



Use of probiotics as an adjuvant to sequential *H. pylori* eradication therapy: impact on eradication rates, treatment resistance, treatment-related side effects, and patient compliance

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ABSTRACT

Background/Aims: To evaluate the effect of probiotics administered as an adjuvant to sequential *Helicobacter pylori* (*H. pylori*) eradication therapy on treatment outcome and patient compliance.

Materials and Methods: In total, 159 patients with *H. pylori* infection receiving sequential *H. pylori* eradication therapy were included in this randomized placebo-controlled study. Starting from day 0 of sequential eradication therapy (ERA), patients in the ERA+probiotic group [n=53, mean (SD) age: 47.7 (14.0) years, 54.7% were females] also received a probiotic supplement with *Bifidobacterium animalis subsp. lactis* B94 (1 capsule/day), patients in the ERA+placebo group [n=52, mean (SD) age: 46.4 (13.4) years, 51.9% were males] received placebo treatment (1 capsule/day), and patients in the ERA-only group [n=54, mean (SD) age: 46.3 (11.9) years, 55.6% were females] received no additional treatments. Eradication rates, patient compliance, and side effects of eradication therapy were recorded in each treatment group.

Results: Significantly higher eradication rates were noted in the ERA+probiotic group (86.8% vs. 70.8%, p=0.025) than in the combined ERA (ERA-only and ERA-placebo) group. Non-compliance with anti-*H. pylori* treatment was noted in 24 (15.1%) of 159 patients. Lower rates of first week treatment non-compliance due to diarrhea (1.88% vs. 12.26%, p=0.036) were noted in the ERA+probiotic group than in the combined ERA (ERA-only and ERA-placebo) group. Treatment resistance (p: 0.389) was similar between the groups, indicating pure antibiotic resistance without any compliance problems. The number needed to treat for an additional beneficial outcome (NNTB) was 6.2 (CI 95%, 3.5 to 28.9) for probiotic use.

Conclusion: In conclusion, adjuvant administration of probiotic (*B. animalis subsp. lactis*) in 2-week sequential *H. pylori* eradication therapy is associated with a higher *H. pylori* eradication rate, lower first week diarrhea-related treatment discontinuation rates, less common self-reported side effects, and higher treatment compliance.

Keywords: *H. pylori*, eradication, sequential therapy, probiotics, side effects, patient compliance

INTRODUCTION

Helicobacter pylori is a common bacterium that colonizes the gastric mucosa and infects 70%–90% of the population in developing countries (1). Given its major contributory role in a wide range of gastrointestinal disorders (1,2), great emphasis has been placed on its successful eradication to ameliorate *H. pylori*-related complications (3,4).

Standard triple antibiotic therapy, including a proton-pump inhibitor (PPI), along with any two out of amoxicillin, clarithromycin, and nitroimidazole, has been used widely as the first approach recommended for *H. pylori* eradication, while it has become associated with increasing antibiotic resistance; thus, eradication failure that ranges from 10% to 45% (5). Bismuth quadruple, sequential, concomitant, and hybrid therapies

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are amongst the alternative options recommended for increasing *H. pylori* eradication by extending the treatment duration to 10–14 days and are shown to be associated with eradication rates of 75%–90% (4,6–9).

Sequential therapy is a 10-day treatment consisting of 5 days of PPI therapy with amoxicillin, followed by further 5 days of PPI with clarithromycin and metronidazole (10). Being recommended particularly in areas with high clarithromycin resistance, including Turkey (11), it has been associated with a success rate of 90%–94% (10).

However, similar tendencies of decline in eradication rates have also been reported in recent studies with these alternative regimens because of increased antibiotic resistance as well as increased incidence of undesirable side effects of longer term treatment, which can lead to patient non-compliance (2,3,6,7,12–14).

Owing to the growing need for newer alternative eradication regimens or adjuvant treatments, the addition of probiotics (administration of live microbial species) has been considered likely to offer beneficial health effects (15) and thus has attracted substantial interest with respect to *H. pylori* eradication therapy (16–19). On the basis of the demonstration of anti-*H. pylori* activity of probiotics such as *Lactobacillus*, *Saccharomyces boulardii*, and *Bifidobacterium*, in in vitro and experimental models of *H. pylori* infection (19), probiotics have been suggested to improve *H. pylori* eradication and reduce side effects during therapy (17,20,21). However, findings from clinical studies on the use of probiotics as an adjuvant therapy to *H. pylori* infection have been inconsistent, with the demonstration of reduced side effects of antibiotics and improved eradication rates in some studies (16,22–30), while no significant effects of adjuvant probiotics on eradication rates (22,31–36) or side effects (26,27,30,31,32,33,35–37) have been reported in other studies.

Therefore, in this study, we aimed to evaluate the effect of probiotics administered as an adjuvant to sequential *H. pylori* eradication therapy on eradication rates, treatment resistance, treatment-related side effects, and patient compliance.

MATERIALS AND METHODS

Study Population

In total, 159 patients, diagnosed with *H. pylori* via endoscopic gastric biopsies, were eligible to be included in this randomized placebo-controlled study. Patients who had previous *H. pylori* eradication therapy, gastric cancer, and known allergic reactions to penicillin therapy were excluded. All patients received 2-week sequential *H. pylori* eradication therapy with amoxicillin (Largopen[®], 1,000 mg, bid)+PPI (Pantoprazol, Pulcet[®], 40 mg, bid) in the first week and then metronidazole (Flagyl[®], 500 mg, t.i.d.)+clarithromycin (Macrol[®], 500 mg, bid) + PPI (Pantoprazol, Pulcet[®], 40 mg, bid) in the second week. Starting from day 0 of 2-week sequential eradication therapy (ERA), patients in the

ERA+probiotic group [n=53, mean (SD) age: 47.7 (14.0) years, 54.7% were females] also received a probiotic supplement with Maflor (7×10⁹ CFU *B. animalis* subsp. *lactis* B94; 1 capsule/day), patients in the ERA+placebo group [n=52, mean (SD) age: 46.4 (13.4) years, 51.9% were males] received placebo treatment (1 capsule/day), and patients in the ERA-only group [n=54, mean (SD) age: 46.3 (11.9) years, 55.6% were females] received no additional treatments. The participants were randomly assigned in a 1:1:1 allocation to the ERA+probiotic, ERA+placebo, or ERA-only groups, using a computer-generated, permuted block randomization, with a block size of six. Personnel involved in the delivery of the treatment and participants were blinded to group allocation. The placebo was administered in a similar way to the probiotic regimen in terms of the daily amount (1 capsule/day), shape and color of the boxes containing the active regimen, with no trademark identifications, and the same number of capsules.

Written informed consent was obtained from each subject after a detailed explanation of the aims and protocol of the study, which was conducted in accordance with the ethical principles stated in the “Declaration of Helsinki” and approved by the institutional ethics committee (Date of Approval: 26/02/2015; Reference number: 55/18).

Study Parameters

Patient demographics (age, gender), treatment outcome (eradication rate, compliance, reasons for treatment discontinuation), and symptoms related to side effects of eradication therapy (loss of appetite, nausea, vomiting, taste alteration, dizziness, abdominal pain, diarrhea, headache, and skin rash) were recorded for each patient and compared with respect to treatment groups. Sub-analysis of treatment outcomes and symptom prevalence in patients with vs. without probiotic treatment via comparison of the combined “ERA-only and ERA+placebo” groups with the ERA+probiotic group was also performed.

Assessments

Side effects were evaluated before the treatment and at the 1st and 2nd weeks of anti-*H. pylori* treatment using a form, including items on the standard side effect scoring system for *H. pylori* treatment. Side effects were recorded based on patients’ statements during direct questioning by a physician.

Compliance was verified based on pill counting in medication containers returned by subjects upon completion of treatment, and asking the subject directly about therapy completion, considering compliance to be satisfactory when the drug intake exceeded 85% (38–40).

The group non-compliance includes patients who quit the treatment due to antibiotic-related side effect/effects. Also, patients who finished the whole treatment (no compliance problem) but were *H. pylori*-positive at the end of the treatment were grouped as treatment resistance (pure antibiotic resistance).

Table 1. Patient demographics and treatment outcome

	Total (n=159)	ERA-only (n=54)	ERA+placebo (n=52)	ERA+probiotic (n=53)	p	Combined ERA (n=106) ¹	p “combined ERA” vs. “ERA+ probiotic”
Age (year), mean (SD)	46.8 (13.1)	46.3 (11.9)	46.4 (13.4)	47.7 (14.0)	0.830 ^a	46.4 (12.6)	0.541 ^c
Gender, n (%)							
Male	75 (47.2)	24 (44.4)	27 (51.9)	24 (45.3)	0.702 ^b	51 (48.1)	0.736 ^b
Female	84 (52.8)	30 (55.6)	25 (48.1)	29 (54.7)		55 (51.9)	
Treatment outcome							
Eradication positive	121(76.1)	39 (72.2)	36 (69.2)	46 (86.8)	0.077 ^b	75 (70.8)	0.025 ^b
Treatment resistance	14 (8.8)	5 (9.3)	6 (11.5)	3 (5.7)	-	11 (10.4)	0.389 ^b
Non-compliance	24 (15.1)	10 (18.5)	10 (19.2)	4 (7.5)	0.170 ^b	20 (18.9)	0.060 ^b
<i>Due to diarrhea</i>	15 (9.4)	6 (11.1)	7 (13.5)	2 (3.8)	0.207 ^b	13 (12.3)	0.084 ^b
<i>Due to skin rash</i>	9 (5.7)	4 (7.4)	3 (5.8)	2 (3.8)	-	7 (6.6)	0.719 ^b

¹combination of ERA-only and ERA+placebo groups^aANOVA, ^bχ²test, ^cindependent samples t-testERA: Sequential *H. pylori* eradication therapy

Four weeks after the end of the eradication therapy, evaluation of the *H. pylori* status was repeated via a 14C urea breath test.

Diagnosis of Helicobacter pylori Infection

Overall, 6 endoscopic biopsies were taken, including the antrum (n=3), corpus (n=2), and lower part of the lesser curvature (n=1), for histopathological examination (HE and Giemsa staining) or rapid urease test (*Hp* fast, GI SUPPLY, Check-Med Systems, Inc. USA). Histopathological evaluation was made by experienced pathologists who were blind to the treatment. Patients were considered to be positive for *Hp* only if both histological examination and rapid urease test were positive.

Statistical Analysis

Categorical data were analyzed using Fisher's exact test and the Pearson chi-square test. The Student's t-test was used for comparison of two groups and one-way ANOVA was used for comparison of three groups with a normal distribution. Relative risks (RR) were calculated, including a 95% confidence interval (CI). Results are expressed as n (%) or mean (standard deviation). Intention to treat (ITT) analyses were done and the number needed to treat for an additional beneficial outcome (NNTB) was calculated. The overall data were analyzed with the Statistical Package for the Social Sciences (IBM SPSS Statistics; Armonk, NY, USA) 20.0 and a p value of <0.05 was considered statistically significant.

RESULTS

Patient Demographics and Treatment Outcome

Treatment groups were homogenous in terms of patient demographics. Treatment resulted in eradication of *H. pylori* in 76.1% of overall patients, with no significant difference between the ERA-only (72.2%), ERA+placebo (69.2%), and ERA+probiotic (86.8%) groups. Non-compliance with anti-*H. pylori* treatment

was noted in overall 24 (15.1%) patients, including 10 patients from both the ERA-only (18.5%) and ERA+placebo (19.2%) groups and 4 patients from the ERA+probiotic (7.5%) group. The reason for treatment non-compliance was diarrhea in 15 (9.4%) patients and skin rash in 9 (5.7%) patients (Table 1).

Sub-analysis of treatment outcomes in patients with vs. without probiotic treatment via comparison of the combined “ERA-only and ERA+placebo” groups with the ERA+probiotic group was also performed. This revealed significantly higher rates for eradication (86.8% vs. 70.8%, p=0.025) and no difference for treatment resistance related to antibiotics (10.4% vs. 5.7%, p=0.389) and non-compliance (7.5% vs. 18.9%, p=0.060) in the ERA+probiotic group compared with the combined ERA group (Table 1).

Among patients experiencing diarrhea-related non-compliance, the percentage of patients from the ERA+probiotic group showed no significant difference compared with patients from the combined ERA group (3.8% vs. 12.3%, p=0.084), and also no significant difference was noted between the treatment groups among patients with skin-rash-related non-compliance (Table 1).

On the other hand, when we compared first week diarrhea-related non-compliance (amoxicillin-related diarrhea), the percentage of patients from the ERA+probiotic group was significantly lower as compared with patients from the combined ERA group (1.88% vs. 12.26%, p=0.036) (Table 2 and 3).

Symptom Prevalence

No significant difference was noted between treatment groups in terms of the prevalence of any symptoms prior to treatment. In the first week of anti-*H. pylori* treatment, patients in the ERA+probiotic group expressed significantly lower rates

Table 2. Non-compliance patient properties

	ERA-only (n=10)			ERA+placebo (n=10)			ERA+probiotic (n=4)		
	Patient Number	Day (quit)	Reason	Patient Number	Day	Reason	Patient Number	Day (quit)	Reason
Week 1	1	6	Diarrhea	5	4	Diarrhea	3	2	Diarrhea
	4	3	Diarrhea	8	6	Diarrhea	27	5	Skin rash
	7	2	Diarrhea	14	2	Diarrhea			
	10	2	Diarrhea	18	2	Diarrhea			
	13	3	Diarrhea	23	4	Diarrhea			
	16	4	Diarrhea	26	5	Diarrhea			
				29	5	Diarrhea			
Week 2	19	9	Skin rash	45	8	Skin rash	6	11	Diarrhea
	22	8	Skin rash	51	8	Skin rash	36	10	Skin rash
	25	11	Skin rash	57	9	Skin rash			
	201	8	Skin rash						

ERA: Sequential *H. pylori* eradication therapy

Table 3. Non-compliance

	ERA-only (n=54)	ERA+placebo (n=52)	ERA+probiotic (n=53)	Combined ERA (n=106) ¹	p value “combined ERA” vs. “ERA+ probiotic”
Week 1					
Diarrhea	6 (11.11%)	7 (13.46%)	1 (1.88%)	13 (12.26%)	0.036 ^a
Skin rash	-	-	1 (1.88%)	-	
Week 2					
Diarrhea	-	1 (1.92%)	1 (1.88%)	-	
Skin rash	4 (7.40%)	3 (5.76%)	2 (3.76%)	7 (6.60%)	

ERA: Sequential *H. pylori* eradication therapy, NA: Not applicable

^aχ² test

for loss of appetite (p=0.044) and diarrhea (p=0.010), compared to patients in the ERA-only and ERA+placebo groups. In the second week, loss of appetite (p=0.009), dizziness (p=0.034), abdominal pain (p<0.001), diarrhea (p=0.009), and headache (p=0.003) were significantly less common in the ERA+probiotic group than in the ERA-only and ERA+placebo groups. In the ERA+placebo group, significantly higher rates for nausea (p<0.001) and skin rash (p=0.009) were noted in the second week of therapy as compared with the ERA-only and ERA+probiotic groups (Table 4).

Sub-analysis of symptom prevalence in patients with vs. without probiotic treatment via comparison of the combined “ERA-only and ERA+placebo” groups with the ERA+probiotic group was also performed. This revealed significantly lower rates for loss of appetite (11.5% vs. 29.2%, p=0.014), diarrhea (37.7% vs. 63.2%, p=0.002), and headache (30.2% vs. 48.1%, p=0.031) in patients with compared to without probiotic supplementation in the first week of eradication therapy (Table 5). Similarly, re-

ceiving probiotic treatment during the second week of eradication therapy was associated with significantly lower rates for loss of appetite (11.3% vs. 31.1%, p=0.002), nausea (26.4% vs. 44.3%, p=0.007), dizziness (20.8% vs. 35.8%, p=0.019), abdominal pain (5.7% vs. 37.7%, p<0.001), diarrhea (1.9% vs. 17.9%, p=0.002), headache (18.9% vs. 41.5%, p=0.001), and skin rash (9.4% vs. 20.8%, p=0.042) when compared with a lack of probiotic therapy (Table 5).

The NNTB using probiotic treatment versus standard treatment was 6.2 (CI 95%, 3.5 to 28.9). A post hoc power analysis using Gpower 3.1 (41) revealed 98% statistical power with 0.05 alpha.

DISCUSSION

Our findings revealed significantly higher *H. pylori* eradication rates (86.8% vs. 70.8%) and lower treatment-related side effects with the use of probiotic (*B. animalis subsp. lactis*) as an adjuvant to sequential *H. pylori* eradication therapy than with the use of sequential therapy without probiotics.

Table 4. Symptoms in overall patients with respect to three treatment groups

Symptoms, n (%)	Pretreatment					Week 1					Week 2				
	Total	ERA-only (n=54)	ERA+ placebo (n=52)	ERA + probiotic (n=53)	p	Total	ERA-only (n=54)	ERA+ placebo (n=52)	ERA+ probiotic (n=53)	p	Total	ERA - only (n=54)	ERA+ placebo (n=52)	ERA+ probiotic (n=53)	p
Loss of appetite	45 (28.3)	17 (31.5)	19 (36.5)	9 (17.0)	0.069	38 (23.9)	15 (27.8)	16 (30.8)	6 (11.3)*	0.044	39 (24.5)	17 (31.5)	16 (30.8)	6 (11.3)**	0.009
Nausea	42 (26.4)	16 (29.6)	21 (40.4)	12 (22.6)	0.103	49 (30.8)	20 (37.0)	16 (30.8)	13 (24.5)	0.375	61 (38.4)	17 (31.5)	30 (57.7)***	14 (26.4)	<0.001
Vomiting	9 (5.7)	3 (5.6)	4 (7.7)	2 (3.8)	NA	15 (9.4)	4 (7.4)	4 (7.7)	7 (13.2)	0.515	19 (11.9)	6 (11.1)	2 (3.8)	8 (15.1)	0.202
Taste alteration	37 (23.3)	10 (18.5)	17 (32.7)	14 (26.4)	0.075	46 (28.9)	12 (22.2)	19 (36.5)	15 (28.3)	0.265	71 (44.7)	22 (40.7)	28 (53.8)	24 (45.3)	0.213
Dizziness	59 (37.1)	19 (35.2)	16 (30.8)	24 (45.3)	0.287	49 (30.8)	16 (29.6)	15 (28.8)	14 (26.4)	0.929	49 (30.8)	19 (35.2)	21 (40.4)	11 (20.8)*	0.034
Abdominal pain	40 (25.2)	18 (33.3)	15 (28.8)	12 (22.6)	0.468	53 (33.3)	22 (40.7)	18 (34.6)	16 (30.2)	0.517	43 (27.0)	18 (33.3)	17 (32.7)	3 (5.7)***	<0.001
Diarrhea	19 (11.9)	7 (13.0)	5 (9.6)	6 (11.3)	0.863	87 (54.7)	34 (63.0)	33 (63.5)	20 (37.7)**	0.010	20 (12.6)	7 (13.0)	9 (17.3)	1 (1.9)**	0.009
Headache	63 (39.6)	28 (51.9)	20 (38.5)	17 (32.1)	0.105	65 (40.9)	26 (48.1)	25 (48.1)	16 (30.2)	0.097	54 (34.0)	28 (51.9)	23 (44.2)	10 (18.9)**	0.003
Skin rash	14 (8.8)	6 (11.1)	4 (7.7)	4 (7.5)	NA	14 (8.8)	4 (7.4)	7 (13.5)	3 (5.7)	-	26 (16.4)	6 (11.1)	15 (28.8)**	5 (9.4)	0.009

ERA: Sequential *H. pylori* eradication therapy, NA: Not applicable*p<0.05, **p<0.01 and ***p<0.001; compared with other groups in the same treatment week (χ^2 test)**Table 5.** Symptoms in overall patients with respect to combined ERA vs. ERA+probiotic treatment groups

Symptoms, n (%)	Week 1					Week 2				
	Combined ERA (n=106) ¹	ERA+probiotic (n=53)	p	RR	95% CI	Combined ERA (n=106) ¹	ERA+probiotic (n=53)	p	RR	95% CI
Loss of appetite	31 (29.2)	6 (11.3)	0.014	0.395	0.122–0.814	33 (31.1)	6 (11.3)	0.002	0.332	0.149–0.738
Nausea	36 (34.0)	13 (24.5)	0.225	0.722	0.420–1.241	47 (44.3)	14 (26.4)	0.007	0.543	0.333–0.886
Vomiting	8 (7.5)	7 (13.2)	0.250	1.75	0.671–4.567	8 (7.5)	8 (15.1)	0.196	1.824	0.728–4.569
Taste alteration	31 (29.2)	15 (28.3)	0.902	0.968	0.575–1.629	50 (47.2)	24 (45.3)	0.441	0.875	0.619–1.238
Dizziness	31 (29.2)	14 (26.4)	0.709	0.903	0.527–1.547	38 (35.8)	11 (20.8)	0.019	0.528	0.296–0.941
Abdominal pain	40 (37.7)	16 (30.2)	0.348	0.800	0.497–1.289	40 (37.7)	3 (5.7)	<0.001	0.137	0.045–0.420
Diarrhea	67 (63.2)	20 (37.7)	0.–02	0.597	0.410–0.869	19 (17.9)	1 (1.9)	0.002	0.096	0.013–0.696
Headache	51 (48.1)	16 (30.2)	0.031	0.627	0.398–0.989	44 (41.5)	10 (18.9)	0.001	0.414	0.228–0.752
Skin rash	11 (10.4)	3 (5.7)	0.389	0.545	0.159–1.872	22 (20.8)	5 (9.4)	0.042	0.414	0.167–1.029

¹ combination of ERA-only and ERA+placebo groupsERA: Sequential *H. pylori* eradication therapy, RR: Relative risk, CI: Confidence interval. χ^2 test

H. pylori eradication rates for rescue regimens has been considered to range from 70% to 90% on a global basis (18). Although the success rate of sequential therapy has been indicated to range from 90% to 94% (10), data from a past study in Turkey have revealed the success rate of eradication therapy to be 53% with standard triple therapy and 78% with sequential therapy (42). Accordingly, the achievement of a favorable eradication rate (86.8%) only via the use of probiotics as an adjuvant to sequential *H. pylori* eradication therapy in our cohort emphasizes on the benefit of using adjuvant probiotics to enable better efficacy of sequential therapy in Turkey, where clarithromycin resistance is high (11,42).

The beneficial effects of *Lactobacillus*- and *Bifidobacterium*-containing probiotics on the eradication rate and the incidence of total side effects during *H. pylori* eradication therapy has also been reported in a meta-analysis by Wang et al. (29), includ-

ing 10 clinical trials with 1,469 patients. Moreover, in a recent meta-analysis of 33 randomized controlled trials (RCTs) by Dang et al. (17), involving a total of 4,459 patients., significantly higher rates for pooled eradication have been reported in probiotic supplementation groups than in controls, although this has been confirmed only for four individual *Lactobacillus* and *Bifidobacterium* strains and for relatively ineffective antibiotic therapies.

In a meta-analysis of 23 trials (n=3,900) by Gong et al. (18), pooled *H. pylori* eradication rates have been reported to be 72.26% for triple therapy alone and 80.74% for triple therapy combined with probiotics. Specifically, a combination of *Lactobacillus* and *Bifidobacterium* as an adjunct to PPI-based 1-week triple therapy has been reported to be associated with an increase in the eradication rate from 78.3% to 91.3% in an open randomized trial (43).

Hence, the eradication rates obtained in the placebo group in our cohort are consistent with the overall eradication rates reported previously in randomized trials (3,39,44), while the benefit of probiotic therapy on eradication rates also seems to be in agreement with data from studies on probiotics (18,25,29,30,39,45-47), including *Bifidobacterium* (17,42,45).

Further, our findings are consistent with data from several meta-analyses concluding the beneficial effects of probiotics on decreasing treatment-related side effects (19,25,29,38,45), thereby increasing the likelihood of higher eradication rates (18,19,25,29,38,45) when used as an adjuvant to *H. pylori* eradication regimens.

Notably, a higher efficacy of probiotic supplementation in increasing eradication rates has been suggested when antibiotic therapies are relatively ineffective (17). In this regard, our finding related to a lower rate for treatment resistance in patients with than without probiotic supplementation, which emphasizes the likelihood of probiotic supplementation eliminating resistant strains and thereby improving the eradication rates of *H. pylori*, particularly when antibiotic therapies are relatively ineffective (17).

While the effectiveness of probiotic supplementation in increasing eradication rates has been more consistently reported in clinical studies as well as meta-analyses (16,17,22-30), there is inconsistency regarding side effects, with demonstration of benefit in terms of overall side effects in probiotics groups in some studies (26,27,30), but a lack of any significant change in others (16,22,23,25,28,29,35,36).

Along with no significant difference being found between patients with and without probiotic supplementation prior to treatment, our findings revealed significantly lower rates for side effects related to sequential therapy with than without adjuvant probiotic administration, considering both the first (loss of appetite, diarrhea, and headache) and second (loss of appetite, nausea, dizziness, abdominal pain, diarrhea, headache, and skin rash) weeks of treatment. This seems consistent with previous clinical studies (24,34,37,40), as well as data from some meta-analyses (17,18,19,25,28,29), which indicated that probiotics have a remarkable effect on reducing adverse reactions related to *H. pylori* therapy.

Qualitative and quantitative alterations in intestinal flora have been blamed for the occurrence of gastrointestinal disturbances experienced during *H. pylori* eradication therapy (40,48), while gastrointestinal side effects have been considered to be more common and more severe with the use of amoxicillin (34) and clarithromycin (48), respectively.

The rationale for the efficacy of probiotics in reducing treatment-related side effects has mostly been based on restoration of the physiological microecology in the intestine and inhibi-

tion of the prokinetic action of macrolides (40,49,50). The concentration of *Bifidobacteria*, members of the normal microbiota with beneficial effects to the host (51), has been indicated to decrease during the eradication of *H. pylori* (52). Thus, the probiotic therapy-associated restoration of *bifidobacteria* concentrations could explain the amelioration of treatment-related symptoms in the present study (46).

Unlike the data from clinical trials and meta-analyses reporting a reduction in the rate of taste disturbance resulting from addition of probiotics (*Lactobacillus GG*; *Saccharomyces boulardii* and a combination of *Lactobacillus spp.* and *Bifidobacteria*) to eradication therapy (18,34,40), our findings revealed taste alteration as the most common 2nd week symptom in all treatment groups, regardless of the use of probiotic supplementation, which may be related to the inclusion of symptomatic patients in our cohort.

Notably, in a past study conducted with symptomatic patients with *H. pylori*, based on evaluation of new or aggravated symptoms within the eradication week relative to the baseline, probiotic and placebo groups were found to be similar in terms of individual symptoms, while the treatment-related symptoms assessed via the total symptom score change were reported to be less common in the probiotic group (46).

Accordingly, given the identification of headache, dizziness, and loss of appetite as the three most common symptoms before the initiation of eradication treatment in our study population, our findings emphasize on the importance of pretreatment evaluation of symptoms in safely and simply avoiding the risk of poor adherence during eradication therapy in symptomatic patients as well as the potential benefit of adjuvant probiotic treatment even in previously symptomatic patients.

Indeed, given the presence of various scoring systems and being dependent on patients' subjective reporting in the assessment of side effects, the evaluation of patients' compliance rather than treatment-related side effects has been suggested to remain of key clinical interest in comparing adverse event profiles of different eradication regimens (53). Antibiotic-associated gastrointestinal side-effects, even in mild cases, have been considered to be a serious drawback of *H. pylori* eradication therapies (53), also leading to possible treatment withdrawal in motivated and dyspeptic patients, and the consequent risk of poor treatment or emergence of antibiotic-resistant strains (40).

However, despite the likelihood of probiotics promoting patient compliance by preventing adverse events (37,54), and the fact that non-compliance may substantially lower the eradication success (55), the influence of probiotics on patient compliance under eradication therapy has seldom been studied (38), although limited data indicates a marked influence of therapy-related side-effects on eradication rates (40,56).

Probiotics alone or combined with lactoferrin were reported to improve compliance, but not eradication, in sequential *H. pylori* eradication therapy (57). Moreover, in a meta-analysis of 45 articles with 6,997 participants by Zhang et al. (38), despite an increased eradication rate and reduced adverse events, no evidence of a lesser non-compliance in the probiotics group was noted.

In our cohort, non-compliance with *H. pylori* eradication treatment was noted in overall 24 (15.1%) patients, which was due to diarrhea in 15 (9.4%) patients and skin rash in 9 (5.7%) patients. The non-compliance rate (1.88% vs. 12.26%) due to first week diarrhea was significantly lower with adjuvant probiotic administration. This finding is in agreement with data from a past study indicating that addition of the probiotic *Lactobacillus GG* to a 2-week triple anti-*H. pylori* regimen was associated with better treatment tolerability as compared with eradication therapy per se (40). Also, addition of probiotics to *H. pylori* eradication triple therapy was shown to be associated with a significant reduction in the frequency of diarrhea in a meta-analysis (25).

Being based on a randomized placebo-controlled design with inclusion of previously symptomatic patients, and consideration of the pretreatment symptom profile in assessing treatment-related side effects seem to be the major strengths of this study. However, certain limitations of this study should be considered. Firstly, the relatively low sample size might preclude us from achieving statistical significance for treatment outcomes in comparison of three individual treatment groups, which indeed necessitated binary sub-analysis by re-grouping patients as a combined "ERA-only and ERA+placebo" group and an ERA+probiotic group. Secondly, despite randomization of subjects to groups, and the use of placebo and baseline-adjusted analyses, total control over possible confounding factors cannot be guaranteed because of the small sample sizes. Thirdly, side-effect evaluation was based on patients' subjective reporting, which is likely to be biased due to patients being aware of the intervention. Nevertheless, despite these limitations, given the inconclusive data available on this subject, our findings provide an important contribution to the literature, indicating promising results.

In conclusion, adjuvant administration of probiotic (*B. animalis subsp. lactis*) to 2-week sequential *H. pylori* eradication therapy was associated with a higher *H. pylori* eradication rate, lower diarrhea-related treatment discontinuation rates, less common self-reported side effects, and higher treatment compliance. The use of adjuvant probiotic treatment appears promising in enabling gastric *H. pylori* eradication and ameliorating most treatment-related adverse events, while the subject deserves further investigation in large-scale clinical studies addressing the mechanism of action, and appropriate timing and duration of probiotic administration, as well as the baseline symptoms and antibiotic resistance profiles of patients.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Antalya Training and Research Hospital (Date of Approval: 26/02/2015; Reference number: 55/18).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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REFERENCES

- Go MF. Review article: natural history and epidemiology of Helicobacter pylori infection. *Aliment Pharmacol Ther* 2002; 16(Suppl 1): 3-15.
- Suerbaum S, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; 347: 1175-86. [\[CrossRef\]](#)
- Vergara M, Vallve M, Gisbert JP, Calvet X. Meta-analysis: comparative efficacy of different proton-pump inhibitors in triple therapy for Helicobacter pylori eradication. *Aliment Pharmacol Ther* 2003; 18: 647-54. [\[CrossRef\]](#)
- Malferteiner P, Meqraud F, O'Morain CA, et al. Management of Helicobacter pylori infection—the Masstricht IV/Florence Consensus Report. *Gut* 2012; 61: 646-64. [\[CrossRef\]](#)
- Chey WD, Wong BC; Practice Parameters Committee of the American College of Gastroenterology: American College of Gastroenterology guideline on the management of Helicobacter pylori infection. *Am J Gastroenterol* 2007; 102: 1808-25. [\[CrossRef\]](#)
- Meqraud F. Basis of the management of drug-resistant Helicobacter pylori infection. *Drugs* 2004; 64: 1893-904. [\[CrossRef\]](#)
- Yokeswaran K, Chen G, Cohen L, et al. How well is Helicobacter pylori treated in usual practice? *Can J Gastroenterol* 2011; 25: 543-6.
- Zagari RM, Bianchi-Porro G, Fiocca R, Gasbarrini G, Roda E, Bazzoli F. Comparison of 1 and 2 weeks of omeprazole, amoxicillin and clarithromycin treatment for Helicobacter pylori eradication: the HYPER Study. *Gut* 2007; 56: 475-9. [\[CrossRef\]](#)
- Kim BG, Lee DH, Ye BD, et al. Comparison of 7-day and 14-day proton pump inhibitor-containing triple therapy for Helicobacter pylori eradication: neither treatment duration provides acceptable eradication rate in Korea. *Helicobacter* 2007; 12: 31-5. [\[CrossRef\]](#)
- Ermis F, Senocak Tasci E. Current Helicobacter pylori treatment in 2014. *World J Methodol* 2015; 5: 101-7.
- Urgesi R, Cianci R, Riccioni ME. Update on triple therapy for eradication of Helicobacter pylori: current status of the art. *Clin Exp Gastroenterol* 2012; 5: 151-7. [\[CrossRef\]](#)
- McLoughlin R, Racz I, Buckley M, O'Connor HJ, O'Morain C. Therapy of Helicobacter pylori. *Helicobacter* 2004; 9: 42-8. [\[CrossRef\]](#)
- Kim N, Kim JM, Kim CH, et al. Institutional difference of antibiotic resistance of Helicobacter pylori strains in Korea. *J Clin Gastroenterol* 2006; 40: 683-7. [\[CrossRef\]](#)
- Bell GD, Powell K, Burrige SM, et al. Experience with 'triple' anti-Helicobacter pylori eradication therapy: side effects and the im-

- portance of testing the pre-treatment bacterial isolate for metronidazole resistance. *Aliment Pharmacol Ther* 1992; 6: 427-35. [\[CrossRef\]](#)
15. FAO/WHO. Report on Joint FAO/WHO Expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Cordoba, FAO/WHO, 2001.
 16. Lionetii E, Indrio F, Pavone L, Borrelli G, Cavallo L, Francavilla R. Role of probiotics in pediatric patients with *Helicobacter pylori* infection: a comprehensive review of the literature. *Helicobacter* 2010; 15: 79-87. [\[CrossRef\]](#)
 17. Dang Y, Reinhardt JD, Zhou X, Zhang G. The effect of probiotics supplementation on *Helicobacter pylori* eradication rates and side effects during eradication therapy: a meta-analysis. *PLoS One* 2014; 9: e111030.
 18. 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.
 19. Lv Z, Wang B, Zhou X, et al. Efficacy and safety of probiotics as adjuvant agents for *Helicobacter pylori* infection: A meta-analysis. *Exp Ther Med* 2015; 9: 707-16. [\[CrossRef\]](#)
 20. Malfertheiner P, Megraud 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. [\[CrossRef\]](#)
 21. Hamilton-Miller JM. The role of probiotics in the treatment and prevention of *Helicobacter pylori* infection. *Int J Antimicrob Agents* 2003; 22: 360-6. [\[CrossRef\]](#)
 22. Tursi A, Brandimarte G, Giorgetti GM, Modeo ME. Effect of *Lactobacillus casei* supplementation on the effectiveness and tolerability of a new second-line 10-day quadruple therapy after failure of a first attempt to cure *Helicobacter pylori* infection. *Med Sci Monit* 2004; 10: CR662-6.
 23. Ozdil K, Calhan T, Sahin A, et al. Levofloxacin based sequential and triple therapy compared with standard plus probiotic combination for *Helicobacter pylori* eradication. *Hepatogastroenterology* 2011; 58: 1148-52. [\[CrossRef\]](#)
 24. Huebner ES, Surawicz CM. Probiotics in the prevention and treatment of gastrointestinal infections. *Gastroenterol Clin North Am* 2006; 35: 355-65. [\[CrossRef\]](#)
 25. Tong JL, Ran ZH, Shen J, Zhang CX, Xiao SD. Meta-analysis: the effect of supplementation with probiotics on eradication rates and adverse events during *Helicobacter pylori* eradication therapy. *Aliment Pharmacol Ther* 2007; 25: 155-68. [\[CrossRef\]](#)
 26. Zou J, Dong J, Yu X. Meta-analysis: *Lactobacillus* containing quadruple therapy versus standard triple first-line therapy for *Helicobacter pylori* eradication. *Helicobacter* 2009; 14: 449-59. [\[CrossRef\]](#)
 27. Sachdeva A, Naqpal J. Effect of fermented milk-based probiotic preparations on *Helicobacter pylori* eradication: a systematic review and metaanalysis of randomized-controlled trials. *Eur J Gastroenterol Hepatol* 2009; 21: 45-53. [\[CrossRef\]](#)
 28. Szajewska H, Horvath A, Piwowarczyk A. Meta-analysis: the effects of *Saccharomyces boulardii* supplementation on *Helicobacter pylori* eradication rates and side effects during treatment. *Aliment Pharmacol Ther* 2010; 32: 1069-79. [\[CrossRef\]](#)
 29. 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. [\[CrossRef\]](#)
 30. Zheng X, Lyu L, Mei Z. *Lactobacillus*-containing probiotic supplementation increases *Helicobacter pylori* eradication rate: evidence from a metaanalysis. *Rev Esp Enferm Dig* 2013; 105: 445-53. [\[CrossRef\]](#)
 31. Yoon H, Kim N, Kim JY, et al. Effects of multistrain probiotic-containing yogurt on second-line triple therapy for *Helicobacter pylori* infection. *J Gastroenterol Hepatol* 2011; 26: 44-8. [\[CrossRef\]](#)
 32. Scaccianoce G, Zullo A, Hassan C, et al. Triple therapies plus different probiotics for *Helicobacter pylori* eradication. *Eur Rev Med Pharmacol Sci* 2008; 12: 251-6.
 33. Ahmad K, Fatemeh F, Mehri N, Maryam S. Probiotics for the treatment of pediatric *Helicobacter pylori* infection: a randomized double blind clinical trial. *Iran J Pediatr* 2013; 23: 79-84.
 34. Cremonini F, Di Caro S, Covino M, et al. Effect of different probiotic preparations on anti-*Helicobacter pylori* therapy-related side effects: a parallel group, triple blind, placebo-controlled study. *Am J Gastroenterol* 2002; 97: 2744-9. [\[CrossRef\]](#)
 35. 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. [\[CrossRef\]](#)
 36. Navarro-Rodriguez T, Silva FM, Barbuti RC, et al. Association of a probiotic to a *Helicobacter pylori* eradication regimen does not increase efficacy or decreases the adverse effects of the treatment: a prospective, randomized, double-blind, placebo-controlled study. *BMC Gastroenterol* 2013; 13: 56. [\[CrossRef\]](#)
 37. Gotteland M, Brunser O, Cruchet S. Systematic review: are probiotics useful in controlling gastric colonization by *Helicobacter pylori*? *Aliment Pharmacol Ther* 2006; 23: 1077-86.
 38. Zhang MM, Qian W, Qin YY, He J, Zhou YH. Probiotics in *Helicobacter pylori* eradication therapy: a systematic review and meta-analysis. *World J Gastroenterol* 2015; 21: 4345-57. [\[CrossRef\]](#)
 39. Kim MN, Kim N, Lee SH, et al. The effects of probiotics on PPI-triple therapy for *Helicobacter pylori* eradication. *Helicobacter* 2008; 13: 261-8. [\[CrossRef\]](#)
 40. Armuzzi A, Cremonini F, Bartolozzi F, et al. The effect of oral administration of *Lactobacillus GG* on antibiotic-associated gastrointestinal side-effects during *Helicobacter pylori* eradication therapy. *Aliment Pharmacol Ther* 2001; 15: 163-9. [\[CrossRef\]](#)
 41. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007; 39: 175-91. [\[CrossRef\]](#)
 42. Nadir I, Yonem O, Ozin Y, Kilic ZM, Sezgin O. Comparison of two different treatment protocols in *Helicobacter pylori* eradication. *South Med J* 2011; 104: 102-5. [\[CrossRef\]](#)
 43. Sheu BS, Wu JJ, Lo CY, et al. Impact of supplement with *Lactobacillus*- and *Bifidobacterium*-containing yogurt on triple therapy for *Helicobacter pylori* eradication. *Aliment Pharmacol Ther* 2002; 16: 1669-75. [\[CrossRef\]](#)
 44. Gispert JP, Khorrami S, Calvet X, Gabriel R, Carballo F, Pajares JM. Meta-analysis: Proton pump inhibitors vs. H2-receptor antagonists – their efficacy with antibiotics in *Helicobacter pylori* eradication. *Aliment Pharmacol Ther* 2003; 18: 757-66. [\[CrossRef\]](#)
 45. Dai C, Zheng CQ, Jiang M. Efficacy of probiotics in *Helicobacter pylori* eradication therapy. *J Clin Gastroenterol* 2013; 47: 814-5. [\[CrossRef\]](#)
 46. Myllyluoma E, Veijola L, Ahlroos T, et al. Probiotic supplementation improves tolerance to *Helicobacter pylori* eradication ther-

- apy—a placebo-controlled, double-blind randomized pilot study. *Aliment Pharmacol Ther* 2005; 21: 1263-72. [\[CrossRef\]](#)
47. Hauser G, Salkic N, Vukelic K, Jajacknez A, Stimac D. Probiotics for standard triple *Helicobacter pylori* eradication: a randomized, double-blind, placebo-controlled trial. *Medicine (Baltimore)* 2015; 94: e685.
 48. Adamsson I, Nord CE, Lundquist P, Sjostedt S, Edlund C. Comparative effects of omeprazole, amoxicillin, plus metronidazole versus omeprazole, clarithromycin plus metronidazole on the oral, gastric and intestinal microflora in *Helicobacter pylori*-infected patients. *J Antimicrob Chemother* 1999; 44: 629-40. [\[CrossRef\]](#)
 49. Tomomasa T, Kuroume T, Arai H, Wakasashi K, Itoh Z. Erythromycin induces migrating motor complexes in human gastrointestinal tract. *Dig Dis Sci* 1986; 31: 157-61. [\[CrossRef\]](#)
 50. Bortolotti M, Brunelli F, Sarti P, Mari C, Miglioli M. Effects of oral clarithromycin and amoxicillin on interdigestive gastrointestinal motility of patients with functional dyspepsia and *Helicobacter pylori* gastritis. *Aliment Pharmacol Ther* 1998; 12: 1021-5. [\[CrossRef\]](#)
 51. Isolauri E, Kirjavainen PV, Salminen S. Probiotics: a role in the treatment of intestinal infection and inflammation? *Gut* 2002; 50(Suppl 3): III54-9.
 52. Buhling A, Radun D, Muller WA, Malfertheiner P. Influence of anti-*Helicobacter* triple-therapy with metronidazole, omeprazole and clarithromycin on intestinal microflora. *Aliment Pharmacol Ther* 2001; 15: 1445-52. [\[CrossRef\]](#)
 53. de Boer WA, Tytgat GNJ. The best therapy for *Helicobacter pylori* infection: should efficacy or side-effect profile determine our choice? *Scand J Gastroenterol* 1995; 30: 401-7.
 54. Franceschi F, Cazzato A, Nista EC, et al. Role of probiotics in patients with *Helicobacter pylori* infection. *Helicobacter* 2007; 12(Suppl 2): 59-63.
 55. der Merwe S, Vaz Coelho LG, Fock M, et al. *Helicobacter pylori* in developing countries. World Gastroenterology Organisation Global Guideline. *J Gastrointestin Liver Dis* 2011; 20: 299-304.
 56. Penston JG, McColl KE. Eradication of *Helicobacter pylori*: an objective assessment of current therapies. *Br J Clin Pharmacol* 1997; 43: 223-43.
 57. Manfredi M, Bizzarri B, Sacchero RI, et al. *Helicobacter pylori* infection in clinical practice: probiotics and a combination of probiotics + lactoferrin improve compliance, but not eradication, in sequential therapy. *Helicobacter* 2012; 17: 254-63. [\[CrossRef\]](#)