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Short Communication

Changes in Intestinal Flora Diversity and Intestinal Immune Barrier in Patients with Ulcerative Colitis

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ABSTRACT

The objective of this study was to analyze the changes of intestinal flora diversity and intestinal immune barrier in patients with ulcerative colitis (UC). The present study included 58 UC patients admitted to the Department of Gastroenterology in Dongguan Kanghua hospital from August 2017 to August 2019 and enrolled into the observation group. According to Mayo scores, 58 UC patients were divided into remission stage(n=25) and active stage (n=33). Another 50 healthy people who did not have intestinal lesions at the same time were selected as controls. The immunohistochemically results demonstrated that occludin positive cells were mainly expressed in the cell membrane and cytoplasm of epithelial cells and glandular cells, with brownish yellow staining particles. The expression of occludin positive cells in the observation group was significantly lower than that in the control group ($P<0.05$). β - defensin positive cells were mainly expressed in the cytoplasm of intestinal epithelial cells and interstitial cells, with a small amount in the nucleus. The expression of SIgA was significantly higher in the observation group than in the control group ($P<0.05$). SIgA positive cells were primarily expressed in epithelial cells and plasma cells in the lamina propria, and its expression level in the observation group was significantly lower than that in the control group ($P<0.05$). It was concluded that the UC destroys the intestinal mechanical barrier and immune barrier resulting in the dysfunction of intestinal mucosa barrier.

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XL and GU conducted the experiments in this study. XL and FZ contributed to the design and interpretation of the current study and wrote the article.

Key words
Ulcerative colitis, Intestinal flora, Diversity, Intestinal immune barrier

Ulcerative colitis (UC) is a chronic non-specific inflammatory illness of the colon and rectum, which mainly invades the colonic mucosa and submucosa. It has a long course and is common in people aged 20-30 years (Yadav and Liu, 2009). The UC can be clinically manifested as bloody diarrhea, abdominal pain, hematochezia, vomiting and weight loss, etc. Its pathogenesis has remained unclear so far. It may be associated with a variety of factors such as infection and immunity, as well as psychological, genetic and environmental factors, etc. The UC is a refractory disease and it is difficult to manage. It may cause long-term considerable pain to the patients (Atta *et al*., 2019). Relevant information shows that intestinal flora disorder and intestinal immune barrier dysfunction are closely related to the pathogenesis of UC (Sugimoto *et al*., 2017). More than a thousand species of bacterial flora exist in the human gut, which constitute an ecosystem with a wide range of biological activities and play a key role in the development of the host's immunity. The intestinal flora disorder may injure the completeness and feature of the intestinal mucosal barrier. Meanwhile, the secretion of pathogenic bacteria in the intestinal tract could enhance the intestinal permeability. Therefore, both intestinal defense function and immune regulation function are reduced. A large number of bacteria and their metabolites enter the body through the intestinal mucosal barrier, causing or aggravating the occurrence of UC (Randlane *et al*., 2017; Ishaq *et al*., 2017). In recent years, along with the further study of the protective effect of the intestinal barrier, more and more attention has been paid to the intestinal mucosal barrier against the diseases caused by the invasion of foreign pathogens. As a consequence, the purpose of the study is to analyze the differences of the intestinal flora and the changes of the intestinal immune barrier in UC patients.

Materials and methods

Fifty-eight UC patients treated in the Department of Gastroenterology from August 2017 to August 2019 were included into the observation group. This study was approved by the hospital Ethics Committee.

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Inclusion criteria are including: All the patients meet the diagnostic criteria of UC specified by the Chinese Society of Gastroenterology, they are confirmed with UC by electronic colonoscopy as well as enteroscopic findings and mucosal histological tests; their conditions are classified as mild to moderate, with a course of > 3 months; none have taken drugs that may alter the intestinal flora; the patients have all completed the clinical data; the patients and family members who have been informed and signed the informed consent form. Exclusion criteria are including: The patients who have complicated with severe cardiac, hepatic, renal and pulmonary function diseases; who have a previous history of gastrointestinal surgery or organic gastrointestinal disease; who have combined with UC complications; the patients who complicated with malignant tumors; who also have co-infection or autoimmune diseases; pregnant and lactating women. Of the 58 patients, there were 31 male and 27 female subjects, with a range of 25-48 years old, mean age of (40.13±6.01), weight of 45-68 kg, median of (57.32±7.82) kg. These 58 patients were divided into remission stage (n=25) and active stage (n=33) according to Mayo scores. In addition, 50 healthy people who underwent the colonoscopy in our hospital in the same period with no intestinal lesions were chosen as controls, including 28 men and 22 women, between 25 and 50 years old, (39.78±6.11) in average, weight of 45-68 kg and (56.89±7.23) kg in average. The general data of the two groups were comparable according to statistical tests (P > 0.05).

The flora of the two groups was analyzed using traditional drop method. The 0.5g of fresh feces was collected from each subject of two groups and put into a bottle containing 4.5ml of anaerobic diluent. It was shaken up on a vortex oscillator until the feces was qualitative, with a dilution ratio of 10:1. The 9ml of diluent was respectively added to seven sterile tubes and numbered from 2 to 8. Take 1ml from the homogenate bottle and put it into the second tube and so forth for the double ratio dilution. The medium plate was dried in a 37°C incubator overnight. An appropriate dropping diluent was taken. Starting with the high dilution, take 20 µl portion of the mixed diluted bacteria solution each time and drop it into the corresponding diluent of each plate. Each dilution was repeated three times for aerobic and anaerobic cultures after the droplets were absorbed. Bacteria count (CFU/g) = mean colonies×50×dilutability

The expression of intestinal occludin, β-defensin and secretory immunoglobulin (sIgA) were tested by immunohistochemistry for two tested groups. Steps: The intestinal mucosal tissues from the two groups of subjects were fixed using 4% paraformaldehyde. Paraffin sections were dewaxed twice with xylene for gradient dehydration using ethyl alcohol. Then the heat-induced epitope retrieval was performed after the endogenous peroxidase was blocked. 5% BSA blocking solution was added in the tissue sections which were blocking at room temperature for 20 min. Primary antibodies (rabbit-anti-human occludin polyclonal antibodies, rabbit-anti-human β-defensin polyclonal antibodies and mouse-anti-human sIgA monoclonal antibodies) were dropped in the tissue sections which were placed at 37°C for about a hour then overnight, and washed 5 times in PBS. Secondary antibodies were dropped in those sections and washed 4 times in PBS. After that the reagent SABC was added in those sections and washed 6 times in PBS. DBA color development was conducted using a DBA color development kit, as well as going through the procedures of counterstaining with hematoxylin, dewatering, clearing and mounting with neutral gum. The results of each section were analyzed by two pathologists under a double-blind method.

All measurement data of the study were expressed by ($\bar{x}$±s). The means were compared between the two groups *via* an independent sample t-test. P<0.05 was considered statistically significant. The research data were analyzed by SPSS20.0 software package.

Results

The test results of this study revealed that the diversity of the flora was reduced in patients of the observation group compared to the control group (Table I). Of the streptococci, enterococci and peptococcus were not found. The number of bifid bacterium, lactobacillus and veillonella was evidently lower (P<0.05) while the number of enterobacterium, bacteroides and fusobacterium was obviously higher in the observation group than in the control group. The difference was statistically significant (P<0.05).

The number of bifidobacterium and lactobacillus in the remission stage of UC patients was clearly greater than that in the active stage (P<0.05; Table II). Whereas the number of enterobacter was significantly smaller than that in the active stage of UC patients. The difference was statistically significant (P<0.05).

The results of immunohistochemistry showed that occludin-positive cells were mainly expressed in the cell membrane and cytoplasm of epithelial cells and gland cells, showing brown and yellow stained particles (Table III). Their expression in the observation group was significantly lower than that in the control group (P < 0.05). β-defensin positive cells were mostly expressed in the cytoplasm of intestinal mucosal epithelial cells and stromal cells, and a small amount in the nucleus. They were highly expressed in the observation group when compared with controls (P < 0.05). The sIgA-positive cells were primarily expressed in epithelial cells and phlogocytes in the lamina propria.

They were lowly expressed in the observed group when compared to controls ($P < 0.05$).

Table I. Comparison of fecal flora content between the two study groups (IgCFU/g) ($\bar{x}$±s).

Bacterial strain	Observation group (n=58)	Control group (n=50)	t	P
Bifidobacterium	5.16±0.09	9.38±0.61	52.071	<0.001
Lactic acid bacillus	3.25±1.51	6.37±0.73	13.324	<0.001
Streptococcus	—	6.58±0.84	—	—
Enterobacter	9.36±1.51	5.02±1.50	14.939	<0.001
Enterococcus	—	8.26±0.68	—	—
Staphylococcus	3.28±0.82	3.52±0.40	1.885	0.062
Yeast	2.62±0.58	2.45±0.36	1.795	0.076
Bacteroides	9.13±0.05	7.62±1.02	11.267	<0.001
Fusobacterium	9.42±0.28	7.29±0.73	20.549	<0.001
Veillonella	5.49±0.13	8.50±0.30	69.277	<0.001
Peptococcus	—	4.80±0.54	—	—

Table II. Relationship between the activity and intestinal flora in U C patients ($\bar{x}$±s).

Groups	Bifidobacterium	Lactic acid bacillus	Enterobacter
UC remission stage (n=25)	6.69±1.04	5.61±0.68	8.52±1.07
UC activity stage (n=33)	5.02±0.11	3.11±1.02	9.23±1.19
t	12.161	15.531	3.379
P	<0.001	<0.001	<0.001

Table III. Expression change in intestinal mucosal occludin, β-defensin and sIgA ($\bar{x}$±s) of study subjects between the two groups.

Groups	Occludin	β-defensin	sIgA
Observation group (n=58)	28.42±13.01	29.02±10.04	19.15±5.66
Control group (n=50)	44.17±10.86	19.68±6.85	28.29±8.63
t	6.765	5.556	6.590
P	<0.001	<0.001	<0.001

Discussion

UC is a chronic non-specific intestinal inflammatory disease and its etiology remains unclear. Lesions may involve the whole colon and even the terminal ileum. The patients condition varies with recurrent attacks frequently for months or even years or more. The current study suggests that the UC is an autoimmune disease. Moreover, its incidence rate has been increasing year by year in recent years (Shinzaki *et al*., 2017). The intestinal tract is the largest bacterial library in the human body, among which the colon is the densest part of the bacteria. Under the physiological state, the intestinal flora is interdependent and restricting each other, maintaining the balance of the immune system in the intestinal tract, thereby creating a biological barrier for the host (Senator, 2017). For the past few years, some studies have proved that the composition of the microbiota in UC patients has changed compared with normal people. The reduction in the number of intestinal beneficial bacteria in UC patients makes the number of pathogens go up significantly due to the opportunity of large-scale reproduction. Intestinal flora disorders may cause intestinal mucosal injury, thereby inducing intestinal mucosal barrier dysfunction and leading to the occurrence of a variety of digestive tract diseases (Malaisé *et al*., 2018; Hayase and Teshima, 2017). The test results of this study reveal that the diversity of the flora goes down in patients of the observation group compared with the control group. Of the streptococci, enterococci and peptococcus are not discovered. The number of bifidobacterium, lactobacillus and veillonella in the observation group is significantly decreased ($P < 0.05$) while the number of enterobacterium, bacteroides and fusobacterium is significantly increased as compared with the control group ($P < 0.05$). Furthermore, the difference of flora dynamics in the active stage is more evident than the remission stage in UC patients. This suggests that there is a great decline in protective flora but growth in pathogenic bacteria in the occurrence and development of UC. The imbalance of intestinal flora causes the chronic intestinal inflammation.

The occludin protein is an integral membrane protein that may control the macromolecular substances passing through the intestinal epithelial cells. It plays an important role in the intestinal tight junction barrier (Elhelw *et al*., 2017). In addition, the occludin protein plays a certain role in regulating the selectivity and permeability of cellular ions, thus maintaining the difference of substances on both sides of epithelial cells. It is important for keeping the integrity of the intestinal mechanical barrier (Dekky *et al*., 2018). β-defensin is an antimicrobial peptide with spectral and efficient antimicrobial effect. It is mainly synthesized and secreted by epithelial cells and epithelial stromal cells. As an important part of the intestinal chemical barrier, it is less expressed in normal intestinal tissues but hugely expressed in the intestinal mucus layer at the time of inflammation (McGlasson *et al*., 2017). Related data indicate that β-defensin not only has a direct resistance effect on the infection of pathogenic microorganisms, but also boosts the acquired immune system to improve the body's resistance (Dilek *et al*., 2017). sIgA is synthetized by local plasma cells of mucosa and exocrine glands. It plays a vital role in intestinal immunity. Mostly, sIgA either effectively neutralizes pathogens, toxins and other harmful substances in the mucosal epithelium or inhibits and blocks the adhesion of pathogens to the mucosa, thus

protecting epithelial cells (Ochoa-Martínez *et al*., 2017). In addition, sIgA raises the killing ability of lymphocytes by enhancing antigen-dependent cells. It has a synergistic effect with many antibacterial substances (Tzanakakis *et al*., 2017). Through immunohistochemistry detection in the study, we have discovered that occludin positive cells are mainly expressed in epithelial cells, cell membrane and cytoplasm of gland cells, having brown-yellow stained granules. β-defensin positive cells are mostly expressed in the cytoplasm of intestinal mucosal epithelial cells and stromal cells, and a small amount of expression in the nucleus. The sIgA-positive cells are majorly expressed in epithelial cells and plasma cells in the lamina propria. The expression of intestinal mucosal occludin and sIgA in the observation group is significantly less than controls, whereas the expression of β-defensin is significantly more than controls ($P < 0.05$), indicating that the UC patients have clearly intestinal barrier dysfunction.

Conclusion

The diversity of intestinal flora is reduced in UC patients and the structure of normal flora is damaged. Besides, it is correlated with the severity of UC. The UC destroys the intestinal mechanical barrier and the immune barrier, and causes the intestinal mucosa damages resulting in the intestinal mucosal barrier dysfunction.

Funding

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IRB approval

This research was carried out with the approval of Research Guidance Workshop Committee (Dongguan Kanghua Hospital).

Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Data availability

The data of the study would be available on fair request to corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

References

Atta, A.H., Mouneir, S.M., Nasr, S.M., Sedky, D., Mohamed, A.M., Atta, S.A. and Desouky, H.M., 2019. *Asian Pac. J. Trop. Biomed.*, **9**: 98-108. https://doi.org/10.4103/2221-1691.254603

Dekky, B., Ruff, M., Bonnier, D., Legagneux, V. and Théret, N., 2018. *Oncotarget*, **9**: 21366-21382. https://doi.org/10.18632/oncotarget.25106

Dilek, F., Emin, Ö., Gültepe, B., Yazıcı, M., Çakır, E. and Gedik, A.H., 2017. *Turk. Pediatri. Ars.,* **52**: 79-84. https://doi.org/10.5152/TurkPediatriArs.2017.4497

Elhelw, D.S., Riad, S.E., Shawer, H., El-Ekiaby, N., Salah, A., Zekri, A., Amleh, A., Esmat, G. and Abdelaziz, A.I., 2017. *Arch. Virol.*, **162**: 3283-3291. https://doi.org/10.1007/s00705-017-3449-3

Hayase, E. and Teshima, T., 2017. *Clin. Calcium*, **27**: 829-833.

Ishaq, H.M., Shahzad, M., Wu, X., Ma, C. and Xu, J., 2017. *J. Ayub Med. Coll. Abbottabad.,* **29**: 3-7.

Malaisé, Y., Ménard, S., Cartier, C., Lencina, C., Sommer, C., Gaultier, E., Houdeau, E. and Guzylack-Piriou, L., 2018. *Arch. Toxicol.,* **92**: 347-358. https://doi.org/10.1007/s00204-017-2038-2

McGlasson, S.L., Semple, F., MacPherson, H., Gray, M., Davidson, D.J. and Dorin, J.R., 2017. *Eur. J. Immunol.*, **47**: 658-664. https://doi.org/10.1002/eji.201646799

Ochoa-Martínez, P.Y., Hall-López, J.A., Martínez-García, M.A., Diaz-Molina, R. and Moncada-Jiménez, J.A., 2017. *Rev. Chil. Infectol.,* **34**: 27-32. https://doi.org/10.4067/S0716-10182017000100004

Randlane, T., Tullus, T., Saag, A., Lutter, R., Tullus, A., Helm, A., Tullus, H. and Pärtel, M., 2017. *Can. J. For. Res.*, **47**: 1202-1214. https://doi.org/10.1139/cjfr-2017-0080

Senator, S.A., 2017. *Biol. Bull. Russ. Acad. Sci.,* **44**: 1208-1214. https://doi.org/10.1134/S1062359017100144

Shinzaki, S., Matsuoka, K., Iijima, H., Mizuno, S., Serada, S., Fujimoto, M., Arai, N., Koyama, N., Morii, E., Watanabe, M. and Hibi, T., 2017. *J. Crohns. Colitis.,* **11**: 84-91. https://doi.org/10.1093/ecco-jcc/jjw132

Sugimoto, S., Naganuma, M., Iwao, Y., Matsuoka, K., Shimoda, M., Mikami, S., Mizuno, S., Nakazato, Y., Nanki, K., Inoue, N. and Ogata, H., 2017. *Gastrointest Endosc.,* **85**: 639-646. https://doi.org/10.1016/j.gie.2016.11.013

Tzanakakis, M.E., Savopoulou-Soultani, M., Oustapassidis, C.S., Verras, S.C. and Hatziemmanouel, H., 2017. *Entomol. Hellenica*, **14**: 1335-1349. https://doi.org/10.12681/eh.13953

Yadav, P.K. and Liu, Z., 2009. *Recent Pat Inflamm. Allergy Drug Discov.,* **3**: 65-72. https://doi.org/10.2174/187221309787158407