Originl article:

Potential Immuno-modulatory activity of Probiotics containing *Lactobacillusacidophilus* and *Lacobacilluscasei* to increase the ratio of IFN γ/IL-4 in patients with Allergic Rhinitis

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Abstract

***Aims**:* This study aimed to determine the effect of probiotic combination on ratio of IFN-γ: IL-4 levels in patients with allergic rhinitis. ***Materials and methods**:* Single blind, randomized controlled trial.This study included 40 patients with severe allergic rhinitis according to WHO criteria. This study used 2 treatment groups with probiotics and placebo control group. IFN γ and IL-4 levels were evaluated using ELISA method. Data were analyzed using paired T test with 95% confidence intervals. ***Results and Discussion*:** In the treatment group, the levels of IFN-γ increased significantly ($p<0.05$).In contrast, IL-4 levels decreased significantly($p<0.05$). There was a significant difference between the IFN-γ ratio: IL-4 treatment and control group ($p<0.05$). ***Conclusion**:* The administration of combination of probiotics can improve the levels of IFN-γ/ IL-4 in patients with moderate to severe allergic rhinitis.

Keywords: allergic rhinitis; Interferon gamma; interleukin-4; Probiotics

Bangladesh Journal of Medical Science Vol. 18 No. 01 January'19. Page : 42-45
DOI: https://doi.org/10.3329/bjms.v18i1.39545

Introduction

Allergic rhinitis is a global health problem that can affect the quality of life and is often associated with multiple co-morbidity.The characteristic of patients with allergic rhinitis include changes in the body normal immune response such as increased serum levels of immunoglobulin E (IgE), local eosinophil infiltration and Th_2 cells and various other response caused by Th_1 and Th_2 imbalance toward Th_2 polarization.[1] Allergic rhinitis therapy using probiotics has been currently developed as it is more effective and specific in modulating the immune response. Specific probiotic strains demonstrated efficacy in prevention and treatment of allergies such as atopic ezcema, reduce allergic inflammation both locally and systemically.[2] There have been few studies on efficacy of probiotics in allergic rhinitis and conflicting results due to the ability of the immune system modulation depending on the species of probiotic used and the target population.[3]Thus, the studies on the potential of probiotics containing *L. casei* and *L. acidophilus*in patients with against allergic rhinitis are needed.

Lactobacillus casei and *Lactobacillus acidophilus* are potential probiotics to be developed as a new alternative immunomodulatory-based therapy of allergic rhinitis.[4]The administration of *L. asidophilus* has been shown to activate Th_1 and increase levels of IFN-γ specifically but did not affect the production of IL-4. Induction of immune responses mediated by Th_1has been also observed after administration of *L. casei* of which include increased production of IFN-γ, IL-12 and IL-4 and decreased the levels of IgE.[5] The previous study has shown that the administration of fermented milk containing *L. casei* Shirota strain can reduce levels of IgE in allergic rhinitis patients.[6]

As an immunomodulatory probiotics containing *L. casei* and *L. acidophilus* are more specific so that it has the potential to be developed as a new alternative method of treatment of allergic rhinitis. This study aimed to determine the effect of probiotic containing *L.casei* and *L.acidophilus* on levels of IFN γ/IL-4 in patients with allergic rhinitis.

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Materials and Methods

Study design

This single blind, consecutive controlled clinical trial study was performed in 30 patients with moderate to severe allergic rhinitis. All the eligible subjects in this study were randomly divided into 2 groups: treatment group and control group. All groups underwent a 4 week treatment. Prick test test was performed to determine allergen distribution triggering allergic rhinitis in the study subject.

Ethics, consent and Permission

The study protocol was approved by bioethic commission of Universitas Islam Sultan Agung, Semarang, Indonesia (reg:149/VII/2013/Komisi Bioetik). All participants were fully informed about all aspects of the research project and consented to complete and return the questionnaire. Moreover, they could ask for their names and addresses to be deleted from the enrollment list, by simply signing an appropriate box on the inform consent form and returning it without costs.

Participant

This study was conducted in otorhinolaryngology, head and neck center and biology laboratory. All participants were recruited from via daily outpatient services and advertisement. The criteria of allergic rhinitis were determined by using a questionnaire according to ARIA criteria. All the patients aged 18-21 years with moderate to severe allergic rhinitis were eligible to participate. Patients with allergic asthma, autoimmune diseases, moderate to severe atopic dermatitis, any severe chronic inflammatory disease, specific immunotherapy during last 2 years, continuous using antihistamines and corticosteroids medication in last 2 weeks, pregnancy and breast feeding were excluded.

Intervention

Patients in treatment group received probiotics containing *L. casei* and *L. acidophilus* in liquid milk based preparation much as 65 mL/day for 4 weeks orally while control group were given placebo in the form liquid milk skim based preparation as much as 65 mL/day for 4 weeks.

Serum cytokines analysis

IFN- γ and IL-4 levels in serum were measured by ELISA kit (DRG cat. EIA-4434 and DRG cat. EIA-4435 respectively) according to the manufacturer's instructions and analyzed using a Luminex 100 instrument. Samples were diluted 1:2 in deionized water.

Statistical Analysis

Data of study results presented in tabulation of mean and deviation standard were subjected to normality test to determine their distribution. The increased mean ratio levels of IFN-γ:IL-4 were determined after administration of probiotics containing *Lactobacillus acidophilus* and *Lactobacillus casei* using a parametric paired t-test with 95% confidence intervals.

Results

The result of prick test showed that all respondents had more than 1 type of allergy with the highest percentage of 73.3% allergy to dust mite, 60% subjects was allergic to cockroach, 26.67% subjects were allergic to house dust, 30% were allergic to the mixed fungi, 10% was allergic to human dander, and 20% of allergy respondents was allergic to dog dander, 3.3% of were allergic to horse dander (figure 1).

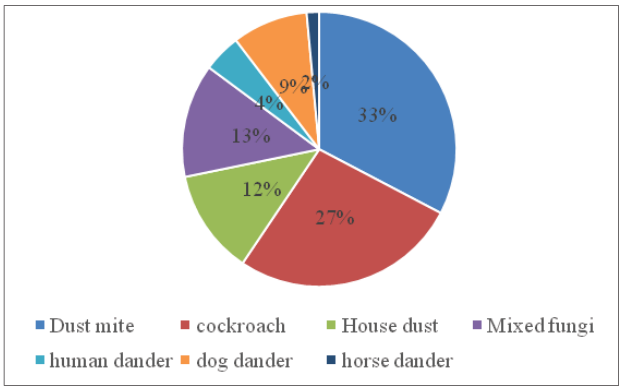

Figure 1. Distribution of allergen in allergy rhinitis.

Table 1 showed no difference in that the characteristics of the respondents of the placebo and probiotics groups.

Table 1: Characteristics of respondents

Characteristics	Groups		sig.
	Placebo	Probiotics	
Gender			
a. Male (%)	40	40	0,67
b. Female*%)	60	60	
Mean of IFN-γ baseline (pg/mL)	143.88±5.32	146.73±5.51	1.000
Mean of IL-4 Level baseline (pg/mL)	29.62±5.76	29.18±6.50	1.000
Mean ratio of IFN-γ/IL-4 Level	5.02±0.94	5.28±1.20	1.000

To elucidate the possible mechamism of probiotics in allergy rhinitis, IFN γ levels were measured using ELISA.The level of IFN-γ was significanly higher in treatment group compared to that of control group (table 2).

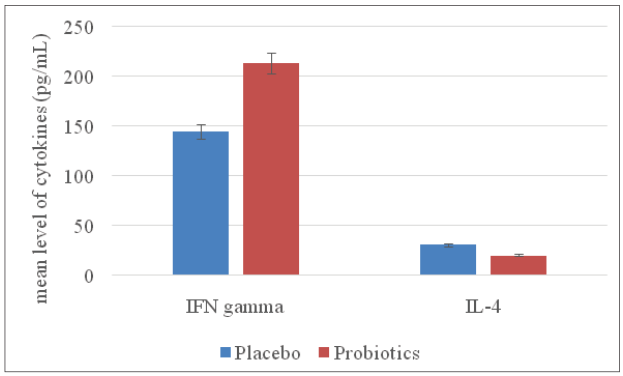

Figure 2. mean level of cytokines after 4 weeks treatment

Figure 1 showed that the administration of milk as a placebo did not increase IFN γ in patients with severe allergic rhinitis. In the probiotics group, IFN γ was by 1.5 times higher after the probiotic treatment (p = 0.000). Increased levels of IFN γ may be due to the effect of the administration of probiotics in patients with allergic rhinitis. This is indicated a significant difference between placebo group and probiotic after treatment (p <0.05).

Interleukin-4 level in treatment group was lower compared to that of control group (p = 0.000). Levels of IL-4 allergic rhinitis patients before and after treatment is presented in table 3. The decreased in levels of IL-4 may be due to the effect of the administration of probiotics in patients with allergic rhinitis for the treatment group. This is supported by the test results of IL-4 levels before and after treatment in the placebo group showing no significant difference (p> 0.05) and significant difference between the placebo group and the probiotic after the treatment (p <0.05).

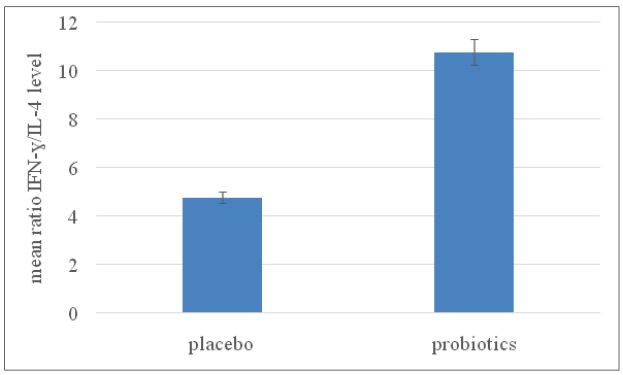

Figure 3. mean ratio of IFN γ/IL-4level after 4 weeks treatment

In order to evaluate the immunodulatory activity, ratio of mean levels of IFN γ/IL-4 was evaluated. Mean ratio of IFN γ / IL-4 significantly increased in probiotics group compared to the control group (figure 2). The increase was 2 times higher after probiotic treatment (p = 0.000).

Discussion

Increased IFN-γ/IL-4 ratio in the probiotic group might have been due to increased IFN-γ and decreased levels of IL-4. This decrease can be caused by increased levels of IL-12 and IL-10. The study by Kaji *et al.* in 2010 showed that *Lactobacillus casei* has the ability to increase the ratio of IL-12 / IL-10 while *Bidifobacterium* has the ability to increase IL-10. Increased IL-10 plays a role in increasing the ratio.[7]

This finding supports a study by Mohamadzadeh in 2005 showing that results that probiotics can activate human dendritic cells tend to Th1 polarization indicated by increased IFN γ. The study used three different types of *lactobacilli i*ncluding *Lactobacillus gasseri, Lactobacillus johnsonii, Lactobacillus reuteri*. All of the three *Lactobacillus* increased IFN γ, but inversely correlated to the IL-2, IL-4 and IL-6.[8] In the probiotic group there was a significant decrease in IL-4 levels after probiotic treatment (p = 0.000).

Previous study showed that IL-4 levels after treatment were lower than before treatment. This was likely due to the stimulation of probiotic bacteria as a strong activator for the innate immune system because their specific molecules on the cell wall of peptidoglycan and lipoteichoic acid which will interact with TLR2 and TLR4 resulting in activation of T cells in the immune system, by polarization toward Th1 and Treg cells [9,10]

Probiotics have a role in the prevention of allergic diseases besides having the ability to inhibit pathogens, probiotics can trigger S-IgA immune mucosal response that systemically trigger T regulator inhibiting Th_2, or overactivity of Th_1 redundant.[11]Wahn *et al.* in 2004 stated that current therapeutic development is directed at improving the homeostasis of allergic biological systems intended to immunomodulatingthe immune response by balancing the Th1 and Th2 immune responses. The advancement of science and technology allows the paradigm shift from preventing allergies in the form of prevention of risk factors toward active induction of immunologic tolerance.[12]

Conclusions and recommendations

The administration of combination of probiotics can improve the levels of IFN-γ/IL-4 in patients with moderate to severe allergic rhinitis. Further studies are needed to determine the effect of *Lactobacillus*

acidophilus and *Lactobacillus casei* treatment on symptom recurrence and the quality of life in allergy rhinitis patients.

Acknowlegment

This research was funded by Indonesian Minister of Research Technology and Higher Education and medical faculty of Universitas Islam Sultan Agung.

Conflict of interest: The authors declared no conflict of interest

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Data gathering and idea owner of this study: Dina Fatmawati

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Data gathering: Wiratn, Dina Fatmawati

Writing and submitting manuscript: Dina Fatmawati,

Editing and approval of final draft: Dina Fatmawati, Wiratno,Andriana Tjitria Widi Wardani

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