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Institusi	: Magister Pharmacy Faculty Of Pharmacy, Universitas Surabaya, Centre For Medicine Information And Pharmaceutical Care, Faculty Of Pharmacy, Universitas Surabaya, Waluyo Jati Regional Public Hospital, Probolinggo Regency

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The Multi-Strain Probiotics Effect as Adjuvant Therapy for HIV Patients at Waluyo Jati Regional Hospital, Probolinggo Regency, East Java, Indonesia

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ABSTRACT

Human Immunodeficiency Virus (HIV) infection causes Acquired Immune Deficiency Syndrome (AIDS) by attacking immune cells, especially T-helper lymphocytes (CD4). This study aims to determine the benefits of multi-strain probiotics as adjuvant therapy in People Live with HIV (PLHIV) with suppressed viral load at Waluyo Jati Regional Hospital with a primary focus on changes in CD4 levels and Opportunistic Infection. This study was conducted using the Quasi-Experimental Design One Group Pretest Posttest method involving 23 PLHIV at Waluyo Jati Regional Hospital. The sample individuals in this study were PLHIV with a history of suppressed viral load and had received ART (antiretroviral) therapy for at least 2 (two) years. Data collection in this study was carried out for 90 (ninety) days. Based on the Shapiro-Wilk normality test value, it is known that the research data obtained is data with a normal distribution ($p > 0.05$). Based on this study, it was found that the administration of adjuvant therapy in the form of multi-strain probiotics show statistically significant effect on increasing CD4 levels in study participants after three months of treatment. Individually, 82,46 % of patients experienced an increase in CD4, while 17,36 % experienced a decrease in CD4 ($p = 0.001$; $p < 0.05$). The supplementation of probiotics has a statistically significant effect on increasing CD4 cell levels in HIV patients undergoing antiretroviral (ART) therapy. The overall occurrence of opportunistic infections (OIs) in the study group was low throughout the three months observation period. Only two OI cases were identified, one case of pneumonia and one case of oral candidiasis. Both of which appeared in the first month, with no additional infections detected in the subsequent months.

***Keyword**: HIV, Antiretroviral, Multi-strain Probiotic, CD4, Opportunistic Infections.*

Efek Probiotik Multi-Strain sebagai Terapi Tambahan untuk Pasien HIV di RSUD Waluyo Jati, Kabupaten Probolinggo, Jawa Timur, Indonesia

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ABSTRAK

Infeksi *Human Immunodeficiency Virus* (HIV) menyebabkan *Acquired Immune Deficiency Syndrome* (AIDS) dengan cara menyerang sel imun, terutama limfosit T-helper (CD4). Penelitian ini bertujuan untuk mengetahui manfaat probiotik multi strain sebagai terapi tambahan pada Orang Dengan HIV (ODHIV) dengan penekanan *viral load* di RSUD Waluyo Jati dengan fokus utama pada perubahan kadar CD4 dan Infeksi Oportunistik. Penelitian ini dilakukan dengan metode *Quasi-Experimental Design One Group Pretest Posttest* yang melibatkan 23 ODHIV di RSUD Waluyo Jati. Individu sampel dalam penelitian ini adalah ODHIV dengan riwayat penekanan *viral load* dan telah mendapat terapi ART (antiretroviral) minimal 2 (dua) tahun. Pengumpulan data pada penelitian ini dilakukan selama 90 (sembilan puluh) hari. Berdasarkan nilai uji normalitas Shapiro-Wilk diketahui bahwa data penelitian yang diperoleh merupakan data yang berdistribusi normal ($p > 0,05$). Berdasarkan penelitian tersebut, ditemukan bahwa pemberian terapi adjuvan berupa probiotik *multi-strain* menunjukkan pengaruh yang signifikan secara statistik terhadap peningkatan kadar CD4 pada subjek penelitian setelah tiga bulan pengobatan. Secara individual, 82,46% pasien mengalami peningkatan CD4, sedangkan 17,36% mengalami penurunan CD4 ($p = 0.001$; $p < 0.05$). Suplementasi probiotik mempunyai dampak yang signifikan secara statistik terhadap peningkatan kadar sel CD4 pada pasien HIV yang menjalani terapi antiretroviral (ART). Kejadian infeksi oportunistik (OI) secara keseluruhan pada kelompok penelitian tergolong rendah selama periode observasi tiga bulan. Hanya dua kasus OI yang teridentifikasi, satu kasus pneumonia dan satu kasus kandidiasis oral. Keduanya muncul pada bulan pertama, dan tidak ada infeksi tambahan yang terdeteksi pada bulan-bulan berikutnya.

Kata Kunci: HIV, Antiretroviral, Probiotik Multi-strain, CD4, Infeksi Oportunistik.

I.INTRODUCTION

Human Immunodeficiency Virus (HIV) is a virus that attacks the immune system and can cause Acquired Immune Deficiency Syndrome (AIDS). HIV can be transmitted through three main routes: sexual, parenteral, and perinatal. Sexual intercourse, particularly anal and vaginal, is the most common mode of transmission (Dipiro, et al., 2023).

HIV infects cells that express the Cluster of Differentiation 4 (CD4) receptor, such as T-helper lymphocytes, monocytes, macrophages, dendritic cells, and brain microglia. Infection occurs through interactions between HIV glycoprotein 160 (gp160) and CD4 (primary interaction) and chemokine co-receptors (secondary interaction) found on the surface of these cells (Dipiro, et al., 2023). The current goal of combination antiretroviral therapy (ART) is to achieve maximal and durable suppression of HIV replication, measured as a plasma HIV-RNA level (viral load) below the lower limit of quantitation, typically 20 to 50 copies/mL (20 × 103–50 × 103/L). Viral load suppression in HIV patients is expected to align with an increase in the patient's CD4 count, indicating an improved immune system (Dipiro, et al., 2023; Kementerian Kesehatan Republik Indonesia, 2022).

In HIV patients, a compromised immune system increases the risk of additional infections (opportunistic infections). The occurrence of opportunistic infections indicates an increased progression of HIV infection. The desired improvement in HIV status is the absence of worsening conditions (immunological, virological, or clinical). Some opportunistic infections that frequently occur in HIV-infected patients include multi-bacterial infections in children under 6 years of age, oropharyngeal candidiasis, esophageal candidiasis, cervical cancer in female patients, extrapulmonary cryptococcal bacterial infections, chronic intestinal cryptosporidiosis, cytomegalovirus retinitis, encephalopathy, herpes simplex virus infections, histoplasmosis, mycobacterial infections, pneumococcal bacterial infections, and salmonella bacterial infections. Therefore, all HIV patients are given ART regardless of their clinical condition. The primary goals of antiretroviral therapy (ART) are to reduce morbidity and mortality, improve quality of life, restore and maintain immune function, and prevent further transmission (Dipiro, et al., 2023).

In the gastrointestinal tract, particularly in the intestines, immune cells and microorganisms communicate and respond to each other in a stable environment to maintain healthy immune activity. Cross-communication between the immune system and the microbiota relies on a complex network of pathways that maintain a balance between immune resistance and immunogenicity. Probiotic bacteria can interact with and stimulate intestinal immune cells and the subsequent gut microbiota to regulate specific immune function and immune cocci. Evidence suggests that probiotic bacteria exhibit important properties that enhance health and immune regulatory factors. Therefore, the use of probiotics could be a promising approach to enhancing immune system activity (Mazziotta, Tognon, Martini, Torreggiani, & Rotondo, 2023).

The intestinal immune system comprises a physical barrier system, underlying epithelium and connective tissue, and a lamina propria containing immune effector cells. The lymphatic tissue associated with the intestinal tract, the

intestinal lymphatic tissue (APT), also has important immune functions. It is contained within the lymphoid tissue and connected to the mucosa (MLT), forming a significant portion of the body's overall immune capacity. It is a major source of T and B cells that travel to effectors to induce immune responses. It also contains diverse dendritic cell (DC) populations. It also comprises Peyer's spots, epithelium associated with local follicles, and mucosal secretory points throughout the gut. Peyer's patches play a crucial immunological role in the surveillance of intestinal bacteria, thus preventing infection by intestinal pathogens. Considering the anatomical structure and composition of intestinal tissue, the epithelial layer can be seen as the front line of external stimulation, but an adaptive immune response is transmitted. DCs capture antigens from the epithelium and microfold (M) and activate T cells upon antigen recognition (Mazziotta, Tognon, Martini, Torreggiani, & Rotondo, 2023).

Research by Lozano, R. and Andrea, M. (2021) found that changes in the normal gut flora in HIV patients lead to bacterial translocation and persistent systemic inflammation. Therefore, probiotic administration is known to be able to address this condition caused by HIV infection. Several other studies have shown that probiotic administration in HIV patients, both on and off ART, can improve immunity, as indicated by an increase in CD4 counts. However, probiotic administration did not significantly reduce viral load (Oyadiran et al., 2024). Some of the bacteria used in previous studies include Lactobacillus rhamnosus, Lactobacillus reuteri, Bifidobacterium bifidum, and Streptococcus thermophillus (Lozano & Andrea, 2021).

Probiotics are essentially Gram-positive bacteria that include species belonging to the genera Lactobacillus and Bifidobacterium. Certain Escherichia coli, Enterococcus, Pediococcus, and yeast species, including Saccharomyces boulardii, are examples of other non-pathogenic species with probiotic properties. Additional gut commensal bacteria, such as Streptococcus oralis and Streptococcus salivarius, have been shown to exert beneficial effects on health. Some of the beneficial effects of probiotics on gut homeostasis have been identified as (i) improvements in innate and adaptive immune responses and associated anti-pathogenic/inflammatory activities, (ii) increased bioavailability of certain natural or metabolic components and essential nutrients, and (iii) reduced food intolerances among susceptible subjects. In other words, similar to the commensal gut microbiota, probiotic consumption has been shown to positively impact the entire organism by improving digestion and immunity (Mazziotta, Tognon, Martini, Torreggiani, & Rotondo, 2023).

Normal intestinal flora plays a vital role in the digestive system and produces several B vitamins and vitamin K. The normal intestinal flora, or commensal microbiota, ferments carbohydrates that cannot be digested by the intestine to form short-chain fatty acids (SCFAs), namely acetate, butyrate, and propionate. SCFAs are then utilized by the microbiota and intestinal epithelial cells as an energy source, enabling proper intestinal function, including increasing mucus secretion as an intestinal barrier. Therefore, probiotic supplements are expected to restore normal intestinal flora and thus improve the immune system of HIV patients (Fidianingsih, 2024).

It is known that administering probiotics in the form of yogurt as adjuvant therapy to HIV/AIDS patients significantly increases body weight compared to

HIV/AIDS patients receiving only ART. Patients receiving adjuvant probiotic therapy were found to be able to work for 8 hours longer than patients receiving only ART (6 hours). In addition, the condition of the digestive tract in HIV/AIDS patients who were given probiotic adjuvant therapy was considered better, as evidenced by the absence of diarrhea during 90 days of probiotic therapy (Reid, 2010).

The multi-strain probiotics used in the intervention are probiotic supplements containing billions of live and active multi-strain microorganisms, thus beneficial for human health. One of the goals of administering multi-strain probiotics is to promote an optimal and balanced immune system. The human immune system is played by antibody cells and immunoglobulin-A, which are largely produced in intestinal lymphoid tissue. Therefore, this study hopes that administering multi-strain probiotics will improve the immunity of HIV patients, as indicated by an increase in CD4 cell counts (Eric, 2019).

Waluyo Jati Regional Hospital is a government hospital in Probolinggo Regency that treats people living with HIV (PLHIV). Based on patient medical records at Waluyo Jati Regional Hospital, 260 patients were undergoing treatment until 2025, and 74 patients had their CD4 counts measured. By 2024, the number of HIV cases recorded in Probolinggo Regency had increased to thousands in recent years. According to published data from the Probolinggo Regency Health Office, the number of HIV cases was recorded at approximately 2,790 people, with 76 new cases discovered per year in 2024 (Badan Pusat Statistik, 2024). Based on the above description, the specific benefits of administering multi-strain probiotics are expected to provide significant benefits to HIV patients at Waluyo Jati Regional Hospital.

II. LITERATURE REVIEW

2.1 HIV (*Human Immunodeficiency Virus*)

HIV is a virus that attacks lymphocytes in the body, resulting in a decrease in human immunity and making the human immune system more susceptible to various diseases, making it difficult to recover from various opportunistic infections and can lead to death. AIDS is a collection of symptoms that arise due to damage to the human immune system caused by the HIV virus (Kementerian Kesehatan Republik Indonesia, 2014). HIV is a type of virus belonging to the retrovirus family. The white blood cells attacked by HIV in infected individuals are T lymphocytes (CD4), which function in the body's immune system. HIV replicates within the infected lymphocytes and damages them, resulting in a compromised immune system and a gradual decline in endurance. AIDS, meanwhile, is an immunosuppressive condition (syndrome) closely associated with various opportunistic infections, secondary neoplasms, and certain neurological manifestations resulting from HIV infection (Dipiro, et al., 2023).

2.2 Probiotics as Adjuvant Therapy

2.2.1 Definition of probiotics

Probiotics are live, consumable organisms that can provide benefits to human health. Recent research has demonstrated the ability of probiotics to enhance human immunity, thereby preventing pathogen colonization and reducing the number and severity of infections. However, the underlying mechanisms by which probiotics fight infectious pathogens remain unknown (Nwosu, Avershina, Wilson, & Rudi, 2014). According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics, when administered in adequate amounts, will promote host cell health. Some of the microbes that play a role in probiotics include the genera Pediococcus, Lactococcus, Enterococcus, Streptococcus, Propionibacterium, and Bacillus (Cohen, 1988).

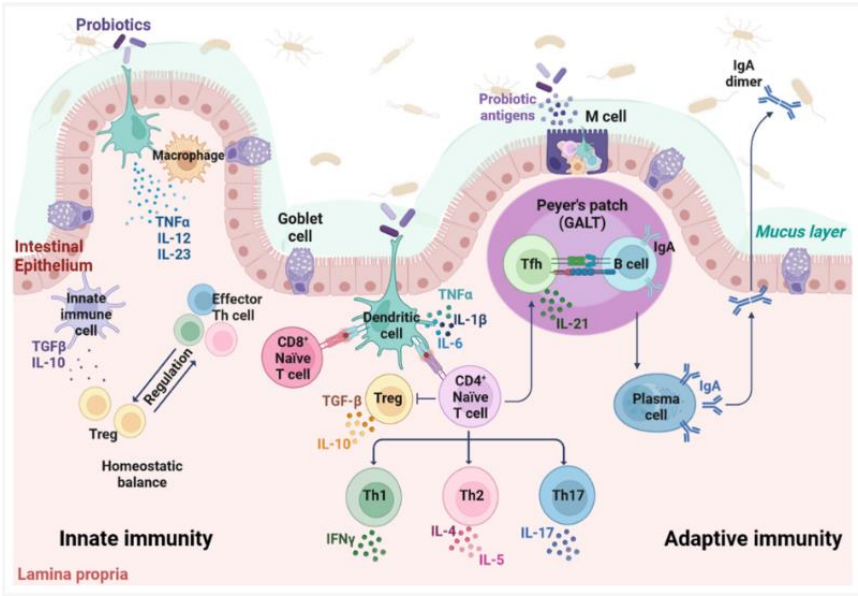

Figure 1. Mechanism of Probiotics Increasing CD4 (modified from Maziotta, 2023)

Probiotics play a crucial role in regulating the host's innate and adaptive immune responses by influencing the activity of various immune cell types, including dendritic cells (DCs), macrophages, and B and T lymphocytes. Interactions between probiotics and host intestinal cells generally occur at the intestinal barrier, specifically in the epithelial layer and the underlying lamina propria. The gut microbiota is separated from the epithelial layer by mucus secreted by goblet cells. Once ingested, probiotic bacteria can bind to intestinal epithelial cells and activate them through pattern recognition receptors (PRRs). This activation triggers the release of cytokines, which in turn stimulate regulatory T cells (Tregs), which play a role in maintaining the balance of the immune system in the intestinal mucosa. Tregs act as effective inhibitors of the immune response, helping to control overactive immune responses. In addition, antigens in the intestine are transferred to DC cells via specialized enterocytes called microfold

cells (M cells), which are found in the epithelial lining of Peyer's patches (Mazziotta, Tognon, Martini, Torreggiani, & Rotondo, 2023).
Probiotics interact directly with dendritic cells located in the lamina propria of the intestinal lumen. These dendritic cells function to activate naive CD8+/CD4+ T cells and direct the differentiation of helper T cells into Th1, Th2, Th17, or Treg subtypes. The Th1 immune response is characterized by the production of interferon (IFN)-γ, which plays a role in cell-mediated immunity, while the Th2 response involves the release of interleukin (IL)-4 and IL-5, which function in humoral immunity. The Th17 response is characterized by the secretion of IL-17, while Treg formation is associated with the release of IL-10 or transforming growth factor (TGF)-ß. Furthermore, probiotics can also stimulate the differentiation of B cells into plasma cells that produce immunoglobulin (Ig)A. Intestinal epithelial cells release cytokines and chemokines that help shape the microenvironment in the lamina propria, supporting the clonal expansion of B cells to produce IgA. This immunoglobulin A then migrates through the epithelium to the mucus layer, where it functions to prevent bacterial adhesion to host tissues (Mazziotta, Tognon, Martini, Torreggiani, & Rotondo, 2023).

2.2.2 Multistrain Probiotics

Probiotics containing a single strain of a specific species are known as monostrains. Strains from multiple probiotic species belonging to one or more genera are called multispecies probiotics. Lactobacillus, Streptococcus, Enterococcus, Lactococcus, and Bifidobacteria, Bacillus, as well as Candida pintolopesii, Aspergillus oryzae, and Saccharomyces remain the most commonly used probiotic agents in livestock.
Multistrain probiotics involve the use of multiple bacterial strains in a single formulation, typically combining strains from different species or genera to achieve a synergistic effect. These probiotics are designed to enhance overall effectiveness by utilizing the unique characteristics and benefits of each strain. Therefore, multistrain probiotics provide a wider variety of health benefits compared to single-strain probiotics (Younas, D.A, Bibi, Ullah, & Rehman, 2025). The probiotic used in this study was Probiotic Brand X which has a composition of Multi-Strain Live and Active Microorganisms including Rhodopseudomonas palustris EMRO 201, Lactobacillus casei EMRO 002, Lactobacillus casei EMRO 213, Lactobacillus plantarum EMRO 009, Lactobacillus fermentum EMRO 211, Lactobacillus rhamnosus EMRO 014, Lactobacillus bulgaricus EMRO 212 each containing >2.0 x 106 cfu/ml. The dose given in the first 3 days of intervention was 3 x 10 mL then 87 days the dose was 3 x 15 mL (D'Ettorre, 2015).

2.3 CD4 (Cluster of Differentiation 4)

Cluster of Differentiation 4 (CD4) is a marker found on the surface of human white blood cells, particularly lymphocytes. CD4 serves as an important indicator of a person's immune status, as a decrease in CD4 count indicates a weakened immune system. When lymphocyte counts decrease, the body's ability to fight

infection also decreases. In individuals with normal immune systems, CD4 levels generally range from 600–1,500 cells/mm^3. Therefore, CD4 count is often used as a parameter to assess immune status, especially in patients with HIV/AIDS (Ayu Setia Anggraeni, 2021).

2.4 Opportunistic Infections

Opportunistic infections are one of the main clinical manifestations that define the advanced stage of Human Immunodeficiency Virus (HIV) infection, known as Acquired Immunodeficiency Syndrome (AIDS). The term "opportunistic" refers to infections caused by microorganisms (viruses, bacteria, fungi, or protozoa) that generally do not cause disease in individuals with healthy immune systems. However, in HIV/AIDS patients, the progressive decline in the number of T-helper (CD4+) lymphocytes due to uncontrolled viral replication creates ideal conditions for these microorganisms to replicate widely and cause serious, even fatal, disease. The primary pathogenesis of opportunistic infections is the failure of the cellular immune system, particularly CD4+ cells, which play a vital role in eliminating intracellular pathogens. Therefore, a decline in the CD4+ cell count is a strong predictor of the occurrence and type of opportunistic infections that will occur. With the advancement of antiretroviral therapy (ART), the incidence of opportunistic infections has decreased drastically, but opportunistic infections remain a major cause of morbidity and mortality in HIV/AIDS patients who are undiagnosed, untreated, or who have experienced ART therapy failure (Pinto & Pereira, 2017).

III. RESEARCH METHODS

3.1 Research Design

This study used a Quasi-Experimental Design with a One Group Pretest-Posttest study, measuring CD4 counts before and after the intervention. This study design was chosen to assess the benefits of Multi-Strain Probiotics administration to the sample individuals, based on CD4 counts achieved at Waluyo Jati Regional Hospital. The distribution of sample data was tested using the Shapiro-Wilk normality test. Statistical analysis of the research data was performed using a paired t-test. The primary objective of using the paired t-test on pre-test and post-test data was to evaluate the effects of the Multi strain Probiotic product intervention. Twenty three patients were recruited using a purposive sampling technique.

3.2 Population And Sample

This study involved 23 individuals living with HIV (PLHIV) at Waluyo Jati Regional Hospital, Kraksaan, aged 18-65 years, with clinical stage 2 - 3. The sample selection criteria included individuals undergoing ART therapy for at least 2 years and with a history of suppressed viral load. The study also considered adherence to visits by individuals with HIV. Those who transferred to another hospital during the study period, had incomplete medical records, discontinued therapy by 2024 (died/stopped therapy/changed therapy regimen), suffered from pancreatic, liver, or kidney disorders, or were pregnant, were excluded from the study.

3.3 Research procedures

The study procedures began with obtaining ethical approval from the Ethics Committee of RSUD Waluyo. Subsequently, patients who met the inclusion criteria were identified. The required data were then extracted from the patients' medical records and entered into a data collection form. Data verification was performed to ensure accuracy and completeness.

In addition, a survey was conducted to further identify eligible participants. The researcher first introduced themselves and explained the study objectives and procedures to potential respondents. Those who agreed to participate were asked to complete an informed consent form. Respondents who had provided consent were then given a questionnaire to complete according to their actual condition. During the questionnaire process, the researcher accompanied the respondents to assist them if there were any questions or unclear items. After completion, each questionnaire was checked to ensure all items were filled in. Every questionnaire was assigned a specific code to facilitate data processing. Finally, the collected data were processed and analyzed, followed by interpretation of the research findings.

3.4 Ethical Test Result Number

Ethical testing was conducted at the Ethics Committee Institution of the University of Surabaya which was published on October 14, 2026 with No. 716/KE/X/2025.

3.5 Instrumen Penelitian

In conducting this research study, research instruments are needed such as patient medical record data and CRF (Case Report Form) to record research data and follow up on subjects.

3.6 Pengolahan Data

Data analysis in this study consisted of descriptive and inferential analysis. Descriptive analysis was used to describe the demographic characteristics of patients, presented in the form of frequency distribution tables and percentages for categorical data, and mean values and standard deviations for continuous data. Furthermore, data regarding patient medication and supplement use were collected using a special form and analyzed descriptively. Furthermore, inferential analysis began with the Shapiro–Wilk normality test to assess whether the data were normally distributed. If the data met the assumption of normality, a paired t-test was performed as a parametric test to compare the mean values before (pretest) and after (posttest) the intervention in the same group.

VI. RESULTS

4.1 Distribution of Characteristics in HIV Patients

The distribution of research data is shown in Table 1, based on the characteristics of the 23 PLHIV who participated in this study. Based on age group, the largest percentage of PLHIV (39.1%) were aged 26-35, 26.1% were aged 36-45, 17.4% were aged 46-55, 13.1% were aged 56-65, and 4.3% were aged 17-25. The

distribution of PLHIV by gender was 43.5% of the sample being male (n=10) and 56.5% being female (n=13). Based on the Population Group, the sample criteria were divided into 43.5% Risk Couples (n=10), 30.4% Men Who Have Sex with Men (MSM) (n=7), 13% Partners of PLHIV (n=3), 8.7% Clients of Sex Workers (n=2), and 4.4% Transgender (n=1). PLHIV based on the type of ART history used, it is known that 87% (n=20) of samples used the combination of TLD-Tenofovir(300)/Lamivudine(300)/Dolutegravir(50) and 13% (n=3) others used the combination of DUVIRAL-Zidovudine(300)/Lamivudine(150) - Nevirapine(200). Based on the research data obtained, it is known that the history of ART treatment of individual samples for 60 - 120 months was 47.8% (n=11), followed by a history of ART treatment > 120 months was 34.8% (n=8) and a history of ART treatment > 24 - 60 months was 17.4% (n=4).

Table 1. Distribution of characteristics in HIV patients

Variabel	*Frekuensi*	*Prosentase (%)*
Age (years)		
• 17-25: late teenager	1	4.3
• 26-35: early adult	9	39.1
• 36-45: late adult	6	26.1
• 46-55: early elderly	4	17.4
• 56-65: late elderly	3	13.1
Gender		
Male	10	43.5
Female	13	56.5
Population		
High-risk couple	10	43.5
MLM	7	30.4
PLHIV couple	3	13
Prostitute	2	8.7
Transgender	1	4.4
ART		
TLD-Tenofovir (300)/Lamivudine (300)/Dolutegravir (50)	20	87
DUVIRAL-Zidovudine (300)/Lamivudine (150) Nevirapine (200)	3	13
Duration of ART (months)		
>24 - 60	4	17.4
60 - 120	11	47.8
> 120	8	34.8

4.2 CD4 Levels Before and After Multi-Strain Adjuvant Probiotic Therapy Intervention Table 3 shows the distribution of CD4 levels in samples of PLHIV before and after multi-strain adjuvant therapy intervention. CD4 levels before intervention ranged from 219 to 477 cells/mm, with a mean of 341.74 cells/mm and a standard deviation of 67.7222. Meanwhile, CD4 levels after intervention

ranged from 142 to 609 cells/mm, with a mean of 420.00 cells/mm and a standard deviation of 141.572.

Table 2. Effect of CD4 levels with multi-strain probiotic adjuvant therapy intervention in HIV/AIDS patients for 3 (Three) months

Before Intervention				***After Intervention***			
CD4 Value	Freq	Percentage (n) (%)	Mean ± SD	CD4 Value	Freq	Percentage (n) (%)	Mean ± SD
299	1	4	341.74 ± 67.722	440	1	4	420.00 ± 141.572
316	1	4		398	1	4	
340	1	4		372	1	4	
310	1	4		313	1	4	
250	1	4		262	1	4	
301	1	4		324	1	4	
408	1	4		533	1	4	
401	1	4		521	1	4	
396	1	4		552	1	4	
431	1	4		601	1	4	
355	1	4		429	1	4	
328	1	4		466	1	4	
219	1	4		219	1	4	
477	1	4		710	1	4	
412	1	4		558	1	4	
409	1	4		531	1	4	
231	1	4		335	1	4	
378	1	4		609	1	4	
262	1	4		432	1	4	
343	1	4		290	1	4	
388	1	4		318	1	4	
292	1	4		142	1	4	
314	1	4		305	1	4	

4.3 Distribution of Opportunistic Infection during Follow-up

Table 3. Distribution of Opportunistic Infection in PLHIV samples for 3 (Three) months

Variable	Month 1	Month 2	Month 3
Participants observed (n)	23	23	23
Any opportunistic infection, n (%)	2 (8.7%)	0 (0%)	0 (0%)
Pneumonia, n (%)	1 (4.3%)	0 (0%)	0 (0%)
Oral candidiasis, n (%)	1 (4.3%)	0 (0%)	0 (0%)

Based on Table 3. above, based on the distribution of opportunistic infection events, data obtained from 23 research subjects who experienced opportunistic infections, two of which occurred in the first month: Candidiasis Oris and Pneumonia.

4.4 Effect Size

In pre–post experimental studies, the effect size value plays an important role in evaluating the strength of the influence of an intervention on the variable being measured. One of the most commonly used effect size measures is Cohen's d, which represents the mean change in standard deviation units of the population.

Cohen's d is calculated using the formula:

$$d_z = \frac{\bar{d}}{\sigma_d}$$

$$= \frac{78.261}{97.486}$$

= 0.8 (Large)

Description:
dz : Cohen's for paired samples
d : Mean difference between measurements before and after intervention
σd : Standard deviation of score differences

Table 4. Cohens'd classification

Nilai d	**Interpretasi**
0,2	*small*
0,5	*medium*

0,8	*large*
>1,0	*very large*

The magnitude of the effect of administering multi-strain probiotics on CD4 levels was analyzed using Cohen's dz effect size in a pretest–posttest design. Based on the results of the paired samples test, the average value of the difference in CD4 levels was 78.26 cells/mm^3 with a standard deviation of 97.49. The results of the effect size calculation show a value of dz = 0.80, which is included in the large effect category, so that the multi-strain probiotic intervention provides a clinically significant immunological impact.

V. DISCUSSION

This study was conducted on 23 subjects in a population of HIV patients with stages 2 and 3. The outcome of the study was the participants' engagement until they completed the three-month treatment period. Twenty-three subjects successfully achieved the outcome, with one subject dropping out due to severe nausea and vomiting, making the intervention impossible. This relatively small dropout rate did not impact the validity of the study results.

The percentage of female and male subjects in this study was 56% and 44%, respectively. This aligns with data from the Ministry of Health, which indicates that approximately 35% of HIV cases occur among housewives, a figure higher than other groups such as husbands of sex workers and men who have sex with men (MSM). Transmission from husbands to wives is estimated to account for approximately 30% of all cases, resulting in an increase of approximately 5,100 new HIV cases annually among housewives (Kemenkes, 2023).

The most common age ranges for HIV patients in this study were 26-35 years (39.1%) and 36-45 years (26.1%). This is related to high levels of sexual activity among those of productive age and a tendency to engage in risky behaviors. These behaviors include unprotected sex, having multiple sexual partners, and sharing unsterile needles for injection drugs, which increases the risk of HIV/AIDS transmission (Sutrasno, 2022).

The study results show that the distribution of HIV patients is dominated by high-risk couples (43.5%), followed by Men Who Have Sex with Men (MSM) (30.4%) in second place. This data aligns with research indicating that there are four key populations or specific groups with high-risk behaviors for HIV. These populations include Men Who Have Sex with Men (MSM), transgender women (transgender), Female Sex Workers (FSW), and injecting drug users (Rahma, 2024).

The results of the study showed that most subjects had a very high level of adherence to the therapy they underwent, both in the use of ART and multistrain probiotics. Almost all respondents had a compliance percentage of 100%, but there were 8 subjects who had compliance below 100% in both therapies. In ART therapy, several subjects were recorded as having compliance of 97.78%, 96.29%, 98.52%, 98.15%, 91.48%, and 99.25%, thus found that the minimum value of

compliance to this therapy is 91.48%. Meanwhile, in the multi-strain probiotic intervention, variations in compliance were also found below 100%, namely 97.78%, 96.29%, 98.85%, 98.14%, 91.48%, and 99.25%, with the same minimum compliance value of 91.48%. These findings indicate that patients were generally able to adhere to the prescribed treatment schedule, ensuring consistent therapy throughout the study period. This high level of adherence is a crucial factor in assessing the effectiveness of the intervention, as it can reduce bias caused by irregular medication or probiotic consumption, which can impact clinical outcomes.

In this study, a multi-strain probiotic supplement was administered to 23 subjects using a quasi-experimental design with a one-group pretest-posttest method to assess the effect of multi-strain probiotic supplementation on CD4 cell counts in HIV-infected patients. This treatment was conducted to evaluate the CD4 counts of the study subjects by observing their impact before and after receiving multi-strain probiotic supplementation without a control group. This was due to the limited number of subjects who failed to meet the inclusion criteria. Observations were made on CD4 counts before and after treatment, followed by observations of changes in CD4 count data.

Based on the distribution of pre- and post-treatment CD4 counts, data showed that 19 patients (82.46%) experienced an increase in CD4 count, while 4 patients (17.36%) experienced a decrease in CD4 count. The distribution of CD4 counts by gender was 7 men (36.82%), 12 women (63.12%), and 3 men (75%) and 1 woman (25%) experienced a decrease in CD4 count.

Table 5. The Shapiro-Wilk normality test was conducted to assess CD4 levels before and after the multi-strain probiotic intervention.

Probiotik Multi strain	*Statistic*	*df*	*Sig*
Pretest	.976	23	.829
Postest	.981	23	.929

Table 5 presents the results of the Shapiro–Wilk normality test for CD4 levels before and after 90 (ninety) days of multi-strain probiotic intervention. The significance value for the pretest data was 0.829 and for the posttest data was 0.929. Both significance values were greater than 0.05 (Sig. > 0.05), indicating that both the pretest and posttest data were normally distributed.

Table 6. The paired t-test (dependent t-test) was used to compare CD4 levels before and after the multi-strain probiotic intervention.

Paired Sample Correlation

Probiotik Multi strain	*N*	*Correlation*	*Sig*

Pretest & Postest	23	.789	.000

Paired Sample Statistics

Probiotik Multi strain	*Mean*	*N*	*Std. Devition*	*Std. Error Mean*
Pretest	341.74	23	67.722	14.121
Postest	420.00	23	141.572	29.520

Probiotic Multi strain	*Mean*	*Std. Deviation*	*Sig (2-tailed)*
Before-after	-78.261	97.486	0.001

Based on Table 6, the Paired Samples Statistics analysis showed that the mean CD4 value before multi-strain probiotic administration (pretest) was 341.74 ± 67.722, while the mean CD4 value after probiotic administration (posttest) increased to 420.00 ± 141.572. The number of subjects measured at each stage was 23. This difference indicates an increase in CD4 levels of 78.26 following multi-strain probiotic supplementation. Furthermore, the Paired Samples Correlations results in Table 5.2 showed a correlation coefficient of r = 0.789 with a significance value of p = 0.000 (p < 0.05). These findings indicate a very strong and statistically significant relationship between CD4 values before and after multi-strain probiotic administration, suggesting that changes in CD4 levels for each respondent were consistent between the pretest and posttest measurements.

The results of the Paired Sample t-Test in Table 5.2 showed a mean difference of -78.261, with a t value of -3.850, degrees of freedom (df) of 22, and a significance value of 0.001. The 95% confidence interval ranged from -120.417 to -36.105, which does not include zero. These results indicate a statistically significant difference between CD4 levels before and after multi-strain probiotic administration.

The findings of this study demonstrate that multi-strain probiotic supplementation significantly increased CD4 levels in the subjects. The increase in CD4 levels after intervention reflects an improvement in immune status, indicating the role of probiotics in supporting immune system function. Multi-strain probiotics are known to modulate the immune system by improving gut microbiota balance and stimulating mucosal immune responses. Probiotic bacteria can bind to intestinal epithelial cells and activate them through pattern recognition receptors (PRRs). This activation triggers cytokine release, which subsequently stimulates regulatory T cells (Tregs), playing a crucial role in maintaining immune balance in the intestinal mucosa. Tregs act as effective suppressors of immune responses, helping to control excessive immune reactions (Mazziotta, Tognon, Martini, Torreggiani, & Rotondo, 2023). The use of multi-strain probiotics is also considered to provide synergistic effects among strains, thereby enhancing immune modulation effectiveness. These findings are

consistent with previous studies indicating that probiotics have the potential to improve immunological parameters. Therefore, multi-strain probiotics may be considered supportive therapy to help increase CD4 levels.

The findings presented in Table 5 indicate a low overall occurrence of opportunistic infections (OIs) among the study over the three-month observation period. Specifically, only two cases of opportunistic infections were detected—one case of pneumonia and one case of oral candidiasis both occurring in Month 1, with no new infections observed in subsequent months. This pattern aligns with evidence suggesting that effective clinical management and maintenance of immune function can significantly reduce the incidence of OIs in patients at risk.

Oral candidiasis and pneumonia have been consistently reported in the literature as among the most common opportunistic infections in immunocompromised individuals, particularly among people living with HIV/AIDS. Oral candidiasis is widely acknowledged as a prevalent fungal manifestation associated with deteriorating immune defense and low CD4+ counts, especially in untreated or advanced cases (e.g., >25% of isolates commonly show persistent infection) (Keyvanfar, 2024). Similarly, pulmonary infections such as Pneumocystis jirovecii pneumonia and severe bacterial pneumonia remain significant contributors to morbidity in immunodeficient populations, particularly in the absence of timely therapeutic intervention (Coelho L. V., 2014).

The marked reduction to zero observed infections in Month 2 and Month 3 likely reflects successful clinical management, including early identification and appropriate treatment strategies, as well as possible immune recovery. Studies evaluating the impact of antiretroviral therapy (ART) in HIV-infected cohorts show that the incidence of opportunistic infections declines significantly within the first year of treatment, with reductions in OI risk ranging from approximately 57% to over 90% compared to ART-naïve individuals, particularly for oral candidiasis and Pneumocystis pneumonia (Low, 2016). This suggests that early treatment and rigorous clinical follow-up—if implemented in this study's context—could have contributed to the absence of new OIs after the initial month.

Additionally, the limited occurrence of opportunistic infections in this cohort might be influenced by factors such as immune status (e.g., CD4+ level), adherence to prophylaxis or treatment, and early intervention, which have been identified as critical determinants of OI risk. Higher CD4+ counts and prompt ART initiation are well-established protective factors that substantially reduce the likelihood of OI development over time (Coelho L. E., 2016).

In summary, the low incidence of opportunistic infections observed in the study supports existing evidence that *effective clinical care*, *immune restoration*, and *early treatment protocols* are key to limiting opportunistic disease events. Continued surveillance and rigorous management protocols remain essential, especially in populations vulnerable to immune suppression.

IV. CONCLUSION

The findings of this study demonstrate a strong correlation between CD4 levels before and after treatment, with multi-strain probiotic supplementation resulting in a statistically significant increase in CD4 counts. This improvement reflects enhanced immune status and supports the role of multi-strain probiotics as an adjunct therapy in strengthening immune system function, potentially through modulation of gut microbiota and immune responses. Furthermore, the low incidence of opportunistic infections observed during the study period aligns with existing evidence that effective clinical management, immune recovery, and early initiation of treatment are crucial in reducing the occurrence of opportunistic diseases in people living with HIV. These results highlight the importance of maintaining high treatment adherence, continuous clinical monitoring, and strict management protocols, particularly among individuals vulnerable to immune suppression, to sustain immune improvement and prevent infection-related complications.

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