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Research Article

Effect of Sijunzi Decoction on Regulating Intestinal Flora and Enhancing Immune Function in Patients with Colorectal Cancer

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Abstract

To study the regulatory effect of Sijunzi Decoction on intestinal flora and immune function in patients with colorectal cancer. 100 patients with colorectal cancer were divided into two groups. The control group was received routine chemotherapy, and the experimental group was taking Sijunzi Decoction based on routine chemotherapy. 16S RNA amplicon sequencing was used to quantitatively detect the intestinal flora, and the immune function of the patients was detected by Flow cytometry analysis. The digestive tract reaction was significantly reduced by Sijunzi Decoction, and the abundance of intestinal Lactobacillus and Bifidoacterium were increased, but the abundance of Enterobacteria and fusobacterium nucleatum were reduced. The proportion of CD4 + / CD8 + cells was increased as shown by Flow cytometry analysis. The species and abundance of intestinal flora were changed, and the immune function of patients with colorectal cancer were significantly improved by Sijunzi Decoction.

Key words: Sijunzi Decoction; Colorectal Cancer; Intestinal Flora; Immune Function.

Introduction

According to the 2020 global cancer burden data released by the international agency for research on cancer (IARC) of the World Health Organization, gastrointestinal malignant tumors account for about 50% of all kinds of cancers, and most of the intestinal tumors are found at the advanced stage, lose the opportunity of surgery, and the side effects of chemotherapy will damage the immune function of patients. As the result, how to reduce the side effects of antitumor therapy and improve the curative effect has become an important topic up to now. Many studies have shown that intestinal flora is closely related to the efficacy of intestinal tumors (1), and antitumor effect will be improved by regulating intestinal flora. Sijunzi Decoction, a classic ancient prescription, has a significant effect on improving immune function, also can improve

intestinal flora in mice found by basic research (2), but there is no relevant clinical report. We intend to take patients with intestinal tumors as the research object, compare the changes of intestinal flora types and abundance, to the immune function between the two groups before and after treatment. The correlation between intestinal flora and immune function were analyzed deeply, to clarify the primary and secondary effects, provide clinical basis for reducing the side effects of antitumor treatment, and improve the curative effect and improving the quality of life of patients with gastrointestinal tumors.

Materials and Methods

1. Data collection

General data collection: 100 patients with colorectal cancer hospitalized in Chongqing Ninth People's Hospital from September 2019 to April 2021 (Confirmed by postoperative pathology, stage II with highly risk, or stage III , but enteroscopy and biopsy are required for those who cannot be operated with stage IV), and the syndrome of spleen and stomach Qi deficiency in traditional Chinese medicine is the research object. The diagnosis of traditional Chinese medicine refers to the relevant contents of traditional Chinese medicine diagnostics [3] in the second edition of the new century. Its syndrome type is deficiency of spleen and kidney Qi , obstruction of dampness and turbidity. The collection time was from September 2019 to April 2021. There were 50 cases in the control group, including 28 males and 22 females, aged 22-75 years, with an average age of (62.3 ± 2.7) years, and 50 cases in the experimental group, including 26 males and 24 females, aged 28-76 years, with an average age of (59.5 ± 6.9) years. There was no significant difference in the clinical data between the two groups, so it is comparable. All subjects did not receive any radiotherapy or chemotherapy before operation, and there was no endocrine, immune or metabolic diseases. This study was approved by the ethics committee, the patients and their families have signed the relevant informed consent form.

2. Inclusion and exclusion criteria

Inclusion criteria: All patients were diagnosed with colorectal cancer, including imaging and pathological examination; All patients received chemotherapy for the first time ; Aged 18-80 years; KPS score is greater than 70; Expected survival time ≥ 6 months; Volunteer to participate in this experiment.

Exclusion criteria: Patients with intestinal obstruction; There are diabetes, hypertension, and serious organ diseases such as heart, liver and kidney. With other autoimmune diseases; With other types of malignant tumors; Or participating in other clinical trials.

3. Main reagents

The main reagents include NK, CD4 +, CD8 +, CD4 + / CD8 + kits are provided by the biomedical company of the Academy of Military Medical Sciences.

4. Method

The patients in the control group were treated with mFolfox4 or Xelox chemotherapy according to 2021CSCO guidelines (Guidelines for diagnosis and treatment of colorectal cancer), combined with acid suppression and antiemesis. 21 days was a cycle, and 4 cycles were a course of treatment. Based on the control group, the patients in the experimental group were supplemented with Sijunzi Decoction. The basic prescriptions were: 15-20g Ginseng, 10-15g Atractylodes macrocephala, 9g Poria cocos and 6g Licorice, and 6g Evodia rutaecarpa for nausea and vomiting; Patients with abdominal pain can take 6 g of Corydalis. All patients in the experimental group were treated with Chinese herbal decoction pieces (No decoction), 1 dose per day, 100ml, warmed, taking it in the morning, and evening, and continuously administered for 4 cycles.

Before and after treatment, the species and abundance of fecal intestinal flora were

quantitatively detected by 16S RNA amplicon sequencing. The changes of intestinal flora, digestive tract symptoms, quality of life and immune function were compared between the two groups before and after treatment.

5. Observation indexes and efficacy evaluation criteria

(1) Evaluation of gastrointestinal symptoms after chemotherapy. The Chinese version of the index of nausea, vomiting and retching (INVR) was used to evaluate, 8 entries in total, including three dimensions: symptom duration, occurrence frequency and severity, to evaluate the occurrence frequency, experience time and severity of the three symptoms of nausea, vomiting and retching in chemotherapy patients in the past 12 hours. The scale is scored with 0-4 points, which represent "none at all", "some", "medium", "very obvious" and "profoundly serious and intolerable". The higher the score of each dimension, the more serious the degree of nausea and vomiting. Items 1, 6 and 7 are scored in reverse.

(2) Cell subsets were detected. Fasting venous blood was collected before and after treatment, plasma was separated. CD4 +, CD8 +, CD4+ / CD8 + and NK cells were detected by American Beckman cytoflex flow cytometry.

(3) Detection of intestinal flora. Fresh feces of patients were collected before and after treatment. DNA of intestinal contents was extracted and 16S rRNA gene sequencing method was used. The average clean reads of each sample were ≥ 100000. PCR amplification products of high variable region were used to build a database, and software was used for flora abundance diversity and composition analysis. The species and abundance of intestinal flora were compared between the two groups. The detection and analysis were completed by Shanghai Jingzhou Gene Technology limited company.

(4) Performance status and quality of life assessment. KPS score and QOL (Quality of life of cancer patients) were used to evaluate before treatment and after 4 cycles of treatment. The full score of KPS scale was 100. The higher the score, the better the physical condition of patients; QOL has 12 items in total, with a full score of 60. The higher the score, the better the quality of life.

6. Statistical analysis

SPSS18.0 and GraphPad prism 5.0 were used for statistical analysis. The data was expressed in mean± standard deviation. One way ANOVA was used for multigroup analysis. Independent sample t-test was used for the comparison between the two groups. $p < 0.05$ was statistically significant.

Results

1. **Comparison of side effects of chemotherapy between the two groups**

The patients in the control group received routine chemotherapy, while the patients in the experimental group took Sijunzi Decoction based on chemotherapy, and the digestive tract symptoms were compared. It can be seen from table 1 that there was no significant difference in symptom scores between the two groups before treatment ($p > 0.05$). The symptoms scores of the two groups after treatment were lower than those before treatment ($p < 0.01$), but the experiement group was significantly lower than that of the control group. See Table 1. The data difference between the two groups was statistically significant ($p = 0.017$).

2. Evaluation of immune function

The comparison of immune function showed that CD4 +, NK and CD4 +/ CD8 + cell ratio increased significantly and CD8 + cells decreased significantly in the experimental group. It can be seen from table 2 that NK cells in the experimental group increased from (8.22 ± 1.45) before treatment to (9.87 ± 1.38); CD4 + increased from (38.27 ± 5.11) to (41.22 ± 3.81) before treatment; CD8 + decreased from (25.62 ± 4.62) to (25.62 ± 4.62) and CD4 + / CD8 + increased from (1.63 ± 0.42) to (1.99 ± 0.62). There was significant

difference between the two groups ($p < 0.05$).

3. Changes of intestinal flora

There was no significant difference in the number of main intestinal flora between the two groups before treatment ($p > 0.05$). After treatment, the number of non-probiotics such as Enterobacter and fusobacterium was decreased and the number of probiotics such as Bifidobacterium and Lactobacillus was increased in the experimental group, which was statistically significant compared with that before treatment ($p < 0.05$). The result of control group was opposite ($p < 0.05$). See Table 3.

4. Performance status and quality of life score

The KPS and QOL scores of the two groups before and after treatment were compared. After treatment, the KPS and QOL scores of the experimental group were significantly higher than those of the control group ($p < 0.05$, table 4).

Table 1. Comparison of Symptom Scores Before and After Treatment ($\bar{x} \pm s$).

Group	n	Treatment time	Symptom scores
Experimental group	50	Before treatment	41.3 ± 16.5
		After treatment	9.6 ± 3.2* △
Control group	50	Before treatment	4.05 ± 15.4
		After treatment	28.9 ± 9.9*

Notes: *, indicates a comparison with that before treatment, $p < 0.05$; △, indicates the comparison with the control group, $p < 0.05$.

Table 2. Comparison of Immune Function Between Control Group and Experimental Group Before and After Treatment ($\bar{x} \pm s$).

NK cell (%)	Before treatment	8.26±1.92	8.22±1.45	0.892	0.496
	After treatment	8.47±1.89	9.87±1.38*	12.769	0.000
CD4+cell (%)	Before treatment	39.68±4.52	38.27±5.11	0.763	0.518
	After treatment	30.35±2.87*	41.22±3.81*	12.721	0.000
CD8+ (%)	Before treatment	25.35±4.21	25.62±4.62	0.046	0.947
	After treatment	31.11±4.10*	25.62±4.62*	12.054	0.000
CD4+/CD8+ (%)	Before treatment	1.42±0.57	1.63±0.42	0.081	0.982
	After treatment	1.31±0.49	1.99±0.62*	6.742	0.000

Notes: *Compared with the same group before treatment, $p < 0.05$.

Table 3. Comparison of Species and Quantity of Intestinal Flora Between the Two Groups Before and After Treatment($\bar{x} \pm s$, lgCFU / g).

Group	n	Time	Lactobacillus	Bifidobacterium	Enterobacter	Fusobacterium
Experimental group	50	Before treatment	5.28 ±0.67	6.43 ±0.70	8.32 ±0.73	3.67 ±0.45
		After treatment	9.46±0.82*△	12.93 1.57*△	4.22±0.65*△	2.30±0.34*△
Control	50	Before treatment	5.36±0.80	6.72±0.67	8.51 ±0.47	3.28 ±0.32

group	After treatment	4.33±0.62*	5.06 ±0.62*	9.68 ±0.59*	4.86 ±0.55*

Notes: *, comparison with that before treatment, $p < 0.05$; △, comparison with the control group, $p < 0.05$.

Table 4 Comparison of KPS Score and QOL Score Between the Two Groups Before and After Treatment (n=50, $\bar{X}$ ±s, score).

Group	KPS score		QOL score	
	Before treatment	After treatment	Before treatment	After treatment
Experimental group	69.89±5.89	75.34±4.78*	46.89±5.78	50.56±4.99*
Control group	69.72±6.33	81.79±6.55*△	45.67±5.43	59.21±5.62*△
T value	0.053	4.973	0.743	6.926
P value	0.895	0.000	0.502	0.000

Notes: *, comparison with that before treatment, $p < 0.05$; △, comparison with the control group, $p < 0.05$.

Discussion

Traditional Chinese medicine is the "Quintessence" inherited by the Chinese nation from generation to generation for thousands of years. Starting from the Holistic view of "Unity of heaven and man," it systematically and comprehensively discusses the anatomical morphology, pathophysiology, etiology and pathogenesis of the body, forming a complete medical theoretical system with the characteristics of Chinese traditional culture. It contains many concepts and theoretical systems closely related to modern microecology(4-5). The overall concept of traditional Chinese medicine, the unity theory of biology and internal and external environment of microecology, the "yin-yang" theory of traditional Chinese medicine, the theory of microecological balance and imbalance, the spleen and stomach theory of traditional Chinese medicine, the theory of digestive tract microecological system, the theory of traditional Chinese medicine adjusting "yin-yang", strengthening health and eliminating evil, and the regulation theory of microecology all have amazing consensus and integration. It shows that they are unified in basic principles and methodology, and there are some common laws.

Sijunzi decoction is a basic prescription for strengthening spleen and Qi. It is composed of Ginseng, Atractylodes macrocephala, Poria cocos and Licorice. Ginseng has a wide range of pharmacological effects on human immune system, cardiovascular system, central nervous system, digestive system and endocrine system. It can enhance appetite, promote blood cell formation, and enhance body adaptability and immune function (6); The therapeutic mechanism of Atractylodes macrocephala on digestive system diseases is reflected in the protection of gastrointestinal motility and gastric mucosa. At the same time, Atractylodes macrocephala also has the functions of promoting hematopoietic function, enhancing cellular immunity, bacteriostasis, anti-inflammatory and anti-tumor (7).

Poria cocos contains Poria cocos polysaccharide and other components, which can significantly regulate and enhance the body's immunity and improve the content of 2 in red blood cells, Diphosphoglyceride (2,3-DPG) level. Other studies have shown that single ginseng and poria cocos can promote the proliferation of bifidobacteria in test tubes or plates and inhibit a variety of bacteria in vitro; Glycyrrhiza has the pharmacological effects of anti-inflammatory, anti-virus and anti-allergy. The flavonoids of Glycyrrhiza have inhibitory effects on Staphylococcus aureus, Streptococcus, Bacillus subtilis, fungi and yeast. Animal experiments

showed that the number of bifidobacteria and Lactobacillus in the intestinal tract of mice increased, while the number of enterobacteria and enterococci decreased after intragastric administration of Sijunzi Decoction for 7 days (8).

The experimental results show that Sijunzi Decoction can significantly reduce the side effects of chemotherapy, enhance the immune function of the body, promote the growth of beneficial bacteria such as bifidobacteria and reduce the growth of harmful bacteria such as enterobacteria and fusobacterium, while the recovery of normal intestinal flora is conducive to the digestion and absorption of sugar, protein, vitamins and micro elements in food, enhance the intestinal barrier and further improve the immune function of the body. The enhancement of immunity is also conducive to the colonization of beneficial bacteria in intestinal mucosa. In addition, the regulation of intestinal flora by oral preparation of Sijunzi decoction may be related to preventing the colonization of pathogenic bacteria on intestinal mucosa. Of course, this needs to be confirmed by further experiments. It is precise because of the regulating effect of Sijunzi Decoction on the whole body, and its regulation is multi-channel and multi-level, which has both direct and indirect effects, so that the body can give full play to its normal functions of resisting and eliminating evil, removing evil, recovering, stopping diarrhea, and restoring the intestinal microecology and immune function to normal.

Although some preliminary results have been obtained, the molecular mechanism of the interaction between intestinal flora and immunomodulatory cells is not clear. We need to further study how intestinal symbiotic flora regulates immunotherapy. Although several research results have been published in recent years, the results are contradictory (9). This also suggests that our research on the relationship between intestinal flora and immune system has just begun. In the future, the research on the functional characteristics of different intestinal flora and the interaction mechanism between intestinal flora and immune system will enable us to further master the characteristics of intestinal flora, better detect them, and finally improve the efficacy of immune checkpoint inhibitors by manipulating them (10).

In addition, although some progress has been made in the research on intestinal flora and tumor treatment at this stage, there are still some deficiencies, such as inconsistent detection technology, small sample size, insufficient sampling depth, lack of flora classification standards and so on. In addition, the existing results are also lack of research on the interaction between different intestinal flora. The impact of the steady-state destruction of intestinal flora and the relationship between fungi, viruses and other microorganisms and tumors are also issues that need to be paid attention to in the future.

Abbreviations

RNA, Ribonucleic acid; CSCO, Chinese Society of Clinical Oncology.

Declarations

1) ***Consent to publication***
We declare that all authors agreed to publish the manuscript at this journal based on the signed Copyright Transfer Agreement and followed publication ethics.

2) ***Ethical approval and consent to participants***
Not applicable.

3) ***Disclosure of conflict of interests***
We declare that no conflict of interest exists.

4) ***Funding***
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5) ***Availability of data and material***

We declare that the data supporting the results reported in the article are available in the published article.

6) Authors' Contributions

XLY designed and managed the whole study. YC wrote the manuscript and completed all figures and tables. JYZ, QY and WJ helped to revise the manuscript. All the authors have read and approved the final manuscript

7) Acknowledgement

None

8) Authors' biography

None

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