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Original Article

Role of sublingual immunotherapy and probiotics in clinical improvements of childhood asthma

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Abstract

Background Sublingual immunotherapy (SLIT) is an effective secondary prevention to improve natural course of asthma. Its efficacy is limited to allergic asthma caused by inhalant allergen. Recent studies showed that probiotic as immunomodulator decreases inflammatory process induced by food allergy. No sufficient clinical evidence reported about the efficacy of combination sublingual immunotherapy and probiotics or probiotics only in clinical improvement of childhood asthma.

Objective To investigate the role of SLIT and probiotic on clinical parameters of childhood asthma (FEV1 reversibility, medication score, and symptom score).

Methods A randomized single blind clinical trial was conducted on 6-17 year-old asthmatic children sensitive to food and aeroallergens. Subjects were allocated to Group A receiving SLIT, Group B receiving probiotics and SLIT, Group C receiving probiotics only. All parameters were evaluated in week 0 until 14.

Results FEV1 reversibility improved in all groups. Medication score and symptom score was also decreased in all groups. The most marked decrease of FEV1 reversibility and symptom score were found in probiotics group. Statistical analysis revealed that the difference of each parameter between groups were not significant.

Conclusion Combination of SLIT and probiotics and probiotics are only similarly effective to improve clinical symptoms as childhood asthma. Hence, information about probiotics as immunomodulator was proved to be an excellent alternative therapy for childhood asthma has been obtained. **[Paediatr Indones 2008;48:261-8]**.

Keywords: *asthma allergy, sublingual immunotherapy, probiotics, medication score, symptom score, FEV1 reversibility*

Sublingual immunotherapy (SLIT) is very effective in the management of asthma in children sensitive to inhalant allergen. The efficacy of SLIT in allergic asthma due to food allergy is not yet proven.[1]

Probiotic bacteria, which affect the host by improving microbial balance, may mediate antiallergenic effects by stimulating production of TH1-cytokines, TGF-β, and gut sIgA.[2] Probiotic may reduce symptoms of allergy in children, but until recently few clinical trials exist. Observational studies in humans also support the importance of the intestinal microbiota in immune development, and have found a relationship with the development of hypersensitivity. Interestingly these differences in load and species composition of *Bifidobacteria* may have anti-inflammatory modality as they have not been found in young children with wheeze and allergic sensitization.[3,4] It is expected that probiotic may act synergistically to improve the clinical efficacy of SLIT that limited to the aero-inhalant allergen.

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The purpose of this study was to investigate the role of SLIT and probiotic on clinical parameters of childhood asthma (FEV_1reversibility, medication score and symptom score).

Methods

Patients

Asthmatic children treated at Allergy and Immunology Outpatient Clinic of Dr. Soetomo Hospital Surabaya, Indonesia from September 2006 to February 2007 were enrolled in this study. A written informed consent form was obtained prior to any study-related procedures after receiving information of the study.

Inclusion criteria of subjects were either gender, 6-17 years of age and had a diagnosis of asthma as defined by the American Thoracic Society; in which the subjects who were 6-11 years of age must have a pre-bronchodilator FEV_1 ≥75% and ≤90% of Polgar predicted normal value at Visit 1 whereas subjects who were 12-17 years of age must have a pre-bronchodilator FEV1 ≥60% and ≤90% of Polgar predicted normal value at Visit 1. For those subjects with an FEV1 >90% and ≤95% predicted might be included if they had an absolute FEV_1/FVC ratio measured on screening spirometry of <80%; FEV1 reversibility ≥12% performed at Visit 1; treatment of orally inhaled corticosteroid for no more than a month (30 days), given immediately proceeding Visit 1.

We excluded subjects with life-threatening asthma including severe asthma, any prior intubations, respiratory arrest or seizures within the past 5 years as a result of an exacerbation of asthma; subjects with ≥2 times hospitalization for asthma within 1 year of Visit 1 or any visit to emergency room due to asthma within six months of Visit 1; used leukotriene modifiers, inhaled long acting β_2 agonist, oral β_2-agonists, theophyllines, anti-cholinergic, cromones or ketotifen (oral), within two weeks prior to Visit 1; history of smoking; acute exacerbation of asthma/respiratory tract infection within 30 days prior to Visit 1; previous randomization into this study; used experimental drug or device within 30 days of visit; and any subject unable to meet the concomitant medication restrictions.

Subjects would be withdrawn if they had: intolerable adverse event, decreased FEV_1≥25 % from Visit 1 or below 40% of predicted; used ≥12 actuations of albuterol or salbutamol pMDI/ day for two days within a three day period; decreased morning PEF>25% from baseline (baseline defined as the mean value of the last 7 days prior to randomization) on three days within a five-day period; 3 night time awakenings requiring treatment with short-acting inhaled β_2-agonist within a five-day period; ≤80% data entry compliance.

The study was designed as true experimental pre- and post-test control group in randomized single blind trial. The patients were enrolled in three groups: Group A (SLIT), Group B (SLIT+Probiotics), and Group C (Probiotics only) using computer software incorporating a random number generator data.

Sublingual immunotherapy (SLIT)

NOVO-HELISEN ORAL (Germany) allergen extract of house dust-mites was used for specific oral immunotherapy. The composition of the allergens was listed on the labels. Novo-Helisen® oral was standardized in TU (therapeutic units) or in PNU (protein nitrogen units). Strength 2 of Novo-Helisen oral was a 1:10 dilution of strength 3, strength 1 was a 1:10 dilution of 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. Treatment begun with 1 drop of the weakest concentration (strength 1). This dose was increased by 1 drop daily. Once the dose of 28 drops a day was reached, treatment was continued with 2 drops of the next highest concentration (strength 2). This dose was again increased by 1 drop daily until the dose of 28 drops of strength 2 were reached. Treatment was 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 preparation

Each capsule contained: *L. johnsonii, L. paracasei,* and *B. lactis* GG 2x40 mg (10^9). A dose of 1010 CFU of each strain was given twice daily. During the 14 week study period, the patients were asked to be abstain from any fermented food products containing live microorganisms.

Symptom scores and medication score

Subjects recorded their daytime asthma symptoms score according to the following scale:

0= None	No symptoms of asthma. Did not require medication.
1= Mild	Asthma symptoms noticeable but were not bad enough to cause trouble with daily routine activities. Did not require albuterol.
2= Moderate	Asthma symptoms often noticed which caused some interference with daily routines activities. Required albuterol.
3= Severe	Asthma symptoms noticed continuously or present most of the day which severely restricted daily routine activities.

Night time asthma symptom score (from going to bed in the evening to waking up the next morning) was based on the following scale:

0= None	No symptoms of asthma. Did not require medication.
1= Mild	Awoke once because of asthma and/or cough but did not use albuterol.
2= Moderate	Awoke at least once because of asthma and/or cough and took albuterol.
3 = Severe	Awake most of the night because of asthma and/or cough.

Subjects answered the following questions in the morning regarding their asthma symptoms from the preceding night:

- Did your asthma wake you up last night?
- If your asthma wake you up last night, did you need to use your rescue medication (albuterol inhaler) before you went back to sleep?

Pulmonary function tests

Pulmonary function tests consisted of triplicate forced expiratory maneuvers in which the subject expires forcefully from total lung capacity to residual volume. A spirometer, which met ATS standards and calibrated daily, was used. Forced vital capacity and FEV1 were obtained from the full expiratory flow-volume-time curve. At least 3 technically satisfactory FVC maneuvers were performed and the largest value was selected and recorded. The difference between the largest and second largest FVC and FEV_1 should not vary by more than 0.2 L. A maximum of eight maneuvers can be performed until the reproducibility criteria met. If the test maneuvers induce bronchoconstriction, consecutive measurements become lower, this trend should be noted and the largest FVC and FEV1 should be reported. The test must be conducted between 6:00 and 9:30 AM + 30 minutes. The timing of the test should then be standardized and remain approximately the same throughout the study, starting with Visit 1. Polgar predicted normal value was used.

Airway reversibility was tested using spirometry before and after administration of inhaled albuterol (90 μg/actuation), 2 actuations, or after up to 2.5 mg of nebulized albuterol (Visit I & XIV). The second spirometry was obtained between 15 to 30 minutes after albuterol administration. An improvement of $\geq$ 12% in FEV1 from baseline was considered diagnostic of reversible airway obstruction.

Treatment protocol

Eligible subjects from Allergy and Immunology Outpatient Clinic were randomized at Visit 2 to receive one of 3 possible treatments for 14 weeks: Group A (SLIT), Group B (SLIT+Probiotics), and Group C (Probiotics only).

Dosing Schedule:

Group:	N (minimal)	Sublingual Immunotherapy	Probiotic
A	9	+	-
B	9	+	+
C	9	-	+

Visiting procedure:

Visit I

All subjects were asked to get certain information and to undergo tests and procedures as indicated below:

- Medical /medication history, documentation of clinical history of asthma.
- Pulmonary function test including reversibility test of FEV_1.
- Complete physical examination including skin test.

- Make an appointment for Visit 2 which should be 7 days (±3 days) after Visit 1.

The subject/guardian will be instructed to record diary information on a daily basis.

Visit II to Visit XIII

- Review diary data to ascertain eligibility.
- The subject/guardian should be queried regarding concomitant medications and adverse events.
- Pulmonary function test without FEV1 reversibility.
- Mouth and throat examination.
- If the subjects had been compliant with the study related procedures and wish to continue, they were then randomized, and were asked to collect and dispense study drug, and were informed regarding the dosing regimen.
- Make an appointment for Visit 3 (and etc.) which should be 1 week (± 3 days) after Visit 2.

Visit XIV

- Complete physical examination including mouth and throat examination.
- Concomitant medication and adverse event query.
- Pulmonary function test (including FEV1 reversibility).
- Review diary data.
- Subject will resume pre-study asthma therapy or receive other appropriate asthma treatment at the discretion of the investigators.

Statistical analysis

Sample size was calculated from a formula with 9 subjects in each treatment group; the study had a power of 80% to detect a difference in change in efficacy and safety between two treatments of 0.05 SD at the 5% level of significance. The analysis for all data was performed using the SPSS version 12.0. Values were expressed as means (SD) unless otherwise noted. Differences between the groups were tested for significance with paired t-test. Differences of baseline characteristics between group were analyzed using Anova, Chi square, and Mann Whitney test. Differences of symptom and medication score between groups were analyzed using Wilcoxon signed ranks test.

Primary outcome was FEV1 reversibility, secondary outcomes were symptom score and medication score.

Results

A total of 34 children with bronchial asthma with the onset less than two years treated in our institution during the study period were included in this study. Of these, 11 patients were treated by SLIT (Group A), 12 patients by combination of SLIT and probiotics (Group B), and 11 patients by probiotics only (Group C). Two patients were dropped out from the study in good condition because their families moved to other city. There were no significant differences in age, gender, body weight, height, and history of atopy between the groups **(Table 1)**. All groups started with the homogenious performance. There was no asthmatic; non asthmatic or medication related

Table 1. Baseline characteristics of subjects from all groups

	Group A (SLIT)	Group B (Combination)	Group C (Probiotics only)
1. Age,mean (SD) yrs	9.09 (2.30)	8.82 (2.18)	9.1 (2.13)
2. Gender			
- ♂	6	8	4
- ♀	5	3	6
3. Body weight, mean (SD) kg	28.6 (11.83)	29.4 (11.73)	28.5 (5.02)
4. Height, mean (SD) cm	131.7 (17.54)	130. 5 (12.77)	131.3 (1.99)
5. Atopy	10	11	9

* Significant difference of $\chi 2$ test when $P<0.05$

adverse event at all groups.

Clinical improvement was proven by decreasing FEV1 reversibility that reflected bronchial hyperreactivity **(Table 2).**

Table 2. Improving of FEV1 reversibility from all groups

	Mean FEV1 reversibility (%)				
	Visit I	Visit XIV	Delta	P	
A	20.25%	4.84%	-16.43%	0.0001	<0.01
B	17.61%	3.83%	-15.75%	0.002	
C	20.59%	2.91%	-14.70%	0.0001	

* Significant difference of Anova when P<0.05

The value of FEV1 reversibility in groups A, B, and C decreased 16.43%, 15.75% and 14.70% respectively. There was significant differences of FE V_1 reversibility between all groups (P=0.01).

The symptom score was also improved significantly in all groups **(Table 3).**

Table 3. Improving of symptom score from all groups

	Mean symptom score			
	Visit I	Visit XIV	P	
A	19.36	2.72	0.018	0.74
B	10.45	2.09	0.009	
C	19.00	2.40	0.021	

* Significant difference of Wilcoxon signed ranks test when P<0.05

The symptom score in group, group B and group C decreased from 19.36% in the first week to 2.72 in the fourteenth week of intervention from 10.45 to 2.09 and from 19.00 to 2.40 respectively. There was no significant difference of symptom score between all groups (P=0.74).

The medication score was also improved significantly in all groups **(Table 4).** The medication score in group A, group B and group C decreased from 9.36 in the first week to 0.68 in the fourteenth week of intervention, from 4.72 to 1.36 and from 6.00 to 1.70 respectively. There was no significant difference of medication score between all groups (P=0.31).

Table 4. Improving of medication score from all groups

	Mean medication score		P	
	Visit I	Visit XIV		
A	9.36	0.68	0.012	0.31
B	4.72	1.36	0.024	
C	6.00	1.70	0.018	

* Significant difference of Wilcoxon signed ranks test when P<0.05

Discussion

There has been some published evidence that gender, age, body weight, and height influence the result of lung function test.[5] The results of our study indicated that there were no significant differences between the groups.

Lung function test was performed in accordance with standardized guidelines. Lung function test with spirometer can be done in a cooperative child to establish the diagnosis accurately, even in mild asthma. Forced Expiratory Volume in One Second (FEV_1) is the amount of air that is forcefully exhaled in the first second of the FVC test. In general, it is common that healthy individuals are able to expell 75%-80 % of their vital capacity in the first second of the FVC test. Hence, FEV_1 has a pulmonary function value that is highly diagnostic in obstructive disease, but FEV_1 only cannot be used to diagnose obstructive and restrictive disorders all by itself. If the patient demonstrates a reduced FEV_1, the patient may repeat the test after inhaling a bronchodilator. Bronchodilator dilates the bronchial passages and reduces airflow obstruction. Post-bronchodilator test often shows a 10%-15% FEV_1 improvement. This simple clinical test of FEV_1 reversibility, strongly suggests that the low FEV1 is due to obstructive phenomenon. If FEV_1 does not change, it suggests that the low FVC is possibly low due to restrictive pathologies. It is expressed as liters. FEV_1 reversibility is accounted from the following formula:

It is a sensitive parameter to estimate the extent

$$\frac{FEV_1 \text{ after bronchodilator} - FEV_1 \text{ before bronchodilator} \times 100\%}{FEV_1}$$

of airflow obstruction and to monitor the result of therapy. This variable showed a progressive result in both intervention.[5]

FEV_1 reversibility in this study decreased significantly. The most marked decrease of FEV_1 reversibility and symptom score were found in SLIT group. Decreased FEV_1 in this group was similar with that of

the study by Bosquet *et al* in 85 children with asthma given SLIT every day for 4 weeks, continued three times a week for 24 months.[6] The similar significant decrease in FEV_1 reversibility in groups of probiotics only and combination of SLIT and probiotics shown in this study had not been noticed in other studies. Therefore this study gave new information that a combination of SLIT and probiotics was also able to improve clinical outcomes in childhood asthma.

The reduction of asthma symptoms (asthma score and medication score) were closely related to the reduction of the degree of bronchoconstriction. It can be measured more accurately by lung-function test. The recommendations of the National Asthma Education Program indicate that such test is essential in the diagnosis and management of asthma because of evidence that both patients and physicians have inaccurate perceptions of the severity of asthma that contribute to delays in treatment. Indeed, underestimation of the extent of airflow (airway) obstruction is associated with increased mortality in asthma. Physicians cannot identify obstructive or restrictive patterns reliably from history taking and physical examination only. Lung function test can be used to predict the possibility of the presence of obstructive pattern by 83% of the time. However, predictions of normal or restrictive patterns are correct only by about half of the time. Besides identifying abnormalities, lung function tests allow the severity of an abnormality to be quantified and the presence of reversible airflow obstruction to be determined.[7,8]

All groups had significant decrease in symptom score ($P<0.05$). SLIT group showed the most marked decrease of symptom score compared to that of other groups, although statistically insignificant. The similar influences of sublingual immunotherapy on clinical symptoms of allergy diseases were formed by 12 previous studies. All these studies described a decrease in asthma symptom score in patients with sublingual immunotherapy.[9-11] Probiotics administration in allergy asthmatic children had never been studied before. A study by Rosenfeldt *et al*[12] and Viljanen *et al*[13] in children with atopic dermatitis showed that probiotics was able to decrease clinical symptoms in accordance to SCORAD criteria.[12,13] Another study by Wang14 in allergic rhinitis patients who were given probiotics (L. paracasei) showed a significant decrease of clinical symptoms.

The potential of mucosal approach via gastrointestinal tract in modulation of broad spectrum systemic hyporesponsiveness has been increasing to modify natural course of allergic disease.[15] Gastrointestinal tract is the largest immunologic organ in the body. It is lined by a single layer of epithelium. It is constantly exposed by large amounts of dietary proteins and other antigens. Despite the large extent of dietary antigenic exposure, only small percentage of individuals has allergic reaction. This is due to development of oral tolerance to antigenic proteins.16 Oral tolerance, refers to a state of active inhibition of immune response to an antigen by means of prior expose of that antigen through oral route. Oral delivery is effective in inducing both systemic and generalized mucosal immune responses. This property has been used to develop antigen-specific therapy in allergy.[4] The exact mechanisms of induction of oral tolerance are still under debate.[17] Recently, the World Health Organization has reported that sublingual-swallow therapy shows evidence of clinical efficacy in the treatment of respiratory allergies. It has been demonstrated that in atopic patients, the allergen can cross the gastrointestinal mucosa, leading to a desensitization of the immune system.[1]

Result of our study indicated clinical improvement of asthma score, medication score, and lung function test described by FEV_1 reversibility. Statistical analysis of efficacy between all groups revealed insignificant differences which meant that sublingual immunotherapy, combination of sublingual immunotherapy and probiotics, or probiotics only had a similar efficacy on the improvement of clinical symptoms of childhood asthma.

Differences in medication score between all study groups were statistically insignificant ($P=0.308$). Several studies about the efficacy of sublingual immunotherapy had been conducted since 1998. A 2-year study by Lilja *et al*[18] on 6-14 years old children with allergic rhinoconjunctivitis due to Parietaria judaica showed that more than 85% subjects experienced improving symptoms compared to only 50% in control group with 30% decreasing symptom score. No significant decrease in medication score was found in both groups. Another study by Bousquet *et al*[6] in allergic asthma children with sublingual immunotherapy revealed insignificant differences between groups in month 11 or 25 for all medications (inhalation cor-

ticosteroid, β-antagonist, anticholinergic, cromones and systemic corticosteroid).1 Several other found also similar results.[19-20]

Medication and symptom scores are useful clinical parameters to assess the clinical efficacy of immunotherapy aside from other immunological parameters such as cell markers and cytokine profiles. Assessment of immunotherapy can be done using objective and subjective parameters. Although objective assessment is more preferable, often it is not practical to be used routinely in clinical setting. Therefore, most experts trust subjective assessment by clinicians, including all complaints of patients about their allergy symptoms.[21]

Several studies showed that sublingual immunotherapy and probiotics are able to decrease clinical symptoms of allergy and asthma.[17,22] Incomplete explanations of its mechanism made the decrease was only seen as a subjective parameter. Many researchers had tried to explain the mechanism of decreasing allergy reactions only through total IgE and IgG, whereas IgA is the most important factor due to its role on mucosal immune system. Previous studies showed that probiotics increased IL-10 and TGF-β as transcriptors for IgA.[23] A study on experimental animals showed that TGF-β, IL-10, serum IgA, mucosal IgA increased after probiotics administration.[24] These studies consistently showed improvements of allergic clinical symptoms, although no decrease in total IgE was found.

To conclude, our study with small number of participants shows that combination of SLIT and probiotics and probiotics only were similarly effective to improve clinical symptoms of childhood asthma. Further studies, preferably with double blind design are needed to establsh a more definitive conclusion.

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