Original Article

Therapeutic Effects, Tolerability and Safety of a Multi-strain Probiotic in Iranian Adults with Irritable Bowel Syndrome and Bloating

Elham Jafari MD MPH[1], Homayoon Vahedi MD•[1,2], Shahin Merat MD[1,2], Shabnam Momtahen MD[1], Aina Riahi MD[1]

Abstract

Background: Irritable bowel syndrome (IBS) is a common disorder in Iran with challenging treatment. Although trials have suggested that probiotics alleviate the complaints of patients with minimal side effects, they have not been investigated in Iranian adults.

Methods: In a randomized double-blind study, 108 eligible IBS patients (Rome III Criteria) aged 20 – 70 years who referred consecutively to a clinical center in Tehran with abdominal bloating from 2010 to 2012 received a combination probiotics or placebo twice daily for 4 weeks. The objective was to evaluate the efficacy and safety of a multi-strain probiotics combination. One week prior to and throughout the treatment, the participants recorded their abdominal symptoms on a daily basis, using visual analogue scale and reported satisfactory relief of general symptoms at the end of each week. Adverse events were evaluated by self-reporting and physical examination. Continuous variables were analyzed by independent t-test and chi-square was used for binomials.

Results: The baseline characteristics were balanced (60% female, mean age 36.7 ± 11.5). A total of 97 (51 intervention, 46 control) completed the treatment. Intention to treat analysis was done on 108 allocated subjects. 85% of the probiotic group reported satisfactory relief of general symptoms compared with 47% in the control group ($P < 0.01$). A reduction in abdominal bloating and pain with probiotic was superior to placebo [-13.0 vs. -3.7 ($P < 0.01$), -8.2 vs. -2.1 ($P = 0.02$), respectively]. No severe adverse drug reaction was seen in either group.

Conclusions: A 4-week period of treatment with the combination probiotics twice daily was safe, well tolerated, and effective in our patients. Further investigation is recommended for other subgroups of IBS. Trial Registration: IRCT.ir IRCT2012071010230N1

Keywords: Adults, bloating, IBS, Iran, probiotic

Cite this article as: Jafari E, Vahedi H, Merat S, Momtahen S, Riahi A. Therapeutic effects, tolerability and safety of a multi-strain probiotic in Iranian adults with irritable bowel syndrome and bloating. *Arch Iran Med*. 2014; **17(7):** 466 – 470.

Introduction

Irritable bowel syndrome (IBS) is a common chronic disease which imposes a heavy burden on the economy of developed countries.[1,2] The global prevalence of IBS is estimated at 11%.[3] In Iran, the prevalence of IBS is reported between 1.1% and 25.0%.[4,5] Its total yearly costs was estimated to be 2.94 billion PPP$ (purchasing power parity dollars) by Roshandel, et al. in 2007, indicating a major need of the Iranian health care system to find a satisfactory treatment for these patients.[6]

The etiology of IBS is unknown. Recurrent abdominal pain, bloating, constipation, and diarrhea are the most common of its symptoms. There is no cure for IBS and the treatments are based on controlling the symptoms. Up to now, several chemical drugs have been introduced for patients with IBS; however, their long-term side effects are not satisfactory.[7]

Probiotics are among recent and favorable approaches which have no severe drug reactions. Probiotics are defined as "viable micro-organisms that confer potential health benefits by preventing or treating specific pathological conditions".[8] A number of different mechanisms have been proposed for probiotic action in IBS. Normalizing intestinal flora, preventing the overgrowth of pathogenic bacteria, and reduction of inflammation in the gastrointestinal tract are among the proposed mechanisms while recent findings suggest the role of "microbiota-gut-brain axis".[9–14] The most commonly evaluated probiotics in previous trials were the lactic acid producing bacteria, particularly *Lactobacillus* and *Bifidobacterium* species (sp.). Consistent gains in these trials were reduction in abdominal bloating and alleviation of general symptoms.[15–17]

IBS is a multi factorial disorder and could be affected by many individual factors in different populations such as genetic factors, the gut microbiota and diet habits. Despite the fact that the safety and effectiveness of probiotics in improvement of IBS has been proven through a number of systematic reviews and meta-analyses, it is necessary to examine the efficacy and tolerability of each strain and combination of probiotics on each symptom of IBS in different populations separately in order to find the most effective combinations of probiotic strains with proper dosage.

While Iran is among the countries with high prevalence of IBS, the efficacy of probiotic therapy has not been investigated in Iranian adults with IBS. We conducted this study to evaluate the efficacy, safety and tolerability of a multi-strain probiotic combi-

Authors' affiliation: [1]Digestive Diseases Research Center, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran. [2]Masoud clinic, Kargar Shomali Avenue, Tehran, Iran.

•Corresponding author and reprints: Homayoon Vahedi MD, Digestive Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran. 14117-13135-North Kargar Ave., Shariati hospital, Tehran-Iran. Tel: +98-21-82414-82415000, Fax: +98-21-82415400, E-mail: vahedi@ams.ac.ir

Accepted for publication: 20 February 2014

nation (Probio-Tec® Quatro-cap-4) in Iranian adult patients with IBS and symptom of bloating. Abdominal pain and bloating are the most frequent and troublesome symptoms of IBS.[18,19] These symptoms, especially bloating, are common between IBS and lactose intolerance (LI). Considering the high prevalence rate of LI among Iranian adults with IBS, and the potential role of some species of probiotics in alleviating LI,[20–23] we planned to exclude all patients with LI from the study to determine the effectiveness of treatment effects of probiotics combination on patients with IBS alone.

Since IBS is a heterogeneous condition, it seems a multi strain product may be more effective than a single strain. The Probio-Tec® Quatro-cap-4 contains *Bifidobacterium animalis* subsp. lactisBB-12®, *Lactobacillus acidophilus* LA-5®, *Lactobacillus delbrueckii* subsp. bulgaricus LBY-27, *Streptococcus thermophilus* STY-31. The first two strains have been used in numerous clinical trials and are suggested to improve gastrointestinal function, fecal properties and microbiota. The two remaining strains are most widely used bacteria in dairy products which help the digestion of lactose and milk protein.

Patients and Methods

The trial was designed as a randomized double blind, placebo-controlled study. The protocol was approved by the ethics committee of the digestive diseases research institute.

From March 2010 to December 2012, patients referring consecutively to a private clinical center of gastroenterology in Tehran, diagnosed with IBS according to Rome III criteria, aged 20 to 70 years and complaining of bloating were evaluated for eligibility. The exclusion criteria were: onset of discomfort above 50 years of age, steady progressive course, symptoms which lead to awakening from sleep, presence of fever or weight loss, dehydration, drug abuse, pregnancy or lactation, dementia or severe mental disease, inflammatory bowel disease (IBD), any cancer, severe systemic disease, diabetes, hypothyroidism, immunodeficiency, history of abdominal surgery (except appendectomy, Caesarean section, tubal ligation, and hysterectomy). Eligible patients who consented to the study underwent lactose breathe hydrogen test. They were considered eligible subjects if their test did not show lactose intolerance (LI).

During the screening visit, they provided their written consent and were asked to record the severity of abdominal symptoms including pain or discomfort, bloating or distention, and feeling of incomplete defecation per each passage in a diary, during one week prior to the documentation of baseline characteristics. The enrolled subjects were randomized to receive either a probiotics combination (Probio-Tec® Quatro-cap-4) or identical looking placebo twice daily for 4 weeks. Subjects were asked to record the same symptoms on a daily basis during the treatment period and report satisfactory relief of general symptoms at the end of each week as yes or no. All participants were followed one month after completing the treatment.

The probiotic combination used was Probio-Tec® Quatro-cap-4: a multi-strain product containing *Bifidobacterium animalis* subsp. lactisBB-12®, *Lactobacillus acidophilus* LA-5®, *Lactobacillus delbrueckii* subsp. bulgaricus LBY-27, *Streptococcus thermophilus* STY-31, with a minimum potency of 4 billion CFU. Both the Probio-Tec® Quatro-cap-4 and the placebo were provided in capsule form for this study by Chr. Hansen Company (Horsholm, Denmark).

The subjects were instructed to avoid laxatives and drugs affecting intestinal motility, anti-diarrhea drugs, symbiotics, other probiotics, antibiotics and any other drugs affecting IBS medication from two weeks before starting the study. Any subject who had been administered antibiotics during the treatment period was asked to discontinue probiotic therapy and postpone it to two weeks after completing the antibiotic treatment.

The efficacy was defined as significant reduction in mean of abdominal bloating score at week four of treatment. Secondary endpoints included description of changes in abdominal pain or discomfort score, reduction in proportion of feeling of incomplete defecation per each passage, determining the proportion of patients reporting relief of symptoms for at least 50% of the time and description of adverse drug reaction in both groups.

Severity of symptoms was determined using Score sheet 100-mm visual analogous scales (VAS), the VAS ranges from 0 to 100 mm (0 = no symptom, 100 = worst symptom). The visual analogue scale (VAS) is the most frequent scale for examining the severity of IBS symptoms in previous trials. The VAS is a reliable scale which contains no words and can be used regardless of language. Validity of the visual analogue scale in determining the severity of IBS symptoms is approved by clinicians and previous studies.[24,25]

In order to assess safety and tolerability, the patients were asked to record any possible complications such as skin rashes, eczema, headache, increased gas, diarrhea, constipation, nausea, heartburn and define its severity as mild (awareness of sign or symptom but easily tolerated), moderate (enough discomfort to cause interference with usual activity), severe (incapacitating with inability to work or perform usual activities), in a diary at baseline and throughout the treatment or report it to the researcher physicians by phone. Physical examination and laboratory testing were performed if needed.

Based on previous studies, a sample size of 106 (53 in each group) was considered. This figure was computed to detect a 30% difference in treatment effect in the intervention group compared with placebo with 90% power at a 0.05 level of statistical significance and considering 20% possible dropout.

Randomization was done by a computer generated randomization list, using the method of randomly permuted blocks (block size 4). For the purpose of blinding, the probiotic and placebo products were packaged in same shape bottles and the true content of each bottle of probiotic or placebo could only be determined by consulting the computer database. This database was kept from researchers and other people involved in assigning or following patients until the end of treatment period.

Analysis was done using SPSS software (version 16, Inc., Chicago, IL) based on intention to treat. The intention to treat population was defined as all participants who consumed probiotics or place before at least one week and visited the interviewer once or more during the treatment. Independent t-test was used for continuous variables and chi-square test was performed for binomials. A *P*-value of less than 0.05 was considered to be statically significant.

Results

349 subjects with IBS (according to Rome III criteria) and bloating were identified; 332 met the inclusion criteria and underwent lactose hydrogen breath test, 214 subjects (64%) were excluded

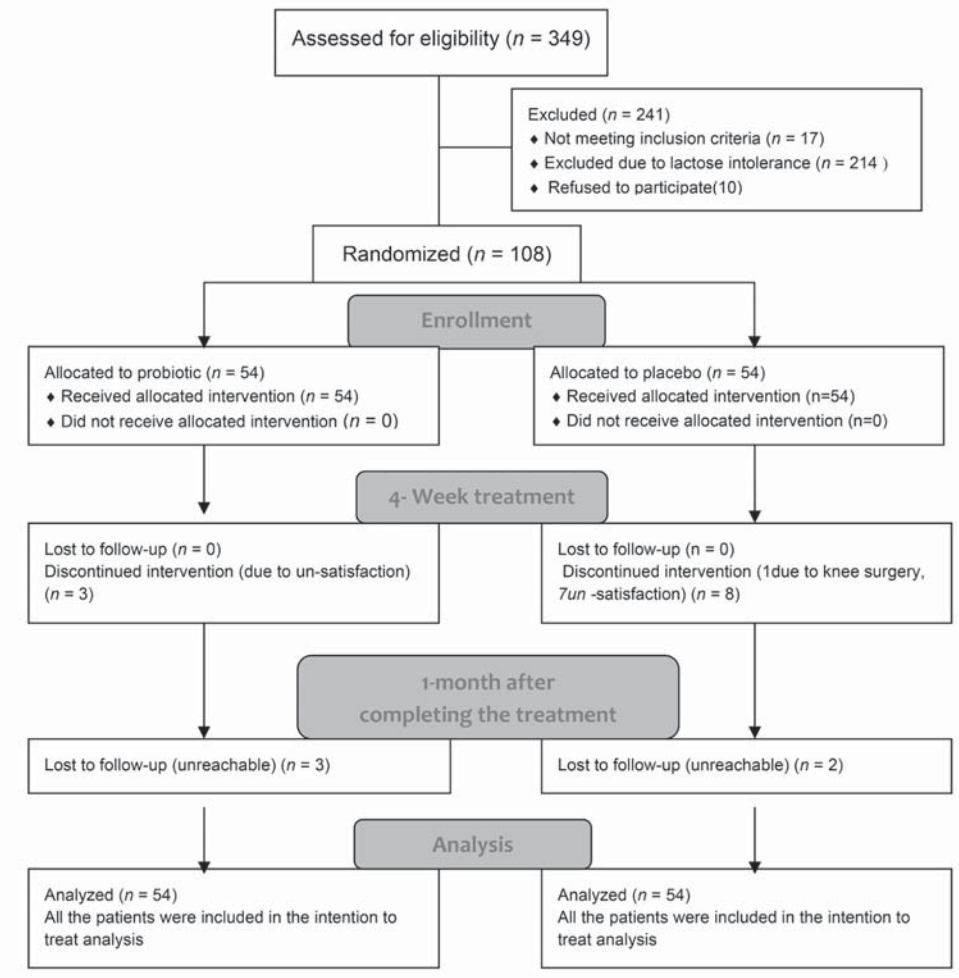

Figure 1. Participant flow diagram.

Table1. Baseline characteristics and symptoms scores of patients

	Probiotic (*n* = 54)	Placebo (*n* = 54)	*P*-value
Females (%)	61.1	59.3	0.84
Mean age (mean ± SD)	36.6 ± 12.1	36.8 ± 11.0	0.93
Baseline VAS score (mean ± SD)			
Abdominal Pain (mm)	18.6 ± 14.7	16.7 ± 14.9	0.50
Abdominal Bloating (mm)	33.1 ± 19.8	29.8 + 19.0	0.39
Feeling of Incomplete Defecation (%) ± SD	57.6 ± 34.8	52.3 ± 29.7	0.40

due to some degree of lactose intolerance, 10 subjects did not consent to the study and the remaining 108 subjects were randomized into equal groups (54 in the probiotic and 54 in the placebo group) (Figure 1).

The baseline characteristics of patients are listed in Table 1. The mean age was 36.7 (±11.5) years and 60% of participants were women.

The severity of abdominal symptoms, namely abdominal pain and bloating, was 88%, 66% mild, 19%, 40% moderate and 1%, 2% severe, respectively. No Significant difference was seen among males and females regarding pain or bloating severity (P = 0.60, P = 0.35, respectively).

After 2 weeks of treatment, 11 of the 108 randomized patients 3 (5%) in the intervention group and 7 (13%) in the control group withdrew from the study due to dissatisfaction and one patient discontinued in the control group due to knee surgery. All of the patients who had at least one visit after starting the treatment were included in the analysis based on the intention-to-treat principles.

The change in the intensity of symptoms after the 4-week intervention period and at the end of one month follow-up after completing the treatment is shown in Table 2.

After completing the treatment, the proportion of patients who reported satisfactory relief of general symptoms with the multi-strain probiotics was 85% compared to 47% among those with placebo ($P < 0.01$).

As listed in Table 3, out of 108 recruited patients, 6 (11%) in the probiotic group and 8 (15%) in the placebo group reported at least increased mild to moderate degree of some gastrointestinal symptoms including abdominal pain, distention, borborygmi, nausea and heartburn. No severe adverse drug reaction or events occurred during the intervention.

Table 2. Change of symptoms on completion of treatment period and one month after

Symptoms	Probiotic (*n* = 54)	Placebo (*n* = 54)	*P*- value	Probiotic (*n* = 54)	Placebo (*n* = 54)	*P*-value
	ΔWeek 4	ΔWeek 4		Δ one month after	Δ one month after	
VAS score(mean, 95% CI)						
Abdominal Pain (mm)	-8.2(-12.5 to -3.9)	-2.1(-5.0 to +0.8)	0.02	-6.9 (-10.9 to -2.9)	+0.1(-3.1 to 3.3)	<0.01
Abdominal Bloating (mm)	-13.0(-17.5 to -8.2)	-3.7(-7.1 to -0.2)	<0.01	-4.7(-10.1 to -0.7)	+2.5(-1.5 to 6.5)	0.03
Feeling of Incomplete Defecation (%) ±SD	-32.9(-40.4 to -24.5)	-12.6(-18.9 to -6.2)	<0.01	-6.0(-9.8 to -3.1)	-3.7(-6.1 to -1.3)	0.25

Table 3. Distribution and quantitative analysis of undesirable symptoms in probiotic and placebo groups during treatment*

	Probiotic (*n* = 54)	Placebo (*n* = 54)
Symptoms	***n* (%)**	***n*(%)**
Nausea	0(0)	2(4)
Heartburn	2(4)	1(2)
Borborygmi	1(2)	3(7)
Abdominal pain	1(2)	1(2)
Abdominal distention	2(4)	1(2)

*None of the differences between the two groups were statistically significant.

Discussion

In this study, we evaluated a multi-strain Probiotics combination (Probio-Tec® Quatro-cap-4) on Iranian adults with IBS and bloating for the first time; after the 4-week treatment period, we observed a superior reduction in abdominal bloating, pain and the percentage of feeling incomplete defecation per each passage with probiotics compared to placebo.

There are numerous studies published on the efficacy of probiotics on IBS; nevertheless, due to their heterogeneity in choosing evaluated outcomes, characteristics of target population and the probiotics examined, it is not easy or justifiable to compare their results. The only study we could find to compare with our results was a meta-analysis by Ortiz-Lucas, et al. which shows that the beneficial effects of Probio-Tec® Quatro-cap-4 on abdominal symptoms are greater than that of each comprising species alone. While the reduction in abdominal pain with Probio-Tec® Quatro-cap-4 was -8.2 (95% CI; -12.5, -3.9), this change was -0.1 (95% CI; -0.2, 0.2) with *Bifidobacterium animalis*, -0.3 (95% CI; -0.6, -0.0) with *Lactobacillus acidophilus* , -0.5 (95% CI; -0.9, -0.1) with *Lactobacillus delbrueckii* subsp. and -0.3 (95% CI; -0.6,-0.0) with *Bulgaricus* and *Streptococcus thermophilus*, the reduction in abdominal bloating with Probio-Tec® Quatro-cap-4 was-13.0 (95% CI; -17.5, -8.2), much more than -0.0 (95% CI; -0.2, 0.2) with *Bifidobacterium animalis*, -0.2 (95% CI; -0.5, -0.2) with *Lactobacillus acidophilus*, -0.1 (95% CI; -0.4, 0.2) *Lactobacillus delbrueckii - Streptococcus thermophiles.*[26]

Satisfactory relief of general symptoms had a response rate of 1.8-fold higher than placebo ($P < 0.01$). This result is consistent with that of a met-analysis by Hoveyda, et al. which estimated the ratio of 1.6 (95%; 1.2, 2.2) for improvement of overall symptoms of IBS with probiotic compared to placebo.[15]

The 45% response rate for satisfactory relief of general symptoms in placebo group was high and more than our previous studies in the Iranian population,[27,28] but it was in the range of a meta-analysis by Dorn, et al. who reported a response rate of 15% – 72% for placebo.[29,30]

Despite the high response rate for satisfactory relief of general symptoms in the placebo group, the difference between proportions of satisfactory relief with Probio-Tec® Quatro-cap-4 remained significant, showing the fact that the sample size was large enough to detect the significant difference.

The strengths of this study include balancing the characteristics of patients at baseline which showed proper randomization and the 90% power of this study which was higher than previous reports.

A novelty of this study is evaluating the beneficial effects of probiotics on pure IBS patients (i.e. those without Lactose Intolerance). Excluding patients with Lactose Intolerance (LI) showed that the effectiveness of Probio-Tec® Quatro-cap-4 in alleviating symptoms of IBS was independent from improvement of lactase deficiency; these results could help to identify the true mechanisms of action of probiotics in improvement of functional problems.

The prevalence of Lactose Intolerance (64%) in our study was consistent with the previous reports of high prevalence of LI in Iran and its overlap with IBS.[31–32]

No severe adverse events were screened in this study. The rare adverse events observed were mild to moderate and tolerable with no difference between probiotics and placebo. Although the safety of probiotics has been established by several previous studies,[33,34] this is the first trial to show safety and tolerability of a multi-strain probiotic combination (Probiotec q cap4) in a subgroup of Iranian patients with IBS.

After one month of follow up, some degree of relapse was seen in all the evaluated symptoms. Although no data is available to compare, we know that IBS is an intermittent and chronic disease and probiotics are used for improving, not curing, it; so, this result was not unexpected, and since there is no report of adverse drug reactions with probiotics in IBS patients, they seem to be a good choice for treatment of IBS in long term. Still, further studies are warranted to determine the optimal dosage and duration of use in order to yield the greatest effectiveness.

Acknowledgments

This study was funded by a grant from the Digestive Disease Research Center, Tehran University of Medical Science. The study medication was kindly provided by Chr. Hansen Company, Horsholm, Denmark.

References

1. Hulisz D. The burden of illness of irritable bowel syndrome: current challenges and hope for the future. Journal of Managed Care Pharmacy. *JMCP*. 2004; **10(4):** 299 – 309.
2. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clinical gastroenterology and Hepatology: The Official Clinical Practice Journal of The American Gastroenterological Association*. 2012; **10(7):** 712 – 721.
3. Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. *Clin Epidemiol*. 2014; **6:** 71 – 80.
4. Jahangiri P, Jazi MS, Keshteli AH, Sadeghpour S, Amini E, Adibi P. Irritable Bowel Syndrome in Iran: SEPAHAN Systematic Review No. 1. *International Journal of Preventive Medicine*. 2012; **3(suppl 1):** 1 – 9.
5. Vahedi H, Ansari R, Mir-Nasseri MM, Jafari E. Irritable bowel syndrome: A review article. *Middle East Journal of Digestive Diseases*. 2010; **2(2):** 66 – 77.
6. Roshandel D, Rezailashkajani M, Shafaee S, Zali MR. A cost analysis of functional bowel disorders in Iran. *International Journal of Colorectal Disease*. 2007; **22(7):** 791 – 799.
7. Lacy BE, Weiser K, De Lee R. The treatment of irritable bowel syndrome. *Therapeutic Advances in Gastroenterology*. 2009; **2(4):** 221 – 238.
8. JMJ HRHIv. Probiotics: A general view. In :Lactic Acid Bacteria in Health and Disease. London: *Elsevior*; 1992.
9. Barbara G, Zecchi L, Barbaro R, Cremon C, Bellacosa L, Marcellini M, et al. Mucosal permeability and immune activation as potential therapeutic targets of probiotics in irritable bowel syndrome. *Journal of Clinical Gastroenterology*. 2012; **46 (suppl 5):** 52 – 55.
10. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*. 2011; **141(2):** 599 – 609.
11. Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; **108(7):** 3047 – 3052.
12. Matricon J, Meleine M, Gelot A, Piche T, Dapoigny M, Muller E, et al. Review article: Associations between immune activation, intestinal permeability and the irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics*. 2012; **36(11 – 12):** 1009 – 1031.
13. Spiller R. Review article: Probiotics and prebiotics in irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics*. 2008; **28(4):** 385 – 396.
14. Walker WA. Mechanisms of action of probiotics. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*. 2008; **46 (suppl 2):** 87 – 91;
15. Hoveyda N, Heneghan C, Mahtani KR, Perera R, Roberts N, Glasziou P. A systematic review and meta-analysis: probiotics in the treatment of irritable bowel syndrome. *BMC Gastroenterology*. 2009; **9:** 15.
16. McFarland LV, Dublin S. Meta-analysis of probiotics for the treatment of irritable bowel syndrome. *World Journal of Gastroenterology (WJG)*. 2008; **14(17):** 2650 – 2661.
17. Ritchie ML, Romanuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. *PloS One*. 2012; **7(4):** e34938.
18. Maxton DG1, Morris JA, Whorwell PJ. Ranking of symptoms by patients with the irritable bowel syndrome. *BMJ*. 1989; **299(6708):** 1138.
19. Chang L, Lee OY, Naliboff B, Schmulson M, Mayer EA. Sensation of bloating and visible abdominal distension in patients with irritable bowel syndrome. *Am J Gastroenterol*. 2001 Dec; **96(12):** 3341 – 3347.
20. Sadre M, Karbasi K. Lactose intolerance in Iran. *The American Journal of Clinical Nutrition*. 1979; **32(9):** 1948 – 1954.
21. Bohmer CJ, Tuynman HA. The effect of a lactose-restricted diet in patients with a positive lactose tolerance test, earlier diagnosed as irritable bowel syndrome: a 5-year follow-up study. *European Journal of Gastroenterology & Hepatology*. 2001; **13(8):** 941 – 944.
22. Levri KM, Ketvertis K, Deramo M, Merenstein JH, D'Amico F. Do probiotics reduce adult lactose intolerance? A systematic review. *The Journal of Family Practice*. 2005; **54(7):** 613 – 620.
23. Almeida CC, Lorena SL, Pavan CR, Akasaka HM, Mesquita MA. Beneficial effects of long-term consumption of a probiotic combination of Lactobacillus caseiShirota and Bifidobacteriumbreve Yakult may persist after suspension of therapy in lactose-intolerant patients. *Nutrition in Clinical Practice: Official Publication of the American Society for Parenteral and Enteral Nutrition*. 2012; **27(2):** 247 – 251.
24. Bengtsson M, Ohlsson B, Ulander K. Development and psychometric testing of the Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS). *BMC Gastroenterol*. 2007; **7:** 16.
25. Bengtsson M, Hammar O, Mandl T, Ohlsson B. Evaluation of gastrointestinal symptoms in different patient groups using the visual analogue scale for irritable bowel syndrome (VAS-IBS). *BMC Gastroenterol*. 2011; **11:** 122. doi: 10.1186/1471-230X-11-122.
26. Ortiz-Lucas M, Tobias A, Saz P, Sebastián JJ.Effects of probiotic species on irritable bowel syndrome symptoms: a bring up to date meta-analysis. *Rev Esp Enferm Dig*. 2013; **105(1):** 19 – 36.
27. Vahedi H, Merat S, Momtahen S, Kazzazi AS, Ghaffari N, Olfati G, et al. Clinical trial: the effect of amitriptyline in patients with diarrhoea-predominant irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics*. 2008; **27(8):** 678 – 684.
28. Vahedi H, Merat S, Rashidioon A, Ghoddoosi A, Malekzadeh R. The effect of fluoxetine in patients with pain and constipation-predominant irritable bowel syndrome: a double-blind randomized-controlled study. *Alimentary Pharmacology & Therapeutics*. 2005; **22(5):** 381 – 385.
29. Dorn SD, Kaptchuk TJ, Park JB, Nguyen LT, Canenguez K, Nam BH, et al. A meta-analysis of the placebo response in complementary and alternative medicine trials of irritable bowel syndrome. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*. 2007; **19(8):** 630 – 637.
30. Ford AC, Moayyedi P. Meta-analysis: factors affecting placebo response rate in the irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics*. 2010; **32(2):** 144 –158.
31. Saberi-Firoozi M, Khademolhosseini F, Mehrabani D, Yousefi M, Salehi M, Heidary ST. Subjective lactose intolerance in apparently healthy adults in southern Iran: Is it related to irritable bowel syndrome? *Indian Journal of Medical Sciences*. 2007; **61(11):** 591 – 597.
32. Adibi P, Mirshahzadeh P, Sadeghizadeh A. Dairy intolerance syndrome in Iranian young adult. Journal of research in medical sciences. *The official journal of Isfahan University of Medical Sciences*. 2009; **14(6):** 357 – 366.
33. Hong KS, Kang HW, Im JP, Ji GE, Kim SG, Jung HC, et al. Effect of probiotics on symptoms in korean adults with irritable bowel syndrome. *Gut and Liver*. 2009; **3(2):** 101 – 107.
34. Mangalat N, Liu Y, Fatheree NY, Ferris MJ, Van Arsdall MR, Chen Z, et al. Safety and tolerability of Lactobacillus reuteri DSM 17938 and effects on biomarkers in healthy adults: results from a randomized masked trial. *PloS One*. 2012; **7(9):** e43910.