Efficacy of Probiotic *Escherichia coli* Nissle 1917 in Patients with Irritable Bowel Syndrome: a Double Blind Placebo-controlled Randomized Trial

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ABSTRAK

Tujuan: *menilai efek potensial probiotik E. coli Nissle 1917 yang dapat membawa perbaikan dalam tatalaksana IBS refrakter pada populasi di Iran.* ***Metode:*** *pendekatan terkontrol plasebo tersamar ganda telah dipakai dalam uji klinis ini. Sebanyak 139 pasien yang dipastikan menderita IBS ikut serta dalam penelitian ini dan diberikan probiotik E.coliNissle 1917 selama 6 minggu. 11 butir pertanyaan dari Birmingham IBS Symptom Questionnaire telah dipakai untuk menilai perubahan gejala setiap 2 minggu.* ***Hasil:*** *enam puluh delapan (49%) subyek adalah laki-laki. Usia rata-rata±SD peserta penelitian 38±13,3 tahun. Sebanyak 49 (35,3%) pasien dominan mengalami diare (diarrhea predominant). Skor total menunjukkan bahwa tidak ada perbedaan bermakna antara kelompok intervensi dibandingkan kelompok kontrol (-6,7±6,8 dibanding -6,7±6,5; p=0,95); tidak ada satu pun butir pertanyaan yang menunjukkan perubahan yang bermakna pada kedua kelompok. Setelah dilakukan stratifikasi pasien berdasarkan tipe IBS yang dideritanya, pasien yang dominan mengalami diare (diarrhea predominant) menunjukkan respons positif terhadap probiotik dan kualitas tidurnya membaik (p=0,05 dan 0,03 masing-masing untuk minggu ke-2 dan ke-6). Pasien IBS dengan gejala dominan konstipasi (constipation-predominant) tidak menunjukkan respons terhadap probiotik; sedangkan pasien IBS dengan kombinasi diare dan kombinasi menunjukkan respons yang kurang baik terhadap probiotik dan butuh mengejan sebelum BAB dibandingkan kelompok plasebo (p= 0,03 dan 0,02 masing-masing pada minggu ke-4 dan ke-6).* ***Kesimpulan:*** *terapi probiotik dengan E.coliNissle 1917 tidak dapat memperbaiki gejala pada pasien IBS tanpa kategori (non-categorized IBS). Meskipun demikian, ketika pasien IBS dikategori ulang menjadi subkelompok berdasarkan gejala utamanya, penilaian efektivitas probiotik atas beberapa butir terpisah dalam daftar gejala ternyata bermakna. Uji klinik prospektif disarankan dilakukan untuk memastikan temuan kami.*

Kata kunci: *probiotik Escherichia Coli Nissle 1917, sindroma iritasi usus (irritable bowel syndrome), uji klinik terkontrol tersamar ganda.*

ABSTRACT

Aim: *to evaluate potential improvement effect for probiotic E. coliNissle 1917 in the management of refractory IBS in an Iranian population.* ***Methods:*** *a double blind placebo controlled approach has been used in the current clinical trial. 139 confirmed IBS patients were included into the study, and were given probiotic E.coli Nissle 1917 for 6 weeks. 11 items Birmingham IBS Symptom Questionnairehas been used for evaluation of changes in the symptoms every 2 weeks.* ***Results:*** *sixty eight subjects (49%) were males. Mean±SD age of the participants*

was 38±13.3 years. 49(35.3%) of the patients were diarrhea-predominant. The total scores showed no significant difference between the intervention vs. control group(-6.7±6.8 vs. -6.7±6.5, respectively; p=0.95); neither did any of the questionnaire items any significant alterations in the two groups. After stratification of patients based on their IBS type, diarrhea-predominant patients showed a positive response to the probiotic improving their sleep (p=0.05&0.03 at weeks 2&6, respectively). Patients with constipation-predominant IBS showed no response to the probiotic; while patients with diarrhea-constipation mixed IBS showed unfavorable response to the probiotic in the need for strain to pass a motion compared to the placebo (p=0.03&0.02 at weeks 4&6, respectively). ***Conclusion:*** *probiotic therapy with E.coliNissle 1917 was not able to induce significant improvement in the symptoms of patients with non-categorized IBS. Nevertheless, when IBS patients were recategorized to subgroups according to their main symptoms, evaluation of the efficacy of the probiotic on some individual items in the symptom list reached the significance level. Prospective clinical trials are recommended to confirm our findings.*

Key words: *probiotic Escherichia Coli Nissle 1917, irritable bowel syndrome, double blind randomized controlled trial.*

INTRODUCTION

Irritable bowel syndrome (IBS) is a complex condition, which is usually very hard to manage.[1] Effects of different therapeutic approaches have been modest or controversial compared to the placebo. Bulking agents and antispasmodics had either no or minimal beneficial effect in the management of IBS.[2] Newer agents such as 5HT3 agonists[3] and 5HT4 antagonists[4] have minimal or controversial efficacy over placebo; moreover, both of the mentioned agent families are now either banned or restricted in general use for their serious health risk enhancement, especially in the cardiovascular system and ischemic colitis.[5] So, the literature does not propose any effective option for the management of IBS, and this urges the scientific community to invest on finding effective treatment approach.

Several alternative therapies have been used for the management of IBS in different populations, which have revealed some prospects, though controversial.[6] In search for pathogenesis of the illness, authors have reported changes in the microflora of the intestine, as a significant factor.[7] Although controversy exists over the significance of these alterations of microflora, which can be a result or reason of IBS, there is overwhelming evidence suggestive of its casual role in the pathogenesis of IBS, with IBS getting inflamed after gastrointestinal infections.[8] Although these studies are highly indicative for the causative role of microbiota in the disease pathogenesis, more indisputable evidence will come out if improvements of gut microflora to the normal state results in the improvement of the illness.

Probiotics, consumable products that contain microbial content similar to that of a normal gutflora, have been used in several randomized controlled trials in hope to observe some therapeutic effects associated with them on the IBS;although the results were controversial with some studies highly suggestive of its beneficial effects[9,10] and some thoroughly disappointing results.[11,12] Different probiotics contain different microbial contents, so it seems logical to pretend different therapeutic effects for different products. Moreover, methodology of the probiotic administration in the IBS patients can also play a key role in the efficacy of the therapy in IBS patients. For the same reason, we conducted a double-blind placebo-controlled randomized trial to evaluate potential improving effect for probiotic *E. coli* Nissle 1917 in the management of refractory IBS in an Iranian population.

METHODS

Study Design and Diagnosis of the IBS

The study was designed using a double-blind randomized placebo-controlled trial. Participants were recruited from the outpatient Gastroenterology Clinic at Rasul-e-Akram Hospital in Tehran from the beginning of March 2010 to the end of October 2014.

Upon attendance to the clinic all the patients undergone full evaluations to exclude organic diseases based on the Kruis score[13]; and finally to define IBS according to the Rome II criteria[14]: Abdominal pain/discomfort for at least 12 weeks (not essentiallyconsecutive) within the last 12 months, besides at least two of the following criteria: (1) relief after defecation; (2) onset associated with alterations in stools frequency and/or (3) onset associated with a change in stool appearance (form).

Randomization and Blinding Protocol

Randomization and blinding was carried out by a member of our research center who was not part of the study team. The boxes were coded as box nA or nB and sealed. Either of the two boxes marked as A or B contained probiotic Escherichia coli Nissle 1917. The labeled boxes were distributed to the patients by one of the authors. The key was stored and sealed beyond the reach of the investigators until the completion of the survey.

Inclusion and Exclusion Criteria

After diagnosis of IBS was confirmed at the Outpatient Clinic of Gastroenterology of Rasul-e-Akram Hospital, only those who had the following inclusion criteria were considered eligible to be included in the study: (1) participants should be between 20 to 50 years of age; (2) they should not have been under probiotic therapy during the last one month; (3) they should eagerly give informed consent for inclusion to the study. Patients would have be excluded if: (1) They were pregnant, or became pregnant during the study period; (2) if they had any significant abdominal surgery except for appendectomy or cholecystectomy; (3)if they had any other disorder with direct influence on the patients' IBS; (4) any other active gastrointestinal disorder; (5) history of hypersensitivity to the cow-milk.

Study Participants

156 people were initially defined with the diagnosis of IBS, and entered the process for getting involved into the study. From these, 17 patients have been excluded from the study because they did not meet the inclusion criteria. Finally 139 patients were left and included into the analysis.At the first follow up session, 118 (84.9%) attended the clinic and filled the questionnaire; at the second follow up, 4 weeks after the study, 108 (77.7%) of the initial participants returned back to the clinic, and at the third follow up, 105 (75.6%) attended the clinic and filled the questionnaire.

Intervention Protocol

Before participants were recruited into the final stage of the study, they were entered a 2 week of run-in period through which they were asked to discontinue use of motility modifiers, antidepressants, opioids, narcotic analgesics and antispasmodic in cases, while use ofloperamide and prokinetic agents were allowed during the study. Also, no antibiotic agent should be used. After entering the study stage, patients who had been randomly assigned to receive either probiotics or placebo were given their agents for one month, and they were asked to fulfill an IBS-symptom-checklist at four time-points: (1) at the baseline; (2) at the second week after the trial starts; (3) at the 4th week after the start of the study; and (4) after the 6th week from the study commencement.

Ethics

All the study participants were asked to sign a consent form, all all of them were given full information about it before. The study has been approved by our local ethics committee in the Iran University of Medical Sciences, by the registration code '18904-30-03-91'.

Measuring Instrument

Birmingham IBS Symptom Questionnaire which consists of 11 symptoms related to IBS was used for evaluations. Each item consists of 5 answers in a scale of 1 to 5 which would be rated by the patients after appropriate description, with higher scores indicative of higher intensity. The overall Birmingham IBS Symptom Questionnaire score was calculated summing scores of all the 11 itemsfor each time-point. The changes in the overall scores were presented as mean± standard deviation (SD).

IBS Subtypes Categorization

IBS patients were categorized into three subgroups based on their main gastrointestinal

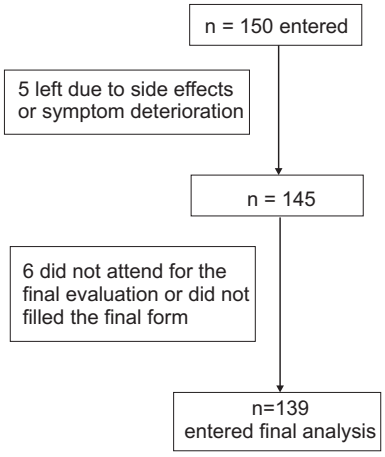

Figure 1. Flowchart indicative of flow of the study participants during the study course

symptoms. Diarrhea-predominant, constipation-predominant, and diarrhea-constipation mixed IBS were used to label IBS patients whose main symptoms were diarrhea, constipation or a mixture of them, respectively.

Other Defnitions

Body mass index (BMI) was defined as weight (Kg)/height (m)*height (m). BMI was defined as low-normal when it was equal or lower than 25 Kg/m^2. It was defined as high, when it was over 25 Kg/m^2. Categorizing response to treatment, we defined an improvement in each item, when the item score decreased from its baseline score, and no improvement when it either increased or changed not at all.

Statistical Analysis

Software SPSS version 17.0 (SPSS Inc, Chicago, Il, USA) has been used for analyses. Chi square test was used for analyzing categorical data. Student's t test was used when comparing changes in questionnaire scores before and after the intervention. One-way ANOVA has been used for comparing continuous variables between the three IBS subtypes, and Tukey's test was used for multiple comparisons, p value ≤0.05 was considered significant.

RESULTS

Demographics

Overall 139 patients entered into the study and filled the questionnaire at the pre-treatment stage. 68 (48.9%) of the participants were males and 71 (51.1%) were females. Mean±SD age of the participants was 38±13.3 years. Mean weight was 69.3±13.9 kgs, mean height, 168.8±9.4 cms and mean BMI was 24.2±3.9 m/kg^2. Forty-nine (35.3%) of the patients were diarrhea-predominant, while 55 (39.6%) were constipation predominant (5 missing data). The baseline characteristics were comparable between the patients in the two groups of the study.

Overall Analysis

At first, we analyzed potential changes in each of the 11 items of IBS symptomatology from the Birmingham questionnaire for the whole population, during each of the evaluation times, each 2 weeks after the study commencement. Evaluation showed no significant differential alterations in any of the items between the two groups. If the change in the overall Birmingham IBS symptom questionnaire scores were determined and compared to each other, despite the significant decrease in each of them, no differential significant difference in the rate of alterations in total questionnaire scores has been detected at the last follow up (-6.7±6.8 vs. -6.7±6.5 for the intervention and placebo groups, respectively; p=0.95).

Table 1. Comparing demographic features of patients in the probiotic and placebo groups

Parameter	Intervention	Placebo
Age (Year)	38.7±14.5	37.3±11.9
Weight (Kg)	69.4±15.1	74.1±51.6
Height (Cm)	169.3±9.2	165.9±21.9
Normal BMI* (%)	35 (53)	38 (55)
IBS** main symptom, n (%)		
- Diarrhea	22 (33)	27 (40)
- Constipation	31 (46)	24 (36)
- Both	14 (21)	16 (24)

*Calculated by the formula: Weight (Kg)/Height (m)^2, **Irritable bowel syndrome

Because of potential confounding effects of some major factors associated with the disease or patients, we conducted re-analyses after stratifying the data for these factors in order to censor these perplexing effects from the original pharmaceutical authority:

Reanalysis After Stratification for IBS Types

At the second step, the analysis was repeated after stratifying the data for the main IBS complaint (diarrhea vs. constipation) to evaluate potential effects of probiotic treatment on different IBS types. In the diarrhea-predominant IBS, probiotic therapy showed a significant improvement in the item 8 (**Table 1**) after 2 weeks (-0.47±0.8 vs. -0.13±0.3; p=0.05) and 6 weeks (-0.78±1.1 vs. -0.1±0.7, p=0.03) of probiotic therapy when they were compared to their counterparts on placebo. Also, probiotic therapy was significantly more effective than placebo in reducing the feeling of urgency (item 10, **Table 1**) in IBS patients with diarrhea-predominant symptoms (-0.84±1.5 vs. -0.04±0.9; p=0.03). Nonetheless, none of the 11 evaluated items had changed significantly in patients with constipation-predominant IBS on probiotics versus those receiving placebo. In IBS patients with both diarrhea-constipation symptoms, probiotic therapy was associated with worse symptoms of item-5 (**Table 1**) after 4weeks (-0.4±0.7 vs. -1.1±0.8; p=0.035) and 6 weeks (-0.36±0.9 vs. -1.4±1.2; p=0.025), compared to placebo therapy.

Reanalysis After Stratification for BMI and IBS Type

Again, a reanalysis of the study results has been conducted after stratifying data for both IBS predominant symptoms and BMI. So, with three group in IBS-type and two in BMI grouping (BMI≤25 & BMI>25), we have six subgroups in which potential effects of probiotic therapy have been compared to that of placebo:[1] Diarrhea-predominant IBS and low-normal BMI: Item 10 (**Table 1**) improved due to probiotic therapy at the first 2 weeks (-1±1.3 vs. 0±0.9; p=0.048); constipation-predominant IBS and low-normal BMI: Item 5 improved after 6 weeks in the case group compared to the placebo (-2.1±1.2 vs. -0.78±1.1; p=0.017); constipation-diarrhea IBS &low-normal BMI: Item 5 experienced worsening after probiotic therapy (vs. placebo) after 4 weeks of treatment start (-0.29±0.5 vs. -0.89±1.3; 0.05); diarrhea-predominant IBS & high BMI: No differential change in the IBS symptoms has been observed in the case versus control patients; constipation-predominant IBS and high BMI: improvement in item 6 two weeks after treatment has been observed in the case group (-0.45±0.8 vs. 0.22±0.8; p=0.04); diarrhea-

Table 2. Improvement rates in Birmingham IBS Symptom Questionnaire during the study period

Questionnaire items	Week 2			Week 4			Week 6		
	Probiotic (%)	Placebo (%)	OR (95% CI)	Probiotic (%)	Placebo (%)	OR (95% CI)	Probiotic (%)	Placebo (%)	OR:95% CI
1	34 (59)	26 (43)	.5 (.3-1.1)	28 (51)	30 (57)	1.3 (.6-2.7)	33 (68)	36 (68)	1.2 (.5-2.7)
2	29 (50)	23 (38)	.6 (.3-1.3)	26 (47)	29 (55)	1.3 (.6-2.9)	25 (48)	32 (60)	1.6 (.8-3.6)
3	26 (45)	26 (43)	.9 (.5-1.9)	24 (44)	27 (51)	1.3 (.6-2.9)	27 (52)	29 (55)	1.1 (.5-2.4)
4	16 (28)	24 (40)	1.7 (.8-3.8)	24 (44)	22 (41)	.9 (.4-2)	28 (54)	23 (43)	.6 (.3-1.4)
5	22 (38)	21 (35)	.9 (.4-1.9)	26 (47)	31 (58)	1.6 (.7-3.4)	29 (56)	30 (57)	1 (.5-2.3)
6	22 (38)	15 (25)	.5 (0.2-1.2)	27 (49)	22 (41)	.7 (.3-1.6)	26 (50)	21 (40)	.7 (.3-1.4)
7	26 (45)	27 (45)	1 (.5-2.1)	27 (49)	28 (53)	1.2 (.5-2.5)	35 (67)	31 (58)	.7 (.3-1.5)
8	19 (33)	15 (25)	.7 (.3-1.5)	24 (44)	21 (40)	.8 (.4-1.8)	22 (42)	20 (38)	.9 (.4-1.9)
9	9 (15)	8 (13)	.8 (.3-2.3)	8 (14)	11 (21)	1.5 (.6-4.2)	11 (21)	12 (23)	1.1 (.4-2.8)
10	17 (29)	17 (28)	1 (.4-2.1)	22 (40)	24 (45)	1.2 (.6-2.7)	22 (42)	20 (38)	.8 (.4-1.8)
11	22 (38)	15 (25)	.5 (.2-1.2)	18 (33)	19 (36)	1.1 (.5-2.5)	19 (36)	23 (43)	1.3 (.6-2.9)

constipation IBS and high BMI: Worsening in items 2 (0.00±0.000 vs. -1.5±1.1; p=0.017) and 4 (0.5±1.1 vs. -0.4±0.79; p=0.05) both after 6 weeks of treatment were detected.

Reanalysis After Categorization of Symptom Changes of IBS

Unlike previous analyses on the absolute change in the scorings of the Birmingham questionnaire, we reanalyzed the data after categorizing the change as improvement and worsening of the scores for each, and then made crosstabs. As can be seen in **Table 2**, no significant change has been observed in the evaluated items in any of the time points. Stratification of data entering the IBS type into the crosstabulation did not change the results.

DISCUSSION

Our study failed to demonstrate a broad therapeutic effect for probiotic therapy with *E. coli* Nissle 1917 in improving symptoms of patients with refractory IBS, in general. Although the efficacy of the probiotic *E-coli* Nissle 1917 was to some degree and in some individual items superior to that of placebo, the significance level has rarely been achieved; and when the questionnaire's scores have been categorized as improved and not improved, none of the analyses reached significance level.In fact, almost all of the questionnaire items in any of the follow-up time points have shown overall improvements; nonetheless these therapeutic effects were seen in both the case and control groups, with no significant difference in most cases. Interestingly, in some cases, the beneficial effect of placebo was shown to be even significantly better than the probiotic, which can be due to potential deleterious effects for the probiotic therapy in particular conditions which would be discussed latter. A number of explanations can be put forward for this observation. The scarcity of significance may be due to the limited sample size. Although compared to the literature of similar topic, our sample size seems comparable to the previous studies[15] or quite larger.[16] Another potential explanation can be the limited follow up time of 6 weeks, through which our patients have been observed. In the only published study about potential therapeutic effects of probiotic *E. coli* Nissle 1917 in IBS, the probiotic was minimally effective only 10 to 11 weeks after the therapy commencement.[17]

Literature suggests that *E.coli* Nissle 1917 inhibits the visceral hypersensitivity associated with trinitrobenzenesulphonic acid (TNBS) colitis.[18] Moreover, inhibitory effects have been observed for probiotic *E. coli* strain Nissle 1917 on adhesion to and invasion of intestinal epithelial cells by adherent-invasive *E. coli* strains isolated from patients with Crohn's disease, suggestive of its preventive or curative role in probiotic therapy of these patients.[19,20] For the same reasons and due to its demonstrated beneficial effects in the clinical trials, *E. coli* Nissle 1917 is used in the management of inflammatory bowel syndrome in the clinical setting.[21]

Despite the lack of obtaining overall negative significant effect for probiotic therapy in this study compared to the placebo, in some of the time points and in individual items, significance level was achieved. Although one may put doubt on the credibility of the found relations, but one another may argue that specific probiotic therapy might only have some beneficial or even deleterious effects on some individual symptoms in particular conditions, including the IBS types. For example, we found differential effects for probiotic therapy in either diarrhea-predominant or constipation-predominant or mixed-symptom IBS; in diarrhea-predominant, both urgency and sleeping disturbances were significantly improved in two evaluation time points at 2 and 6 weeks after treatment commenced, while in constipation-predominant IBS, no improvement in any of the evaluated factors has been detected. Nonetheless, in patients with diarrhea-constipation IBS, probiotic therapy was associated with deleterious effects in needing strain in passing stool in two time points, which may be considered confirmative to each other. So, according to these findings, we recommend IBS patients with mixed diarrhea-constipation symptoms should avoid using probiotic *E. coli* Nissle 1917. Similar conclusions can be made in other founded significance levels in the results section.

Literature also presents contradictory results for the therapeutic value of probiotic therapy in IBS. A comprehensive systematic

review on different probiotic used in IBS by different trials concluded that only the probiotic Bifidobacteriuminfantis 35624 was associated with a significant efficacy.[22] Another reason behind the controversial results about probiotic treatment in IBS patients is that IBS is not a single entity but rather aset of not well defined disorders.[23] Therefore, the fact that there is no well known effective treatment for all these patients can also be explained. Evidence suggests that a given therapeutic approach in different patient populations corresponds to a large dissimilarity in the result (discussed above). In the current study, it was shown that even in the subpopulations of a unique study, profound disparities observed in the responses to treatment.While the probiotic *E.coli* Nissle 1917 was, though minimally, effective in diarrhea-predominant IBS, it was destructive in diarrhea-constipation mixed IBS symptomatology. So, maybe different presentations of IBS would, to some degree, reveal different pathogenesis, necessitating customization of treatment protocols for each specific type of IBS; in fact, several authors have already suggested this approach.[24,25]

There are several mechanisms proposed to explain therapeutic effects of probiotics in IBS. Through cross talk, quorum sensing systems, probiotics can alter microflora of the intestine directly; or indirectly through immunomodulatory, antiinflammatory and barrier activities.[26] It has also been demonstrated that in IBS patients, persistent changes in proinflammatory (IL-12) and antiinflammatory (IL-10) cytokines occur[27]; in which probiotics have been shown to improve IL-12/IL-10 ratio concomitant to clinical improvement. More particularly about *E.coli* Nissle 1917, evidence suggests that it stimulates IL-10 production of peripheral mononuclear cells[28], improves intestinal motility[29], prevents the invasion of pathogens into the mucosa[30], and induces the synthesis of antimicrobial peptides including human β-defensins and also synthesis of tight-junction proteins in intestinal epithelial cells.[31]

CONCLUSION

Probiotic therapy with *E.coli* Nissle 1917 was not able to induce significant improvement in the symptoms of patients with non-categorized IBS. Nevertheless, when IBS patients were recategorized to subgroups according to their main symptoms, evaluation of the efficacy of the probiotic on some individual items in the symptom list reached the significance level. However, due to some limitations of the current study including the limited sample size, and borderline p values achieved through analyses, prospective clinical trials are recommended for confirming our findings.

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