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Original Article

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The Efficacy of *Saccharomyces boulardii* CNCM I-745 in Addition to Standard *Helicobacter pylori* Eradication Treatment in Children

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Purpose: This study aims to investigate *Saccharomyces boulardii* CNCM I-745 during *Helicobacter pylori* eradication in children.

Methods: One hundred ninety-four *H. pylori* positive children were randomized in two groups. Therapy (omeprazole+clarithromycin+amoxicillin or omeprazole+clarithromycin+metronidazole in case of penicillin allergy) was given to both groups during two weeks. In the treatment group (n: 102) *S. boulardii* was added to the triple therapy, while the control group (n: 92) only received triple therapy. The incidence, onset, duration and severity of diarrhea and compliance to the eradication treatment were compared. A ^{13}C urea breath test was done 4 weeks after the end of eradication therapy in two groups of 21 patients aged 12 years and older to test the *H. pylori* eradication rate.

Results: In the treatment group, diarrhea occurred in 12 cases (11.76%), starting after 6.25±1.24 days, lasting 3.17±1.08 days, and compliance to eradication treatment was 100%. In the control group, diarrhea occurred in 26 cases (28.26%), starting after 4.05±1.11 days, lasting 4.02±0.87 days, and in six cases eradication treatment was stopped prematurely ($p < 0.05$). The ^{13}C urea breath test showed successful *H. pylori* eradication in 71.4% of the patients in the treatment and in 61.9 % in the control group (not significant).

Conclusion: *S. boulardii* has a beneficial effect on the prevention and treatment of diarrhea during *H. pylori* eradication in children. Although *S. boulardii* did only slightly increase *H. pylori* eradication rate, compliance to eradication treatment was improved.

Key Words: Child, Compliance, *Helicobacter pylori*, Diarrhea, Prevention & control, Probiotics, Yeasts

INTRODUCTION

Epidemiological data suggest that up to 50% to 80% of the children in developing countries are *Helicobacter pylori* carriers [1]. Standardized eradication treatment of *H. pylori* requires a combination

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of antibiotics, usually administered during one to two weeks. Therefore, diarrhea develops frequently during *H. pylori* eradication. This diarrhea not only increases the morbidity, but also increases the economic burden of the treatment. *Saccharomyces boulardii* CNCM I-745 has been shown to be an effective probiotic in several types of acute diarrhea. In this study, we aimed to demonstrate the efficacy of *S. boulardii* in the prevention of diarrhea during *H. pylori* eradication treatment in children.

MATERIALS AND METHODS

Children presenting with chronic abdominal pain or chronic vomiting were investigated for organic disease. Two hundred and five children in whom *H. pylori* infection was diagnosed were included in this study (Fig. 1). Since 11 patients were lost in follow-up, data of 194 patients, between 22 months and 16 years old were collected. All patients were diagnosed with *H. pylori* infection through serological quantification of *H. pylori* immunoglobulin G (IgG) antibodies (122 cases) or histological evaluation (72 cases). In a subgroup of 42 children between 12 and 16 years old (mean age of 13.33±1.26 years) a ^{13}C urea breath test (UBT) was done four weeks after the end of the eradication therapy to confirm eradication.

All patients were given oral triple eradication therapy during two weeks (omeprazole+amoxicillin+clarithromycin, omeprazole+metronidazole+clarithromycin for participants with a penicillin allergy). The treatment group received two sachets of *S. boulardii* orally per day, starting on the first day of the antibiotic therapy (250 mg per sachet; Biocodex, Paris, France, Chinese brand name: YiHuo) during two weeks. If diarrhea occurred, patients in either group received montmorillonite powder (3 g three times daily, Smecta; Beaufour Ipsen Pharmaceuticals, Paris, France) orally without interrupting the *S. boulardii* treatment.

The primary outcome was the incidence of diarrhea defined as the percentage of patients developing a diarrhea. Diarrhea was defined as an increase in the frequency of bowel movements (>3/day) or decrease in stool consistency (Bristol stool scale 5 or 6). Secondary outcomes were: i) the onset of diarrhea, which was defined as the time between the inclusion of the patient and the onset of diarrhea; ii) the duration of diarrhea, which was defined as the

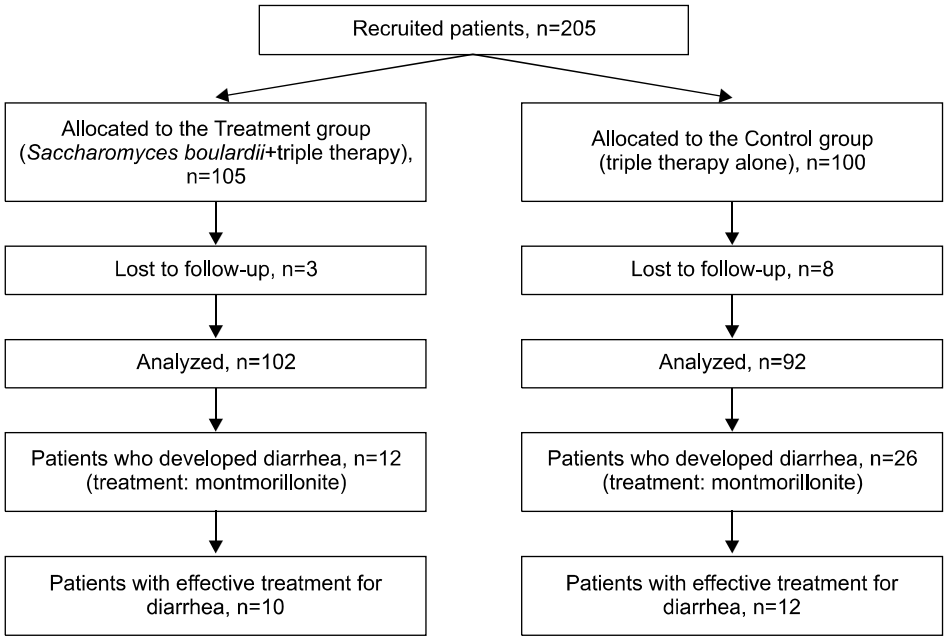

Fig. 1. Flow diagram of patients.

number of days with diarrhea (the end of the diarrhea was defined as the moment when consistency (Bristol stool scale <4) or frequency (<3 stools/day) of the stools returned to normal); iii) the eradication rate; iv) compliance, defined as the number of patients who completed the entire course of the triple eradication therapy; v) efficacy of the diarrhea treatment ; vi) severity of diarrhea [2] (Table 1). The efficacy of the treatment was defined as the percentage of patients in whom the eradication treatment resulted in a negative ^{13}C UBT.

The sample size was calculated using the following formula $N=Z^2\times[p\times(1-P)]/E^2=174$ where N is the sample size, Z the confidence interval (=1.96), E the sampling error (=0.05) and P the incidence of AAD (=0.13). Data analysis was performed using SPSS 11.5 statistical software (Softonic; Softonic International S.A., http://en.softonic.com/s/spss-11.5-statistical-software-free-download). The onset time and duration of diarrhea were analyzed using two-tailed t-tests; $p<0.05$ was considered statistically significant. This study received approval from ethics committee of Daping Hospital of The Third Military Medical University (No. 2003009).

RESULTS

Data of 194 (90 male, 104 female) patients were analyzed. In the treatment group, 12/102 (11.8%) patients developed diarrhea, while in the control group 26/92 (28.3%) patients had diarrhea ($p<0.05$) (Table 2). The onset of diarrhea in the treatment group was 6.25±1.24 days, what was later than in the control group (4.05±1.11 days; $p<0.001$). The duration of diarrhea in the treatment group was 3.17±1.08 days, which was significantly shorter

Table 1. Efficacy of Treatment and Severity of Diarrhea

Terminology	Meaning
Significantly effective	The appearance and frequency of the stool return to normal within 72 hours of treatment, and systemic symptoms disappear.
Effective	The appearance and frequency of the stool markedly improve within 72 hours of treatment, and systemic symptoms markedly improve.
Ineffective	The appearance and frequency of the stool and systemic symptoms do not improve, or even worse within 72 hours of treatment.
Mild diarrhea	Diarrhea without dehydration or toxic symptoms
Severe diarrhea	Diarrhea with moderate to severe dehydration, or with obvious toxic symptoms and signs (irritability, restlessness, pale, low body temperature, high white blood cell count).

Table 2. Patient Characteristics

Characteristic	Total	Treatment group	Control group	*p*-value*
Patients (n)	194	102	92	NS
Age (y)	8.51±3.60	8.41±3.66	8.63±3.54	NS
Diarrhea	38 (19.6)	12 (11.8)	26 (28.3)	0.004
Mild diarrhea		11 (92)	21 (81)	
Severe diarrhea		1 (8)	5 (19)	
Onset diarrhea (day)		6.25±1.24	4.05±1.11	<0.001
Duration (day)		3.17±1.08	4.02±0.87	<0.001
Eradication		15/21 (71.4)	13/21 (61.9)	0.513
Compliance		102 (100)	86 (93.4)	0.027

Values are presented as number only, mean±standard deviation, or number (%).
NS: not significant.
*By two-tailed t-test.

Table 3. Comparison of Treatment Efficacy for Diarrhea (Montmorillonite)

Group	Patient	Significantly effective	Effective	Ineffective	Efficacy rate (%)
Treatment	12	4	6	2	83.33
Control	26	4	8	14	46.15
p-value*					0.040

*By Fisher's exact test.

than in the control group (4.02±0.87 days; $p<0.001$) (Table 2).

H. pylori was eradicated in the treatment group in 15/21 cases (eradication rate: 71.4%) and in 13/21 cases (eradication rate: 61.9%) in the control group. The difference in *H. pylori* eradication rate was not statistically significant ($p=0.513$, Table 2). No patient discontinued *H. pylori* treatment prematurely in the treatment group while six patients in the control group stopped *H. pylori* treatment without medical advice, resulting in a statistically significant difference ($p=0.027$, Table 2). In the treatment group, we observed 11 cases of mild diarrhea and 1 case of severe diarrhea. In the control group, there were 26 cases with diarrhea, of whom 21 were mild and 5 severe ($p<0.05$, Table 2).

In the treatment group, montmorillonite powder was effective in 10/12 (83.3%) cases that developed diarrhea. In the control group, montmorillonite powder was effective in only 46.1% ($p<0.05$, Table 3).

DISCUSSION

The normal microbiome contributes to digestion, stimulates the immune system and provides a mechanical barrier called "colonization resistance" [3,4]. This involves the interaction of many bacterial microorganisms and results in a barrier effect against colonization by pathogenic organisms. Factors such as fasting, surgery and antibiotics disrupt this protective barrier. *H. pylori* eradication treatment includes proton pump inhibitor and two antibiotics out of three: amoxicillin, clarithromycin and metronidazole [5]. Proton pump inhibitors increase intragastric pH levels, what contributes to dysbiosis [6]. Due to these adverse effects on the intestinal micro-ecosystem environment, patients receiving *H. pylori* eradication treatment are prone to a series of gastrointestinal disorders such as bloating, anorexia, diarrhea and constipation [7]. One study reported an incidence of 30.9 % of antibiotic associated diarrhea in children during *H. pylori* eradication [8]. According to a meta-analysis, the incidence of diarrhea during *H. pylori* eradication is 12.2% [9]. Our study evidenced that *S. boulardii* is an effective option to prevent diarrhea during *H. pylori* eradication treatment in children. This effect is observed for the incidence, delay in onset and duration of the diarrhea. The results of this study indicate that treatment with *S. boulardii* has the potential to significantly shorten the duration of diarrhea [10,11]. Antibiotics result in host susceptibility to pathogen colonization until the normal microbiome has recovered. Probiotics are uniquely qualified to fit into this window of susceptibility and may act as surrogate normal microflora until recovery is achieved [12].

Studies reported that the mechanisms of action of *S. boulardii* in the treatment of diarrhea could include the following 4 aspects: i) effects of anti-bacterial toxins; ii) direct or indirect inhibition of growth of intestinal pathogens, such as *Candida albicans*, *Salmonella*, *Yersinia*; iii) increase of short chain fatty acids concentrations; iv) immunomodulatory effects such as an increase in sIgA [13]. *H. pylori* interacts with the host immune response by upregulation of various cytokines, such as tumor necrosis factor-α, interferon-γ, interleukin (IL)-1, IL-6 and IL-8 [14]. These cytokines interact through a complex inflammatory immune regulatory network of paracrine and endocrine pathways and act on B lymphocytes, natural killer cells, macrophages [14]. This results in a specific and non-specific immune response [15,16]. The level of IL-8 is positively correlated with the severity of inflammation [17]. Studies in a mouse ileum loop model showed that *S. boulardii* inhibits the activation of ERK1/2 and MAPkinase which inhibit IL-8 production, and limits the activation of T-helper lymphocytes in order to inhibit the

inflammatory response [18,19]. *S. boulardii* inhibits the inflammatory signaling pathways, by inhibiting the translocation of NF- κB in inflammatory signaling pathways, exhibiting anti-inflammatory properties [20,21].

There has been widespread controversy whether probiotics can be used in the treatment of *H. pylori* infection. Gotteland et al. [22] demonstrated that *S. boulardii* increases the eradication rate with 12 %, which is similar to our finding. Szajewska et al. [9] reported in a meta-analysis that *S. boulardii* given along with triple therapy significantly increased the eradication rate and reduced the overall risk of *H. pylori* therapy-related adverse effects, particularly of diarrhea. It is not clear if the higher eradication rate is due to a specific probiotic activity on *H. pylori*, by improving the efficacy of the eradication treatment or by improving compliance.

In our study, diarrhea was treated with montmorillonite powder, a natural clay [23]. The major mode of action is as an absorbans of water [23]. A meta-analysis showed that this product is effective and safe in the treatment of diarrhea [23]. The efficacy of the product is also acknowledged in the recommendations for the treatment of acute gastroenteritis by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition [24]. In our study, montmorillonite was found to be more effective in the treatment group that received concomitant *S. boulardii*. This may be related to the fact that the diarrhea in this group started later and was less severe. *S. boulardii* contributes to the decrease of diarrhea by providing a restoration of the gastro-intestinal flora.

Our study does have some limitations as it is an open study. The diagnosis of *H. pylori* was not according to standard techniques since serology is not accepted as a reliable diagnostic tool in children [5]. However, the proportion of patients diagnosed by serology in the treatment group and control group did not show any statistical difference. The proportion of patients diagnosed by serology in the subgroups of those who developed diarrhea and those who had conducted UBTs also did not show any statistical difference. Therefore, although bias regarding the inclusion of false positive patients in our study may have possibly existed, bias regarding allocation among groups was resolved. Moreover, this study focusses on the decrease of adverse effects linked to *H. pylori* eradication and not on *H. pylori* pathology and diagnostic techniques.

In conclusion, the yeast probiotic did prevent diarrhea associated with *H. pylori* standard eradication treatment with a proton pump inhibitor and two antibiotics. Also, when diarrhea developed, it was less severe and of shorter duration in the *S. boulardii* group. *S. boulardii* increased the compliance to *H. pylori* eradication therapy which may be related to a small increase of *H. pylori* eradication with 10 percent. However, about 65% of the children were diagnosed on the basis of serology. A comparable percentage of those developing diarrhea were diagnosed on serology, as was the case in those in whom a UBT was performed. The findings of this trial need to be confirmed with a prospective double blind study in which diagnosis is based on histology and culture.

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