Clinical Efficacy of *Saccharomyces boulardii* and Metronidazole Compared to Metronidazole Alone in Children with Acute Bloody Diarrhea Caused by Amebiasis: A Prospective, Randomized, Open Label Study

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**Abstract.** The aim was to evaluate the efficacy of *Saccharomyces boulardii* (Sb) in addition to metronidazole in amebiasis. A prospective, randomized, open clinical trial was performed in 50 children presenting with acute bloody diarrhea caused by *Entamoeba histolytica*. Group A and B (each \( N = 25 \)) was treated with metronidazole, but Sb (250 mg, twice daily) during the 7 days was added to Group B patients who were re-evaluated 2, 3, 5, 10, and 30 days after diagnosis. Duration of bloody diarrhea was significantly longer in Group A (72.0 ± 28.5 versus 42.2 ± 17.4 hours, \( P < 0.001 \)). On day 5, amebic cysts had disappeared in all children in Group B, whereas in Group A, amebic cysts were still present in 6 children \( (P < 0.05) \). On day 10, all children were cured and cysts had disappeared in all. The addition of Sb to metronidazole in amebiasis significantly decreases duration of (bloody) diarrhea and enhances clearance of cysts.

**INTRODUCTION**

Diarrheal diseases are a worldwide leading cause of childhood morbidity and mortality. The etiology of acute diarrhea in children includes gastrointestinal tract and systemic infections, malabsorption disorders, nutritional deficiency, food allergy, etc. Amebiasis, caused by *Entamoeba histolytica*, affects worldwide more than 50 million people per year. Although the majority of cases remain asymptomatic, over 100,000 deaths caused by amebiasis are annually reported. Probiotics are living microorganisms that, when ingested, survive in the gastrointestinal tract and have beneficial effects on the host. The most commonly used probiotics are lactic acid bacteria, such as lactobacilli or bifidobacteria, and yeast such as *Saccharomyces boulardii* (S. boulardii). Little is known about the efficacy of *S. boulardii* against protozoal infections. In adults, co-administration of lyophilized *S. boulardii* with conventional treatment in acute amebic colitis significantly decreased the duration of symptoms and cyst carriage after 4 weeks. To the best of our knowledge, there are no published data on the efficacy of *S. boulardii* in children with acute bloody diarrhea caused by amebiasis. The aim of this prospective study was to compare the efficacy of the addition of *S. boulardii* to the standard metronidazole therapy of acute bloody diarrhea resulting from amebiasis.

**MATERIALS AND METHODS**

This prospective, randomized, open label clinical trial was performed in the Eskisehir Osmangazi University Faculty of Medicine Hospital, Turkey. Children who were presented with fever and acute macroscopic bloody diarrhea with clinical findings suggesting (fever, abdominal pain) and laboratory findings confirming acute intestinal amebiasis were eligible for inclusion. The diagnosis was confirmed by the detection of typical *E. histolytica* trophozoites containing red blood cells in the stool with microscopic examination of the fresh stool in children presenting with fever (> 38.5°C) and abdominal pain. Children presenting without fever and children with amebiasis needing hospitalization were not eligible for inclusion. Exclusion criteria were the use of medication for any underlying disease, antibiotic use during the previous month, and the objectivation of another pathogen causing (bloody) diarrhea. Fecal culture for bacteria, including *Salmonella* and *Shigella*, Rotavirus rapid antigen test, and microscopic examination for other protozoa and parasitologic infection were performed. Stool culture for *Campylobacter jejuni* could not be performed. If the result was positive for another microorganism that was *E. histolytica*, the patient was not eligible for inclusion. All stool studies were performed by a blinded co-author (N.D.). The microscopic examination of the stool specimen for the presence of ova and parasites consists of three separate techniques: direct wet smear, concentration, and permanent stained smear (Trichrom). It is not possible to perform molecular diagnosis of amebiasis in the institute.

Randomization was performed by alternating the inclusion of each patient to one of both treatment groups. Group A was treated with metronidazole (30 mg/kg twice daily) alone, and Group B was treated with metronidazole (same dose) and lyophilized *S. boulardii* (250 mg twice daily, Reflur, Sanofi-Aventis, Turkey) during 7 days. Patients were re-evaluated at the second, third, fifth, tenth, and thirtieth days for clinical findings and the fifth, tenth, and thirtieth days for the presence of blood in the stool. At Days 5, 10, and 30, stool samples were examined microscopically for the presence of cysts; this was performed blinded for the treatment.

The primary endpoint was the duration of bloody diarrhea (disappearance of macroscopic and microscopic blood from the stools). Microscopic detection for the presence of blood in the stools was performed by a blinded investigator. Secondary end points were the duration of the diarrhea and follow-up for microscopic examination of the stool for amebiasis. The Bristol criteria were applied to measure the duration of diarrhea. At inclusion, all infants had a Bristol-stool score Type 7; diarrhea was considered to have stopped when the stools had a Bristol score of 5 or less. Statistical analysis was performed with SPPS for Windows (version 13.0, SPPS, Chicago, IL). Independent \( t \) test, \( \chi^2 \) test,

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Table 1
Demographic and clinical findings of study patients at admission*

<table>
<thead>
<tr>
<th></th>
<th>Group A Metronidazole (N = 25)</th>
<th>Group B Metronidazole plus Saccharomyces boulardii (N = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>11.7 ± 2.1 years</td>
<td>10.9 ± 2.2 years</td>
<td>NS</td>
</tr>
<tr>
<td>Gender</td>
<td>12 girls, 13 boys</td>
<td>14 girls, 11 boys</td>
<td>NS</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>25 (100%)</td>
<td>25 (100%)</td>
<td>NS</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>17 (68%)</td>
<td>15 (60%)</td>
<td>NS</td>
</tr>
<tr>
<td>Fever</td>
<td>25 (100%)</td>
<td>25 (100%)</td>
<td>NS</td>
</tr>
<tr>
<td>Headache</td>
<td>7 (28%)</td>
<td>6 (24%)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of bloody diarrhea</td>
<td>41.2 ± 2.6</td>
<td>46.2 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of abdominal pain</td>
<td>71.2 ± 2.8</td>
<td>74.2 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of fever</td>
<td>28.2 ± 1.8</td>
<td>25.2 ± 1.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS = not significant.

and McNemar’s test were used for comparisons. The $P < 0.05$ was considered as statistically significant. This study was approved by the local ethical committee; and informed consent was obtained from at least one parent.

RESULTS

In the study period (January 2006–September 2007), 53 children with bloody diarrhea, fever, abdominal pain, and presence of amebic cysts were included. Three children had to be excluded: one from Group A and two from Group B because of the lack of stool samples at Day 5 and Day 10.

Group A was composed of 25 children (12 girls, 13 boys, mean age 11.7 ± 2.1 years) treated with metronidazole during 7 days. Group B contained also 25 children (14 girls, 11 boys, mean age 10.9 ± 2.2 years) and was treated with metronidazole (same dose, same duration) and lyophilized $S. boulardii$ (250 mg twice daily PO for 7 days, Reflor, Sanofi-Aventis).

All children presented with bloody diarrhea, fever, and abdominal pain. There was no statistically significant difference between Group A and Group B for age and gender ($P > 0.05$). There was no statistical significant difference at inclusion for the duration of fever, abdominal pain, vomiting, and headache between the two groups (Table 1).

The duration of bloody diarrhea was significantly longer in Group A compared with Group B (72.0 ± 28.5 hours versus 42.2 ± 17.4 hours, $P < 0.001$). Cessation of diarrhea (first stool with Bristol score ≤ 5) in Group B was 46.1 ± 18.2 hours and 73.9 ± 32.4 hours in Group A ($P = 0.001$).

Although statistically not significant, after 48 hours of treatment, both the number of watery and bloody stools was higher in Group A. After 3 days, diarrhea was still present in 19 children (76%) and blood in the stools in 11 children (44%) in Group A compared with only 6 children (24%) with diarrhea and 3 with blood in the stools (12%) in Group B ($P < 0.001$ for both). The microscopic examination of stool specimen on Day 5 showed the disappearance of blood in the stools in all children, whereas 6 children from Group A still had diarrhea. Furthermore, on Day 5, 6 children still (have also blood in the stools) had amebic cysts in the stools, whereas all patients in Group B were negative for trophozoites and cysts ($P < 0.05$) (Table 2). On Day 10, all children were clinically and parasitologically cured and cysts had disappeared. After 1 month, amebic cysts were detected in one asymptomatic child in Group A.

DISCUSSION

Amebiasis is one of the most common parasitic infections worldwide, especially in developing countries and can cause acute non-bloody diarrhea, bloody diarrhea, necrotizing enterocolitis, ameboma, and liver abscess. In this study, only children with bloody diarrhea, fever, and the presence of trophozoites were included.

$Saccharomyces boulardii$ is a saprophytic yeast, which is recommended for the prevention and treatment of gastroenteritis. There are several randomized, placebo-controlled studies showing the efficacy of $S. boulardii$ in the management and prevention of acute childhood diarrhea. The shortening of the duration of diarrhea results in social and economic benefits. Only one study suggested the efficacy of $S. boulardii$ in the treatment of acute diarrhea because of amebiasis in adults. This study showed that addition of $S. boulardii$ to metronidazole decreased the mean duration of diarrhea with an almost 25% positive effect on fever and abdominal pain. In our study group, cessation of diarrhea in children that received the combination of $S. boulardii$ and metronidazole was more rapid than in children that received metronidazole alone. Another difference between both studies is that the adult patients were treated with iodoquinol, whereas this was not the case in the children.

Besirbellioglu and others compared the efficacy of $S. boulardii$ in addition to metronidazole in patients with giardiasis. The combination therapy resulted in a disappearance of the $Giardia$ cyst 2 weeks after start of the treatment. However, 17.1% of the patients treated with 10 days metronidazole as monotherapy still had $Giardia lamblia$ cysts in the stool.

The data reported in this study confirmed the findings in adults with $Giardia lamblia$ and amebiasis: cysts, blood in the stool, and diarrhea disappeared more rapidly. Metronidazole does not treat cysts as such. Although the mechanism by which $S. boulardii$ might exert its activity remains unclear, several possible mechanisms have been proposed: inhibition of pathogen adhesion, strengthening of enterocyte tight junctions, neutralization of bacterial virulence factors, and enhancement.

Table 2
Follow-up parameters of study patients after treatment*

<table>
<thead>
<tr>
<th></th>
<th>Group A Metronidazole (N = 25)</th>
<th>Group B Metronidazole plus Saccharomyces boulardii (N = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of bloody diarrhea</td>
<td>72.0 ± 28.5 hours</td>
<td>42.2 ± 17.4 hours</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>Duration of diarrhea</td>
<td>73.9 ± 32.4</td>
<td>46.1 ± 18.2 hours</td>
<td>$P = 0.001$</td>
</tr>
<tr>
<td>At Day 3 bloody diarrhea</td>
<td>6 (24%) 19 (76%)</td>
<td>3 (12%) 11 (44%)</td>
<td>$P &lt; 0.001$ $P &lt; 0.001$</td>
</tr>
<tr>
<td>At Day 5 bloody diarrhea cyst passage</td>
<td>0 (%) 6 (24%) 6 (24%)</td>
<td>0 (0%) 0 (0%) 0 (%)</td>
<td>NS $P &lt; 0.05$ $P &lt; 0.05$</td>
</tr>
</tbody>
</table>

*NS = not significant.
of the mucosal immune response. Rigothier and others concluded/postulated that the possible mechanism of S. boulandii on amebic dysentery is to decrease the number of ameba bearing red cells and a reduction in the number of red cells adhering to amoebae.

We have some limitations of this study. We could not perform a diagnostic test for Campylobacter jejuni, which is another important cause of bloody diarrhea. A newer generation test, including polymerase chain reaction (PCR) and E. histolytica stool antigen, could be used to clarify the differentiation of the cysts of E. histolytica and E. dispar in further studies. Furthermore, our patient group includes children with abdominal pain and fever, which present a minority of patients with intestinal amebiasis and further larger studies about the efficacy of S. boulandii in children with amebiasis with or without systemic findings.

Our data results showed that the addition of S. boulandii to metronidazole for the treatment of acute bloody diarrhea because of intestinal amebiasis significantly decreases the duration of (bloody) diarrhea and enhances the gastrointestinal clearance of the amebic cysts as compared with metronidazole alone.

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