EFFECTS OF PROBIOTICS ON INTESTINAL MICROFLORA
OF PATIENTS WITH HIV

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HIV-1 infection results in structural damage to the intestinal mucosa and changes of gut microflora following dysfunction of the gastrointestinal system, including compromised barrier function. Known properties of probiotics suggest that they may be useful tools in restoring normal intestinal flora. Our study goal was to determine whether the use of a probiotics can recover normal gut flora in chronically HIV-infected adults. Standard bacteriological methods were used to exam the gut microbial content of the HIV-infected patients. Changes of intestinal microbiota were found in all of the patients. In the most cases the decrease of obligatory microorganisms, especially Bifidobacterium spp. (in 90% of patients) was found. Overgrowth of major opportunistic pathogens (S. aureus and Candida spp.) was registered in only a minority of patients. The probiotic interventions resulted in significantly elevated levels of beneficial bacteria load (such as Bifidobacterium spp., Lactobacillus spp.) and a decrease in pathogenic bacteria load (such as Clostridium, Candida spp.). Probiotic preparations can successuflly augment the levels of beneficial species in the gut during chronic HIV-1 infection. These findings may help inform future studies aimed at testing pre- and probiotic approaches to improve gut function and mucosal immunity in chronic HIV-1 infection.

Key words: HIV-infection, intestinal microflora, probiotics.

Objective. It is known that the total number of microorganisms inhabit the human gut ($10^{14}$), on two orders exceeds total number of the cells. Besides, a large number of exogenous xenobiotic including pathobionts and food antigens passes through the intestine daily. It’s not a surprise that up to 80% of the lymphoid tissue is associated with the intestine region (GALT).

Indigenous intestinal microflora has a symbiotic relationship with the intestinal mucosa and is an integral part of the gastrointestinal tract. Close interaction between the microbiota and mucosa is a major imperative of intestinal homeostasis [6, 12]. It has been found out recently that dysbiotic changes in the gut (dysbiosis) accompany not only various intestinal disorders, but are also associated with a wide range of multi-organ pathologies, including HIV infection [5, 17].

HIV has been established to infect and destroy vast amount of GALT CD4+ T cells and dendritic cells, as well as affect directly enterocytes: HIV tat protein inhibits glucose uptake by enterocytes, impairing their function, gp120 protein increases the amount of calcium in the cells, which causes depolymerization of tubulin and, consequently, dysfunction of cytoskeleton. This leads to disruption of intercellular interaction and increased permeability of the intestinal barrier. At the same time, the expression of genes that control the integrity of epithelium is suppressed [9].

Villus atrophy, crypt hyperplasia, malabsorption of several important nutrients, apoptosis of enterocytes, and increased permeability of epithelium characterize enteropathy accompanied by HIV. Mass deaths of the immune effector cells in the lamina propria, destruction of Peyer’s patches, and a sharp reduction of secretory IgA and defensins levels create favorable conditions for the breeding of excessive microflora including pathogenic one in the intestinal lumen [16].

These factors lead to the penetration of LPS and other bacterial components through the intestinal barrier into the blood circulation although bacteremia is not observed as a rule. Translocation of LPS and chronic exposure to peripheral lymphocytes result in persistent systemic immune response accompanied by high level of proinflammatory cytokines, which fairly soon leads to the depletion of the immune system. It is believed that translocations and chronic immune activation play a key role in the development and progress of opportunistic complications [1, 11].

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Methods. This opens up the prospect of influence on the infectious process by correcting dysbiotic changes in HIV-infected patients. In this regard, the aim of the present study was to evaluate changes in microflora of the large intestine in chronic HIV infection and the possibility of correction by means of bacterial preparations (probiotics).

Materials and methods. The study involved 40 HIV-1-infected patients of the regional center of prevention and control of AIDS in Kharkov. All the patients were informed about the purpose and plan of study and gave their written agreement to participate in the study. All the patients had been diagnosed according to the criteria of WHO with the III-IV stage of HIV infection. During the month before the survey the patients did not take any antibiotics. Dysbiosis correction circuit was designed for one month taking of probiotic preparations. Six weeks later the follow-up study was conducted to investigate gut microflora of 20 HIV-infected patients.

Bacteriological methods. Stool samples were placed on solid media (HiMedia Lab., India) and analyzed with accordance to standard procedures. Summarized data of healthy adults microflora contents served as a normal standard [13–15].

Statistical methods. The results are presented in the form of averages, standard deviation and median assuming a normal distribution of data. The Shapiro – Wilk test, verified normal distribution of quantitative traits. The research results are processed using “STATISTICA 10.0” (StatSoft Inc., USA, version 10.0.1011.6) and spreadsheet editor Microsoft Excel 2013.

Results and discussion. 27 of 40 (67.5 %) HIV-infected patients participated in the study were women and 13 (32.5%) men. The average age of patients was (35.6 ± 8.2) years. The average number \((M \pm m)\) of CD4+ T cells before the study was 426 ± 264 in 1 µl (table 1).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Sex, n/%</th>
<th>Age</th>
<th>CD4+T (cells/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-infected (n = 40)</td>
<td>13/32.5</td>
<td>27/67.5</td>
<td></td>
</tr>
<tr>
<td>(M \pm m)</td>
<td></td>
<td>35.65 ± 8.20</td>
<td>426 ± 264</td>
</tr>
<tr>
<td>median</td>
<td></td>
<td>34</td>
<td>416</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>All ((n = 40))</td>
</tr>
</tbody>
</table>

As it can be seen from table 2, the quantitative and qualitative composition of the normal microflora of the large intestine has been broken in all patients. Changes are identified among anaerobic and facultative anaerobic bacteria. It should be noted that violations of microbiota not followed emerging opportunistic pathogens. Reduced number of organisms concerned primarily bifidobacteria, which dominated in the anaerobic flora and accounted for about 95% of the intestinal microbiota. According to our data, in (90.00 ± 4.74) % of cases the number of bifidobacteria was less than \(10^6\) CFU/g and in (10.00 ± 4.74) % of cases it was about \(10^7\) CFU/g. The number of very important lactobacilli at HIV infection is significantly reduced against healthy controls [17] accounting less than \(10^5\) CFU/g in (87.5 ± 5.2) % and \(10^6–10^7\) CFU/g in (12.5 ± 5.2) % of patients against \(10^7–10^8\) CFU/g respectively (P < 0.05).

The group of anaerobic bacteria, Bacteroides, wasn’t detected in patients. The leading representative of the facultative anaerobic bacteria belonging to the indigenous microflora is \(E.\ coli\). The number of bacteria of this type in about half of virus carriers remained constant while the other were reduced by 1–2 orders of magnitude. Hemolytic \(E.\ coli\) strains in small concentrations detected in only (5.0 ± 3.4) % investigated.

The same trend is observed in relation to other pathobionts: \(S.\ aureus, S.\ epidermidis,\) and \(C.\ albicans\) in low titers are found in only a minority of infected patients (P < 0.05). Only in one patient \(Clostridia\) were isolated in very low concentrations. In addition, a case of a serious intestinal dysbiosis in HIV-infected patients was accompanied by falling down on 1–2 orders of the obligate commensals \(E.\ faecalis,\) and \(E.\ faecium\) presented in large numbers in the faeces of healthy adults.
Thus, HIV infection, regardless of the duration of the course, clinical stage of the disease and antiviral managing manifests a profound violation of the gut homeostasis accompanied by a simultaneous decrease in quantitative anaerobic (Bifidobacteria and Lactobacilli) and facultative anaerobic flora (E. coli).

Other researchers have identified serious changes of the intestine microflora in chronic HIV infection. Deep changes of intestinal microbiota is accompanied by the appearance of enteropathogenic bacteria capable of converting tryptophan to kynurenine immunomodulatory derivatives, which correlates with the progression of the disease and contributes to the violation of mucosal immunity.

At the same time ART-naïve patients increases the levels of some bacterial taxa, and the suppression of 45 taxa. The most significant enrichment was mentioned for Erysipelothricaceae, which often accompanies obesity and is associated with increased incidence of cardiovascular diseases. Erysipelothricaceae, Escherichia coli, Enterobacter aerogenes, and Bacillus subtilis were found to be significantly enriched in the gut microbiota of patients with HIV infection.

Table 2. Composition of gut microbiota in HIV-1 infected patients

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>The normal level of intestinal microorganisms CFU/g</th>
<th>The level of microorganisms before the correction of probiotic preparations</th>
<th>The level of microorganisms after the correction of probiotic preparations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Concentration of bacteria CFU/g</td>
<td>The number of patients (n = 40)</td>
</tr>
<tr>
<td>Bifidobacterium spp.</td>
<td>10^9–10^10</td>
<td>&lt; 10^6</td>
<td>36</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>10^7–a</td>
<td>&lt; 10^5</td>
<td>35</td>
</tr>
<tr>
<td>E. coli (lac+)</td>
<td>10^9</td>
<td>&lt; 10^5–10^9</td>
<td>5</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>10^9</td>
<td>&lt; 10^5</td>
<td>25</td>
</tr>
<tr>
<td>E. faecium</td>
<td>10^9</td>
<td>&lt; 10^5</td>
<td>15</td>
</tr>
<tr>
<td>E. coli Hly</td>
<td>–</td>
<td>&gt; 10^5</td>
<td>6</td>
</tr>
<tr>
<td>S. aureus</td>
<td>–</td>
<td>&lt; 10^5</td>
<td>33</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>&lt; 10^4</td>
<td>&lt; 10^4–10^5</td>
<td>27</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>&lt; 10^4</td>
<td>&lt; 10^4–10^5</td>
<td>32</td>
</tr>
<tr>
<td>Cl. perfringens</td>
<td>&lt; 10^3</td>
<td>&lt; 10^3–10^4</td>
<td>38</td>
</tr>
</tbody>
</table>

*P < 0.05 versus normal.
system disorders. Such types as *Proteobacteria* are part of the most enriched genera of ART-naïve patients. Among them is the species included in the genera of *Salmonella*, *Escherichia*, *Serratia*, *Shigella* and *Klebsiella* of the *Enterobacteriaceae* family, known as pro-inflammatory pathobionts. The gut content of ART-naïve HIV-carriers is enriched with *Staphylococcus*, *Pseudomonas*, *Campylobacter* spp., *Candida albicans*, which often cause opportunistic infections and bacteremia, with a significant decrease in the content of bifidobacteria and lactobacilli, *Clostridia* and *Bacteroides* with particularly strong suppression of *Bacteroides* and *Alistipes* genera [2, 8].

At the same time, Saxena et al. concluded that HIV infection might be more diversity of microbes in the intestinal level of the genus, but had only limited quantitative and qualitative impact on the overall microbiome [5].

In our study, the use of probiotic bacterial preparations on the background of the microbiome dysbiosis in HIV-infected patients resulted in a significant mitigation of these violations, but complete restoration was not also observed (Table 2).

Probiotic preparations used for the dysbiosis correction contained such strains of microorganisms as:

1. *Lactobacillus casei*, *L. rhamnosus*, *L. acidophilus*, *L. bulgaricus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *B. longum*;
2. *Bifidobacterium bifidum*, *Escherichia coli*, strain M-17;
3. *Saccharomyces boulardii*.

Recently it was shown the combination of probiotics in the model system can increase the level of Treg-cells, and suppress the development of the disease [10].

Activity had only a mixture of several species of Lactobacilli. Suppressive activity was accompanied by increased secretion of IL-10 Treg-cells, which led to a weakening of the secretion of pro-inflammatory cytokines by cells Th1 and Th17.

The model system also proved that the administration of *L. acidophilus*, *L. casei*, *L. reuteri*, *Bifidobacterium bifidum* and *Streptococcus thermophilus* induced a low response of T and B cells, reduced the secretion of Th1, Th2, and Th17 cytokines, inhibited apoptosis and caused migration of Treg cells in the inflammatory focus [3]. Probiotics have a beneficial effect on the HIV-infection. [4]. This is accompanied by a simultaneous increase of the levels of bifidobacteria and decrease levels of *Clostridium coccoides*, *Eubacterium rectale*, *Clostridium lituseburense* and *Clostridium histolyticum* [7]. These findings thus suggest that the correction of dysbiosis can have desirable effects in the restoration of intestinal function and repair. Thus, the mechanism of probiotic bacteria effect on the immune status and HIV infection may influence on the translocation and activation of the immune balance of regulatory T-lymphocyte subpopulations.

**References**


ВПЛИВ ПРОБІОТИКІВ НА МІКРОФЛОРУ КИШЕЧНИКУ ВІЛ-ІНФІКОВАНИХ

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Наведено оцінку стану мікрофлори товстої кишки хворих на хронічну ВІЛ-інфекцію до та після прийому бактерійних препаратів. Стан мікрофлори кишечнику 40 ВІЛ-інфікованих визначали звичайним бактеріологічним методом. Дослідження показало, що у всіх обстежених був порушенний якісний та кількісний склад мікрофлори кишечнику. Зміни виявили як серед анаеробних (бифідо- і лактобактерій), так і серед факультативно-анаеробних бактерій (E. coli). Надмірний ріст умовно-патогенної мікрофлори (S. aureus, C. albicans) спостерігали лише у деяких пацієнтів. Після корекції дисбіозу виявлено збільшення кількості бактерій облігатної мікрофлори (Bifidobacterium spp., Lactobacillus spp.) та зниження рівня умовно-патогенної мікрофлори. Отже, дослідження показали, що пробіотичні препарати позитивно впливають на якісний і кількісний склад мікрофлори кишечнику ВІЛ-інфікованих, що проявилося у збільшенні рівня пробіотичних видів бактерій в кишечнику та зменшенні кількості умовно-патогенної і патогенної мікрофлори.

Ключові слова: ВІЛ-інфекція, мікрофлора кишечнику, пробіотики.