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Dissociation Effect of Curing Irritable Bowel Syndrome with Probiotics and its Function of Flora Equilibrium

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The irritable bowel syndrome (IBS) is a kind of disease syndrome composed of such characteristics as abdominal pain, abdominal distension, bowel habit and abnormal defecate character, symptom persistence or intermittent attacks, abnormal biochemical indicators without morphology. It is a disease of bowel function disorder with higher morbidity, whose clinical manifestation is complex and varied. And it is divided into three clinical syndromes of diarrheic type, constipation type and alternating diarrhea and constipation type. The changing condition of intestinal flora has been analyzed for IBS patients through research and observation. This paper has studied the dissociation effect of applying probiotics in curing irritable bowel syndrome of IBS patients and its function of balancing microflora. This paper has found that: 1. there exists abnormal intestinal flora among IBS patients, and it represents the phenomenon of decreasing anaerobic probiotics such as bifid bacterium and lactobacillus etc, the increase of aerobic bacteria such as enteric bacilli, as well as the weakening colonization resistance of intestinal tracts, which suggests that there exists alteration of intestinal flora among IBS patients, and dysbacteriosis maybe one of the important causes of IBS.

Key words: Probiotics; strains of microorganisms; dissociation effect; the function of balancing flora**.**

The microecological balance is a dynamic physiological combination during different developmental stages of normal microbiota and its host which are formed in long-term evolution process. This combination refers to the normal and interactional physiologic entity of corresponding ecological space structure in normal microbiota ecological structure at all levels, as well as the body and body surface of host under the influence of common macro environment conditions. And the internal structure and existing condition of this entity is the microecological balance. There are more than 400 kinds of microorganism in human intestinal, where Bifidobacterium, lactobacillus acidophilus, streptococcus faecalis accounts for more than 98%, and the rest of them are the bacteria and harmful bacteria in intermediate state. Under normal condition, these bacteria are in the state of relative balance. When the organism body is influenced by factors like environment, climate, food, medicine and mood etc, the germ in the intermediate state of intestinal tract will transform into pathogenic bacteriumÿmeanwhile pathogenic bacterium is of mass propagation, thus makes the intestinal micro ecology system lose equilibrium, as a result, the dysbacteriosis appears, then occurs the intestines problem. The microorganism in intestinal tract has played an important role in

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providing nutrition for intestinal mucosa cells. When the intestinal flora is in the state of microecological balance, the population of beneficial bacteria group in intestinal tract is dominant. The probiotic has a variety of physiological functions such as anti- infection, nourishment, activating the immune system, antitumous effect and anti-agingetc. Xiaohua Hou etc has performed analysis on the bacterial flora changes of IBS faeces in diarrheic type in 1990, and found that there exists dysbacteriosis in the faeces of such patients.

The research for clinical data

Case Selection

All the disease cases are selected from 26 patients who looked at the clinic in our digestive internal medicine from March 2011 to June 2011 (age 42.88±13.40 years old, male14/female 12), meanwhile 25 people without digestive tract symptom in normal control group are selected according to the ratio of age and sex (age 38.76±9.33 years old, male 13/female 12)[1]. There is no significant difference on the age between two groups, Pÿ0.05 (P=0.21). All entrants are inquired about their living habits such as disease history, diet, and sport and so on in detail, and offered physical examination. The general data of research subjects in two groups is shown in Table 1.

Inclusion Criteria

Conforming with Roman b IBS diagnostic criteria: recurrent episodes of abdominal pain or discomfort. There are at least 3 attacks within 3 months recently, accompanying with following 2 or more than two points: 1. The symptom is relieved after defecating.2.The change of defecation frequency is accompanied with breaking out[2]. 3. The change of fecal's character is accompanied with attack through physical examination including blood, urine, dung routine, blood biochemistry and abdomen ultrasound[3]. The intestinal organic disease is excluded through examination of electronic colonoscope or barium enema. Abdominal discomfort refers to the discomfort which is difficult to describe with pain[4].

Exclusion Criteria

Patients' age is smaller than 18; people who has the intestinal organic disease or other serious illnesses such as cardiovascular, respiratory, kidney and so on, or people who has previous abdominal surgery during pregnancy or lactation, and the patients who have not used medicines such as expansive agent, antidiarrheal, spasmolysis medicine, probiotics, and antibiotics etc within recent one month, people who are allergic to probiotic preparations[5].

Materials

Specimen Collection

About 10g fresh dejection will be extracted naturally from all entrants after being selected, then it is put into the box of excrement specimen which is sterile with ethylene oxide and waits for inspection after seal immediately. The required time should be controlled within 30 minutes strictly[6]. One specimen is extracted from each patient with IBS within 4 weeks' treating and 2 weeks after withdrawal respectively according to above method, the living habits keep unchanged during this period[7].

The Choice of Medium

Selective cultivating of bifidobacterium MRS, selective medium of lactobacillus LBS, bacteroid (it is mainly weak brittle bacteroides) selective mediumBBE, enterococcus (it is mainly enterococcus faecalis) selective mediumPSE agar, enteric bacilli) it is mainly escherichia coli) selective medium EMB[8]. The composition of each culture medium is shown as following:

Usage: 37.4g ingredient is taken from the products, it is heated, mixed and dissolved into 1000ml distilled water, then it is put into triangular flask respectively, finally it is performed with autoclaved sterilization for 15 minutes at 121°C for standby application.

The Main Situation of Instrument and Equipment

(1) BHC-1300aA2 biohazard safety equipment: Sujing antai air Technology Company;
(2) GSKP-01Ba Thermostatic water- jacket incubator: medical instrument factory in Huangshi city of Hubei province;
(3) YQX-a YQX-II: Planted medical equipment manufacturing co., LTD in Shanghai;
(4) Sterile plastic bag and waste collector are purchased from equipment department of provincial hospital in Anhui province;
(5) OlympusCX40 optical microscope: The products of Japan Olympus Company;
(6) BCD-216 refrigerator: RongShiDa co., LTD;
(7) All Electronic scales, High- Pressure Steam Sterilization Pot, Quantitative liquid-moving machine, Sterile tubes, Sterility petri dish,

Glass Beads, inoculating loop, L-type bar, microscope, marking pen are provided by Microbiology teaching and research section of Anhui Medical University[9].

The Source of Drug Reagents

(1) High pure nitrogen is provided by Microbiology teaching and research section of Anhui Medical University.
(2) All the selective medium is purchased from Hai bo biological technology co., LTD in a high-tech park of Qingdao.
(3) Gram stain is provided by Microbiology teaching and research section of Anhui Medical University.
(4) Bacteria tube of biochemical identification is purchased from Microbial reagents co., LTD in Hangzhou.
(5) Bifico: it is purchased from SINE in Shanghai, specification: Each capsule contains 210mg powder, component: bifidobacterium, eosino-
(6) lactobacillus, Enterococcus sanlian living bacteriumÿthe number of living bacterium contained in them is not less than 1.0×10000000 CFU respectively; Patients buy it from provincial hospital pharmacy of Anhui province.

EXPERIMENTAL

Produce Stool Specimens

1g fresh dejection in the center part of specimen is taken sterilely, then it will be put into sterile erlenmeyer flask which contains a few glass beads, 9ml diluent is added into itÿthis is the first dilution degrees (1:10), full and rapid shock could blend them as far as possibleÿthen 0.5ml specimen and 4.5mldiluent are added into sterile tubes for shock and blending, then it is diluted to 10^{-8} ^{10} continuously according to the method of 10 times serial dilution.

The Vaccination of Specimen

50uL of 10^{-3},10^{-4},10^{-5} at different dilutability are respectively taken and vaccinated into MRS, BBE, LBS culture medium, PS Eagar culture-medium, 20uL of 10^{-3},10^{-4},10^{-5} at different dilutability are respectively taken and vaccinated into EMB culture medium, then they are evenly coated with L-form SS-Spreader[11].

The Cultivation of Specimen

After inoculating MRS, BBE, LBS culture medium, they are put into anaerobic tank of glove type for cultivating 48h-72h respectively, after PS Eagar culture-medium and EMB culture medium are inoculated, they are put into conventional incubator at constant temperature for cultivating 24h-48h respectively[12].

The Identification of Bacteria

The bacteria are authenticated to the level of genera through applying the method of three-level bacteria identification method. Identifying steps: 1.Morphological observation: first it needs to observe whether the size, color, shape, edge of cultivated bacterial colony is neat, transparent and smooth, and other representation such as its purity and living infectious microbe; 2.Separation and purification: the bacterial clump grown in culture medium is selected to extend the purification culture, and the purification colony obtained from passage will be in morphological observation, then it is of gram stain, the composition germ of bacterial colony is clear and definite to be Gram-positive or Gram-negative bacterium, and its bacillus or coccus shape, size of thallus under light microscope will be clear, whether it has capsule, flagella and spore etc.3. Biochemical identification (fermentation experiments of sugar alcohol): the separated and purified aerobic bacteria should be inoculated into biochemical identification tube, and then it is put into calorstat for 24 h's cultivating, the separated and purified anaerobic bacteria will be inoculated into assessor, which is then placed in anaerobic chamber for 24 h cultivating at 37°C, the situation of changing liquid will be observedÿit is positive if its color changes, otherwise, it is negative. Finally the results could be determined according to control table of direction for use through applying assessor of microscale biochemistry[13].

The Count of Bacterial Colony

Proper number of culture medium between 30-300 with appropriate dilution degrees and uniform growth is selected to perform colony counting, the average number is taken at the same dilutability, and the bacteria content in per gram samples is calculated, formula CFU/g) (ml)specimen=the average number of bacterial colony at dilution degrees* dilution ratio*50. Finally all the results take the form of common

numerical value log10CFU/g. Colonization resistance refers to the resistance of host to exotic bacteria's settling down in the gut, normal value is bigger than 1)[14].

The Evaluation of Living Quality and Anxiety, Depression

Eight dimensions including physiological function (PF), role physical (RP), body pain (BP), general health (GH), vitality (VT), social function (sF), role emotion (RE) and mental health (MH) are evaluated through applying Dungeon Hunter sF-36 BSRS, scoring method: through applying Li ke accumulative method, raw score is calculated according to the last topic value, then transmuted score is calculated with standard formula. The scoring range of each dimension is from 0 (the poorest) -100 (the best)[15], which is compared with normal control group's matching; the anxious degree of patients with IBS is evaluated through applying SAS manifest anxiety scale, and the results conforms with Chinese norms, the boundary value of SAS standard score is 50, where 50-59 score is regarded as mild anxiety, 61-70 score is regarded as moderate anxiety, more than 70 score is regarded as severe anxiety; the depression degree is evaluated through applying SDS depression scale for patients with IBS, the results are defined according to Chinese norms, the standard boundary value is 53 score, 53-62 score is mild depression; 63-72 score is moderate depression; More than 72 score is major depressive disorder[16].

Therapeutic Method and Curative Effect Observation

Patients with IBS are given three pill of bifid triple viable (bifico) for three times every dayÿthe course of treatment is four weeks. The rating scale of intestinal symptoms is filled for patients with IBS before and after treatment respectively (Table 2). All the symptoms before and after treatment are evaluated for patients with IBS respectively according to rating scales .The total score is compared and observed.6 aspects including the time of abdominal pain, frequency of abdominal pain, abnormal defecation shape, abnormal defecation process, the situation of mucous stool and defecation are evaluated, each is counted as 0~3 score, the total score is 0~l8 score. Conspicuous effect: transference cure or symptoms integral has decreased more than 80%. In effect: the decrease of symptoms integrale ≥50 %80~<%; nullity: the decrease of symptoms integral is less than 50ÿ.The total effective rate= (the number of conspicuous effect case + the number of effective case)/the total number of cases*100%. defecation shape refers to: the proportion of unshapen, pasty, watery stool, sheep-dung in faeces; the abnormal proportion in defecating process: the proportion of urgency, endless, strenuosity feeling in defecating.

Statistical Approach

All the survey data is inputted into computer for setting up database, statistic analysis is performed through applying statistical software spss16.0, the difference between two groups is tested with Chi-squared test and t-test according to data distribution characteristics, P"ÿ0.05 is regarded as that the difference has statistical significance.

RESULTS

Bacterial Culture and Identification Result

Enteric bacilli: Violet black circular colonies and transparent colony with colourless periphery and black center is formed in Eosin-Methy Blue Agar Medium, the edge of the colony is finishing, slightly protruding the surface of the medium, and the surface is metallic luster. Microscopically: the basic morphological characteristics of escherichia coli: the bacteria is Gram-negative and curtobacterium with both blunt ends, most of them are individualism or germination, but it does not present permutation of long chain,some strains have capsule or microcapsule; germ will not be formed. Biochemical reaction and oxidase reaction (-). Glucose glucose oxidation test is fermentation, nitrate reduction test (+), dynamic test (+). 2. Enterococcus: it decomposes esculoside in PSE culture medium, and represents protuberant colonies of white dot, its periphery is accompanied with clear halo; microscopically: Gram-positive, round or oval aureus, most of them are arranged in gemination or short chain; biochemical test indicates: enzyme touching experiment (-), bile esculoside experiment (+), which could grow in 65g/l sodium chloride broth, pyrrolidone aryl amides enzymes (PYR) experiment (+). 3. Bacterium lacticum: Round and milky large colonies are formed on LBS culture medium, the

edge is smooth and neat, the surface is rough, petri dish emits special sour. Microscopically: Short gram positive bacillus, arranged in single row, pairing or gridÿmost of which are in orderliness. Biochemical test: it could ferment yeast glucose, lactose and sucrose, esculoside experiment (+), indole test (-), Catalase test (-), nitrate reduction test (-), ,not liquefied gelatin.4.Bifidobacterium: Cheese colony which is light yellow, circular, dry and has neatly smooth edge is formed on Mupirocin lithium salt improved MRS culture medium; Microscopically: Gram positive non-spore- forming long coli, it is of diverse morphology , most of them are bending and furcation shape,

Table 1. The general data of research subjects in two groups

General data	IBS group (n=26)	Control group (n=25)
Age(year old)	42.88±13.40	38.76±9.33
Sex(male/female)	14/12	13/12

Table 2. Eosin-Methylene Blue Agar (EMB)

Formula: (g/L)	
Peptone	10.0
Milk sugar	10.0
Dipotassium phosphate	2.0
Agar	15.0
Eosin	0.4
Methylene blue	0.065
pH value 7.1 ± 0.2	25°C

Table 3. Rating scales of intestinal symptoms among patients with IBS

Symptom	0 score	1 score	2 score	3 score
stomachache/the occurring times of abdominal discomfort (Hours/day)	none	<1	2-8	>8
stomachache/the occurring times of abdominal discomfort (Days/week)	none	1-2	3-5	6-7
The proportion of abnormal defecation shape	none	<1/4	1/4-3/4	>3/4
The abnormal proportion of defecation process	none	<1/4	1/4-3/4	>3/4
The proportion of mucous stool	none	<1/4	1/4-3/4	>3/4
The ration of abdominal distension in defecation	none	<1/4	1/4-3/4	>3/4

Table 4. The comparison for the number of flora and B/E value between patients with IBS and normal control group(x ±s, Log10 CFU/g muck)

Group	IBS group(n=26)	Control group(n=25)	P value
enteric bacilli	8.66±0.82	8.20±0.73	P<0.05
enterococcus	6.59±1.17	6.76±0.95	Pÿ0.05
lactobacillus	6.21±0.97	7.07±1.14	P<0.01
bifidobacterium	8.36±1.03	8.92±0.88	P<0.05
bacteroid	8.64±0.75	8.43±0.53	Pÿ0.05
B/E value	0.97±0.15	1.09±0.13	P<0.01

Table 5. The comparison results of living quality between patients with IBS and normal control group

The dimensions of living quality	Control group(n=25)	IBS group(n=26)	P value
PF	94.80± 8.84	81.73±19.89	P<0.01
RP	91.00±24.87	61.54±43.72	P<0.01
BP	87.76±14.96	58.42±16.09	P<0.01
GH	76.60±13.43	39.92±16.09	P<0.01
SF	85.50±13.35	66.83±23.17	P<0.01
VT	72.60±11.83	52.12±18.82	P<0.01
RE	76.00±31.21	44.87±39.93	P<0.01
MH	73.12±13.19	60.31±17.12	P<0.01

representing the arrange of Y, V character. Biochemical test: biochemical reaction is not active, and could ferment glucose and galactose, dynamic test (-), indole test (-), nitrate reduction test (-), catalase test (-), not liquefied gelatin. 5. Bacteroides : protuberant colony of white dot is formed from decomposing esculoside on BBE culture medium, its periphery is accompanied with large and black halo; microscopically: Gram-negative bacillus coli, uneven dyeing, there is no color or lighter dyeing in the center of thallus, both ends are blunt and engrain with different length. Biochemical test: it could ferment glucose, maltose and sucrose, esculoside experiment (+) is able to bear or endure 20% bile and grow exuberantly, catalase test (+), indole test(-), nitrate reduction test (-), not liquefied gelatin.

The Analysis for the Number of Flora and Customization Resistance for Patients with IBS and Normal Control Group

The number of customization in faeces of 26 patients with IBS is higher than normal control group, the difference between them is significant ($P<0.05$); the number of bifidobacterium is lower than normal control group, the difference is significant ($P<0.05$); the number of lactobacillus is significantly lower than normal control group, the difference between them is extremely obvious ($P<0.01$); while the difference among enterococcus, bacteroid and normal control group has no significance (Pÿ0.05) and statistical significance; B/E value is significantly lower than normal control group ($P<0.01$). See Table 4.

The Comparison for the Living Quality, Anxiety and Depression State between Patients with IBS and Normal Control Group

Compared with normal control group, the average product of living quality among 26 patients with IBS has declined obviously on 8 dimensions (all $P<0.01$), the general health (GH) has declined the most obviously among them (average integration 76.60±13.43VS39.92 ±16.09), the average integration of other dimensions has declined to different degree, which are role emotional (RE) average score: 44.87±39.93, vitality (VT) average score: 52.12±18.82, bodily pain (BP) average score: 58.42±16.09, mental health (MH) average score: 60.31±17.12, role physical (RP) average score: 61.54±43.72, social function (SF) average score: 66.83±23.17, physiological function (PF) has lowest influence, the average score is 81.73±19.89 respectively. See Table 5.

The research discussion

The research has verified that there exists alteration of intestinal flora among IBS patients, including the decrease of bifidobacterium and lactic acid bacillus, as well as the increase of bacteroides fragilis, enteric bacilli and enterococcus when compared with healthy people. While the stool specimens could not represent the flora state of colon completely, and the increase of anaerobic bacteria, enterohemorrhagic escherichia coli and bacteroides fragilis has been found through colonic mucosa biopsy among IBS patients. According to the theory of microecology, there exists feasibility in curing or preventing the morbidity of IBS through restoring intestinal flora by supplementing probiotics. Bifidobacterium species and lactic acid bacillus could balance the intestinal flora through directly supplementing beneficial and normal physiology bacteria; prevent the invasion and reproduction of pathogenic bacterium. The bifidobacterium species and lactic acid bacillus has such functions as the inhibition and promotion of biological chemistry, competition for nutrients, immune elimination, the competition of adhesion receptor. They could reduce the PH value of intestinal tract and redox reduction potential through inhibting other harmful bacteria with bacteriocin secretion, whose advanced glycosylation end product is short-chain fatty acid, which could produce antagonism effect to other microorganism and directly inhibit the growth of harmful pathogens through competing for limited special nutrients with other bacteria. Meanwhile it also has the function of anti-infection, antitumous effect, various synthesis protein and vitamin, as well as cleaning virus.

The research conclusion

At present, there are many reports about curing IBS with probiotics, most of the curative effect is good. The result indicates the lower number of bifidobacterium species and lactic acid bacillus in faeces of IBS patients in diarrheic type and higher number of bacteroides fragilis, enteric bacilli, enterococcus and saccharomycetes when compared with healthy control groupÿwhich is similar with reports at home and abroad. After 4 weeks' treatment, the number of flora in group A and B has recovered to the level of health control

group. But after 10 d's treatment, the curative effect of group is not significant, but the effect of group B is significant. Meanwhile, after 4 weeks of drug withdrawal, there is no statistical significance between the intestinal tract constitution of group B and control group (P>0.05), while there is statistical significance between the flora constitution of intestinal tract in group A and control group (P<0.05). This indicates that taking probiotics could obviously improve the imbalance condition of intestinal flora in diarrhea group, and the intake of larger doses could rapidly and durably improve the imbalance condition of intestinal flora among IBS patients in diarrheic type. The earlier stage of micro ecological imbalance in human body is reversible, timely adjusting living habit, dietary pattern and using all kinds of probiotics or biostime preparation could eliminate and reverse the condition of micro ecological imbalance, thus keep away from disease and return to health. The pathogenesis of IBS is not completely clear at present. We have found that there generally exists intestinal microecological imbalance among IBS patients from previous research. The micro ecological imbalance is involved in the occurrence and development of IBS symptom in all aspects,and probiotics treatment has made certain curative effect in some research, but it could not pinpoint the causal relationship between intestinal microecological imbalance and IBS because of the methodology limitation and incomplete knowledge about intestinal flora. As this paper has short observation time and less diseases cases, the forwarding effect is still difficult to know. In order to explore the more effective method of curing diarrhea-predominant IBS, thus the research about observation cases and long course of treatment should be further expandedÿas a result, it could provide new train of thought for the pathophysiology and remedy of IBS.

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