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RESEARCH ARTICLE

Probiotics Effect on Interleukin-4 (IL-4) and Imunoglobulin E (IgE) Levels on Asthmatic Patients

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ABSTRAK

Pendahuluan: Probiotik berperan penting dalam pematangan sistem imun, dan dapat mengurangi resiko alergi dan asma pada anak. Namun, peran probiotik sangat dipengaruhi oleh berbagai faktor antara lain jenis bakteri dan dosis. **Tujuan:** untuk mengetahui efek pemberian probiotik (Lactobacillus acidophilus, Bifidobacterium longun, Streptococcus; LactoB®) terhadap kadar immunoglobulin E (IgE) dan interleukin-4 (IL-4) pada penderita asma anak.

Metode: Penelitian ini menggunakan rancangan pre-post randomized control trial dengan sampel sebanyak 40 anak penderita asma berumur 1-5 tahun. Sampel dibagi menjadi 2 kelompok. Kelompok kontrol (KK) yang diberi obat standar dan plasebo, sedangkan kelompok perlakuan (KP) diberi obat standar dan probiotik Lacto B®. Plasebo dan probiotik diberikan secara oral 2 kali sehari selama 8 minggu. Kadar IgE dan IL-4 ditentukan dengan metode ELISA. Perbedaan antara kelompok perlakuan dianalisis menggunakan uji T berpasangan dengan interval kepercayaan 95%.

Hasil: Pada KP, rerata kadar IgE setelah pemberian probiotik mengalami penurunan secara signifikan dibandingkan dengan sebelum pemberian probiotik (148,18 pg/mg ; 283,20 pg/mg; p<0,05). Demikian juga rerata kadar kadar IL-4 setelah pemberian probiotik mengalami penurunan secara signifikan dibandingkan dengan sebelum pemberian probiotik (111,03 pg/mg ; 142,08 pg/mg; p<0,05). Sedangkan pada KK, rerata kadar IgE pada setelah diberi plasebo tidak berbeda secara signifikan dibandingkan dengan sebelum perlakuan (292,39 pg/ml; 286,94 pg/ml; p>0,05). Rerata kadar IL-4 KP setelah diberi plasebo tidak berbeda secara signifikan jika dibandingkan dengan sebelum perlakuan (136,76 pg/ml; 139,56 pg/ml; p>0,05).

Kesimpulan: terdapat perbedaan efek pemberian probiotik terhadap kadar IgE dan IL-4 penderita asma anak.

Kata kunci : Probiotic, Immunoglobulin E (IgE), Interleukin (IL)-4, asma anak

ABSTRACT

Introduction: *Probiotics may play a role in immune system maturation and may reduce the risk of allergies and asthma in childhood. However, the therapeutic benefits of probiotics in asthma depend on various factors such as strain of probiotics and dosing regimen.* ***Objectives:*** *The aims of this study was to evaluate the effect of probiotic (LactoB®) on Immunoglobulin E (IgE) and Interleukin (IL-4) serum level in childhood Asthma.*

Methods: *Forty children aged 1-5 years with asthma were recruited into a randomized controlled trial. The children were assigned into a probiotic (Lactobacillus acidophilus, Bifidobacterium longun, Streptococcus; Lacto B®) or an equivalent volume of placebo, twice daily orally for 8 weeks. The IgE and IL-4 serum level were determined by ELISA. The differences between groups were analyzed by t-test dependent with confidence interval of 95%.*

Results: *In intervention group, mean of IgE serum levels after the probiotics treatment was significantly lower compared to that of before the treatment (148.18 pg/mg; 283.20 pg/mg; respectively). Mean of IL-4 serum levels after the treatment was significantly lower compared with that of before the treatment (111.03 pg/mg; 142.08 pg/mg respectively). In control group, there were no significant differences between IgE serum levels mean before the administration of placebo and after the intervention (292.39 pg/ml; 286.94 pg/ml respectively). There were no significant differences between IL-4 serum levels mean before and after the treatment (136.76 pg/ml; 139.56 pg/ml).*

Conclusion: *there was an effect of probiotics supplementation on IgE and IL-4 serum levels in childhood asthma.*

Keywords: *Probiotic, Immunoglobulin E, Interleukin (IL)-4, Childhood asthma*

INTRODUCTION

Asthma represents a global health problem that can affect the quality of life and is often associated with multiple comorbidities (Hoecke et al., 2007). Asthma is characterized by an increase of immunoglobulin E (IgE) serum levels, local eosinophil infiltration and Th2 cells, and a variety of other immune responses caused by an imbalance between Th1 and Th2 resulting in polarization toward the Th2 (Bousquet et al., 2001). Probiotics, either in the form of monobacterial strains or in combination, are alternative therapeutic options for asthma (Baken, 2006). Previous studies has suggested that the administration of probiotic *Lactobacillus gasseri* can improve the clinical symptoms of asthma (Baken 2006). However, IgE profile following administration of the combination of probiotics on asthmatic children has not been established.

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Probiotics are non-pathogenic bacteria that may affect the innate immune system and able to enhance macrophage phagocytosis (Baken, 2006) and the of natural killer cell (NK) cytotoxicity (Nagao et al., 2000; Matsuzaki et al., 2005). Induction of Th1-mediated-immune responses are also shown following an administration of probiotics, including increased production of IFNγ and IL-12, as well as lower the levels of IL-4 and IgE (Baken 2006). The purpose of this study was to determine the levels of IL-4 and IgE after administration of probiotics in asthmatic children.

METHODS

This study was designed as pre-post test randomized control trial with an intervention period for 8 weeks at the UNISSULA Faculty of Medicine and Sultan Agung Islamic Hospital of Semarang. The inclusion criteria were: being 1-5 years of age, and met the diagnostic criteria for asthma as defined by Global Initiative for Asthma (GINA). The exclusion criteria used in this study was if the subject withdraw the informed consent. The combination of probiotic used is LactoB® administered orally as much as two sachets per day. Each sachet of LactoB® contains 1x107 CFU/g *(Lactobacillus acidophilus, Bifidobacterium longun, Streptococcus thermophillus)*, 10 mg of vitamin C, vitamin B1 0.5 mg, 0.5 mg of vitamin B2, vitamin B6, 0.5 mg, 2 mg niacin, 0:02 g protein, fat 0.1 g. IgE and IL-4 serum level were measured using ELISA. Before participating in the study, the guardians of the participating patients signed informed consent forms prior to involvement in the study. The study was stopped if the patient refuse to continue the study, or having any side effects or adverse drug. This study was approved by the ethics committee of the Faculty of Medicine, Sultan Agung Islamic University. The statistical analysis was performed using a paired t-test with 95% confidence intervals.

RESULTS

Based on the results of the questionnaires, 40 subjects met the inclusion and exclusion criteria. The mean IgE and IL-4 level before and after the treatment for the control and intervention group is presented in table 1.

Table 1 shows the difference in mean levels of IgE and IL-4 before and after the treatment in intervention and control group. In intervention group, means IgE and IL-4 levels after probiotics treatment showed a significant decrease compared with those prior to administration of probiotics. While in control group, there were no significant differences in mean IgE and IL-4 serum level before and after treatment.

Table 1. IgE and IL-4 serum levels mean before and after the treatment for intervention and control groups.

Variables	Mean	
	Before	After
IgE (pg/ml)		
Intervention	283.20	148.18
group		
control	286 94	292 39
IL-4 (mg/dL)		
Intervention	142.08[a]	111.03
control	139.56[ab]	136.76[b]

[ab] **p>0.05**

DISCUSSIONS

The use of probiotics in the treatment of allergic diseases is based on the ability of probiotics to modulate toll-like receptor (TLR) and the recognition of antigen that activates dendritic cells and increase the response of Th1 cells leading to Th2 cell responses (Cha et al., 2012). Statistical test results presented in Table 1 shows a significant difference in levels of IL-4 before and after the intervention with the p-value of 0.010 ($p<0.05$). This might have been due to a protein or antigen binding to TLRs leading to the activation of macrophages. Active macrophages produce IL-12-inducing Th1 cells and NK cells to produce IFN-γ. This cytokine inhibits Th2 cells preventing the formation of IgE by B cells. By this way, the production of chemical mediators such as histamine, which cause allergy symptoms, decreases (Abbas, 2012). The concept is supported by research conducted by Kim JY et al. (2008), which conducted treatment on rats using the probiotic Lactobacillus acidophilus AD031, Bifidobacterium lactis AD011 to suppress the production of IgE OVA-specific IgG1 and IgA, showed that the levels of IL-4 was significantly lower and the level of interferon IFN-γ and IL-10 were significantly higher compared with untreated rats (Kim et al., 2008). Probiotic treatment for allergy therapy leads to homeostasis improvement of biological systems in allergic patients by balancing the Th1 and Th2 immune responses, so that allergic reactions can be improved (Wang, 2004).

Tsai et al. (2012) showed that administration of multi probiotics is more effective than mono probiotics. The finding of a 4-week study showed that higher IFN-γ and lower IL-4 levels in the group treated with multi probiotics than that of only mono probiotics. IFN-γ is a proinflamatory cytokine that can inhibit the production of Th2 cytokines in the immune response. IL-4 as Th2 cytokines have the opposite effect with IFN-γ (Tsai et al., 2012).

Limitations of this study includes the difference in the trigger of moderate and severe allergic rhinitis - that can affect the distribution of the data has not been controlled. In addition, the effect of multi probiotics in certain type of allergens has not been established.

The results showed a significant effect of probiotic on IgE serum levels in children with asthma. Research conducted has controlled the factors affecting changes in levels of IgE, such as, subjects did not complaining any indigestion disorder, and did not consuming corticosteroids and antihistamines. Decreased levels of IgE by probiotic through induction of response to immunologic starting the innate immune system and led to the return of the Th1-Th2 balance. The bacteria are also the normal flora in the gastrointestinal tract that able to control the balance of intestinal microflora of bacterial pathogens.

Streptococcus thermophilus, Lactobacillus bulgaricus, Bifidobacterium, Lactobacillus acidophilus are gram positive bacteria with lipotechoic acid (LTA) cell wall and peptidoglycan (PGN), which then recognized by TLRs. It help host cells to recognize the pathogens. The four walls of bacteria bind to TLR-2 TLR 1/6 in dendritic cells and then will balance Th2 leading to reduced production of IgE (Kim et al., 2008).

This is supported by the finding of study conducted by Torii et al. (2007) in Japan, explaining that the species *Lactobacillus acidophilus* strain L-92 could balance the immune response of Th1 and Th2 through the induction of cytokines Th1, i.e. IFN-γ mediated by Th0 which will suppress the Th2 cytokines, namely IL-4 ($p< 0.05$). The results of this study showed a significant higher levels of mean IFN-γ and IL-4 in the group induced by L-92 compared with the control group (Torii et al., 2007).

Wang et al. (2004) explained that current therapies aim at improving the biological system homeostasis in allergic patients, especially in immunodulation by balancing the Th1 and Th2 immune responses. The advancement of science and technology has shifted the paradigm from prevention of allergies, such as avoidance of risk factors, toward active induction of immunologic tolerance (Wang et al., 2004).

Widjaja (2010) showed that nutritional deficiencies lead to decreased immune response, phagocyte function, cytokine production and the complement system. This finding is supported by research conducted by Chandra and Bloom, providing evidence that rats fed a low protein diet (2%) are more susceptible to pulmonary tuberculosis infection than that of fed adequate protein diet (20%). Rats fed a low protein diet showed decreased levels of IFN-γ, TNF-γ, and nitric oxide. It can be concluded that an adequate intake of nutrients, especially protein, increases the immune response (Widjaja, 2010).

A number of factors affecting IgE levels should be controlled in order to determine the single effect of probiotics. Limitations of this study include the absence of measurement of levels of IL-10 and IL-17. Cytokines indicate the pattern of the Th1 response (IL-2, IFN-γ, IL-12) or Th2 (IL-4, IL-5, IL-13). Th2 response pattern is associated with allergic inflammation reaction, whereas Th1 response pattern associated with delayed hypersensitivity and an inflammatory reaction to infection. Th2 cells will increase the synthesis of IL-4 and IL-13, which in turn increase the production of IgE. IL-4 is important in the process of primary antigen sensitization. IL-4 when given to animals that have undergone sensitization has less effect in reducing the production of Th2 cytokines, eosinophil reflux and bronchial hyper responsiveness. In contrast, IL-13 plays more role compared with IL-4 after secondary exposure to the antigen (Kips, 2001).

CONCLUSION

Probiotics administration have been shown to lower the levels of IL-4 and IgE in children with asthma.

SUGGESTION

Further studies on effect of probiotics on various allergens in which blood sample should be taken at the presence IL- 4 in the allergic process.

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