

# Clinical Efficacy of Combined Probiotics and Immunotherapy in Childhood Allergic Rhinitis

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Sublingual immunotherapy (SLIT) is considered a safe and beneficial treatment for allergic rhinitis (AR). Probiotics are unusual treatment options for AR and have lately created great concern in the scientific community. The aim of this study was to investigate the efficacy of combined probiotics plus SLIT on nasal symptoms in AR children. In this study, the SLIT only group (n = 15) received SLIT for 6 months, combined treatment group (n =15) received probiotics plus SLIT for 2 months and then SLIT for another 4 months. After 6 months of therapy, all symptoms of AR were significantly improved in both groups. The nasal obstruction was significantly resolved in both groups,  $p < 0.001$ . Total nasal symptom score (TNSS) was significantly decreased after treatment in both groups ( $p < 0.001$ ). However, a significant difference was observed between the percent change in TNSS between both groups ( $P = 0.031$ ). Also statistically significant difference was found between the studied groups regarding decrease use of anti-allergic medications ( $P = 0.034$ ). In Conclusion: combined probiotics plus SLIT showed efficacy in improving symptoms of AR in children.

Allergic rhinitis (AR) is a kind of inflammation in the nose that occurs when the immune system overreacts to allergens in the air. The distinctive symptoms of allergic rhinitis are: rhinorrhea, nasal itching, sneezing, nasal congestion and obstruction [1]. AR currently affects up to 40% of the population worldwide [2]. There is no apparent sex selectivity [3]. In susceptible individuals, exposure to certain foreign proteins in can lead to allergic sensitization. Allergen stimulates Th2 proliferation in allergic subjects that leads to the release of their specific set of cytokines including IL-3, IL-4, IL-5, IL-9, IL-10, and IL-13. These cytokines stimulate IgE release and mast cell production [4].

Allergen immunotherapy is the practice of repeated administration of specific allergens to a patient with an IgE-mediated condition to give safety against the allergic symptoms and inflammatory reactions that

occur on natural exposure to these antigens [5].

Sublingual immunotherapy (SLIT) does not need injections and has a much lower risk of adverse systemic allergic reactions compared with subcutaneous immunotherapy (SCIT). Also, self-administration of SLIT is an easy practice by patients [6]. When we give allergen extracts by the sublingual route, they are primarily taken up by dendritic cells in the mucosa and presented to T cells in the draining lymph nodes. Regulatory T cells (Tregs) activation and downregulation of mucosal mast cells occur. Within the oral and sublingual mucosa, effector cells, such as mast cells, are less abundant. This distinguishing character of the oral mucosa is an essential factor in the lower rates of anaphylaxis seen with SLIT [7].

Probiotics are dietary supplements that contain useful bacteria such as *Lactobacillus GG* (LGG) and are effective in protection

against early childhood atopy through the modification of gastrointestinal flora [8]. Oral administration of probiotics can reduce allergen specific IgE production and modify systemic cytokine production in animal models of asthma (9). Certain probiotics such as *Bifidobacterium lactis* and *Lactobacillus reuteri* have been found to reduce airway hyper-responsiveness and inflammation by promoting regulatory mechanisms [9].

Probiotics encourage the steadiness of Th1 and Th2 immune responses, and promotes Tregs to prevent excessive Th1 and Th2 reactivity. It also can modify the molecules and immune response in the gut mucosae [10]. Probiotic supplementation was found to have a benefit in reducing nasal eosinophil percentages in allergic rhinitis children [11].

In this study, we aim to evaluate the efficacy of combined probiotics plus SLIT on the clinical condition of children with AR to pollen.

## Study design and Subjects

This randomized controlled clinical trial was carried out in the Allergy and Immunology Unit, Medical Microbiology and Immunology Department and Otorhinolaryngology and Head and Neck Surgery, Faculty of Medicine, Zagazig University from June 2018 to December 2018.

Thirty children aged 5-18 years with established AR and positive skin test to pollen were included and randomly divided into 2 groups. Group A included 15 children who received SLIT only. Group B included 15 children who received probiotics plus SLIT.

We excluded children with active upper respiratory tract, infection within 1 month before the study, patients with any previous immunotherapy, patients who have clinically significant diseases or malignancies and chronic treatment with systemic corticosteroids or immune suppressive drugs.

## Materials and Methods

The study protocol was reviewed and approved by the Ethics Committee of Zagazig University Hospitals (3894/13-8-2017). Both a verbal assent from children and an informed consent from each participant's parents were taken after explaining the purpose of the study.

All patients were subjected to the following:

-Complete history taking.

-Total nasal symptom score (TNSS) was measured at the start of the study, after 2 months and after 6 months. TNSS is the sum of scores for each of nasal obstruction, sneezing, nasal itching, and rhinorrhea at each time point, using a four point scale (0–3), where 0 indicates no symptoms, a score of 1 for mild symptoms that are easily tolerated, 2 for awareness of symptoms which are bothersome but tolerable and 3 for severe symptoms that are hard to tolerate and interfere with daily activity TNSS is calculated by adding the score for each of the symptoms to a total out of 12 [12].

-SLIT: The patients of both groups received SLIT. Coca's extracted antigens were prepared as aqueous solution in a concentration of 1/10 Wt/Vol. A potency of 1:10 indicates that 1g of dry allergen (date palm pollen) was added to 10 cc of a buffer for extraction. The allergen was eluted for a time, and then the solid material was filtered out, leaving an aqueous solution [13]. Dilutions were prepared using Glycerin 50% (Al-Gomhorria Medical Industries Company, Egypt) in 20 ml sample bottles with glassy droppers which were prepared under complete aseptic conditions under the laminar flow at the Allergy & Immunology Unit, Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University. The patients were given 3 different concentrations, each was identified according to the guidelines from (American Academy for Allergy, Asthma and Immunology (AAAAI) by using different colors [14] as in Table 1.

### Route of administration

Allergen immunotherapy extract was given as sublingual drops which were kept under the tongue for two minutes and then swallowed. The sublingual drops were administered in the morning on an empty stomach [15].

Probiotics: a drug, lacteol fort (Rameda Pharmaceutical Company, Egypt), was given to subjects in group B as a sachet once daily on empty

stomach for 2 months. Each sachet contained 10 billion lactobacillus acidophilus.

#### Statistical Analysis

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20. Quantitative variables were described using their means and standard deviations. Categorical variables were described using their absolute frequencies and to compare the proportion of categorical data, chi square test was used when appropriate. To compare means of two groups, independent sample t test (used with normally distributed data) was used to compare means of two groups or Mann Whitney test (for non-parametric data). To compare change at two points of time within the same group, paired sample t test (for normally distributed data) and Wilcoxon signed rank test (for non-parametric data) were used. The receiver operating characteristic (ROC) curve was used to determine best cutoff of the studied parameters in diagnosis of certain health problem. The level statistical significance was set at 5% ( $P < 0.05$ ).

Table 1. SLIT dilutions and labeling colors.

Dilutions	Labeling Color
1/200 W/V	RED [Starting dose]
1/100 W/V	YELLOW
1/50 W/V	GREEN [Maintenance dose]

Table 2. Comparison between treatment of the studied groups regarding total nasal symptom score (TNSS) before, and 2 months and 6 months after treatment.

TNSS	Sublingual immunotherapy only group	Combined probiotic and sublingual immunotherapy group	P value
	N=15	N=15	
Baseline:			
Mean $\pm$ SD	8.733 $\pm$ 1.87	8.667 $\pm$ 1.175	NS
Range	4 – 12	7 - 11	
After 2 months:			
Mean $\pm$ SD	7.73 $\pm$ 1.75	7.6 $\pm$ 0.99	NS
Range	3 – 10	6 – 9	
After 6 months:			
Mean $\pm$ SD	6.867 $\pm$ 1.642	6.333 $\pm$ 0.817	NS
Range	1 – 6	3 – 6	
P value	<0.001**	<0.001**	

Paired t Paired t test t Independent sample t test  
 $P > 0.05$  in not significant (NS)

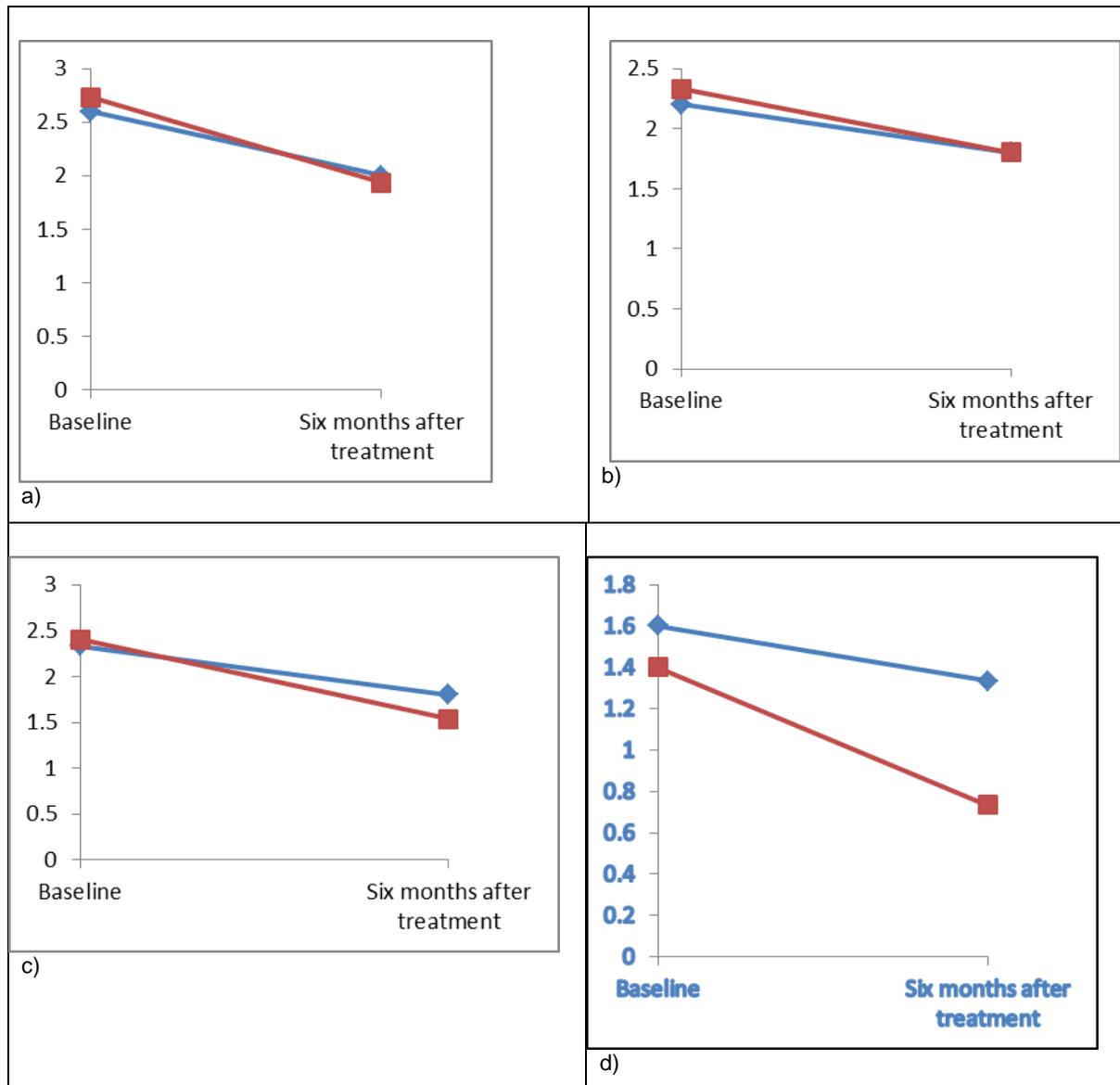
## Result

### Demographic characteristics

In the present study, the mean age in the SLIT group (group A) was  $9.6 \pm 3.38$  compared to  $9.27 \pm 2.94$  in combined treatment group (group B). In addition, females represent 60% of the subjects in group A while 53.3% in group B. There was no difference between the studied groups as regard to demographic characteristics.

### Clinical effects

All the nasal symptoms were significantly improved after treatment in both groups, with significant change in nasal obstruction  $P \leq 0.001$ . There were significant improvements in rhinorrhea, sneezing and nasal itching severity  $P < 0.05$  after 6 months of therapy in both groups (Figure 1). There was no difference between the studied groups regarding TNSS at 2 months and 6 months after treatment. A statistically significant change in TNSS was observed in each group over time ( $P < 0.05$ ) (Table 2).



—◆— sublingual immunotherapy only group,  
 —■— combined probiotic and sublingual immunotherapy group

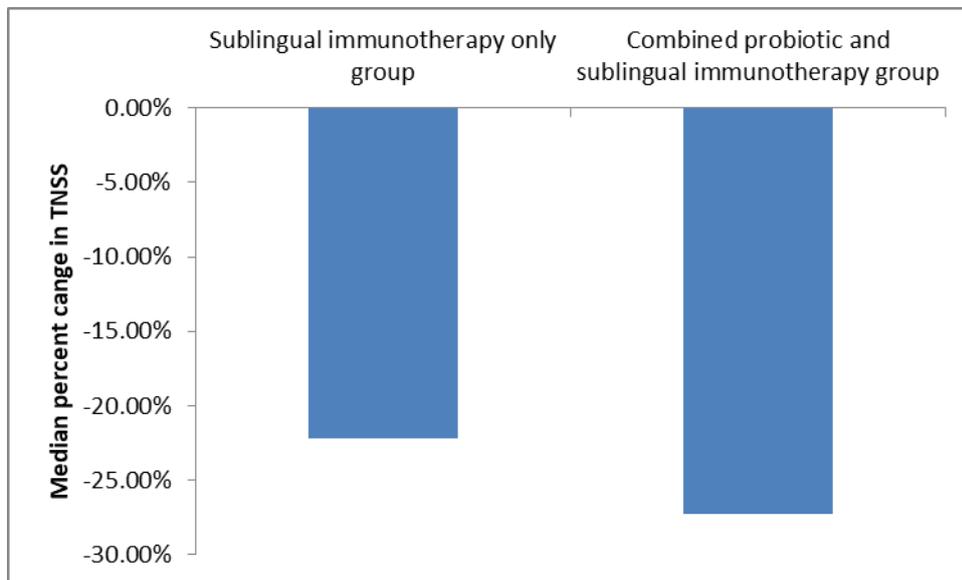
**Figure 1.** Line graphs showing comparison between treatment of the studied groups regarding a) nasal obstruction, b) sneezing, c) rhinorrhea, d) nasal itching baseline and six months after treatment

**Change of TNSS**

The percent change of TNSS was higher in the group with combined treatment (group B) compared to the group with single treatment (group A); (27.27% in group B versus 22.22% in group A) with significant difference between the studied groups ( $P=0.031$ ) (Figure 2).

**Anti-allergic medications use at six months**

The reduction in use of anti-allergic medications in group A (46.7%), after six months, was significantly lower than that in group B (86.7%),  $P=0.034$  (Table 3).



**Figure 2.** Bar chart showing comparison between treatment of the studied groups regarding percent change in TNSS.

**Table 3.** Comparison between the studied groups regarding anti-allergic medications at 6 months

	Sublingual immunotherapy only group N=15 (%)	Combined probiotic and sublingual immunotherapy group N=15 (%)	P value
Antiallergic drugs:			
Decrease use	7 (46.7)	13 (86.7)	0.034*
Increase use	4 (26.7)	1 (6.7)	
similar use	4 (26.7)	1 (6.7)	

$P>0.05$  is significant

## Discussion

The present study investigated the efficacy of SLIT and combined probiotics plus SLIT on the clinical condition of children with AR and positive skin test to pollen. We observed a statistically significant decrease in severity of all symptoms of AR in combined treatment group as nasal obstruction, rhinorrhea, sneezing and nasal itching significantly improved. There was also statistically significant change in TNSS 6 months after combined treatment. These results agree with a study carried out by Rossi *et al.*, 2016 [16], reported that patients who were administered SLIT plus probiotics showed a significant improvement of TNSS.

Other researchers reported comparable findings with different probiotic strains that have parallel or even more distinct features. By the way, Ouwehand *et al.*, 2009 [17] used a mixture of *Lactobacillus acidophilus* NCFM (ATCC 700396) and *Bifidobacterium lactis* Bl-04 (ATCC SD5219) and showed that the administration of probiotics is valuable in avoiding the pollen-induced infiltration of eosinophils into the nasal mucosa, and found a tendency toward a decrease of nasal symptoms.

Also, Manzotti *et al.*, 2014 [18] used *Lactobacillus acidophilus* NCFM/ *Bifidobacterium lactis* BL-04 / fructooligosaccharide. After the treatment with two multi-strain symbiotic preparations, a significant decrease of TNSS and a reduction in consumption of orally administered corticosteroids and antihistaminic drugs. Lin *et al.*, 2012 [19] found that a marked reduction of the symptom scores was observed during treatment with *Lactobacillus salivarius*, with differences in anti-allergic drug intake in patients.

In our study there was no difference in TNSS 6 months after therapy between SLIT group and combined treatment group. This observation could be due to the small size of the study population. However, there was statistically significant difference between the studied groups regarding the percent change in TNSS ( $P= 0.031$ ) and it was higher in combined treatment group. This finding agrees with that of a study conducted by Rossi *et al.*, 2016 [15], found that both groups (SLIT group and combined treatment group) showed a marked improvement of TNSS during the study period, with the combined treatment group showing a tendency to an even better improvement with respect to the SLIT group (71.4% versus 66.7%, without statistically significant difference).

Also agrees with a study conducted by Irmawati *et al.*, 2008 [20] who tested the treatment efficacy in asthma patients and indicated clinical improvement of asthma score, medication score, and lung function test after combined treatment. Statistical analysis of efficacy between SLIT and combined treatment groups revealed insignificant differences.

In this study there was statistically significant difference between the studied groups regarding decrease use of anti-allergic medications ( $P<0.05$ ). These results agree with Rossi *et al.*, [15] who found that patients belonging to the combined treatment group had a significant reduction of the medication score (-32.6%) versus SLIT group ( $P = 0.02$ ).

In conclusion, combined treatment with probiotics and SLIT may be beneficial in children with AR and positive skin test for pollen.

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