Research Protocol

The Role of Probiotics L. plantarum, S. thermophiles, B. bifidum on Gut inflammation, Bacterial Translocation, and CD4+ Cell Count in HIV Patients with Immunological Non-Responder

(PIONIR)

INVESTIGATORS:

Principal : Erni J. Nelwan, MD, Ph.D

Others : Rina Agustina, M.Sc, Ph.D

Prof. Marcellus Simadibrata, MD, Ph.D

FACULTY OF MEDICINE

UNIVERSITY OF INDONESIA

Approved May 1st, 2018

I. Background

Human Immunodeficiency Virus (HIV) infected patients treated with anti retroviral drugs may experiencing immunological failure known as immunological non responders (INR). In this particular group of patient, the level of CD4 still below normal limit even though the HIV virus is suppressed (undetected). Immunological non responders (INR) were associated with high morbidity.[1] Hypothetically, INR patients have decreased gut integrity and immunity due to increased gut inflammation and further causing microbial translocation.[2] Probiotics may improved gut inflammation condition, however the direct benefits of probiotics supplementation for INR HIV patients have not been studied.[3] Growing number of INR HIV patients will be associated with higher economical burden and decreased quality of life.

Microbial translocation was thought as the underlying mechanism of persistently immune activation which may lead to immunological failure condition in virally suppressed HIV patients.[2] Decreased number of T- helper 17 (Th17) in HIV patients may facilitated microbial translocation. HIV Virus destroyed the CD4 T memory cells which mainly found in *gut associated lymphoid tissue* (GALT), thus resulting in lower production of Th17 in gut mucosal tissue.[2,4] One of the microbial translocation marker is 16S ribosomal DNA (16S rDNA) bacteria blood level.[5] Gut inflammation may be represented by fecal calprotectin level.[6]

Probiotics supplementation in HIV patients was proposed may increase gut integrity and immunity through several mechanisms: improving tight junction gut mucosal, increasing mucin production, regulating Th17; therefore decreasing gut inflammation and microbial translocation. Improved gut inflammation and decreased microbial translocation may increased the immune recovery in INR HIV patients [7-9]

This clinical trial is intended to evaluate the immune recovery effect of Probiotics supplementation in INR HIV patients.

II. Objectives

A. General Objectives

To determine the effect of Probiotic in immune response for immunological non responder HIV patients

B. Specific Objectives

a. Primary Objectives

i. To determine the average CD4 value in immunological non responder HIV patients treated with Probiotic compared to placebo

ii. To determine the average T helper 17 value in immunological non responder HIV patients treated with Probiotic compared to placebo

iii. To determine the average 16S rDNA value in immunological non responder HIV patients treated with Probiotic compared to placebo

iv. To determine the fecal calprotectin value in immunological non responder HIV patients treated with Probiotic compared to placebo

b. Secondary Objectives

To determine the effect of Probiotic supplementation towards quality of life improvement in immunological immunological non responder HIV patients, using HIV symptom index.

III. Methods

A. Study Design

This study is double-blind randomized control trial design

B. Time and Place

This study will be conducted in Cipto Mangunkusumo National Central General Hospital, especially in Human Infectious Disease polyclinic. Enrollment will start on May 2018, and recruited until minimum sample size was fulfilled

C. Study Population

1. Targeted Population: HIV patients with Immunological Non-responder condition in Indonesia
2. Sample: HIV patients with Immunological Non-responder condition in Indonesia
3. Inclusion Criteria:
    a. HIV positive patients aged 18-55 years old
    b. had already treated with first line Highly Active Anti Retroviral Therapy for at least 6 months
    c. had CD4 level of 200-410 cell/μL
    d. undetectable Viral Load (<34 copies/ml blood)
    e. agreed to participate in the study and signed the informed consent

4. Exclusion Criteria:
    a. Pregnant patients
    b. Lactating patients
    c. Known *Lactobacillus* allergy
    d. Body Mass Index (BMI) <16 kg/m^2
    e. Under Tuberculosis treatment or other acute illness, acute diarrhea,
    f. routinely taking selenium containing vitamin in last 1 month,
    g. routinely consuming probiotics containing product in last 1 month
5. Estimation Sample Size: 80 samples. Which will be divided into two groups equally.
6. Study Procedure

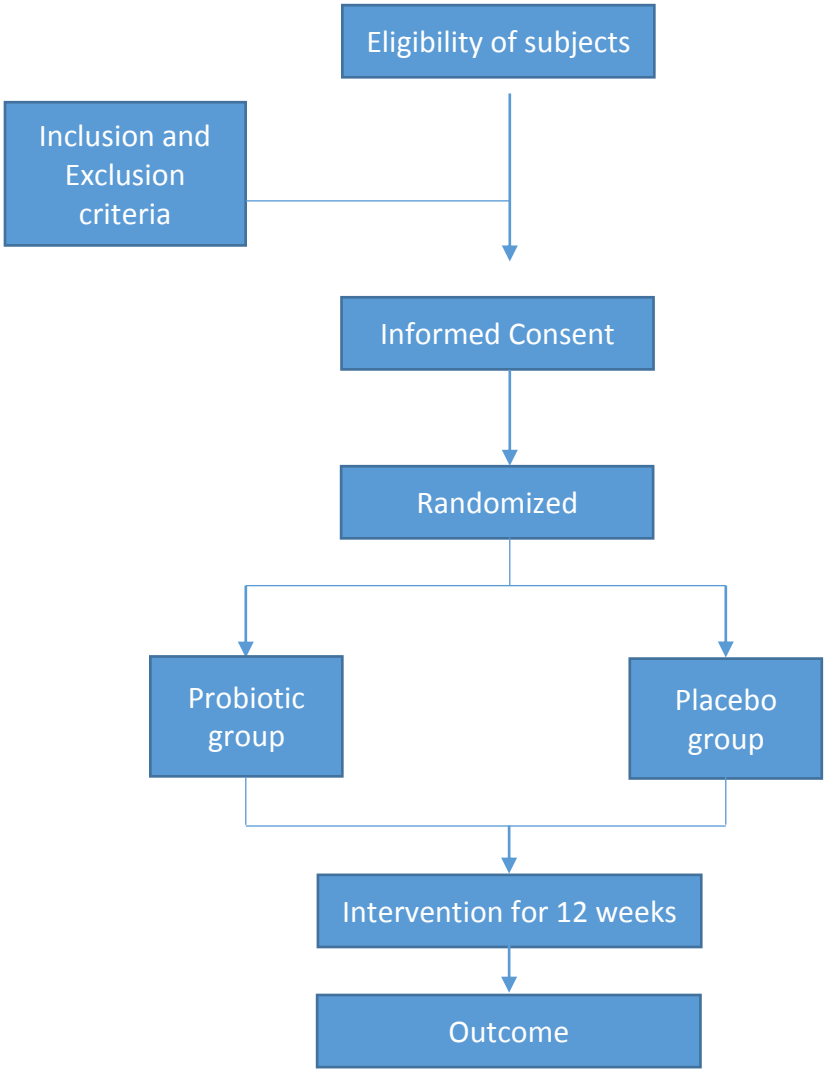
    Eligible patients will be approached to be enrolled in the clinical trial. Informed consent will be obtained. Patients will be randomized and divided into two groups, intervention and placebo group. Intervention group will receive Probiotics (Chewable tablet) containing *Lactobacillus plantarum, Stretococcus thermophiles, Bifidobacterium bifidum* once daily. While, Placebo group will receive placebo which had identical shape, flavor, and smell as the interventional drug.

Intervention will be held for 12 weeks. CD4, Th17, 16S Ribosomal RNA, and fecal calprotectin level will be checked at the 0 week and repeated after 12 weeks of intervention. Self administered HIV Symptoms Index questionnaire will be filled by the respondent on 0,4,8,12 weeks of intervention. Food frequency questionnaire will be filled by the respondents with the help of nutritionist on 0,4,8,12 weeks of intervention.

7. Side effects

   All side effects that may arise and related to probiotics intake will be assessed and reported. Appropriate medical advice will be provided.

D. Research Flow



E. Ethics

This research has been approved by Ethics Committee of the Faculty of Medicine Universitas Indonesia (Approval number: 981/UN2.F1/ETIK/2017)

IV. Statistical Analysis Plan

All Data will be analyzed using SPSS 24.0 (SPSS) for Windows. Laboratory parameters will be compared between two different times and also compared between two groups by using paired and independent t test (if normally distributed) or using Wilcoxon and Mann Whitney (if not normally distributed). Statistically significance was determined by p value <0.05. Intention to Treat analysis will be used. HIV symptom index and food frequency will be reported descriptively.

V. References

1. Wilson EMP, Sereti I. Immune restoration after antiretroviral therapy: the pitfalls of hasty or incomplete repairs. Immunological reviews. 2013;254(1):343-54.

2. Gaardbo JC, Hartling HJ, Gerstoft J, Nielsen SD. Incomplete Immune Recovery in HIV Infection: Mechanisms, Relevance for Clinical Care, and Possible Solutions. Clinical and Developmental Immunology. 2012;2012. 3. d'Ettorre G, Ceccarelli G, Giustini N, Serafino S, Calantone N, De Girolamo G, et al. Probiotics Reduce Inflammation in Antiretroviral Treated, HIV-Infected Individuals: Results of the "Probio-HIV" Clinical Trial. PloS one. 2015;10(9):e0137200.

4. Brenchley JM, Douek DC. The mucosal barrier and immune activation in HIV pathogenesis. Current opinion in HIV and AIDS. 2008;3(3):356-61.

5. Harris KA, Hartley JC. Development of broad-range 16S rDNA PCR for use in the routine diagnostic clinical microbiology service. Journal of medical microbiology. 2003;52(Pt 8):685-

91.

6. Xiang JY, Ouyang Q, Li GD, Xiao NP. Clinical value of fecal calprotectin in determining disease activity of ulcerative colitis. World journal of gastroenterology. 2008;14(1):53-7.

7. Karczewski J, Troost FJ, Konings I, Dekker J, Kleerebezem M, Brummer RJ, et al. Regulation of human epithelial tight junction proteins by Lactobacillus plantarum in vivo and protective effects on the epithelial barrier. American journal of physiology Gastrointestinal and liver physiology. 2010;298(6):G851- 9.

8. Anderson RC, Cookson AL, McNabb WC, Kelly WJ, Roy NC. Lactobacillus plantarum DSM 2648 is a potential probiotic that enhances intestinal barrier function. FEMS microbiology letters. 2010;309(2):184- 92.

9. Lopez P, Gonzalez-Rodriguez I, Gueimonde M, Margolles A, Suarez A. Immune response to Bifidobacterium bifidum strains support Treg/Th17 plasticity. PloS one. 2011;6(9):e24776.