ORIGINAL ARTICLE

APPLICATION OF THE SACCHAROMYCES BOULARDII PROBIOTIC COMPLEX IN THE CORRECTION OF INTRAINTESTINAL HOMEOSTASIS IN PATIENTS WITH DYSBIOTIC DISORDERS DUE TO ANTIBIOTIC THERAPY

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Adrian D. Kvit[1], Mykhaylo M. Tutka[1], Oksana V. Laba[2], Volodymyr V. Kunovskiy[1]
[1] LVIV CLINICAL EMERGENCY HOSPITAL, LVIV, UKRAINE
[2] LVIV REGIONAL CLINICAL PRENATAL CENTER, LVIV ,UKRAINE

ABSTRACT

The aim: Evaluation of the effectiveness of the biotherapeutic agent Saccharomyces boulardii in the treatment complex of patients with clinical manifestations of dysbiosis and/or signs of enteric insufficiency refractory to previous therapy, to develop ways for their medical correction.

Materials and methods: The study included 209 patients, treated during 2018-2020, grouped into main and the comparison group. The main group was divided into subgroups depending on the nosological form of the underlying disease: surgical – 36, general therapeutic – 58, gynecological – 47 patients. Main group treatment was supplemented with Saccharomyces boulardii for 10-14 days from the first day of inclusion of patients in the observation group.

Results: The analysis of motor-evacuation disorders verified flatulence in 89.47%, localized epigastric pain in 22.48%, diarrhea in 55.02%, and constipation in 11.48% of cases. Based on the results of laboratory parameters, an absolute increase in the number of leukocytes in the range from 12.4 ± 1.8 x 109 / l to 14.7 ± 2.8 x 109 / l with a neutrophilic formula shift, bilirubinemia to 54.4 ± 12.2 mmol / l , elevated levels of urea to 14.7 ± 3.9 and creatinine to 0.199 ± 0.07 mmol / L.

Conclusions: After completion of the treatment course (main group), a significant ($p < 0.05$) improvement in the general condition (decrease in stool frequency within 1–4 times (2.3 ± 0.28) per day, the absence of rumbling, flatulence and pain in abdomen) in 131 (92.9%) of 141 patients included in the study was stated. Clinical improvement was confirmed by bacteriological studies of copro-culture.

KEY WORDS: antibiotic therapy, dysbiotic disorders, intra-intestinal homeostasis, probiotic complex Saccharomyces boulardii

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INTRODUCTION

The human intestine contains on average about 50 trillion microorganisms, which is about 1.3 times more than the total number of cells in the body [1]. The "relationships" between the intestine and its microflora is not just harmless coexistence, but rather a form of mutualism, which means, mutually beneficial relationships [2].

The species composition of the colon microflora is divided into three groups: the main (Bifidobacterium, Bacteroides), which makes up up to 70% of all bacteria)), the concomitant (Lactobacillales, Escherichia coli, Enterococcus faecalis) and the residual (Staphylococcus aureus, Proteus mirabilis).

The composition of the digestive tract microflora can change under the influence of various factors and adverse effects that weaken the body's defense mechanisms (acute and chronic intestinal diseases, diseases of the pancreas and biliary tract, extreme climatic and geographical conditions, pollution of the biosphere with industrial waste, various chemicals, and infectious diseases).

Every practicing physician encounters a problem of choosing a complex of medicines, which are necessary for adequate medication treatment of a patient. It becomes especially relevant when carrying out "massive" antibacterial therapy or during "aggressive" therapy of neglected and/or erased forms of diseases. In attenuated patients, especially those with immune disorders, self-healing of the intestinal ecology does not occur, which leads to the development of the clinical picture of dysbiosis and, as a result, the formation of enteric insufficiency syndrome [3, 4, 5].

At this stage of treatment, most patients experience symptoms associated with a change in the intestinal microbiota, interpreted as dysbiotic disorders of the digestive tract – dysbiosis [6,7,8]. The degree of the disbalance of the correlation of microbial composition is diverse and can be observed both in the biotope of the small (syndrome of increased bacterial seeding of the small intestine) and the large intestine (a change in the quantitative/qualitative composition of microorganisms,

accompanied by an increase in their invasiveness and aggressiveness with respect to the patient's macroorganism).

THE AIM

The aim of our study was to evaluate the effectiveness of the biotherapeutic agent Saccharomyces boulardii in the complex treatment of patients with clinical manifestations of dysbiosis and/or signs of enteric insufficiency refractory to previously used therapy.

MATERIALS AND METHODS

In a prospective multicentric study, the condition and the results of complex treatment of a sample group of 209 patients who were hospitalized in the Lviv City Municipal Clinical Emergency Hospital during 2018-2020 were analyzed and assessed.

Patients were attracted to the study according to the following criteria: consent of the patient to participate, confirmed diagnosis requiring inpatient treatment with antibiotic therapy, signs of digestive tract motility disorder clinically verified, the absence of background concomitant pathology, that may affect the formation of dysbiotic digestive tract disorders and propulsive bowel ability.

All patients were conditionally devided into two groups – the main and the comparison group. The main group was divided into three subgroups depending on the nosological form of the underlying disease: the first (A) subgroup – patients with a surgical profile (acute pancreatitis, acute appendicitis, intestinal obstruction) – the total number of patients – 36 (19 men and 17 women aged 63, 3 ± 4.5 years); the second (B) subgroup of 58 patients of general therapeutic profile (23 men and 35 women aged 57.4 ± 3.3 years); the third (C) subgroup – gynecological patients (47 women aged 30.2 ± 4.7 years). The average age indicator for patients of the main group is 52.4 ± 6.1 years. The division of patients into groups is presented in Table I.

The comparison group consisted of 68 patients, equivalent both in nosological forms of diseases and in the severity of clinical manifestations of dysbiotic disorders.

The diagnosis of the disease was established on the basis of an assessment of general clinical, laboratory, biochemical examinations, as well as ultrasound CT and endoscopy data and results. All patients received comprehensive conservative treatment in accordance with the protocols of the Ministry of Health of Ukraine.

The duration of the disease at the time of hospitalization ranged from 2 to 9 days. The inclusion of patients in the observation group was regulated by the duration of preventive antibiotic therapy at the prehospital stage (at least 5-7 days).

In patients of the main group, in order to correct dysbiotic disorders, treatment was supplemented with a probiotic complex, from the moment when patients were included in the observation group (5-7 days of antibacterial therapy) and was carried out during 10-14 days. The daily dose of Saccharomyces boulardii was 750 mg divided into 3 doses.

The comparison group also included patients with diseases of the surgical, general therapeutic and gynecological profile in a correlation identical to the patients of the main group. The division of patients into groups (the main and comparison groups) were carried out using the «blind envelope» method. The average age for patients in the comparison group was 56.1 ± 4.3 years. All 68 patients underwent similar basic drug therapy (in accordance with the protocols of the Ministry of Health of Ukraine), but without the inclusion of the probiotic complex Saccharomyces boulardii in the treatment algorithm.

Evaluation of the effectiveness of conservative drug therapy was carried out by dynamic monitoring of changes in the clinical condition of patients, the results of laboratory and biochemical examinations, as well as computed tomography and ultrasonography results and analysis of the results of digestive tract motor-evacuation function indicators examination in dynamic.

Evaluation of the treatment effectiveness was based on the dynamics of clinical symptoms, data from general clinical laboratory and instrumental studies, results of a co-cytogram and bacteriological coproculture examination for the presence of dysbiotic disorders.

The tonus and contractility of the digestive tract monitoring in patients with acute panctreatitis was carried out according to the graphic control technique, developed in the clinic with computer analysis of the observation results – peripheral electrogastroenterography.

An important component for confirming the enteric insufficiency syndrome formation in surgical profile patients, as complication following the dysbiotic small intestine biotope microflora disorders, were the results of mucous membrane of the upper small intestine studies (histological and morphometrical studies).

Material sampling (biopsy of the initial part of the small intestine wall) was carried out during endoscopy, as well as during the suboperative formation of ejunostomy by excision of a small piece of the small intestine wall.

The gained material was fixed in a 10% formalin solution with buffer (according to Lilly). Samples were passed through ethanol of various concentrations and embedded in paraffin, from which preparations were cut, followed by hematoxylin and eosin staining. Sections were examined using optical light microscopy. The method of linear integration was used to determine the height of the villi and the depth of the crypts of the mucous membrane of the small intestine in five fields of view on each of the preparations with a magnification of 630 times using light microscopes.

To quantify the changes in the small intestine mucosa, morphometric studies were performed (the number of villi per unit area, their height, crypt depth, and the correlation coefficient between the villus height and crypt depth) were evaluated.

RESULTS

The use of antibacterial agents on the background of a pronounced violation of the gastrointestinal tract propulsive ability, as a rule, was accompanied by a

Table I. Division of patients by nosological forms of the disease (main group)

	Nosological forms	Age	Patients amount	Gender	
				male	female
1 Group (n=36)	Acute appendicitis	52,4 ± 8,3	17	7	10
	Acute pancreatitis	44,1±5,2	8	5	3
	Iliac passion	68,4±4,2	11	7	4
		63,3±4,5	36	19	17
2 Group (n=58)	Pneumonia (of various etiologies)	59,4±2,4	35	14	21
	Inflammatory Kidney Diseases	48,6±1,1	23	9	14
		57,4±3,3	58	23	35
3 Group (n=47)	Bacterial vaginosis	28,3±3,2	12		12
	Vulvovaginitis	32,3±1,3	11		11
	Cervicitis	26,4±2,1	24		24
		30,2±4,7	47		47
	TOTALLY	52,4±6,1	141	42	99

significant risk of dysbiotic local intestinal biotopes microflora disorders.

Most patients develop a number of complications during long-term antibiotic therapy, namely sensitization of the body, there is a risk of allergic complications, and prerequisites are formed for a significant immunological reactivity background decrease. The basis for the formation of these complications is an disbalance in the intestinal microflora, accompanied by a high probability of developing dysbiotic disorders and diarrheal syndrome.

The analysis of motor-evacuation disorders of the digestive tract in patients, included in the study, was accompanied by the formation of certain complaints, namely: a feeling of heaviness in the epigastric region and nausea were verified in 209 patients (100%), flatulence in 187 (89.47%), local epigastric pain in 47 (22.48%), diarrhea in 115 (55.02%), constipation in 24 (11.48%). The formation of clinical symptoms of impaired bowel ability due to prolonged antibiotic therapy (from 10 to 14 days) in combination with a background disease significantly affected the general condition of the patient and were one of the key prerequisites for the formation of pain and intoxication syndromes.

Based on the results of laboratory observations, it was stated: an absolute increase in the number of leukocytes in the range from 12.4 ± 1.8 x 10^9/ l to 14.7 ± 2.8 x 10^9/ l with a neutrophilic shift in the formula, bilirubinemia to 54.4 ± 12 , 2 μmol / L, elevated levels of urea up to 14.7 ± 3.9 and creatinine up to 0.199 ± 0.07 mmol / L.

It is also worth noting that the most pronounced clinical signs of dysbiotic disorders were observed in patients with a surgical profile (acute pancreatitis and intestinal obstruction).

In this group of patients, the negative dynamics of laboratory parameters (general clinical studies – blood analysis, indicators of carbohydrate and protein metabolism), as well as the formation of a picture of clinically expressed symptoms (paresis, nausea, flatulence) correlated with the results of endoscopic studies (pathomorphological microstructural changes in the small intestine mucosa) and served as confirmation of antibiotic-associated complications – dysbiotic disorders of local biotopes at the level of the small intestine and enteric insufficiency attentiveness.

Conducting histological studies in patients with acute pancreatitis (Fig.1) allowed to state the revealed pronounced pathomorphological microstructural changes in the small intestine (qualitative / quantitative structural changes in enterocytes). Primary necrosis of the villi tops of the intestinal mucosa was verified in 14 patients (82.35%) with acute pancreatitis and in 6 patients (54.5%) with intestinal obstruction.

In 25 patients (89.28%) from a cohort of patients with a surgical profile, edema of the distal villi was observed, as a result of enterocyte ischemia, accompanied by an increase in the intervals between lamina propria. In 13 patients (46.42%), rejection of necrotic enterocytes into the intestinal lumen and a change in the structure of myocytes were detected.

A similar picture of microstructural changes in the small intestine mucosa was observed in patients with intestinal obstruction (Fig. 2).

In accordance with the bacteriological studies, microbiocenosis disorders were detected in most patients of the main (77; 55.32%) and control (43; 63.23%) groups. Patients of both groups showed a decrease in the quantitative and qualitative content of bifidobacteria and lactobacilli, enterococci and a decrease in the total number of *Escherichia coli* in 166 cases (79.42%) on the background of the formation of microbial associations of *Proteus vulgaris, Proteus mirabilis* (127; 60.76%). *Klebsiella granulomatis*, *Klebsiella terrigena* (38; 18.18%), *Enterobacter* (59; 28.22%), *Candida albicans* (42; 20.09%) and *Staphylococcus aureus* (72; 34.44%).

In the study of coprocytograms in patients of the main group (with clinical dysbiotic manifestations and / or signs of

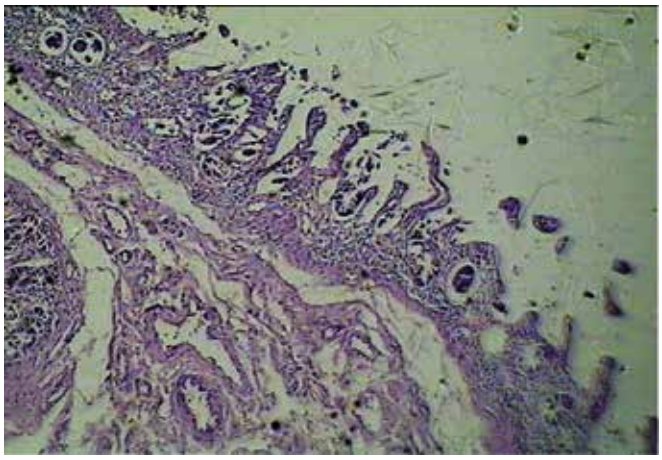

Fig. 1. Fragment of the small intestine mucous membrane (decrease in the number of enterocytes per unit area)

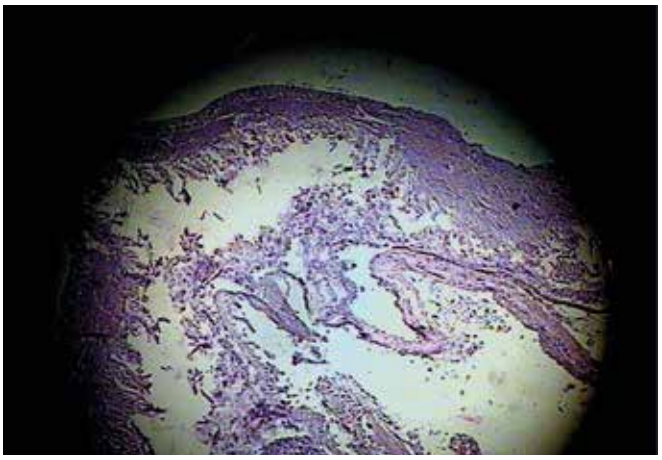

Fig. 2. A fragment of the small intestine mucous membrane (detachment of the apical membrane, a decrease in the number of enterocytes per unit area, detachment of the submucosal layer).

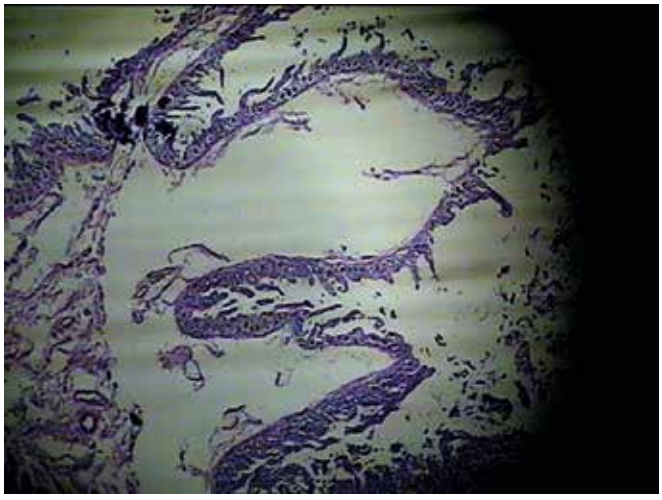

Fig. 3. A fragment of the small intestine mucous membrane with acute pancreatitis at the end of treatment (an increase in the number of microvilli in the viewing area, an increase in their height, the absence of apical necrosis and the presence of an adequate correlation between the number of villi and crypts)

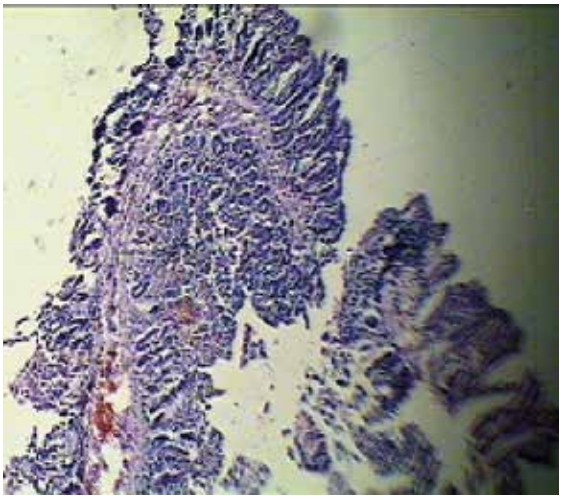

Fig. 4. A fragment of the small intestine mucous membrane with acute pancreatitis at the end of treatment (an increase in the number of microvilli in the viewing area, an increase in their height, the absence of apical necrosis and the presence of an adequate correlation between the number of villi and crypts)

prolonged (up to 2-3 days of diarrhea) quantitative growth of muscle fibers (47; 33.3%), neutral fat (26; 12.4%), starch and digestible fiber (65; 46.09%). Similar changes were observed in 54 (79.41%) patients of the comparison group.

It should be noted that among the total number of patients in the main group (141 patients), in the third group (group C – gynecological patients), dysbiotic disorders of the digestive tract were minimal and were found only in 14 cases (29.78%). This is primarily due to the minimum (over the duration) period of antibiotic therapy (on average 7.3 ± 1.1 days) and the young age of the patients (30.2 ± 4.7 years).

After completion of the (main group) treatment course, a significant ($p < 0.05$) improvement in the general condition was found (a decrease in stool frequency within 1–4 times (2.3 ± 0.28) per day, no rumbling, flatulence and epigastric pain syndrome) in 131 (92.9%) of 141 patients included in the study. Clinical improvement was confirmed by bacteriological studies of copro-culture.

In 47 patients (69.11%) of 68 comparison group patients, an improvement in the general condition was also noted, however, in the group of patients receiving the Saccharomyces boulardii probiotic complex, a noticeable improvement in the clinical condition was observed already on the 6–8 day of treatment, while in patients from the comparison group not earlier than 10-12 days.

In 89 main group patients (63.12%), it returned to normal on the 10-th day of treatment, and in 27 (19.14%), the coprocytogram improved notably.

In the comparison group, pathological changes in the coprocytogram on the 14-th day from the treatment start persisted in 39 patients (57.35%).

The most significant were changes in the intestine microbial flora. So in 118 (83.68%) main group patients, a reliable normalization of the quantitative/qualitative microflora composition correlation was observed within two to three weeks from the start of the treatment, while in 14 (20.6%) comparison group patients dysbiotic disorders were observed over 4-5 weeks.

A good result of the complex treatment with the inclusion of the probiotic complex Saccharomyces boulardii was also found in the group of patients with acute pacreatitis (Fig. 3-4).

Since there is an increase in the uncontrolled use of antibacterial drugs, the question of the medical correction of digestive tract microbiocenosis local disorders is becoming increasingly relevant and timely. The proposed method for leveling dysbiotic disorders using Saccharomyces boulardii expands the range and increases the effectiveness of conservative treatment.

DISCUSSION

Cascade disorders of the digestive system as permanent manifestations of acute pancreatitis, according to E.A. Deitch, turn the "intestinal tube" into an undrained abscess – the main source of endogenous intoxication [9, 10] .

Significant violations of motor-evacuation functions of the intestine, according to many authors [11, 12], lead to significant dysbiotic disorders of intestinal microecology with the initiation of excessive colonization syndrome and rapid destruction of the intestinal mucosa, causing the development of pathological bacterial translocation, which is most clearly observed [13].

The role of intestinal microbiota in the formation of resistance to infectious agents is constantly evolving. Practitioners are increasingly recognizing the importance of the microbiota interacting with the immune system and intestinal epithelial cells in providing additional barriers to infectious agents [14-16].

A well-known side effect of antibiotic therapy is antibiotic-associated diarrhea (AAD), defined as diarrhea that occurs during antibiotic treatment and cannot be explained by another cause [17-22].

Systematic literature reviews confirm that most of the studied probiotics have been clinically proven to be effective in reducing the risk of AAD in the general (mostly adult) population. Multicenter studies state that the potential effects of probiotics can occur at the level of intestinal microbiota, intestinal epithelium and associated immune properties of the mucous membrane and at the systemic level [23-28].

Similar results were obtained when using probiotic formations for the prevention and treatment of *C. difficile-associated* diarrhea. The authors concluded that the available data (all obtained for adults) indicate the feasibility of prescribing probiotics with antibiotics to prevent or treat *C. difficile-associated* diarrhea using probiotics (*Saccharomyces boulardii, L. rhamnosus GG, Lactobacillus casei* DN 114 001) appropriate [29-32].

The obtained data formed the basis for our clinical study.

CONCLUSIONS

1. Antibiotic-associated intestinal lesions are among the most common complications of antibiotic therapy, and therefore their prevention and treatment are a priority in practical public health.
2. The inclusion of the probiotic complex Saccharomyces boulardii in the complex of drug therapy in patients with background antibacterial therapy helps to level dysbiotic disorders, widens the range and increases the effectiveness of conservative treatment in this group of patients.

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ORCID and contributionship:
Adrian D. Kvit: 0000-0002-5036-3606 [A,E,F]
Mykhaylo M. Tutka: 0000-0003-4395-8406 [B,D]
Oksana V. Laba: 0000-0002-1237-796X [B,D]
Volodymyr V. Kunovskiy: 0000-0003-2796-4814 [C,E]

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CORRESPONDING AUTHOR
Adrian D. Kvit
Lviv Clinical Emergency Hospital
9 Mykolajchuka, 79000, Lviv, Ukraine
tel: +380505324454
e-mail: adrian_kvit@yahoo.pl

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