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IMMUNOLOGICAL DETECTION OF DESTRUCTIVE CHANGES IN ORGANS AND EVALUATION OF DEPENDENCE ON INDICATORS OF INTESTINAL DYSBIOSIS

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Abstract:

Qualitative and quantitative indicators of the local intestinal microbiota and bacteria of the facultative group were studied using the classical bacteriological method. The susceptibility of tissues to degradation in the study was determined immunologically using quantitative indicators of antigen-binding lymphocytes. Representatives of the intestinal microbiota analyzed the statistical correlation between changes in quantitative indicators and the frequency of antigen-binding lymphocytes

Keywords: students, intestinal dysbiosis, tissue antigen, antigen-binding lymphocytes, prepathology

INTRODUCTION. Currently, the intestinal microbiocenosis (IM) is called the latent metabolic "organ" of man, and there is no doubt that it plays a key role in maintaining human health. This is inextricably linked to the great influence of the intestinal microbiota directly on physiological processes, metabolism of the macroorganism, the nervous endocrine and immune systems [4]. The intestinal microbiota modulates the innate and adaptive immune responses of the intestinal mucosa in infections, inflammation and autoimmune diseases [10, 12]. S.V. Bulgakova and co-authors concluded that the development of mitochondrial dysfunctions, cellular aging, neurodegenerative and age-related chronic diseases is largely due to a violation of the microbiota of the symbiotic intestine. [5, 11].

It turns out that depending on the degree of disorders in the intestinal microbiota, the biochemical parameters of the blood deteriorate, these indicators are manifested by a decrease in the total protein content, a decrease in the relative amount of albumin, creatinine [8]. This is the action of substances formed as a result of the interaction of different populations of the microflora of the human body, which supports the biochemical, metabolic and immunological balance necessary to maintain human health [13]. In conditions of disruption of the intestinal microbiota and changes in the intestinal barrier function, the intake of immunogenic molecules into the systemic bloodstream increases, including food antigens, bacterial toxins and pathogens that cause chronic inflammation in tissues [2].

One of the manifestations of immune reactions when proteins of endogenous or exogenous origin enter the internal environment is the appearance of specific sensitized antigen - binding lymphocytes (ABL) in peripheral blood in relation to the same tissue antigen (tag) [3]. In particular, in patients with escherichiosis, high endogenous intoxication in the liver, intestines and kidneys was determined using tag relative AIL, and in this AIL clearly demonstrated in which organ the foci of endogenous intoxication are located [7].

THE PURPOSE OF THE RESEARCH. To identify destructive changes in the organs and tissues of students at the stage of acquired pathology, the correlation between the number of anti-inflammatory lymphocytes against tissue antigen and indicators of the intestinal microbiota is evaluated.

RESEARCH MATERIALS AND METHODS. The study was conducted in the problem laboratory "Clinical Microbiology, Mycology and Immunology" at the Department of Microbiology, Virology and Immunology of the Tashkent Medical Academy in the period 2020-2023. The study used a special questionnaire to assess the health status of 149 students enrolled in the 2nd year of the Tashkent Medical Academy, and laboratory tests were conducted. In this case, peripheral blood (5 ml) from the student's vein was used for immunological examination, and a stool sample (1 g) was used for microbiological examination. The average age of students is 21.0±0.2 years**.**

Immunological studies revealed the levels of sensitized antigen-binding lymphocytes (ABL) to tissue antigens (TAg) of various organs in the blood of students (brain, liver, lungs, endocardium, myocardium, kidneys, pancreas, prostate). Determination of quantitative indicators of AIL relative to TAg in blood Garib F.Yu. et al. The method was studied using an indirect rosette formation reaction (IRFR) [6]. At the same time, lymphocytes that formed

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a rosette with the attachment of at least 3 antigens within every 100 lymphocytes under the microscope were assessed as pathology – when counting 0-3% (1.5±0.7%) – the **normaly**, when counting in the range of 4-6% (4.4±1.4%), when counting 7% (8.4±2.8%) – the **prepathology** and more lymphocytes were counted as **pathological** conditions.

The total number of asporogenic anaerobes, *Bifidobacterium spp.,* for the study of intestinal microbiota in bacteriological studies., *Lactobacterium spp., E.coli* and other *Enterobacter spp., Enterococcus spp., Staphilococcus spp., Streptococcus spp.* and *Candida spp.,* the amounts were determined in lg IRFR/g. When identifying microorganisms by morphological, cultural, tinctorial, and biochemical properties, the Bergi classification is taken as a basis. It is based on Ministry of Health order 177 on quantitative and qualitative assessment of the intestinal microbiota [1]. The degree of dysbiosis Odilov Sh.K., Narbayeva I.E., Garib F.Yu. was evaluated according to the proposed method [9].

Statistical processing of the obtained data was carried out in the practical software package of the personal computer "Statistica for Windows 10.0", using formulas for estimating the correlation coefficient of the arithmetic mean (M), the error of the arithmetic mean (m), the reliable interval (r), the standard deviation index (σ) and the correlation between the results obtained.

THE RESULTS AND THEIR DISCUSSION. 22.8% (34/149) of the students who participated in the study were absolutely healthy according to the results of intestinal microbiota and immunological analysis. Pathology was detected in 25.5% of students (38/149) in accordance with the amount of ABL against tissue antigen when examining students with observed intestinal dysbiosis.

In a total of 209 cases, one or more of the 67.8% of the students who participated in the study had an increase in TAg compared to AIL compared to the norm. In 28.8% (43/149) of students who had normal intestinal microflora, an increase in ABL from the norm was observed in 40.2% (84/209) compared with tissue antigens. In 59.8% of cases (125/209), it was found that the ABL level was higher than normal, while intestinal dysbiosis occurred in combination with grade I-II.

Specific sensitized antigen-binding lymphocytes to tissue antigen accounted for 3-6%, and 25.5% of female students diagnosed with grade I-II dysbiosis could not compare them due to changes in the tissues of the skull and endometrium and the lack of variation in the quantitative indicators of bacteria causing microflora disorders. The correlation relationship between the remaining tissues and representatives of the colon microflora showed the following results (Table 1).

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Table 1.
The results of the correlation analysis of indicators of acquired pathology in dysbiosis of the colon and tissues in students

Representatives of the intestinal microbiota	Tissues involved in the research											
	Liver	Lung		Myocard		Endocard		Kidney	Pancreas		The prostate gland	
	**C	*M.s	**C	*M.s	**C	*M.s	**C	*M.s	*M.s	**C	*M.s	**C
Bacteroides spp.	-0,4	0,0	0,56	0,11	-0,54	-0,99	-1,0	0,45	0,31	0,60	0,23	-0,22
Clostridium spp.	0			0,44				-0,20	0,05	0,94		-0,53
Bifidobacterium spp.	0,44	-0,87	0,00	0,77	-0,18	-1,0	0,65	-0,20	-0,18	-0,28	-0,24	-0,53
Lactobacterium spp.	-0,08	-0,87	-	0,0	0,24	1,0	-0,33	0	0,08	0,13	-0,3	-0,85
E.coli.	0,39	-0,99	0,0	-0,76	0,76	-1,0	-0,33	-0,30	-0,05	0,60	-0,46	0,22
lactose-negative *E. coli*				0,21		-0,5		0,3	0,42		-0,16	
Hemolyzing of *E.coli.*		0,87						-0,12	0,04		0,18	
Pathogenic enterobacteria				-0,4		1,0						
Enterobacter spp.				0,32		0,38	-0,33	-0,33	0,07	0,13	0,43	0,8
Enterococcus spp.	-0,26			0,40	0,54	0,50	-0,33	0,14	0,24	0,94	0,17	0,13
Staphylococcus spp.		-0,87						-0,20	0,35		-0,24	
Candida spp.	-0,20	0,87		0,15	-0,54	0,63		0,32	-0,08	-0,27	0,50	

*Изох: **M.s – Mushy stool *C– Predominance of constipation in stool*

The amount of ABL relative to the pulmonary TAg (4.6±0.15) was 3.1 times higher than normal. In pathology, changes were found in the lung tissue involving *Bifidobacterium spp.* (6.6±0.12), *Lactobacterium spp.* (5.7±0.11) and *E coli.* (7.0±0.04) a strong feedback (from r =-0.87 to r=-1.0), *hemolytic* of *E coli,* exists between a decrease in quantitative indicators. (3.9±0.21) and *Candida spp.* There was a strong direct relationship (r=+0.87) with an increase in the number (5.0±0.14), while students who observed constipation had an average direct relationship (r=+0.56) with quantitative indicators of *Bacteroides spp* (7.8±0.11).

The amount of ABL in relation to liver TAg (4.5±0.16) was 3 times higher than normal. In pathology, *Bacteroides spp*. were found in liver tissue with indicators indicating changes (7.8±0.11) and *Bifidobacterium spp.* (6.6±0.12), an average feedback (r =-0.4), E, between decreasing quantitative indicators. The advantage of constipation in the incidence of constipation was noted, based on the medical history of all students, while an average correct attachment (r =+0.39) to a decrease in the amount of *E. coli* was observed.

The amount of ABL in relation to endocardial TAg (4.5±0.21) was 3 times higher than normal. The pathology of the endocardium acquired changes in *Bacteroides spp., Bifidobacterium spp., E of coli.* a strong feedback (r =-1.0) between a decrease in quantitative indicators, a strong direct relationship with an increase in the number of pathogenic enterobacteria (r =+1.0), *Enterobacter spp.* (3.26±0.06), *Enterococcus spp.* (5.5±0.1) and *Candida spp.,* the average correct association with an increase in the amount of (r =+0.38; r =+0.63), *Bacteroides spp.* students with constipation had a strong feedback with quantitative indicators (r =-1.0), *Bifidobacterium*

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spp., an average direct relationship (r =+0.65). A decrease in the number was found.

The amount of ABL in relation to myocardial TAg (4.9±0.17) was 3.3 times higher than normal. Pathology in myocardial tissue has undergone changes with *Bifidobacterium spp.,* a strong direct relationship (r =+0.77) between a decrease in the amount of *Clostridium spp.* (3.3±0.27), the average correct relationship with an increase in quantity (r =+0.44), i.e. a strong feedback (r =-0.76), *Enterobacter spp.,* to a decrease in *E coli., Enterococcus spp.,* an increase in quantity was followed by a moderate direct relationship (r =+0.32 and R=+0.4), weak connection with other representatives of the microbiota. *Bacteroides spp.* students with observed constipation. and *Candida spp.,* the average feedback with an increase in quantity (r =-0.54), *Enterococcus spp.,* with normal indicators of quantity, the average correct relationship (r =+0.54) and strong direct relationship (r =+0.76) before the decrease in the amount of *E. coli* was assessed as existing.

The amount of ABL relative to the renal TAg (4.5±0.16) was 3.0 times higher than normal. Kidney pathology with acquired changes, lactosanegative *E.coli* (4.3±0.12), *Candida spp. and Bacteroides spp.,* the average correct relationship between quantitative indicators (r=+0.3-r=+0.45), weak feedback in relation to other triggers, was canceled.

The amount of ABL versus pancreatic TAg (3.6±0.16) was 2.4 times higher than normal. *Bacteroides spp.* with acquired pathological changes in pancreatic tissue., lactosanegative *E. coli* and *Staphylococcus spp.* (4.3±0.15), an average direct relationship between quantitative indicators (r=+0.31-r=+0.42), a weak relationship was found compared with other triggers. *Clostridium spp* with AIL vs tag results, students had constipation. and *Enterococcus spp.,* quantitative indicators include a strong direct relationship (r=+0.94), *Bacteroides spp.* and the average correct correlation (r=+0.6) with quantitative indicators of *E. coli* was determined.

The amount of ABL in relation to the prostate gland TAg (3.7±0.21) was 2.5 times higher than normal. Acquired pathology in prostate tissue (3.7±0.21) with changes in *Enterobacter spp.* and *Candida spp.,* quantitative indicators included an average direct relationship (r=+0.43; r=+0.5), weak relative to other triggers, and an average feedback *Enterobacter spp* with ABL leads to constipation in the observed students. Correlation results were obtained showing a strong positive correlation between the quantitative indicator (r=+0.8), and a strong inverse correlation (r=-0.85) between the quantitative indicator of lactobacilli.

CONCLUSIONS. Among students with pre-pathological conditions in their organs and tissues, there is a difference between changes in the lung tissue and an increase in the amount of *Candida spp., hemolyzing E.coli* in the microbiota (r=+0.87), between changes in the myocardium and a decrease in the amount of *E.coli* and *Bifidobacterium spp.* (r=+0.77), between the changes in the pancreatic tissue and the increase in the amount of *Clostridium spp.* (r=+0.94) a strong direct correlation, between the changes in the endocardium and the increase in the amount of pathogenic enterobacteria absolute (r=+1.0) correct connections were detected.

Also, between the changes in the liver and endocardial tissue with the decrease in the amount of Bifidobacterium spp. (r=+0.44 and r=+0.65), the decrease in the amount of E.coli (r=+0.6) with the changes in the pancreas tissue and lactose-negative E.coli (r=+0.42), with changes in the prostate gland, Enterobacter spp. (r=+0.43) and Candida spp., (r=+0.5) moderately correct correlations were found.

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