ENHANCED EFFICACY OF SUBLINGUAL IMMUNOTHERAPY IN CHILDHOOD ALLERGIC ASTHMA BY PROBIOTICS

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ABSTRAK

Probiotik diharapkan dapat berperan secara sinergis untuk meningkatkan efektifitas klinis imunoterapi sublingual (SLIT). Tujuan penelitian ini adalah untuk mengetahui peran probiotik dalam peningkatan efektivitas imunoterapi sublingual pada asma alergi anak. Penelitian klinis acak buta tunggal ini dilakukan pada anak-anak asma alergi yang berusia 6-17 tahun yang sensitif terhadap alergen debu rumah. Subyek dialokasikan ke dalam kelompok A yang menerima SLIT saja, kelompok B yang menerima SLIT bersama probiotik, dan kelompok C yang hanya menerima probiotik. Parameter klinis (reversibilitas FEV_1, skor pengobatan, dan skor gejala) serta parameter imunologis (jumlah IgE, eosinofil, IFN-γ, IL-2, IL-4, IL5, IL10, dan TGF-β) dievaluasi pada minggu ke-0 sampai 14. Hasil analisis statistik menunjukkan bahwa tidak ada perbedaan signifikan antar kelompok dalam parameter klinis (reversibilitas FEV_1, skor pengobatan, dan skor gejala). Perbedaan antar kelompok dalam parameter imunologis dalam hal IgE total, IFN-γ, IL-2, IL-4, IL5, IL10, dan TGF-β juga tidak signifikan. Jumlah eosinophil menurun pada subyek yang mendapat kombinasi SLIT dengan probiotik. Kesimpulan dari penelitian ini adalah probiotik dapat meningkatkan efektivitas imunoterapi sublingual pada asma alergi anak melalui penurunan jumlah eosinofil. (FMI 2018;54:64-74)

Kata kunci: Alergi asma; imunoterapi sublingual; probiotik; efektivitas

ABSTRACT

It is expected that probiotics may act synergistically to improve the clinical efficacy of sublingual immunotherapy (SLIT). The aim of this study was to investigate the role of probiotics in enhancing efficacy of sublingual immunotherapy in childhood allergic asthma. This was a randomized single blind clinical trial conducted on 6-17 year-old asthmatic children sensitive to house dust mite allergens. Subjects were allocated to Group A receiving SLIT, Group B receiving probiotics and SLIT, Group C receiving probiotics only. Clinical parameters (FEV_1 reversibility, medication score, and symptom score) and immunological parameters (total IgE, eosinophil count, IFN-γ, IL-2, IL-4, IL5, IL10, and TGF-β were evaluated in week 0 until 14. Statistical analysis revealed that the difference of clinical parameters (FEV_1 reversibility, medication score, and symptom score) between groups were not significant. The difference of the immunological parameters of total IgE, IFN-γ, IL-2, IL-4, IL5, IL10, and TGF-β were also not significant. Eosinophil count decreased in subjects who received combination SLIT with probiotics. In conclusion, probiotics enhanced the efficacy of sublingual immunotherapy in childhood allergic asthma by decreasing the eosinophil count. (FMI 2018;54:64-74)

Keywords: Asthma allergy; sublingual immunotherapy; probiotics; efficacy

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INTRODUCTION

The prevalence of IgE-mediated allergic diseases has increased more than two-fold during the last decades. Currently, more than 30% of the population in industrialized countries are allergic to aeroallergens such as house dust mites (Pfaar et al 2012, Upton et al 2000). Allergen-specific immunotherapy (AIT) is an effective therapy for IgE-mediated respiratory allergic diseases and has a potentially sustained effect after completed treatment (Durham 2011, Moingeon & Mascarell 2012). Sublingual immunotherapy (SLIT), demonstrates safety and clinical efficacy with improvements on adverse events (Durham 2011, Moingeon & Mascarell 2012, Canonica et al 2003, Durham et al

2012, Dahl et al 2006). SLIT has been shown to induce a shift from the allergy promoting Th2 cells (IL-4, IL-5, and IL-13) toward TH1 cells (IFN-γ), induce allergen-specific regulatory T-cells, and produce allergen-specific IgA and IgG, mainly IgG4 (Allam et al 2011, Moingeon et al 2006, Radulovic et al 2008, Scadding et al 2010). Concomitantly with an increased efficacy, the number and severity of adverse events also increase with dose of SLIT. The target profile for an improved SLIT treatment is therefore improved safety with maintained or increased clinical efficacy, and increased patient convenience.

Pre-clinical studies have shown that probiotics could modulate the global immune response of the host, thus

reducing sensitization and allergic inflammation (Sudo et al 1997, Geuking et al 2011). Many studies have suggested the hypothesis that probiotics might be protective for asthma. Specifically, in pediatric asthma LGG was reported to reduce the concentration of exhaled nitric oxide among 4- to 7-year-olds. Treatment with LGG has been shown to decrease MMP9 expression in lung tissue, to inhibit inflammatory cell infiltration, and to decrease the number of infiltrating inflammatory cells and Th2 cytokines in broncho-alveolar lavage fluid and serum (Wu et al 2016). Similar results have been reported with other probiotics (Elazab et al 2013). Bifidobacterium bifidum, B. lactis, and Lc. lactis were shown to have a good IL-10-inducing capacity and to exert a significant inhibition of Th2-related cytokines IL-5 and IL-13 (Gorissen et al 2014, Niers et al 2005, Niers et al 2007). It is expected that probiotics may act synergistically to improve the clinical efficacy of sublingual immunotherapy.

The purpose of this study was to investigate the role of probiotics in enhancing efficacy of sublingual immunotherapy in childhood allergic asthma on clinical parameters (FEV_1 reversibility, medication score, and symptom score) and immunological parameters (total IgE, eosinophil count, IFN-γ, IL-2RB, IL-4, IL5, IL10, and TGF-β).

MATERIALS AND METHODS

Participants and study design

This clinical trial was a randomized study conducted at an allergic clinic of the Child Health Department, Soetomo General Hospital from September 01, 2008 to March 31, 2012. Informed written consents were obtained from each patient or their legal guardians. Subject has a willingness to participate as indicated by a signed and dated informed consent form, by parent or guardian, prior to undergoing any study-related procedures or medication withdrawal. A written informed assent form, signed by the child, if old enough to understand the research nature of the study, would also be obtained, where practical. This study was approved by the Hospital Ethics Committee.

The investigation took place during fourteen clinical visits (screening, and visits 1 to 14). During the screening visit, general data, including name, address, age, gender, body weight, body height and telephone number, were collected and recorded for all the participants. A comprehensive medical and allergy history was obtained for all subjects, especially pertaining to the allergic asthma and its duration, as well as details of family allergies and control medications. Blood samples

were taken before the study for measurement of total IgE and specific IgE skin test to five allergens. The subjects, of both sexes, were 6-17 years of age and had a diagnosis of asthma as defined by the American Thoracic Society (ie, "a disease characterized by increased responsiveness of the trachea and bronchi to various stimuli and manifested by widespread narrowing of the airway that changes in severity, either spontaneously or as a result of therapy") (1) for ≥ 3 months. Subjects 6-11 years of age must have a pre-bronchodilator forced expiratory volume in 1 second (FEV_1) of $\geq 75\%$ and $\leq 90\%$ of Polgar predicted normal value at Visit 1. Subjects 12-17 years of age, must have a pre-bronchodilator forced expiratory volume in 1 second (FEV_1) of $\geq 60\%$ and $\leq 90\%$ of Polgar predicted normal value at Visit 1. Subjects with predicted FEV_1 of $>90\%$ and $\leq 95\%$ may be included if they have an absolute FEV_1/FVC ratio measured on screening spirometry of $<80\%$. Reversibility criteria were defined as an increase in FEV_1 $\geq 12\%$ from pre-bronchodilator FEV_1, 15-30 minutes after 2 actuations of a salbutamol pMDI (100 μg/actuation), performed at Visit 1. All patients showed sensitization to house dust mites (DP), as evaluated by skin testing (wheal ≥ 3 mm).

Exclusion criteria for the subjects consisted of life-threatening asthma; two or more hospitalizations for asthma within 1 year of Visit 1 or any emergency room; Visit for asthma within 6 months of Visit 1; using steroids during the month (28 days) prior to Visit 1 or at any time during the trial, using leukotriene modifiers or ketotifen (oral), within 2 weeks prior to Visit 1; with any history of smoking, with any clinically relevant respiratory diseases such as cystic fibrosis, broncho-pulmonary dysplasia (BPD), interstitial lung disease or active or untreated pulmonary tuberculosis; have past or present diseases of the cardiovascular, endocrine, renal, hepatic, gastrointestinal, neurological, musculoskeletal, or metabolic systems or other medical conditions considered clinically significant; with a history of malignancy in the past 5 years; with a planned hospitalization during the study; with known hypersensitivity to salbutamol and excipients; has severe asthma, as judged by the investigators; and previously diagnosed with neuropsychiatric or congenital immunodeficiency or probiotics allergy.

Specific reasons for discontinuing a subject from this study are: withdrawal of informed consent; intolerable adverse event (AE); use of disallowed medication; pregnancy; incorrect randomization; decrease in FEV_1 (L) $\geq 25\%$ from Visit 1 or to below 40% of predicted; use of 12 or more actuations of salbutamol pMDI per day for 2 days within a 3 day period; three nighttime awakenings requiring treatment with short-acting

inhaled β_2-agonist within a 5-day period; and not maintaining at least 80% data entry compliance.

After enrollment at visit 1 (baseline), subjects were randomly assigned to one of three groups (A=SLIT alone, B=Probiotics together with SLIT and C=Probiotics alone). Each subject should be seen at approximately the same time of day for each visit, starting with Visit 1. For each clinical assessment and individual subject, the assessment should be performed using the same equipment, relevant for the assessment, at all Visits. Lung function tests should be performed between 6:00 9:30 AM for ±30 minutes, and for the individual subject, each test should be performed ±60 minutes from the time the test was performed at Visit 1. If necessary due to scheduling, sites may conduct Visit 1 Pulmonary Function Tests on a separate day than the remainder of Visit 1 procedures. The Pulmonary Function Tests should not be more than 3 days before or after the remainder of the Visit 1 procedures.

Visit 1

All subjects were asked to supply certain information and underwentstudy tests and procedures as follows: reading and signing informed consent/assent (with parental or guardian assistance, as needed); providing oral consent/assent; medical/medication history; pulmonary function testing; clinical chemistry, hematology, cytokine, urinalysis; and complete physical examination.

Visit 2 to visit 13

If the subject metall inclusion criteria and none of the exclusion criteria previously described, the following tests and procedures were verified that the subject did not meet the discontinuation criteria established; concomitant medication and adverse event query; review diary data to ascertain eligibility; pulmonary function testing; mouth examination; dispense study drug, and instruct the subject regarding the dosing regimen.

Visit 14

The following tests and procedures were performed at Visit 14 verifying that the subject did not meet the discontinuation criteria; had completed physical examination including mouth examination; clinical chemistry, hematology, cytokine and urinalysis; concomitant medication and adverse event; pulmonary function testing; and diary data.

Assessment of symptoms and drug scores

Patients' diary cards recording symptoms and medication scores were used during the study for 14 weeks. The symptoms scores for asthma (cough and breathlessness) were separately recorded by each patient's parent and rated according to the following scale: 0=no symptoms; 1=mild symptoms; 2=moderate symptoms; 3=serious symptoms. For each patient, the total of medications taken daily (systemic antihistamines, nasal chromoglycate, ocular cromoglycate, and beta-2-agonist) were recorded in daily diary cards. Symptoms and medication scores were considered in each patient as monthly cumulative values obtained by summing-up the scores recorded daily.

The efficacy variables

The primary efficacy variable was the change from baseline in FEV_1 reversibility in % of predicted normal value measured at the clinic Visits. Secondary efficacy variables were nighttime and daytime asthma symptom scores, rescue medication use (number of puffs per day and number of days used), change from baseline in total IgE, eosinophil count, IFN-γ, IL-2RB, L-4, IL5, IL10, and TGF-β.

Safety variables

Safety variables included the incidence of adverse events, discontinuations due to adverse events, serious adverse events, laboratory evaluations, and physical examinations.

Sublingual Immunotherapy Drug and Dose

NOVO-HELISEN ORAL (Germany) allergen extract of house dust mite was used for sublingual immunotherapy. The composition of the allergens is listed on the labels. Novo-Helisen® oral is standardized in TU (therapeutic units) or in PNU (protein nitrogen units). Strength 2 of Novo-Helisen oral is a 1:10 dilution of strength 3, strength 1 is a 1:10 dilutionof strength 2 and strength 0 is a 1:10 dilution of strength 1.Initial treatment set: 3 vials of strength 1, 2, 3 vials of strength 1, 2, 3 with 30 ml each. Maintenance treatment set: 3 vials of strength 3 or 1 vial of strength 3 (on special request) with 30 ml each.Treatment begins with 1 drop of the weakest concentration (strength 1). This dose is increased by 1 drop daily. Once the dose of 28 drops a day has been reached, treatment is continued with 2 drops of the next highest concentration (strength 2). This dose is again increased by 1 drop daily until 28 drops of strength 2 are reached. Treatment is then continued with 2 drops of strength 3, again increased daily by 1 drop until the maximum dose of 28 drops of strength 3 is reached.

Probiotics

Each capsule contained *Lactobacillus rhamnosus* GG 5×10^9 cfu, *Bifidobacterium longum* 2×10^9 cfu, and *Bifidobacterium bifidum* 2×10^9 cfu. The study preparation was manufactured by Outpatient Allergy Clinic Dr. Soetomo General Hospital. Each dose was stored in airtight alu-bags at -20°C until the first day of the intervention period. During the 14 weeks study period, the patients were asked to abstain from any fermented food products containing live microorganisms and none of the patients changed diet during the study period. Issues regarding taste of the test preparation and the child's preferred way to ingest the powder were discussed at each scheduled visit.

Statistical analysis

The primary outcome measure was change in symptom score from baseline following treatment. The sample size of 30 patients provided sufficient power (90%) to detect a difference of 10% between 3 groups (α=0.05). Statistical analysis was performed by means of multivariate analysis. for intra- and inter-group analysis, respectively. All lost rates, missing values and reasons for drop out or premature termination of the study was recorded. The SPSS software was used for data management and statistical analysis. Data were presented as mean ± SD and p values of 0.05 or less were considered as statistically significant.

RESULTS

Demographic Characteristics

In total, 50 subjects were enrolled during their screening visit; 32 fulfilled the inclusion criteria and were randomized into three groups, A, B, and C, receiving SLIT alone and SLIT combined with probiotics and probiotics alone, respectively. Patient demographics and baseline characteristics were similar for the three groups (Table 1). Although intra-group comparisons showed some differences, these differences did not reach statistical significance. There were no significant between-group differences in sensitization rate to the five allergens. No participants left the trial prematurely due to adverse effects or other factors (such as poor compliance or moving away) or failed to finish the diary book questionnaires.

Clinical scores and efficacy

Symptom scores for asthma and drug usage were not different at baseline visit (pre treatment) and at the post treatment between group A, B and C (Table 1). Similar-

ly, all of therapy groups had no differences on the decreasing of the symptoms, drug usage and FEV_1 reversibility in the post treatment (Table 2).

Laboratory parameters

The reduction of total IgE, eosinophil count, IL-4 and IL-5 observed in the group Awere also observed in the group B and group C. There were no differences in the reduction of total IgE, IL-4 and IL-5 between group A, B and C. The decreasing of eosinophil count was significantly different between group A, B and C. IFN-γ, IL-2, IL-10 and TGF-β increased in all of the groups and there was no significant difference between them (Table 3).

DISCUSSION

We have shown prospectively that probiotics enhance the efficacy of sublingual immunotherapy (SLIT). SLIT is a safe and effective alternative to injective immunotherapy (Tari et al 1990, Troise et al 1995, Sabbah et al 1994, Clavel et al 1998, Feliziani et al 1995, Quirino et al 1996, Passalacqua et al 1998, Vourdas et al 1998, Bousquet et al 1999, Canonica et al 2003, Bousquet et al 1998). World Health Organization (WHO) and Allergic Rhinitis and its Impact on Asthma (ARIA) recognized SLIT as an efficacious treatment for seasonal allergies (Bousquet et al 1998, Bousquet et al 2001).

The original rationale for administering immunotherapy sublingually was that of achieving a prompt and rapid absorption of the vaccine to avoid possible gastro-intestinal degradation. Although it was recently demonstrated that no relevant direct absorption through the sublingual mucosa occurs, SLIT proved effective in a great number of trials; therefore, it is presently the most widely used non-injection route for immunotherapy in Europe. About 22 DBPC trials (Tari et al 1990, Sabbah et al 1994, Feliziani et al 1995, Troise et al 1995, Hirsch et al 1997, Clavel et al 1998, Horak et al l998, Vourdas et al 1998, Hordijk et al 1998, Passalacqua et al 1998, Passalacqua et al 1999, Bousquet J et al 1999, Purello et al 1999, Pradalier et al 1999, La Rosa et al 1999, Guez et al 2000, Pajno et al 2000, Caffarelli et al 2000, Ariano et al 2001, Bahcecilier et al 2001, Voltolini et al 2001, Lima et al 2002) conducted with adequate methods and analysis have been published in peer-reviewed journals and the magnitude of the clinical efficacy ranged between about 20% and 50% reduction of symptom or medication scores, thus quite superior to the placebo effect and close to the effect of subcutaneous immunotherapy (SCIT). SLIT exerts its effect on asthma symptoms. The results from some studies (Bousquet et al 1999, Purello

et al 1999, Pradalier et al 1999, La Rosa et al 1999, Guez et al 2000, Pajno et al 2000) showed a reduction of the days with asthma symptoms (Pradalier et al 1999), of the use of 2-agonists (Bousquet et al 1999, Pradalier et al 1999) of the intake of systemic steroids (Guez et al 2000), and of clinical symptoms (Bousquet et al 1999, Pradalier et al 1999). One single study also demonstrated the measurable effect of SLIT on the quality of life of patients (Bousquet et al 1999). In our study, we have shown that SLIT alone and SLIT combined with probiotics could decrease asthma symptoms and drug usage including symptomatic and 2-agonists.

The immunological aspects of SLIT are not still clear (Lichtenstein et al 1973, Creticos et al 1985, Secrist et al 1995, Varney et al 1993). Many studies on SLIT have focused on possible decrease of serum IgE or/and increase of IgG1 and IgG4, but these changes were not constant and reproducible (Allam et al 2011, Moingeon et al 2006, Radulovic et al 2008, Scadding et al 2010, Fanta et al 1999). SLIT has been proved to reduce intercellular adhesion molecule 1 expression on nasal-epithelial cells and to decrease methacholine respons-iveness (Silvestri et al 2002). Our study showed that SLIT could decrease serum IgE, eosinophil count, TH2 cytokines level (IL-4 and IL-5) and increase TH1 cyto-kines level (IFN-γ and IL-2) and T regulator cytokines (IL-10 and TGF-β).

Table 1. Demographic characteristics at the baseline visit

Variables	Group A (SLIT) n=11	Group B (SLIT+Probiotics) n=11	Group C (Probiotics) n=10	p value
Age, mean (SD) yrs	9.09 (2.30)	8.82 (2.18)	9.10 (2.30)	0.945
BW, mean (SD) kg	28.55 (11.83)	29.36 (11.73)	28.50 (5.02)	0.976
BH, mean (SD) cm	131.73 (17.55)	130.45 (12.77)	131.30 (11.99)	0.978
Total-IgE, mean (SD)IU/ml	414.91 (358.15)	220.45 (244.30)	426.70 (277.49)	0.213
Eosinophil Count, mean (SD) /cmm	508.64 (249.22)	440.45 (224.56)	532.50 (281.75)	0.685
FEV 1 reversibility, mean (SD) %	17.71 (4.52)	17.61 (4.96)	20.59 (12.03)	0.618
Gender				
Male	7 (63.6)	3 (27.3)	4 (40.0)	0.219
Female	4 (36.4)	8 (72.7)	6 (56.3)	
FH of Allergy				
Yes	1 (9.1)	2 (18.2)	1 (10.0)	0.779
No	10 (90.9)	9 (81.8)	9 (90.0)	
Feather				
Yes	2 (18.2)	2 (18.2)	1 (10.0)	0.840
No	9 (81.8)	9 (81.8)	9 (90.0)	
Egg white				
Yes	5 (45.5)	6 (54.5)	7 (70.0)	0.521
No	6 (54.5)	5 (45.5)	3 (30.0)	
Cow's Milk				
Yes	10 (90.9)	9 (81.8)	8 (80.0)	0.757
No	1 (9.1)	2 (18.2)	2 (20.0)	
Shrimp				
Yes	9 (81.8)	10 (90.9)	8 (80.0)	0.757
No	2 (18.2)	1 (9.1)	2 (20.0)	
Der p 1 ∅wheal, mean (SD) mm	10.18 (3.97)	7.18 (4.21)	9.40 (3.24)	0.185
∅flare, mean (SD) mm	23.09 (10.75)	15.55 (11.51)	25.20 (12.30)	0.143
Der f 1 ∅wheal, mean (SD) mm	8.09 (3.67)	6.09 (2.07)	7.50 (1.72)	0.211
∅flare, mean (SD) mm	18.91 (8.50)	14.00 (5.35)	19.10 (6.57)	0.172

Table 2. Clinical score at the baseline visit and at the post treatment

| Variables	Group A (SLIT) n=11	Group B (SLIT+Probiotics) n=11	Group C (Probiotic) n=10	P value
Symptom Score				
Pre, mean (SD)	51.73 (42.57)	31.91 (28.21)	46.40 (37.70)	0.434
Post, mean (SD)	17.64 (17.33)	13.09 (16.26)	13.60 (11.11)	0.751
Pre-post difference, mean (SD)	34.09 (36.09)	18.82 (21.02)	32.80 (39.53)	0.496
Drug Score				
Pre, mean (SD)	26.36 (29.28)	13.18 (11.80)	14.30 (10.74)	0.233
Post, mean (SD)	5.64 (9.84)	6.55 (8.32)	7.80 (7.44)	0.848
Pre-post difference, mean (SD)	20.73 (25.42)	6.64 (13.03)	6.50 (13.15)	0.134
FEV_1 reversibility				
Pre, mean (SD) %	17.71 (4.52)	17.61 (4.96)	20.59 (12.03)	0.618
Post, mean (SD) %	3.83 (1.79)	2.91 (1.83)	4.85 (3.54)	0.852
Pre-post difference, mean (SD) %	13.88 (4.97)	14.70 (4.60)	15.74 (11.42)	0.218
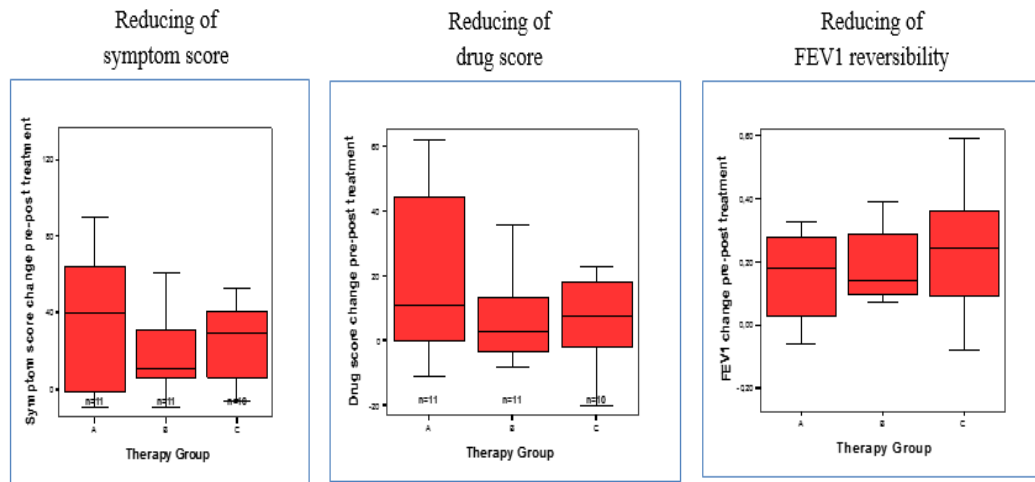
Fig. 1. Clinical outcome of three groups therapy (Group A receiving SLIT alone; Group B receiving SLIT combined with probiotic; and Group C receiving probiotics alone). Reducing of symptom scores, drug usage and FEV_1 reversibility were not different at baseline visit (pre treatment) and at the post treatment between these groups.

Many investigators have reported that probiotics and related products have beneficial effects for allergy sufferers (Isolauri 2001, Matricardi et al 2003). Our results also demonstrate that live probiotics may alleviate the symptoms caused by allergic asthma. Many previous studies reported that probiotics bacteria, which beneficially affect the host by improving its microbial balance (Fuller 1991), mediate antiallergenic effects by stimulating production of Th1-cytokines (Lombardi et al 2001, Lichtenstein et al 1973), TGF-B (Isolauri et al 2000, Paganelli et al 2002) and gut IgA (Fukushima et al 1999, Kirjavainen et al 1999). Our study also have shown that probiotics may mediate antiallergenic effects as good as sub-lingual immunotherapy (SLIT) or SLIT combined with probiotics by stimulating Th1-cytokines, IL-10 and TGF-β and by decreasing Th2-cytokines (Matsuzaki et al 2000).
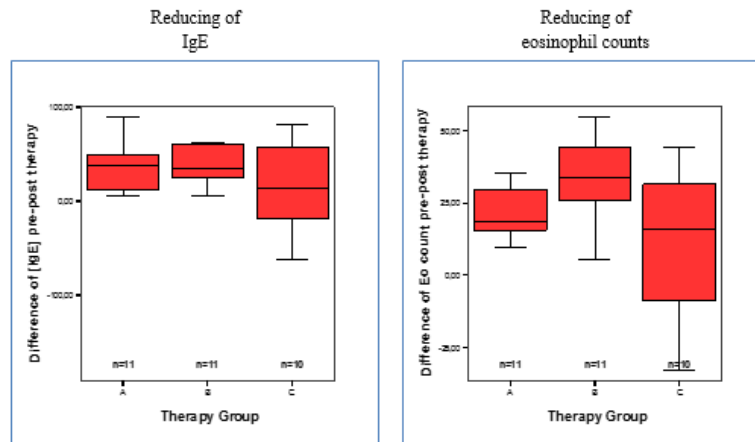
Fig. 2. Reducing IgE and eosinophil counts of three groups of therapy (Group A receiving SLIT alone; Group B receiving SLIT combined with probiotic; and Group C receiving Probiotics alone). Reducing of IgE were not different at baseline visit (pre treatment) and at the post treatment between these groups. There was significant difference in eosinophil count between Group B (SLIT combined with probiotic) and Group A or between Group B and Group C.
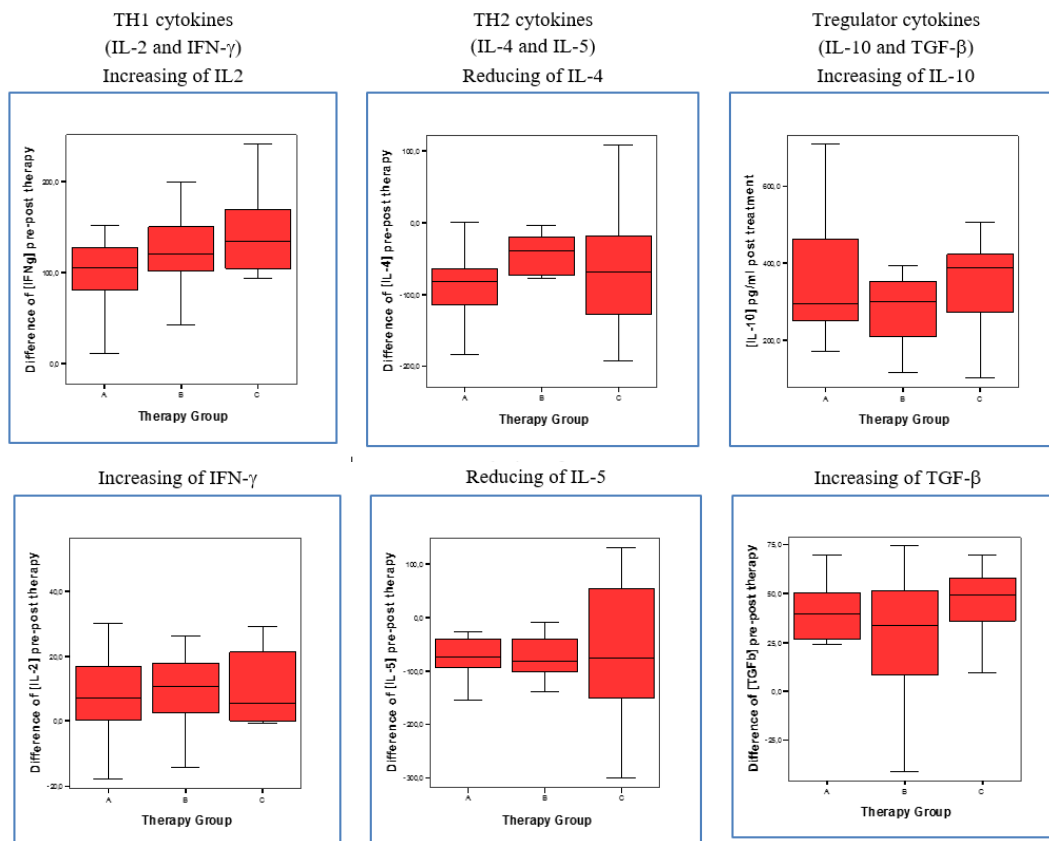

Fig. 3. Profile of TH1, TH2 and T-regulator change of three groups therapy (Group A receiving SLIT alone; Group B receiving SLIT combined with probiotic; Group C receiving Probiotics alone). Reducing ofTH2 cytokine (IL-4 and IL-5), increasing of TH1 cytokine (IFN-γ and IL-2) and T-regulator (IL-10 and TGF-β were not different at baseline visit (pre treatment) and at the post treatment between these groups.

Table 3. Laboratory parameters at the baseline visit and at the post treatment

Variables	Group A (SLIT) n=11	Group B (SLIT+Probiotics) n=11	Group C (Probiotic) n=10	P value
IgE Total				
Pre, mean (SD)IU/ml	414.91 (358.15)	220.46 (244.30)	426.70 (277.49)	0.213
Post, mean (SD)IU/ml	276.82 (251.17)	142.91 (164.50)	496.40 (580.91)	0.105
Pre-post difference, mean (SD)	20.89 (71.71)	38.91 (19.88)	3.83 (68.67)	0.396
Eosinophil count				
Pre, mean (SD)/cmm	669.82 (349.35)	679.46 (365.40)	588.70 (281.44)	0.798
Post, mean (SD)/cmm	508.64 (249.22)	440.46 (224.56)	532.50 (281.75)	0.685
Pre-post difference,mean(SD)/cmm	23.00 (17.23)	34.38 (15.55)	9.81 (26.91)	0.033
IFN-γ				
Pre, mean (SD)IU/ml	35.67 (27.07)	37.20 (26.53)	39.92 (17.01)	0.921
Post, mean (SD)IU/ml	133.03 (61.44	150.67 (61.84)	185.26 (39.11)	0.111
Pre-post difference,mean(SD)IU/ml	97.36 (44.49)	113.47 (57.44)	145.34 (46.12)	0.100
IL-2				
Pre, mean (SD)IU/ml	19.00 (18.05)	21.18 (10.62)	21.38 (10.24)	0.903
Post, mean (SD)IU/ml	29.76 (19.29)	30.78 (10.88)	31.78 (16.08)	0.958
Pre-post difference,mean (SD)IU/ml	10.76 (19.30)	9.60 (11.40)	10.40 (12.23)	0.982
IL-4				
Pre, mean (SD)IU/ml	165.95 (108.64)	149.16 (91.70)	203.63 (95.99)	0.450
Post, mean (SD)IU/ml	77.49 (92.54)	84.84 (74.39)	144.20 (50.26	0.104
Pre-post difference,mean (SD)IU/ml	-88.46 (49.45)	-64.33 (70.55)	-59.43 (92.42)	0.613
IL-5				
Pre, mean (SD)IU/ml	187.16 (91.04)	173.75 (81.47)	243.84 (131.81)	0.273
Post. mean (SD)IU/ml	110.12 (92.66)	101.31 (62.79)	176.36 (71.46)	0.069
Pre-post difference,mean (SD)IU/ml	-77.04 (44.13)	-72.44 (44.58)	-67.48 (130.81)	0.965
IL-10				
Pre, mean (SD)IU/ml	114.36 (120.38)	76.89 (87.93)	141.40 (159.29)	0.499
Post, mean (SD)IU/ml	369.00 (183.91)	282.04 (90.45)	347.66 (117.14)	0.316
Pre-post difference,mean (SD) IU/ml	254.64 (111.44)	205.15 (143.70)	206.26 (231.63)	0.737
TGF-β				
Pre, mean (SD)IU/ml	52.04 (28.85)	34.91 (29.60)	24.34 (19.83)	0.071
Post, mean (SD)IU/ml	89.89 (27.35)	63.47 (23.78)	69.96 (17.86)	0.035
Pre-post difference,mean (SD)IU/ml	37.86 (22.50)	28.56 (35.730)	45.62 (20.15)	0.368

We have also shown prospectively that probiotics enhanced the efficacy of sublingual immunotherapy (SLIT) by decreasing blood eosinophil count. Asthma patients with greater blood eosinophil experience have more severe exacerbations and have poorer asthma control. Furthermore, a count-response relation exists between blood eosinophil counts and asthma-related outcomes. Blood eosinophil counts could add predictive value to Global Initiative for Asthma control-based risk assessment (Price et al 2015).

CONCLUSION

Probiotics enhanced the efficacy of sublingual immuno-therapy in childhood allergic asthma by decreasing blood eosinophil count. This finding is important to prevent more severe asthma exacerbation and poorer asthma control in the future.

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