

Effect of Probiotics on Symptoms in Korean Adults with Irritable Bowel Syndrome

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Background/Aims: Irritable bowel syndrome (IBS) is a troublesome disease. Some strains of probiotics reportedly exert remarkable immunomodulatory effects, and so we designed a prospective double-blind randomized placebo-controlled clinical study to assess their effects in Korean adults with IBS. **Methods:** IBS patients who met Rome III criteria were randomly assigned to receive composite probiotics or placebo. A total of 20 billion lyophilized bacteria were administered twice daily for 8 weeks. Primary outcome variables were symptom scores consisting of abdominal pain, flatulence, defecation discomfort, and sum of symptom scores. A visual analogue scale was used to quantify the severity. Secondary outcome variables consisted of the quality of life and bowel habits including defecation frequency and stool form. **Results:** Thirty-six and 34 patients were randomized to the probiotics and placebo groups, respectively. Intention-to-treat analysis showed significant reductions in pain after 8 weeks of treatment: -31.9 and -17.7 in the probiotics and placebo groups, respectively ($p=0.045$). The reductions in abdominal pain, defecation discomfort, and sum of scores were more significant in 58 patients with a score of at least 3 on the baseline stool-form scale. **Conclusions:** Composite probiotics containing *Bifidobacterium bifidum* BGN4, *Lactobacillus acidophilus* AD031, and other species are safe and effective, especially in patients who excrete normal or loose stools. (*Gut and Liver* 2009;3:101-107)

Key Words: Probiotics; Irritable bowel syndrome; *Bifidobacterium bifidum*

INTRODUCTION

Irritable bowel syndrome (IBS) is one of the most troublesome diseases, which has the high prevalence as well as the chronic and recurrent course. In United States, IBS is known as the most common gastrointestinal disease and comprises 25 to 50% of all referrals to gastroenterologists,¹ and the prevalence of IBS has estimated to range 9% to 22% of the population.² In Korea, Park *et al.*³ reported the prevalence of IBS increased up to 16.8%. A few effective medicines such as cisapride, tegaserod and alosetron have been withdrawn from the market because of their serious adverse drug reactions. There is no specific treatment that has proven to be effective and safe in the patients with IBS.

Through previous studies, IBS is known to be associated with low-grade inflammation of the intestinal mucosa regardless of whether to be the post-infectious subtype or not.^{4,5} Although a few clinical studies to evaluate the immunomodulatory effect of probiotics showed symptom relief in IBS patients, probiotics are not yet used widely in daily practice.⁶⁻⁸ As global competition for searching more potent strain was heating up, a well-designed clinical study became necessary to validate the effect of promising probiotics. Two *in vitro* studies using some strains of *Bifidobacterium bifidum* demonstrated that they were effective in inhibiting lipopolysaccharide (LPS)-induced inflammation, and the later study showed high LPS-binding capacity and inhibition of inflammatory cytokine.^{9,10} Kim *et al.*¹¹ reported *Bifidobacterium bifidum* BGN4 strain had the significant immunomodulatory effect

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on the control of inflammatory bowel disease (IBD) using a mouse model. With composite probiotics including *Bifidobacterium bifidum* BGN4 and other promising strains, we designed a prospective double-blind randomized placebo-controlled clinical study to prove the effect in the Korean adults with IBS.

MATERIALS AND METHODS

1. Study population

Patients were recruited from the outpatient department of Seoul National University Hospital. The study protocol was approved by the institutional review board. The inclusion criteria were as follows: age of 19-75 years, both male and female, and the presence of previous gastrointestinal symptoms suggestive of IBS using the Rome III criteria regardless of its subtypes. All participants gave a written informed consent form that had been approved by the institutional review board.

The exclusion criteria were as follows: previous abdominal surgery except appendectomy and hernia repair, history of IBD, current use of medications that may alter gastrointestinal motility, antibiotics or probiotics within 2 weeks prior to the 1-week run-in period, severe co-morbidity such as cancer, heart or renal failure, gynecologic disease etc., and pregnant or breast-feeding female.

2. Study design

We performed a parallel-group, double-blinded, randomized, placebo-controlled clinical study. A 1-week run-in observation period was followed by an 8-week treatment period. During this entire 9-week period, participants were required to record a daily diary of bowel habits consisting of frequency and consistency. A questionnaire on irritable bowel symptoms such as abdominal pain, flatulence and defecation discomfort was recorded at baseline, 4th and 8th week after treatment. A questionnaire on quality-of-life (QOL) was recorded at baseline and 8th week.

Primary outcome variables were symptom scores that consisted of abdominal pain, flatulence, defecation discomfort and the sum of these three symptom scores. Laborious evacuation, tenesmus and urgency were included in questionnaire for defecation discomfort. A 100 mm visual analogue scale (VAS) was used to measure the severity of each symptom as scores ranging from 0 to 100. When participants were asked to mark VAS after treatment, they could look at his or her previous marks. Secondary outcome variables were bowel habits that recorded using a validated Bristol stool form scale and QOL that recorded using a RAND 36-item health survey.¹²⁻¹⁴

3. Administration of probiotics

Composite probiotics were composed of 4 viable lyophilized bacteria species: *Bifidobacterium bifidum* BGN4; *Bifidobacterium lactis* AD011; *Lactobacillus acidophilus* ADO31; and *Lactobacillus casei* IBS041. Each probiotic packet with equal doses of 4 strains contained total 20 billion lyophilized bacteria in a powder form. A placebo packet containing skim milk powder looked identical to the composite probiotics. Both probiotics and placebo were supplied by BIFIDO Co., Ltd., Hongchun, Korea. Each participant in both treatment groups received one packet orally with water within 10 minutes after a meal, twice daily (40 billion lyophilized bacteria per day) for 8 weeks.

4. Randomization

We used blocked randomization method with block size 4 or 6 and generated permutations at random using SPSS for Windows 12.0.1 (SPSS Inc., Chicago, IL, USA) provided by the medical research collaborating center at our institution that was independent of medical care. All participants were assigned an allocation number in regular sequence after confirmation of enrollment. For adherence to double-blind design, the allocation number was matched to a randomization code successively by a clinical trial pharmacist. Till completion of the study, the clinical trial pharmacist kept the randomization table sealed off.

5. Sample size calculation and statistical analyses

On the ground of previous reports, we assumed the response rates would be 70% in probiotics group and 40% in placebo group.⁸ The response was defined as reduction of symptom score by at least 50% after treatment. Other assumptions for sample size calculation were as follows: alpha error 0.05; statistical power 0.8; drop-out rate 0.05; and one-sided test. We used the equation below, the sample size was estimated as 35 per group in view of drop-out rate.

$$N = \frac{\left(Z_{\alpha} \sqrt{2p(1-p)} + Z_{\beta} \sqrt{p_1(1-p_1) + p_2(1-p_2)} \right)^2}{d^2}$$

$$p_1 \text{ (response rate in probiotics group)} = 0.7$$

$$p_2 \text{ (response rate in placebo group)} = 0.4$$

$$p = (p_1 + p_2) / 2 = 0.55$$

$$d = p_2 - p_1 = 0.3$$

$$Z_{\alpha} = 1.65 \text{ (alpha error} = 0.05)$$

$$Z_{\beta} = 0.84 \text{ (statistical power} = 0.8)$$

All data were collected by a single trained interviewer who was a clinical research coordinator. Week 0 (the end

of run-in phase) was considered as baseline in all statistical analyses. The “intent-to-treat” (ITT) population was defined as all participants who received probiotics or placebo for at least one week and visited our hospital for the interview once or more. Efficacy analysis was performed in the ITT population. χ^2 -test was performed to test response rates. As symptom score, QOL score, consistency and frequency were all continuous variables, two-sided T-test was performed using the 0.05 significance level.

RESULTS

Between 1 November 2007 and 29 February 2008, 76 patients were screened. 5 patients (6.5%) were ineligible as they did not meet the inclusion criteria, and 1 withdrew consent. A total of 70 patients were enrolled, 36 were randomized to probiotics group and 34 to placebo. Demographic and clinical characteristics were similar between the two groups (Table 1).

1. Compliance and concomitant medications

Of 36 participants assigned to probiotics, 35 completed treatment as planned. One participant withdrew from the study due to an exacerbation of abdominal pain associated with IBS. Another one participant, who was assigned to placebo, withdrew from the study due to an exacerbation of constipation associated with IBS. Both withdrawn participants were included in efficacy analysis

Table 1. Characteristics of Subjects

Characteristics	Probiotics (n=36)	Placebo (n=34)
Age (years)		
Mean (\pm SE)	36 \pm 2	38 \pm 3
Range	21-69	22-72
Females (n)	11	12
Baseline score (\pm SE)		
Pain	50.3 (\pm 3.5)	46.9 (\pm 3.4)
Flatulence	49.9 (\pm 3.6)	49.3 (\pm 4.8)
Defecation	53.5 (\pm 4.0)	47.0 (\pm 5.1)
Sum	153.6 (\pm 8.9)	143.2 (\pm 9.0)
QOL	104.6 (\pm 1.1)	104.7 (\pm 1.4)
Defecation frequency*	8.1	7.1
Stool consistency [†]	4.2	4.0
IBS subtype [‡]		
Diarrhea	19	13
Constipation	7	7
Mixed	2	4
Unsubtyped	8	10

QOL, quality-of-life.

*Bowel movements per week, [†]Bristol stool form scale; average for a week, [‡]Rome III criteria.

based on the definition of ITT population; symptom scores and the bowel habit were imputed using the mean value of the group. A total of 68 participants completed the study. Overall compliance was more than 98% in both groups.

Eleven of 70 participants required concomitant medications during the study, 3 in probiotics group and 8 in placebo. Ten participants, except one in probiotics group who was prescribed a common cold medication, used loperamide, prokinetics, pain killers, histamine 2 receptor antagonists, proton pump inhibitors or laxatives due to an exacerbation of bowel symptoms. According to ITT principle, all the eleven patients were included in analyses.

2. Symptom scores, QOL and bowel habits

ITT analyses showed significant reductions of pain score after 8 weeks of treatment (−31.9 in probiotics group vs. −17.7 in placebo [p=0.045]) and defecation discomfort after 4 weeks of treatment (−29.2 vs. −13.5, respectively [p=0.043]). Subgroup analyses in 58 patients whose baseline Bristol stool form scales were 3 or more

Table 2. Symptom Scores Analyzed over 4 and 8-week Treatment Period (Two-sided T-test Using the 0.05 Significance Level)

		Probiotics	Placebo	p-value
ITT population (n=70)				
Pain	Baseline	50.3	46.9	0.487
	Δ 4 week	−23.9	−10.9	0.061
	Δ 8 week	−31.9	−17.7	0.045
Flatulence	Baseline	49.9	49.3	0.928
	Δ 4 week	−18.5	−18.4	0.982
	Δ 8 week	−27.0	−21.3	0.437
Defecation	Baseline	53.5	47.0	0.311
	Δ 4 week	−29.2	−13.5	0.043
	Δ 8 week	−30.5	−18.4	0.122
Sum	Baseline	153.6	143.2	0.413
	Δ 4 week	−71.7	−42.8	0.115
	Δ 8 week	−89.5	−57.5	0.064
Subgroup* (n=58)				
Pain	Baseline	51.2	46.2	0.315
	Δ 4 week	−26.9	−5.8	0.004
	Δ 8 week	−33.9	−13.3	0.006
Flatulence	Baseline	49.2	47.8	0.826
	Δ 4 week	−19.6	−13.0	0.463
	Δ 8 week	−26.7	−15.9	0.175
Defecation	Baseline	53.2	45.0	0.826
	Δ 4 week	−30.4	−10.6	0.013
	Δ 8 week	−30.0	−14.5	0.064
Sum	Baseline	153.6	138.9	0.288
	Δ 4 week	−76.9	−29.4	0.010
	Δ 8 week	−90.6	−43.6	0.010

*Baseline Bristol stool form scale \geq 3.

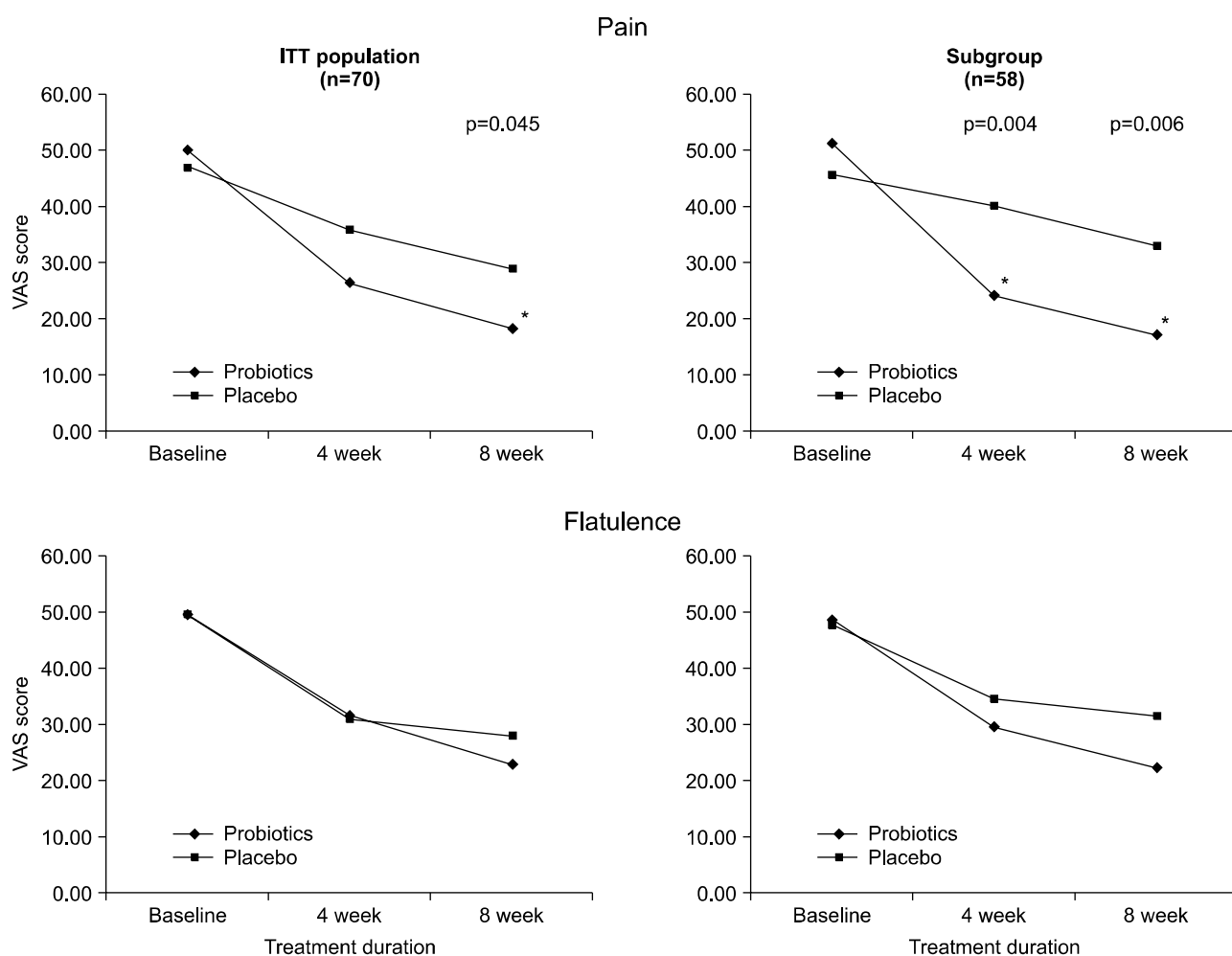


Fig. 1. Pain and flatulence scores analyzed over 4- and 8-week treatment periods.

showed more significant reductions of pain score after 8 weeks of treatment (-33.9 in probiotics group vs. -13.3 in placebo [$p=0.006$]), defecation discomfort score after 4 weeks of treatment (-30.4 vs. -10.6 , respectively [$p=0.013$]), and sum of scores after 8 weeks of treatment (-90.6 vs. -43.6 , respectively [$p=0.010$]) (Table 2, Figs. 1 and 2). Subgroup analyses in 10 patients, whose baseline Bristol stool form scales were below 3, did not show any significant changes. Response rate evaluation through χ^2 -test failed to show significant changes as follows: response rate in pain were 64% in probiotics group vs. 44% in placebo ($p=0.248$), response rate in defecation discomfort were 58% vs. 41% ($p=0.317$), and response rate in sum of scores were 56% vs. 50% ($p=0.750$), respectively. There was no significant change of QOL and bowel habits including defecation frequency and stool consistency in both groups (Table 3).

3. Adverse events

There was no serious adverse event associated with treatments. Twelve of 70 participants reported mild adverse events including common cold, headache, cystitis, low back pain etc. The number of adverse events per group was 8, same in both groups.

DISCUSSION

Symptoms of IBS are subjective and there is no objective test that can measure severity of IBS. Symptom scores are popular methods for assessing severity of IBS, but these can be influenced by an interviewer as well as patients themselves. Strict double-blind design is essential to assess the effect of probiotics on symptoms in IBS patients. We could perform a strict double-blinded study by the help of MRCC and clinical trial pharmacists for random code generation and code-matching. Patel *et al.*¹⁵

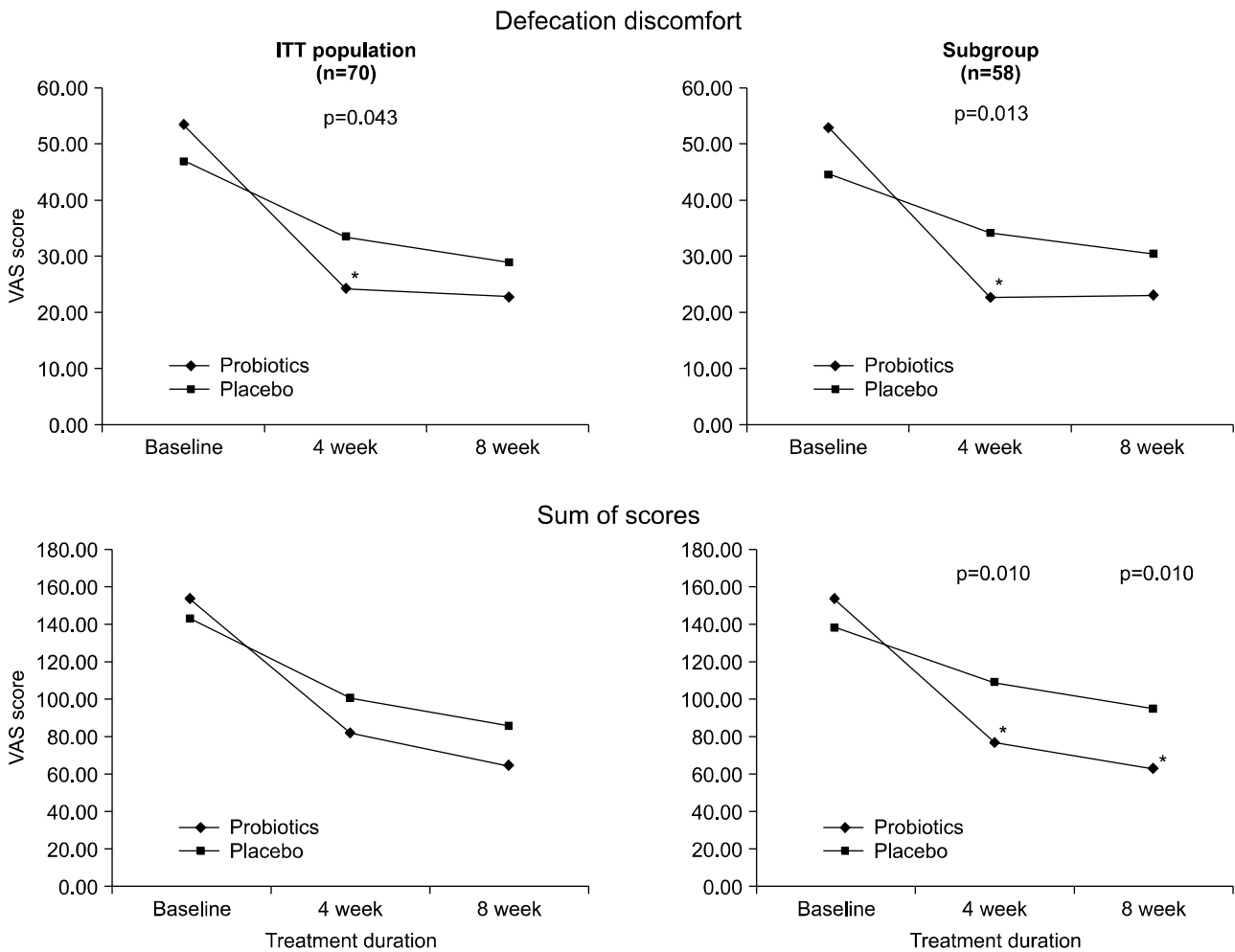


Fig. 2. Defecation discomfort and sum of scores analyzed over 4- and 8-week treatment periods.

Table 3. Analysis of Quality of Life, Defecation Frequency and Stool Consistency over 8-week Treatment Period (Two-sided T-test Using the 0.05 Significance Level)

		Probiotics	Placebo	p-value
ITT population (n=70)				
QOL	Baseline	104.6	104.7	0.957
	Δ 8 week	-1.3	-1.1	0.887
Frequency [†]	Baseline	8.1	7.1	0.414
	Δ 8 week	-0.1	0.1	0.817
Consistency [‡]	Baseline	4.2	4.0	0.453
	Δ 8 week	0.2	-0.1	0.430
Subgroup* (n=58)				
QOL	Baseline	104.4	104.8	0.876
	Δ 8 week	-1.5	-1.7	0.919
Frequency [†]	Baseline	8.7	7.9	0.553
	Δ 8 week	-0.6	-0.2	0.689
Consistency [‡]	Baseline	4.6	4.4	0.374
	Δ 8 week	-0.1	-0.4	0.241

QOL, quality-of-life.

*Baseline Bristol stool form scale ≥ 3 , [†]Bowel movements per week, [‡]Bristol stool form scale; average for a week.

reported that placebo response in IBS studies ranged from 16% to 71% via meta-analysis. In our study, the overall placebo effect was 35% that is comparable with many other IBS studies and seems to be a matter of course in double-blinded study.¹⁶⁻¹⁸ Strong points of our study design include strict double-blind design, data collection by a single trained interviewer, relatively large number of study population (n=70) and no need of data processing including adjusting and standardization.

Although Kim *et al.*^{6,7} reported that VSL #3 reduced flatulence scores in the patients with IBS, probiotics have not been the standard treatment of IBS due to the following reasons: need of huge dose of probiotics (VSL #3, 4.5×10^{11} bacteria/packet), relatively low efficacy (10 mm difference from placebo on 100 mm scale) and no effect in abdominal pain and urgency. A recent report showed that low dose of one strain (*Bifidobacterium infantis* 35624, 1×10^8 bacteria/capsule) reduced symptom scores in IBS patients,⁸ and the result suggested that the effect of probiotics for IBS was dependent on a specific strain as well

as dose. Some strains of *Lactobacillus* were known to be effective in controlling IBS symptoms.¹⁹ A paper studying an alteration of gut microbiota reported that *Lactobacillus* sequences were absent in stool from IBS patients, contrary to healthy control.²⁰ Regarding the reduction of abdominal pain by probiotics, a recent study demonstrated that one strain of *Lactobacillus acidophilus* induced the expression of mu-opioid and cannabinoid receptors in intestinal epithelial cells of rodents and mediated analgesic functions in the gut.²¹ As described in introduction, considering the immunomodulatory effect of *Bifidobacterium bifidum* BGN4 and potential benefit of *Lactobacillus* sp. we selected study medication as composite probiotics composed of *Bifidobacterium bifidum* BGN4, *Bifidobacterium lactis* AD011, *Lactobacillus acidophilus* AD031 and *Lactobacillus casei* IBS041. All of them are original strains that were collected from Koreans and have been never used in clinical study.

We demonstrated that selected composite probiotics were effective in IBS patients (ITT population, n=70) as follows: pain reduced by 64% in probiotics group vs. 38% in placebo (p=0.045), and defecation discomfort reduced by 55% vs. 29% (p=0.043), respectively. In agreement to a previous report, probiotics were more effective in patient who excreted mainly normal or loose stool (baseline Bristol stool form scale ≥ 3 , n=58) as follows: pain reduced by 66% in probiotics group vs. 29% in placebo (p=0.006), defecation discomfort reduced by 57% vs. 24% (p=0.013), and sum of scores reduced by 59% vs. 31% (p=0.010), respectively.⁸ In contrast to many reports that probiotics showed minimal or no effect on abdominal pain, we demonstrated the beneficial effect in the treatment of IBS symptoms including abdominal pain using composite probiotics containing *Bifidobacterium bifidum* BGN4 and *Lactobacillus acidophilus* AD031.^{6-8,16} Although Sinn *et al.*²² reported that two strains of *Lactobacillus acidophilus* reduced abdominal pain by 20% more than placebo in IBS patients, we could demonstrate superior effect of probiotics on abdominal pain up to 37% over placebo. As compared with VSL #3 that showed effectiveness on overall score up to 16% over placebo, our composite probiotics were more effective on overall score up to 28% over placebo.⁶

χ^2 -test was performed to test the response which was defined as reduction of symptom score by at least 50% after treatment in this study, but it could not show significant change. Data loss was inevitable in the process of converting symptom scores into responder status which was classified as yes or no, and it seemed to be the reason of low sensitivity of χ^2 -test. By simultaneously doing parametric analyses over changes of individual scores after

treatment, we could demonstrate the effect of probiotics accurately.

Although the analyses on bowel habits showed slight decrease of frequency, there was no statistically significant change between two groups, which might be due to low power of this study. A previous study showed normalization of frequency in *Bifidobacterium*-treated group through *post hoc* analyses (n=182) using data stratified by baseline bowel movements per day.⁸ On the basis of further large-scale studies, probiotics are expected to be revealed as effective in correction of bowel habits.

In conclusion, composite probiotics containing *Bifidobacterium bifidum* BGN4, *Bifidobacterium lactis* AD011, *Lactobacillus acidophilus* AD031 and *Lactobacillus casei* IBS041 were safe and effective, especially in patients who excreted mainly normal or loose stool.

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