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Efficacy of probiotics in *Helicobacter pylori* eradication therapy

Probiyotiklerin *Helikobakter pilori* eradikasyon tedavisindeki etkinliği

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Background/aims: *Triple therapy with a proton pump inhibitor, amoxicillin and clarithromycin in Helicobacter pylori eradication is widely accepted, but this combination fails in a considerable number of cases. Our aim was to evaluate whether probiotic-containing yogurt affects the success of eradication. The second aim was to investigate the efficacy of probiotics in the prevention of the side effects related to eradication therapy.* ***Methods:*** *A total of 76 histopathologically proven H. pylori-positive patients enrolled in this study were randomized into two groups. The following regimens were recommended: Group A: pantoprazole (40 mg, b.i.d.), amoxicillin (1000 mg b.i.d.), clarithromycin (500 mg b.i.d.), and 125 ml of probiotic-containing yogurt (Bifidobacterium DN-173 010-1010 cfu/g) before breakfast for 14 days; and Group B: pantoprazole (40 mg, b.i.d.), amoxicillin (1000 mg b.i.d.) and clarithromycin (500 mg b.i.d.) for 14 days. Subjects were asked to report any side effects of therapy during the treatment period. H. pylori status was rechecked four weeks after the completion of the eradication therapy by 13C-urea breath test.* ***Results:*** *H. pylori eradication was achieved in 25 of the 38 patients in Group A (66%) and in 20 of the 38 patients (53%) in Group B. Although the success rate was higher in Group A than in Group B, the difference was not significant (p=0.350). The addition of probiotics to the triple therapy significantly lessened the frequency of stomatitis and constipation (p=0.037 and p=0.046, respectively).* ***Conclusions:*** *The addition of probiotic-containing yogurt to the triple therapy did not increase the H. pylori eradication rates for the evaluated dosage and model; however, it decreased the frequency of stomatitis and constipation.*

Key words: *Helicobacter pylori*, probiotic-containing yogurt, eradication, triple therapy

Amaç: *Proton pompa inhibitörü, amoksisilin ve klaritromisin içerikli üçlü tedavi, Helikobakter pilori eradikasyonunda sıkça kullanılmakta olmasına rağmen önemli bir oranda hastada başarısızlıkla sonuçlanmaktadır. Bu çalışmada, antibiyotik tedavisine probiyotik eklenmesinin H. pilori eradikasyonu üzerine etkisini araştırmayı amaçladık. İkinci amacımız ise probiyotiklerin, eradikasyon tedavisinin yan etkilerini önlemedeki etkilerini incelemek idi.* ***Yöntem:*** *Histopatolojik olarak H. pilori pozitif saptanmış 76 hasta 2 gruba ayrıldı ve her bir kişiye rastgele olarak iki tedavi protokolünden birisi uygulandı; grup A: Pantoprazol 40 mg 2x1, amoksisilin 1 gr 2x1, klaritromisin 500 mg 2x1 ve probiyotik içerikli yoğurt 125 ml (Bifidobacterium DN-173 010-1010 cfu/gr) 1x1, kahvaltı öncesi, 14 gün, grup B: Pantoprazol 40 mg 2x1, amoksisilin 1 gr 2x1 ve klaritromisin 500 mg 2x,14 gün. Hastalardan tedavi boyunca karşılaştıkları yan etkileri bildirmeleri istendi. Tedavi bitiminden 4 hafta sonra H. pilori durumu 13^C-üre nefes testi ile kontrol edildi.* ***Bulgular:*** *Group A'da ki 38 hastanın 25'inde (%66), group B'de ki 38 hastanın 20'sinde (%53) H. pilori eradikasyonu sağlanmıştır. Group A'da başarı oranı group B'den daha yüksek olmakla birlikte, bu fark istatistiksel olarak anlamlı bulunmamıştır (p=0.350). Klasik üçlü tedaviye probiyotik eklenmesi stomatit ve kabızlık sıklığında anlamlı şekilde azalmaya yol açmıştır (sırasıyla, p=0.037 ve p=0.046).* ***Sonuç:*** *Üçlü tedaviye probiyotik eklenmesi, verilen doz ve modelde H. pilori eradikasyonunu arttırmamış ancak stomatit ve kabızlık görülme sıklığını azaltmıştır.*

Anahtar kelimeler: *Helikobakter pilori*, probiyotik içerikli yoğurt, eradikasyon, üçlü tedavi

INTRODUCTION

Helicobacter pylori (H. pylori) infection is a major cause of chronic gastritis and peptic ulcers and is a risk factor for gastric malignancies, adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. It affects nearly 50% of the world's population (1). *H. pylori* was classified as a type 1

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carcinogen by the World Health Organization in 1994 (2). A remaining question is the high prevalence of *H. pylori* in developing countries, while its prevalence is decreasing in Western countries (3, 4). Antibiotic resistance due to frequent and uncontrolled use and the high prevalence of antibiotic side effects are the most common causes for treatment failure. To increase the proton pump inhibitor (PPI)-based first-line triple therapy eradication rates, as defined in the Maastricht III Report (5), several clinical trials have been initiated involving extended treatment duration, the use of new antibiotics (quinolone), the use of quadruple therapy as a first option, or the addition of probiotics to triple therapy.

A probiotic is defined as a living microbial species that, upon administration, may have a positive effect on bowel microecology and improve health conditions (6). Probiotics have several mechanisms by which they can affect *H. pylori* in vitro (7). The first is that the lactic acid produced by probiotics inhibits *H. pylori* urease by lowering the gastric pH. The second is that the bacteriocins produced by the lactic acid bacteria kill *H. pylori*. The third mechanism is the inhibition of the adhesion of *H. pylori* to gastric epithelial cells. Other mechanisms include the stabilization of the gastric barrier function due to mucin production and an immunologic response (8). In addition, several studies have shown that probiotics can reduce the side effects of antibiotics and improve the treatment tolerability (9, 10).

In this study, we aimed to determine whether the addition of yogurt containing the probiotic Bifidobacterium DN 173-010 to PPI-based triple therapy affects *H. pylori* eradication rates and the side effects of antibiotics.

MATERIALS AND METHODS

Patients

This study was designed as a prospective and randomized trial and included 78 patients who presented to the Haydarpaşa Numune Training and Research Hospital Outpatient Clinic with dyspeptic complaints such as heartburn, dyspepsia, nausea, and epigastric pain. Exclusion criteria were: (1) age under 18; (2) previous gastric surgery; (3) allergy to any of the drugs used in the study; (4) receipt of antibiotics, PPIs, H2 receptor blockers, bismuth salts, or probiotics within the previous four weeks; (5) presence of clinically significant conditions such as hepatic, cardiorespiratory, renal, or neoplastic diseases or coagulopathy; and (6) pregnancy or lactation. The protocol was approved by the Ethics Committee of Haydarpaşa Numune Training and Research Hospital, and it was concordant with the principles of the Helsinki II Declaration. Informed consent was obtained from all patients.

Study Method

All patients found to be eligible for the study underwent esophagogastroduodenoscopy (EGD). The patients who were determined to have esophagitis, gastric or duodenal ulcers, erosion, or tumors were excluded from the study. Two samples were taken from the gastric antrum and corpus for histologic assessment, and the biopsy specimens were fixed in 10% formalin solution. Preparations were stained with hematoxylin-eosin and modified Giemsa stains and were evaluated according to updated Sydney classification.

H. pylori-positive patients were randomized into two therapy regimens: patients in Group A (n:38) were given pantoprazole (40 mg, b.i.d., before breakfast), amoxicillin (1000 mg b.i.d., after meals), clarithromycin (500 mg b.i.d., after meals), and 125 ml of probiotic-containing yogurt (Bifidobacterium DN-173 010-10^{10} cfu/g) per day before breakfast for 14 days, whereas patients in Group B (n:38) were given pantoprazole (40 mg, b.i.d., before breakfast), amoxicillin (1000 mg b.i.d., after meals), and clarithromycin (500 mg b.i.d., after meals) for 14 days. Subjects were asked to report any side effects of therapy during the treatment period and were given a possible side effect list, such as epigastric pain, diarrhea, taste disturbance, constipation, and stomatitis. They were also asked to grade each side effect according to severity: mild (effect observed, but could be disregarded), moderate (effect sometimes interfered with daily activities), or severe (effect continuously interfered with daily activities). Patient compliance was evaluated at the end of the treatment by pill count and was considered as completed if >80% of the medication had been taken. Successful eradication was defined as a negative ^{13}C-urea breath test *(Helicobacter test INFAI*, Bochum, Germany) result four weeks after discontinuation of the therapy.

Statistical Analysis

Statistical analyses were performed using the GraphPad Prisma Version 3 packet program. Va-

lues were reported as mean ± standard deviation. Non-parametric t-test, chi-square test, and Fisher's exact test were used to compare two independent groups. All of the reported P-values were two-tailed, and those less than 0.05 were considered to be statistically significant.

RESULTS

A total of 76 patients (25 males [33%] and 51 females [67%]) were initially included in the study. The patients were randomized into Group A (triple therapy plus probiotic; n:38) and Group B (triple therapy; n:38) for *H. pylori* eradication. All of the patients in both groups used more than 80% of the therapies, and no patients were lost to follow-up. There were no statistically significant differences between the two groups regarding age and gender (p=0.586, p=0.625, respectively) (Table 1). *H. pylori* eradication was achieved in 25 of the 38 (65.8%) patients in Group A and in 20 of the 38 (52.6%) patients in Group B. Although the success rate for *H. pylori* eradication was higher in Group A than in Group B, the difference was not statistically significant (p=0.350) (Table 2).

The frequencies of stomatitis (inflammation of the mucosa of the mouth) and constipation, from among the side effects recorded during the therapy period, were significantly lower in Group A than in Group B (p=0.037 and p=0.046, respectively). The differences between the remaining side effects in both groups demonstrated no statistical significance (Table 3).

DISCUSSION

The recommended first-line treatment for *H. pylori* eradication in the published guidelines in Europe and North America is PPI combined with amoxicillin and clarithromycin (5), and this is also the most popular treatment regimen in Turkey. Alarmingly, the rates of eradication with this regimen are falling due to a combination of antibiotic resistance and a poor compliance with therapy, which is primarily due to the side effects of the antibiotics. In a large, randomized, controlled trial performed in Turkey, *H. pylori* eradication rates achieved with an omeprazole+clarithromycin+amoxicillin regimen for 7 and 14 days were 24% and 43%, respectively (11). A metanalysis by Aydın et al. (12) documented that the average *H. pylori* eradication rate with PPI-based triple regimens was 93.3% in 1996 and decreased to 47.1% in 2004. Decreases in eradication rates may be attributed to antibiotic resistance, especially clarithromycin resistance, which is approximately 28% in Turkey (13). Antibiotic related side effects during anti-*H. pylori* treatment are common and usually affect the gastrointestinal system. Poor patient compliance due to the side effects and discontinuation of the therapy in the stated time intervals impair the efficiency of the therapy and increase the possibility of resistance development against the antibiotics (14). Additionally, antibiotics can not exhibit their real effects due to insufficient diffusion to the bacteria.

It has been well documented in studies published since 2000 that some lactobacillus and bifidobacteria species inhibit *H. pylori* proliferation by means of the secretion of bacteriocin and organic acids (15–18). It has also been established that probiotics interfere with *H. pylori* adhesion to epithelial cells, increase gastric barrier stabilization and decrease mucosal inflammation. It was reported that the antioxidant and anti-inflammatory effects of probiotics can contribute to gastric mucosa healing (19). Canducci et al. (27) documented that the addition of probiotics to the PPIs and antibiotics increased the eradication rates in 120 *H. pylori*-positive patients with dyspeptic symptoms, but it did not improve the side effects associated with the therapy. On the other hand, in another study conducted by Armuzzi et al. (21), the addition of probiotics to the first-line therapy did not change the eradication rates, but side effects such as diarrhea, bloating and abnormal taste were lower in the probiotic group than in the control group. The common point in both studies was the use of the lactobacillus species as probiotic. In another study conducted with only clarithromycin by Felley et al. (22), the addition of lactobacillus-containing milk to the clarithromycin decreased the bacterial den-

Table 1. Demographic features of patients

		Probiotic group (n=38)	Placebo group (n=38)	p
Age		38.32±10.66	36.95±8.62	0.586
Gender	Male	28.9% (n=11)	36.8% (n=14)	
	Female	71.1% (n=27)	63.2% (n=24)	0.625

Table 2. Comparison of *Helicobacter pylori* eradication rates between groups

Hp eradication	Probiotic group (n=38)	Placebo group (n=38)	p
Successful	65.8% (n=25)	52.6% (n=20)	0.35
Unsuccessful	34.2% (n=13)	47.4% (n=18)	

Table 3. Side effects observed during treatment

Side effects during treatment	Grade	Probiotic group % (n)	Placebo group % (n)	p
Nausea	Absent	73.68 (28)	52.6 (20)	0.3
	Mild	15.79 (6)	31.6 (12)	
	Moderate	7.89 (3)	13.2 (5)	
	Severe	2.63 (1)	2.6 (1)	
Vomiting	Absent	92.11 (35)	86.3 (33)	0.4
	Mild	5.26 (2)	10.5 (4)	
	Moderate	0 (0)	2.6 (1)	
	Severe	2.63 (1)	0 (0)	
Metallic taste	Absent	18.42 (7)	18.4 (7)	0.09
	Mild	23.68 (9)	5.3 (2)	
	Moderate	26.32 (10)	23.7 (9)	
	Severe	31.58 (12)	52.6 (20)	
Stomatitis	No	94.74 (36)	76.3 (29)	**0.046**
	Yes	5.26 (2)	23.7 (9)	
Abdominal pain	Absent	76.32 (29)	68.4 (26)	0.4
	Mild	15.79 (6)	13.2 (5)	
	Moderate	7.89 (3)	13.2 (5)	
	Severe	0 (0)	5.3 (2)	
Constipation	Absent	92.11 (35)	76.3 (29)	**0.037**
	Mild	0 (0)	15.8 (6)	
	Moderate	7.89 (3)	7.9 (3)	
	Severe	0 (0)	(0)	
Diarrhea	Absent	63.16 (24)	60.5 (23)	0.5
	Mild	26.32 (10)	60.5 (23)	
	Moderate	5.26 (2)	15.8 (6)	
	Severe	5.26 (2)	2.6 (1)	

sity and inflammation in the stomach. In studies made only with probiotics, it was shown that probiotics alone could not completely eradicate *H. pylori*, but they could significantly decrease the bacteria number (16, 17). These findings support the idea that probiotics have inhibitory effects against *H. pylori*. In light of all these studies, we postulated that the addition of a probiotic-containing yogurt to the *H. pylori* eradication therapy could increase patient compliance by decreasing the antibiotic-related side effects and could increase the success rates, but there is no absolute consensus on this issue.

In the present study, the success rate of *H. pylori* eradication with first-line triple therapy was 53%, and it rose to 66% with the addition of probiotic-containing yogurt. Clearly, the success rate achieved with standard triple therapy is far from the rates that are accepted as ideal. In the studies published in western countries, the success rate of *H. pylori* eradication therapy was reported as approximately 70%, which is near the ideal values (23). In countries in which frequent and uncontrolled antibiotic utilization is common, as in Turkey, this difference in eradication rates is due to the higher antibiotic resistance.

One notable point is that most of the studies used the lactobacillus species. In a literature review, we did not find any eradication study using only bifidobacteria, as was used in the present study. The reason for the lactobacillus choice may be their ability to remain 80% alive in the gastric environment for two hours and their stability in acid as compared with other bacteria. In our study, we selected the use of a bifidobacteria-containing product, because bifidobacteria have properties similar to those of lactobacillus and have not yet been used alone in such a study.

We postulated that giving the probiotic therapy in the morning with the triple therapy might be more useful in keeping the effects of the bacteria at high levels, as the optimum reported pH of the environment should be between 6.5–7.0 for the proliferation of bifidobacteria, and their proliferation is inhibited in a pH below 4.5 (24). For this purpose, the gastric pH would be increased to the desired levels by the PPI, and an optimum environment would be produced for the probiotic proliferation. Therefore, the low eradication rates can be attributed to a low bifidobacteria concentration.

Another aim in our study was to investigate the efficacy of probiotics for the prevention of the side

effects related to the therapy. As mentioned in the Maastricht III Consensus Report, the 14-day treatment is superior to the 7-day treatment. Because of the higher antibiotic resistance rates, developing countries such as Turkey prefer 14-day treatment regimens. This increased duration of therapy carries the burden of increased side effects. It has been shown that the large doses of antibiotics used in the triple therapy change the normal bowel flora. This may account for the adverse events in the gastrointestinal tract (25). It can be speculated that probiotic supplementation may resist this change or diminish the overgrowth of harmful bacteria or yeast induced by the antibiotic treatment. Some previous studies have shown that probiotics have a positive impact on *H. pylori* eradication therapy-related side effects (26-28). In our study, we did not observe the probiotic therapy to have any effect on the metallic taste, nausea, vomiting, epigastric pain, or diarrhea. However, stomatitis and constipation were significantly lower in the probiotic arm (Group A) than in Group B (p=0.046, p=0.037, retrospectively). This effect is important for those countries in which anti-*H. pylori* regimens are applied for longer periods (14 days).

In conclusion, our study suggests that the addition of a probiotic-containing yogurt to triple therapy did not increase the *H. pylori* eradication rates in the evaluated dosage and model; however, it significantly decreased the frequency of stomatitis and constipation. This suggests a possibility that the addition of probiotic-containing yogurt, commercialized as a kind of food, might be an option for increasing patient compliance, thus encouraging more research in this field.

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