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Bifidobacterium lactis Fermented Milk Was Not Effective for *Helicobacter pylori* Eradication: A Prospective, Randomized, Double-Blind, Controlled Study

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***Abstract*—**The management of *Helicobacter pylori* (*H. pylori*) eradication is still a matter of discussion, full effectiveness is rarely achieved, and it has many adverse effects. The use of probiotics may be associated with better eradication rates and possibly prevention of adverse events due to antibiotic therapy. The present clinical study was undertaken to evaluate the efficacy of a specially designed fermented milk product, containing *Bifidobacterium lactis* B420, on the eradication of *H. pylori* infection in a prospective, randomized, double-blind, controlled study in humans. Four test fermented milks (FM) were specially designed in which counts of viable cells in all products were 10^{10} Log CFU. 100 mL^{-1} for *Bifidobacterium lactis* - *Bifidobacterium* species 420. 190 subjects infected with *H. pylori*, with previous diagnosis of functional dyspepsia according to Rome III criteria entered the study. *Bifidobacterium lactis* B420, administered twice a day for 90 days was not able to eradicate *H. pylori* in Brazilian patients with functional dyspepsia.

Trial registration: Current Controlled Trials ISRCTN22923997

***Keywords*—**Antibacterial Therapy, Bifidobacteria Fermented milk, *Helicobacter pylori*, probiotics.

I. Introduction

FUNCTIONAL dyspepsia is a clinical syndrome that biochemical examinations and endoscopy reveal no organic lesions or findings. The primary symptoms are epigastric pain, early satiety and post-prandial fullness [1].

The management of functional dyspepsia is a challenge for gastroenterologists in clinical practice. *Helicobacter pylori (H. pylori)* eradication is one of the main strategies to treat this disease [2].

H. pylori is a gram negative, microaerophilic, spiral bacterium that colonizes human stomach [3]. The ability to produce urease is a central biochemical characteristic and could be used to determine positive subjects [3]. *H. pylori* infection affects more than 50% of the world's population. Its prevalence in the development countries is around 80%, initially infected during childhood [4], [5].

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H. pylori eradication is achieved usually with antibiotics associated with a proton pump inhibitor (PPI). In Brazil, the recommended the first line treatment is composed by a PPI plus clarithromycin and amoxicillin [6], [7].

Eradication rates with this scheme in countries such as ours, with low clarithromycin resistance, are around 90%. However, combination of two antibiotics is frequently associated with adverse effects and contributes to the increase in antibiotic resistance [7]. In this context, probiotics have emerged as potential candidates as an alternative therapy [8]-[10].

Animal, human and *in vitro* studies that evaluated *H. pylori* and probiotics could demonstrate an *in vitro* inhibitory effect of probiotics on *H. pylori*. Those animal studies demonstrated that some probiotic strains are effective in reducing *H. pylori* – associated gastric inflammation [10]. Moreover, an improvement of *H. pylori* gastritis and decrease *H. pylori* density after administration of probiotics was also observed in human studies [9]-[11].

A meta-analysis by [11] of randomized controlled trials on the efficacy of probiotics in *H. pylori* eradication therapy in children, concluded that the probiotics were beneficial in decreasing side effects secondary to triple therapy for *H. pylori* eradication, particularly diarrhoea. However, the efficacy of probiotics for *H. pylori* eradication in adults remains controversial [11].

Most studies used lactobacilli species like *L. johnsonii* La1, either in a fermented milk preparation containing live bacteria [10], [12]-[15] or as a free-cell culture supernatant [12], [16], [17]. On the contrary, few studies used bifidobacteria [18], [19].

Bifidobacteria have been extensively used in various food formulations due to its strong adhesion to human intestinal mucosa and its high capacity for colonization [20]. Some authors have shown that some species are capable of stabilizing the intestinal microbiota during and after antibiotic therapy [21], modulate the immune system, protect against chemically induced inflammatory bowel disease and reduce symptoms of colitis [22]. It may have beneficial effects in increasing eradication rates of H. pylori in the adults and also reducing the incidence of side effects [19].

The present clinical study was undertaken to evaluate the efficacy of a specially designed fermented milk product containing *Bifidobacterium lactis* B420 on the eradication of

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H. pylori infection in a prospective, randomized, double-blind, placebo-controlled study in humans.

II. Material and Methods

A. Patients

Patients with dyspepsia symptoms were invited to participate in the study [23].

Inclusion criteria included: more than 18 years old, no previous *H. pylori* treatment, no chronic decompensate disease, no use of anti-inflammatory or antibiotic drugs within 4 weeks prior to enrolment.

Exclusion criteria were: pregnant or breastfeeding women, patients over 80 years of age, history of gastrointestinal surgery and gallbladder stones. Patients with difficulty to understand the treatment or to report disease symptoms and adverse effects or who had participated in other clinical studies in the last two months were also excluded.

All patients were followed at the Gastroenterology out-patient clinic of Hospital das Clínicas of the University of São Paulo, in São Paulo, Brazil and were submitted to an upper digestive endoscopy to exclude organic dyspepsia, being classified as functional dyspepsia according to the Rome III criteria [24].

The study was approved by the ethics and research committee of the institution: Ethics Committee for Analysis of Research Projects (Protocol Number 0602/11) - CAPPesq – of the Clinical Board of Clinics Hospital, Faculty of Medicine, University of São Paulo, by the ethics committee of DuPont, and registered in the Clinical Trials database ISRCTN22923997.

B. Fermented Milk Production

Four specially designed fermented milk products were studied: (i) FM1: *Streptococcus thermophilus* strain TA040; butter oil; (ii) FM2: *Bifidobacterium lactis* - Bifidobacterium species 420 together with the one classical yoghurt starter, *Streptococcus thermophilus* strain TA040; butter oil; (iii) FM3: *Bifidobacterium lactis* - Bifidobacterium species 420 plus *Streptococcus thermophilus* strain TA040; fibre from passion fruit power peel and plant oil; and (iv) FM4: *Bifidobacterium lactis* - Bifidobacterium species 420 plus *Streptococcus thermophilus* strain TA040; fibre from passion fruit power peel and butter oil. The FM1 formulation was used as control group.

Counts of viable cells in all products were 10^7 Log colony forming unit (CFU) per mL for *Bifidobacterium lactis* - Bifidobacterium species 420, and 10^9 Log CFU per mL for *Streptococcus thermophilus*. All products had similar appearance, colour, texture, taste, and gross composition. Each serving, corresponding to one pot, contained 100 g of fermented milk. All products were specifically prepared for the study and provided by Brasil Foods (BRF) (Carambeí, Paraná, Brazil).

C. Diagnosis

Two hundred and forty-seven patients initially recruited had physical examination (visit 0 - V0) and underwent ^{13}C - urea breath test (UBT) and upper digestive endoscopy. PPI or H_2 blockers were discontinued 14 days before the beginning of the study. The symptomatic use of aluminum hydroxide was allowed. During endoscopy, mucosal fragments were collected (two specimens from antrum and two from the body of the stomach) to perform histological assessment with HE and Giemsa staining and rapid urease test to evaluate *H. pylori* infection. Twelve weeks after treatment completion (Visit 5 – V5), patients underwent urea breath test to check for *H. pylori* eradication.

D. Randomization and Study Performance

Randomization was carried out using a list obtained by computer. Patients received their numbers in ascending order according to study enrolment and were randomized in four different groups. Subjects and investigators were blinded during the whole study. The need to disclose the randomization always results in patient exclusion.

Each subject received the test product, which was placed into a thermal sac in quantities sufficient for 30 days (V1). They returned every 30 days for physical examination and product delivering, for a 90-day period (V1-V4). During the study, all patients maintained a stable and common diet. Subjects consumed the fermented milk product (100 g/day), twice a day. Twelve weeks after treatment completion another urea breath test was performed to check for *H. pylori* eradication (V5).

E. Statistical Analysis

The frequencies of the variables, the percentage of tests and measurements at V1 and V5 were carried out using Statistical Package for the Social Sciences, 10.0 Version (SPSS Inc., Chicago, IL, EUA). In order to compare the eradication rates per product in each visit, the non-parametric test of McNemar was used with 95% confidence interval (P<0.05).

III. Results and Discussion

Two hundred and forty-seven patients entered the study, 190 patients had the profile that met the inclusion criteria; 20 patients did not complete the protocol (10.5 %).

Patients were randomly divided into four groups being 93% women who mean age was 41 years. There were no significant differences between groups in terms of sex and age (significance level of 5%).

According to Table I, the administration of fermented milk did not change the *H. pylori* status, i.e., *H. pylori* negative at visit 1 remained *H. pylori* negative in the visit 5. The same happened with *H. pylori* positives.

TABLE I
COMPARISON OF *H. PYLORI* IN TIME

H.pylori V1 \ *H. pylori* V5	Negative	Positive	Total	*P*-value*
Negative	94	0	94 (55.3)	1,000
Positive	0	76	76 (44.7)	
Total	94 (55.3)	76 (44.7)	170 (100.0)	

*Non-parametric test of McNemar.
V1: before consume; V5: after 90 days of fermented milk consume (100g, twice a day)

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Also, no statistical significance was observed in *H. pylori* status in the different FM preparations (Table II).

The fermented milks were well tolerated, and no adverse effects or any other complications were observed during the study. There was an improvement in dyspepsia symptoms (data not shown) after supplementation.

H. pylori is a very common pathogen, been linked to potential serious diseases such as peptic ulcer disease, gastric cancer and MALT lymphoma. Its eradication is indicated in the clinical setting above and many others [6], [24]. Until now the only accepted treatments are antibiotic based. The antibiotic use is linked to some adverse events that include allergies, alterations of gut motility and of course, dysbiosis [25]. This modification of gut microbiota leads to a great amount of intestinal and extra-intestinal symptoms. The problem is even worse, since antibiotic use could lead to bacterial resistance.

Thus, is necessary to look for different strategies to decrease adverse effects secondary to antimicrobial use. One possible way of doing that, is probiotic consumption. These friendly bacteria can help through many ways: bacteriocin production, immunomodulation, anti-inflammatory properties, modification of gut motility, productions of short chain fat acids, etc. [26].

TABLE II
COMPARISON OF *H. PYLORI* ACCORDING TO THE FERMENTED MILKS (FM) WITH *BIFIDOBACTERIUM LACTIS* B420 AT EACH VISIT TIME

H.pylori\ FM	FM2	FM4	FM3	FM1	Total	*P*-value *
			H. pylori V1			
Negative	29 (63)	21 (44.7)	30 (62.5)	26 (53.1)	106 (55.8)	0.225
Positive	17 (37)	26 (55.3)	18 (37.5)	23 (46.9)	84 (44.2)	
Total	46 (100.0)	47 (100.0)	48 (100.0)	49 (100.0)	190 (100.0)	
			H. pylori V5			
Negative	25 (64.1)	19 (45.2)	26 (60.5)	24 (52.2)	94 (55.3)	0.309
Positive	14 (35.9)	23 (54.8)	17 (39.5)	22 (47.8)	76 (44.7)	
Total	39 (100.0)	42 (100.0)	43 (100.0)	46 (100.0)	170 (100.0)	

P-value for Chi-square test.
V1: before consume; V5: after 90 days of fermented milks consume (100g, twice a day)

Bifidobacteria are potentially very good candidates for *H. pylori* eradication. Unfortunately, the specific strain studied in our protocol, the *Bifidobacterium lactis* B420, was not able to eradicate *H. pylori*. New strains, as well as, different bacteria should be tested.

The addition of a probiotic to the *H. pylori* antibiotic treatment can be helpful in terms of reducing collateral effects.

IV. Conclusion

The probiotic fermented milk used in the present study (*Bifidobacterium lactis* B420), administered twice a day for 90 days did not show an increase in *H. pylori* eradication in Brazilian patients with functional dyspepsia. However, more research to standardize the dosages; timing of taking probiotics and the strain specificity to reduce *H. pylori* infection in humans are indispensable.

Authors' Contributions

All authors contributed to the design of the study. Acquisition of data and quality control: TNR, NPP, CH, RCB, PB, CSBB, JNE, MNO. Analysis and interpretation of data: TNR, RCB, JNE, NPP, CSBB, MNO. Endoscopic examinations: RCB, TNR. All authors have read and approved the final manuscript.

Competing Interests

The authors declare they have no financial or non-financial competing interests

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