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The Effect of Probiotic Dietary Supplements on Oral Candida Albicans Colonization in AIDS Patients

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ABSTRACT

Background and Objective: Oral candidiasis is one of the most common oral manifestations of acquired immunodeficiency syndrome (AIDS). This condition affects the patients' quality of life. This study aims to assess the effect of probiotic dietary supplements on oral Candida albicans (C. albicans) colonization in AIDS patients.

Methods: This randomized clinical trial was conducted on 30 AIDS patients with less than 100 CD4 cells per mm^3, who were under anti-retroviral therapy. The patients were then randomly divided into two groups of intervention and control (n=15), based on their medical file number (even, odd). The intervention group received probiotic tablets (2 tablets/day for 1 month) along with anti-retroviral therapy (ART). The control group only received ART. One mL of saliva was collected from each patient on the first day of study and after intervention and was cultured on Sabouraud dextrose agar to quantify the number of C. albicans colonies in colony forming units/milliliter (CFUs/mL).

Findings: The two groups were initially identical in terms of CD4 count. Nine patients were withdrawn from the control, and 8 patients from the intervention group. Thus, 13 patients were analyzed (4 females, 2 males in the control group, and 4 females, and 3 males in the intervention group). The C. albicans colony count was significantly higher in the test group before the intervention (CFUs/mL=47714±40372, p=0.038). The difference in C. albicans colony count was not significant between the two groups after the intervention. In the control group, the C. albicans colony count did not change significantly (7833±1722 before, 7666±2160 after) while in the intervention group, the number of C. albicans significantly decreased after the intervention (47714±40372 before, 14571±25683 after, p=0.02).

Conclusion: According to the results of this study, it appears that probiotic dietary supplements can decrease C. albicans colonization.

Keywords: *Probiotics, Candidiasis, AIDS.*

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Introduction

Human immunodeficiency virus (HIV) is a human retrovirus that causes the breakdown of the immune system, and leads to development of AIDS (1). Immunological failure is defined as CD4 count at or below 250 cells/mm^3 following a clinical failure or persistent CD4 levels below 100 cells/mm^3 (2). These patients are at higher risk of developing oral candidiasis compared to others due to a low CD4 count (3). Candidiasis is the most common opportunistic fungal infection of the oral cavity caused by Candida albicans (C. albicans). Approximately one-third of HIV-positive patients and over 90% of AIDS patients suffer from oral candidiasis (4, 5).

Probiotics are viable microorganisms consumed as a dietary supplement, which improve the microbial balance of the gastrointestinal system, airway infections, and allergy (6). Probiotics have antimicrobial effects exerted by the production of organic acids such as lactic acid and acetic acid, hydrogen peroxide, bacteriocin, and low molecular-weight materials with non-specific antifungal effects. They can also prevent the growth and metabolic activity of oral C. albicans (6, 7). Therefore, probiotics have been proposed for the prevention and treatment of bacterial infections due to their safety and efficacy. However, little is known about their potential role regarding prevention of fungal infections, or their potential use as an alternative/complementary therapy against Candida infections, along with fluconazole, or independently (4, 8, 9).

Salari et al. reported that both L. acidophilus and L. plantarum probiotics were able to inhibit the growth of most of the oral Candida spp, except for C. albicans in HIV patients (10). In a study concluded by Jørgensen et al., both L. reuteri strains exhibited good inhibitory effects on the growth of most of the tested Candida species. Jiang et al. reported that the lactobacilli failed to inhibit C. krusei (11). Contrary to the findings of Salari et al., C. albicans was the most susceptible yeast to lactobacilli (12).

Due to the controversies in the literature, this study was conducted to assess the effect of probiotic dietary supplements on oral C. albicans colonization in HIV-positive patients with immunological failure.

Methods

This cross-sectional study was conducted after being approved by the Ethics Committee of the Dental Unit of Tehran Islamic Azad University with the code IR.IAU.DENTAL.REC.1395.8 and registered in the Iranian Clinical Trials System with the code IRCT20170618034619N2.

The sample consisted of 30 HIV-positive patients who referred to West Tehran Health center with immunological failure and persistent CD4 levels below 100 cells/mm^3. The patients were under the supervision of an infectious disease specialist, and had fully received anti-retroviral therapy (ART). They were briefed about the study and signed the informed consent forms prior to enrollment. Initially a past medical history was obtained form the patients, followed by a clinically examination by a trained senior dental student under the supervision of an oral medicine specialist to ensure absence of oral candidiasis.

The inclusion criteria were HIV positive patients with immunological failure, based on the flow cytometry test (CD4<100 cell/µl), who had not used any antibiotics, probiotic products, and antifungal medication in the last 30 days prior to sampling. All patients were under ART, and did not have oral candidiasis. The patients were then randomly divided into two groups of test and control, based on their

medical file number (odd and even), and were standardized in terms of gender and CD4 count. Patients with poor cooperation, allergic reaction to probiotics, severe infections during the study period were excluded from the study. The test group received probiotic tablets (Lactocare, Zist Takhmir, Tehran, IRAN) twice a day with breakfast and lunch for 30 days under the supervision of an infectious disease specialist. The microorganisms present in probiotic tablets included Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium longum, and Streptococcus thermophiles. Each tablet had 10^{10} CFUs/mL of the abovementioned microorganisms. The control group did not receive probiotics. Saliva samples were collected from all patients before breakfast, initially and after 30 days. The patients were instructed to refrain from eating and drinking for 90 minutes prior to sampling. They were asked to brush their teeth without a toothpaste and drink a glass of water prior to sampling. After one hour, unstimulated saliva samples were collected by spitting method (a minimum of 1 mL) and transferred into microtubes. The saliva samples were sent to a laboratory on the same day for microbiological culture and assessment. Sabouraud dextrose agar along with chloramphenicol was used for C. albicans culture.

Saliva samples were cultured and the plates were incubated at 37°C for 48 hours, and were then stored at room temperature for 5 days. The colonies were then stained, and number of C. albicans colonies was counted and reported as colony forming units per milliliter (CFUs/mL) by a microbiologist. On day 1, saliva samples were collected from both the test group (prior to taking the probiotic tablet) and the control group as explained earlier. After 30 days, saliva samples were collected again from both groups and underwent microbiological assessment. The colony count was measured and reported for both groups as colony forming units per milliliter (CFUs/mL).

The effect of probiotic food supplements on oral colonization of Candida albicans in HIV-positive patients with immunological failure who did not receive any antibiotics (interventional variable) was investigated. The sample size was calculated based on the study of Ohshima et al. (13) assuming alpha=0.05 and beta=0.07, and the power of the study was determined using two-way fixed-effects ANOVA and SPSS 22 software.

All saliva microplates and culture plates were coded in such a way that the experimenter was blinded to their allocation group. The statistician who analyzed the data was also blinded to the data allocation group. Changes in the number of Candida albicans colonies and the difference between the two groups were analyzed using the Mann-Whitney U test and Fisher's exact test, respectively, and $p<0.05$ was considered significant.

Results

Of a total of 30 patients that were initially assigned to the test and control groups (n=15), 9 were withdrawn from the control, and 8 were withdrawn from the test group (Figure 1). Thus, 13 patients were analyzed (4 females, 2 males in the control group, and 4 females, and 3 males in the intervention group). The C. albicans colony count was significantly higher in the test group before the intervention (CFUs/mL=47714±40372, p=0.038) by accident. In the control group, the C. albicans colony count did not change significantly (7833±1722 before, 7666±2160 after), as shown by Mann Whitney U test, while in the intervention group, the number of C. albicans significantly decreased after the intervention (47714±40372 before, 14571±25683 after, p=0.02). The difference in C. albicans colony count was not significant between

the two groups after the intervention (Table 1). After one month, all 6 patients in the control group (100%) were positive for Candida albicans, while in the experimental group, 4 patients did not have Candida albicans ($p<0.05$).

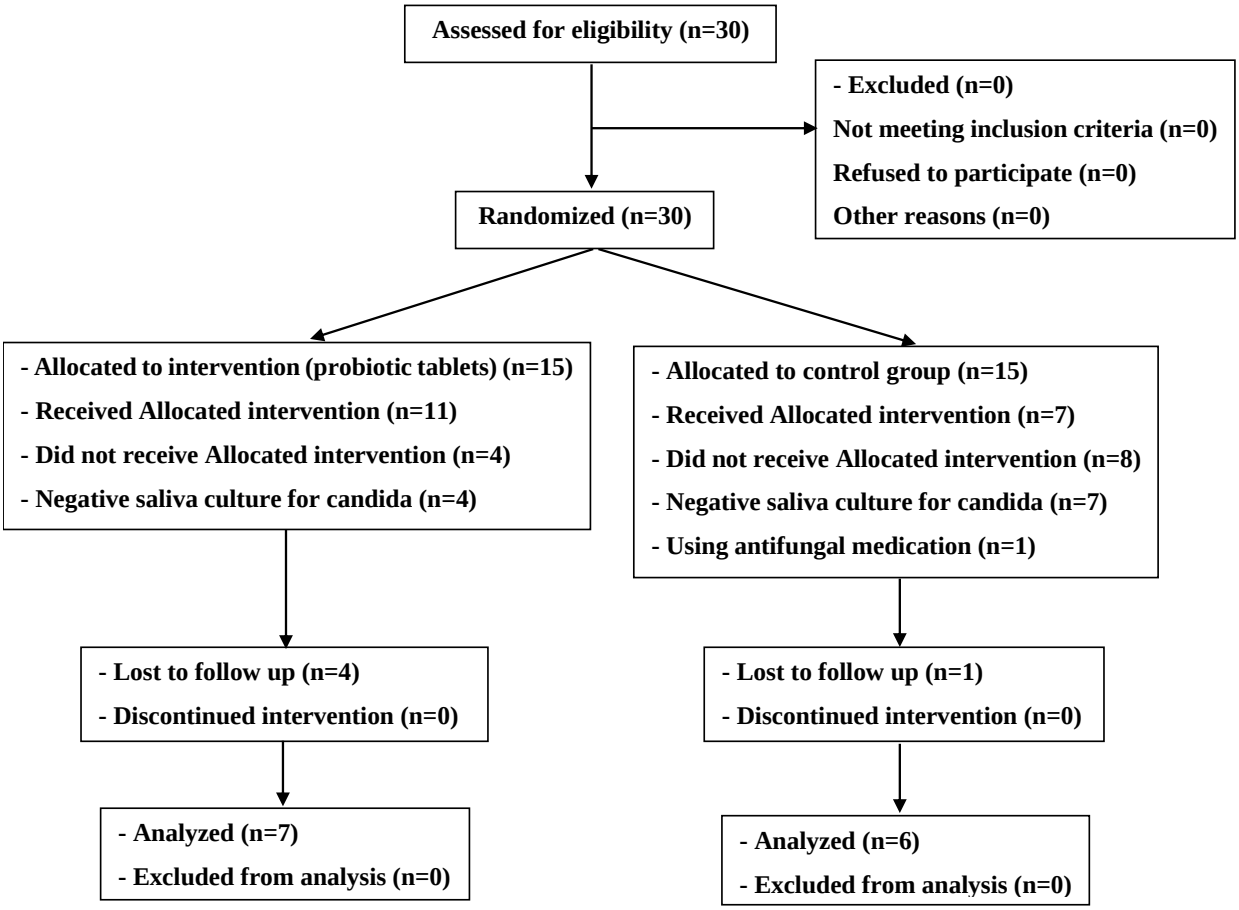

Figure 1. CONSORT flow diagram of the study

Table 1. C. albicans colony count before and after the intervention in the two groups and their trend of change

Groups	Day 1 (Cfu/ml) Mean±SD	Day 30 (Cfu/ml) Mean±SD	p-value*
Control	1722±7833	2160±7666	0.713
Test	40372±47714	25683±14571	0.018
p-value	0.038	0.663	-

*Mann Whitney U test

Discussion

This study assessed the effect of probiotic dietary supplements on oral C. albicans colonization in HIV-positive patients with immunological failure. The results showed that the difference in C. albicans colony count was not significant between the two groups after the intervention. In the control group, the C. albicans colony count did not change significantly while in the intervention group, the number of patients with C. albicans significantly decreased after the intervention. Also, all 6 patients had oral candidiasis after 1 month in the control group while this rate was 3 out of 7 patients in the test group.

Yang et al. showed that although ART can cause a reduction in C. albicans colony count by increasing the number of CD4 cells, it had no such effect on patients with low CD4 count (14), which was in line with the present findings. Although C. albicans colony count was significantly higher in the test group at baseline, it decreased over time, such that the two groups had no significant difference in this regard after 1 month. This reduction in C. albicans colony count might be attributed to the use of probiotic tablets by the test group. Similarly, Hatakka et al. showed that probiotic intervention decreased the C. albicans colony count from 30% to 21%. It also decreased the risk of high yeast counts by 75% (15). Kraft-Bodi et al. indicated that consumption of probiotic tablets decreased the prevalence of oral candida from 72% to 51%. They added that daily consumption of probiotic tablets can control oral candidiasis (16). Mendonça et al. evaluated the effect of probiotics on the prevalence of oral candidiasis in the elderly. They demonstrated that following the consumption of probiotic products, the prevalence of oral candidiasis decreased from 92.9% to 85.7% (17). Hu et al. assessed the effect of probiotic yogurt on oral and vaginal candidiasis and showed that HIV-positive females who consumed probiotic yogurt experienced a significant reduction in vaginal Candida colonization compared with periods of no probiotic use (29% versus 54%) (18). Ishikawa et al. evaluated the short-term effects of different types of probiotics on oral Candida colonization in denture wearers. They found that reduction of Candida species in probiotic consumers had no significant correlation with candidiasis, colony species, and denture age (19). Their results were somehow in line with the present findings.

Salari et al. reported that both L. acidophilus and L. plantarum probiotics were able to inhibit the growth of most of the oral Candida spp, except for C. albicans in HIV patients (10). Jiang et al. and Zhao et al. reported that the lactobacilli failed to inhibit C. krusei (12). Contrary to Salari's findings, C. albicans was the most susceptible yeast to lactobacilli (10).

Based on our study and previous works, it appears that probiotics decrease the C. albicans colony count and thus, their products might prevent or control oral candidiasis in HIV-positive patients. The present study was conducted on patients with immunological failure and low CD4 count, who do not often favorably respond to ART. However, the results showed optimal efficacy of probiotic dietary supplement for reduction of oral C. albicans colony count. Thus, probiotic tablets can be used for this purpose, along with fluconazole. Fluconazole is a low-cost triazole with benefits in terms of compliance, minimal medication interactions, and low rates of adverse effects (1). Small sample size due to patient dropouts was a limitation of this study. Also, number of HIV-positive patients with virological success, immunological failure, and absence of oral candidiasis was low. We had to exclude patients with oral candidiasis since they had to use nystatin. All these factors contributed to the small sample size. Further studies with a larger sample size are required to confirm the present results. Also, the effect of different types of probiotics and different probiotic products on oral candidiasis in immunocompromised patients should be investigated in future studies.

It appears that probiotic dietary supplements can decrease oral C. albicans colonization. However, further studies are required on this topic.

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