ISSN: 2582-4686 SJIF 2021-3.261, 2022-2.889, 2023-5.384, 2024-6.875 ResearchBib IF: 9.948 / 2024

THE MULTIDISCIPLINARY JOURNAL OF SCIENCE AND TECHNOLOGY

VOLUME-5, ISSUE-7

THE STATE OF INTESTINAL MICROBIOME IN HIV-INFECTED CHILDREN WITH INFECTIOUS DIARRHEA.

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Research objective: To study the state of intestinal microbiota in HIV-infected children with infectious diarrhea.

Materials and methods of research. The study was conducted from 2020 to 2025 based on the examination of 499 HIV-infected children aged 3 to 18 years with acute diarrhea. The patients were divided into two groups: Group 1 consisted of 261 HIV-infected children with acute infectious diarrhea, and Group 2 comprised 238 HIV-infected children without acute diarrhea.

Research results: Grade IV intestinal dysbiosis developed in 26.4% of children in the main group, while this grade of intestinal dysbiosis occurred 2.4 times less frequently in children of the comparison group. At the IV clinical stage of HIV infection, no significant differences were found between the groups for grade III intestinal dysbiosis ($P>0.05$); however, the significant difference between the groups for grade IV intestinal dysbiosis was 1.9-fold ($P<0.05$). In children with severe immunodeficiency, grade III intestinal dysbiosis developed 2.4 times less frequently in the main group compared to the comparison group, while grade IV developed 3 times more frequently. In children with acute infectious diarrhea, when the viral load in HIV infection was below 1000 copies, significant differences were found in intestinal dysbiosis of grades I, II, III, and IV compared to the comparison group of children without infectious diarrhea. However, with a high viral load in HIV infection, the differences between grades III and IV intestinal dysbiosis were not significant. In children of the main group with HIV viral load ranging from 10,000 to 100,000 copies, grade IV intestinal dysbiosis developed 1.6 times more frequently than in children of the comparison group.

Conclusion: The incidence of acute infectious diarrhea in HIV-infected children is associated with profound disturbances in intestinal dysbiosis, which are related to the clinical stage of HIV infection, the degree of immunodeficiency, and the viral load, compared to HIV-infected children who did not have acute infectious diarrhea.

Keywords: HIV infection, children, acute infectious diarrhea, intestinal microbiome

Relevance of the problem. At present, HIV infection remains the most important medical and social problem worldwide [1]. It is known that there is a close relationship between the immune system and intestinal microflora, and nearly 90% of the world's population suffers from dysbiosis to some degree. Intestinal dysbiosis is considered a secondary manifestation or complication of any disease [2]. In turn, specific dysbiotic changes worsen the course of the underlying disease and treatment outcomes, and can become a pathological factor in functional disorders of the human body. With the development of dysbiosis, colonization resistance decreases, the body's immune system weakens, and susceptibility to infectious diseases increases. Patients with intestinal microbiocenosis disorders, even those with clinical manifestations, are considered a risk group for the development of functional disorders of the gastrointestinal tract [3].

Microflora performs a protective function in the body, stimulating its immune reactivity.

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Disruption of the quantitative and qualitative composition of the intestinal microflora negatively affects the body's protection against infections and the state of local immunity [9]. Immunodeficiency in HIV infection is most closely related to immune disorders. It is known that the main effect of HIV is directed at one of the subpopulations of T-lymphocytes, namely CD4+ T-lymphocytes [10]. A characteristic feature of HIV infection is an increase in CD4+ subpopulation, which should be considered as a manifestation of progressive disorders of the immune system and its morphological structure [8].

Damage to the intestinal mucosa and dysbiosis of the intestinal microbiota are important factors in the development of HIV and interact with each other. In addition to bacteria, fungal microbiota, which is an important component of the intestine, plays a key role in this disorder. The intestinal mucosal barrier protects against pathogens and their metabolites, epithelial cells, mucins, and microbes. It restricts penetration through proteins and immune cells and promotes the coexistence of the host and microorganisms, which play an important protective role for the organism [2]. However, due to the high concentration of CCR5 (HIV co-receptors) expressed by CD4+ T cells in the intestine, this, in turn, makes the intestinal mucosa the main target of HIV [3]. Damage to the intestinal mucosa associated with HIV infection leads to local and systemic inflammatory reactions in infected patients [1, 7], which increases intestinal permeability, subsequently enhancing the translocation of intestinal microbiota and thereby constantly stimulating immune activation and the development of chronic inflammation [6]. The microbial barrier is an important component of the intestinal mucosa, Changes in the intestinal microbiota are closely related to the stability of the intestinal mucosa. Previous studies have confirmed that HIV-infected individuals experience significant changes in the composition and metabolic functions of the intestinal bacterial microbiota, which affects immune homeostasis and exacerbates chronic inflammation [4, 11].

Numerous studies indicate that microecological disturbances in the intestine are often characterized by a decrease in the population levels of local microorganisms and an increase in the intensity of intestinal mucosal colonization by opportunistic microorganisms [5]. In HIV-infected children, opportunistic microsymbionts frequently serve as the cause of secondary bacterial infections [10].

Research objective: To study the state of intestinal microbiota in HIV-infected children with infectious diarrhea.

Research materials and methods. The study was conducted from 2020 to 2025 at the Specialized Infectious Diseases Clinic of the Republican AVDS Center, Uzbekistan An examination of 499 children aged 3 to 18 with HIV infection and acute diarrhea was conducted in the "HIV Infection" department of the Research Institute of Virology at the Scientific and Practical Medical Center of Epidemiology, Microbiology, Infectious and Parasitic Diseases of the Ministry of Health of the Republic of Uzbekistan. The sick children were divided into two groups for the study: the 1st group consisted of 261 HIV-infected children with acute infectious diarrhea, and the 2nd group comprised 238 HIV-infected children without acute diarrhea. The diagnosis of "HIV infection" in children was made based on the order of the Ministry of Health of the Republic of Uzbekistan No. 270 dated 19.08.2023 "On protocols for the prevention and treatment of human immunodeficiency virus infection" and No. 122 dated 25.03.2015 "On improving measures to combat typhoid fever, paratyphoid, salmonellosis and acute intestinal diseases among the

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population of the republic." According to these, the clinical stage II of HIV infection was observed in 105 children of group 1 and 95 children of group 2, the clinical stage III in 123 children of group 1 and 114 children of group 2, and the clinical stage IV in 33 children of group 1 and 29 children of group 2. Based on the number of CD4+ cells in 1 ml of blood of a patient with HIV infection, 4 degrees of immunodeficiency and viral load were distinguished. In severe immunodeficiency, the CD4+ count was less than 200 cells; in pronounced immunodeficiency - 200-349 cells; in moderate - 350-499 cells; in mild - more than 500 cells. Viral load was categorized into 4 groups: more than 100,000 copies, 10,000-100,000 copies, 1,000-10,000 copies, and less than 1,000 copies.

The material for studying intestinal dysbiosis was feces, which included bifidobacteria, lactobacilli, typical and lactose-negative *Escherichia coli*, opportunistic and pathogenic enterobacteria, non-fermenting gram-positive bacteria, staphylococci, hemolytic forms of microorganisms, fungi similar to enterococci, and yeast genes were isolated. Identification of anaerobic microorganisms, opportunistic and pathogenic enterobacteria was carried out on the VITEK 2 Compact device using identification cards: ANC (244147910), GN (bioMerieux France). Fecal examination included bacteriological method and polymerase chain reaction (PCR) with determination of nucleic acids *of Campylobacter* spp., Salmonella spp., Shigella spp., Escherichia coli, Y.pseudotuberculosis, Y.enterocolitica, Norovirus, Adenovirus, Atrovirus, Rotavirus . The study was conducted using Xpect Clostridium difficile Toxin A/B (Oxoid) test systems. Data processing was carried out using parametric and non-parametric statistical methods using Excel computer programs. The reliability of the differences was determined by calculating the Chi-square and proportional error at $P<0.05$.

Research results and their discussion. The degrees of microbiocenosis (dysbiosis) reflect changes in the composition of microorganisms in a specific location, most often in the intestine. Several degrees are distinguished, including significant changes in the quantitative and qualitative composition of the microflora, as well as disorders ranging from mild to severe, characterized by clinical manifestations.

Degree 1 compensated (latent) dysbiosis - quantitative changes in microflora composition, decrease in the number of normal microorganisms, but the ratio of bifido- and lactobacilli remains within the normal range. Clinical manifestations are usually absent.

Degree 2 subcompensated (localized) dysbiosis - slight decrease in bifidobacteria, increase in opportunistic pathogenic microorganisms, inflammatory processes in the intestine (enteritis, colitis). Clinical signs may be mildly present.

Degree 3 widespread dysbiosis - significant changes in the composition of the microflora, intestinal dysfunction of varying degrees.

Degree 4 disseminated (decompensated) dysbiosis - sharp decrease or absence of bifido- and lactobacilli, increase in E. coli, clinically manifested by severe intestinal dysfunction, bacteremia, septic bacteremia, septic complications, and dystrophic changes in internal organs.

1-Table. Degrees of intestinal dysbiosis in HIV-infected children with CHD.

Microbiocenosis degree	Degree I		Degree II		Degree III		Degree IV	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Main group HIV+ AVD+ **n=261**	13	5.0	45	17.2	134	51.3	69	26.4

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Comparison group HIV+ AVD- **n=238**	49	20.6	102	42.9	61	25.6	26	10.9

As can be seen from the table, in children of the main group (HIV+AVD+), grade I intestinal dysbiosis occurred 4.1 times less frequently than in children of the comparison group (HIV+AVD-), and grade II intestinal dysbiosis occurred 2.5 times less frequently (5.0%; 20.6% and 17.2%; 42.9% of cases, respectively, P<0.05). Grade III intestinal dysbiosis was observed in more than half of the children in the main group, which was 2 times significantly higher than in children of the comparison group (P<0.05). While grade IV intestinal dysbiosis developed in 26.4% of children in the main group, this grade of intestinal dysbiosis developed 2.4 times less frequently in children of the comparison group (P<0.05). The incidence of acute infectious diarrhea in HIV-infected children leads to more profound disorders in intestinal dysbiosis compared to HIV-infected children who did not have these infectious diarrheas.

2-Table Dependence of the degree of intestinal dysbiosis in children with AVD on the clinical stage of HIV infection

	Clinical stage II				Clinical stage III				Clinical stage IV			
	HIV+AVD + n =105		HIV+ AVD- n=95		HIV+AVD + n=123		HIV+ AVD- n=114		HIV+AVD + n= 33		HIV+ AVD- n= 29	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Grade I	11	10.5	29	30.5	2	1.6	19	16.7	0	0.0	1	3.4
Grade II	31	29.5	47	49.5	14	11.4	48	42.9	0	0.0	7	24.1
Grade III	45	42.9	14	14.7	69	56.1	34	29.8	20	60.6	15	51.7
Grade IV	18	17.1	5	5.3	38	30.9	13	11.4	13	39.4	6	20.7

2-In Table 0, we examined the state of intestinal microbiocenosis disorders in relation to the clinical stages of HIV infection in the children under our observation. According to the findings, in the clinical stage II of HIV infection, children in the main group showed significantly lower rates of grade I intestinal dysbiosis (almost 3 times less) and grade II intestinal dysbiosis (2.5 times less) compared to children in the control group. Conversely, grade III and IV intestinal dysbiosis were detected almost 3 times more frequently in the main group (P<0.05). In the clinical stage III of HIV infection, only 2 children (1.6%) in the main group and 16.7% of children in the control group developed grade I intestinal dysbiosis. The difference between the groups in the incidence of grade II intestinal dysbiosis was 3.8 times, which was statistically significant (P<0.05). In children of the main group with clinical stage III HIV infection, grade III intestinal dysbiosis was observed 1.9 times more frequently, and grade IV was 2.7 times more frequent compared to the control group (56.1% vs 30.9% and 29.8% vs 11.4%, respectively, P<0.05). 30.9% and 29.8%; While only 2 (1.6%) children in the main group with the III clinical stage of HIV infection and 16.7% of children in the comparison group developed grade I intestinal dysbiosis, the significant difference between the groups in the indicators of grade II intestinal dysbiosis was 3.8 times (P<0.05). In children of the main group, at the III clinical stage of HIV infection, the III degree of

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intestinal dysbiosis was 1.9 times and the IV degree 2.7 times significantly more often than in children of the comparison group (56.1%; 30.9% and 29.8%; 11.4% of cases, respectively, P<0.05).

At the IV clinical stage of HIV infection, intestinal dysbiosis of I and II degrees was not observed in children of the main group. In the comparison group, only one child (3.4%) developed intestinal dysbiosis of I degree, and 24.1% developed intestinal dysbiosis of II degree (P<0.05). At this clinical stage of HIV infection, no significant differences were found between the groups for grade III intestinal dysbiosis (P>0.05). However, the significant difference between the groups for grade IV intestinal dysbiosis was 1.9 times higher (P<0.05).

As the clinical stage of HIV infection progressed, an increase and aggravation of intestinal dysbiosis were observed. Intestinal dysbiosis of III and IV degrees was more common in the main group than in the comparison group, especially in the III and IV clinical stages. At stage IV, significant differences were observed only for grade IV intestinal dysbiosis. 2-table presents an analysis of intestinal dysbiosis levels in relation to the degree of immunodeficiency in HIV infection. In cases of severe and pronounced immunodeficiency, intestinal dysbiosis of grades I and II was not observed in children of either comparative group. Grade III intestinal dysbiosis occurred 2.4 times less frequently in children of the main group with severe immunodeficiency compared to the comparison group, while grade IV dysbiosis developed 3 times more frequently (33.3% vs. 80% and 66.7% vs. 20.0% of cases, respectively, P<0.05). In cases of pronounced immunodeficiency, although no significant differences were found between the comparative groups for grade III intestinal dysbiosis (P>0.05), grade IV intestinal dysbiosis was recorded 1.8 times more frequently in children of the main group compared to the comparison group (P<0.05). 20.0% of cases, respectively, P<0.05).

In HIV infection with moderate immunodeficiency, grade I intestinal dysbiosis was not observed in children of the main group, while in the comparison group, this indicator was detected in only 2 (2.9%) children. Grade II intestinal dysbiosis with moderate immunodeficiency in HIV infection was detected in 2.7% of children in the main group, while it was reliably noted in 1/3 of children in the comparison group (P<0.05). Although no significant differences were observed between the indicators of both comparative groups for grade III intestinal dysbiosis at a moderate level of immunodeficiency, the significant difference for grade IV was 2.1 times higher (P<0.05).

Degrees of intestinal dysbiosis in children of the comparison group versus children of the main group with a low level of immunodeficiency in HIV infection showed reliable differences between indicators: For grade I - 3.9 times, for grade II - almost 2.0 times, for grade III - 4.7 times, and for grade IV - 3.2 times (P<0.05).

In HIV-infected children with acute infectious diarrhea, intestinal dysbiosis of grades III and IV developed more frequently compared to HIV-infected children without diarrhea, at a high degree of immunodeficiency. At a low level of immunodeficiency, significant differences were observed between grades I, II, III, and IV of intestinal dysbiosis. 2-An analysis of intestinal dysbiosis levels in relation to viral load in HIV infection was conducted in the table. In both groups, patients with HIV infection with a viral load of more than 100,000 copies and in the range from 10,000 to 100,000 copies did not develop intestinal dysbiosis of I and II degrees. However, no significant differences were observed in the indicators of intestinal dysbiosis of III degree, as well as in the indicators of intestinal dysbiosis of IV degree in children of both groups with HIV infection with a viral load of more than 100,000 copies (P>0.05). In children of the main group with a viral load in

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the range of 10,000 to 100,000 copies of HIV infection, intestinal dysbiosis of the IV degree developed 1.6 times more often than in children of the comparison group, showing a significant difference (P<0.05).

In children of the main group with a viral load from 1000 to 10000 copies of HIV infection, grade I intestinal dysbiosis was noted in only one (1.8%) patient, while in patients of the comparison group, this degree of dysbiosis did not develop. In this range, in children of the main group with a viral load of HIV infection, grade II intestinal dysbiosis developed 3.4 times less frequently than in patients of the comparison group, while grade IV, on the contrary, developed almost 1.7 times more frequently, showing a significant difference (P<0.05). In patients of both groups, intestinal dysbiosis of III degree showed no significant difference between the indicators (P>0.05).

Significant differences were observed in intestinal dysbiosis levels between children in the main group with HIV viral load below 1000 copies and children in the comparison group. The differences were 4.7 times for grades I and IV, 2.3 times for grade II, and 3.2 times for grade III (P<0.05).

In children with acute infectious diarrhea and HIV viral load below 1000 copies, significant differences were found in grades I, II, III, and IV of intestinal dysbiosis compared to the comparison group. However, with high HIV viral load, the differences between grades III and IV of intestinal dysbiosis were not significant.

Conclusions:

1. The incidence of acute infectious diarrhea in HIV-infected children led to the development of more severe intestinal dysbiosis compared to HIV-infected children without infectious diarrhea. Grade IV intestinal dysbiosis developed in 26.4% of children in the main group, while this grade of intestinal dysbiosis occurred 2.4 times less frequently in children of the comparison group.
2. In children with acute infectious diarrhea, a significant increase in the degree and severity of intestinal dysbiosis was observed in many cases as the clinical stage of HIV infection progressed, compared to children without infectious diarrhea. At the IV clinical stage of HIV infection, no significant differences were found between the groups for grade III intestinal dysbiosis (P>0.05), however, the significant difference between the groups for grade IV intestinal dysbiosis was 1.9 times higher (P<0.05).
3. Intestinal dysbiosis of the III degree in severe immunodeficiency was 2.4 times less frequent in children of the main group compared to children of the comparison group. In contrast, grade IV intestinal dysbiosis developed 3 times more frequently in the main group.
4. In children with acute infectious diarrhea, when the viral load in HIV infection was less than 1000 copies, significant differences were found in intestinal dysbiosis of I, II, III, and IV degrees compared to the comparison group. However, with a high viral load in HIV infection, the differences between intestinal dysbiosis of III and IV degrees were not significant. In children of the main group with a viral load in the range of 10,000 to 100,000 copies of HIV infection, intestinal dysbiosis of the IV degree developed 1.6 times more often than in children of the comparison group.

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