Effect of probiotic consumption on increasing the CD4+ T cell counts among Iranian patients living with HIV

A double-blind randomized clinical trial

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Abstract

Purpose – During the ART era, persistent immune activation remains a significant challenge in people living with HIV (PLWH). Microbial translocation play an essential role in this setting. Probiotics have several immunological benefits which can reverse this process. The purpose of this paper is to investigate the safety and efficacy of probiotics on CD4 counts among Iranian PLWH.

Design/methodology/approach – In total, 50 PLWH with CD4 counts above 350 cells/mm^3 did not receive ART participated in a randomized, double-blind trial and underwent 24 weeks of treatment with either LactoCare® or placebo twice daily. CD4 counts of the patients were measured at baseline, 12 weeks and 24 later in the two groups. Side effects were measured monthly using a specific checklist.

Findings – The mean CD4 count of the patients showed a significant difference between the two groups after six months. Through six months follow up, the mean CD4 count of the patients showed a significant reduction as compared to the baseline in the placebo group; however, it did not show a significant difference in the probiotic group. Repeated Measures Anova test showed a significant effect for time × treatment interaction on the CD4 count during the trial course. No significant difference between the two groups concerning adverse events was reported.

Originality/value – It seems the use of probiotics in PLWH with a CD4 count above 350 cells/mm^3 who are not receiving antiretroviral drugs is safe and can reduce the devastating process of CD4+ T cells in these patients.

Keywords HIV, Probiotic consumption, CD4 count, Iran

Paper type Research paper

Introduction

In spite of three decades since the rise of the HIV infection in the USA, no curative treatment or effective vaccine has been discovered. With respect to newly antiretroviral therapy (ART), AIDS-related deaths fell from 1.9m in 2004 to 940,000 in 2017. It is

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estimated that more than 36m people were infected by the end of 2017, reflecting the enormous burden of disease on the international community[1]. In Iran, by the beginning of 2018, a total of 36,571 people were diagnosed with HIV. However, the true figure is estimated to be much higher. Therefore, any attempts to understand the mechanisms of disease sustainability or progression as well as the strategies to contain and deal with the disease is essential[2].

The universal use of ART in the past decades has led to a change in the face of HIV infection from a deadly disease to a chronic one. Therefore, non-infectious complications such as cardiovascular disease, diabetes, metabolic syndrome, obesity and premature aging, all mediated by chronic inflammation, are more common in patients[3]. Immune system activation persists despite using ART. Growing evidence supports the findings that intestinal mucosa breakdown during SIV/HIV infections might lead to the release of microbial products into the bloodstream that stimulates the immune system. This phenomenon could be due to the structural, microbial and immunological changes in the intestine during the infection[4]. Low-level viremia and other simultaneous viral infections, such as cytomegalovirus (CMV) and hepatitis C virus (HCV), are also associated with inflammation persistence[5].

The shift in intestinal microbial flora is common in all HIV disease stages, regardless of the presence of opportunistic infection. In one study, the most frequent changes were found in the number of essential intestinal microorganisms, especially in bifidobacteria. Increased growth of pathogenic organisms, mainly Staphylococcus aureus, Candida and Klebsiella, was observed in 57.1 percent of the patients[6].

Normally, microbes and microbial products pass through phagocytosis in the laminopropria and mesenteric lymph nodes. The level of lipopolysaccharides (LPS) in HIV infected patients, especially in advanced stage (CD4 count < 200 cells/mm^3), is significantly higher than non-infected patients, suggesting a higher rate of microbial translocation. Furthermore, there is a direct correlation between the level of LPS in plasma and the level of intrinsic and acquired immune system activity[7, 8].

LPS of gram-negative bacteria mediates C-C chemokine receptor Type 5 (CCR5) coreceptor overexpression and facilitates infection of the laminopropria CD4 + T cells (LP CD4 T) without simultaneously activating a large number of other T cells, hence ultimately lowering the LP CD4 T. This phenomenon could indicate a new mechanism that potentially links intestinal dysbiosis to mucosal pathogenesis of HIV[9].

On the other hand, a subgroup of T helper (TH) cells, TH17 cells and Interleukin (IL)-17 play a critical role in host immunity and protect the intestinal mucosal integrity. Therefore, their reduction during HIV infection along with the microbial products translocation through damaged mucosal epithelium provides immune stimulation and a poor long-term prognosis for people living with HIV (PLWH). Furthermore, ART treatment initiation could not improve the TH17 population in the intestinal mucous membrane completely[10].

Recently, Valiathan and Asthana revealed that an increase in circulatory TH17 (cTh17) had a reverse relation to the percentage and absolute count of CD4 + T cells and direct relation to microbial translocation and immune activation[11].

Probiotics are defined as living organisms that benefit the host when adequately administered. Commonly, lactobacillus or bifidobacterium species are used in various illnesses with promising results in some conditions, such as reducing the duration of acute infectious diarrhea, and the severity of infantile necrotizing enterocolitis, or as an alternative treatment for bacterial vaginosis[12]. During HIV infection, reduced intestinal CD4 + T cells and dendritic cells damage the intestine. In addition, ART side effects such as gastrointestinal (GI) symptoms like diarrhea impact on the quality of life and can even result in ceasing treatment. A novel hypothesis suggests that probiotics protect

the GI tract reducing diarrhea and postpone the HIV progression to AIDS for several years (Figure 1)[13].

Moreover, probiotics can reduce intestinal inflammation and permeability, increase mucosal immunity response, balance the T helper cells response as well as increase Immunoglobulin A (IgA) polymer secretion (Figure 2)[15, 16].

It is also shown that probiotics can inhibit the HIV-1 pseudovirus *in vitro* through CD4 receptors expression on the lactobacillus cell surface, and increase CD4 counts[18].

Concerning the side effects of probiotics, there is a case report of lactobacillus acidophilus bacteremia that has been used as a probiotic in a patient with AIDS[19]. Another study showed an increased mortality rate in the probiotic group that received six different lactobacillus species and bifidobacterium, with a total of 10 CFU daily dose, for infectious complications of acute pancreatitis. There were also reports of bacteremia, endocarditis and liver abscess caused by lactobacillus species (including L.hamnosus GG) particularly in patients with small intestinal syndrome, central venous catheter, intestinal feeding tubes, or severe underlying disease[20].

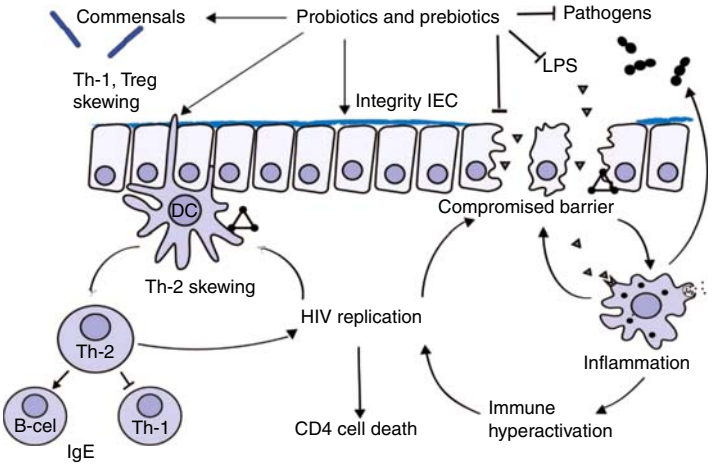
However, it seems that the use of these agents, especially lactobacillus species, is safe and well-tolerated in patients without AIDS, although the number of studies and tested species is limited[15, 21].

Therefore, for the first time in Iran, we investigated the safety and efficacy of probiotics on CD4 + T cell counts among Iranian patients living with HIV.

Materials and methods

Participants

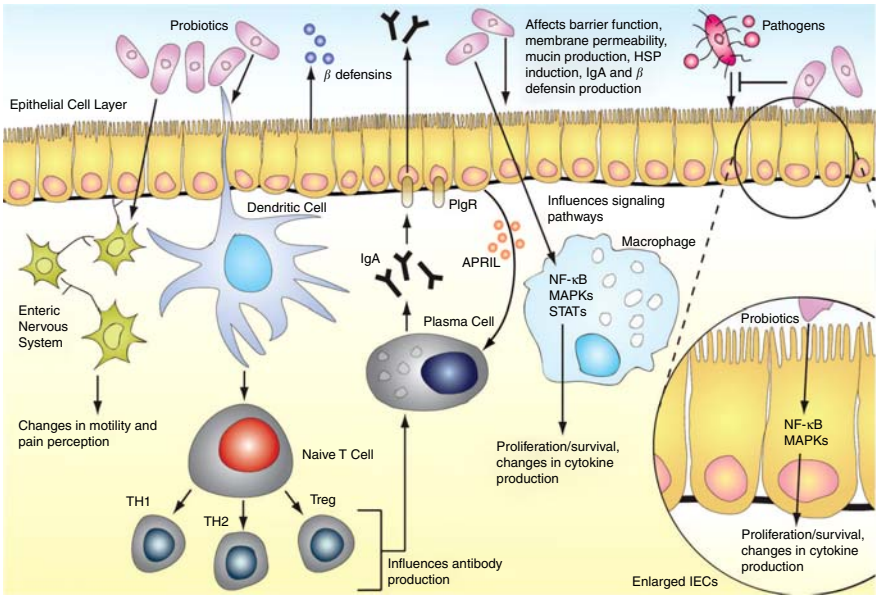
In total, 50 patients out of a total of 207 HIV infected patients (male and female), who were referred to the voluntary counseling and testing (VCT) center at Imam Khomeini Hospital in Tehran, Iran were selected to participate in this trial between February 2016 to October 2017. To participate, eligible patients had to be aged 18 to 65 years old with CD4 count



Notes: Pro- and prebiotics may ameliorate the HIV-induced intestinal problems through effects on the microbiota and its metabolism, on various cells of the immune system (as represented by the arrow pointing at the sampling DC), and on intestinal epithelial cells (IEC)

Source: Reproduced from Hummelen *et al*. in 2010 [14], with permission

Figure 1.
Potential benefits of probiotics and prebiotics in HIV-induced intestinal pathogenesis: HIV infection induces effects and positive feedback mechanisms that induce a loss of intestinal homeostasis and promotes the replication of the virus (triangles)



Notes: APRIL, a proliferation-inducing ligand; HSP, heat shock protein; IEC, intestinal epithelial cell; Ig, immunoglobulin; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor-kappaB; pIgR, polymeric immunoglobulin receptor; STAT, signal transducers and activator of transcription; Treg, T regulatory cell. Intestinal epithelial cell (IEC) barrier function is enhanced through probiotic modulation of tight junctions as well as enhanced mucin production. Probiotics interfere with pathogens by increasing β defensin secretion from IECs and IgA from plasma cells and by directly blocking the signaling pathways hijacked by pathogens. Cytokine secretion by IECs, macrophages and dendritic cells are regulated by probiotics through modulation of key signaling pathways such as NFκB and MAPKs. Changes in these pathways can also affect the proliferation and survival of target cells. Through interactions with dendritic cells, probiotics can influence T cell subpopulations and skew them towards a Th1, Th2 or Treg response. Probiotics can also cause changes in gut motility and pain perception by modulating pain receptor expression and secreting potential neurotransmitter molecules
Source: Reproduced from Thomas *et al*. in 2010 [17] with permission

Figure 2.
Probiotics benefit the host by communicating with a variety of cell types

greater than 350 cells/mm^3 who did not receive any ART. Those selected had to attend and complete a 24-week randomized, double-blind and placebo-controlled trial (Figure 3).

Random allocation

Following simple randomization with a computerized random number generator, eligible patients received either a probiotic (LactoCare®, Zist Takhmir Pharmaceutical Company, Tehran, Iran) or a placebo (provided by Zist Takhmir Pharmaceutical Company, Tehran, Iran) capsule twice daily for 24 weeks (allocation ratio 1:1). A third-party assignment was used for the allocation concealment. The probiotic and placebo capsules were identical regarding their size, shape, color, texture and odor. The patients and investigators were blinded to the treatment assignment.

Measurements and procedures

The CD4 count was measured using the Partec kit (Partec GmbH, Münster, Germany) for all participants at baseline, 12 and 24 weeks. The primary outcome was the assessment of

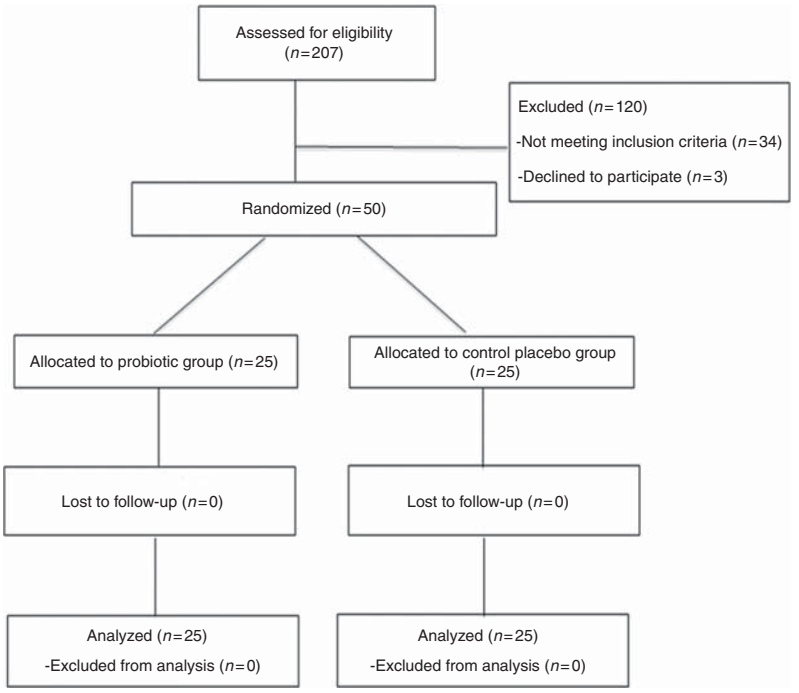


Figure 3.
The flow of participants through study

probiotic safety and efficacy to increase CD4 count compared to placebo in PLWH who were not treated with ART using general linear model repeated measures. Side effects were measured monthly during the study using a specific checklist. In addition, participants were warned to immediately alert the researchers to any unexpected symptom during the duration of the study.

This trial was a proof of concept study; however, we assumed a significant difference of 15 on the CD4 count, an SD of 10, a two-sided significance level of 0.05, a power of 80 percent based on a previous study[22], and a loss to follow up rate 20 percent. Thus the sample size was calculated 50 (25 patients in each arm).

Ethical considerations

The authors considered the declaration of Helsinki and subsequent revisions and obtained written informed consent from all patients before participation in the study. Participants knew they could leave the study when they wanted without any interruption in their standard care. The study protocol was approved by the institutional review board of Tehran University of Medical Sciences.

Statistical analysis

All patient data were decoded and analyzed. Quantitative and qualitative variables were reported as mean ± SD and number (%). Baseline, at 12 weeks and at 24 weeks CD4 count were compared between the two groups using an independent *t*-test. The independent *t*-test was also used to compare the reduction in CD4 count from baseline to each time point in both groups. Anova's repeated-measure analysis was used to compare the CD4 count between the two groups during the trial course. Qualitative variables were analyzed using Fisher's exact test. All tests

were in two domains and a p-value less than 0.05 was considered statistically significant. All statistical analyses were performed with the statistical package of social science (SPSS) software (version 22; IBM Company, Armonk, New York, USA). The graphs of repeated-measure tests were drawn with Sigma plot (version 12; Systat Software Inc., San Jose, California, USA).

Results

Demographic characteristics

Baseline demographic characteristics of the participants, as well as their baseline CD4 count, were not significantly different between the two groups (Table I).

Outcomes

There was no significant difference in the baseline CD4 count between the probiotic and the placebo groups (710.2 ± 143.37 vs 694.4 ± 213.80, respectively, mean difference (MD) (95% confidence interval) = −15.8 (−119.32 to 87.72), $t = -0.37$, $p = 0.7$). The mean CD4 count was not significantly different at weeks 24 compared to the baseline in the probiotic group and it remained relatively constant in contrast to the probiotic group. In the probiotic group, the independent t-test showed no significant difference regarding the mean CD4 count from baseline to weeks 12 in the probiotic group compared to the placebo group ($p = 0.06$), but after 24 weeks the mean CD4 count in the placebo group was significantly lower than the probiotic group ($p = 0.001$). The mean CD4 count in the probiotic group on week 12 dropped significantly ($p = 0.04$) compared to the baseline. At week 24, the mean CD4 count in the probiotic group increased substantially ($p = 0.02$) from week 12; however, compared to the beginning of the trial, this was not statistically significant ($p = 0.7$) (Table II).

In the probiotic group, the independent analysis showed that the change from baseline to week 24 in the CD4 count was significantly different to the other group ($p = 0.001$) and also,

	Placebo group ($n = 25$)	Probiotic group ($n = 25$)	p-value
Age (years) (mean ± SD)	35.68 ± 7.77	34.76 ± 6.86	$p = 0.9$
Sex M: F n (%)	10 (40.0), 15 (60.0)	11 (44.0), 14 (56.0)	$p = 0.7$
Educational level			
Illiterate	1 (4.0)	0 (0)	$p = 0.2$
Diploma	10 (40.0)	5 (20.0)	
Under the diploma	9 (36.0)	8 (32.0)	
Master's degree and higher	4 (16.0)	11 (44.0)	
PhD	1 (4.0)	1 (4.0)	
Transmission routes			
I.V. drug	8 (32.0)	9 (36.0)	$p = 0.7$
Maternal	1 (4.0)	0 (0)	$p = 0.9$
Sexual	13 (52.0)	15 (60.0)	$p = 0.6$
Unknown	3 (12.0)	1 (4.0)	$p = 0.7$

Table I. Baseline characteristics of the patients in the trial groups, Tehran, 2016–2017

	Baseline	Week 12	Week 24	p-value
Probiotic	710.2 ± 143.37	687.4 ± 144.56	718.0 ± 136.13	0.001
Placebo	694.4 ± 213.80	607.7 ± 148.87	537.7 ± 143.78	0.387

Table II. Comparison of mean CD4 count between the two groups using the independent t-test, Tehran, 2016–2017

the increased CD4 count percent from baseline to week 24 was greater in the probiotic group during study ($p < 0.001$) (Table III).

ANOVA repeated measurement analysis demonstrated significant effect for time × treatment interaction on the CD4 count from the beginning of the study to 24 weeks $p < 0.05$ (Figure 4).

The mean CD4 count in the placebo group at baseline, week 12, and week 24 in patients who acquired HIV infection via intravenous (I.V) drug transmission route was 633.5 ± 138.4, 593.2 ± 194.6 and 540.6 ± 131.7 cells/mm^3, respectively. This variable in the sexual transmission route was 726.6 ± 171.4, 635.1 ± 109.7 and 513.5 ± 116.6 cells/mm^3, respectively (p-value in each part was $p = 0.1$, $p = 0.3$ and $p = 0.1$, sequentially).

The mean CD4 count in the probiotic group at baseline, week 12 and week 24 in patients who acquired HIV infection via I.V drug transmission route was 617 ± 117.8, 635.5 ± 136.8 and 684.2 ± 118.2 cells/mm^3, respectively. This variable was in the sexual transmission route 773.4 ± 128.6, 724.3 ± 146.2 and 742.8 ± 146.1 cells/mm^3, sequentially (p-value in each part was $p = 0.1$, $p = 0.2$ and $p = 0.4$, respectively).

Evidently, the I.V drug and sexual transmission routes and the comparison of mean CD4 count between the placebo and probiotic groups did not show any significant difference during each time point of the study (at baseline, week 12 and week 24 was $p = 0.1$, $p = 0.2$ and $p = 0.4$, respectively).

Adverse effects

No significant adverse events occurred between the two groups ($p =$ ns). Additionally, no mortalities were recorded (Table IV).

Table III. Comparison of CD4 count changes between the two groups, Tehran, 2016–2017

	Placebo group	Probiotic group
Increase of CD4 count percent from baseline to week 12	4 (20.0)	19 (79.2)
Increase of CD4 count percent from baseline to week 24	0 (0)	16 (66.7)

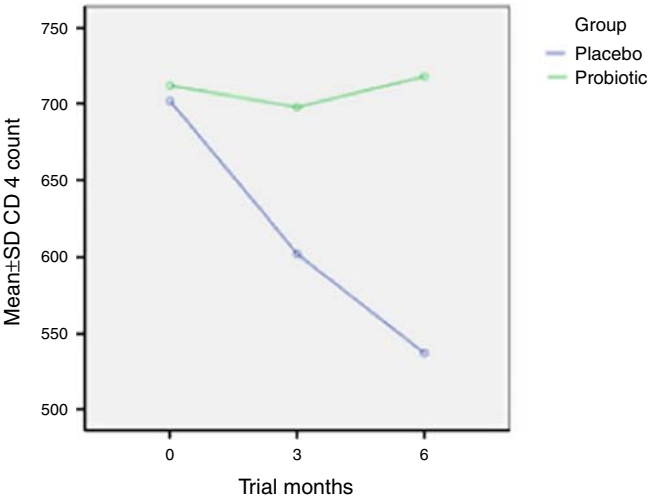


Figure 4. Repeated measure for comparison of two study groups on the CD4 count

Discussion

This double-blind randomized clinical trial showed that for PLWH, one probiotic capsule consumed twice daily for 6 months significantly prevented CD4 count reduction compared to the placebo group. Since the baseline clinical characteristics in both probiotic and placebo groups were not significantly different, this effect in the treatment group can be attributed to the improvement of the immune system caused by probiotic consumption. As the side effects were not significantly different in the two groups, it could be concluded that probiotic use was well tolerated, and no significant complications were observed in this study.

For HIV patients, in addition to the intestinal lymphoid tissue (GALT) infection, a major lymphocyte reservoir in the body, the accumulation of other triggered CD4+ T cells in the intestine as a consequence of the microbial translocation leads to an increase in infected CD4+ T cells and subsequently increased viral load as well as progression of the infection[23]. Several studies showed probiotics could play a role in interrupting this faulty cycle. Probiotic products such as probiotic yogurt are readily available and inexpensive. Furthermore, food fermentation mediated by probiotics can effectively lead to the essential fatty acids release in repairing the intestinal mucosal wall and improving the barrier between pathogenic and beneficial microorganisms. In addition to CD4 count improvement, probiotics can alleviate common ART side effects associated with low compliance such as GI upset[23].

Our study was in line with the review of Miller *et al.*[23] in which 13 studies were analyzed to evaluate the effect of probiotic consumption on the CD4 count of HIV infected patients between 2004 and 2015. Ten studies showed a statistically significant increase or stability in the CD4 count. Therefore, the authors concluded that probiotics have a potential role in enhancing the immune system of HIV infected adults and children.

In the present study, although the mean CD4 count of patients in the probiotic group after three months of the trial did not significantly increase compared to the placebo group; after six months of probiotic administration, the mean CD4 count was significantly higher than the placebo group. Considering the nature of HIV infection, it was expected that through the first three months of the trial, the mean CD4 count dropped off significantly in the probiotic and placebo groups. Surprisingly, this trend reversed in the probiotic group compared to the placebo group in the remaining follow up period. The emergence of the probiotic effect might illustrate this phenomenon. Therefore, probiotics administration longer than three months as an adjunct therapy might decelerate HIV progression. Heiser *et al.*[24] showed probiotic consumption for three months improved diarrhea associated with ART, but it was not effective in preserving the CD4 count. The latter finding could be due to a relatively shorter duration of taking probiotics. Gonzalez-Hernandez *et al.*[25] also demonstrated that the CD4 count increased significantly only in the symbiotic group compared to the probiotic or placebo groups during a short period of 16 weeks therapy. Synbiotics are a combination of probiotics and prebiotics which contain no digestible fiber compounds stimulating the growth of probiotic microorganisms.

Although it was shown that the loss of lactobacillus species in vaginal flora could increase the risk of HIV acquisition from an HIV infected woman, we observed no impact of probiotic consumption on the mean CD4 count concerning HIV acquisition via the sexual contact vs I.V drug use. This suggests that HIV interacts with the human gut microbiota ignoring the route of transmission[26].

Table IV. Prevalence of adverse events in the trial groups, Tehran, 2016–2017

Adverse event	Placebo group (%)	Probiotic group (%)	*p*-value
Mild diarrhea	1 (4.0%)	2 (8.0%)	0.9
Weight gain	1 (4.0%)	6 (24.0%)	0.1
None	23 (92.0%)	17 (68.0%)	–

In our study, the side effects of probiotic consumption, including sepsis and gastrointestinal symptoms, were not statistically significant in the two groups. Of course, none of the participants had the risk factors for bacteremia associated with Lactobacillus species, such as CD4 counts below 50 cells/mm^3, alcoholic liver disease, gastrointestinal disorder (active infection, malignancy and recent surgery) which Haghighat *et al.*[19] purposed. Likewise, Carter *et al.*[27], did not find any bacteremia or fungemia associated with probiotic consumption in the analysis of 39 studies.

We used LactoCare® containing lactobacillus and bifidobacterium species as well as Streptococcus thermophilus in this trial. In the literature, lactobacillus or Bifidus species administration was associated with a moderate increase in the mean CD4 count in all papers where their primary outcome was the CD4 count. However, the use of Saccharomyces boulardii was not helpful in one study[27].

To the best of our knowledge, this is the first study to evaluate the immune-boosting benefit of probiotics on Iranian PLWH. While in the majority of papers analyzed in the previous review papers treatment duration was less than six months[21, 24], we followed up patients for 6 months preparing a more accurate comparison between the probiotic and placebo group. In addition, a double-blind, randomized, placebo-controlled trial design provided a reliable comparison of probiotic effects on CD4 count. However, the limitations of this study included its relatively small sample size, lack of inflammatory factors evaluation such as erythrocyte sedimentation rate (ESR), C – reactive protein (CRP), IL-1β, IL-6, TNF-α, and other markers related to bacterial translocation such as CD14, LPS, plasma 16SrRNA and bacteria in feces. Unfortunately, we could not measure the HIV viral load in the plasma due to a lack of financial support.

In conclusion, probiotic consumption twice daily amongst HIV-infected patients with a CD4 count greater than 350 cells/mm^3 who are not receiving antiretroviral drugs is safe and well-tolerated. It also might preserve CD4+ T cell populations in these patients and slow progression of the disease. Further studies with larger sample sizes in patients receiving ART by different probiotic strains in combination with prebiotics are warranted.

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