

Immunological Assessment of Changes in Various Organs and Tissues in Students with Observed Intestinal Dysbiosis

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Abstract 149 young students who were considered practically healthy were selected for the study. Bacteriological analysis of the intestinal microbiota of students, 48% of whom had dysbiosis of 1-2 degrees. When studying the quantitative indicators of specifically sensitive antigen-binding lymphocytes (ABL) to various tissue antigens (TAg) in the blood of these students, there was pre-pathology and pathology in the tissues, and it was found that pathological changes were 1.5 times (125/84) higher than changes in the organs and tissues of students with normal microflora.

Keywords Student youth, Intestinal microbiocenosis, Antigen-binding lymphocytes, Tissue antigens

1. Introduction

According to the World Health Organization (WHO), approximately 95.0% of the world's population suffers from intestinal dysbiosis. The intestinal microbiota (IM) is a separate element of the human body and has not been studied enough. The intestinal microbiota performs physiological and biochemical functions that seriously regulate the "host" organism, and are not considered as a set of symbiotic living bacteria only in the intestine [7,9]. Modern studies show that IM has protective, metabolic, trophic and immunological functions and is able to create "reciprocal relationships" that include cellular and soluble elements of mucosal immunity [6]. L.P. According to Knyshova and co-authors, the ability of the intestinal microbiota to detoxify can be compared with the ability of the liver. Modern studies show that disorders of the intestinal microbial-tissue complex are primary not only in the formation of liver steatosis, but also in the formation of pancreatic pathologies [3]. IM interactions in immune system interactions include homeostasis and inflammation in the intestine and, in some cases, in extra-intestinal tissues. Translational microbiome therapy can be used to treat chronic diseases by balancing the relationship between the macroorganism and microflora [12]. T- and B-lymphocytes make up the bulk of antigen-binding lymphocytes (ABL). Due to the pathological process in the body, the higher the level of cell destruction and necrosis in organs, the higher the level of sensitized ABL in the blood compared to tag [8,10]. In immunological screening, the use of ABL is an extremely effective method, in particular, the experiments of Yu. A.

Kuzmin showed that ABL can be detected in the blood 12 hours after the antigenic stimulus, i.e. long before the formation of antibodies [4].

Stress observed in adolescence, diet, alcohol consumption, and irregular use of antibiotics lead to changes in the gut microbiota [14]. The preservation of health, its management in the interests of life achievements is an urgent problem of national importance for the protection of youth health, although it is a personal matter for each of us, but as the main labor potential of society in the near future [2,11,13].

2. The Purpose of the Study

The method of immunological screening of changes in organs and tissues of students with observed intestinal dysbiosis in the analysis of the health status of students.

3. Material and Methods of Research

The research work was carried out in the problem laboratory "Clinical Microbiology, Mycology and Immunology" at the Department of Microbiology, Virology and Immunology of the Tashkent Medical Academy. Our study involved 149 "practically healthy" young people aged 18 to 25 years by their consent. Students received material for bacteriological (1 g of feces) and immunological (5 ml of peripheral venous blood) tests. The average age of students is 21.0 ± 0.2 years.

Based on a special survey, data were collected on the absence of acute or chronic diseases of the digestive system in the student's anamnesis, information about the nutrition of students, mild complaints characteristic of intestinal microflora disorders, and the fact that Ham had not taken

antibiotics for the last 1 month. Based on the collected data from practically healthy students, material for bacteriological taxa was obtained. Sampling and seeding it into the food medium was carried out on the basis of Order 177 MHO and on the basis of other regulatory documents [5]. All the studied biological samples were planted by the Gold method in bloody agar, monitol-solitol-agar, Saburo and Endo mucous media, and it was found that the concentration of more than 103 CFU/ml was significant. The isolated cultures were identified according to their morphological, tinctorial, cultural and biochemical characteristics.

For an immunological study, 5 ml of micror blood was taken from the ulnar vein in the morning on an empty stomach and placed in test tubes with an isotonic solution consisting of 2.0 ml of sodium chloride and 2-3 drops of heparin. Lymphocytes from heparinized blood, 2 ml of ficolla-verografin gradient (density $d=1,077$ g/ml) were poured into test tubes and centrifuged at a speed of 1500 ro/m. the district attorney was suspended for 30 minutes. Quantitative indicators of antigen-binding lymphocytes (ABL) in relation to tissue antigens in the blood (brain-control, liver, lungs, endocardium, myocardium, kidneys, endometrium, pancreas, prostate gland) F.Yu. were studied by the method of Garib and co-authors by indirect rosette formation (IRF) reaction [1]. The reaction results were visualized under an immersion microscope. The normal ABL index of $1.5 \pm 0.7\%$ compared to the tissue antigen, the range of $4.4 \pm 1.4\%$ of the acquired pathological condition, $8.4 \pm 2.8\%$ was estimated as a predisposition to the development of pathological processes.

4. Results and Their Analysis

In the IM analysis, out of 149 students who participated in the study, students diagnosed with dysbiosis accounted for 72 (48.3%), students with an ABL frequency higher than $1.5 \pm 0.7\%$ in immunological analysis accounted for 101 (67.8%), and absolutely healthy students in both analyses accounted for 34 (22.8%). IM tests were normal, the pathology obtained by ABL indicators and pathological indicators observed by students were observed in 43 patients (28.8%), of which the obtained pathology indicators were observed in 28 students (18.8%), pathological indicators were observed in 15 students (10.0%).

According to the dysbiosis index (DI) proposed by Sh.K. Adilov and co-authors, a total of 72 students (48.3%) were found to have I-II levels of dysbiosis. Of these, 63 (42.3%) received a grade of DI from students and 9 (6%) received a grade of DI II from students. The results of the immunological taxil of 72 students who had dysbacteriosis showed the frequency of ABL with pathology (25.5%) and pathological indicators (13.4%) in 58 of them (38.9%). In 14 students (9.4%) who were diagnosed with dysbacteriosis, the frequency of ABL in immunological analyses in the studied tissues was normal.

During the immunological examination of students diagnosed with dysbiosis of the I degree, ABL received pathology in one and several tissues - 23.5% of the occurrence in pathogens, 2.0% of the occurrence in only 1 tissue according to pathological indicators, received pathology in several tissues, and the joint occurrence of pathological changes was observed in 9.4% of the number of students. Taking into account the presence of constipation in 23 students (15.4%) according to the results of a special survey conducted before laboratory tests among students who had dysbiosis I, we decided to highlight changes in the organs and tissues of these students in Table 1 separately.

In 6% of students diagnosed with dysbiosis of the II degree, based on the analysis of the intestinal microbiota, the amount of ABL versus tagas returned the following indicators: while in 2% of students, ABL indicators did not exceed the norm, in another 2% pathology received the frequency of ABL, indicating that the condition was observed. Dysbiosis of the II degree revealed the remaining 2% of pathological conditions, and the obtained ABL indicators, indicating only a pathological condition, were not observed when determining that changes occur in different tissues simultaneously (Table 1).

Against the background of dysbiosis, the rate of joint and/or individual development of pathology and pathological changes in various tissues of students observed at indicators exceeding the norm was analyzed. In Class I, DI observed that the range of TAg values in students was $4.4 \pm 1.4\%$ ABL compared to 17 in 1 participant, 12 in 2 participants, 2 in 3 participants and 4 in 4 participants. And indicators of pathological status were recorded only in one of 3 students (2.0%) in this group.

Table 1. Results of intestinal microbiocenosis and immunological assessment of students

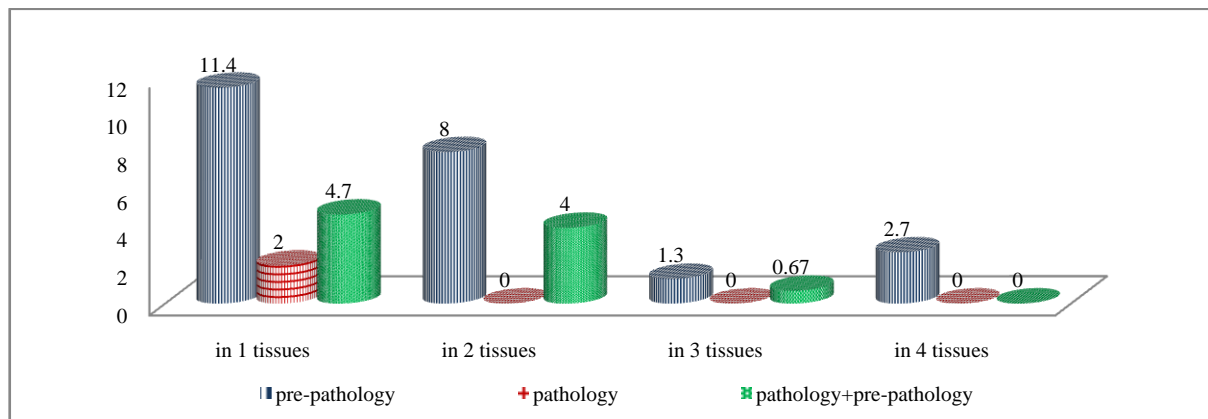
№	Intestinal microbiota	Results of immunological analysis				Total
		Healthy	Pre-pathology	Pathology	Pathology+pre-pathology	
1.	Healthy	34	28	6	9	77
2.	Dysbiosis I	11	35	3	14	63
	Constipation	1	13	1	8	23
	Liquid feces	10	22	2	6	40
3.	Dysbiosis II	3	3	-	3	9
Total		48	66	9	26	149

Note: norm – ABL= $1,5 \pm 0,7\%$; pre-pathology – ABL= $4,4 \pm 1,4\%$; pathology – ABL= $8,4 \pm 2,8\%$; pathology+pre-pathology – the joint appearance of ABL indicators above the norm in different tissues in 1 student.

Table 2. Comparative characteristics of the results of bacteriological analysis of intestinal microbiota and immunological screening indicators

Results of the study of the intestinal microbiota		Healthy		Dysbiosis I				Dysbiosis II		Total:
				Liquid feces		Constipation				
Indicators of ABL		p.p	p	p.p	p	p.p	p	p.p	p	
Organs and tissues during examination	Brain TAG	6	5	2	-	1	4	-	-	18
	Liver TAG	5	2	-	1	11	4	-	-	23
	Lunges TAG	12	3	3	4	4	-	-	1	27
	Endocardium TAG	8	3	1	4	4	2	2	1	25
	Myocardium TAG	6	2	3	-	6	-	3	-	20
	Kidney TAG	4	-	12	-	1	2	2	1	22
	Pancreas TAG	10	1	9	2	6	-	1	2	31
	Prostate gland TAG	10	1	10	1	5	-	1	-	28
Endometrium TAG	5	1	3	-	3	1	2	-	15	
Total:		66	18	43	12	41	13	11	5	209

Note: p.p – pre-pathology, p – pathology.

**Figure 1.** Indicators of progression of grade I dysbiosis by TAg opposite ABL in one or more members and tissues of the observed students

With dysbiosis of the II degree, there was an increase in the level of ABL in relation to the members and tissues of students who received pathology, an increase in the indicators observed when compared with 2 different tissue antigens, in 2 out of 3 students, the frequency of ABL did not exceed $4.4 \pm 1.4\%$. (Fig. 1).

Pathological changes in tissues were observed in 58 students (38.9%) in cases of occurrence in 2 or more organs, with damage amounting to a maximum of 7 tissues.

Based on the results of the analysis of the intestinal microbiota, the degree to which the organs noted during a separate examination, TAg compared with ABL indicators exceed the norm was studied.

According to the analysis of the intestinal microbiota, in healthy students, the frequency of ABL in 4.0% of cases was recurrent in the range of $4.4 \pm 1.4\%$, compared with liver and kidney indicators - from 2.7%, lungs - from 8.0%, endocardium - from 6.0%, pancreas - from 6.4%, prostate gland was detected in 6.7% cases, and the endometrial mark - in 3.3% of cases. In this group, ABL was detected in 3.3% of cases compared with the brain TAg with indicators higher than $8.4 \pm 2.8\%$, 2.0% compared with the lung TAg, 1.3% compared with the liver TAg, endocardium, myocardium (see Table 2).

According to the results of the study of the intestinal microbiota in students diagnosed with dysbiosis of the I degree, the predominance of the facultative group was observed in the indicators of ABL $4.4 \pm 1.4\%$ against the indicators of the brain TAg - 1.3%, kidney TAg - 8.0%, pancreatic TAg - 7.4%, prostate TAg - 6.7%, lung tag, myocardium, endometrium - from 2.0%, the endocardial label - from 0.06%, the label observed in the liver relative to sensitive lymphocytes was not found. The frequency of ABL was observed at the 2.7% position, against the pancreatic TAg - from 0.06%, respectively, against the lung and endocardial TAg - against the liver and prostate TAg with indicators above $8.4 \pm 2.8\%$. According to the results of the intestinal microbiota, dysbiosis was observed in students diagnosed with a predominance of level I – bifido and a decrease in the number of lactobacilli (constipation), according to the indicators that ABL received pathology in relation to the liver TAg - 8.0%, in relation to the myocardial TAg - 4.0%, in relation to the pancreas and prostate - 3.3%, accordingly, with respect to the lungs and endocardial TAg - 2.7%, the frequency of ABL was detected from 2.7%, with respect to the brain and liver TAg with indicators above $8.4 \pm 2.8\%$, endocardial and renal TAg - 1.3%.

In female students with confirmed grade II dysbiosis, ABL was observed with a frequency of $4.4 \pm 1.4\%$ compared

with myocardial TAg, respectively, with 1.3% compared with endocardial, kidney and endometrial tag with a frequency of 2.0%. At a frequency exceeding $8.4 \pm 2.8\%$ ABL, indicating the presence of a pathological condition, a pancreatic label was found in relatively 1.3%, respectively 0.06% of students compared with lungs, endocardium, kidney soles (see Table 2).

5. Conclusions

1. According to the results of bacteriological analysis, the degree of organ and tissue damage of students who were diagnosed with dysbiosis was 80.5% (58/72), and in students who did not have dysbiosis, the degree of organ and tissue damage was 55.8% (43/77). Also, in all students who were diagnosed with dysbiosis, the ABL level in 2 or more people exceeded the norm.
2. In one or more of the 101 students who participated in the study, a total of 209 cases showed an increase in the tag counter ABL index from the norm, while injuries in the extremities (125) on the background of dysbiosis were 1.5 times higher than injuries in the extremities (84) on the background of normal microflora.
3. Pre-pathology of dysbiosis and pathology against the background of changes in levels I - II were found mainly in 16 cases in the pancreas and prostate gland of the prostate gland, in 15 - in the kidneys, in 12 - in the myocardium, in 11 - in the liver, as well as pathological changes in the endocardium, in 7 - in the liver and lungs and 4 in the pancreas.

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