ANALYSIS OF INTESTINAL FLORA DISORDER AND RELATED INFLUENCING FACTORS IN PATIENTS WITH COLORECTAL CANCER

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ABSTRACT

Objective: *To analyze intestinal flora disorder and related influencing factors in patients with colorectal cancer.*

Methods: *85 patients with colorectal cancer who presented in our cancer center between 2018 and September 2019 were selected to form the colorectal cancer study group, and 60 healthy individuals who visited the hospital for physical examination were selected to form the normal control group Logistic regression analysis was used to analyze the risk factors of intestinal flora abnormalities in patients with colorectal cancer.*

Results: *The number of fecal Escherichia coli, Enterococcus faecium, and yeast in the colorectal cancer group was significantly higher than in the control group, while the number of Bifidobacterium and Lactobacillus in the colorectal cancer group was significantly lower than in the control group (P<.05). The relative content of Bacteroides and the number of butyrate-producing bacteria in the colorectal cancer group were significantly lower than in the control group (P<.05). There was no significant difference in total bacterial gene copy number, Bacteroides gene copy number, and coenzyme A (CoA) gene copy number between the two groups (P>.05). Binary logistic regression analysis showed that abnormal body mass index and medication history were risk factors for intestinal flora abnormalities in colorectal cancer patients (OR = 1.609 and 6.059, respectively; P = .003 and .036, respectively).*

Conclusion: *The number of Escherichia coli, Enterococcus faecium and yeast in the intestinal flora of patients with colorectal cancer is significantly higher, indicating that abnormal changes in flora may be an important factor in the occurrence and development of colorectal cancer. Abnormal body mass index and medication history are risk factors for intestinal flora abnormalities in patients with colorectal cancer, and corresponding intervention measures are of great significance for the clinical treatment of colorectal cancer.*

Keywords: *Colorectal cancer, intestinal flora disorder, influencing factors.*

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Introduction

Colorectal cancer is a malignant tumor occurring in the colon or rectum of the digestive tract, with the third highest incidence and the second highest mortality rate in the world, posing a great threat to human health. Patients with colorectal cancer have no obvious symptoms in the early stages, but as the tumor progresses, it may present with different clinical symptoms and signs such as changes in stool habits, abdominal pain, hematochezia, and intestinal obstruction, based on the site of the disease[1]. In recent years, studies have confirmed that 70-90% of colorectal cancer cases are related to dietary factors, and the influence of dietary components on cancer is generally realized through the metabolic activities of human intestinal bacteria[2]. The human intestinal flora in the rectum, comprising about 1014 bacteria, is one of the most important components of the intestinal microbial ecology; mainly by using the nutrients to survive in the human body and thereby playing a role in metabolism, digestion

and absorption, immune regulation, maintenance of intestinal metabolic health, and other processes. However, factors that lead to changes in the amount and type of human intestinal flora can cause intestinal flora disorder as well as changes to and imbalance in the intestinal microenvironment.

Such an imbalance in the composition and function of intestinal flora is closely associated with damage to human health and the occurrence and development of diseases[3, 4]. In recent years, many studies have found that microecological dysregulation not only comprises imbalances in the intestinal environment in the disease state, but also plays a role in the occurrence and development of colorectal cancer; thus, the subject of microecological dysregulation is receiving more and more attention[5]. In order to further understand changes in the clinical indicators of microbiota dysregulation in disease and the related risk factors in patients with colorectal cancer, 85 patients with colorectal cancer admitted to our hospital were evaluated in this study.

Materials and methods

General information

A total of 85 patients with colorectal cancer admitted to our tumor center from 2018 to September 2019 were included in the colorectal cancer group.

Inclusion criteria were as follows:

• Patients diagnosed with colorectal cancer by colonoscopy and pathological examination;

• Patients had first onset;

• Patients in good general condition with an expected survival period of 6 months or more;

• Complete clinical examination data was available;

• All patients and their family members gave informed consent to participate in this study.

The exclusion criteria were as follows:

• Age over 80 years;

• Patients used antibiotics, antacids, or probiotics in the 3 months before the study;

• Patients with inflammatory bowel disease and other gastrointestinal diseases;

• Patients had complications of infectious diseases, metabolic diseases, preoperative radiotherapy, and chemotherapy;

• Intestinal preparation within 2 weeks;

• Patients with other severe internal organ diseases.

The selected patients included 51 males and 34 females aged 30-70 years with an average age of 48.25±5.27 years. The group comprised 58 cases of stage I, 27 cases of autumnal stage, 70 cases of histologically graded adenocarcinoma, 5 cases of undifferentiated carcinoma, and 10 cases of adenosquamous carcinoma. Eight patients had a history of abdominal visceral surgery or a related history of digestive system diseases, and 24 patients had a history of long-term use of antibiotics, non-steroidal anti-inflammatory drugs, and other drugs related to the intestinal flora metabolism.

In addition, 60 healthy people including 32 males and 28 females aged 30-70 years with an average age of 49.45±5.32 years were selected to form the normal control group. All control subjects had no obvious intestinal lesions after colonoscopy, and those who had used antibiotics, antacids, and probiotics in the past 3 months were excluded. There was no statistically significant difference in age and gender between the two groups (P>.05).

Methods

Intestinal flora type culture and test: Fecal samples were collected from the subjects in the 2 groups. Fecal samples were extracted from the patients in the colorectal cancer group before treatment, and microflora analysis was performed by traditional drip seed method as follows. 0.5 g of fresh feces was added to 4.5 ml diluent of anaerobes and shaken on a vortex oscillator until the feces homogenized and the dilution concentration was 10-1.

Seven sterile test tubes were numbered 2-8 and 9 ml diluent was added to each. 1 ml of the homogenate was added to 2 tubes and dried overnight at a dilution of 10-8 in an incubator at 37 °C. The appropriately diluted droplet species was chosen, beginning with a high dilution. 20 μl of the mixed diluted bacterial solution was taken and dropped onto each plate at the corresponding dilution.

The droplet was absorbed and the plate turned over for aerobic culture and anaerobic culture, respectively. Escherichia coli, Bifidobacterium, Enterococcus faecium, Lactobacillus, yeast, and other microbes were cultured and qualitatively and quantitatively analyzed.

DNA analysis of intestinal flora: The same fresh stool samples were stored in the lab in a –4 °C refrigerator. Genomic DNA was extracted using the Tianamp Stool DNA Kit, and all extracted DNA samples were refrigerated at –80 °C for subsequent analysis. The extracted fecal DNA was analyzed by real-time quantitative PCR, and the specificity of the PCR products was analyzed. Escherichia

coli, Bifidobacterium, Enterococcus faecium, Lactobacillus and yeast were detected in the samples by real-time quantitative PCR.

Statistical Methods

The data in this study were analyzed using the SPSS21.0 software package, and the measurement data were expressed as mean ± SD. The t-test was used to compare data from the study group and control group, and the dichronistic logistic regression was used to analyze the risk factors of intestinal flora abnormalities in patients with colorectal cancer. A P value <.05 was considered statistically significant.

Results

Comparison of fecal flora in colorectal cancer patients and healthy population

The number of Escherichia coli, Enterococcus faecium and yeast in the feces of the colorectal cancer group was significantly higher than in the control group, while the number of Bifidobacterium and Lactobacillus in the feces of the colorectal cancer group was significantly lower than in the control group, with the difference being statistically significant (P<.05). The results are displayed in Table 1.

Bacterial species	Colorectal cancer patients	Normal controls		
Escherichia coli	5.57 ± 0.23	4.25 ± 0.26	32.239	< 0.001
Bifidobacterium	4.51 ± 0.28	9.40 ± 0.35	93.311	< 0.001
Enterococcus faecium	5.60 ± 0.29	4.87 ± 0.16	17.680	< 0.001
Lactobacillus	5.59 ± 0.21	7.24 ± 0.13	53.968	< 0.001
Yeast	6.75±0.40	4.41±0.35	36.504	< 0.001

Table 1: Comparison of fecal flora in colorectal cancer patients and healthy population.

Comparison of fluorescence from quantitative PCR of intestinal flora in colorectal cancer patients and healthy population

The fluorescence from the quantitative PCR showed that the relative content of Bacteroides and the number of butyrate-producing bacteria in the colorectal cancer group were significantly lower than in the control group, and the differences were statistically significant (P<.05).

There were no significant differences in the total bacterial gene copy number, Bacteroides gene copy number, and coenzyme A (CoA) gene copy number between the 2 groups (P>.05). See Table 2.

Bacterial species	Colorectal cancer patients	Normal controls		
Total bacterial gene copy number	7.73 ± 1.32	8.16 ± 1.58	1.780	0.077
Bacteroides gene copy number	6.88 ± 1.35	7.22 ± 0.90	1.701	0.091
CoA gene copy number	5.90 ± 0.76	6.12 ± 0.87	1.616	0.108
Relative content of *Bacteroides* (%)	10.87 ± 1.45	17.25 ± 2.34	20.242	< 0.001
Relative content of butyrate-producing bacteria (%)	1.30 ± 0.45	3.08 ± 0.68	18.968	< 0.001

Table 2: Comparison of fluorescence from quantitative PCR of intestinal flora in colorectal cancer patients and healthy population.

Binary logistic regression analysis of risk factors for intestinal flora abnormalities in patients with colorectal cancer

The results of the binary logistic regression analysis showed that abnormal body mass index and medication history were risk factors for intestinal flora abnormalities in patients with colorectal cancer (OR = 1.609 and 6.059, respectively; P=.003 and .036, respectively). See Table 3.

Risk factors	Regression coefficient	Standard error	Wald	OR	*P*	95% CI
Age	8.576	0.626	5.713	0.979	0.738	0.713~1.539
Gender	0.269	20.815	0.116	1.841	0.468	0.341~11.058
Abnormal body mass index	0.404	13.130	0.325	1.609	0.003	1.124~13.541
Medication history	0.358	0.854	8.543	6.059	0.036	1.032~1.634
Medical history	0.278	1.716	4.329	1.112	0.874	1.027~1.687
Pathological type	–8.824	2.185	0.038	0.884	0.842	0.231~1.865
Clinical stages	–0.506	5.814	0.082	0.546	0.523	0.566~5.642

Table 3: Binary logistic regression analysis of risk factors for intestinal flora abnormalities in patients with colorectal cancer.

Discussion

Colorectal cancer is one of the most common malignant tumors in clinical practice. The morbidity and mortality gradually increase with increase in age, but its pathogenesis and specific factors are not very clear. At present, the prevalence of colorectal cancer in China is about 70 per 100,000 population, with an increasing trend in recent years. (6) Epidemiological studies have shown that about 70%-90% of the incidence of cancer is related to lifestyle and environmental factors, among which it has been confirmed that dietary factors

are the most closely associated with the incidence of colorectal cancer[7]. There are a wide variety of microorganisms on the surface and inside the body of humans and animals, most of which exist in the digestive tract. Human health is closely related to the activities of the intestinal flora. The microbial cells in the human gut constitute an ecosystem of interdependence and mutual restriction amongst bacteria and between bacteria and host[8]. Normal intestinal flora can maintain a stable microecological environment in the intestinal tract and resist foreign pathogenic microorganisms, and the dynamic balance of the ecosystem plays an important role in maintaining human health[9]. Relevant data show that in normal human intestinal flora, some bacteria can inhibit inflammation whereas others can cause it. Therefore, intestinal flora have the potential to both inhibit and promote inflammation. According to relevant data, there is a correlation between the occurrence of colorectal cancer and the disorder of intestinal flora[10]. Therefore, in order to improve the treatment effect of colorectal cancer and reduce the incidence of colorectal cancer, we need to strengthen our understanding of the factors affecting colorectal cancer and explore changes in the intestinal flora of patients with colorectal cancer to provide help for future treatment.

Under normal circumstances, the flora and the host maintain a relative balance to form an effective bacterial barrier and resist foreign bacterial infection. Moreover, the normal flora can synergistically promote the metabolism and absorption of endogenous proteins and other substances and play an irreplaceable physiological role in the immune regulation, growth, and development of the host. The flora also participate in the synthesis of vitamins, amino acids, and carbohydrates needed by the host to jointly maintain the processes of life[11,12]. The results of this study showed that the quantities of Escherichia coli, Enterococcus faecium, and yeast in the feces of colorectal cancer patients were significantly higher than in that of controls, while the quantities of Bifidobacterium and Lactobacillus were significantly lower than in controls (P<.05).

Colorectal cancer patients with fecal Escherichia coli and Bifidobacteria in present expressed to the contrary, says aerobe number increases, the decrease in the number of anaerobic bacteria, normal human intestinal anaerobic bacteria accounted for most of the flora, whereas aerobes were fewer. The results of patients with colorectal cancer indicate that the relative balance between the host and flora is disturbed. Thus, the body's immunity and resistance will be reduced to varying degrees. Related data showed that high-fat and high-protein diets can lead to imbalance in the relative composition of anaerobic and aerobic bacteria, which is closely related to the occurrence of colorectal cancer[13]. Aerobic bacteria such as Escherichia coli contain nitro reductase, which converts intestinal contents into nitrite and nitramide, increasing the risk of cancer. Bifidobacterium and other anaerobic bacteria can promote the metabolic activity of intestinal flora and reduce the activity of carcinogenic bacterial enzymes such as nitro reductase; in addition, they can promote the release of certain cytokines and induce the apoptosis of tumor cells[14].

The fluorescence results from the quantitative PCR showed that the relative content of Bacteroides and the number of butyrate-producing bacteria in the colorectal cancer group were significantly lower than in the control group (P<.05). There were no significant differences in the total bacterial gene copy number, Bacteroides gene copy number and CoA gene copy number between the two groups (P<.05). Butyrate can maintain the integrity of the intestinal epithelium, provide energy for cellular respiration, and reduce DNA oxidative damage, and sufficient butyrate in the human intestinal tract is of great importance for intestinal health[15]. The results suggested that the number of butyrate-producing bacteria beneficial to intestinal health decreased significantly in patients with colorectal cancer, and the number of pathogenic bacteria increased. The results of the binary logistic regression analysis showed that abnormal body mass index and medication history were risk factors for intestinal flora abnormalities in patients with colorectal cancer (OR=1.609 and 6.059, respectively; P=.003 and .036, respectively), and appropriate treatment measures could significantly improve the clinical treatment effect of colorectal cancer.

The number of Escherichia coli, Enterococcus faecium, and yeast in colorectal cancer patients is significantly high. Abnormal flora changes may be an important factor for the development of colorectal cancer, while abnormal body mass index and drug history are risk factors for intestinal flora abnormalities in colorectal cancer patients; thus, the development of corresponding intervention measures is of great importance for the effective clinical treatment of colorectal cancer.

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