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The Effect of House Dust Mite Immunotherapy, Probiotic and *Nigella sativa* in The Number of $CD4^+IL\text{-}4^+$ Cell, Total IgE level and Asthma Control Test (ACT) Score

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Abstract : *Ashtma is a chronic inflammatory disorder of the airways dominated by Th2. Immunotherapy was beneficial for ashtma patients. Its long duration was oftenly caused patients to drop out treatment . Probiotic and Nigella sativa as immunomodulator for asthma expectantly could increase the efficacy of immunotherapy . The aims of this study was to evaluate the therapeutic efficacy of immunotherapy combined with probiotics and Nigella sativa in the number of $CD4^+IL\text{-}4^+$ cells, Total IgE level and Asthma Scoring Test. A total of 31 children with mild asthma were evaluated and then randomized to receive immunotherapy or immunotherapy plus Nigella sativa or immunotherapy plus probiotic or immunotherapy plus Nigella sativa plus probiotic openly for 14 weeks. Statistical analysis found no significant difference in the mean number of $CD4^+IL\text{-}4^+$cells pre and posttest in all three treatment group. The Total IgE level was decreased significantly in the immunotherapy+ probiotic + Nigella sativa group (p = 0,022). The ACT score were increased in the immunotherapy + Nigella sativa group (p = 0.001), in the immunotherapy + probiotic group (p = 0.004), and immunotherapy + Nigella sativa + probiotic group (p = 0.000). Correlation test found a significant association between the number of $CD4^+IL\text{-}4^+$cells, Total IgE level and ACT score in all groups. The combination immunotherapy, Nigella sativa and probiotics could decrease the Total IgE level thus improve the clinical symptom.*

Keywords – *Immunotherapy, House dust mite, Probiotics, Nigella sativa, $CD4^+IL\text{-}4^+$, Ig E, Asthma Control test*

I. INTRODUCTION

Asthma is one of the most prevalent chronic illness in children and one of the leading cause of death in children worldwide. The prevalence of asthma in children is 8-10%. [1-3]. The inflamation process in ashtma was due to release of mediator that activated target cell in airway causing bronchoconstriction, microvascular leakage, edema and mucuos hypersecretion. In ashtma, Thelper2(Th2 cell) have a central role in the alergic inflamation process. Cytokines released by Th2 cells such as Interleukin-4 (IL-4) will induce inflammatory cells such as mast cell, eosinophil and neutrophil. IL-4 have the ability to control the differentiation of T cell differentiation from Th0 to Th2 and also causing eosinophilic inflamation by chemotaxis and eosinophil activation. IL-4 along with IL-13 will induce the production og IgE by B cells. [4] Most asthma patient have increase of IgE levels. IgE will bind to its receptor in mast cell and stimulate the release of inflammatory mediator such as histamin, prostaglandin, leucotrien and cytokines then causing bronchoconstriction, mucous hypersecretion and ashtma symptoms. [5, 6]

The domination of Th2 is an important factor in asthma patophisiology, thus the recent management of ashtma target to return of the homeostasis of biologic system. The function of Treg is to maintain the balance of immune system in airway. [7, 8]. This immunomodulator function of Treg has become the target of ashtma therapy.[9] Immunotherapy is a vaccination of allergen to create tolerance to allergen by immunotherapy. Many allergens can be used in immunotherapy, including House Dust Mites, the major cause of respiratory allergy worldwide. [1] Many studies have shown modulation of Tcell responsivenss with variable ihibition of Th2 responses (IL-4 and IL-5) to allergen and induction of more Th-1 like responses with increased allergen induced interferon (IFN)ɣ. Others suggest immunotherapy induced Treg and its immunoregulatory cytokines, IL-10 . Immunotherapy can decrease the inflamation by inhibiting the inflamation cells recruitment and the release of inflammatory mediators [10, 11].

Probiotic are live microorganism that when administrated in adequate amount, confer a health benefit to the host. Probiotic can modulate mucosal and sistemic immune response. In allergic disease, probiotic can act as immunomodulator by balancing the immune response of Th2 and Th1. Probiotic also induced Treg cells. In animal studies, probiotic *Bifidobacterium lactis(BB-12)* dan *Lactobacillus rhamnosus Gg* able to inhibit allergy sensititation by decrease the production of IgE and Th2 cytokines. A variety of human studies on the effects of probiotic administration in ashtma and other allergic disease showed conflicting result. [12-16].

Nigella sativa (NS) is one of the herbal medicine showing anti-inflamation and immunomodulator effect. Traditionally, people in Middle East, South Asia and South Europe used it as asthma medication. Many in vivo and in vitro studies showed beneficial effect of administration of NS in ashtma. Animal study by Barlianto et al (2012) showed NS able to decrease the number of CD4+ T lymphocytes, increase the number of Treg cells, prevent airway remodelling and modulate immune response. Other study show Nigella sativa had relaxation and anti histamin effect in the ashmatic mice trachea. Clinical trial by Kalus et al showed NS can reduce the subjective complain in rhiitis allergica, ashtma and atopic eczema children. Study by Ahmad *et al* in 5-15 years old pediatric patient with wheeze show improvement of a *peak expiratory flow rate* (PEFR) after treatment with NS. [17-20]

Immunotherapy can improve 60-70% clinical score in ashtmatic patients. However, immunotherapy in ashtmatic patients seldom results in complete alleviation of symptoms, and its long duration of treatment cause many patients drop out before completing it. [21, 22] Therefore, adjuvant for immunotherapy is considered to improve the efficacy of immunotherapy. Probiotic and Nigella sativa have immunomodulatory and anti inflammation effect that expectantly will improve the efficacy of immunotherapy. The objective of this study is to evaluate the efficacy of the combination of HDM specific immunotherapy, probiotics, and *Nigella sativa* in influencing the number of $CD4^{+}IL\text{-}4^{+}$cells, total IgE level and clinical scoring of asthma in children.

II. Methods

This study was an experimental research, randomized clinical trial (RCT), single-blind, pre and post test control group. A total of 31 children with mild asthma were evaluated and then randomized to 4 groups, there are group that receive immunotherapy, group that receive immunotherapy plus *Nigella sativa*, group that receive immunotherapy plus probiotic and group that receive immunotherapy plus *Nigella sativa* plus probiotic openly for 14 weeks. The samples were pediatric patients diagnosed with asthma and underwent outpatient Allergy and Immunology clinic of dr. Saiful Anwar hospital, Malang during the period August to December 2012 that meet the inclusion and exclusion criteria.

The inclusion criteria for this study sample include children diagnosed with asthma based on national guidelines for childhood asthma for children aged ≥ 5 years and asthma predictive index (IPA) for children <5 years, and classified the degree of intermittent or mild persistent asthma based on global criteria initiative for asthma (GINA), aged 4-14 years, allergic to house dust mite by skin prick test results., had never received previous immunotherapy, the patient's parents volunteered to follow the study and signed an informed consent.

The exclusion criteria were patients with asthma who take cytostatic medicine, accompanied by co-morbid asthma patients such as sinusitis, otitis media, tuberculosis, pneumonia, nasal polyps, gastro-esophageal reflux, or other anatomical abnormalities, patients suffering from immunodeficiency, autoimmune diseases, or have cardiovascular abnormalities, a history of severe allergies such as asthma attacks and anaphylactic shock, life-threatening asthma , had suffered from respiratory failure or been intubated in the last 5 years, patients taking therapeutic doses of corticosteroids during the 1 - 2 weeks, vitamin D3 doses of more than 2000 IU / day for 3-4 months, β blockers, angiotensin-converting enzyme (ACE), leukotriene antagonists, teophillin, anti-cholinergic, cromolyn and ketotifen in the 2 weeks before and during the study, patients with asthma who smoked actively or passive smoking, asthma exacerbations or respiratory tract infection within the last 30 days.

The drop out criteria in this study are: patients who did not come in immunotherapy schedule for 3 consecutive weeks during the induction phase of immunotherapy and should repeat the initial dose, patients do not take probiotics and/or *Nigella sativa* as recommended by the researchers, patients were eligible exclusion for observational studies, and patients who experience severe side effects such as anaphylactic shock due to treatment delivery.

During the build-up phase, an injection at a dose of allergen is increased in order to obtain the target dose. In this study, immunoalergen HDM was produced by Soetomo General Hospital Pharmacyst. Each vial contains 5 ml and contained 5 mg / ml of HDM. House dust mite specific immunotherapy was given to asthmatic patients with results of skin prick test proved HDM allergic. Immunotherapy is given every week for 14 weeks using liquid III (mixture of HDM allergens and Cocca filtra) with increased dose per week. Dilution immunotherapy extracts given in this phase is 1:100. The dose given during this phase increased gradually each week until the maintenance dose is reached. This protocol is based on immunotherapy guidelines of Saiful Anwar General Hospital Malang.

Table 1. Immunotherapy dose in buid up phase

Week	Dose(ml)	Week	Dose (ml)
1	**0,1**	**8**	**0,1**
2	**0,15**	**9**	**0,15**
3	**0,22**	**10**	**0,22**
4	**0,32**	**11**	**0,32**
5	**0,48**	**12**	**0,48**
6	**0,72**	**13**	**0,72**
7	**1**	**14**	**1**

Probiotics which used in this study is PROBI ® of Medifarma, containing $2x10^9$ cfu / g mixed bacteria *Lactobacillus acidophilus* LA life-5^{TM} and *Bifidobacterium lactis* Bb-12^{TM}, vitamin premix (vitamin $B_1$0, 1 mg, vitamin $B_2$0, 1 mg, vitamin B_6 0.1 mg, vitamin C 3 mg), and selenium yeast1 mg with the dose is 1 sachet per day. *Nigella sativa* were used in this study was *Nigella sativa* in powder form and in capsules prepared and was given at a dose of 15 mg / kg / day [33]. Each capsule contains 150 mg of *Nigella sativa*. Each 10 kg of weight will receive 1 capsule of *Nigella sativa* . The first examination of $CD4^+IL4^+$, Total IgE level and clinical score was obtained in the first day of the immunotherapy before injection and the second was a week after the 14th injection of immunotherapy. The examination of the number of $CD4^+IL4^+$ cells by flowcytometry using PMBC isolation from peripheral blood. The number of $CD4^+IL4^+$cells were counted subsequently analyzed using BD Cell quest Pro software. The examination of Total IgE level by using Enzyme Chemiluminescece Immunoassay by Roche Elecsys 2010. The clinical score of ashtma is measured by Ashtma Control Test (ACT). The asthma children answered the questionare accompanied by their parents.

In this study, data analysis techniques will be performed six stages of counting, respectively: (1) test the validity and reliability of the questionnaire, (2) test the normality of the data sample with Kolmogorov-Smirnov test, (3) one-way ANOVA test for sample data pretest, (4) paired sample t test for pretest-posttest sample data, (5) one-way ANOVA test to post-test sample data, and (6) Pearson correlation test. All calculations performed with the software of SPSS for Windows 19.0.

III. Results

Thirty two pediatric asthma patients were randomly allocated to receive immunotherapy only (8 patients), immunotherapy plus NS (8 patients), immunotherapy plus probiotics (8 patients) and immunotherapy plus *Nigella sativa* plus probiotics (8 patients) . One sample from immunotherapy group (group 1) drop out of the research because absent for 3 weeks during the treatment. During our research, there were no side effects from immunotherapy, probiotic neither NS. Characteristics of the sample data are presented in Table 1. There were no statisticaly significant differences of the number of $CD4^+IL\text{-}4^+$, Total IgE level and ACT scores between each group before the treatment. This means that the condition in the beginning of the study is the same in all groups. (Table 2).

Table 1. Baseline characteristics of sample

Characteristics	Group 1 (n=7)	Group 2 (n=8)	Group 3 (n=8)	Group 4 (n-8)
a. Age				
< 5 years	1(14.3%)	3 (37.5%)	2 (25%)	1 (12.5%)
≥ 5 years	6 (85.7%)	5 (62.5%)	6 (75%)	7 (87.5%)
b. Sex				
-Boy	4 (57.1%)	4 (50%)	4 (50%)	5 (62.5%)
-Girl	3 (42.9%)	4 (50%)	4 (50%)	3 (37.5%)
c. History of atopy				
-No	3 (42.9%)	2 (25%)	3 (37.5%)	3 (37.5%)
-Yes	4 (57.1%)	6 (75%)	5 (62.5%)	5 (62.5%)
d. Skin prick test result				
-HDM	3 (42.9%)	3 (37.5%)	5 (62.5%)	3 (37.5%)
-HDM + food	4 (57.1%)	3 (37.5%)	1 (12.5%)	4 (50%)

-HDM + pet	0 (0%)	1 (12.5%)	0 (0%)	0 (0%)
-HMD + food + pet	0 (0%)	1 (12.5%)	2 (25%)	1 (12.5%)
e. Status gizi				
-gizi baik	7 (100%)	8 (100%)	8 (100%)	8 (100%)
-gizi kurang	0 (0%)	0 (0%)	0 (0%)	0 (0%)
f. Chief complain				
-Cough	3 (42.9%)	1 (12.5%)	1 (12.5%)	2 (25%)
-Shortness of breath	1 (14.2%)	1 (12.5%)	0 (0%)	0 (0%)
-Cough + rhinitis	0 (0%)	0 (0%)	0 (0%)	2 (25%)
-Cough + shortness of breath	3 (42.9%)	6 (75%)	3 (37.5%)	1 (12.5%)
-Cough++shortness of breath+rhinitis	0 (0%)	0 (0%)	4 (50%)	3 (37.5%)
g. Diagnosis				
- Intermitten asthma	4 (57.1%)	1 (12.5%)	1 (12.5%)	0 (0%)
-Mild persistent asthma	1 (14.3%)	6 (75%)	4 (50%)	3 (37.5%)
-Intermitten asthma	1 (14.3%)	0 (0%)	0 (0%)	1 (12.5%)
-asthma intermitten + moderate severe intermitten rhinitis	0 (0%)	1 (12.5%)	0 (0%)	2 (25%)
-Mild persistent asthma +mild intermitten rhinitis	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)
-Mild persistent asthma + moderate-severe intermitten rhinitis	1 (14.3%)	0 (0%)	3 (37.5%)	1 (12.5%)

Table 2. The comparation of $CD4^+IL\text{-}4^+$, Total IgE levels and ACT score between group before treatment

Treatment Group	$CD4^+IL\text{-}4^+$cells (%) Mean ± SD	Total IgE Level (IU/ml) Mean ± SD	ACT Score Mean ± SD
Immunotherapy	0.22±0.15	917± 772.18	15.86±5.12
Immunoterapi+*Nigella sativa*	0.42±0.31	1,120± 890.58	16.00±2.73
Imunoterapi+probiotic	0.44±0.34	1,232.81±453.48	15.75±1.67
Imunoterapi+*Nigella sativa*+probiotic	0.23±0.19	1,450.9±653.97	15.38±1.60
ANOVA	p value : 0.216	p value : 0.537	p value : 0.980

Table 3. The comparation of the number of $CD4^+IL4^+$ cellss before and after treatment

Treatment Group	Pretest $CD4^+$ $IL4^+$ (%) Mean ± SD	Postest $CD4^+$ $IL4^+$ (%) Mean ± SD	p-value (paired sample t test)
Immunotherapy	0.22±0.15	0.36±0.22	0.317
Immunotherapy + Nigella sativa	0.42±0.31	0.48±0.22	0.728
Immunotherapy+ probiotic	0.44±0.34	0.52±0.31	0.678
Immunotherapy+Nigella sativa+probiotic	0.23±0.19	0.32±0.24	0.034

Table 3 shows the changes of the number of $CD4^+IL\text{-}4^+$cells before and after the 14 weeks of treatment.There were no significant differences of the number of CD4+IL4+ cells(0.22±0.15)before treatment and after treatment (0.36±0.22) in the immunotherapy group (p-value=0.183). In the other groups, there were no significant differences of the number of $CD4^+IL4^+$ cells before and after treatment (immunotherapy plus *Nigella sativa* 0.42±0.31 vs 0.48±0.22 p=0.317 *;* immunotherapy plus probiotic 0.44±0.34 vs 0.52±0.31 p= 0.678. There was significant differences in the immunotherapy plus *Nigella sativa* plus probiotic group 0.23±0.19 p=0.034 .

The comparation of Total IgE levels before and after the treatment was shown in Table 4. In the immunotherapy group, there were decrease of total IgE levels but not statistically significant (917± 772.18 vs

578.46±489.61, p=0.133). The Immunotherapy plus Nigella sativa and immunotherapy plus probiotic group also showed decrease of IgE level but not significant (1,120± 890.58 vs 876.13±667.07, p= 0195; 1,232.81±453.48 vs 952±522.02, p = 0.122)). In the immunotherapy plus Nigella sativa plus probiotic, the difference of Total IgE level before and after treatment were significant (1,450.9±653.97 vs 1,076±546.86, p=0,022).

Table 4. The comparation of Total IgE levels (IU/ml) before and after treatment

Treatment Group	Pretest Total IgE (IU/ml) Mean ± SD	Postest Total IgE (IU/ml) Mean ± SD	p-value (paired sample t test)
Immunotherapy	917± 772.18	578.46±489.61	0.133
Immunotherapy + Nigella sativa	1,120± 890.58	876.13±667.07	0.195
Immunotherapy+ probiotic	1,232.81±453.48	952±522.02	0.122
Immunotherapy+Nigella sativa+probiotic	1,450.9±653.97	1,076±546.86	0.022

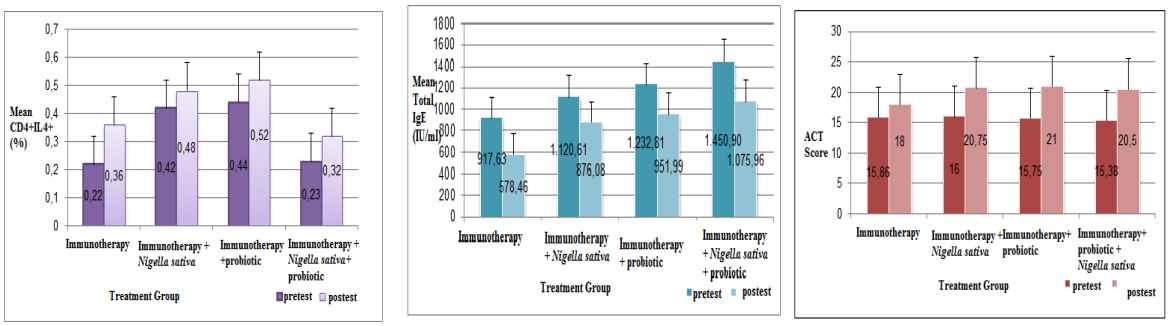

Figure 1. The comparation of $CD4^{+}IL4^{+}$, Total IgE level and ACT score before and after treatment.

Table 5 shows the comparation of ACT score before and after treatment. There was improvement of ACT score but not statistically significant in the immunotherapy group (15.86±5.12 vs 18.00±7.42, p: 0.062). The ACT score improved significantly before and after treatment in the immunotherapy plus Nigella sativa group (16.00±2.73 vs 20.75±3.37, p= 0,001) immunotherapy plus probiotic (15.75±1.67 vs 21.00±3.82, p= 0.004) and immunotherapy plus probiotic and Nigella sativa (15.38±1.60 vs 20.50±2.07 p=0.000). This means that the provision of immunotherapy treatments + *Nigella sativa* + probiotics in patients with mild asthma children will affect the ACT, which is able to increase the ACT.

Table 5. The comparation of ACT score before and after treatment

Treatment Group	Pretest ACT Mean ± SD	Postest ACT Mean ± SD	p-value (paired sample t test)
Immunotherapy	15.86±5.12	18.00±7.42	0.062
Immunotherapy + Nigella sativa	16.00±2.73	20.75±3.37	0.001
Immunotherapy+ probiotic	15.75±1.67	21.00±3.82	0.004
Immunotherapy+Nigella sativa+probiotic	15.38±1.60	20.50±2.07	0.000

The correlation between the number of $CD4^{+}IL\text{-}4^{+}$cells, total IgE level and ACT score in the immunotherapy group is shown in Table 6. The correlation between the number of CD4+IL4+cells and total IgE level was very significant (*p-value*=0.001<) with r = 0.949. The positive value showed parallel relationship between $CD4^{+}IL\text{-}4^{+}$and IgE level. Table 6 also shows that there was a significance relationship between the number of $CD4^{+}IL\text{-}4^{+}$cells with ACT score (p-value = 0.016 <α) in the immunotherapy group, indicated level of closeness of relationship (correlation coefficient) which was very powerful (-0.850). The level of IgE also shows negative relationship with ACT score. (r=-0.936, p=0.002) .Negative values indicate the opposite relationship exists, ie if there is an increase in the number of $CD4^{+}IL\text{-}4^{+}$cells there will be a decrease in the ACT in children with mild asthma, or vice versa. Table 7 shows the correlation between the number of $CD4^{+}IL\text{-}4^{+}$cells, total IgE level and ACT score in the immunotherapy plus *Nigella sativa* group. There was positive relationship between the number of $CD4^{+}IL\text{-}4^{+}$cells and total IgE level (r=0.968, p=0.00) and a negative relationship between the number of $CD4^{+}IL\text{-}4^{+}$cells and ACT score (r=-0.903, p=0.02). The correlation between Total IgE and ACT is also significant (r= -0.849, p= 0.008)

Table 6. The correlation between the number of CD4+IL4+ cells, Total IgE level and ACT score in the immunotherapy group

Corelation between		Correlation coefficient (r)	*p-value*
CD4+IL4+	Total IgE	0.949	0.001
	ACT	-0.850	0.016
Total IgE	ACT	-0.936	0.002

Table 7. The correlation between the number of CD4+IL4+ cells, Total IgE level and ACT score in the immunotherapy plus *Nigella sativa* group

Correlation between		Correlation coefficient (r)	*p-value*
CD4+IL4+	Total IgE	0.968	0.000
	ACT	-0.903	0.002
Total IgE	ACT	-0.849	0.008

Table 8 show the correlation between the number of $CD4^+IL\text{-}4^+$cells, total IgE level and ACT score in the immunotherapy plus probiotic group The correlation between the number of CD4+IL4+celss and total IgE level was significant (*p-value*=0.004) with r = 0.880. There was a significant relationship between the number of CD4+IL4+cells with ACT score (r=-0.79 p=0.04). The level of IgE also shows negative relationship with ACT score. (r=-0.858, p=0.006) .Negative values indicate the opposite relationship exists, ie if there is an increase in the number of $CD4^+IL\text{-}4^+$cells there will be a decrease in the ACT in children with mild asthma, or vice versa. Table 9 shows the correlation between the number of $CD4^+IL\text{-}4^+$cells, total IgE level and ACT score in the immunotherapy plus *Nigella sativa* plus probiotic group. There was positive relationship between the number of $CD4^+IL\text{-}4^+$cells and total IgE level (r=0.773, p=0.024) and a negative relationship between the number of $CD4^+IL\text{-}4^+$cells and ACT score (r=-0.911, p=0.002). The correlation between Total IgE and ACT is also significant (r= -0.926, p= 0.001)

Table 8. The correlation between CD4+IL4+, Total IgE level and ACT score in the immunotherapy plus probiotic group

Correlation between		Correlation coefficient (r)	*p-value*
$CD4^+IL\text{-}4^+$	Total IgE	0.880	0.004
	ACT	-0.879	0.004
Total IgE	ACT	-0.858	0.006

Table 9. The correlation between CD4+IL4+, Total IgE level and ACT score in the immunotherapy plus *Nigella sativa* group

Correlation between		Corelation coefficient (r)	*p-value*
$CD4^+IL\text{-}4^+$	Total IgE	0.773	0.024
	ACT	-0.911	0.002
Total IgE	ACT	-0.926	0.001

IV. Discussion

The result of this study shows there were no significant difference in the number of CD4+IL4+ cells in all treatment group. This result differs from other previous studies showing immunotherapy can decrease the number of $CD4^+IL4^+$ cells. From 19 patients who had increase of the number of $CD4^+IL4^+$ cells , 12/19 patients had mutiple allergy from their skin prick test result, not only HDM allergy. Therefore, the multiple allergy condition might be the reason why the number of $CD4^+IL4^+$ cells increased. The domination of Th2 in the multiple allergy asthma patients represented by the number of $CD4^+IL4^+$ cells could not be reduced by immunotherapy.

Previous study by Ohashi et all (1997) showed that immunotherapy less than 3 years show no significant change in IL-4, specific IgE and IFN-ɣ. The modulation of IL-4 was not detected in patient who had short term immunotherapy. Other study stated that the decrease of IL-4 in immunotherapy was depend on the duration of the treatment. [23, 24]. In this study, the immunotherapy was given for 14 weeks (build up phase). This duration might not be long enough to produce immunomodulation effect.

In this study, the addition of probiotic did not decrease the number of $CD4^+IL4^+$ cells. The result of previous studies of probiotic use in ashtma patients show various results. Many factors can influenced the effect of probiotic in allergic patients. Different condition of host factor (genetic differences on microbial response, allergic predisposition), enviroment factor and probiotic results in different immune response. [25-27]

In this study, there were no significant difference of the number of $CD4^+IL4^+$ cells before and after the addition of *Nigella sativa.* This result is differing from previous study that showed Nigella sativa can decrease the level of IL-4.[28] This difference could be caused by different dosage of Nigella sativa. One of the limitation of this study is no examination of the number of $CD4^+IL4^+$ cells in healthy control children. Therefore, we could not know whether the number of $CD4^+IL4^+$ cells is increased or within normal limit.

In the immunotherapy only treatment group,the decrease of IgE level and the increase of ACT score were not significant. Earlier immunotherapy studies showed that immunotherapy could decrease the IgE level and improved asthma symptoms. [29, 30]. Study by Douglass et all showed that the immunomodulation effect of immunotherapy happened after 12 months. [31] Thus, the duration of immunotherapy for 14 weeks in this study might be the cause of the result.

There was a significant decrease of total IgE level in immunotherapy plus *Nigella sativa* plus probiotic treatment group. This result showed that the combination of probiotic and *Nigella sativa* could improve the efficacy of immunotherapy in ashtma patients. The mechanism might be from the effect of immunotherapy in switching of B cells to produce IgG4. The production of IgG4 could be influenced by IL-10 and TGFβ produced by Treg cells. Previous studies about probiotic and Nigella sativa in ashtma showed that they can induced Treg cells and production of IL-10.[18, 27, 32].

In this study, there were significant improvement in ACT score in the immunotherapy plus Nigella sativa, immunotherapy plus probiotic and immunotherapy plus probiotic and Nigella sativa group. This result is constant with previous studies showing that Nigella sativa and probiotic could reduce the ashtma symptoms, improve lung function in ashtmatic children[14, 17, 20, 26, 33-35]. This effect could be caused by the effect of Nigella sativa and probiotic as anti inflamation, anti histamin and immunomodulator. This result supported also by the strong correlation between the number of $CD4^{+}IL\text{-}4^{+}$cells, Total IgE level and ACT score. A decrease of $CD4^{+}IL\text{-}4^{+}$and Total IgE level will increase the ACT score.

V. Conclusion

The administration of combination immunotherapy plus *Nigella sativa* plus probiotics did not reduce the number of peripheral blood $CD4^{+}IL\text{-}4^{+}$cells asthmatic children, but could decrease the total IgE level and improve clinical symptoms proved by the increase of ACT. Further studies needed to discover the mechanism of the immunotherapy combined with Nigella sativa and probiotic in improving the clinical symptoms of ashtma patients and decrease the IgE level.

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