ТАКТИКА ВЕДЕНИЯ ПАЦИЕНТОВ С ЯЗВЕННЫМ КОЛИТОМ С УЧЕТОМ МИКРОБИОЛОГИЧЕСКОГО ИССЛЕДОВАНИЯ БИОПТАТОВ СТЕНКИ ТОЛСТОЙ КИШКИ

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Язвенный колит (ЯК) – распространенное заболевание, с тенденцией к ежегодному увеличению случаев выявления. Преимущественно поражаются лица молодого и трудоспособного возраста. Пик заболеваемости приходится на 20-29 и 50-55 лет. *Цель исследования* – оптимизация диагностики и тактики лечения пациентов с ЯК путем коррекции антибактериальной терапии на основании данных микробиологического исследования микрофлоры стенки толстой кишки. *Материалы и методы.* Обследовано 35 пациентов с язвенным колитом в возрасте от 28 до 61 года, средний возраст пациентов 37,6 лет, которые находились на амбулаторном и стационарном лечении в отделениях колопроктологии и гастроэнтерологии Клиник СамГМУ с января по май 2017 года. Из них 48,6% мужчин. *Результаты.* Зарегистрировано значительное видовое разнообразие микрофлоры, требующее точной видовой идентификации и разработки стандартных процедур выделения микроорганизмов из биоптатов у пациентов с ЯК с целью назначения антибактериальной терапии. При анализе чувствительности к антибиотикам выделенных штаммов было выявлено, что 45% штаммов микроорганизмов имеют признаки резистентности к 1-2 группам лекарственных препаратов, 33% – к 3 и более группам. Только 22% штаммов оказались чувствительными ко всем тестируемым препаратам. Эрадикация флоры с множественной антибиотикорезистентностью представляет сложности и требует назначения комбинированной терапии после исследования биоптата. *Заключение.* Всем пациентам с ЯК необходимо исследовать микробный состав кишечной стенки с целью оптимизации диагностики и тактики проводимого лечения. Микроорганизмы в титре 10^5-10^6, их большое видовое разнообразие могут поддерживать воспаление в толстой кишке и препятствовать его купированию. Необходимо продолжить работу по изучению микробиологического состава стенки толстой кишки в сравнительном аспекте для оптимизации диагностики и тактики лечения пациентов с ЯК.

Ключевые слова: язвенный колит, микрофлора, биопсия, антибиотикотерапия.

MANAGEMENT OF PATIENTS WITH ULCERATIVE COLITIS WITH ACCOUNT OF MICROBIOLOGICAL EXAMINATION OF BIOPTATE OF COLON WALL

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Ulcerative colitis (UC) is a common disease with the evident tendency to annual increase in incidence. The disease mostly affects young individuals of active working age. The peak of incidence of the disease is observed at the age of 20-29 and 50-55 years. ***The aim of study* was** optimization of diagnostics and management of patients with ulcerative colitis by correction of antibacterial therapy on the basis of the data of microbiological examination of the microflora of the wall of colon. ***Materials and Methods.*** 35 Patients with ulcerative colitis from 28 to 61 years of age with the average age 37.6 years who underwent outpatient and stationary treatment in colonoproctology and gastroenterology departments of SamSMU clinics in the period from January to May 2017 were examined. Of them, 18 were males (48.6%) and 17 females (51.4%). ***Results.*** Significant species diversity of microflora was identified that requires exact species identification and development of standard procedures for isolation of microorganisms from bioptates of patients with ulcerative colitis with the aim of administration of antibacterial treatment. In analysis of sensitivity of the isolated strains to antibiotics 45% of the isolated microorganisms were found to have signs of resistance to 1-2 groups of medical drugs, and 33% showed signs of resistance to 3 and more groups. Only 22% of strains were found to be sensitive to all tested preparations. Eradication of such flora presents certain difficulties, and in our opinion, requires administration of combined therapy after examination of bioptate. ***Conclusions.*** All patients with ulcerative colitis require examination of the microbial composition of the intestinal wall for optimization of diagnostics and treatment. 10^5-10^6 titer of microorganisms, their wide species diversity may support inflammation in the colon and prevent relief. It is necessary to continue study of microbiological composition of the colonic wall in the comparative aspect for optimization of diagnostics and management of patients with ulcerative colitis.

Keywords: ulcerative colitis, microflora, biopsy, antibiotic therapy.

Ulcerative colitis (UC) is a common disease with the observed tendency to increase in annual incidence. Young individuals of the active working age are mostly affected [1]. The peak of the disease occurs at the age from 20 to 29 and from 50 to 55 [2,3]. In case of ineffective therapy or development of complications surgery is performed in 10-12% of patients with 12-50% of lethality. The etiology of ulcerative colitis still remains unclear [1,4]. One of important factors complicating its treatment is microflora of the colon that may play a role in exacerbation of the disease. Patients with inflammatory diseases of the intestine show reduced immunological tolerance to bacteria that inhabit the gastrointestinal tract [5,6]. UC frustrates the barrier function of mucosa of the colon in result of which bacterial agents may penetrate deep into the layers of the gut and launch a cascade of inflammatory and immune reactions [5-8]. Breakage of the integrity of the mucous membrane creates favorable conditions for contamination of the damaged area with transitory microflora [9].

Microorganisms existing in the mucous membrane and on the surface of ulcerative-necrotic formations directly participate in development of complications of UC. To decrease bacterial inflammation antibacterial drugs are administered [1-4], however, administration of antibacterial therapy without determination of antibiotic resistance of the microflora participating in inflammation of the intestinal wall, may appear ineffective.

Aim of study: optimization of diagnostics and treatment of patients with ulcerative colitis through correction of antibacterial therapy on the basis of the data of microbiological examination of the microflora of colonic wall.

Materials and Methods

35 Patients with ulcerative colitis at the age from 28 to 61 years with the average age 37.6 years who underwent outpatient and stationary treatment in colonoproctology and gastroenterology departments of SamSMU clinics in the period from January to May 2017 were examined. Of them, 18 were males (48.6%) and 17 females (51.4%) (Table 1). Criteria for inclusion into the study: patients of both genders of the age from 20 to 70 years inclusive with the newly found ulcerative colitis or with not more than two attacks of the disease in the past; left-sided or total levels of damage; absence of biological therapy in treatment. All patients signed the informed consent. Criteria of exclusion from study: the age under 20 or over 70; diabetes mellitus; symptoms of decompensated heart failure, existence of systemic autoimmune diseases or of oncopathology; mental diseases preventing conduction of study; pregnancy or breastfeeding; incapability of a patient; chronic non-specific infections – tuberculosis, HIV-infection, etc., refusal from cooperation and ignorance of medical recommendations.

In all patient standard clinical and laboratory examinations, irrigoscopy, rectoramanoscopy, fibrocolonoscopy with biopsy were conducted.

Severity of UC was evaluated by criteria of J.C. Truelove and L.I. Witts (1955) with additions of E.A. Belousova [4,10]. A mild form was found in 4 (11.4%) of patients, moderately severe form – in 18 (51.5%) patients and severe form in 13 (37.1%) patients (Tab. 2).

Table 1

Distribution of Patients by Age and Gender
(n=35)

Gender	Age				
	21-40 years, n (%)	41-50 years, n (%)	51-60 years, n (%)	61-70 years, n (%)	In total, n (%)
Males	13 (37.1%)	3 (37.5%)	2 (60.0%)	-	18 (51.4%)
Females	10 (28.5%)	5 (62.5%)	1 (40.0%)	1 (100.0%)	17 (48.6%)
In total	23 (65.7%)	8 (22.9%)	3 (8.6%)	1 (2.8%)	35 (100.0%)

Table 2

Distribution of Patients by Extent of Severity of Disease
(n=35)

Form of UC	Age				
	21-40 years, n (%)	41-50 years, n (%)	51-60 years, n (%)	61-70 years, n (%)	In total, n (%)
Mild	2 (5.7%)	1 (2.9%)	1 (2.9%)	-	4 (11.4%)
Moderately severe	12 (34.3%)	3 (8.6%)	2 (5.7%)	1 (2.9%)	18 (51.5%)
Severe	10 (28.5%)	2 (5.7%)	1 (2.9%)	-	13 (37.1%)
In total	24 (68.5%)	6 (17.2%)	4 (11.4%)	1 (2.9%)	35 (100.0%)

Distal colitis was identified in 16 (45.7%), subtotal in 11 (31,4 %), total in 8 (22.9%) patients.

Therapy included basic preparations containing 5-ASA (5-aminosalicylic acid), folic acid, steroid hormones (prednisolone, hydrocortisone) and symptomatic medications – spasmolytics: papaverine, drotaverine. Patients with moderately severe and severe forms were given infusion therapy, amino acids, potassium chloride, parenteral nutrition, antibacterial therapy in accordance with the data of microbiological examination of the microflora of the gut wall bioptate.

Biopsy material of ulcerative lesions of the gut wall mucosa was taken in fibrocolonoscopy or rectoramanoscopy before treatment and after coloproctectomy (n=7).

Material was taken in accordance with methodical instructions МУ 4.2.2039-05 "Techniques of Taking and Transportation of Biomaterials to Microbiological Laboratories" and with clinical recommendations. The biopsy material of ulcerative lesions of the colonic mucosa was taken during fibro-colonoscopy or rectoramanoscooy. Identification of the isolated cultures was carried out using MALDI-TOF mass-spectrometry. Antibiotic resistance of all isolated cultures was determined by disk-diffusion method in accordance with clinical recommendations and methodical instructions МУК 4.2.1890-04 "Determination of Sensitivity of Microorganisms to Antibacterial Drugs". In all enterobacteria phenotypes of production of extended spectrum beta lactamase were additionally determined using a method of double disks with cephalosporins of III generation and amoxicillin/clavulanic acid [6,8].

Results and Discussion

From biopsy material of 35 patients 65 strains of microorganisms of 21 species were isolated and identified. Among the isolated microflora representatives of *Enterobacteriaceae* family, enterococci, streptococci, corine-bacteria and representatives of *Comammonas* species were identified. The species composition of the isolated microorganisms is given in Table 3.

2. Микроорганизмы, не относящиеся к нормальной микрофлоре толстой кишки, а In 6 patients (20%) microorganisms were isolated in monoculture, in 24 patients (68%) 2-4 strains were isolated, in 3 patients (4%) 5 strains of microorganisms were determined. The growth of microflora was not isolated from 2 samples of biopsy material of patients (8%).

In semi-quantitative estimation it should be noted that 36 strains were isolated in the titer 10^5 CSU in the bioptate, 5 strains – in the titer 10^6 CSU in the bioptate, 16 strains – in the titer 10^4 CSU in the bioptate, 8 strains – in the titer 10^3 CSU in the bioptate.

On the basis of species peculiarities, pathogenicity factors and data of participation of the microorganisms in inflammatory processes in the colon, the isolated microflora was conventionally divided into 3 groups:

1. Representatives of the normal microflora of the colon – 16 strains (lactose-positive non-hemolyzing *E. coli,* representatives of *Enterococcus* species).

2. The second group included microorganisms that were not referred to the normal microflora of the colon, and also microorganisms isolated in titers exceeding those permissible in the quantitative evaluation of the microflora of intestinal contents, having factors of pathogenicity, providing potential participation of microorganisms in purulent-

Table 3

Species Composition of Microflora Isolated from Biopsy Material (n=65)

Species name of microorganism	n	%
Escherichia coli	22	33.8
Klebsiella pneumonia	6	9.2
Klebsiella oxytoca	1	1.5
Citrobacter braakii	2	3.0
Morganellam organii	2	3.0
Hafnia alvei	1	1.5
Enterobacter cloacae	2	3.0
Enterobacter kobei	1	1.5
Enterobacter asburiae	1	1.5
Aeromonas caviae	1	1.5
Enterococcus faecium	6	9.2
Enterococcus faecalis	8	12.3
Enterococcus casseliflavus	2	3.0
Enterococcus asburiae	2	3.0
Enterococcus galinarium	1	1.5
Streptococcus lutetiencus	1	1.5
Streptococcus galloluticus	2	3.0
Streptococcus sabivarius	1	1.5
Streptococcus haemalyticus	1	1.5
Corynebacteriu margentor.	1	1.5
Comamonasker stersii	1	1.5
In total	65	100%

necrotic processes of different localization – 40 strains (*E.coli* with hemolyzing activity, representatives of geni *Klebsiella, Hafnia, Citrobacter, Morganella, Enterobacter*).

3. Microorganisms whose participation in the inflammation process was unclear – 9 strains.

In analysis of sensitivity of the isolated strains to antibiotics it was found that 45% of isolated microorganisms possessed signs of resistance to 1-2 groups of medical drugs, 33% of them showed signs of resistance to 3 and more groups. Only 22% of strains appeared to be sensitive to all tested preparations. Eradication of such flora presents difficulties and, in our opinion, requires administration of combined therapy after examination of bioptate [11].

In evaluation of the quantitative composition of the microflora isolated from patients with a mild degree of the disease, microflora in the wall of colon was either absent, or the inoculated strains were determined in low titers (10^2-10^3 CSU per bioptate). In patients with a moderately severe and severe course of the disease, evident microbial contamination of the submucosal layer (with the quantity of microorganisms 10^5-10^6 per bioptate), and high diversity of species (more than 3) were found in 50.0% and 53.8% of cases, respectively. According to clinical recommendations, antibacterial therapy is indicated to patients with a severe fever or on suspicion of intestinal infection, but, in our opinion, it is necessary to take into consideration species composition of the microflora and its sensitivity to antibacterial preparations. In 50% of cases of a moderately severe course of the disease and in 46.2% of cases with a severe

attack, the microbial contamination of the wall of the colon was either absent or poorly expressed (with the amount of microorganisms 10^2-10^3 per bioptate) and was represented by strains of the normal flora. In patients in whom participation of the microflora in support of inflammation was not identified, administration of antibacterial microflora may aggravate dysbiotic manifestations and, in our opinion, is unreasonable [11].

In comparative analysis of the results of microbiological examinations of bioptates taken in fibrocolonoscopy before surgery (n=28) and after coloproctectomy (n=7) identical data were obtained from bioptates of the removed part of the colon (p<0,05).

In our opinion, detailed examination of the species composition of enterococci in patients with UC is important in connection with a high risk of their spread in the intrahospital conditions and with formation of resistance to medical drugs, which is in agreement with literature data [8,12].

Conclusions

1. In all patients with ulcerative colitis examination of microbial composition of the intestinal wall should be conducted with the aim of optimization of diagnostics and therapeutic approach.

2. Microorganisms in titer 10^5-10^6, their wide diversity can support inflammation in the colon and prevent relief.

3. It is necessary to continue studying of microbiological composition of the colonic wall in a comparative aspect for optimization of diagnostics and therapeutic approach in patients with ulcerative colitis.

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