Double strain probiotic effect on Helicobacter pylori infection treatment: A double-blinded randomized controlled trial

Abstract

Background: A decreased rate of successful helicobacter pylori (H. pylori) infection treatment has revealed serious demand for more effective regimens to eradicate infection. Therefore, probiotics have recently been considered to increase the rate of antibiotic regimens efficacy in H. pylori infections. In current randomized controlled trial, we evaluated the effect of double strain probiotic combination with standard triple therapy (STT), in the eradication rate of H. pylori infection.

Methods: In current randomized placebo-control study, all patients (176 subjects) underwent the STT for 10 days. However, the study group received triple therapy for the eradication of H. pylori with supplement of Lactobacillus probiotic for 4 weeks and placebo was administered to control group, as well. Adverse effects of the antibiotic regimen were recorded for all patients. Six weeks after the cessation of probiotic intake, all patients underwent H. Pylori with fecal antigen of test, followed by a recurrence evaluation six months later.

Results: There was no significant difference in demographic data and presenting symptoms between the study groups. The eradication rate of H. pylori infection was significantly higher in probiotic group (78.4%), compared to that of placebo group (64.8%) (P=0.033). In addition, adverse events were significantly less prevalent in patients that received probiotic (P=0.047). Nonetheless, there was no significant difference in terms of infection recurrence during a 6-month follow-up (P=0.07).

Conclusion: Double strain probiotic in combination with STT increased the eradication rate of H. pylori infection, while the adverse events due to antibiotic therapy decreased.

Keywords: Helicobacter pylori, Standard triple therapy, Probiotic, Lactobacillus, Recurrence.

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During recent years, despite the triple therapy efficacy and high compliance, a constant decline has been reported in eradication rates to less than 55%, subsequent to standard therapy in both adults and pediatrics (8). The falling rate in treatment success is mostly based on bacterial resistance to prescribed antibiotics, yet, antibiotic-associated adverse effects, including diarrhea, nausea, vomiting, abdominal pain, and bloating, decrease the therapy compliance limiting the use of antibiotics (7, 9, 10). Therefore, there is a great interest over the development of a new treatment regimens including administrating of probiotic supplements besides standard protocol to improve eradication rate.

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) defined probiotics as live microorganisms, conferring a health benefit on the host (11). Although, there are several clinical trials which have been included in reviews and meta-analyses suggesting efficacy of probiotics as an adjuvant treatment to be of several benefits, in improving the treatment tolerability and reducing the side effects of triple therapy. Some reviews reported no significant improvement in H. pylori infection treatment, subsequent to of consumption probiotics (12-15).

Thus, with due attention to current controversies on the effect of probiotics on the eradication of H. Pylori infection and reducing the side effects of therapies, we aimed to evaluate the accuracy of probiotics on the eradication of infection, as well as to investigate the efficiency of probiotics in the recurrence rate after 6 months.

**Methods**

During a one-year period, between November 2015 to November 2016, a prospective randomized placebo-control trial was conducted in infectious diseases wards of Sina and Imam Reza Hospitals affiliated to Tabriz University of Medical Sciences, Tabriz, Iran, to compare the efficiency of adding probiotics to investigate the modification of H. pylori eradication therapy during the past 5 years, 4) the use of NSAIDs and corticosteroids, 5) severe diseases such as malignant disease or history of upper gastrointestinal (GI) problems, such as diaphragmatic hernia, barrette esophagus, and achalasia, 6) pregnancy or lactation, 7) history of immunosuppressive drugs consumption, 8) patients with hereditary immunodeficiency, 9) patients suffering from poorly controlled diabetes, 10) individuals not capable to adhere therapy protocol. All patients provided written informed consent. This study was approved by the Ethics Committee of Tabriz University of Medical Sciences and registered in Iranian Registry of Clinical Trials, (IRCT) (IRCT2016060628304N1).

Randomization was centrally conducted in all groups using standard methodology with a computer-generated list, subsequent to patients’ enrollment. To prevent bias in participant selection and follow-up, we used a combination of both intention-to-treat (ITT) and per protocol method. Prior to drugs administration, all patients presenting symptoms were recorded. Patients received the standard triple therapy (STT) including, pantoprazole (40 mg daily, before breakfast), amoxicillin (1000 mg b.i.d., after meals), clarithromycin (500 mg b.i.d., after meals) for 10 days. Although, the probiotic group received the triple therapy for the eradication of H. pylori with supplement of Prodigest® (500 mg b.i.d.) (Gostaresh Milad Pharmed Co. Tehran, Iran) and the control group received triple therapy with placebo. The Prodigest® capsules contain two bacteria strain including Lactobacillus and Bifidobacterium, with total viable count (TVC) of $15 \times 10^8$ CFU/ per capsule.

The Prodigest® and placebo were continued up to 4 weeks after triple therapy. At least 6 weeks after the cessation of probiotic intake all patients were tested for the eradication by measuring fecal antigen of H. pylori (Using TOYO H.PYLOREG ANTIGEN Test Stool). Also, six months after cessation of probiotic supplement, H. pylori status was rechecked to determine relapse rate, respectively.

All data were analyzed using SPSS Version 23 Software (SPSS Inc. Chicago, IL). Student’s t-test was used to determine the nonparametric proportions between the two study groups. Moreover, Pearson’s chi-square test and Fisher’s exact test were carried out to determine the efficacy of the two treatments. Furthermore, we used direct-regression test for analyzing the effects of variations on the obtained results. The results with p<0.05 were considered to be statistically significant.
Results

In current randomized controlled trial, 176 patients with *H. pylori* infection diagnosis were enrolled and randomly allocated to study (88 patients) and control (88 patients) groups (figure 1). Of these, 95 (53.9%) patients were males, and the mean age was 28.34±5.78 year. Patient demographic data are summarized in table 1, considering the case (A) and control (B) groups. There were no significant differences in terms of age and gender between the study groups. While comparing the primary severity of the symptoms, there was no significant difference in terms of prevalence of presenting symptoms between the study groups (P: 0.073).

Patients underwent *H. pylori* fecal antigen test, in which a month later, it showed positive results in 50 (28.4%) patients. Though, *H. pylori* eradication during a one-month follow-up after treatment was significantly higher in the group that received probiotic supplements (P=0.033). The prevalence of adverse events subsequent to antibiotic consumption including epigastric pain, diarrhea and vomiting is listed in table 1. Although, there was only...
significant difference in the prevalence of epigastric pain between the study groups (P=0.040), the overall prevalence of adverse effects was significantly lower in patients that received combined STT and probiotic regimen (P=0.047).

To evaluate rate of recurrence during a 6-month follow-up, the patients who had positive *H. pylori* antigen in the first month after treatment (19 and 31 patients in groups A and B, respectively), were excluded. Subsequent to patients’ exclusion, we compared age and gender distribution between reformed study groups, which showed no significant difference (table 2.). Fecal antigen test revealed, infection recurrence in 6 (8.6%) patients in group A and 11 (19.2%) patients in group B suffered *H. pylori* at the end of a 6-month follow-up, however, there was no significant difference between the study groups (P=0.070).

Linear regression was used to evaluate the effect of age, gender and treatment protocol on the eradication of infection and rate of treatment success, whereas, treatment protocol was the only variable to significantly affect the successful eradication rate in *H. pylori* infection (P=0.038).

### Table 1. Patient demographic data, presenting symptoms and habitual history.

| Groups            | STT (n=88) | Probiotic (n=88) | P value |
|-------------------|------------|------------------|---------|
| **Gender (%)**    |            |                  |         |
| Male              | 42 (46)    | 47 (53.4)        | 0.766   |
| Female            | 46 (54)    | 41 (46.6)        |         |
| **Presenting Symptoms (%)** |          |                  |         |
| Indigestion       | 25 (28.4)  | 27 (30.6)        | 0.434   |
| Heartburn         | 21 (23.8)  | 26 (29.5)        | 0.248   |
| Regurgitation     | 12 (13.6)  | 8 (9)            | 0.238   |
| Nausea            | 6 (6.8)    | 8 (9)            | 0.391   |
| GI bleeding       | 7 (7.9)    | 4 (4.5)          | 0.268   |
| **Smoking History (%)** |        |                  |         |
| STT: Standard triple therapy

### Table 2. Eradication rate of *H. pylori* and therapy adverse events in patients.

| Groups            | STT(n=88) | Probiotic(n=88) | P value |
|-------------------|-----------|-----------------|---------|
| **Eradication**   | 57 (64.8) | 69 (78.4)       | 0.033   |
| **Adverse Events**|           |                 |         |
| Epigastric Pain   | 10 (11.3) | 3 (3.4)         | 0.04    |
| Vomiting          | 10 (11.3) | 4 (4.5)         | 0.81    |
| Diarrhea          | 3 (3.4)   | 7 (7.9)         | 0.165   |
| **Gender***       |           |                 |         |
| Male              | 22 (48.2) | 28 (40.6)       | 0.483   |
| Female            | 35 (61.4) | 41 (59.4)       |         |
| Recurrence after 6 months* | 11 (19.2) | 6 (8.6)         | 0.07    |

* After exclusion of infected patients

### Discussion

*H. pylori* infection is a common condition that imposes financial burdens in health systems, considering its impact on the development of gastric ulcers as well as metaplastic changes (16-20). In late twenties, the SST (including amoxicillin, clarithromycin, and PPIs of the *H. pylori* infection provided the successful treatment rate up to 95% (17, 21, 22). Nevertheless, according to recent studies, increasing antibiotic resistance and treatment side effect leading to poor compliance with therapy has led to reduced rate of infection to less than 75% (23, 24). Consequently,
several studies have been conducted to evaluate the efficacy of novel therapies, such as hybrid and concomitant regimen and probiotic compounds including regimen (17, 25, 26).

In current double-blind randomized controlled trial, we compared the efficacy of probiotic compounds on STT for *H. pylori* infection. Our results showed a significant increase in the rate of infection eradication in patients that received probiotic combined with STT comparing with the patients that received STT regimen. Subsequent to consumption of probiotic for 4 weeks, infection eradication increased to 78.4%, compared to 64.8% in STT group. During a 6-month follow-up, although, the patients’ symptoms including dyspepsia and epigastric pain were resolved in both groups, the prevalence of symptoms was significantly lower in the probiotic group. Nonetheless, there was significantly lower prevalence of regimen adverse effects in probiotic groups. Yet, *H. pylori* fecal antigen test revealed no difference in the rate of infection recurrence between the study groups. In a recent systematic review, Zhang et al. have reported probiotics administration without regarding their strain, combined with standard *H. pylori* treatment, which has led to approximately 10% increase in the rate of *H. pylori* infection, as well as reduction in the prevalence of adverse events (23).

Similarly, McFarland et al. reviewed 19 randomized controlled trials and reported significant improvement in terms of *H. pylori* eradication and adverse events subsequent to combined administration of eradication therapy and multi-strain probiotics (27). However, some meta-analyses suggested no significant difference between STT regimen alone and STT regimen combined with probiotics, in the rate of eradication in *H. pylori* infection, but the strain of probiotics and administration dosage has not been taken into consideration (28, 29).

There are several studies discussing the efficacy of single strain probiotics on therapeutic outcomes of the STT in the eradication of *H. pylori* (30, 31). In a review, the administration of *Lactobacillus* containing probiotics found to raise *H. pylori* eradication rate more than multi-strain probiotics (32, 33). While the results of recent studies including a randomized placebo-controlled study have shown an inhibitory effect of single strain (*Lactobacillus reuteri*) probiotic on *H. pylori* growth, as well as a 9.1% decrease in the rate of antibiotic related adverse effects during the standard regimen prescription (34, 35). In the current study, we found that STT in combination with *Lactobacillus* and *bifidobacterium* containing probiotic was significantly helpful, not only in increasing the rate of *H. pylori* eradication, but also, in reducing STT regimen administration adverse events, which was consistent with previous study results. We found no significant effect of probiotic administration on infection recurrence after 6 months. To our knowledge, there are few studies that evaluated the efficacy of probiotics combined with conventional STT regimen in the treatment of *H. pylori* infection in Iran. In a randomized trial, *Saccharomyces boulardii* that contained probiotics improved the incidence of STT adverse events, but with no significant effect on *H. pylori* eradication rate (20). Shavakhi et al. discussed the effect of adding combined quadruple (PPI, bismuth citrate, amoxicillin and clarithromycin) and multi-strain probiotic regimen (36). Nonetheless, they report no significant difference in the treatment of *H. pylori* infection by adding probiotic compounds to quadruple regimen. In the present study, we administered standard triple therapy for 10 days and the study group received probiotic compound containing *Lactobacillus* strains for 28 days. The results showed a significant higher eradication rate for this regimen as compared to probiotic regimen in Shavakhi’s study.

To compare our results with previous studies, it can be concluded that double-strain probiotics are more effective than multi-strain probiotic in improving the rate of *H. pylori* infection eradication. Neverthess, in a recent review, McFarland et al. have reported that few probiotic strains including *Lactobacillus* may be effective in the improvement of *H. pylori* infection eradication and reduction of adverse events (27). As a result, they suggested probiotic strain type as the most effective factor in predicting the efficacy of regimen.

Some studies have suggested that the efficacy of probiotic compounds may depend on several factors, such as dosage and its components (36). Though considering the abovementioned studies and our findings, we hypothesized that double-strain probiotics seem to be more effective than multi-strain probiotics, not only in enhancing the rate of *H. pylori* eradication, but also in terms of adverse events. Although, we detected no significant difference between probiotic containing regimen and STT in the rate of infection recurrence, probiotics role should be evaluated in further studies. In the current study, fastidious study design, scrupulous patient enrolment and careful follow-up were employed to evaluate single strain probiotic effect on the
improvement of STT regimen efficacy. In spite of that, our study had some weaknesses and limitations, which as follows: first, due to lack of standardized protocol for probiotic supplementation, administration performed based on previous study methods, second, our patients received STT, despite that, bismuth quadruple therapy has been suggested by the Iranian Association of Gastroenterology and Hepatology (IAGH) to be more effective in Iranian population. In conclusion Administration of Lactobacillus probiotics combined with standard triple therapy increased the rate of H. pylori infection eradication to 78%. Since probiotic consumption reduces adverse events due to antibiotic regimen, it enhances therapy compliance significantly. However, even though it revealed a lower rate of infection recurrence at the end of six-months follow-up, it was not statistically significant.

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References
1. Malfertheiner P, Mégraud F, O’Morain C, et al. Current concepts in the management of Helicobacter pylori infection--the Maastricht 2-2000 Consensus Report. Aliment Pharmacol Ther 2002; 16: 167-80.
2. Tokudome S, Hosono A, Suzuki S, et al. A Helicobacter pylori infection as an essential factor for stomach cancer. Asian Pac J Cancer Prev 2006; 7: 163.
3. Ertem D. Clinical practice: Helicobacter pylori infection in childhood. Eur J Pediatr 2013; 172: 1427-34.
4. Zamani M, Masrour-Roudsari J, Zamani V. Hematologic disorder: A manifestation of helicobacter pylori infection. Caspian J Intern Med 2017; 8:131-2.
5. Uemura N, Okamoto S, Yamamoto S, et al. Helicobacter pylori infection and the development of gastric cancer. N Engl J Med 2001; 345: 784-9.
6. Tonkic A, Tonkic M, Lehours P, Mégraud F. Epidemiology and diagnosis of Helicobacter pylori infection. Helicobacter 2012; 17: 1-8.
7. Megraud F. H pylori antibiotic resistance: prevalence, importance, and advances in testing. Gut 2004; 53: 1374-84.
8. Kutluk G, Tutar E, Bayrak A, et al. Sequential therapy versus standard triple therapy for Helicobacter pylori eradication in children: any advantage in clarithromycin-resistant strains? Eur J Gastroenterol Hepatol 2014; 26: 1202-8.
9. Perri F, Qasim A, Marras L, O’Morain C. Treatment of Helicobacter pylori infection. Helicobacter 2003; 8: 53-60.
10. Fischbach L, Evans EL. Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for Helicobacter pylori, 2007. Aliment Pharmacol Ther 2007; 26: 343-57.
11. Araya M, Morelli L, Reid G, et al. Guidelines for the evaluation of probiotics in food. Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada, April 30 and May 1, 2002.
12. Homan M, Orel R. Are probiotics useful in Helicobacter pylori eradication? World J Gastroenterol 2015; 21: 10644-53.
13. Zhang MM, Qian W, Qin YY, He J, Zhou YH. Probiotics in Helicobacter pylori eradication therapy: a systematic review and meta-analysis, 2015. World J Gastroenterol 2015; 214: 4345-57.
14. Zhu R, Chen K, Zheng YY, et al. Meta-analysis of the efficacy of probiotics in Helicobacter pylori eradication therapy. World J Gastroenterol 2014; 20: 18013-21.
15. Ebahrimpour S, Esmaeili H, Ghadimi R. Food bioactive components, a possible adjuvant for H. pylori eradication. Caspian J Intern Med 2017; 8: 131-2.
16. Tokudome S, Ghadimi R, Suzuki S, et al. Helicobacter pylori infection appears the prime risk factor for stomach cancer. Int J Cancer 2006; 119: 2991.
17. Dajani AI, Abu Hammour AM, Yang DH, et al. Do probiotics improve eradication response to Helicobacter pylori on standard triple or sequential therapy? Saudi J Gastroenterol 2013; 19: 113-20.
18. Tokudome S, Ando R, Ghadimi R, et al. Are there any real Helicobacter pylori infection-negative gastric cancers in Asia? Asian Pac J Cancer Prev 2007; 8: 462-3.
19. Zoja H, Ghobakhloo M, Rajabalinia H, et al. The efficacy and safety of adding the probiotic Saccharomyces boulardii standard triple therapy for
eradication of H. pylori: a randomized controlled trial. Gastroenterol Hepatol Bed Bench 2013; 6: S99-104.
20. Sotuneh N, Hosseini SR, Shokri-Shirvani J, Bijani A, Ghadimi R. Helicobacter pylori infection and metabolic parameters: Is there an association in elderly population? Int J Prev Med 2014; 5: 1537-42.
21. Wang ZH, Gao QY, Fang JY. Meta-analysis of the efficacy and safety of Lactobacillus-containing and Bifidobacterium-containing probiotic compound preparation in Helicobacter pylori eradication therapy. J Clin Gastroenterol 2013; 47: 25-32.
22. Garza-González E, Perez-Perez GI, Maldonado-Garza HJ, Bosques-Padilla FJ. A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. World J Gastroenterol 2014; 20: 1438-49.
23. Zhang MM, Qian W, Qin YY, He J, Zhou YH. Probiotics in Helicobacter pylori eradication therapy: a systematic review and meta-analysis,
24. Yaşar B, Abut E, Kayadibi H, et al. Efficacy of probiotics in Helicobacter pylori eradication therapy. Turkish J Gastroenterol 2010; 21: 212-7.
25. Heep M, Kist M, Strobel S, Beck D, Lehn N. Secondary resistance among 554 isolates of Helicobacter pylori after failure of therapy, 2000. Eur J Clin Microbiol Infect Dis 2000; 19: 538-41.
26. Toracchio S, Marzio L. Primary and secondary antibiotic resistance of Helicobacter pylori strains isolated in central Italy during the years 1998–2002. Dig Liver Dis 2003; 35: 541-5.
27. McFarland LV, Huang Y, Wang L, Malfertheiner P. Systematic review and meta-analysis: Multi-strain probiotics as adjunct therapy for Helicobacter pylori eradication and prevention of adverse events. United European Gastroenterol J 2016; 4: 546-61.
28. Zhu R, Chen K, Zheng YY, et al. Meta-analysis of the efficacy of probiotics in Helicobacter pylori eradication therapy. World J Gastroenterol 2014; 20: 18013-21.
29. Lu C, Sang J, He H, et al. Probiotic supplementation does not improve eradication rate of Helicobacter pylori infection compared to placebo based on standard therapy: a meta-analysis. Sci Rep 2016; 6: 23522.
30. McFarland LV, Malfertheiner P, Huang Y, Wang L. Meta-analysis of single strain probiotics for the eradication of Helicobacter pylori and prevention of adverse events. World J Meta-Analysis 2015; 3: 97-117.
31. Gong Y, Li Y, Sun Q. Probiotics improve efficacy and tolerability of triple therapy to eradicate Helicobacter pylori: a meta-analysis of randomized controlled trials. Int J Clin Exp Med 2015; 8: 6530-43.
32. Li BZ, Threapleton DE, Wang JY, et al. Comparative effectiveness and tolerance of treatments for Helicobacter pylori: systematic review and network meta-analysis. BMJ 2015; 351: h4052.
33. O’Connor A, Fischbach W, Gisbert JP, O’Morain C. Treatment of Helicobacter pylori infection. Helicobacter 2016; 21: 55-61.
34. Francavilla R, Polimen L, Demichina A, et al. Lactobacillus reuteri strain combination in Helicobacter pylori infection: a randomized, double-blind, placebo-controlled study, 2014. J Clin Gastroenterol 2014; 48: 407-13.
35. Dore MP, Soro S, Rocchi C, Loria MF, Bibbò S, Pes GM. Inclusion of Lactobacillus reuteri in the treatment of Helicobacter pylori in Sardinian patients: a case report series. Medicine (Baltimore) 2016; 95: e3411.
36. Shavakhi A, Tabesh E, Yaghoutkar A, et al. The effects of multistrain probiotic compound on bismuth-containing quadruple therapy for helicobacter pylori infection: a randomized placebo-controlled triple-blind study. Helicobacter 2013; 18: 280-4.