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Andrii Protsyk
Department of Infectious Diseases and Epidemiology, Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine

Correspondence
Andrii Protsyk
Department of Infectious Diseases and Epidemiology, Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine

Usage of probiotics in therapy of patients with combined invasions of *Giardia* and *Ascaris*

Andrii Protsyk

Abstract
Recently, more and more cases of concomitant invasion have been reported. The affection of the gastrointestinal tract in patients is often accompanied by dysbiotic changes. The aim of research is to study the state of intestinal microflora in patients with combined invasion of *Giardia* and *Ascaris*. 28 patients with mixed invasion of *Giardia* and *Ascaris* were examined and divided into 2 groups. The 1st group consisted of patients who received traditional basic treatment, the 2nd group - traditional basic treatment with probiotic. The index of dysbiosis was used to determine the degree of intestinal dysbiosis. During the study of the state of intestinal microflora it was discovered that the following changes were identified in all of patients before treatment. Among aerobic spectrum, a reduced level of lacto- and bifidobacteria, an increased level of bacteroides and peptococci were observed. The total number of E. coli was decreased, while the number of E. coli a with weak fermentative properties increased, and hemolytic E. coli was present. Among anaerobic spectrum, an increase in number of enterococci, opportunistic bacteria and the presence of fungal flora was noted. There was a significant improvement in intestinal microflora in patients, who received treatment with probiotic, compared to results before treatment and the ones in the same group that didn't take probiotic.

Keywords: *Giardia* sis, ascariasis, mixed invasion, dysbiosis, probiotic

1. Introduction
Parasitic diseases have a high level of morbidity in the structure of infectious diseases. In Ukraine, the most common intestinal parasitic diseases are *Giardia* sis and ascariasis. Recently, more and more cases of mixed-invasion of these pathogens are noted [7, 11].
Giardia sis belongs to protozoal diseases, caused by protozoa Lamblia (*Giardia*) intestinalis, and is practically spread all over the world [4]. The penetrability of the intestinal wall increases for large molecular antigens with the development of malabsorption and triggering of mechanisms of food allergies, micronutrient deficiencies and multivitamin deficiency, biliary disorders, which is one of the mechanisms of intestinal peristalsis disorders, abdominal syndrome, dyskinesia of the gastrointestinal tract (GIT) and biliary tract [2, 9]. Changes in chemical parameters of chyme and the disturbance of microbiological background of different parts of the intestine leads to formation of intestinal dysbiosis [6, 12].
Many scientists have proved that intestinal dysbiosis is not only a related clinical and laboratory syndrome of the main somatic pathology of the gastrointestinal tract but it can independently be a manifestation of many pathological conditions [4, 6].
Ascariasis is one of the most common intestinal nematodoses in adults [1]. Due to background of prolonged lesions of the gastrointestinal tract, patients with ascariasis may also experience quantitative and qualitative changes in species-specific content of intestinal microflora [10].
With a combined invasion of *ascaris* and lamblia, the clinical manifestation is characterized by a more severe course and an increase in incidence of complications in patients, including rising of changes in intestinal microflora [8, 9].
Nowadays the main method of pathogenetic treatment of dysbiotic changes in the intestine is the use of drugs from the group of probiotics. There is an experience in using of probiotics in monoinvasion, but there is not enough data on their effectiveness in adults with mixed intestinal infections [10].

1.1. The aim of the research
To study the effectiveness of the use of probiotic in the complex therapy of patients with combined invasions of lamblia and ascarids.

2. Materials and methods

28 patients with mixed invasion of lamblia and ascarids aged 18 to 47 years (average age 30.71 ± 1.01 years) underwent observation. There were 13 (46.4%) men and 15 (53.6%) women. Diagnosis of *Giardia* sis and ascariasis was confirmed by detecting a pathogen with parasitoscopy of feces. All patients received etiotropic treatment with albendazole and ornidazole.

Patients were divided into 2 groups, equally to age and gender. The first group (n = 14) included patients with mixed invasion of lamblia and ascarids, who received traditional basic treatment (enterosorbents, antihistamines, vitamins). The second group (n = 14) consisted of patients with mixed invasions of lamblia and ascarids, who received traditional basic therapy and probiotic containing saccharomyces boulardii. It was used internally in 1 capsule 2 times a day for 14 days. It contains active ingredient: 1 capsule contains saccharomyces boulardii CNCM I-745 (lyophilized cells) 250 mg and auxiliary substances: lactose monohydrate, magnesium stearate.

The control group consisted of 20 practically healthy persons.

The condition of intestinal microflora was determined with using of classical microbiological investigation of feces with measurement of population level of the microflora after VA Znamensky [5]. The investigations were conducted twice: before treatment and on 14th day after treatment.

The index of dysbiosis (ID) was used to evaluate the degree of dysbiosis. The ID was calculated by formula as the ratio between the autochthonous and allochthonous microorganisms of the intestinal content:

$$\frac{(\text{bif.}+\text{lact.}+\text{tot.esh.}+\text{ent.})}{(\text{bact.}+\text{pept.}+\text{WFE}+\text{HE}+\text{CPE}+\text{staph.}+\text{strep.}+\text{Cand.})}$$

Where bif. - Bifidobacterium, lact. - Lactobacilli, tot. esh. - total number of E. coli, ent. - Enterococci, bact. - bacteroides, pept. -Peptococci, WFE-weak fermentative E. coli, HE - hemolytic E. coli, CPE - conditionally pathogenic enter bacteria, staph. - Staphylococci, strep. - Streptococci, Cand. - Fungi of the genus Candida, (quantity, indicators in lg CFU/g) [3].

The statistical analysis of obtained results was performed through the using of variational-statistical method of analysis.

3. Results and Discussion

According to microbiological investigation of feces, quantitative and qualitative changes of the intestinal microflora were detected in two groups of patients with combined invasion.

Table 1: Quantitative composition of microflora in patients with *Giardia* sis and ascariasis, M ± m

Groups of microorganisms	Number of microorganisms, lg COD / g			
	Before treatment (n=28)	After treatment		Control group (n=20)
		I group (n=14)	II group (n=14)	
Bifidobacteria	4,61±0,19*	6,93±0,27** #	7,93±0,22***	8,05±0,15
Lactobacilli	4,57±0,20*	6,79±0,19** #	7,64±0,23***	8,10±0,18
Peptococci	8,11±0,29*	6,21±0,15**	5,86±0,18***	5,65±0,20
Bacteroides	9,71±0,22*	7,93±0,37** #	7,07±0,22***	6,85±0,20
E. coli (total number)	5,11±0,17*	6,57±0,17** #	7,21±0,21***	7,50±0,20
Weak fermentative E. coli	1,32±0,15*	1,07±0,13** #	0,57±0,14***	0,45±0,11
Hemolytic E. coli	2,21±0,25*	0,57±0,14** #	0,14±0,10***	0
Enterococci	5,75±0,20*	6,93±0,25** #	7,64±0,23***	7,95±0,14
CPE	7,11±0,36*	5,21±0,21** #	4,21±0,21***	4,05±0,27
Fungi of the genus Candida	5,32±0,33*	2,86±0,35** #	1,36±0,20***	1,25±0,18
Staphylococci	3,96±0,20*	2,64±0,31**	2,36±0,20***	2,20±0,12

Note.

* p <0,05 the reliability of the difference between the similarity indicators between the group before treatment and the control group;

** p <0,05 the reliability of the difference between the similar indicators between the group before treatment and the 1st group (without a probiotic);

*** p <0,05 the reliability of the difference between the similar indicators between the group before treatment and the 2nd group (with the addition of a probiotic);

# p <0.05 the reliability of the difference between the similar indicators between the 1st and 2nd groups.

Analyzing Table 1, we see that changes in intestinal microflora are observed in the group of persons with a combined invasion before treatment. The content of bifidobacteria (4,61 ± 0,19 lg CFU / g) was reduced, which is lesser than in control group (p<0.05); the amount of lactobacilli was reduced to 4,57 ± 0,20 lg COD / g (p<0.05). In addition, the appearance of many peptococci (8,11 ± 0,29 lg CFU / g) and bacteroides (9,71 ± 0,22 lg CFU / g) (p<0,05) was observed.

Total number of E. coli also decreased, and it was 5.11 ± 0.17 lg CFU / g (p<0.05). At the same time, level of E. coli with weak fermentative properties was increased (1.32 ± 0.15 lg CFU / g) (p<0.05). There was presence of hemolytic E. coli (2.21 ± 0.25 lg CFU / g), which was not found in a group of practically healthy individuals (p<0.05). Number of enterococci in this group was 5.75 ± 0.20 lg CFU / g (p<0.05).

The presence of conditionally pathogenic bacterias and representatives of the fungal flora were detected. The content of CPE in patients with mixed-parasitosis was 7.11 ± 0.36 lg CFU / g (p<0.05). The proteuses, hafnies, enteriobacterias, klebsielles, and citrobacters were in the structure of the UPP. Among the fungal flora there were fungi of the genus Candida (5.32 ± 0.33 lg CFU / g) (p<0.05). The level of staphylococci (3.96 ± 0.20 lg CFU / g) was quite high, comparing with results of the control group (p<0.05).

After treatment, in the both group, there were positive changes in the aerobic and anaerobic spectrum of intestinal microflora. In the 1st group (treatment without probiotic use), the results were compared with the data before treatment. The content of bifidobacteria was lower and fell to 6.93 ± 0.27 lg CFU / g (p<0.05). The quantitative content of lactobacilli was 6,79 ± 0,19 lg CFU / g, but it was higher than this one before treatment (p<0,05). Also, a small number of peptococci (6.21

± 0.15 lg CFU / g) and bacteroides (7.93 ± 0.37 lg CFU / g) (p<0.05) were observed. The total amount of the E. coli was decreased (6.57 ± 0.17 lg CFU / g), but it was higher than before starting of treatment (p<0.05). The elevated level of the E. coli with weak fermentative properties, which is 1.07 ± 0.13 lg CFU / g (p<0.05), was detected. There was a presence of hemolytic c E. coli (0.57 ± 0.14 lg CFU / g), which is not normally registered (p<0.05), as well as the number of enterococci (6.93 ± 0.25 lg CFU / g) (p< 0.05).

Despite the etiotropic and base pathogenetic treatment, the presence of UPP and fungal flora was detected. The number of CPE in patients was 5.21 ± 0.21 lg CFU / g, mainly due to proteuses (p<0.05). Among the fungal flora there were fungi of the genus Candida in the amount of 2.86 ± 0.35 lg CFU / g (p<0.05). Decreasing of the level of staphylococci was not reliabile (2.64 ± 0.31 lg CFU / g), compared with this one of control group (p<0.05).

In 2nd group of patients (treatment with the addition of probiotics), there are more significant indications of improving of condition of intestinal microflora both in relation to the results obtained before the treatment and in comparison with the data of selected groups. The content of bifidobacteria was 7.93 ± 0.22 lg CFU / g, which was significantly higher in relation to other two groups. The quantitative content of lactobacilli was slightly decreased and reduced to 7.64 ± 0.23 lg CFU / g (p<0.05). Among other representatives of intestinal anaerobic flora, the amount of bacteroides (7,07 ± 0,22 lg CFU / g) (p<0,05) positively changed, and the number of peptococci (5,86 ± 0,18 lg CFU / g) was significantly lower than this one in selected first group (p> 0.05). In this group, the number of E. coli also was changed: the total content of the E. coli (7.21 ± 0.21 lg CFU / g) was increased because of reducing the dose and treatment without the use of probiotic (p<0.05), the decrease in the content of the E. coli with weak fermentative properties (0.57 ± 0.14 lg CFU / g) (p<0.05) was observed. In addition, the presence of hemolytic E. coli was detected only in 2 patients (0.14 ± 0.12 lg CU / g), which is significantly lower than before treatment (p<0.05) and compared with the number of the first group (p<0.05).

Analyzing the content of other representatives of the anaerobic spectrum of the intestinal microflora, we observe almost complete normalization of the enterococci level (7.64 ± 0.23 lg CFU / g (p<0.05)).

In addition, in the treatment with probiotic, a decrease in CPE content was found; it was 4.21 ± 0.21 lg CFU / g (p<0.05). The level of fungal flora of the genus Candida was minimal (1.36 ± 0.20 lg CFU / g), which gives results, similar to other groups (p<0.05). Also, there was a decrease in the staphylococcal content (2.36 ± 0.20 lg CFU / g), although the results were not reliable in relation to the group without administration of probiotic (p> 0.05).

In all patients, the degree of dysbiosis was calculated according to the ID. When the value of ID is above 1 unit, it means that signs of dysbiosis are not detected. 1st degree of dysbiosis corresponded to value of ID from 1 to 0.75 units, 2nd degree - from 0.75 to 0.5 units, 3 degrees - from 0.5 to 0.3 units. Lower values were interpreted as dysbiosis of the IV degree. In the control group, the average value of the ID was 1.33 ± 0.14 units and was not lower than 1 unit.

In all patients with combined invasion before the treatment, signs of intestinal dysbiosis were detected. Dysbiosis of the I-st degree was established in 9 patients (32,2%), of the II–nd degree - in 10 (35.7%), and dysbiosis of the III-rd degree – in 7 (25.0%) persons. In 2 patients (7.1%), the dysbiosis of the IV-th degree was detected.

After the treatment in both groups, there was a decrease in degree of intestinal dysbiosis. In group of patients with combined invasion without the use of probiotics (group 1), signs of intestinal dysbiosis were found in most patients (12 patients - 93.33%), dysbiosis of the I-st degree - in 6 patients (42, 9%), the II-nd degree dysbiosis - in 4 (28.6%) and dysbiosis of the III-rd degree - in 2 persons (14,3%). Patients with IV-th degree dysbiosis were not detected.

Patients of the 2nd group had a significant improvement of intestinal microflora. In 8 people, signs of dysbiosis were not detected (57.1%), since the ID was above 1 unit. Dysbiosis of the I-st degree was detected in 5 patients (35.7%), and dysbiosis of the II-nd degree was observed only in 1 (7.1%) patient. Patients with dysbiosis of the III-rd and IV-th degrees were not detected.

4. Conclusions

1. Dysbiotic changes in the intestinal microflora with combined invasions of liamblia and ascarids are characterized by decreasing in the level of normal intestinal microflora, an increase in the content of conditionally pathogenic enterobacteria and the appearance of pathogenic microorganisms, as well as fungal flora.
2. Usage of probiotic containing saccharomycetes boulardii in the complex therapy of patients with mixed invasions of *Giardia* and *Ascaris* leads to significant improvement in intestinal microflora, compared to results before treatment and these ones in the same group without the use of a probiotic.
3. Usage of probiotic containing saccharomycetes boulardii in the complex therapy of patients with combined invasions of lamblia and ascarids was characterized by decreasing of index of dysbiosis.

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