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Evaluation value of intestinal flora detection for intestinal mucosal inflammation and immune response in patients with ulcerative colitis

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ABSTRACT

Objective: To study the evaluation value of intestinal flora detection for intestinal mucosal inflammatory response and immune response in patients with ulcerative colitis. **Methods:** The patients who were diagnosed with ulcerative colitis in Zigong Fifth People's Hospital between March 2015 and February 2017 were selected as the UC group, and those who were diagnosed with colonic polyps were selected as the control group. Fresh excreta were collected to detect the number of intestinal flora, and the diseased intestinal mucosa tissue was collected to detect the expression of inflammatory response molecules and immune cell transcription factors. **Results:** enterococcus contents in intestinal tract and TLR4, NF-kB, TNF-α, HMGB-1, T-bet and RORC mRNA expression levels in intestinal mucosa of UC group were significantly higher than those of control group while bifidobacteria contents in intestinal tract and SOCS2, SOCS3, Foxp3 and GATA-3 mRNA expression levels were significantly lower than those of control group; TLR4, NF-kB, TNF-α, HMGB-1, T-bet and RORC mRNA expression levels in intestinal mucosa of UC patients with grade II and grade III flora disturbance were significantly higher than those of UC patients with normal flora and grade I flora disturbance while SOCS2, SOCS3, Foxp3 and GATA-3 mRNA expression levels were significantly lower than those of UC patients with normal flora and grade I flora disturbance; TLR4, NF-kB, TNF-α, HMGB-1, T-bet and RORC mRNA expression levels in intestinal mucosa of UC patients with grade III flora disturbance were significantly higher than those of UC patients with grade II flora disturbance while SOCS2, SOCS3, Foxp3 and GATA-3 mRNA expression levels were significantly lower than those of UC patients with grade II flora disturbance. **Conclusion:** The intestinal flora disturbance in patients with ulcerative colitis can result in inflammatory response activation and immune response disorder.

1. Introduction

Ulcerative colitis (UC) is a kind of inflammatory bowel disease with unknown etiology, its main lesion characteristic is colonic mucosa inflammation or ulceration, and serious cases can involve the entire colon and affect patients' daily life. Abnormal activation of the inflammatory response and the unbalance of immune response are thought to be closely related to the occurrence of UC, and the differentiation disorder of Th1, Th2, Th17, Treg and other CD4+T lymphocyte subsets in UC patients can change the local immune response and activate the inflammatory reaction to participate in the pathological process of UC[1,2]. However, the regulatory mechanism of inflammatory response and immune response in UC patients is not yet clear at present. In recent years, research on UC shows that intestinal flora disturbance is one of the important characteristics of UC, and the suppression of bifidobacterium, bacteroides and other physiological dominant bacteria and the excessive reproduction of enterobacter, enterococcus and other conditioned pathogens can cause intestinal local micro-ecology unbalance, and then result in disorder of inflammatory response and immune response[3,4]. In the following studies, we analyzed the evaluation value of intestinal flora detection for intestinal mucosal inflammatory response and immune response in patients with ulcerative colitis.

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2. Subjects and methods

2.1 Research subjects

A total of 58 patients who were diagnosed with ulcerative colitis in Zigong Fifth People's Hospital between March 2015 and February 2017 were selected as the UC group of the study, and 40 patients who were diagnosed with colonic polyps in Zigong Fifth People's Hospital over the same period were selected as the control group. Both groups underwent colonoscopy and were diagnosed by pathology biopsy, and patients combined with autoimmune diseases, diabetes and tuberculosis were excluded. UC group included 32 men and 26 women that were 38-59 years old; control group included 22 men and 18 women that were 36-58 years old. There was no statistically significant difference in general information between the two groups (P>0.05).

2.2 Research methods

2.2.1 Intestinal flora testing

Before colonoscopy, 1-2 g fresh excreta tissue was collected from two groups of patients, added in 10 mL PBS buffer, mixed evenly and centrifuged to separate supernatant, the steps were repeated for 3 times, the 3 times of supernatant was collected and centrifuged for 15 min at a speed of 13 000 r/min, the precipitation was collected to extract the genome DNA, the specific primers of bifidobacteria and enterococcus were designed, the amplification was continued, and the number of bacteria was calculated according to the amplification curve and in terms of lg n/g.

2.2.2 Molecule expression testing in intestinal mucosa

During colonoscopy, the right amount of diseased intestinal mucosa tissue was collected from two groups of patients and added in Trizol lysate to separate RNA and synthesize it into cDNA by reverse transcription, then fluorescence quantitative PCR reaction was conducted, the primers were for TLR4, NF-kB, TNF-α, HMGB-1, SOCS2, SOCS3, T-bet, GATA-3, Foxp3 and RORC, and the relative mRNA expression of above genes were calculated after the amplification curve was obtained.

2.3 Statistical methods

SPSS 19.0 software was used to input and analyze data, measurement data comparison between two groups was by t test, comparison among three groups was by variance analysis and P< 0.05 indicated statistical significance in differences.

3. Results

3.1 Number of intestinal flora

The number of bifidobacteria and enterococcus in intestinal tract of UC group were (5.23±0.67) lg n/g, (9.84±1.03) lg n/g respectively, and the number of bifidobacteria and enterococcus in intestinal tract of control group were (7.85±0.98) lg n/g, (6.31±0.89) lg n/g respectively. After t test, the number of bifidobacteria in intestinal tract of UC group was significantly lower than that of control group while the number of enterococcus was significantly higher than that of control group. Differences in the number of bifidobacteria and enterococcus in intestinal tract were statistically significant between the two groups of patients (P<0.05).

3.2 Inflammatory response molecule expression in intestinal mucosa

Analysis of inflammatory response molecules TLR4, NF-kB, TNF-α and HMGB-1 expression in intestinal mucosa between two groups of patients was as follows: TLR4, NF-kB, TNF-α and HMGB-1 mRNA expression levels in intestinal mucosa of UC group were significantly higher than those of control group. Differences in TLR4, NF-kB, TNF-α and HMGB-1 mRNA expression levels in intestinal mucosa were statistically significant between the two groups of patients (P<0.05).

Analysis of inflammatory response molecules TLR4, NF-kB, TNF-α and HMGB-1 expression in intestinal mucosa among UC group of patients with different degree of flora disturbance was as follows: TLR4, NF-kB, TNF-α and HMGB-1 mRNA expression levels in intestinal mucosa of UC patients with grade II and grade III flora disturbance were significantly higher than those of UC patients with normal flora and grade I flora disturbance; TLR4, NF-kB, TNF-α and HMGB-1 mRNA expression levels in intestinal mucosa of UC patients with grade III flora disturbance were significantly higher than those of UC patients with grade II flora disturbance. Differences in TLR4, NF-kB, TNF-α and HMGB-1 mRNA expression levels in intestinal mucosa were statistically significant among UC group of patients with different degree of flora disturbance (P<0.05).

Table 1.

Comparison of inflammatory response molecule expression in intestinal mucosa between two groups of patients.

Groups	n	TLR4	NF-kB	TNF-α	HMGB-1
UC group	58	2.31±0.35	1.95±0.23	2.94±0.32	2.67±0.35
Control group	40	1.02±0.15	1.04±0.14	0.98±0.13	0.97±0.11
T		13.289	9.127	19.304	20.318
P		<0.05	<0.05	<0.05	<0.05

Table 2.

Comparison of inflammatory response molecule expression in intestinal mucosa among UC group of patients with different degree of flora disturbance.

UC patients	n	TLR4	NF-kB	TNF-α	HMGB-1
Normal flora and grade I flora disturbance	17	1.44±10.7	1.39±0.16	1.65±0.20	1.62±0.19
Grade II flora disturbance	26	2.23±0.31*	2.03±0.28*	2.88±0.36*	2.52±0.32*
Grade III flora disturbance	15	2.94±0.42*#	2.78±0.32*#	3.85±0.51*#	3.35±0.38*#

*: compared with UC patients with normal flora and grade I flora disturbance, P<0.05; #: compared with UC patients with grade II flora disturbance, P<0.05.

Table 3.

Comparison of immune cell transcription factor expression in intestinal mucosa between two groups of patients.

Groups	*n*	SOCS2	SOCS3	T-bet	GATA-3	Foxp3	RORC
UC group	58	0.42±0.06	0.33±0.05	2.38±0.31	0.38±0.05	0.25±0.03	2.71±0.37
Control group	40	1.02±0.13	0.97±0.11	1.04±0.15	0.95±0.12	1.07±0.12	1.03±0.15
T		13.281	19.338	14.209	16.528	26.877	18.791
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 4.

Comparison of immune cell transcription factor expression in intestinal mucosa among UC group of patients with different degree of flora disturbance.

UC patients	*n*	SOCS2	SOCS3	T-bet	GATA-3	Foxp3	RORC
Normal flora and grade I flora disturbance	17	0.67±0.08	0.61±0.08	1.65±0.20	0.58±0.06	0.70±0.08	1.78±0.22
Grade II flora disturbance	26	0.48±0.07*	0.40±0.05*	2.33±0.28*	0.42±0.05*	0.46±0.06*	2.42±0.32*
Grade III flora disturbance	15	0.31±0.04*#	0.25±0.03*#	3.28±0.40*#	0.23±0.04*#	0.19±0.02*#	3.81±0.51*#

*: compared with UC patients with normal flora and grade I flora disturbance, $P<0.05$; #: compared with UC patients with grade II flora disturbance, $P<0.05$.

3.3 Immune cell transcription factor expression in intestinal mucosa

Analysis of immune cell transcription factors SOCS2, SOCS3, T-bet, GATA-3, Foxp3 and RORC expression in intestinal mucosa between two groups of patients was as follows: T-bet and RORC mRNA expression levels in intestinal mucosa of UC group were significantly higher than those of control group while SOCS2, SOCS3, Foxp3 and GATA-3 mRNA expression levels were significantly lower than those of control group. Differences in SOCS2, SOCS3, T-bet, GATA-3, Foxp3 and RORC mRNA expression levels in intestinal mucosa were statistically significant between the two groups of patients ($P<0.05$).

Analysis of immune cell transcription factors SOCS2, SOCS3, T-bet, GATA-3, Foxp3 and RORC expression in intestinal mucosa among UC group of patients with different degree of flora disturbance was as follows: T-bet and RORC mRNA expression levels in intestinal mucosa of UC patients with grade II and grade III flora disturbance were significantly higher than those of UC patients with normal flora and grade I flora disturbance while SOCS2, SOCS3, Foxp3 and GATA-3 mRNA expression levels were significantly lower than those of UC patients with normal flora and grade I flora disturbance; T-bet and RORC mRNA expression levels in intestinal mucosa of UC patients with grade III flora disturbance were significantly higher than those of UC patients with grade II flora disturbance while SOCS2, SOCS3, Foxp3 and GATA-3 mRNA expression levels were significantly lower than those of UC patients with grade II flora disturbance. Differences in SOCS2, SOCS3, T-bet, GATA-3, Foxp3 and RORC mRNA expression levels in intestinal mucosa were statistically significant among UC group of patients with different degree of flora disturbance ($P<0.05$).

4. Discussion

The intestinal tract is where the microbes gather in the body, and more than 500 species of bacteria locate in the intestinal tract and mainly concentrate in the colon and the end of small intestine[5]. In the physiological condition, obligate anaerobes, such as bifidobacterium and Bacaeroides, are the physiological dominant bacteria in the intestinal tract, account for 99% of the total intestinal flora, and participate in the maintenance of the integrity of the intestinal mucosal barrier; enterobacter, enterococcus and other facultative anaerobes are the conditioned pathogens in the intestinal tract and account for 1% of the total intestinal flora, and their reproduction is in a relatively restrained state. When external factors such as antibiotics, hormones and immune preparation act on the intestinal tract, intestinal flora balance will be broken, and conditioned pathogen reproduction activity increases significantly, which can increase the synthesis of bacterial metabolites and affect local inflammatory response and immune response. The intestinal flora also changes in the pathogenesis of ulcerative colitis[6]. In the study, analysis of the differences in intestinal flora contents between patients with ulcerative colitis and patients with colon polyps showed that the number of bifidobacteria in intestinal tract of UC group was significantly lower than that of control group while the number of enterococcus was significantly higher than that of control group. It means that the decreased Numbers of physiological dominant bacterium bifidobacterium and the increased Numbers of conditioned pathogen enterococcus are associated with the pathogenesis of ulcerative colitis, and the flora disturbance may affect the inflammatory response and immune response to participate in the occurrence of the disease.

Inflammatory response activation is an important pathologic feature of local intestinal mucosa in patients with ulcerative colitis, and TLR4 signaling pathway is an important pathway in vivo to identify pathogen pattern molecules and regulate inflammatory response[7,8].

The intestinal flora disturbance in the intestinal tract of patients with ulcerative colitis can cause the abnormal reproduction of a variety of conditioned pathogens, and TLR4, as pattern recognition receptor, can identify abnormally reproductive conditioned pathogen, pass inflammatory signals by downstream adaptor molecules such as MyD88, and cause transcription factor NF-kB to be activated and transferred into the nucleus; the NF-kB in the nucleus can initiate the transcription of TNF-α, HMGB-1 and other genes and increase the expression of corresponding genes[9,10]. The expression products of TNF-α and HMGB-1 have pro-inflammatory activity, and can promote the infiltration and activation of various inflammatory cells in the intestinal mucosa, and thereby activate the inflammatory response in the intestinal mucosal tissue[11,12]. In the study, analysis of the inflammatory response molecule expression in intestinal mucosa between patients with ulcerative colitis and patients with colon polyps showed that TLR4, NF-kB, TNF-α and HMGB-1 mRNA expression levels in intestinal mucosa of UC group were significantly higher than those of control group. This indicates that the high expression of the inflammatory reaction molecules caused by the activation of TLR4/NF-kB signaling pathway is related to the pathogenesis of ulcerative colitis. Further analysis of the effect of intestinal flora disturbance on inflammatory response molecule expression in intestinal mucosa of ulcerative colitis showed that TLR4, NF-kB, TNF-α and HMGB-1 mRNA expression levels significantly increased in intestinal mucosa of UC patients with grade II and grade III flora disturbance, and the more severe the flora disturbance degree, the more significant the increase of inflammatory response molecule mRNA expression. This confirms that intestinal flora disturbance can activate the TLR4/NF-kB signaling pathway to increase the expression of inflammatory reaction molecules, which leads to inflammatory response of intestinal mucosa and participates in the occurrence of ulcerative colitis.

Abnormal activation of the inflammatory response in the local intestinal mucosa of patients with ulcerative colitis is not only associated with pathogen pattern molecule recognition by TLR4, but also closely related to the immune response disorder and abnormal immune cell differentiation[13]. CD4+T cell subsets Th1, Th2, Th17 and Treg are the important cell mass involved in cellular immune response regulation, the cytokines secreted by Th1 and Th17 cells can mediate local inflammation enhancement and mucosal tissue injury, and the cytokines secreted by Treg and Th2 have anti-inflammatory and negative immunoregulation activity[14-16]. T-bet, GATA-3, RORC and Foxp3 are the specific transcription factors that regulate the differentiation of these four types of T cell subsets, and they are directly related to the differentiation and maturation of cells[17,18]; SOCS2 and SOCS3 are the SOCSs family members that negatively regulate the JAK/STAT signaling pathway activation, and they can induce immune response disorders by affecting the activation of corresponding signaling pathways[19,20]. In the study, analysis of the immune cell transcription factor expression in intestinal mucosa between patients with ulcerative colitis and patients with colon polyps showed that T-bet and RORC mRNA expression levels in intestinal mucosa of UC group were significantly higher than those of control group while SOCS2, SOCS3, Foxp3 and GATA-3 mRNA expression levels were significantly lower than those of control group. This indicates that Th1/Th2/Th17/Treg immune response disorders are associated with the onset of ulcerative colitis. Further analysis of the effect of intestinal flora disturbance on Th1/Th2/Th17/Treg immune response in the intestinal mucosa of ulcerative colitis showed that T-bet and RORC mRNA expression levels in intestinal mucosa of UC patients with grade II and grade III flora disturbance were higher while SOCS2, SOCS3, Foxp3 and GATA-3 mRNA expression levels were lower, and the more severe the flora disturbance degree, the more significant the change of immune cell transcription factor expression. This confirms that intestinal flora disturbance can promote the differentiation of Th1 and Th17 cells and inhibit the differentiation of Th2 and Treg cells so as to cause the intestinal mucosal immune response disorder and participate in the occurrence of ulcerative colitis.

In the above study, we analyzed the correlation of intestinal flora disturbance with inflammatory response and immune response in patients with ulcerative colitis, and it can be preliminarily concluded from the result analysis and discussion that there is intestinal flora disturbance in patients with ulcerative colitis, the number of physiological dominant bacterium bifidobacterium decreases, and the number of conditioned pathogen enterococcus increases; intestinal flora disturbance can lead to inflammatory response activation and immune response disorders.

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