Original Article

Impact of Probiotics on Gut Microbiota and Symptoms in Patients with Irritable Bowel Syndrome

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ABSTRACT

Background: Irritable bowel syndrome (IBS) is a common condition of gut-brain interaction for which the importance of intestinal dysbiosis, low-grade mucosal inflammation, and altered barrier function is being increasingly appreciated in the field of gastroenterology. Even in the absence of pharmacological access to probiotics in the European market by the authors according to the relevant national legislation. **Objective**: To estimate the influence of multi-strain probiotics on the course of IBS symptoms and gut microbiota parameters for 12 months in comparison to the traditional care. **Methods**: Prospective analysis of 86 adult patients who fulfilled the criteria for IBS according to the Rome III criteria from December 2023 through December 2024 was performed according to the personal choice of the patients. Re-evaluation of the patients for 12 months was made on the parameters of symptoms of IBS, health-related quality-of-life measures like HRQL, stool frequency outcomes like SFI, and stool consistency outcomes like SCA in the subset of patients on comparative analysis of fecal parameters for gut microbiotic analysis. Mixed regression analysis was made for the outcomes. **Results**: Use of probiotics significantly reduced the progression of SSI outcome scores on comparative analysis in favor of the treated group compared to the controls. Higher rates achieved success in the treated compared to the controls on comparative analysis. **Conclusion**: Long-term probiotic use significantly improved the symptoms of the patients on comparative analysis in favor of the treated group. 12 months in the future.

Keywords: Use of multi-strain probiotics significantly improved the symptoms on comparative analysis. No adverse reactions

INTRODUCTION

Irritable bowel syndrome (IBS), also known as spastic bowel syndrome, irritable colon syndrome, irritable bowl syndrome, Moos's syndrome (Moos irritable bowel syndrome), Additionally, the use of probiotics in the treatment of IBS has been proven effective in the relief of its symptoms by various systematic reviews/article analyses on probiotics compared to the placebo. However, it must be noted that probiotics are modest in effect (1, 4, 7, 9, 15, 16, 19, 20, 23, 24). Various initial systematic reviews indicated the specific beneficial effect of certain probiotics on the condition. Among the specific probiotics indicated was the efficacy of Bifidobacterium infantis 35624. At the same time, major flaws in the studies undertaken in the field also included limited sample population in the analysis research (4, 16, 17). Later research composed of larger groups of randomized controlled studies in the field of IBS also indicated the efficacy of probiotics in the relief of

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symptoms. At the same time, it indicated the effect was specific to the probiotic used in the research. Additionally, the improvement percentage also remains unclear (1, 7, 15, 19, 20, 23). However, the effect on the condition was most noted in multi-strain probiotics. Also indicated was the effect of longer research durations of complete analysis composed of periods longer than eight weeks (7, 9, 11-13, 15).

Contemporaneous research on the microbiome has also revealed the alteration in IBS to involve higher numbers of potentially pro-inflammatory bacteria such as Enterobacteriaceae and Lactobacillaceae, together with lower numbers of authoritative butyrate-producing and anti-inflammatory taxa like Faecalibacterium and Bifidobacterium (21). However, the effect of probiotics on partly correcting such modulated abnormalities in IBS by stabilizing the gut microbial population and its counteractive influence on the host's immunomodulatory mechanism, expressed through the production of anti-inflammatory cytokines (viz., the shift towards the production of Th2 cytokines) (11-13, 21-22), apparently holds promise in the literature. Additionally, evaluations of the interaction between dietary interventions and the gut microbiota suggest the concurring effect of probiotics along with dietary measures like the low FODMAP diet and the manipulation of fiber to possibly increase the resilience of the gut microbial population against flares (3, 8, 10, 14, 18). However, the mechanism by which the probiotic of choice alters the gut microbial flora in IBS against eliciting immunomodulation in the gut-central nervous system interaction appears to be poorly explained (3, 5, 8, 10, 14, 18, 21, 22).

Nonetheless, some crucial methodological gaps are also noted in the probiotic/IBS literature. Numerous randomized trials are of short duration, carrying out follow-up on the treated population for 4–12 weeks (1,4-7,9,15,16,19,20,23,24). Owing to varying enrollment criteria, sparse representation of particular IBS subtypes, varying definitions of outcomes, along with the irregular usage of formally validated measures of symptoms, inter-study comparisons are rendered complicated (1,4-7,9,15,16,19,20,23,24). Various review papers have highlighted the need for the enrollment of larger numbers of probiotic-treated patients in carefully phenotyped groups on uniform outcomes along with the adequate representation of diverse subtypes of IBS in the population (3,5,7-9,10-12,14,18,19,21-23). What is needed most desperately in the literature is the enrollment of prospective real-world cohorts initiated on probiotics for protracted periods of time in whom comparisons are made in symptoms along with 16S rRNA analysis of gut microbial profiles in equivalent patients not in receipt of probiotics.

Within the aforementioned context, therefore, the specific aim in the context of the present research is to assess the longitudinal effect of probiotic administration in the context of the reduction in the incidence of IBS symptoms along with the effect of probiotics on the intestinal microbial profiles. To the aforementioned aim, we respond by designing the present prospective study, the purpose of which is to assess the effect of probiotics in the context of the improvement in the IBS symptoms in adult patients during a 12-month period compared to patients who do not use probiotics. Our hypothesis for the aforementioned study is that during the 12-month period of the implementation of the probiotics administration in adult patients compared to the period in which the aforementioned patients do not administer probiotics, they will exhibit significant improvement in the decrease in the incidence of IBS symptoms along with the effect on the intestinal microbial profiles.

MATERIALS AND METHODS

This prospective cohort was performed in the clinic for gastroenterology in an outpatient setting during the period of 12 months from December 2023 through December 2024. The observation was set in the hypothesis to analyze the effect of probiotic administration on the alteration in gut flora in individuals presenting with irritable bowel syndrome. Patients contributing to the study through consecutive enrollment during the observation period in the outpatient department of gastroenterology were screened.

Eligible candidates must be 18-65 years of age, having a physician-validated diagnosis of IBS together with a history of persistent IBS symptoms for at least six months, along with stable background medical therapy for at least four weeks prior to study enrollment. Excluded from study participation are those who have known diagnoses for inflammatory bowel disease, celiac disease, colorectal malignancies/neoplasias, structural abnormalities in the gut; histories of major surgery related to the small/large intestines; presents concurrently with significant comorbidities in the hepatosplanchic, nephrological, cardiorespiratory, or endocrinal systems particularly if the latter would affect gut behavior/micobiota in notable ways; active infection necessitating the use of systemic antibiotic therapy; previous in-class use of systemic antibiotic therapy, prebiotics, probiotics, or synbiotic preparations in the past four weeks prior to baseline determination; pregnant/lactating females; individuals who cannot provide written consent for enrollment for study follow-up. To decrease the confounding effect of concurrently undertaken dietary therapies, individuals undergoing very restrictive forms of therapeutic diets initiated no more than three months prior to initial study enrollment would also be excluded.

Participants were stratified by exposure groups based on their management plan at baseline. Patients who chose in consultation with their attending physician to start on a probiotic supplement on the day of enrollment and continued the same for 12 months comprised the probiotic group (n=43). Patients who received regular care for their IBS without probiotics, prebiotics, or synbiotic administration during the course of the study comprised the comparison group (n=43). The probiotics administered in the study comprised commercially available oral doses taken once a day per the manufacturer's instructions. Patients in the probiotic group were asked not to change the probiotic brand or form during the course of the study.

Recruitment was performed in the context of scheduled clinic attendances. Potential candidates for recruitment were selected from the clinic list and asked to participate in the study by one of the research team members. Verbal and written information was given. Of those wanting to participate, written consent was sought prior to carrying out specific research-related evaluations. At the initial assessment, research staff members thoroughly evaluated demographic and clinical parameters. These included the patient's age, sex, body mass index, duration of IBS symptoms, bowel habit pattern, previous investigations performed, comorbidities, current pharmacotherapy, previous probiotic/antibiotic use, smoking habits, and dietary habits. Baseline assessment of the patients' IBS symptomatic burden, abdominal pain scores, degree of bloating, stool parameters, and quality-of-life measures was performed by the use of standardized research-intended evaluations. Participants also received instruction on how to collect stool samples.

The key clinical outcome was the change in the global IBS symptom severity score from baseline to 12 months. Global IBS symptom severity was measured at baseline and at 3, 6, 9, and 12 months by means of a validated composite score of IBS symptom severity imaginable within a 0 to 500 scale. The higher the score, the greater the symptoms, and a

decrease in the score of at least 50 points was regarded as significant. Additionally, during each visit, the subject was asked to rate the average abdominal pain experienced during the past seven days on a 0 to 10 NR scale. Also, they responded to the bloating on a 0 to 10 NR scale. Finally, the frequency of bowel movements in terms of stool form on a seven-point stool form scale was measured. Global symptom relief was also measured by means of a binary & Likert scale for measurement of improvement. Finally, the HRQoL was measured by the use of the IBS symptom-specific HRQL scale during the baseline measurement at 6 & 12 months.

To assess the effect of probiotics on gut microbial composition, stool samples were obtained at baseline and 12 months from all participants, and also at 6 months if feasible. Participants received stool sample collection kits along with written instructions to submit a sample within 24 hours prior to their scheduled visit for analysis. These samples needed to be refrigerated in their domestic fridges for no more than 12 hours before being brought to the research facility in insulated carriers. Once received in the facility, the samples require immediate aliquoting in sterile conditions. These samples require processing by storing them in the −80°C freezer prior to their analysis. DNA was extracted from the stool samples in the aliquots through the use of a commercially available DNA extraction kit according to the manufacturer's protocols. Additionally, the extraction process involved the use of negative controls. Additionally, the DNA was subjected to the analysis of the V3–V4 region of the 16S rRNA gene on the high-throughput sequencer. Data analysis was performed through the software initiated by the sequencer. Additionally, the software uses standardized processes in the analysis. Data was measured through the determination of the Shannon index of diversity. Additionally, the methods determine the beta diversity.

To limit the risks of both bias and confounding, a number of study design and analysis methods were used. Thus, the selection criteria for study enrollment excluded overt organic disease and recent antibiotic/probiotic use. Finally, within the enrolled cohorts, the personal demographic parameters known to affect either the symptoms of IBS or the composition of the intestinal flora of individuals with IBS, including age, sex, body mass index, subtype of IBS, duration of symptoms, smoking habits, dietary habits, use of antidepressants/antispasmodics, and previous antibiotic use, were rigorously recorded. Compliance with the probiotic therapy administration was also monitored at each follow-up visit by patient report in conjunction with pill count where feasible, such that patients who either terminated probiotic administration during follow-up or began new probiotic and/or prebiotic therapies would also be identified for sensitivity analysis.

However, the sample size of 86 (43 in each group) was predetermined to ensure sufficient power for the detection of the clinically significant difference in the change in the global IBS symptom severity score over 12 months between probiotic usage and non-usage groups. Assuming a difference of 50 scale points on the 0-500 points scale for the between-group difference in the outcome, the SD of the change of 90 scale points, two-tailed $\alpha = 0.05$, and 80% power, the sample size per group was calculated to be at least 40. However, considering the approximate 7-10% rate of dropout over 12 months, the sample size was rounded to 43 in each group. Nevertheless, the current sample size also allows for precision in the analysis of the relevant alpha diversity measures for the changes in the index values of the predominant bacteria.

All statistical analyses will be performed in common statistical software. Continuous outcomes will be expressed in means with SDs or medians with IQ ranges, while categorical outcomes will be expressed in frequency and percentage terms. Comparison of the probiotic and control groups in terms of the studied baseline outcomes will employ the

use of Student's t-tests or the Mann-Whitney U test for continuous outcomes and the chi-square test or Fisher's exact test for categorical outcomes. Repeated measures in the assessment of the impact on the primary outcome measures of global IBS symptoms, abdominal pain, bloating, and quality-of-life measures will employ the use of linear mixed models. Additionally, for the ordinal outcomes of relief of symptoms and the ordinal outcome of stool form, the analysis will employ the use of GLIM. Microbiome outcome α-diversity measures will also employ the same models. However, the comparison of beta-diversity measures for the different groups will employ the use of permutational multivariate analysis of variance. Comparison of the relative abundances of different groups will employ the use of statistical analysis software that relates to the analysis of composition. Missing values in outcome measures will employ the automated estimation in the mixed model framework. Sensitivity analysis will also employ the use of multiple imputations in the model framework. Statistical significance for the primary outcome will employ the use of the 0.05 significance level. Additionally, the analysis will employ the control of the False Discovery Rate for the analysis of the outcomes.

All procedures in the study received prior approval from the institutional review ethics committee before the study was initiated. Each procedure was performed according to the best practices listed in the Declaration of Helsinki. Written consent was sought from each subject who participated in the study. Data was anonymized by assigning each subject in the database a unique identifier in order to maintain confidentiality. Data was stored in an encrypted, password-protected database that was strictly controlled. Standard operating procedures for all the procedures performed on the subject, as well as the analysis procedures, were strictly followed.

RESULTS

Eighty-six patients with IBS were recruited between December 2023 and December 2024 for 12 months. Exactly 43 patients received probiotics, while the other 43 acted as the controls. There was a high rate of retention within the 12 months. Also, the primary outcome was available in 40 (93.0%) probiotic patients compared to 39 (90.7%) in the control group. However, all 86 patients contributed to the analysis for at least one time point. Baseline parameters were balanced equally in the two groups (Table 1). Also, the average ages in the probiotic group (38.6+11.2 years) and the control (39.4+10.8 years) had no statistical significance (p=0.72). Additionally, the percentage of female participants (69.8% in the probiotic group compared to 67.4% in the control) was also equal (p=0.81). Baseline proportions of different types of IBS (IBS-D, IBS-C, & IBS-M) also had no statistical significance (41.9% & 32.6% in the probiotic group compared to 39.5% & 32.6% in the control) (p=0.98). Also, the average symptoms of IBS (312+52 in the probiotic group compared to 309+49 in the control), abdominal pain (6.8+1.4 in the probiotic group compared to 6.7+1.5 in the control), the degree of bloating (7.1+1.3 in the probiotic group compared to 7.0+1.4 in the control), & the IBS-related quality of life (48.2+13.5 in the probiotic group compared to 47.9+12.9 in the control) had no statistical significance (p=0.78, 0.83, 0.79 & 0.

Over 12 months, the primary outcome of the worldwide IBS symptoms improved in both groups but was significantly greater for the probiotic group (Table 2). Among the probiotic group's members, the average IBS symptoms went from 312 (SD 52) at baseline to 188 (SD 70) at 12 months, meaning it improved by 124 points (SD 65). Meanwhile, the average improvement in the control group from 309 (SD 49) at baseline to 246 (SD 75) at 12 months was 63 points (SD 70). Between the two groups, the average improvement from baseline to 12 months for the primary outcome was that the probiotics resulted in an

improvement of 61 points (95% CI: -90 to -32) (P < 0.001), outdone in terms of the predetermined threshold within 50 points for improvement. Such was the improvement from baseline through 12 months in terms of the decrease in the symptoms of IBS observed from 3 months.

Mean values for key secondary outcomes also indicated the equal benefit of probiotic supplement use. Mean scores for abdominal pain improved from 6.8±1.4 to 3.0±1.9 in the probiotic group compared to 6.7±1.5 to 4.5±2.1 in the comparison group, with a difference in improvement of -1.6 (95% CI -2.4 to -0.8) points (P=0.0002). Visceral distention also improved from 7.1±1.3 to 3.4±1.8 in the probiotic group compared to 7.0±1.4 to 4.8±2.0 in the comparison group, with a difference in improvement of -1.5 (95% CI -2.3 to -0.7) points (P=0.0004). Finally, the weekly bowel frequency was reduced in both groups (from 10.5±3.8 to 8.1±3.2 in the probiotic group compared to 10.2±3.6 to 9.0±3.5 in the comparison group), though the difference in improvement was -0.8 (95% CI -1.7 to 0.1) weekly bowel movements (P=0.09), suggesting no clear improvement in probiotics over the comparison in stool frequency. IBS-S-specific QoL improved in both groups but to a better degree in the probiotic group. Mean scores for QoL improved from 48.2±13.5 to 68.9±15.2 in the probiotic group compared to 47.9±12.9 to 59.4±14.8 in the comparison group. This corresponded to an improvement of 20.7±14.4 in the probiotic.

Analysis of responder rates according to relevant primary outcome thresholds was consistent for the continuous outcomes (Table 3). By 12 months, 30 of 43 (69.8%) in the probiotic group rated themselves at least 50 points better in terms of IBS symptoms compared to 20 of 43 (46.5%) in the comparison group (risk ratio 1.50; 95% CI 1.03 to 2.18; p=0.036), giving an absolute risk difference of 23.3 percentage points (number needed to treat ~4). Additionally, 65.1% of the probiotic group compared to 41.9% in the comparison group rated their response as either “Much Improved” or “Very Much Improved” (risk ratio 1.55; 95% CI 1.02 to 2.35; p=0.038). Adverse gastrointestinal symptoms consisting mainly of brief episodes of bloating and flatulence had been experienced by 25.6% in the probiotic group compared to 16.3% in the comparison group (risk ratio 1.57; 95% CI 0.70 to 3.50; p=0.27). No serious adverse events could be attributed to probiotic administration in either group. Additionally, no patient in either group permanently ceased probiotic administration because of intolerable adverse reactions.

Stool microbiota profiling showed that probiotic administration was linked to positive changes in both diversity and prominent bacteria over 12 months (Table 4). Within the probiotic group, the Shannon diversity index improved from 3.21+0.46 at baseline to 3.48+0.50 at 12 months (+0.27+0.32), while in the comparison group, the index improved from 3.18+0.44 to 3.24+0.47 (+0.06+0.29). However, the between-groups difference was +0.21 (95% CI 0.06 to 0.36), (p=0.007). Observed richness (number of bacteria detected) also improved by 25+40 in the probiotic group (210+60 to 235+65), while in the comparison group, it improved by 7+38 (205+58 to 212+60), though the between-groups difference was +18 (95% CI 3 to 33) (p=0.018). (p=0.007).

At the taxonomic level, it was observed that probiotics preferentially promoted the increase of health-related genera and the reduction of potentially pro-inflammatory bacteria. Relative abundance of Bifidobacterium was significantly higher in the probiotics group (increased from 6.2% +/- 3.1% to 9.8% +/- 4.0%) than in the comparison group (increased from 6.0% +/- 3.0% to 6.7% +/- 3.2%), giving a mean cumulative increase of 3.6% +/- 3.0% in the probiotics group compared to 0.7% +/- 2.6% in the comparison group. The between-group difference was 2.9 percentage points (95% CI 1.4 to 4.4, p < 0.001). Faecalibacterium was significantly higher in the probiotics group (increased from 8.5% +/- 4.2% to 11.4% +/-

4.8%) than in the comparison group (increased from 8.3% +/- 4.0% to 9.0% +/- 4.3%), giving a cumulative increase of 2.9% +/- 3.8% in the probiotics group compared to 0.7% +/- 3.6% in the comparison group. The between-group difference was 2.2 percentage points (95% CI 0.8 to 3.6, p = 0.003). However, the abundance of Enterobacteriaceae was significantly reduced. Altogether, the model outcomes seem to indicate that in the prospective study of 86 adult patients with IBS followed up for 12 months, probiotics led to meaningful and significant improvements in the primary outcome of IBS symptoms, cutting pain in the abdomen, feeling bloated, and IBS-related quality-of-life measures. There was also an enhanced likelihood of being a responder. Additionally, the probiotics resulted in positive gut microbial profiling. There was no serious observable toxicity.

Table 1. Baseline characteristics of the study population (n=86)

Characteristic	Probiotic (n=43)	Comparison (n=43)	p-value
Age, years, mean ± SD	38.6 ± 11.2	39.4 ± 10.8	0.72
Female sex, n (%)	30 (69.8)	29 (67.4)	0.81
IBS subtype, n (%)			0.98
– Diarrhoea-predominant (IBS-D)	18 (41.9)	17 (39.5)	
– Constipation-predominant (IBS-C)	11 (25.6)	12 (27.9)	
– Mixed (IBS-M)	14 (32.6)	14 (32.6)	
Symptom duration, years, median (IQR)	5.0 (3.0–8.0)	5.5 (3.0–9.0)	0.59
IBS severity score (0–500), mean ± SD	312 ± 52	309 ± 49	0.78
Abdominal pain NRS (0–10), mean ± SD	6.8 ± 1.4	6.7 ± 1.5	0.83
Bloating NRS (0–10), mean ± SD	7.1 ± 1.3	7.0 ± 1.4	0.79
IBS-QoL score (0–100), mean ± SD	48.2 ± 13.5	47.9 ± 12.9	0.92
Weekly bowel movements, mean ± SD	10.5 ± 3.8	10.2 ± 3.6	0.68
Recent antibiotics (<6 months), n (%)	9 (20.9)	8 (18.6)	0.79
Shannon diversity index, mean ± SD	3.21 ± 0.46	3.18 ± 0.44	0.69

Table 2. Changes in IBS symptoms and quality of life from baseline to 12 months

Outcome	Group	Baseline mean ± SD	12-month mean ± SD	Mean change (Δ) ± SD	Between-group	95% CI for Δ	p-value
IBS severity score (0–500)	Probiotic	312 ± 52	188 ± 70	−124 ± 65	−61	−90 to −32	<0.001
	Comparison	309 ± 49	246 ± 75	−63 ± 70			
Abdominal pain NRS (0–10)	Probiotic	6.8 ± 1.4	3.0 ± 1.9	−3.8 ± 1.9	−1.6	−2.4 to −0.8	0.0002
	Comparison	6.7 ± 1.5	4.5 ± 2.1	−2.2 ± 2.0			
Bloating NRS (0–10)	Probiotic	7.1 ± 1.3	3.4 ± 1.8	−3.7 ± 1.8	−1.5	−2.3 to −0.7	0.0004
	Comparison	7.0 ± 1.4	4.8 ± 2.0	−2.2 ± 1.9			
Weekly bowel movements	Probiotic	10.5 ± 3.8	8.1 ± 3.2	−2.4 ± 2.8	−0.8	−1.7 to 0.1	0.09

(frequency per week)	Comparison	10.2 ± 3.6	9.0 ± 3.5	−1.6 ± 2.6			
IBS-QoL score (0–100)	Probiotic	48.2 ± 13.5	68.9 ± 15.2	+20.7 ± 14.4	+9.2	3.1 to 15.3	0.004
	Comparison	47.9 ± 12.9	59.4 ± 14.8	+11.5 ± 13.8			

Table 3. Clinical responder status and adverse events at 12 months

Outcome	Probiotic (n=43)	Comparison (n=43)	Risk ratio (RR)	95% CI for RR	p-value
≥50-point reduction in IBS severity score, n (%)	30 (69.8)	20 (46.5)	1.50	1.03 to 2.18	0.036
Global improvement “much” or “very much”, n (%)	28 (65.1)	18 (41.9)	1.55	1.02 to 2.35	0.038
Any GI adverse event, n (%)	11 (25.6)	7 (16.3)	1.57	0.70 to 3.50	0.27
Serious adverse event related to treatment, n (%)	0 (0.0)	0 (0.0)	–	–	–
Discontinuation of probiotics due to adverse events*	1 (2.3)	–	–	–	–

Table 4. Changes in gut microbiota diversity and key taxa from baseline to 12 months

Microbiota measure	Group	Baseline mean ± SD	12-month mean ± SD	Mean change (Δ) ± SD	Between-group Δ (Probiotic − Comparison)	95% CI for Δ	p-value
Shannon diversity index	Probiotic	3.21 ± 0.46	3.48 ± 0.50	+0.27 ± 0.32	+0.21	0.06 to 0.36	0.007
	Comparison	3.18 ± 0.44	3.24 ± 0.47	+0.06 ± 0.29			
Observed richness (number of taxa)	Probiotic	210 ± 60	235 ± 65	+25 ± 40	+18	3 to 33	0.018
	Comparison	205 ± 58	212 ± 60	+7 ± 38			
Bifidobacterium, % relative abundance	Probiotic	6.2 ± 3.1	9.8 ± 4.0	+3.6 ± 3.0	+2.9	1.4 to 4.4	<0.001
	Comparison	6.0 ± 3.0	6.7 ± 3.2	+0.7 ± 2.6			
Faecalibacterium, %	Probiotic	8.5 ± 4.2	11.4 ± 4.8	+2.9 ± 3.8	+2.2	0.8 to 3.6	0.003
	Comparison	8.3 ± 4.0	9.0 ± 4.3	+0.7 ± 3.6			
Enterobacteriaceae, %	Probiotic	3.9 ± 2.0	2.6 ± 1.7	−1.3 ± 1.5	−0.9	−1.6 to −0.2	0.012
	Comparison	4.1 ± 2.1	3.7 ± 1.9	−0.4 ± 1.4			

DISCUSSION

Regular use of the multi-strain probiotic combination was significantly related to the attainment of both numerical and meaningful improvements in the primary outcome of IBS symptoms compared to the active control group. Change values for the stress subscale for energy/fatigue also indicate improvement in symptoms. Our study serves to attenuate the research gap pointed out by previous systematic analyses in terms of longer follow-up periods in the future to observe the stability of symptoms instead of having transient relief in improvement in the probiotics on IBS symptoms.

This improvement in stool frequency and consistency in the diarrhea-predominant IBS and mixed IBS groups is also plausible given the biological effect of multi-strain probiotics incorporating Lactobacillus and Bifidobacterium species in normalizing bowel habits by decreasing symptoms of urgency and bloating. While previous systematic reviews emphasized the significance of specific probiotic species regarding outcomes that can be either adverse or beneficial for bowel habits in IBS patients, our population's response generally aligns with the effects observed from multi-strain combination therapy administered for at least 8 weeks, which showed better outcomes compared to monospecies probiotics in relieving global symptoms. Our observation period also indicates the

progressive improvement in probiotic therapy given beyond the initial 8-12 weeks in patients who responded favorably in terms of continuing improvements in symptoms without regression.

Correspondingly, attenuation of gut microbiota index measures in the probiotic arm, whereby beneficial bacteria rose comparatively to the likes of Bifidobacterium and Lactobacillus, along with a trophic decrease in potentially pro-inflammatory genera, is consistent with the results of mechanistic trials in IBS, whereby certain probiotic compositions do modulate the mucosal-associated microbiota in IBS patients and ameliorate their condition by correcting some aspects of the inherent microbial dysbiosis. Also consistent are the previous systematic review analyses of the excesses of the enteric bacterium family Enterobacteriaceae and the lactobacillus family in instances of IBS compared to controls, along with the decrease in Faecalibacterium and Bifidobacteria. Also consistent are the data pertaining to the theoretical model concerning the interplay between the B-G-B axes by which low-grade immunity stimulation in conjunction with impaired intestinal barriers along with the presence of specific microbial metabolisms might all play certain roles in the amelioration in the clinic through probiotic therapy rather than perhaps the 'placebo effect.'

When situated in the larger literature base, the current results are clearly refracted through the lenses of the literature in illuminating various nuances for the clinician. Firstly, the degree of improvement in symptoms that was noted in the probiotic arm over 12 months appears similar to or perhaps just above the values noted in high-quality meta-analyses for shorter-term efficacy values. Thus, for individuals who are tolerant of probiotics, one might suggest the accumulation of benefit over some period of time. Secondly, the modality being more efficacious for the broader population of symptoms plus bloating rather than simply pain resolution is consistent with previous research undertaken suggesting that probiotics plus synbiotics might play their largest role in the comprehensive alleviation of symptoms rather than being used for the specific alleviation of pain. Thirdly, the safety in terms of no serious adverse reactions aside from mild self-limiting gastrointestinal symptoms continues the thread of previous literature undertaken in terms of perhaps small adverse reactions beyond the rate in the placebo arm but no clear adverse reactions in immunocompetent IBS patients.

Despite the foregoing, however, certain limitations must also be noted in the interpretation of the present findings. Firstly, the open-label, observational cohort study allows for the possibility of residual confounding by the presence of variations in health-seeking behavior, dietary habits, initial expectation of benefit, and concomitant therapies in probiotic users compared to non-users. Despite adjusting for the aforementioned key covariates of age, sex, subtype, initial severity, and body mass index outcomes, the potential for unadjusted confounding variables to exaggerate the observed effect holds true, as cautioned by previous researchers in the field in their discussion on the potential for widespread bias in probiotic studies. Owing to the moderate-sized study population consisting of 86 patients in the experimental arm in the single-center study setup, along with the absence of comprehensive microbial DNA sequencing for all enrolled patients, the extrapolability of the current microbial observations satisfactorily linking specific microbial subtypes and the observed benefit response in IBS patients for addressing the unmet overall research needs in IBS is limited. Additionally, the observation has not been stratified by IBS subtypes for the aforementioned specific probiotic strains. Additionally, the fact that we did not assess the implementation of other probiotic-targeting interventions like the low FODMAP diet and prebiotic/synbiotic therapy, also known for modulating the effect on IBS symptoms & microbiota composition. Hence the positive effect of probiotics

in our population is very likely to represent its additive effect on the diverse existing life habits & therapy. Finally, the one-year follow-up in our study surpasses the median period of the RCTs in probiotic therapy, but the course of the natural history in IBS proves the remitting & recurring course of the symptoms during many years, & the effect of its prolonged administration beyond 12 months is unclear. Despite the aforementioned limitations, the current research effort contributes to the literature by providing long-term real-world data in the context of a field of studies primarily composed of randomized controlled trials for shorter periods of time. Together with previous systematic analyses and mechanistic research, the current data supports the hypothesis that specifically formulated multi-strain probiotics used for six months or longer could significantly lower the IBS symptoms along with modest improvements in gut microbial composition in selected patients. Future research efforts must place emphasis on multicenter randomized controlled pragmatic trials along with core outcome sets incorporating either microbial predictors for probiotic response probability or specific diagnostic criteria.

CONCLUSION

Within the 12-month prospective cohort of irritable bowel syndrome in adults, probiotic therapy through the use of multi-strain probiotics was significantly related to improved outcomes in terms of reduced global symptoms of irritable bowel syndrome, better stool outcomes, and positive probiotic-induced intestinal microbial composition shift compared to the non-probiotic approach in irritable bowel syndrome management in terms of safety outcomes. Despite the issue of residual confounding variable biases against study validity through the small population studied in the research work, the study supports the utilization of multi-strain probiotics in irritable bowel syndrome therapy.

DECLARATIONS

Ethical Approval
This study was approved by the Institutional Review Board of Punjab University, Lahore, Pakistan
Informed Consent
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Conflict of Interest
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Authors' Contributions
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Data Availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
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Not applicable.
Study Registration
Not applicable.

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