Effects of Probiotics on Gut Microbiota in Patients with Inflammatory Bowel Disease: A Double-blind, Placebo-controlled Clinical Trial

Mahdi Shadnoush, Rahebeh Shaker Hosseini, Ahad Khalilinezhad, Lida Navai, Hossein Goudarzi and Maryam Vaezjalali
National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Department of Immunology, Medical School, Shahid Beheshti University of Medical Sciences, Department of Clinical Nutrition and Dietetics, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Department of Microbiology, Medical School, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background/Aims: Several clinical trials have revealed various advantages for probiotics in inflammatory bowel disease (IBD). The aim of this study was to further investigate the effects of probiotic yogurt consumption on gut microbiota in patients with this disease.

Methods: A total of 305 participants were divided into three groups; group A (IBD patients receiving probiotic yogurt; n=105), group B (IBD patients receiving placebo; n=105), and control group (healthy individuals receiving probiotic yogurt; n=95). Stool samples were collected both before and after 8 weeks of intervention; and population of Lactobacillus, Bifidobacterium and Bacteroides in the stool specimens was measured by Taqman real-time PCR method.

Results: By the end of the intervention, no significant variations in the mean weight and body mass index were observed between three groups (p>0.05). However, the mean numbers of Lactobacillus, Bifidobacterium, and Bacteroides in group A were significantly increased compared to group B (p<0.001, p<0.001, and p<0.01, respectively). There were also significant differences in the mean numbers of either of three bacteria between group A and the healthy control group; however, these differences between two groups were observed both at baseline and the end of the intervention.

Conclusions: Consumption of probiotic yoghurt by patients with IBD may help to improve intestinal function by increasing the number of probiotic bacteria in the intestine and colon. However, many more studies are required in order to prove the concept.

(Key Words: Inflammatory bowel diseases; Microbiota; Probiotics)

INTRODUCTION

Inflammatory bowel disease (IBD) occurs in one of two forms, ulcerative colitis (UC) and Crohn’s disease (CD), mainly in the second-to-fourth decade of life, and with unknown etiology. The two forms of the disease closely resemble each other, so that it is difficult to distinguish between them, even pathologically; however, they are sufficiently different so that they are regarded as independent entities. First, higher prevalence and incidence of IBD were reported in northern areas of Europe and America, and lower prevalence of the disease was reported among Asian people. However, recent data show a rapid increase in prevalence of IBD, worldwide; particularly in Asia and Iran, which is presumably the result of changes in the life style and nutritional habits of the inhabitants of these areas, high smoking, and development of new diagnostic tools for the disease.

Human gut microbiota consists of more than one thousand species of bacteria, among which 99% belong to Firmicutes (such as, Lactobacillus), Bacteroidetes, Proteo-
bacteria, Actinobacteria.\textsuperscript{14,15} Anaerobic microorganisms, including Lactobacillus, Bifidobacterium, Clostridium, Porphyromonas, and Bacteroides, are the most common bacteria residing in colon.\textsuperscript{16,17} Recently, researchers have suggested two theories regarding the role of bacteria in pathogenesis of IBD. First, malfunction of the immune system against the bacteria of the intestinal natural flora. Second, alteration in gut microbiota or malfunction of mucosal barrier resulting in harmful immunological responses against mucosa may be implicated in the pathogenesis of IBD.\textsuperscript{18,19} Indeed, it seems that combination of these two mechanisms leads to inflammation and abnormal immune responses, involving alterations in gut microbiota and epithelial-cell function.\textsuperscript{20,21} In addition, clinical evidences have revealed a significant role of gut microbiota, especially flora of distal ileum and colon, in the pathogenesis of IBD.\textsuperscript{19,21}

Probiotics, which are live microbial dietary supplements, have beneficial effects on host health, probably by improving its intestinal microbial balance. Lactobacilli, Bifidobacteria and Streptococci are commonly used probiotics.\textsuperscript{22,23} Many clinical trials have been conducted in order to clarify the effects of probiotics on inflammatory and non-inflammatory conditions, including IBD, allergies, rheumatoid arthritis, infections, gastrointestinal microbiota, etc., and revealed various advantages of probiotics.\textsuperscript{24-27}

Several studies have reported that the use of probiotic products, particularly probiotic yogurt, has beneficial effects on intestinal function and gut microbiota in patients with UC and CD.\textsuperscript{28-31} For example, it is suggested that the probiotics, containing different strains of Lactobacillus and Bifidobacterium, are efficient in maintaining microbiota balance in the intestine.\textsuperscript{20,32} In addition, Ishikawa et al.\textsuperscript{33} found that consumption of Bifidobacterium-fermented milk by patients with UC for one year decreased the stool concentration of Bacteroides; however, some controversial data have been reported.\textsuperscript{34,35}

We conducted a clinical trial in order to investigate the effects of probiotic yogurt consumption on gut microbiota in patients with IBD using molecular approaches.

**SUBJECTS AND METHODS**

1. Subjects

Subjects consisted of 210 IBD patients with no manifestation of acute inflammation during histological examination (198 UC patients in remission status defined as a UC clinical activity index score \(\leq 4\), an endoscopic index score \(\leq 4\), and 22 CD patients in remission status defined as a CD clinical activity index score \(\leq 150\) referring to the hospitals or liver and gastrointestinal research center of Shahid Beheshti University of Medical Sciences, confirmed by the gastroenterologist after a comprehensive physical examination and considering the patient’s history and manifesting symptoms, such as diarrhea, constipation, anemia, pain, abdominal cramps, rectal bleeding, bowel movement urgency, etc. The eligibility of participants was evaluated based on specific criteria.

The patients were divided into two groups; group A or probiotic yogurt (n=105), and group B or placebo (n=105). A group of 95 healthy volunteers, who met inclusion criteria without IBD history, were also followed as the control group. Various types of medications were used by the patients, including mesalazine, sulfasalazine, and budesonide. The participants had not used prebiotics, probiotics, antioxidants and omega 3 supplements and also antibiotics within the 3 months before the investigation. Patients with rheumatoid arthritis, diabetes, infectious and other gastrointestinal diseases, and lactating and pregnant women were excluded from the study. In addition, all participants were instructed to maintain the previous lifestyle, such as exercise, diet, and smoking during the period of the intervention.

Fiber and energy intake among subjects was assessed by a nutritionist through a three-day dietary recall, in which all changes in nutritional regime of the subjects were recorded at baseline and at the end of intervention. It was hypothesized that any changes in fiber and energy intake might be affected by intervention and or might affect the results.

2. Intervention

Both probiotic yogurt and placebo with 1.5% fat, in identical 250-g packages marked A and B, respectively, and with 20-day shelf time, were kindly provided from Pegah Dairy Industries (Tehran, Iran). Each 250 g of probiotic yogurt contained Lactobacillus acidophilus La-5 and Bifidobacterium BB-12 with the mean concentration of 106 colony-forming units (CFU)/g of yogurt. The total numbers of bacteria were controlled before each intervention. Group A and B received probiotic and placebo 250 g/day, respectively; for 8 weeks.
In line with double-blind design, neither the patients nor the technicians knew the type of yogurt given to each patient. The healthy control group also consumed 250 g from probiotic yogurt for the same duration.

3. DNA extraction and real-time PCR assay

For evaluation of gut microbiota, before and at the end of the intervention, stool samples were collected from all participants and preserved at \(-20^\circ\text{C}\). For homogenizing, 1 g of each stool sample was suspended in 9 mL of phosphate-buffered saline and centrifuged for 2 minutes, and then 200 \(\mu\text{L}\) of each sample was taken for DNA extraction, using a QiAamp DNA stool mini kit according to the manufacturer’s guidelines (Qiagen, Hilden, Germany). The obtained total DNA was preserved at \(-20^\circ\text{C}\).

Real-time PCR (Taqman method) was performed using the manufacturer’s protocol (Qiagen). Briefly, 100 ng DNA and 600 nM of each primer were added to 12.5 \(\mu\text{g}\) of Taqman master mix (Fermentase, Waltham, MA, USA), and then DNA was amplified. The primers and probes (Table 1) were designed using the Primer Express software version 2.0 (Applied Biosystems, Foster City, CA, USA) and were constructed by Takapouzist Company under supervision of experts from the microbiology department of Shahid Beheshti University of Medical Sciences.

4. Statistical analysis

Nutritionist IV software (The Hearst Corp., San Bruno, CA, USA) was used for analysis of the total nutrient intake. In all groups, for the data following normal and non-normal distribution, mean values before and after intervention were compared by paired \(t\)-test and Wilcoxon- signed ranks, respectively. The baseline mean value among groups was compared by one-way ANOVA test for normal data, and by Kruskal-Wallis test for non-normal data. Linear regression test was used for monitoring the energy effect on nutrient intake. Data were expressed as mean±SD and \(p\)-value < 0.05 was considered statistically significant.

5. Ethics

The ethics committee of Shahid Beheshti University of Medical Sciences approved the use of the clinical information and collection of samples for research purposes (89/01/100/7384/5091). All patients and control subjects signed a written informed consent letter. The patients were not asked to stop their medications during the intervention.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Type</th>
<th>Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bifidobacterium</strong></td>
<td>F_bif IS</td>
<td>GGG ATG CTG GTG TGG AAG AGA</td>
</tr>
<tr>
<td></td>
<td>R_bif IS</td>
<td>TGC TCG CGT CCA CTA TCC AGT</td>
</tr>
<tr>
<td></td>
<td>P_bif IS</td>
<td>FAM-TCA AAC CAC CCG CCA-BHQ1</td>
</tr>
<tr>
<td><strong>Lactobacillus</strong></td>
<td>F_lact</td>
<td>TGG ATG CCT TGG CAC TAG GA</td>
</tr>
<tr>
<td></td>
<td>R_lact</td>
<td>AAA TCT CCG GAT CAA AGC TTA CTT AT</td>
</tr>
<tr>
<td></td>
<td>P_lact</td>
<td>FAM-TAT TAG TTC CGT CCT TCA TC-BHQ1</td>
</tr>
<tr>
<td><strong>Bacteroides</strong></td>
<td>HuBacf</td>
<td>GGG TTT AAA GGG AGC GTA GG</td>
</tr>
<tr>
<td></td>
<td>HuBac594Bhqf</td>
<td>CTA CAC CAC GAA TCC CGC CT</td>
</tr>
<tr>
<td></td>
<td>HuBac594Bhqf</td>
<td>FAM-TAA GTC AGT TGT GAA AGT TTG CGG CTC-BHQ1</td>
</tr>
</tbody>
</table>

*Bifidobacterium, Lactobacillus, and Bacteroides load in stool of the participants by Taqman real-time PCR method.*

**Fig. 1.** Flow chart of the present experiment. Group A, inflammatory bowel disease (IBD) patients receiving probiotic yogurt; Group B, IBD patients receiving placebo; Control group, healthy individuals receiving probiotic yogurt.
and none of the participants had any problem with yogurt consumption.

**RESULTS**

As shown in Fig. 1, a total of 86 patients (32 females and 54 males) in group A, 90 patients (40 females and 50 males) in group B, and 84 healthy individuals (56 females and 28 males) in the control group continued their participation until the end of study; 19 patients from group A, 15 patients from group B, and 11 healthy individuals from the control group were dropped out due to personal issues, any changes in the dose of medications according to the physician’s discretion, or failure to follow the required schedule of the study. No complication such as abdominal discomfort, nausea, vomiting, bloating, diarrhea, etc. was observed following consumption of the probiotic yogurt.

1. **Anthropometric features**

The mean age and height of group A were 36.63±9.07 years old and 1.7±0.07 m, respectively; and no significant (p > 0.05) difference was found in comparison with group B (placebo) and the healthy group (Table 2). The mean weight and BMI of the three groups are shown in Table 3. No significant (p > 0.05) variations in the mean weight and BMI were observed between three groups, both at baseline and at the end of the intervention (Table 3).

2. **Nutrients intake**

The results of mean energy and nutrient intake assessment via two recalls showed insignificant differences in the intake of protein, total fat, saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid, cholesterol, carbohydrate, calcium, and vitamin D between and also within the groups, both at baseline and at the end of the intervention (p > 0.05). Although the fiber intake was significantly different between group A and group B at the end of the intervention (p < 0.05), the energy intake showed no significant variation between two groups (p > 0.05).

3. **Changes in Populations of Lactobacillus, Bifidobacterium, and Bacteroides**

All the designed primers were completely specified for the target bacteria, not interfering with other microorganisms residing in the intestinal. As shown in Table 4, at baseline there was no significant difference in the mean number of either Lactobacillus or Bifidobacterium or Bacteroides between groups A and B (p > 0.05), while at the end of the intervention the mean numbers of Lactobacillus, Bifidobacterium, and Bacteroides in group A were significantly increased compared to group B (p < 0.001, p < 0.001, and p < 0.01, respectively). This increase might be due to consumption of...
Table 4. Stool Concentrations of Lactobacillus, Bifidobacterium and Bacteroides in Group A and Group B before and after the Intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>End</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus (CFU/g)</td>
<td>6.1±0.4</td>
<td>8.3±0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>5.9±0.3</td>
<td>6.1±0.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>p-value</td>
<td>0.079</td>
<td>&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>Bifidobacterium (CFU/g)</td>
<td>7.3±0.3</td>
<td>10.5±0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>7.1±0.3</td>
<td>6.8±0.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>p-value</td>
<td>0.099</td>
<td>&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>Bacteroides (CFU/g)</td>
<td>1.7±0.1</td>
<td>1.1±0.2</td>
<td>0.034</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>1.9±0.1</td>
<td>2.2±0.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>p-value</td>
<td>0.079</td>
<td>0.005</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are presented as mean±SD.
Group A, inflammatory bowel disease (IBD) patients receiving probiotic yogurt; Group B, IBD patients receiving placebo; CFU, colony-forming unit.

Table 5. Stool Concentrations of Lactobacillus, Bifidobacterium and Bacteroides in Group A and Control Group before and after the Intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>End</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus (CFU/g)</td>
<td>6.1±0.4</td>
<td>8.3±0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>6.2±0.4</td>
<td>7.9±0.3</td>
<td>0.009</td>
</tr>
<tr>
<td>p-value</td>
<td>0.033</td>
<td>0.021</td>
<td>-</td>
</tr>
<tr>
<td>Bifidobacterium (CFU/g)</td>
<td>7.3±0.3</td>
<td>10.5±0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>8.2±0.2</td>
<td>9.1±0.2</td>
<td>0.009</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>-</td>
</tr>
<tr>
<td>Bacteroides (CFU/g)</td>
<td>1.7±0.1</td>
<td>1.1±0.2</td>
<td>0.034</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>3.1±0.2</td>
<td>3.9±0.3</td>
<td>0.037</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are presented as mean±SD.
Group A, inflammatory bowel disease patients receiving probiotic yogurt; Control group, healthy individuals receiving probiotic yogurt; CFU, colony-forming unit.

probiotic yogurt by patients in group A. There was also a significant difference in the mean numbers of either Lactobacillus or Bifidobacterium or Bacteroides between group A and the healthy control group (p < 0.05, p < 0.05, and p < 0.001, respectively); however, these differences between two groups were observed both at baseline and the end of the intervention (Table 5). These findings demonstrate that the numbers of Lactobacillus, Bifidobacterium, and Bacteroides in patients with IBD might not change compared to healthy individuals, although regarding the well-known beneficial effects of such microorganisms, the observed increase in their numbers, following consumption of probiotic yogurt, might help to improve gut function in patients with IBD.

In addition, the increase in the numbers of either Lactobacillus or Bifidobacterium or Bacteroides at end of the intervention compared to the baseline were statistically significant within group A (p < 0.001, p < 0.001, and p < 0.01, respectively) and also within the healthy control group (p < 0.01, p < 0.01, and p < 0.05, respectively); however, such changes within group B (placebo) were statistically insignificant (p > 0.05). These findings further support the incremental effects of probiotic yogurt on gut populations of Lactobacillus, Bifidobacterium, and Bacteroides in patients with IBD.

DISCUSSION

During life, microbial colonization of intestine and colon is affected by many factors, including nutrition, environment, host physiological events, anatomical and physiological structure of the gastrointestinal lumen. Probiotics are live microbial dietary supplements that have beneficial effects on host health. Although IBD is mainly curable by surgery and is controlled by some medications, several studies have revealed that the use of probiotic products, particularly yogurt probiotic has beneficial effects on gut function and microbiota in patients with UC and CD, although some controversial data have been reported.

Our results indicated that probiotic yogurt consumption by patients with remission course of IBD and also by healthy control subjects significantly increased the stool concentration of Lactobacillus and Bifidobacterium, and decreased stool concentration of Bacteroides, while the consumption of placebo did not result in significant change in stool concentration of these bacteria.

These findings are in accordance with those reported by Cui et al., where the consumption of capsules, containing Bifidobacterium, by IBD patients for 8 weeks, significantly increased the stool concentration of Bifidobacterium and Lactobacillus, compared to placebo. Venturi et al. also observed that the consumption of probiotics by patients with UC, containing various strains of Bifidobacterium, lactoba-
cillus and Streptococcus, for 12 months resulted in significant increase in stool concentration of these bacteria, but showed no significant change in concentration of Bacteroides, Clostridiums, coli forms and other aerobic and anaerobic microorganisms.

In addition, there are many studies in agreement with our investigation. For example, in the study of García-Albiach et al., the use of probiotic yogurt by healthy individuals resulted in predominance of lactic acid bacteria and reduction of Bacteroides strains in stool. Tannock et al. found that the intestinal concentration of Lactobacillus was higher in healthy people consuming Lactobacillus-rich milk, compared to consumers of plain milk. Uyeno et al. also observed an increase in intestinal population of Lactobacillus in healthy individuals consuming Lactobacillus-rich probiotic yogurt.

In the current clinical trial, the consumption of probiotic yogurt caused a weak, yet significant reduction in the concentration of Bacteroides, which was in line with the findings of Ishikawa et al., who observed significant decrease in Bacteroides vulgatus stool population caused by one-year consumption of Bifidobacterium-fermented milk in patients with UC. However, in the study of Kato et al., consumption of Bifidobacterium-fermented milk for 12 weeks did not significantly change the concentration of stool Bacteroides. Therefore, it seems that the amount of duration of probiotic yogurt consumption might be one of the factors determining its effect on stool concentration of Bacteroides, and perhaps other microorganisms.

Presumably, gastrointestinal microorganisms affect the host by different mechanisms, such as fermentation, intestinal motility, colonization and limiting pathogenic microorganisms, production of some vitamins, butyrate and short chain fatty acids, mineral uptake, and transformation of bile acids, steroids, etc. For example, it is reported that Bifidobacterium and Clostridium residing in the gastrointestinal tract convert the nutritional fibers into short chain fatty acids that provide 10% of the body energy. In addition, in patients with CD the removal of carbohydrates from the nutrition schedule improved the disease outcome, suggesting a role for microbial fermentation in the pathogenesis of the disease. In the current study, we observed a significant difference in fiber intake between the studied groups at the end of the intervention; however, the energy intake showed no significant difference. This result indicates that the consumption of probiotic yogurt might have affected the metabolism of fibers, which led to the body requiring further absorption of the fibers.

In conclusion, our findings indicate that consumption of probiotic yogurt by patients suffering from IBD may help to improve intestinal function by increasing the number of helpful bacteria such as Lactobacillus and Bifidobacterium in the intestine and colon. However, the probable side effects of these bacteria and the mechanisms by which they affect human health remains to be well-elucidated.

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REFERENCES


