The effect of oral probiotics on CD4 count in patients with HIV infection undergoing treatment with ART who have had immunological failure

Masoud Mortezazadeh[1], Saeed kalantari[2], Nooshin Abolghasemi[3], Mitra Ranjbar [2], Saeedeh Ebrahimi[4], Abbas Mofidi[2], Babak pezeshkpour[2], Ensieh Sadat Mansouri[1], seyed zia tabatabaei[1], and MEHDI KASHANI[1]

[1]Tehran University of Medical Sciences
[2]Iran University of Medical Sciences
[3]Islamic Azad University
[4]Alborz University of Medical Sciences

January 30, 2024

Abstract

Introduction: Probiotics are live microorganisms that, when administered in appropriate colonies, can delay the destruction of the immune system and contribute to the maintenance of immunity in HIV patients. Probiotics play an important role in stimulating natural killer T cells, strengthening the functional gut barrier, and reducing systemic inflammation. Methods: This study was a randomized double blind clinical trial involving 30 patients treated with ART who had experienced immunological failure despite HIV viral suppression. Patients were divided into two equal groups of 15, the first group received 2 probiotic capsules daily with a colony count of 109CFU per capsule containing seven strains ,after three months they were examined for CD4+ counts by flow cytometry, after a one month washout period the participants who had received probiotics were switched to placebo, and the participants who had received placebo were given probiotics for three months, and they were examined for CD4+ counts seven months after the start of the study. Results: In the first group, administration of the placebo resulted in a decrease in CD4 count in the first three months (from 202.21 to 181.79, p-value < 0.001),which may be due to the natural history of the disease. After probiotics administration, CD4 count increased significantly (from 181.79 to 243.86, p-value < 0.001). Overall, after 7 months of study, there was a significant increase in the mean CD count from 202.21 to 243.86 (p-value < 0.001). In the second group, administration of probiotics in the first three months of the study resulted in a significant increase in the mean CD4 count (from 126.45 to 175.73, p-value < 0.001).Termination of treatment with probiotics resulted in a significant decrease (from 175.73 to 138.9, p-value < 0.001)but overall the CD4 count at the end of the study was significantly higher than at baseline (p-value < 0.001).

Introduction :

Antiretroviral therapy (ART) is the basis of treatment for adults with a CD4+ count of less than 350 cells per microliter or with a CD4+ count that has any of the following conditions: Concurrent hepatitis B or a sexual partner with this disease, HIV-induced nephropathy, age > 50, active tuberculosis, a viral load greater than 100000, or malignancy associated with HIV or malignancy requiring chemotherapy/radiotherapy.[1]

Viral load and CD4+ count are two factors that determine treatment success.

virologic failure is defined as incomplete response or no response from HIV RNA to ART or virologic recurrence, as defined below:

Incomplete virologic response: more than 400 copies HIV RNA per milliliter after 24 weeks of treatment with HAART or more than 50 copies/ml at week 48 of treatment in a patient receiving treatment for the first time.

Virologic relapse: HIV RNA up to 400-1000 copies/ml, four to eight weeks after viral suppression, in two sessions.[2]

Immunologic failure: an increase in CD4 cell count of 150 per cubic millimeter is expected in the first year of HIV treatment. If this increase is less than 25 to 50 in the first year, or if the CD4 count does not increase from baseline despite decreased viral load, or if CD4 cells are consistently below 100 cells per cubic millimeter or decrease by 50% of the maximum CD4 count during treatment, immunologic failure is present.

Generally, immunologic failure follows virologic failure, and then clinical exacerbations occur. However, these may occur months to years apart and do not necessarily occur in the order mentioned. [3]

In the patients in our study, viral load was minimized and the desired virologic response was achieved, but despite this success, we observed immunologic failure , so according to previous studies we try to increase the gut microflora by probiotics especially with high count of lactobacilli to reduce intestinal inflammation and achieve immunologic response .

Probiotics are live microorganisms that, when administered in adequate numbers of colonies, can delay immune destruction and help maintain immunity in HIV patients. A study by Ruben Hummeln et al.(9) investigated the effect of probiotics on the immune system of patients with HIV. The results indicate that at the end of week 25, the group that received placebo in combination with ART had an increase of mean 19 CD4 cells per microliter and the group that received probiotics in combination with ART had an increase of mean 46 CD4 cells per microliter. [4]

In another study of the effect of probiotics on two groups of HIV patients, 19 patients received probiotics with a colony count of $2x10^9$ CFU/day and 25 patients received placebo. At the end of week 25, the CD4 count of patients in the probiotics and placebo groups increased by 19 and 46 cells, respectively. [5]

Several other studies have shown the effect of probiotics on increasing CD4 cell counts. Based on these results, we designed this study to investigate the effect of probiotics in patients who have immunologic failure despite virologic success.

Method :

This study was a randomized double-blind clinical trial involving 30 patients treated with ART at West Medical Center, Tehran, Iran, who had experienced immunological failure despite HIV viral suppression.

Patients were divided into two groups of 15. The first group received 2 probiotic capsules daily with a colony count of 109CFU per capsule containing seven strains (including Lactobacillus casei , Lactobacillus acidophilus, Lactobacillus rhamnosus , Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium longum , Streptococcus thermophiles with prebiotic froctooligosaccharide .

By the end of the month 3, patients were tested for CD4+ count using flow cytometry and were asked to fill questionnaires concerning the history of diarrhea or intake of the antibiotic, especially Cotrimoxazol .

after a one- month washout period the participants who had received probiotics were switched to placebo, and the participants who had received placebo were given probiotics for three months, and they were examined for CD4+ counts seven months after the start of the study.

Figure 1: patients intervention tree diagram .

Inclusion criteria included:

1. patients with a plasma level HIV RNA of less than 200 HIV-1 RNA copies/ml or a decrease in plasma viral load of more than 1 log10

2. patients with immunologic failure if CD4 count decreased to pre-therapy baseline (or below) or decreased by 50% from peak during treatment (if known) or CD4 count remained below 100 cells/mm3 6 months after ART initiation

The exclusion criteria included:

1. patients taking antibiotics during the study
2. patients with concurrent hepatitis B and C
3. patients not adhering to pill regimen
4. pregnancy during the study
5. active infectious diseases
6. surgical procedures 6 months prior to or during the study on the gastrointestinal tract
7. severe liver or kidney failure**.**

Determination of CD4+ count:

The sample needed for this test is whole blood collected with EDTA anticoagulant. The measurement of CD4+ cells is based on the flow cytometry method. In this method, the blood is first incubated with specific amounts of antibodies conjugated with fluorescent substances such as phycoerythrin, the anti-marker to be measured; the cell suspension is then analyzed in a flow cytometer and the values are recorded.

Statistical analysis :

Chi-square test was used for comparison of qualitative variables and t-test or Mann-Whitney U test was used for analysis of quantitative variables. The effect of probiotics was studied using the logistic regression model.

Result :

Thirty patients participated in the study, 5 women and 25 men, and were randomly assigned to two groups. During the first 3 months, five patients voluntarily withdrew from the study (4 in the experimental group, one in the placebo group). We continued the study to the end with 25 patients, 5 women (20%) and 20 men (80%). Our groups included 56 and 44 percent of patients.

The average age of the remaining subjects was 41.4 years (30.8 years for women, 43.9 years for men) and the average duration of treatment with ART was 4.4 years.

Chart 1 shows how participants participated in each part of the study.(figure 1)

The mean CD4 count before administration of probiotics was 168.88 (SD =71.072) in all patients. After our first intervention, administration of placebo and probiotics for groups A and B, we observed a significant increase in CD4 count in group A compared with group B (mean of CD4 count changes: Group A=-20.4286, Group B=49.27, difference(A-B)=-69.70, CI(95%): (-97.96 ˜ -41.43), p-value < 0.001).

In our second intervention, we exchanged the two groups and administered probiotics for group B and placebo for group A. Again, we observed a significant increase in CD4 counts in the probiotic group compared to the previous phase (mean of CD4 count changes: Group A=62.071, Group B=-36.81, Difference(A-B)=98.88, CI(95%): (70.66˜127.11), p-value < 0.001).

Next, we examined the effect of probiotics on each group separately. We wanted to see how the beginning and the end of taking probiotics affected the CD4 count. In group A, administration of the placebo resulted in a decrease in CD4 count in the first three months (from 202.21 to 181.79, p-value < 0.001), which could be due to the natural history of the disease. After probiotics administration, CD4 count increased significantly (from 181.79 to 243.86, p-value < 0.001). Overall, after 6 months of study, there was a significant increase in mean CD count from 202.21 to 243.86 (p-value < 0.001).

In group B, administration of probiotics in the first three months of the study resulted in a significant increase in mean CD4 count (from 126.45 to 175.73, p-value < 0.001). Termination of treatment with probiotics resulted in a significant decrease (from 175.73 to 138.9, p-value < 0.001), but overall the CD4 count at the end of the study was significantly higher than at baseline (p-value < 0.001). (table 1)

Our analyses on other variables such as age, gender, duration of treatment with ART and duration of disease did not reveal significant associations.

Table 1: mean CD4 changes in group A&B

Groups	Mean CD4 difference	Standard deviation	P-value
Placebo group (A) first three months (n=14)	-20.4286	36.35237	0.001
Probiotic taken group(B) first three months (n=11)	49.2727	30.44697	0.001
Probiotic group(A) 2^{nd} three months (n=14)	62.0714	40.48070	0.001
Placebo group(B) 2^{nd} three months (n=11)	-36.8182	22.53361	0.001

Discussion :

The intestinal microbiota plays an essential role in stimulating the development and maintenance of the intestinal immune system .

HIV studies showed that HIV can wreak havoc in the gut, where there are many CD4 cells , This apparently happens fairly soon after infection with HIV.[6]

In addition, modern antiretroviral drugs, although well tolerated, cause severe gastrointestinal symptoms such as nausea, vomiting, bloating, or diarrhea in mild to severe forms.[7]

Previous studies demonstrated that Yoghurt enrichment with probiotics as a snack may increase CD4 levels and protect against some HIV-related gastrointestinal infections and local inflammations .

This local inflammation can lead to new collagen formation and fibrosis, which contribute to the deletion of CD4+ T cells and limit the recovery of the immune response. An increased predisposition to collagen neogenesis may be related to a decreased population of virgin CD4+ T lymphocytes whose phenotype is not activated before antiretroviral therapy. Fibrosis may be one of the causes of failure of immune recovery during treatment despite suppressed viral replication.[8]

After the HIV virus infects the CD4 cells, it plants itself in several organs, including the MALT system of the digestive system. In this organ, the HIV implants itself in up to ten times the amount than in the bloodstream.

At this stage, the virus begins to mix its genome with that of the intestinal lymphoid cells. Treatment with ART can combat the virus before this fusion, but after genetic fusion and entry into the silent phase, the virus is inaccessible to the immune system and antivirals.

Probiotics with their effect on enteric translocations bring these infected cells out of their silent phase and expose them to the immune system and pharmacological agents, thereby reducing the burden on the infected reservoir.

In addition to restoring the balance of the gut microbiota in terms of competition with pathogens and improving the intestinal barrier, probiotics also play an important role in restoring mucosal immune function through the Th17/Treg ratio, which reduces systemic and local inflammation in the gut .[9]

Probiotics also may promote epithelial healing by altering intestinal flora and reduce the risk of viral transmission and hospitalization for coinfection by preventing the decline in CD4+ cell count. ART -treated patients who do not show an immunological response (CD4 < 200) have lower lactobacilli levels, increased LPS and sCD14 levels, and increased inflammatory markers such as IL -6 and sCD14.[10]

So in this study we administered kind of probiotics with high count of lactobacilli then this intervention can lead to reducing inflammation in intestinal mucosa and make a good response for HIV patients with immunologic failure .

Our results show that administration of probiotics increased CD4 cell counts compared with placebo. After discontinuation of probiotics, CD4 counts approached baseline values again, but were still significantly higher.

Probiotic administration resulted in an average increase of 62 and 49 CD4 cells per microliter in both groups, respectively.

our study showed that the number of CD4+ cells increases by taking probiotics but decreases back to baseline levels after stopping probiotics. The results could also mean that by administering probiotics for a longer time, we could expose more infected cells and achieve a better result.

On the other hand previous studies showed that a CD4 count of < 50 cells/mm3 was a main predictor of treatment failure. This finding may be due to the fact that patients with baseline CD4 counts of [?]100 cells/mm3 have reduced immunity and response to initial treatment ART may be unsatisfactory . [3]

Therefore longer period probiotic intervention in this group of study can help them to overcome immunologic failure in persistent CD4 counts above 100 cells/mm3 .

It is also recommended to consider other Reasons cited for immunologic failure include the role of the thymus gland, which declines with age, and drug toxicity of zidovudine, which clinically causes anemia, neutropenia, pancytopenia, and granulocytopenia in 45% of recipients of this drug. [11]

In this type of failure, it is also recommended to first rule out other causes of immunosuppression, such as HIV2, HTLV1, HTLV2, and drug toxicity, and the combination of didanosine and tenofovir is also among the causes that lead to a decrease in CD4 increase and a lack of appropriate immunological response.[12]

In our study we excluded all the mentioned causes of immunologic failure .

In another study by Livia Trois, conducted on 77 HIV-infected children aged 2-12 years, they were divided into two groups: one group received probiotics containing Bifidobacterium bifidum with Streptococcus thermophilus 2.5 x $10^{(10)}$ CFU for two months, and the other group received a standard diet (control group). CD4 counts were collected at baseline and at the end of the study. The mean CD4 count increased in the probiotic group (791 cells/mm3), whereas it decreased slightly in the control group (538 cells/mm3). The change in mean CD4 cell count from baseline was +118 cells/mm3 versus -42 cells/mm3 in the children receiving the probiotic diet and the control group, respectively ($p = 0.049$).

. [13]

In a similar study from Tanzania After initiation of yogurt enriched probiotic consumption, an additional increase of 0.28 cells/μL/day (95% CI; 0.10-0.46, P=0.003) was observed. After adjustment for duration of antiretroviral drug use, the additional increase explained by yogurt consumption remained at 0.17 cells/μL/day (95% CI; 0.01-0.34, P=0.04) which was also significant**.[14]**

compared to previous studies, the patients we studied suffer from immunologic failure and given the low CD4 count of the patients in our study, most are classified as stages 3 and 4 of AIDS. This shows the importance of CD4 count, as their low levels can cause numerous clinical adverse events and increasing CD4 count can significantly improve patients' quality of life.

conclusion: the use of probiotics to prevent and attenuate various gastrointestinal manifestations and improve immunity of intestinal-associated lymphoid tissue in HIV infection by modulating epithelial barrier functions and microbiota composition can result in CD4+ count rise in hiv patients with immunologic failure

Daily administration of probiotics at appropriate doses-two 109CFU capsules in our study-in HIV patients who had immunologic failure despite virologic response lead to a transient increase in CD4+ counts.

Declarations:

1. Funding : No funding were used to support this study

2. Conflicts of interest/Competing interests: The authors declare that there are no conflict of interest to declare.

3. Ethics approval: In this study, no additional costs were imposed on the patients. We maintained the patients privacy, and their written consent were obtained.

The trial registered in ethical committee of Iran university of medical sciences with ethical number of (IR.IUMS.REC 1395.8821215220)

4. Consent to participate:The patients had consented to the participation of this article.

5. Consent for publication : The participants had consented to the publication of this article.

6. Availability of data and material :The data that support the findings of this study are available from the corresponding author, [MM], upon reasonable request.

7. Code availability (Not applicable)

8. Authors' contributions :M.M, S K . and N.A . contributed to data collection, writing, drafting of the manuscript and critical appraisal of the manuscript. A.M, B.P, S.E, M.R, M.K, Sz.T , E.M. contributed to scientific writing and final revision

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