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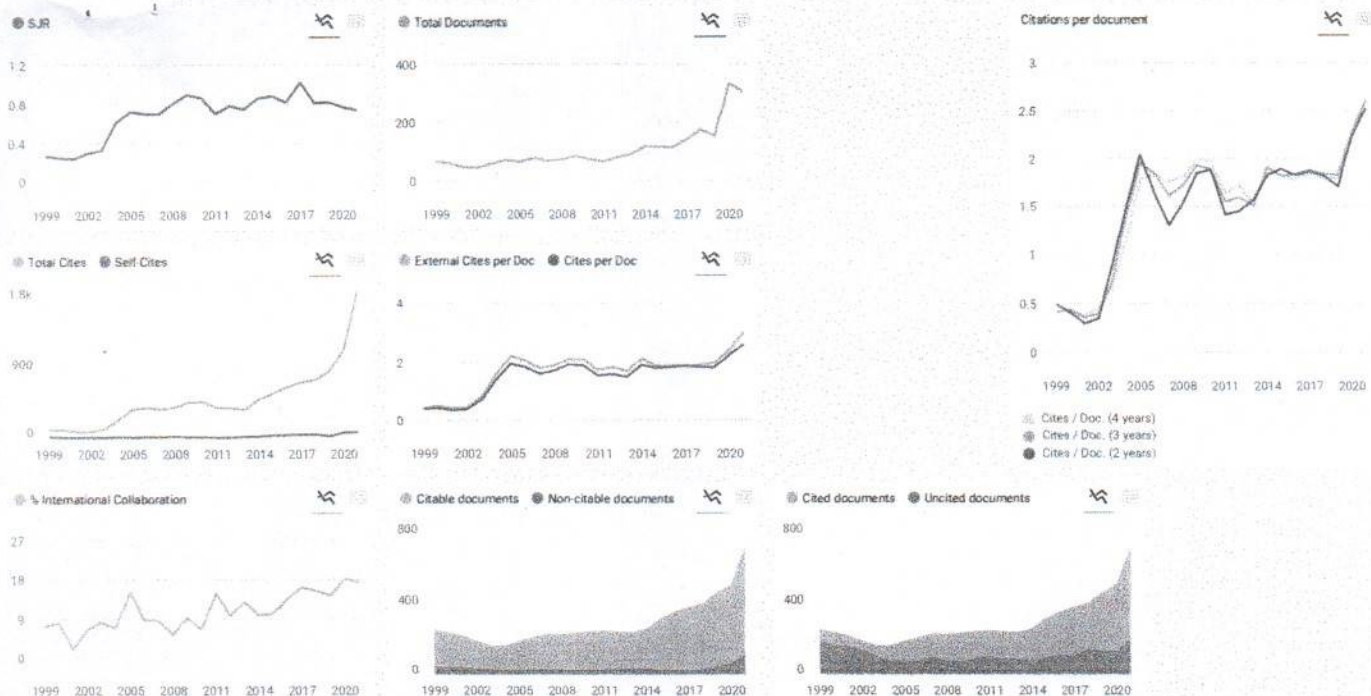
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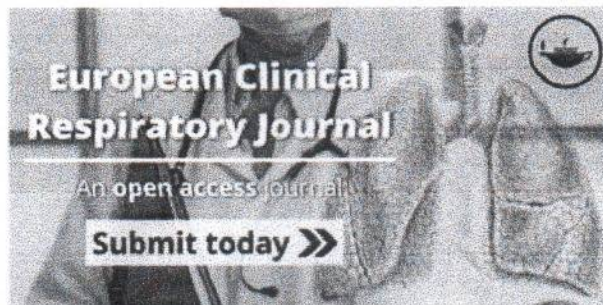
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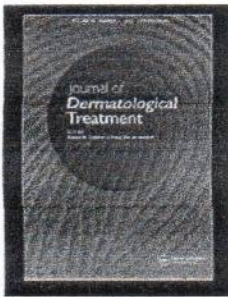
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Beneficial effect of *Lactobacillus plantarum* IS-10506 supplementation in adults with atopic dermatitis: a randomized controlled trial

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ARTICLE



Beneficial effect of *Lactobacillus plantarum* IS-10506 supplementation in adults with atopic dermatitis: a randomized controlled trial

C. R. S. Prakoeswa^a , L. Bonita^a, A. Karim^a, N. Herwanto^a, M. A. Umborowati^a , T. Setyaningrum^a, A. N. Hidayati^a and I. S. Surono^b 

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ABSTRACT

Introduction: Although the therapeutic effects of probiotics in atopic dermatitis (AD) are known, the limited findings in adults are inconsistent. *Lactobacillus plantarum* (LP) IS-10506 was found to improve AD symptoms due to its immunomodulatory effects.

Objective: To assess the Scoring Atopic Dermatitis Index (SCORAD), the serum immunoglobulin E (IgE), interleukin (IL)-4, interferon-gamma (IFN- γ), forkhead box P3 (Foxp3+), and IL-17 levels in adults with mild and moderate AD after LP IS-10506 supplementation.

Methods: A randomized double-blind placebo-controlled trial comparing the microencapsulated probiotic (2×10^{10} CFU/day) and placebo (skim milk-Avicel) was conducted at an outpatient clinic on 30 adults with mild and moderate AD. The patients were divided into 2 groups with 15 patients each: intervention and control.

Result: The SCORAD score was significantly lower in the probiotic than the placebo group on the 8th week. The IL-4 and IL-17 levels were significantly lower in the probiotic than the placebo group. The IFN- γ and Foxp3+ levels were significantly higher in the probiotic than the placebo group. However, the IgE levels remained significantly unchanged.

Conclusion: The administration of LP IS-10506 is effective for alleviating AD symptoms in adults owing to its immunomodulatory effects.

ARTICLE HISTORY

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Atopic dermatitis; adult; probiotic; *Lactobacillus plantarum*

Introduction

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease that is a significant cause of morbidity, a decrease in the quality of life (QOL), and psychological and economic burden (1). The disease mainly affects infants and children, and it disappears in 50% of the cases at adolescence, although it can persist or even begin in adulthood. AD is associated with other atopic symptoms such as allergic rhinitis, allergic conjunctivitis, and asthma (2).

AD prevalence has rapidly increased with industrialization. The worldwide prevalence of AD is 1–20% in children, which is more than that in adults (approximately 1–3%) (2,3). According to the National Health Interview Survey, the epidemiological studies in the USA in 2013 suggested that the prevalence of adult AD was 10.2% in 1 year (4). In an agricultural country such as China, Eastern Europe, and Central Asia, the prevalence of AD is much lower (5,6). In a retrospective study conducted in the Division of Allergy Immunology Outpatient Unit Dermatology and Venereology of Dr. Soetomo Teaching Hospital (2013–2015), 7.3% AD patients were recorded belonging to the most common age group of 15–24 years (33.3%) (7).

The pathogenesis of AD is multifactorial, including immunological factors. AD is chronic dermatitis that is characterized by the dominant expression of T-helper (Th) 2 cells associated with increased cellular infiltration of the skin and increased IgE.

These T cells can induce the apoptosis of keratinocytes and affect the function of the skin barrier, which can lead to further infection and the occurrence of complications (8). Approximately 70–80% of adult AD cases are of extrinsic types of AD, showing an increase in serum IgE specific to environmental allergens or food (8,9). In extrinsic AD, memory T cells express skin-homing receptor 2 cutaneous lymphocyte-associated antigen (CLA), which increases the levels of cytokines from Th2. This T-cell CLA+ also produces abnormal IFN- γ , which is a Th1 cytokine that can inhibit Th2 cell functions (9–11).

AD is characterized by increased production of cytokines Th2, interleukin (IL)-4, IL 5, and IL-13 associated with eosinophilia and an increase in the serum IgE levels. The increase in IgE and eosinophil responses reflects an increase in the expression of cytokines Th2 (6,8). Th1 will mainly produce IFN- γ and IL-12, which will suppress IgE production and increase the regulation of IgG antibodies. IL-17 is another important component of allergic and inflammatory diseases, and the infiltration of IL-17 produces T cells (Th17), which are also known to be higher in acute AD skin lesions (6). The discovery of the Th17 pathway and regulatory T cells (Treg) alter the simple concept of Th1/Th2 balance and becomes a 4-way balance system. Th17 cells and Foxp3+ Treg produce T cells (TR1), which play an important role locally and systemically in controlling the symptoms of AD (6). Therefore, the balance of the 4-way pathway among Th1,

Th2, and Th17 cytokines and Treg cells is needed for controlling the pathogenesis of AD occurrence.

Several approaches to AD treatment have been proposed, including skin hydration, emollients, allergen avoidance, and the use of antihistamines or corticosteroids during the exacerbation phase. This treatment can relieve symptoms, but is often ineffective and causes side-effects when used over a long period of time (10). AD patients often require long-term systemic treatment, especially in adults who are more resistant to adequate topical treatment.

The dysregulation of cellular immunity due to the imbalance of Th1 and Th2 cells and Th17 and Foxp3⁺ Treg cells is the basis of AD occurrence, and, until date, no therapy has focused on this imbalance. Probiotics can be a promising treatment in improving this dysregulation and can be considered as an alternative or adjuvant therapy in AD because of its immunomodulatory effects in both children and adults (12,13). However, there is a lack of strong evidence to support the use of probiotics for future AD clinical treatment (14,15).

Although the effectiveness of probiotics in AD remains elusive, probiotics have been searched as an alternative treatment and as a prevention option for AD management in several studies, especially in the pediatric population, albeit it remains extremely rare in adults (15). The consumption of probiotics helps stimulate the intestinal microbiota by modulating toll-like receptors, and the introduction of proteoglycan proteins in enterocytes can activate dendritic cells and Th1 responses. This Th1 stimulation suppresses the Th2 response, which in turn leads to improvements in the balance between Th1 and Th2 (16,17). To synthesize the evidence of the effectiveness of probiotics in the treatment or prevention of AD in adults, studies conducted by experts have shown that the use of probiotics in adult AD can increase the production of IL-10 and IFN- γ and reduce the IgE levels and secretion of TNF- α , IL-5, and IL-17 (16,18).

For AD patients, in addition to focusing on the treatment of the current diseases, improvement in the QOL of patients should be considered. Severe pruritic complaints, frequency of sleep disturbances, and skin dryness are relevant predictors for assessing the QOL of adult AD patients (4). The mean QOL tends to increase with age in AD patients (4,5,19).

This study determined the efficacy of probiotic use in mild to moderate AD adults at Dr. Soetomo Teaching Hospital Surabaya. Probiotic administration is expected to restore the Th1/Th2 balance by suppressing Th2 cytokines (represented by IL-4), which in turn will increase the regulation of Th1 cell cytokines (represented by IFN- γ). Similarly, the balance of the Th17 (IL-17) and Foxp3 pathways + regulator T lymphocytes (20). This relationship can be evaluated using SCORAD. The probiotics used belong to the *Lactobacillus* sp. group, *L. plantarum* (LP) IS-10506, originated from dadih, traditional fermented buffalo milk of West Sumatera (21) and were identified by 16S rRNA gene sequencing (Gene Bank accession no. DQ860148) (22). As an indigenous probiotic from Indonesia, the microencapsulated strain is proven to be resistant to acidic and bile salts conditions (23) and has the potential in reducing SCORAD index in AD children (24).

Materials and methods

This was a randomized, double-blind, controlled, and parallel analytical experimental clinical trial that compared the LP

probiotic therapy and placebo. The study was conducted at the Division of Immunology Allergy Outpatient Unit Dermatology and Venereology Department of Dr. Soetomo Teaching Hospital Surabaya among adult patients with mild and moderate AD (both old and new patients). The subjects included 30 consecutive adult AD patients (aged >14 years) who met the AD diagnosis criteria according to the Hanifin-Rajka criteria and had serum IgE levels >100 IU/ml, generally good condition, and the willingness to participate in the study. Patients using systemic corticosteroids and phototherapy in the past 1 month, systemic immunosuppression drugs in the last 3 months, probiotics and their products in the past 4 weeks or with immunosuppressed conditions and other serious illnesses such as clinical cases of skin diseases or other systemic diseases were excluded from the study. The subjects were divided into 2 groups of 15 each by simple randomized technique to either received LP therapy or placebo.

Patients who meet the criteria for receiving samples were examined for IL-4, IFN- γ , IL-17, and Foxp3⁺ Treg cells, and their SCORAD index were determined. Next, the patients were divided into 2 groups to receive either LP probiotics or placebo. The researchers and the subjects were blinded about the drugs given to the study subjects (Figure 1).

Probiotic microencapsulation of LP IS-10506 at a dose of 2×1120 g (10^{10} CFU)/day or placebo (skim milk-Avicel) was given for the 8-week intervention phase. The placebo resembled the test drug in terms of shape, packaging, smell, color, and taste. LP and placebo were provided by the Department of Food Technology, Faculty of Engineering, Bina Nusantara University, Jakarta.

Total IgE from all blood samples was assessed using an ELISA kit (Advia Centaur XPT[®], Siemens, Wiesbaden, Germany) with the Advia Centaur IgE Reagent, and T-cell IL-4, IFN- γ , IL-17, and Foxp3⁺ cytokines were assessed by flow cytometry (Facs Calibur[®], Singapore, Singapore). Purified cytokines (IL-4, IFN- γ , IL-17, and Foxp3⁺) were purchased from BD Plasminogen (San Jose, CA).

To read flow cytometry results, gating is performed on lymphocyte populations based on size and granularity. We also conducted gating on CD4⁺ lymphocytes to produce CD4⁺ expression for IL-4, IFN- γ , IL-17, and Foxp3⁺ Treg cells. The baseline readings of these parameters were recorded before the start of supplementation and after the intervention. The SCORAD index was evaluated and recorded at weeks 4 and 8.

Results

The mean age in the 2 groups of this study was 37.87 ± 14.214 years, and the age group of patients between the 2 groups did not differ with a p -value of .940. A history of atopy was obtained from the patients and their families, including a history of bronchial asthma, allergic rhinitis, and AD. The mean duration of the illness was 15.4 ± 11.94 days, with no difference between the groups. Moreover, there were no significant differences between the groups in sex ($p = 1.000$), age ($p = .940$), history of atopy ($p = .674$), disease onset ($p = .944$), and duration of illness ($p = .633$). There was also no significant difference between groups in baseline SCORAD and laboratory parameters, such as serum IgE, IL-4, IFN- γ , IL-17, and Foxp3⁺ level with p -values > .05 (Table 1).

Supplementation with probiotics LP IS-10506 was found to decrease Th2 and increase Th1 cytokines. Th2 modulation of the

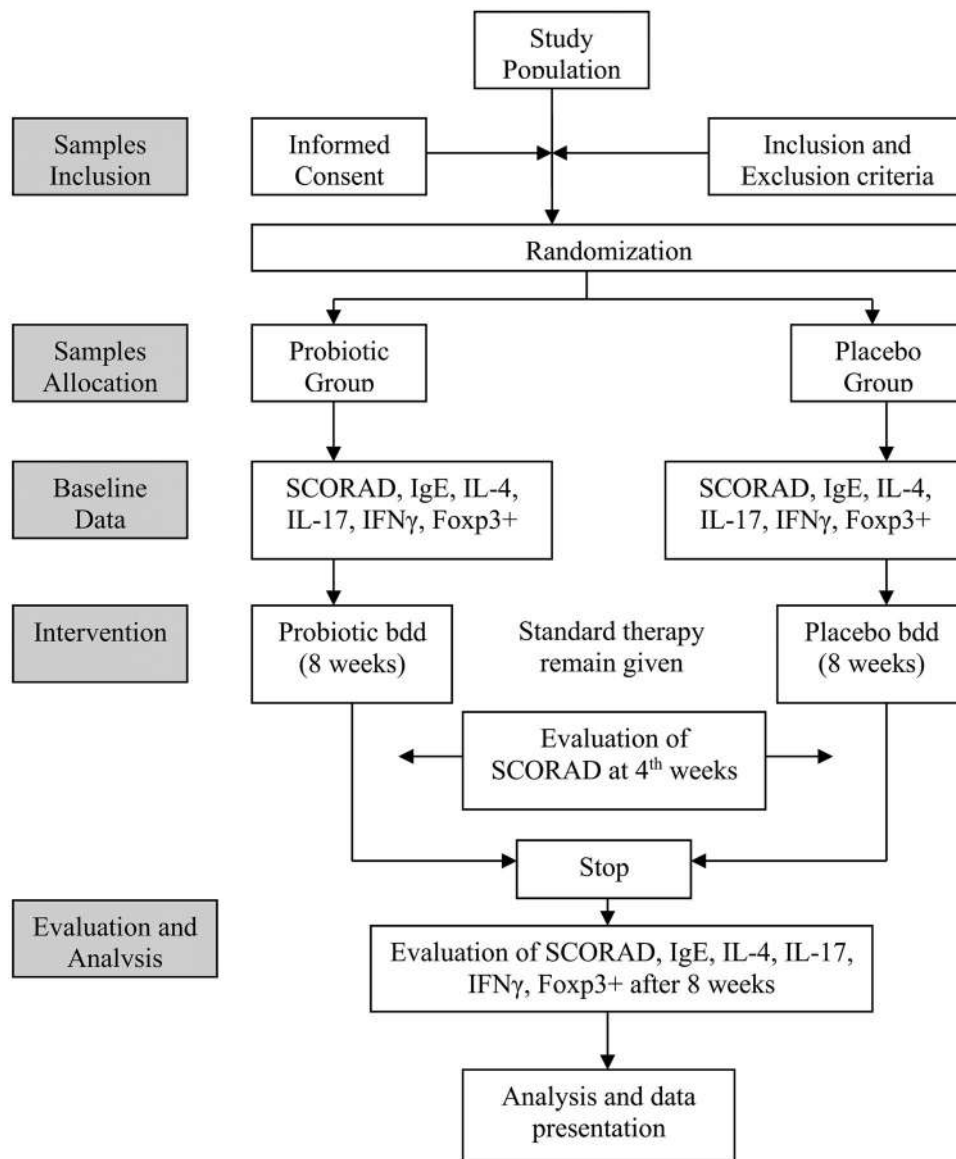


Figure 1. Research algorithm.

Table 1. Baseline characteristics of the adult atopic dermatitis subjects in the Immunology Allergy Division outpatient unit of the Dermatology and Venereology Department of Dr. Soetomo Teaching Hospital Surabaya.

Variable	Probiotic LP (n = 15)	Placebo (n = 15)	p-Value
Sex			
Men, n (%)	4 (26.7)	5 (33.3)	1.000
Women, n (%)	11 (73.3)	10 (66.7)	
Age (Years) mean ± SD	37.67 ± 15.92	38.07 ± 12.837	.940
Atopy history			
Yes, n (%)	12 (80.0)	12 (80.0)	.674
No, n (%)	3 (20.0)	3 (20.0)	
Onset (years), mean ± SD	2.46 ± 1.45	2.5 ± 1.11	.944
Morbidity (day), mean ± SD	14.4 ± 9.04	16.5 ± 14.53	.633
SCORAD (IU/ml), mean ± SD	34.79 ± 12.41	31.55 ± 12.76	.349
Serum IgE (IU/ml), mean ± SD	522.71 ± 832.65	260.79 ± 206.10	.340
IL-4 (IU/ml), mean ± SD	3.32 ± 0.52	3.00 ± 2.03	.662
IFN-γ (IU/ml), mean ± SD	0.91 ± 0.89	1.63 ± 1.40	.191
IL-17 (IU/ml), mean ± SD	5.50 ± 2.36	4.31 ± 1.95	.143
Foxp3 (IU/ml), mean ± SD	1.23 ± 0.69	1.47 ± 1.31	.492

Note. Foxp3+: forkhead box P3; IFN-γ: interferon gamma; IgE: immunoglobulin E; IL: interleukin.

adaptive immune response observed in this study developed through the expression of IL-4 and modulation of the Th1 adaptive immune response developed through IFN-γ expression. The level of IL-4 produced by CD4⁺ T lymphocytes was significantly reduced in both the placebo and probiotics groups (Figure 2 and 3), and the IL-4 levels in CD4⁺ T lymphocytes in the probiotic group were significantly lower than those in the placebo group (Figure 4).

IFN-γ produced by CD4⁺ T lymphocytes was significantly higher in the probiotic group (Figure 2). Probiotic supplementation resulted in a significant reduction in the IL-4 levels of CD4⁺ T lymphocyte (p = .000). The mean IFN-γ CD4⁺ T lymphocyte levels appeared to be higher in the probiotic group than in the placebo group at the end of the treatment with p = .003 (Figure 4).

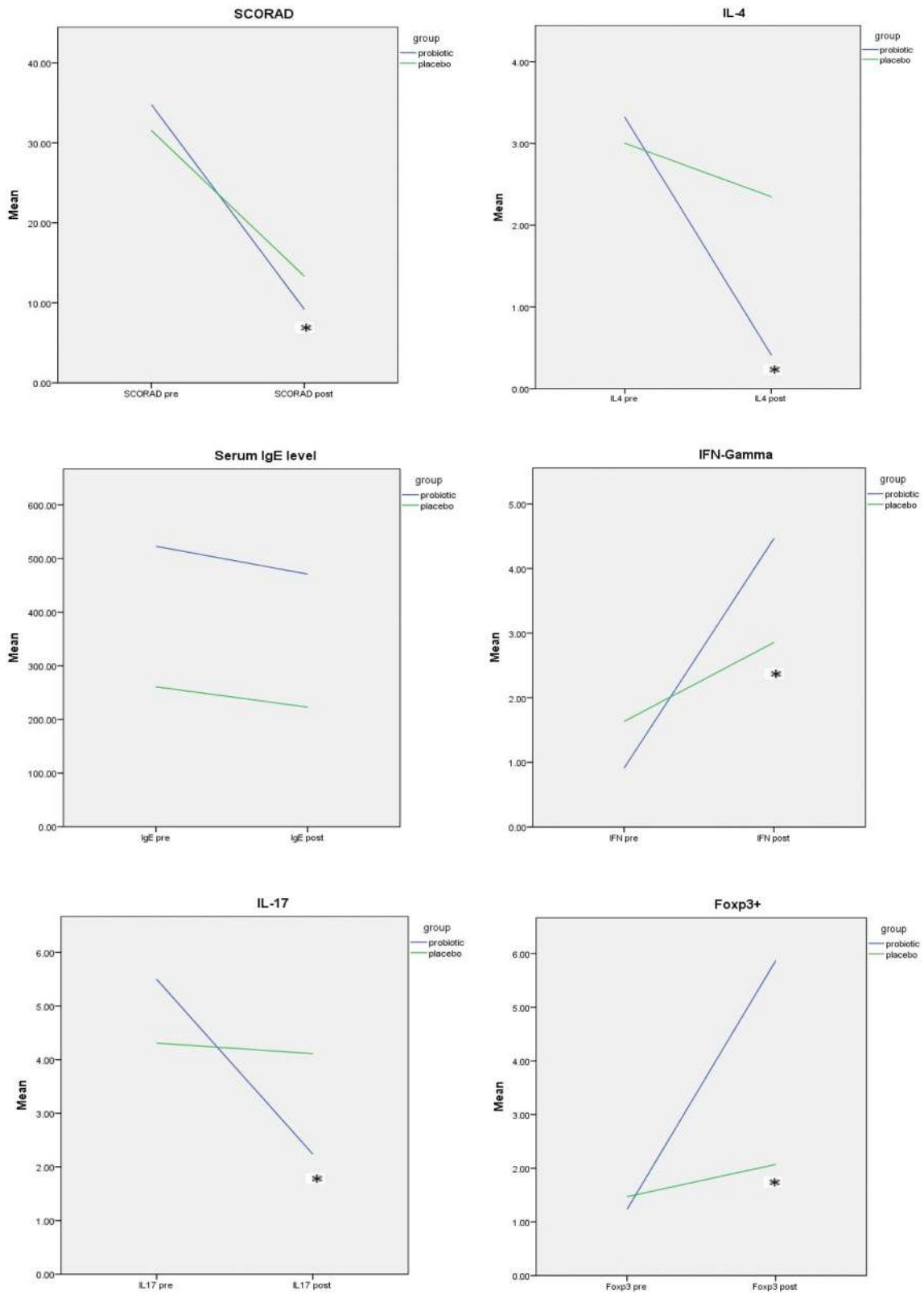


Figure 2. The profile of immune parameters in the probiotic group as measured by flow cytometry. (A) Interleukin (IL)-4, before; (B) IL-4, after; (C) interferon (IFN)- γ , before; (D) IFN- γ , after; (E) IL-17, before; (F) IL-17, after; (G) forkhead box P3 (Foxp3)+, before; (H) Foxp3+, after.

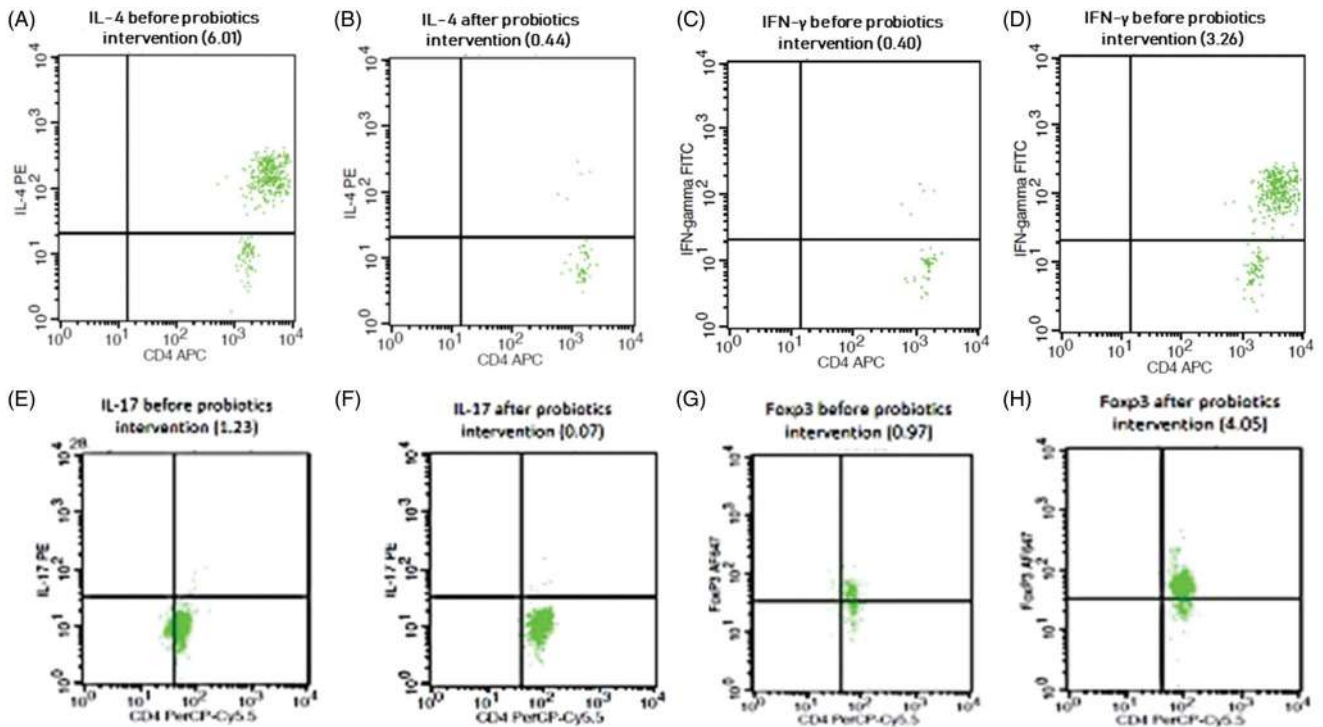


Figure 3. The profile of immune parameters in the placebo group as measured by flow cytometry. (A) Interleukin (IL)-4, before; (B) IL-4, after; (C) interferon (IFN)- γ , before; (D) IFN- γ , after; (E) IL-17, before; (F) IL-17, after; (G) forkhead box P3 (Foxp3)⁺, before; (H) Foxp3⁺, after.

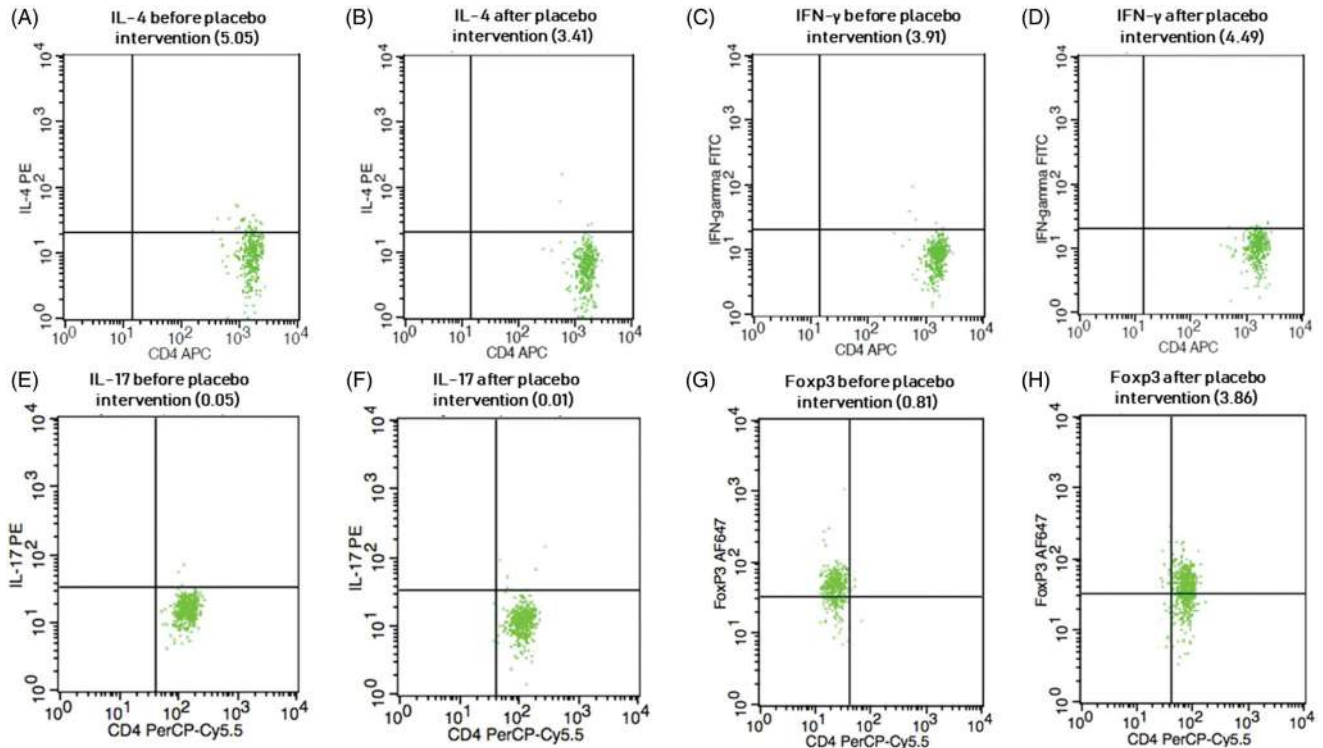


Figure 4. Comparison of the changes in the laboratory parameters between the probiotic LP-1S10506-administered group and the placebo group. The ratios of change are shown for the levels of SCORAD score, IL-4, serum IgE, IFN- γ , IL-17, and Foxp3⁺ in both groups. Data were analyzed by the Mann-Whitney *U* test. * $p < .05$ was statistically significant.

Immunoglobulin E level

No alteration was noted in the total serum IgE level after 8 weeks of therapy in both the groups, but the mean total IgE level in the probiotic group tended to be lower than in the placebo group (Table 2).

Activation of regulatory T cells from the adaptive immune response

Treg activates the adaptive immune system, as analyzed from Foxp3 levels. In this study, a significant increase in the Foxp3 levels was noted in the probiotic group ($p = .000$) after 56 days of taking probiotics (Figure 4).

Activation of IL-17 from the adaptive immune response

The activation of IL-17 from the adaptive immune response was observed through the ratio of IL-17 and CD4⁺ T lymphocyte cells. The ratio of IL-17/CD4⁺ lymphocyte T cells decreased significantly ($p = .009$) in the probiotic group as compared to that in the placebo group.

Discussion

Homeostasis abnormalities occur in AD due to an imbalance in the Th1-Th2 response as a result of allergen exposure, which in turn causes the development of mild to severe clinical symptoms (8). In this study, improvements in clinical manifestations calculated by the SCORAD index in the probiotic group differed significantly as compared with that in the placebo group (Figure 4). The administration of LP for 8 weeks significantly reduced the SCORAD index as compared to the placebo and the mean SCORAD index for probiotics and placebo ($p = .000$). Clinical improvements noted in the placebo group was derived from the standard therapy using antihistamines, emollients, and corticosteroid.

In this study, the use of probiotics improved the AD clinical symptoms. The clinical symptoms decreased significantly, but not with respect to the total IgE level. Vakirlis et al. demonstrated that IgE was strongly correlated with the tumor necrosis factor (TNF), IL-4, and eosinophil and was associated with the severity of the disease, as indicated by the SCORAD index (10).

The result of the comparative test between the treatment groups revealed that the results of IL-4 levels decreased significantly in both the groups. Our results conform to those of a trial study by Inoue et al. who tested 49 AD patients aged ≥ 16 years and administered with *Lactobacillus acidophilus* L-92 for 8 weeks. AD lesions were assessed using the SCORAD index before the intervention, and then at weeks 4 and 8 after the intervention. The serum cytokine levels were measured in both the groups at 8 weeks after the start of the intervention. Our results suggested that the probiotic group had lower SCORAD values than the controls ($p = .002$), as well as decreased eosinophil ratio in the peripheral blood ($p = .03$), although the ratio of serum TGF- β changes increased significantly ($p = .04$) in this group. The administration of LP-92 was found to be effective for alleviating AD symptoms in adults as a result of the dominant suppression of Th2 inflammatory cytokines (25).

Another study, also a randomized, double-blind, placebo-controlled study, evaluated the clinical effectiveness of the

Table 2. Levels of SCORAD, IgE, IL-4, IFN- γ , IL-17, and Foxp3[±] in both the groups after 8 weeks of intervention.

Variable	Probiotic group (n = 15)	Placebo group (n = 15)
SCORAD	9.6133 ± 2.552	13.133 ± 5.029
Ig E (IU/ml)	470.833 ± 751.329	222.826 ± 181.681
IL-4 (IU/ml)	0.414 ± 0.2336	2.349 ± 1.787
IFN- γ (IU/ml)	4.466 ± 0.847	2.856 ± 1.698
IL-17 (IU/ml)	2.236 ± 2.000	4.110 ± 1.685
Foxp3+ (IU/ml)	5.868 ± 1.521	2.071 ± 1.681

Note. Foxp3+: forkhead box P3; IFN- γ : interferon gamma; IgE: immunoglobulin E; IL: interleukin.

intake of *L. salivarius* LS01 probiotic strains in the treatment of adult AD patients (26). They treated a total of 38 patients with probiotics or placebo (maltodextrin) for 16 weeks. The probiotic group showed a statistical increase in the clinical parameters (SCORAD, $p < .0001$ and DLQI, $p = .021$) at the end of the treatment period in comparison with the placebo group. Subsequently, after treatment, a significant reduction was noted in the level of Th1 cytokines (IL-12 + IFN- γ) ($p = .03$) and the Th1/Th2 ratio (IL-12 + IFN- γ /IL-4 + IL-5) ($p = .019$) only in the placebo group (26).

Harima et al. assessed whether the daily intake of citrus juice containing LP YIT-0132 can reduce the symptoms in mildly moderate adult AD patients after intervention for 8 weeks. The before and after intervention comparisons revealed showed a significant reduction in the Skindex-16 scores. The authors, therefore, concluded that the daily intake of fermented juice containing LP has a beneficial effect on the symptoms and QOL of AD patients because of the juice's immunomodulatory effects through reduced IgE and cationic eosinophil (ECP) protein levels (27).

Probiotics function through several pathways, and it is believed that every probiotic strain has a specific function or effect. As antihistamine therapy administered in this study affected the humoral immunity, it could have contributed to the study outcomes. No significant side-effects were reported during the study. Some studies on *Lactobacillus* sp. have reported the effectiveness of AD in children, but only a few reports have studied its effect in adulthood. This probiotic strain can play an important role in modulating Th1 and Th2 cytokine profiles and should therefore be considered as an important adjuvant therapy in the treatment of adult AD.

Inoue et al. state that standard therapy should be continued in all patients treated with topical corticosteroids (potent/very strong potential steroids for the body, including mild extremities and steroids for the face and neck) with moisturizers as well as 1–2 oral antihistamines, according to the management's guidelines (28).

An important finding of this study is the reduction in the clinical symptoms by probiotics via suppression of the Th2 adaptive immune response and increase in the Th1 adaptive immune response. The homeostasis mechanism was involved in increasing the Treg immune response and decreasing Th17 response. Treg cells can be activated by the mucosal immune system as a tolerance for inductors when processing allergens (13).

The probiotic LP IS-10506 can alleviate clinical symptoms in adult AD patients, as indicated by a decrease in the SCORAD index, IL-4, and IL-17, as well as an increase in the Foxp3[±] levels. An important finding from this study is the reduction in the

clinical symptoms by probiotics through the regulation of the Th2 adaptive immune response and by regulation of the Th1 adaptive immune response. Probiotic LP IS-10506 is an additional treatment with the potential to prevent recurrence or development of AD. In the future, further study with larger sample sizes and longer observation periods are warranted to strengthen the evidence about the beneficial role of the probiotic LP IS-10506 in adult AD.

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