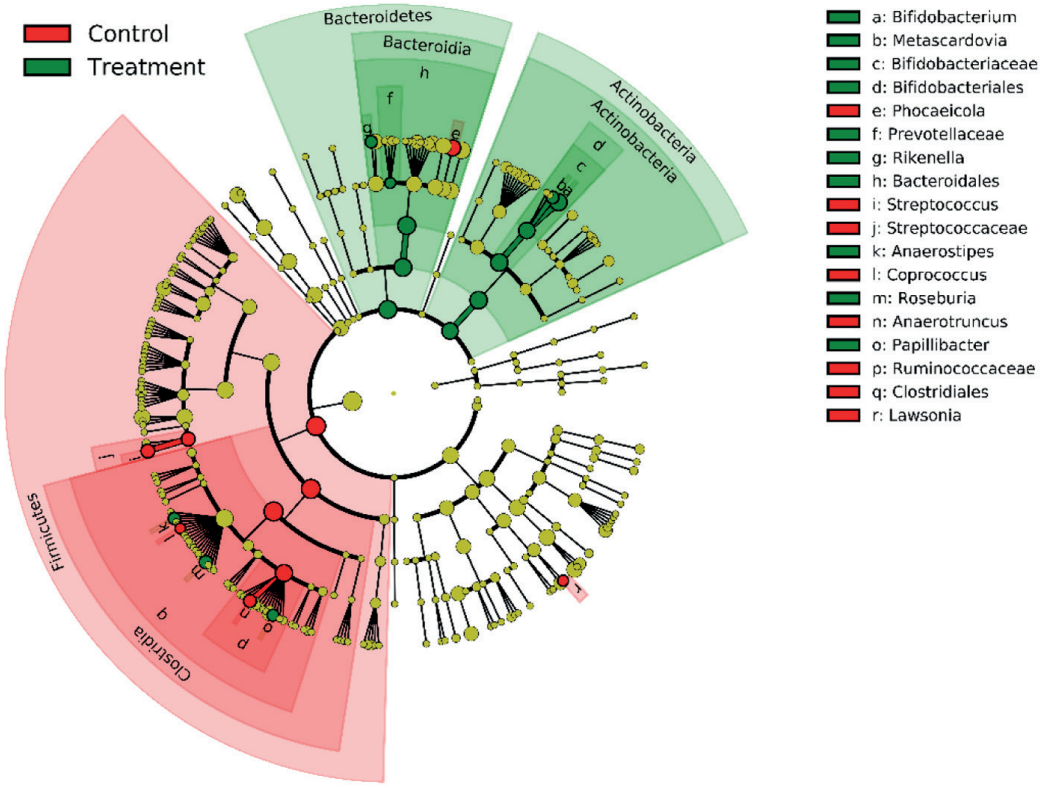

Figure 1. LEfSe analysis of intestinal flora of UC patients in two groups.

natant was finally taken to detect the levels of Cal, MMP-9, and MPO using the ELISA kit, and the levels of these indicators in feces were obtained by conversion.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM Corp., Armonk, NY, USA) was used for data statistics and analysis, Chi-square test was chosen to for the enumeration data, and *t*-test was adopted for the measurement data. $p<0.05$ was considered a statistically significant difference.

Results

Comparisons of Therapeutic Effects of Patients in the Treatment Group and the Control Group

Therapeutic outcomes of patients in treatment group and control group were shown in Table I. The overall response rate of treatment group (91.11%) was significantly higher than that of control group (68.89%) (p=0.000). Among them, the proportion of cases with a curative effect in treatment group (0.67) was remarkably higher than that in control group (0.46) (p=0.004). In addition, the proportion of cases without a curative effect in control group (0.32) was notably higher than that in treatment group (0.09) (p=0.000).

Analysis of Intestinal Flora of Patients in the Treatment Group and the Control Group

LEfSe analysis results of intestinal microorganisms of patients in treatment group and control group were shown in Figure 1, and LDA scores were shown in Figure 2. The abundances of intestinal microflora, including *Bifidobacterium, Actinomycetes, Rikenellaceae, Genus VI of Acidaminococcaceae,* and *Metascardovia* in treatment group were significantly higher than that in control group. The abundances of intestinal microorganisms such as *Phocaeicola, Law-*

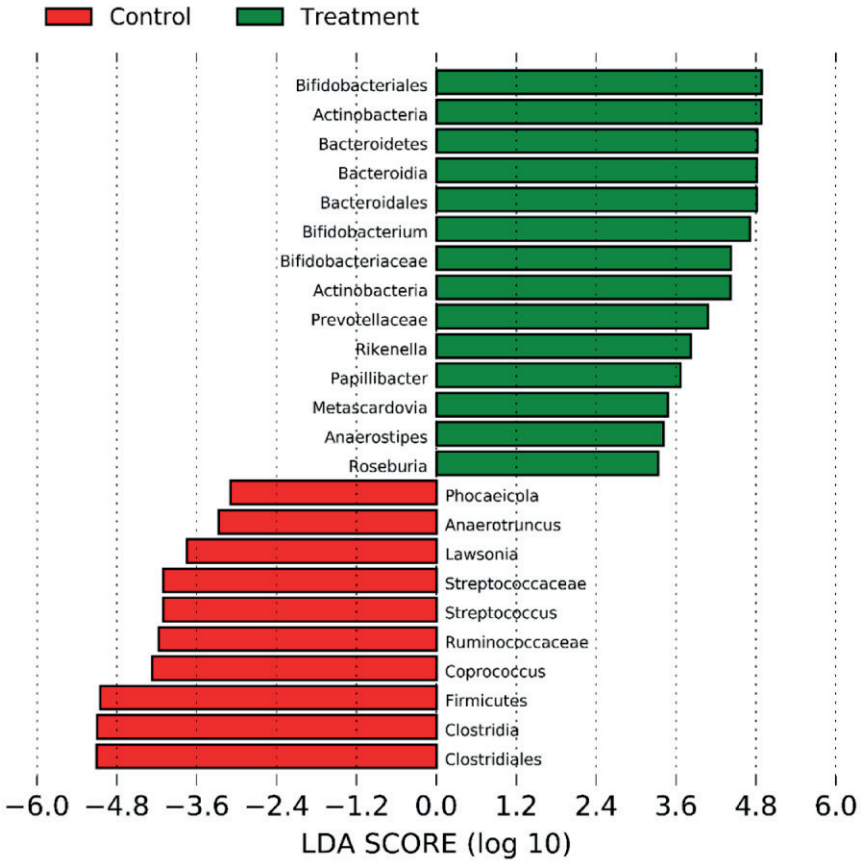

Figure 2. LDA scores of intestinal flora of UC patients in two groups.

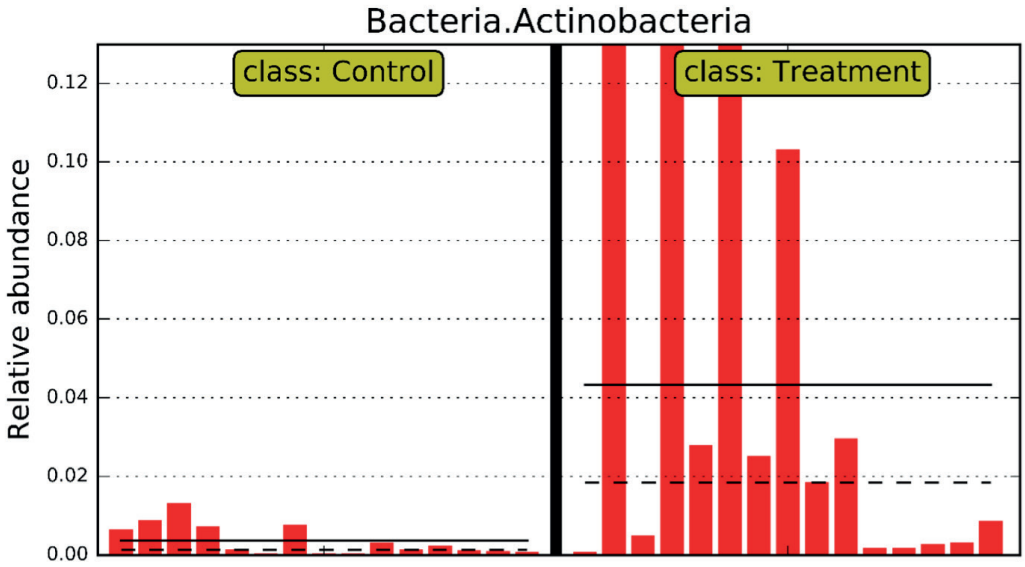

Figure 3. Comparisons of *Actinomycetes* levels of UC patients in two groups.

sonia intracelluaris, Streptococcus, Ruminococcaceae, and *Clostridium* in control group were remarkably higher than that in treatment group. The levels of *Actinomycetes* in the intestinal tract of patients in treatment group and control group were shown in Figure 3.

Comparison of Ig of UC Patients in the Treatment Group and the Control Group Before and After Medication

The content of Igs in the serum of UC patients before and after medication in the two groups were shown in Table II. Serum levels of IgG, IgM, and IgA in UC patients presented no statistical difference between treatment group and control group before treatment (p>0.05). After treatment, serum levels of IgG and IgM in patients of treatment group were significantly lower than those in control group, with IgG of (15.14±0.98) (p=0.031) and IgM of (1.50±0.18) (p=0.000). The IgA level of patients demonstrated no statistical difference between treatment group and control group after treatment (p=0.871).

Table I. Comparisons of therapeutic effects of UC patients in two groups.

Group	No.	Complete response	Effective	Ineffective	Overall response rate (%)
Control group	90	20 (0.22)	41 (0.46)	29 (0.32)	68.89%
Treatment group	90	22 (0.24)	60 (0.67)	8 (0.09)	91.11%
X^2		1.32	9.21	42.18	54.07
p		0.250	0.004	0.000	0.000

Table II. Ig level in the serum of UC patients before and after medication in the two groups (g/L).

Group	No.	IgG		IgM		IgA	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group	90	21.14±1.13	18.36±2.16	1.49±0.19	1.59±0.12	2.11±0.09	2.08±0.11
Treatment group	90	22.15±2.14	15.14±0.98	1.51±0.22	1.50±0.18	2.13±0.05	2.09±0.12
p		0.871	0.031	0.719	0.002	0.931	0.871

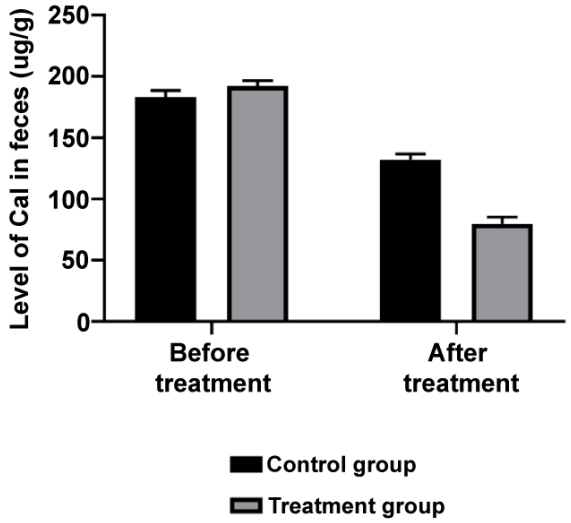

Figure 4. Level of Cal in feces of UC patients in two groups before and after medication.

Levels of Cal, MMP-9, and MPO in Feces of UC Patients in the Treatment Group and the Control Group Before and After Medication

The levels of Cal, MMP-9, and MPO in feces of UC patients before and after medication in the two groups were shown in Table III, Figure 4, Figure 5, and Figure 6, respectively. The Cal, MMP-9, and MPO levels in feces of UC patients showed no significant difference between treatment group and control group before treatment (p>0.05). After treatment, the levels of Cal [(79.81±5.42)] and MMP-9 [(4.89±0.98)] in feces of patients in treatment group were significantly lower than those in control group (p=0.000, p=0.000). There was no statistical difference in the MPO level between treatment group and control group after treatment (p=0.871).

Table III. Levels of Cal, MMP-9 and MPO in feces of UC patients in two groups before and after medication.

Indicator	Before treatment			After treatment		
	Control group (n=90)	Treatment group (n=90)	*p*	Control group (n=90)	Treatment group (n=90)	*p*
Cal (ug/g)	183.13±5.23	192.45±4.13	0.769	132.19±4.68	79.81±5.42	0.000
MMP-9 (ng/mL)	21.65±1.23	20.98±2.87	0.871	16.78±2.78	4.89±0.98	0.000
MPO (U/mL)	1.31±0.11	1.25±0.13	0.713	0.54±0.16	0.61±0.09	0.217

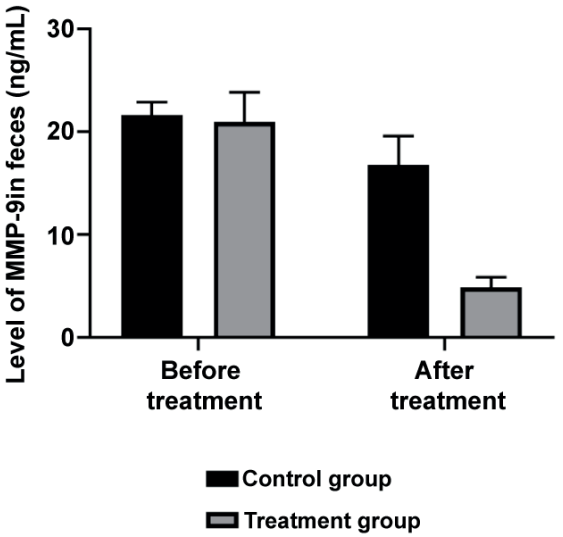

Figure 5. Level of MMP-9 in feces of UC patients in two groups before and after medication.

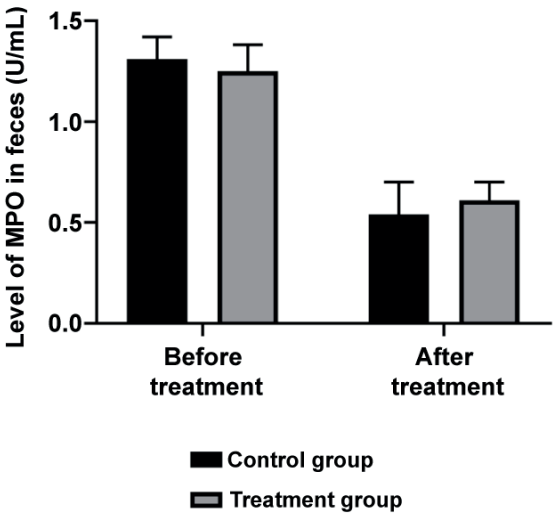

Figure 6. Level of MPO in feces of UC patients in two groups before and after medication.

Discussion

About 15% of UC patients suffer from severe disease conditions, of which 30% require surgeries[10,11]. Drug therapy can alleviate intestinal and systemic inflammation and reduce the damage, thus gradually relieving the severity in UC patients. 5-ASA, mainly mesalazine, is preferred for UC, which can mitigate the symptoms of the disease by oral administration[12]. Other drugs include steroids and immunosuppressive drugs, such as 6- mercaptopurine (6-MP), whose effectiveness has been supported by clear evidence[13-15]. However, 20% to 40% of UC patients do not respond to conventional drugs (e.g., mesalazine), or have relatively serious adverse effects, such as allergy and systemic collapse[16], which may be explained by changes in intestinal flora composition of patients caused by mesalazine. Therefore, it is of great significance to study the combination of auxiliary drugs that can regulate intestinal flora and mesalazine to improve conditions of UC patients.

Bifid triple viable is mainly made of three probiotics, which has an excellent regulatory effect on the intestinal microenvironment. It not only plays an important role in inhibiting harmful bacteria like *Staphylococcus aureus*, but also increases and replaces high-quality microorganisms in the human intestinal tract, changes the composition ratio of microorganisms in the intestinal tract, thus regulating flora balance and enhancing the immunity of the body[17,18]. Long-term inflammation greatly changes the amount and composition of microorganisms in the intestinal tract of UC patients, potentially affecting the development of the disease and the immune system[19,20]. Mesalazine treatment can slow down the progression of the disease and relieve symptoms, but it also affects intestinal flora of UC patients, which may be attributed to adverse effects. Bifid triple viable regulated the intestinal microflora of UC patients treated with mesalazine and notably increased the abundances of intestinal microflora of patients such as *Bifidobacterium, Actinomycetes, Rikenellaceae, Genus VI of Acidaminococcaceae,* and *Metascardovia*, decreased the abundances of intestinal microflora such as *Phocaeicola, Lawsonia intracelluaris, Streptococcus, Ruminococcaceae,* and *Clostridium* (Figure 1 and Figure 2). The overall response rate (91.11%) of patients in treatment group was significantly higher than that of the patients in control group (68.89%) (p=0.000), indicating a better therapeutic effect (Table I). A relevant study has indicated that the *Bifidobacterium* can be adopted as a landmark microorganism in the intestinal tract of UC patients, which is significantly related to the development of UC[21].

Mesalazine combined with bifid triple viable not only can regulate the intestinal flora, but also can change the immune system of patients. Serum levels of IgG and IgM in patients of treatment group were significantly lower than those in control group, with IgG of (15.14±0.98) (p=0.031) and IgM of (1.50±0.18) (p=0.000) (Table II). Therefore, compared with the administration of mesalazine alone in the treatment of UC, the combination of the two drugs can significantly reduce the body's systemic immune response to improve the curative effect.

Moreover, the levels of Cal and MMP-9 in feces of UC patients treated with mesalazine combined with bifid triple viable were significantly lower than those in control group, with Cal of (79.81±5.42) (p=0.000) and MMP-9 of (4.89±0.98) (p=0.000) (Table III). It is suggested that the enhancement of therapeutic efficiency of bifid triple viable may be achieved by regulating the activity or quantity of neutrophil in inflammatory local tissues. Cal and MMP-9 in feces are mainly secreted by neutrophils in intestinal tissues, which can damage intestinal epithelial cells, and their increases represent an increase in the activity of UC. Mesalazine combined with bifid triple viable can reduce the release of Cal and MMP-9 in intestinal neutrophils of UC patients, thus alleviating local inflammation and advancing curative effect.

Conclusions

Briefly, mesalazine combined with bifid triple viable was able to enhance the curative effect for UC, improve the composition of intestinal flora, weaken the immune response, and reduce levels of Cal and MMP-9 in the intestinal tract.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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