Can probiotics improve efficiency and safety profile of triple Helicobacter pylori eradication therapy? A prospective randomized study

Mogu li probiotici poboljšati efikasnost i bezbednosni profil trostrukih eradikacije terapije za Helicobacter pylori? Prospektivna randomizirana studija

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Abstract

Background/Aim. Some studies suggest the benefit of applying different probiotic strains in combination with antibiotics in the eradication of Helicobacter pylori (H. pylori) infection. The aim of this study was to evaluate the effect of co-administration of multiple probiotic strains with triple H. pylori eradication therapy. Methods. This prospective study included 167 patients with dyspeptic symptoms and chronic gastritis who were diagnosed with H. pylori infection and randomized into two groups. The group I of 77 patients underwent triple eradication therapy, for 7 days, with lansoprazole, 2 × 30 mg half an hour before the meal, amoxicillin 2 × 1.000 mg per 12 hours and clarithromycin 2 × 500 mg per 12 hours. After the 7th day of the therapy, lansoprazole continued at a dose of 30 mg for half an hour before breakfast for 4 weeks. The group II of 90 patients received the same treatment as the patients of the group I, with the addition of the probiotic cultures in the form of a capsule comprising Lactobacillus Rosell-52, Lactobacillus Rosell-11, Bifidobacterium Rosell-1755 and Saccharomyces boulardii, since the beginning of eradication for 4 weeks. Eradication of H. pylori infection control was performed 8 weeks after the therapy by rapid urease test and histopathologic evaluation of endoscopic biopsies or by stool antigen test for H. pylori. Results. Eradication of H. pylori infection was achieved in 93.3% of the patients who received probiotics with eradication therapy and in 81.8% of patients who were only on eradication therapy without probiotics. The difference in eradication success was statistically significant, (p < 0.05). The incidence of adverse effects of eradication therapy was higher in the group of patients who were not on probiotic (28.6%) than in the group that received probiotic (17.7%), but the difference was not statistically significant. Conclusion. Multiple probiotic strains addition to triple eradication therapy of H. pylori achieves a significantly better eradication success, with fewer side effects of antibiotics.

Key words: helicobacter pylori; helicobacter infection; disease eradication; clinical protocols; probiotics; treatment outcome.

Apstrakt

Uvod/Cilj. Pojedine studije ukazuju na dobrobit primene različitih probiotičkih sojeva u kombinaciji sa antibioticima u eradicaciji infekcije prouzrokovane bakterijom Helicobacter pylori (H. pylori). Cilj ove studije bio je da se proceni efekat koadministracije muliplicit probiotičkih sojeva i trostrukih eradikacionih terapija za H. pylori. Metode. U ovu prospektivnu studiju bilo je uključeno 167 bolesnika sa dispeptičkim simptomima i brončanim gastritism kod kojih je dijagnostikovana H. pylori infekcija i koji su randomizirani u dve grupe. Grupa I, od 77 bolesnika, podvrgnuta je trostrukoj eradikacionoj terapiji u trajanju od 7 dana, sa lansoprazolom 2 × 30 mg pola sata pre obroka, amoksicilinom 2 × 1 000 mg na 12 sati i klaritromicinicom 2 × 500 mg na 12 sati. Posle 7. dana nastavljena je terapija lansoprazolom u dozi od 30 mg pola sata pre doručka još 4 nedelje. Grupa II, sastavljena od 90 bolesnika, podvrgnuta je istoj terapiji kao i bolesnici grupa I, uz dodatak kulture probiotika u vidu jedne kapsule, koja je sadržala Lactobacillus Rosell-52, Lactobacillus Rosell-11, Bifidobacterium Rosell-1755 i Saccharomyces boulardii, od početka eradikacione terapije, u trajanju od 4 nedelje. Kontrola eradicacije H. pylori infekcije izvršena je 8 nedelja nakon terapije brzim urezom i patohistoloskom procenom endoskopskih biopsija ili testom antigena u stolici na H. pylori. Rezultati. Eradicacija H. pylori infekcije postignuta je kod 93,3% bolesnika koji su dobijali probiotik uz eradikacionu terapiju i kod 81,8% bolesnika koji su bili samo na eradikacionoj...
**Introduction**

*Helicobacter pylori* (*H. pylori*) is a Gram-negative, microaerophilic bacterium that colonizes the gastric mucosa. Since the discovery of *H. pylori* in 1983, numerous studies have shown that this bacterium is a major risk factor in the development of peptic ulcer, chronic gastritis, gastric cancer and mucosa associated lymphoid tissue (MALT) lymphoma. The prevalence of infection in developed countries is 20–25%. While in developing countries it reaches up to 80%.

However, the majority of infected people, despite the existence of chronic gastritis, has no symptoms, while 10–20% obtain peptic ulcer. In 1–2% of infected persons there is the risk of developing gastric cancer during the lifetime, and in less than 1% the risk of developing gastric lymphoma. Therefore, elimination of infection is a good strategy for the prevention of gastric malignancy. In addition, indications for eradication of *H. pylori* infection are certain extragastric diseases, such as idiopathic thrombocytopenic purpura, vitamin B12 deficiency and unclear iron deficient anemia.

Standard triple eradication therapy with a proton pump inhibitor (PPI) and two antibiotics (clarithromycin, amoxicillin or metronidazole) is still the most frequent first line therapy. The rising resistance to clarithromycin requires the introduction of sequential or concomitant therapy as the first option, especially for the areas with high resistance to clarithromycin. Levofloxacin in combination with different antibiotics showed a good therapeutic effect as the first, second or third line therapy but arriving problem is the emergence of resistance to fluoroquinolones. In a study from Japan newer fluoroquinolones sitafloxacin, which shows the lowest minimum inhibitory concentration for *H. pylori*, proved to be effective in combination with PPI, amoxicillin and metronidazole as third line therapy. Quadruple therapy with bismuth, like fluoroquinolones, has proved effective as a first line therapy or as rescue therapy. The main reason for the increased resistance to antibiotics is point mutations which accumulate in the *H. pylori* DNA.

The main principle of treating *H. pylori* infection is based on the introduction of newer therapeutic regimes which would achieve better therapeutic effects and reduce side effects of antibiotics. A number of studies suggest that lactic acid bacteria, such as *Lactobacillus* and *Bifidobacterium*, increase the effect of eradication of *H. pylori* and reduce side effects when combined with antibiotics. These bacteria inhibit the growth of *H. pylori* by means of the secretion of protein components, or organic acids, reduce the capacity of adherence of *H. pylori* on the gastric epithelial cells, reduce the mucosal inflammation, and stabilize the gastric barrier.

Many preparations of probiotics in addition to the strains of *Lactobacillus* and *Bifidobacterium* contain probiotic yeast, such as *Saccharomyces boulardii*. Unlike the studies that support the co-administration of probiotics with the standard therapy, sequential therapy and therapy based on levofloxacin, other studies do not support co-administration of probiotics and multiple strains. The literature has evaluated the use of individual probiotic strains (usually *Lactobacillus spp*, *Saccharomyces spp*, *Bifidobacterium spp* and *Bacillus clausii*) and multiple strains. The reason for conflicting results of particular studies is the lack of placebo-controlled trials, a significant heterogeneity in probiotics treatment duration and the time of administration of probiotics with respect to the use of antibiotics and the use of different probiotic strains.

The aim of this prospective randomized study was to evaluate the effect of co-administration of multiple probiotic strains (*Lactobacillus spp*, *Bifidobacterium spp* and *Saccharomyces spp*) and triple *H. pylori* eradication therapy.

**Methods**

This prospective randomized study included a total of 167 patients with endoscopic and histological findings of chronic gastritis (41.3% or 69 males and 98 or 58.7% females), diagnosed with *H. pylori* infection in the period of one year (during 2014). The patients had symptoms of upper dyspepsia (nausea, epigastric pain, postprandial bloating, belching, heartburn), without alarming symptoms (bleeding, anemia, weight loss). The criteria for exclusion of patients from the study were: younger than 18 years, the use of antibiotics, proton pump inhibitors (PPI) and H2 receptor antagonists in the last two weeks (according to Maastricht IV consensus report), allergy to penicillin and any other administered drugs, previous eradication of *H. pylori*, pregnancy, lactation, previous gastric surgery, gastric malignancy, peptic ulcer, peptic pyloric stenosis, reflux esophagitis and significant comorbidity with the presence of malignant disease and/or bad general condition.

Patients were randomized into two groups. The group I of 77 patients (27 or 35.1% males and 50 or 64.9% females), average age 56.2 ± 14.8 years (range 21 to 80 years) were treated with triple eradication therapy of *H. pylori* infection, within 7 days, with a proton pump inhibitor lansoprazole 2 × 30 mg half an hour before a meal, amoxicillin 2 × 1.000 mg at 12 hours and clarithromycin 2 × 500 mg per 12 hours. After the 7th day of the therapy, lansoprazole was continued in a dose of 30 mg for half an hour before breakfast for 4 weeks. The group II of 90 patients (42 or 46.7% males and
of the patients of the group I, and 13 or 14.4% of the patients of the group II) assessment of the success of eradication was carried out by stool antigen test for *H. pylori*. Stool antigen test is a qualitative immunochromatography test type CER-TEST BIOTEC SL. The test relies on the presence of nitrocellulose membranes coated with mouse monoclonal antibodies against *H. pylori* in the test line, in the field of results, and with rabbit polyclonal antibodies to a specific protein, in the control line. Anti-*H. pylori* antibodies present on the membrane (test line) bind dye conjugate and the red line used to read the results becomes visible.

Statistical analysis of the results was carried out with the help of tests for the arithmetic mean, standard deviation, Student’s *t*-test, Fisher’s exact test and *χ²* test. Differences between individual parameters were considered significant at *p* values less than 0.05.

**Results**

Among the groups of patients there were no statistically significant differences found in gender, age, smoking status, use of nonsteroidal anti-inflammatory drugs (NSAIDs) and acetyl salicylic acid (ASA), as well as in terms of comorbidity (Table 1). Regarding the comorbidity in the group I there was hypertension (14 or 18.2%), diabetes mellitus 3 (3.9%), hypothyroidism (3 or 3.9%), hyperthyroidism (1 or 1.3%) and chronic obstructive pulmonary disease (3 or 3.9%).

Within the group II of the patients there was hypertension (22 or 20%), diabetes mellitus (8 or 8.9%), hypothyroidism (2 or 2.2%) and chronic obstructive pulmonary disease (COPD) (1.1%).

*H. pylori* eradication infection was achieved in 63 out of 77 (81.8%) patients of the group I and in 84 out of 90 (93.3%) patients of the group II. The difference in the success of eradication between the groups of patients was statistically significant, *χ² = 5.16 > χ² (1 and 0.05) = 3.84, p < 0.05*, adds ratio (OR) = 3.1 (1.04 < OR < 9.63), confidence interval (CI) 95%; relative risk (RR) = 1.14 (1.01 < RR < 1.28), CI 95% (Table 2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (n = 77)</th>
<th>Group II (n = 90)</th>
<th><em>p</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female), n (%)</td>
<td>27 (35.1)/50 (64.9)</td>
<td>42 (46.7)/48 (53.3)</td>
<td>ns</td>
</tr>
<tr>
<td>Age, x ± SD</td>
<td>56.2 ± 14.8</td>
<td>56.3 ± 14.8</td>
<td>ns</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>10 (13)</td>
<td>13 (14.4)</td>
<td>ns</td>
</tr>
<tr>
<td>NSAID users, n (%)</td>
<td>24 (31.2)</td>
<td>26 (28.9)</td>
<td>ns</td>
</tr>
<tr>
<td>Comorbidity, n (%)</td>
<td>24 (31.2)</td>
<td>31 (34.5)</td>
<td>ns</td>
</tr>
</tbody>
</table>

**NSAID** – non-steroidal anti-inflammatory drugs; **Group I** – patients treated with triple eradication therapy of *Helicobacter pylori* infection (lansoprazole, amoxicillin, clarithromycin); **Group II** – patients treated with the same triple eradication therapy as patients of the Group I + probiotic cultures; **n** – number of patients; **p** – significance (*χ²*-test).

<table>
<thead>
<tr>
<th>Comparison of success of eradication of <em>Helicobacter pylori</em> (<em>H. pylori</em>) infection between the examined groups of patients</th>
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<tr>
<td>Group</td>
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<tr>
<td>Group I</td>
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<td>Total</td>
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For explanations see under Table 1, *p < 0.05 (χ² test).
Comparing the success of eradication between the genders in the group I, infection eradication was achieved in 23/27 (85.2%) women and in 40/50 (80%) men. The difference in the success of eradication by gender was not statistically significant in the group I of patients, \( p < 0.05, \) OR = 1.44 (0.35 < OR < 6.21) CI 95%; RR = 1.06 (0.86 < RR < 1.31) 95% CI. In the group II \( H. pylori \) infection eradication was achieved in 39/42 (92.8%) males and 45/48 (93.7%) women. The difference in the success of eradication of \( H. pylori \) by gender was not statistically significant in the group II, \( p < 0.05, \) OR = 0.89 (0.13 < OR < 5.94), CI 95%; RR = 0.99 (0.89 < OR < 1.11), 95% CI.

While taking eradication therapy in the patients of the group I there were nausea (in 9 11.6% the patients), metallic taste in the mouth (5 or 6.5%), headache (3 or 3.9%), diarrhea (3% or 3.9) and epigastric pain (3 or 3.9%). Also, in the patients of the group II during eradication therapy there were nausea (7 or 7.7%), metallic taste in the mouth (2 or 2.2%), headache (2 or 2.2%), diarrhea (1 or 1.1%) and epigastric pain (3 or 3.3%). With individual comparative analysis of the incidence of adverse effects there was no statistically significant difference observed between the group I and the group II. In the total of 22 (28.5%) patients of the group I and in 15 (16.7%) patients of the group II adverse effects of the therapy occurred. The total difference in respect of adverse effects of eradication therapy was also not statistically significant between the groups of patients, \( \chi^2 = 3.35 < \chi^2 \) (1 and 0.05) = 3.84, \( p > 0.05, \) OR = 1.99 (0.89 < OR < 4.46), CI 95%, RR = 1.16 (0.98 < RR < 1.37), 95% CI (Table 3). In both groups of the patients adverse effects in any case did not lead to discontinuation of the therapy.

We compared the frequency of lagging dyspeptic symptoms after eradication therapy. Dyspeptic symptoms maintained in 19 (24.6%) of the patients of the group I and in 21 (23.3%) of the patients of the group II. The difference was not statistically significant, \( \chi^2 = 0.04 < \chi^2 \) (1 and 0.05) = 3.84, \( p > 0.05, \) OR = 0.93 (0.43 < OR < 2.01) CI 95%, RR = 0.95 (0.55 < RR < 1.63), CI 95%.

**Discussion**

In recent years, many alternative treatments of \( H. pylori \) infection have been studied because of the phenomena of resistance to antibiotics and occurrence of adverse effects of the application of several antibiotics simultaneously, as in case of concomitant therapy. One attempt to solve this problem is the use of probiotic cultures. Certain initial studies had promising results, but many issues remained unresolved. Namely, we do not know the exact mechanism of probiotics’ action. Different probiotic strains can cause different host responses, depending on the immune status of the host. Studies on animal models suggest that probiotic bacteria establish immune regulation by controlling the balance of proinflammatory and anti-inflammatory cytokines, which can lead to reduction of the activity of inflammation in the stomach. Previous studies had shown that *Lactobacillus salivarius* inhibits the secretion of gastric epithelial cells stimulated by *H. pylori* over the interleukin-8. It also leads to increased production of secretory IgA in the intestinal epithelium, which enhances the mucosal barrier. The non-immunological mechanisms of probiotics action are the product of antimicrobial substances, competition with *H. pylori* to adhesion receptors, stimulation of mucus production and stabilization of the mucosal barrier. Some strains, such as *Lactobacillus plantarum* 299V and *Lactobacillus rhamnosus* GG induce mucin gene expression. Certain strains of *Bifidobacterium* release antimicrobial protein substances that act against *H. pylori*.

Latest studies show that the strain of *Lactobacillus pentosus* LPS16 through the production of lactic acid achieves the bactericidal effect against *H. pylori* and that the bactericidal effect is identical to the antibiotics sensitive and resistant strains of *H. pylori*. It has been shown that lactic acid has a higher bactericidal activity than the acetic acid and hydrochloric acid. Therefore, the application of *Lactobacillus pentosus* LPS16 proved to be useful in prevention and in the treatment of *H. pylori* infection, especially in cases of *H. pylori* resistance to many antibiotics.

In most studies on animal and human models different strains of *Lactobacillus* were tested (*L. Jahnsoni* La1, *L. rhamnosus* GG, *L. casei*, *L. acidophilus*, *L. brevis*, *L. gasseri* OLL2716, *L. reuteri*), *Bifidobacterium* strains (*B. lactis*, *B. animalis*, *B. breve*) and probiotic yeast *Saccharomyces boulardii*. The diagnosis of *H. pylori* infection and the effect of probiotics on *H. pylori* gastritis is usually assessed by serological tests, rapid urease test, urea breath test, stool antigen test and histological examination of gastric biopsies.

In our study we examined the effect of probiotic cultures to *H. pylori* infection, which contained *Lactobacillus Rosell-52*, *Lactobacillus Rosell-11*, *Bifidobacterium Rosell-175*.
and Saccharomyces boulardii. For the initial diagnosis of H. pylori infection, we used rapid urease test and histological examination of gastric biopsies. In order to assess the eradication success we used rapid urease test, histological examination of gastric biopsies and stool antigen test for H. pylori. Qualitative immunochromatography test antigen in stool, which we used, has high sensitivity, over 94%, and specificity over 99%.

Our study showed a significantly higher success in infection eradication in the group of patients treated with triple eradication therapy combined with probiotics than in the group without probiotics (93.3% vs 81.8%), p < 0.05. The success of eradication by gender was not statistically significantly different in the examined groups of patients. Despite the fact that in a small number of patients in the group with probiotic there were side effects of eradication therapy manifested (17.8%) compared to the group without probiotics (28.6%), the difference was not statistically significant (p > 0.05).

One of the first clinical studies on the effects of probiotics on H. pylori eradication is a study by Canducci et al. 18, which shows that Lactobacillus acidophilus LB significantly enhances the effect of eradication, but does not diminish the adverse effects of antibiotic therapy. The open and uncontrolled study by Sheu et al. 19 shows that Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12 increase the effect of eradication and decrease adverse effects of triple eradication H. pylori therapy. Studies of some authors show that the application of Lactobacillus acidophilus La5 together with Bifidobacterium lactis Bb12 before quadruple secondary line therapy after failure of triple eradication therapy enhances the effect of eradication. The benefit of the application of probiotic strains seems not to depend on the type of applied strains 1.

A recent meta-analysis of 14 randomized controlled trials has shown that the addition of probiotics to standard triple therapy improves eradication effect, established by both intention-to-treat (ITT) analysis (OR = 1.67, 95% CI: 1.38 to 2.02) and per-protocol (PP) analysis (OR = 1.68, 95% CI: 1.35 to 2.02) and per-protocol (PP) analysis (OR = 1.68, 95% CI: 1.35 to 2.02). However, studies have shown no adverse effects of probiotics, considering that some strains of probiotics such as Lactobacilli and Bifidobacteria are part of a normal gastrointestinal microbiota.

Conclusion

Our study shows that triple H. pylori eradication therapy achieves a statistically significantly better eradication success combined with probiotic strains Lactobacillus Rosell-52, Lactobacillus Rosell-11, Bifidobacterium Rosell-175 and Saccharomyces Boulardii. Also, there are fewer adverse effects of antibiotic therapy by using probiotics although the difference is not statistically significant. To come to more accurate conclusions about the effects of probiotic strains in the treatment of H. pylori infection further studies and standardization of studies in terms of the type of the applied probiotic strain is required, as well as the number of probiotic strains (single use of a single strain or multiple strains), and the length and time of administration of probiotics in relation to antibiotic therapy.

R E F E R E N C E S


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