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A trial of adding *Lactobacillus johnsonii* EM1 to levocetirizine for treatment of perennial allergic rhinitis in children aged 7–12 years

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ABSTRACT

Background: Supplementary consumption of probiotics may temporarily alter the intestinal microflora of infants and children, thereby preventing and treating allergic disorders.

Objective: To compare the clinical efficacy of levocetirizine with that of levocetirizine plus *Lactobacillus johnsonii* EM1 (*Lj* EM1) for treating perennial allergic rhinitis (PAR) in children.

Methods: Sixty-three children aged 7–12 years fulfilled the entry criteria for the study and had moderate to severe PAR of at least 1 year's duration. The treatment followed a randomized, open-label crossover design: all subjects were randomized to 2 crossover treatment regimens of levocetirizine with *Lj* EM1 (group 1) or levocetirizine alone (group 2) for 12 weeks; subsequently, treatments were reversed for a further 12 weeks. The effects of the 2 regimens were compared using the Pediatric Rhinoconjunctivitis Quality of Life Questionnaire (PRQLQ) and the total symptom score (TSS) from diary cards. The parameters evaluated were nasal peak expiratory flow rate (nPEFR), FVC, FEV1, serum immunoglobulin E (IgE), mite-specific IgE, eosinophilic cationic protein (ECP), resistin, blood eosinophils, eosinophil percentage in nasal smears, IL-4, IL-10, interferon- γ (IFN- γ), and transforming growth factor- β (TGF- β). **Results:** After the first 12 weeks of treatment, TSS in both groups had improved progressively compared with that in the run-in period. Both groups had improved TSS at weeks 4, 8, and 12 ($P < 0.05$), and group 1 was more efficacious than group 2 at week 4 ($P = 0.014$), week 8 ($P = 0.011$), and week 12 ($P < 0.009$). During the second 12-week period, group 2 showed continual and progressive improvement, while group 1 did not. The PRQLQ scores were significantly decreased in both groups ($P < 0.05$), but there was no statistically significant difference between the 2 groups ($P = 0.446$). The eosinophil percentage in nasal smears decreased in both groups compared with that in the run-in period, and significant differences were detected in groups 2 and 1 at 16 and 24 weeks of treatment, respectively ($P < 0.05$). Both groups showed significant improvement in nPEFR at weeks 4, 8, 12, 16, and 24 ($P < 0.01$), and the treatment for group 1 appeared to be more efficacious than that for group 2 at weeks 12, 16, and 20 ($P < 0.05$). FVC and FEV1 were improved in both groups at weeks 8 through 24 ($P < 0.05$), but there was no significant difference between the 2 groups. In cytokine measurements, IFN- γ and IL-10 increased significantly and IL-4 decreased significantly in both groups, while elevation of TGF- β was seen only in group 1 at 12 weeks ($P < 0.001$). However, the difference in TGF- β disappeared after 24 weeks treatment. There was no difference in serum resistin levels. No serious adverse events were recorded in either treatment group. **Conclusion:** The 24-week, 2-phase, crossover treatment program showed that levocetirizine plus *Lj* EM1 was more effective for PAR than levocetirizine and that this difference persisted for at least 3 months after discontinuation of *Lj* EM1.

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1. Introduction

Allergic rhinitis (AR) is one of the most common allergic diseases and affects between 5% and 50% of the world's population [1]. According to current estimates, the incidence of AR is about 20–30% [2], and the prevalence of physician-diagnosed and self-diagnosed AR may be higher than 50% in Taiwanese children [3].

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The disease is chronic and persists for life if untreated and may result in the impact of symptoms of AR on sleep, quality of life, cognitive function, and school/workplace productivity [4], there are high costs related to the disorder [5].

Histamine is the most well-known allergy mediator and has been shown to cause AR symptoms. Non-sedating antihistamines have become the standard first-line therapy for treatment of AR. Among them, levocetirizine is the R-enantiomer and active form of cetirizine [6]. It has a smaller volume of distribution than cetirizine, which confers improved safety because of low passage through the hematoencephalic barrier and low cerebral receptor binding [7]. The exclusion of the S-enantiomer leads not only to enhanced peripheral receptor binding compared with that of cetirizine but also to improved specificity for the H1 receptor [8].

Probiotics may bias toward a T helper cell type 1 (Th1) response and predominance of regulatory T cells (Tr) by changing the composition of the host's intestinal microflora through direct or indirect action on gut-associated lymphoid tissue (GALT) [9]. Previous *in vitro* studies have suggested that *Lactobacillus* strains might modulate the balance of Th1/Th2 response in the immune system [10]. Several *Lactobacillus* strains including *L. casei* strain Shirota, *L. plantarum* L137, and *L. acidophilus* strain L-92 are used as probiotics and have been reported to modify the levels of antigen-specific serum immunoglobulin E (IgE) in animal models [11–13]. From the review, Michail mentioned that it is likely that in the practice other factors play a more important role and that the paradigm Th1/Th2 is only one part of the problem to explain the allergic cascade [14]. In contrast, only a few studies have examined the effects of probiotics on allergic airway diseases such as rhinoconjunctivitis and asthma, and the results of these studies have been controversial [15–18]. In recent report showed that probiotic supplementation can protect infants in childcare against episodes of diarrheal illness and fever, but not against respiratory illness [19]. Further, *L. rhamnosus* GG (LGG) did not alleviate symptoms in patients with birch pollen allergy [20]. However, it has been shown that *L. paracasei* might improve the quality of life of adolescents with PAR [15,16], and others studied the effects of long-term consumption of probiotic milk and suggested that lactobacilli could be beneficial for the respiratory tract [21,22]. Vliagoftis et al. studied from the review of the literature that probiotics may have a beneficial effect in AR by reducing symptom severity and medication use [23]. However the data addressing the effect of probiotics in AR is even less robust [14].

Supplementary consumption of *Lactobacillus johnsonii* EM1 (*Lj* EM1) has been suggested to temporarily alter the intestinal microflora of infants and children, and thus may be beneficial in treating or preventing allergic disorders, including those of the gastrointestinal tract [24,25]. There is only limited information on treating AR with levocetirizine combined with the probiotic *Lj* EM1. Therefore, this clinical trial was designed to assess the efficacy of *Lj* EM1 as a supplement to levocetirizine in treatment of AR. No previous studies have used this approach in children aged 7–12 years old. In this study, we aim to evaluate the clinical efficacy and safety of levocetirizine combined with *Lj* EM1 in children aged 7–12 years old with PAR.

2. Materials and methods

2.1. Patients

Of the 65 patients screened, 63 patients aged between 7 and 12 years fulfilled the inclusion criteria for the study, which included moderate-to-severe PAR [according to the Allergic Rhinitis and its Impact on Asthma (ARIA) classification] of at least 1-year duration. All subjects were allergic to the house dust mite, which was confirmed by a skin prick-test response and a positive

reaction to mite-specific IgE (Pharmacia, Uppsala, Sweden). Exclusion criteria were any of the following: deformities of the ear, nose, or throat; infection during the 2 weeks preceding the initial visit; consumption over the previous 7 days of medication such as antihistamine and decongestant that may have affected any allergy symptoms; consumption of any form of steroid or sodium cromoglycate within the 4 weeks prior to the study. Participants were excluded if they had a respiratory tract infection or received any of the above medications during the study period. A washout period of 7 days was required for patients taking leukotriene antagonists. The study was approved by Chung Shan Medical University Hospital Institutional Review Board. Written parental informed consent was obtained before commencing the study.

2.2. Study design

In the initial screening visit, a comprehensive medical and allergy history was obtained for all participants, and they were administered skin prick tests. Daily activity diaries were given to participants with instructions to record all symptoms every day starting 7 days before treatment. Medication histories were reviewed to ensure that participants had not taken an H1-antagonist, decongestant, or any form of steroid during the preceding 7 days.

The trial used a randomized, open-label crossover design, where 65 patients were screened at visit 1. At visit 2, 63 patients met the study criteria, and all subjects were randomized to a 2-arm crossover treatment regimen, whereby each was administered levocetirizine or levocetirizine plus *Lj* EM1 (TTY Biopharm Com. Taiwan) for 12 weeks, then vice versa for a further 12 weeks. For the first 12 weeks, 32 subjects (group 1) took levocetirizine (5 mg) combined with *Lj* EM1 (1×10^{10} cfu/capsule), and 31 subjects (group 2) took levocetirizine alone (5 mg). The assigned medication was taken once daily at bedtime for 12 weeks, beginning after the baseline visit. All patients were followed up every 4 weeks during the first 12-week period and during the 12 weeks after crossover to the second treatment arm. Corren reported that patients with AR and no clinical evidence of asthma commonly exhibit nonspecific bronchial hyperresponsiveness [26]. At each visit, the subjects' activity diaries were reviewed, and a physical examination, FVC, FEV1, nPEFR, and nasal smear were conducted. In addition, levels of peripheral blood eosinophils and total serum IgE, mite-specific IgE, ECP, resistin, IFN- γ , IL-4, IL-10, and TGF- β were evaluated at the baseline, 12-week, and 24-week visits. During the study, guardians were requested to complete the PRQLQ. The effects of the 2 regimens were then compared between the 2 groups.

2.3. Daily diary of total symptom score and sleep quality

A diary card was given to each patient 7 days before the start of the treatment. Guardians and patients were instructed to record the patient's daily symptoms, which included 4 nasal symptoms (nasal itching, sneezing, rhinorrhea, and nasal congestion) and 5 non-nasal symptoms (throat itching, coughing, conjunctival itching, conjunctival hyperemia, and eye tearing). Symptoms were evaluated using a 4-point scale as follows: 0, none (symptom not noticeable); 1, mild (symptom noticeable but not bothersome); 2, moderate (symptom noticeable and bothersome some of the time); and 3, severe (symptom bothersome most of the time and/or very bothersome some of the time). The total symptom score (TSS) was the sum of the 9 recorded symptom scores. The baseline TSS was calculated as the mean of the daily scores during the baseline study period of 7 days. Subsequently, TSS was based on the scores for the preceding 28 days at the 4th, 8th, 12th, 16th, 20th, and 24th week after the treatment. We also incorporated sleep quality in the

diary card, in which symptoms were scored as follows: 0, slept well and did not wake up; 1, did not sleep too well or woke up once; 2, slept poorly or woke up 2–3 times; 3, slept very poorly or woke up more than 3 times.

2.4. Pediatric Rhinoconjunctivitis Quality of Life

The Pediatric Rhinoconjunctivitis Quality of Life (PRQLQ) has 23 items in 5 areas: nasal symptoms, ocular symptoms, practical problems, other symptoms, and activity limitations. Each item is scored using a 7-point scale from 0 to 6 [27]. The questionnaire could be completed within 10 min by children. Some of the younger children recorded the scores with the help of their parents.

2.5. Nasal peak expiratory flow rate

Nasal peak expiratory flow rate (nPEFR) was performed with a Mini-Wright peak expiratory flow meter. The meter was equipped with a well-sealed purpose-built facemask that was placed on the patient during tests. Patients were sitting and were instructed to inspire deeply and then blow the nose forcefully with the mouth firmly closed. This test was performed 3 times and the highest value recorded [28].

2.6. Nasal smear

The underside of the patient's inferior turbinate was rubbed with a cotton swab, and the collected cells were smeared on a glass slide. The slides were stained with Leu stain and examined with a light microscope. An experienced cytologist, who was blinded to the subjects' clinical status, performed this assay. The nasal smear was evaluated by counting a minimum of 100 leukocytes, and the eosinophil count was expressed as a proportion of the total number of leukocytes.

2.7. Peripheral blood eosinophils, total serum IgE, mite-specific IgE, ECP, resistin, IL4, IL-10, IFN- γ , and TGF- β

Total serum IgE, ECP, and mite-specific IgE were measured by fluoroimmunoassay using the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden). Peripheral blood eosinophils were counted using a hemocytometer and were recorded at the baseline, 12-week, and 24-week visits. At the same visits, resistin, IL4, IL10, IFN- γ , and TGF- β levels were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kits that employ the quantitative sandwich enzyme immunoassay technique, according to the manufacturer's instructions (R&D Systems, Minneapolis, Minnesota, USA). Cytokine levels (pg/ml) were calculated from standard curves generated with recombinant cytokines. The differences between duplicate wells were consistently less than 10% of the mean values.

2.8. Statistical analysis

A standard PC with SPSS 12.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analyses. All data are expressed as the mean $\pm$ standard deviation. One-way analysis of variance, *t*-test, and paired *t*-test methods were used to compare the demographics and baseline characteristics between the 2 treatment groups. $P < 0.05$ was regarded as significant.

3. Results

Of the 65 patients screened, 63 patients aged 7–12 years fulfilled the criteria for the study and completed the study.

Table 1

Demographics and baseline characteristics of the treatment groups.

	Levocetirizine + Lj EM1 (Gr.1; n = 32)	Levocetirizine (Gr.2; n = 31)
Male/female	26/6	20/11
Age (yr)	8.82 $\pm$ 1.64	8.88 $\pm$ 1.54
Body weight (kg)	32.72 $\pm$ 9.94	32.68 $\pm$ 9.27
Height (cm)	133.37 $\pm$ 10.45	132.64 $\pm$ 10.50
TSS	13.64 $\pm$ 8.82	12.66 $\pm$ 7.52
PRQLQ	53.34 $\pm$ 33.94	55.48 $\pm$ 27.22
Serum IgE (IU/ml)	638.22 $\pm$ 898.54	770.52 $\pm$ 983.29
ECP level (ng/L)	50.22 $\pm$ 38.82	56.58 $\pm$ 43.72
Blood eosinophil counts (per μ l)	519.22 $\pm$ 365.22	435.87 $\pm$ 242.10
Cytokine (pg/ml)		
IL-4	578.14 $\pm$ 217.52	498.74 $\pm$ 202.17
IL-10	83.73 $\pm$ 28.62	85.74 $\pm$ 28.75
IFN- γ	27.44 $\pm$ 4.75	28.11 $\pm$ 6.30
TGF- β	2.51 $\pm$ 0.66	2.64 $\pm$ 0.72
Resistin	8.20 $\pm$ 3.88	8.21 $\pm$ 4.27
nPEFR	70.25 $\pm$ 12.55	68.74 $\pm$ 8.22
Eosinophil % in nasal smear	44.34 $\pm$ 28.72	38.34 $\pm$ 34.72
FEV1 (%)	97.48 $\pm$ 12.22	97.12 $\pm$ 14.44
FVC (%)	88.32 $\pm$ 12.11	89.22 $\pm$ 12.22

Values are mean $\pm$ standard deviation; Lj EM1: *Lactobacillus johnsonii* EM1; Gr: group; n: number; TSS: total symptom score; PRQLQ: Pediatric Rhinoconjunctivitis Quality of Life; ECP: eosinophil cationic protein; nPEFR: nasal peak expiratory flow rate; FEV1: forced expiratory volume at 1 s; FVC: forced vital capacity.

Thirty-two patients assigned to treatment group 1 took levocetirizine (5 mg) combined with Lj EM1 (1×10^{10} cfu/capsule) daily, and 31 patients assigned to group 2 took levocetirizine (5 mg daily). Six of the original patients were excluded from the study because their TSS recordings had not been completed during the treatment period (2 in group 1; 4 in group 2). There were no significant differences in baseline demographics and characteristics between the patients of the 2 groups, including age, sex, height, body weight, and disease characteristics in terms of total IgE, total eosinophil count, ECP, resistin, IFN- γ , IL-4, IL-10 and TGF- β , eosinophil proportion of nasal smear, nPEFR, and TSS (Table 1).

Table 2 shows that TSS in both groups had improved progressively after the first 12 weeks of treatment compared with that in the run-in period. Both groups improved at weeks 4, 8, and 12 ($P < 0.05$), and treatment for group 1 was more efficacious than that for group 2 at week 4 ($P = 0.014$), week 8 ($P = 0.011$), and week 12 ($P < 0.009$). During the second 12-week period, group 2 improved continually and progressively, while group 1 did not (Table 2 and Fig. 1).

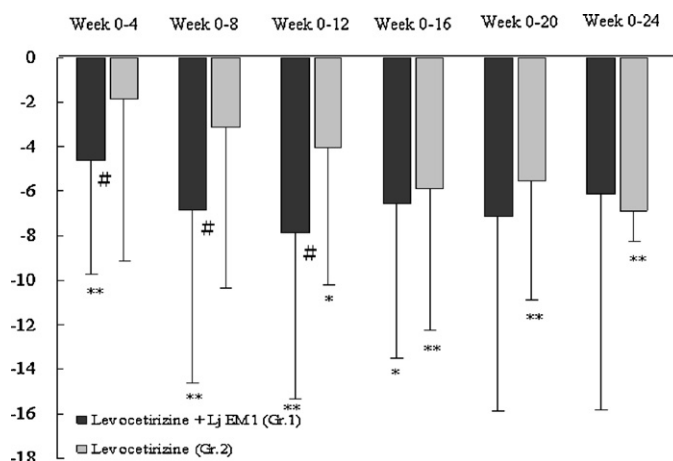


Fig. 1. Change from baseline in mean total symptom score. Significant change compared with baseline in both groups (* $P < 0.05$, ** $P < 0.001$). Gr. 1 significantly reduced TSS scores compared with Gr.2 at weeks 4, 8, and 12 (# $P < 0.05$).

Table 2

Change from baseline.

	Levocetirizine + Lj EM1 (Gr.1, n = 30)	Levocetirizine (Gr.2, n = 27)	P-value [®]
TSS			
Day 4 w	−5.73 ± 4.97**	−1.63 ± 7.17	0.014*
8 w	−7.73 ± 6.85**	−2.81 ± 7.16	0.011*
12 w	−8.45 ± 7.10**	−3.78 ± 5.83*	0.009*
16 w	−6.54 ± 6.99	−5.87 ± 6.40**	0.740
20 w	−7.18 ± 8.71*	−5.51 ± 5.39	0.433
24 w	−6.13 ± 9.72	−6.87 ± 6.36**	0.767
PRQLQ			
Day 4 w	−14.53 ± 21.16*	−13.19 ± 18.15*	0.798
8 w	−21.00 ± 29.41*	−18.37 ± 21.41**	0.704
12 w	−27.40 ± 28.44**	−24.33 ± 17.58**	0.623
16 w	−29.15 ± 22.58**	−25.28 ± 16.35**	0.509
20 w	−25.90 ± 19.94**	−32.28 ± 19.07**	0.281
24 w	−23.95 ± 22.74**	−28.76 ± 19.23**	0.446
Serum IgE (IU/ml)			
Day 12 w	14.25 ± 216.42	−8.04 ± 197.89	0.721
24 w	117.95 ± 695.98	−61.64 ± 300.53	0.251
ECP (ng/L)			
Day 12 w	−3.63 ± 20.21	−6.06 ± 39.43	0.791
24 w	19.17 ± 98.99	−12.31 ± 46.62	0.202
Blood eosinophil count			
Day 12 w	−28.00 ± 226.47	87.20 ± 398.03	0.256
24 w	−55.00 ± 350.93	22.40 ± 252.86	0.395
Cytokine (pg/ml)			
IL-4			
Day 12 w	−287.17 ± 205.22**	−309.84 ± 239.89**	0.710
24 w	−297.84 ± 253.54**	−261.54 ± 223.09**	0.580
IL-10			
Day 12 w	210.03 ± 65.24**	217.08 ± 105.02**	0.767
24 w	225.05 ± 100.61**	169.09 ± 56.07**	0.015*
IFN-γ			
Day 12 w	26.99 ± 13.22**	22.20 ± 14.71**	0.214
24 w	16.30 ± 11.35**	14.96 ± 18.77**	0.751
TGF-β			
Day 12 w	0.51 ± 1.07*	−0.56 ± 1.89	0.012*
24 w	0.45 ± 1.03*	0.02 ± 1.44	0.213
Resistin (pg/ml)			
Day 12 w	−0.05 ± 2.67	−0.67 ± 3.14	0.509
24 w	−0.36 ± 2.20	−0.59 ± 1.74	0.711
nPEFR			
Day 4 w	5.17 ± 5.49**	3.33 ± 3.10**	0.123
8 w	10.67 ± 10.56**	6.30 ± 4.30**	0.044*
12 w	17.17 ± 10.88**	10.19 ± 4.90**	0.003*
16 w	18.25 ± 12.80**	11.00 ± 4.33**	0.024*
20 w	19.25 ± 11.61**	12.40 ± 4.36**	0.020*
24 w	19.75 ± 13.61**	14.60 ± 5.39**	0.124
Eosinophil % in nasal smear			
Day 4 w	1.19 ± 27.24	−10.61 ± 30.28	0.127
8 w	−7.54 ± 35.61	−9.67 ± 40.81	0.843
12 w	−11.62 ± 39.47	−16.49 ± 43.85	0.660
16 w	−13.06 ± 38.98	−19.87 ± 38.96*	0.563
20 w	−17.17 ± 42.91	−12.01 ± 36.34	0.665
24 w	−23.81 ± 31.50*	−13.04 ± 35.57	0.295
FEV1 (%)			
Day 4 w	1.55 ± 3.71*	2.70 ± 3.56*	0.237
8 w	2.92 ± 3.61**	3.13 ± 6.34*	0.875
12 w	2.52 ± 4.29*	3.67 ± 4.65**	0.336
16 w	3.68 ± 6.83*	3.94 ± 5.63*	0.887
20 w	4.93 ± 5.75*	5.96 ± 8.01*	0.629
24 w	5.58 ± 5.65**	6.82 ± 8.34**	0.571
FVC (%)			
Day 4 w	1.85 ± 3.73*	1.65 ± 4.86	0.860
8 w	3.73 ± 4.54**	3.02 ± 4.61*	0.558
12 w	3.44 ± 5.27*	4.30 ± 5.98*	0.569
16 w	6.22 ± 7.49*	3.74 ± 6.55*	0.243
20 w	7.67 ± 8.51*	6.32 ± 6.82**	0.556
24 w	7.75 ± 7.64**	7.30 ± 8.25**	0.852

Values are mean ± standard deviation; Lj EM1: *Lactobacillus johnsonii* EM1; TSS: total symptom score; PRQLQ: Pediatric Rhinoconjunctivitis Quality of Life; ECP: eosinophil cationic protein; nPEFR: nasal peak expiratory flow rate; FEV1: forced expiratory volume at one second; FVC: forced vital capacity; P-value[®]: treatment effect by 2-sample *t*-test.

* *P* < 0.05.** *P* < 0.001.**Table 3**

Change from baseline of individual symptom scores.

	Levocetirizine + Lj EM1 (Gr.1, n = 30)	Levocetirizine (Gr.2, n = 27)	P-value [®]
Rhinorrhea			
Day 4 w	−0.73 ± 0.98**	−0.43 ± 1.08	0.277
8 w	−0.72 ± 1.14*	−0.49 ± 1.34	0.457
12 w	−0.99 ± 1.32**	−0.49 ± 1.37	0.163
16 w	−0.66 ± 0.82*	−0.92 ± 1.17*	0.375
20 w	−0.98 ± 1.17*	−0.71 ± 1.52*	0.536
24 w	−0.85 ± 0.99*	−1.01 ± 1.51*	0.657
Nasal congestion			
Day 4 w	−0.84 ± 0.32*	−0.14 ± 1.42	0.060
8 w	−1.18 ± 1.45**	0.06 ± 1.85	0.007*
12 w	−1.25 ± 1.21**	−0.22 ± 1.54	0.007*
16 w	−0.75 ± 1.09*	−1.01 ± 1.30*	0.489
20 w	−0.81 ± 1.46*	−0.97 ± 1.01**	0.683
24 w	−0.84 ± 1.59*	−1.21 ± 1.25**	0.391
Nasal itching			
Day 4 w	−0.88 ± 1.00**	−0.52 ± 1.33	0.256
8 w	−1.27 ± 1.33**	−0.67 ± 1.27*	0.088
12 w	−1.27 ± 1.45**	−0.81 ± 1.33*	0.213
16 w	−0.90 ± 1.38*	−0.82 ± 1.44*	0.859
20 w	−0.65 ± 0.31	−0.87 ± 1.09*	0.554
24 w	−0.60 ± 0.33	−0.88 ± 1.49*	0.527
Sneezing			
Day 4 w	−0.81 ± 1.16*	−0.44 ± 1.18	0.246
8 w	−1.07 ± 1.32**	−0.71 ± 1.21*	0.299
12 w	−1.15 ± 1.65*	−0.80 ± 1.34*	0.395
16 w	−0.85 ± 1.10*	−1.14 ± 1.29**	0.421
20 w	−0.76 ± 1.36*	−1.03 ± 1.35**	0.497
24 w	−0.70 ± 1.65	−1.23 ± 1.49**	0.259
Throat itching			
Day 4 w	−0.43 ± 0.81*	−0.14 ± 0.72	0.159
8 w	−0.47 ± 0.62**	−0.14 ± 0.59	0.047*
12 w	−0.50 ± 0.82*	−0.25 ± 0.75	0.227
16 w	−0.46 ± 1.19	−0.28 ± 0.64*	0.510
20 w	−0.55 ± 1.12*	−0.26 ± 0.82	0.327
24 w	−0.43 ± 1.22	−0.50 ± 0.62*	0.807
Cough			
Day 4 w	−0.36 ± 1.06	−0.04 ± 1.29	0.309
8 w	−0.73 ± 1.04*	−0.43 ± 0.96*	0.272
12 w	−0.82 ± 1.27*	−0.28 ± 0.76	0.061
16 w	−0.67 ± 1.19*	−0.18 ± 1.42	0.230
20 w	−0.57 ± 1.16	−0.28 ± 0.87	0.331
24 w	−0.72 ± 1.07*	−0.51 ± 0.81*	0.465
Conjunctival itching			
Day 4 w	−0.66 ± 0.86**	0.06 ± 1.27	0.016*
8 w	−0.90 ± 1.22**	0.00 ± 1.06	0.004*
12 w	−0.91 ± 1.06**	−0.15 ± 0.90	0.006*
16 w	−1.10 ± 1.34*	−0.39 ± 1.13	0.062*
20 w	−1.37 ± 1.42**	−0.27 ± 1.24	0.009*
24 w	−0.81 ± 1.63*	−0.28 ± 1.05	0.195
Tearing			
Day 4 w	−0.23 ± 0.43*	−0.19 ± 0.75	0.791
8 w	−0.30 ± 0.51*	−0.31 ± 0.61*	0.939
12 w	−0.30 ± 0.51*	−0.28 ± 0.60*	0.842
16 w	−0.19 ± 0.51	−0.35 ± 0.73	0.416
20 w	−0.28 ± 0.66	−0.36 ± 0.83	0.717
24 w	−0.16 ± 0.77	−0.36 ± 0.76	0.393
Conjunctival hyperemia			
Day 4 w	−0.28 ± 0.44*	0.31 ± 1.19	0.015*
8 w	−0.23 ± 0.53*	0.02 ± 0.91	0.207
12 w	−0.31 ± 0.43**	0.13 ± 1.39	0.106
16 w	−0.30 ± 0.61*	−0.27 ± 0.55*	0.912
20 w	−0.36 ± 0.54	−0.13 ± 0.85	0.307
24 w	−0.24 ± 0.70	−0.14 ± 0.66	0.645

Values are mean ± standard deviation; Lj EM1: *Lactobacillus johnsonii* EM1; P-value[®]: treatment effect by two-sample *t*-test.

* *P* < 0.05.** *P* < 0.001.

The changes from baseline in the diary card individual symptom scores are illustrated in Table 3. Group 1 showed significant reduction in nasal congestion at weeks 8 and 12; throat itching at week 8; conjunctival itching at weeks 4, 8, 12, 16, and 20; and conjunctival hyperemia at week 4. Comparing between

Table 4

Change from baseline of nasal symptom score, non-nasal symptom score and sleep quality.

	Levocetirizine + LjEM1 (Gr.1, n = 30)	Levocetirizine (Gr.2, n = 27)	P-value ^a
Nasal symptom scores			
0–4	−3.26 ± 3.19**	−1.46 ± 3.62*	0.052
0–8	−4.29 ± 4.26**	−1.48 ± 4.59	0.020*
0–12	−4.60 ± 4.54**	−2.22 ± 3.92*	0.040*
0–16	−3.14 ± 3.39	−3.79 ± 4.02*	0.479
0–20	−3.15 ± 4.73	−3.41 ± 3.48**	0.738
0–24	−2.29 ± 4.66	−4.27 ± 4.68**	0.289
Pharynx symptom scores			
0–4	−0.81 ± 1.60*	−0.20 ± 1.68	0.163
0–8	−1.17 ± 1.44**	−0.59 ± 1.33*	0.122
0–12	−1.31 ± 1.65**	−0.53 ± 1.32*	0.056
0–16	−1.13 ± 1.79	−0.44 ± 1.09	0.253
0–20	−1.12 ± 1.85	−0.55 ± 1.32*	0.252
0–24	−1.12 ± 1.81	−1.00 ± 1.25*	0.857
Eye symptom scores			
0–4	−1.20 ± 1.20**	0.52 ± 3.69	0.027*
0–8	−1.50 ± 1.74**	−0.33 ± 2.19	0.029*
0–12	−1.51 ± 1.47**	−0.55 ± 1.68	0.024*
0–16	−1.73 ± 2.15*	−1.05 ± 2.07*	0.312
0–20	−2.04 ± 2.09**	−0.76 ± 2.61	0.092
0–24	−1.25 ± 2.59*	−0.76 ± 2.05	0.529
Day sleep			
0–4	−0.16 ± 0.48	−0.17 ± 0.31*	0.954
0–8	−0.36 ± 0.58*	−0.16 ± 0.49	0.159
0–12	−0.47 ± 0.56**	−0.17 ± 0.35*	0.019*
0–16	−0.25 ± 0.38*	−0.10 ± 0.42	0.226
0–20	−0.41 ± 0.69*	−0.17 ± 0.47	0.164
0–24	−0.35 ± 0.63*	−0.25 ± 0.38*	0.460
Sleep quality			
0–4	−0.32 ± 0.56*	−0.27 ± 0.71	0.750
0–8	−0.43 ± 0.65*	−0.21 ± 0.76	0.237
0–12	−0.59 ± 0.70**	−0.26 ± 0.50*	0.045*
0–16	−0.41 ± 0.52*	−0.26 ± 0.42**	0.289
0–20	−0.45 ± 0.74*	−0.33 ± 0.55**	0.541
0–24	−0.43 ± 0.66	−0.36 ± 0.54**	0.698

Values are mean ± standard deviation; Lj EM1: *Lactobacillus johnsonii* EM1; P-value^a: treatment effect by 2-sample t-test.

* P < 0.05.

** P < 0.001.

groups, group 1 showed more reduction in most symptoms than did group 2 during the first 12-week treatment period, especially in nasal congestion and conjunctival itching, but this difference gradually disappeared during the second 12-week treatment period. Table 4 shows that both groups showed significantly improved nasal and non-nasal symptom scores and sleep quality compared with the baseline, but group 1 was superior in the first 12-week treatment period, especially in terms of nasal symptom and eye symptom scores and sleep quality. However, the difference between the groups gradually disappeared during the second 12-week treatment period.

The PRQLQ score showed a significant decrease over the study period in both groups ($P < 0.05$), but there was no statistically significant difference between the 2 groups ($P = 0.446$) after 24 weeks of treatment (Table 2 and Fig. 2).

The eosinophil proportion in nasal smears decreased in both groups, and a significant difference compared to the run-in period was observed in group 1 at 24 weeks and in group 2 at 16 weeks ($P < 0.05$). This result suggests that recruitment of eosinophils to the nasal mucosa is further inhibited after cetirizine plus Lj EM1 treatment (Table 2).

Both groups showed significant improvement in nPEFR at weeks 4, 8, 12, 16, and 24 ($P < 0.01$), and treatment for group 1 was more efficacious than that for group 2 at weeks 8, 12, 16, and 20 ($P < 0.05$, Table 2 and Fig. 3). FVC and FEV1 were improved in both groups between weeks 8 and 24 ($P < 0.05$), but there was no significant difference between the 2 groups (Table 2).

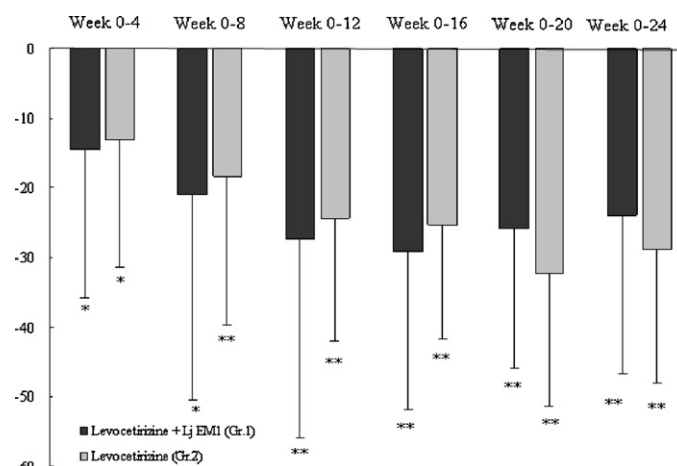


Fig. 2. Change from baseline of mean PRQLQ value in the 2 groups. Significant change compared with baseline in both groups (* $P < 0.05$, ** $P < 0.001$). There was no statistically significant difference between the groups.

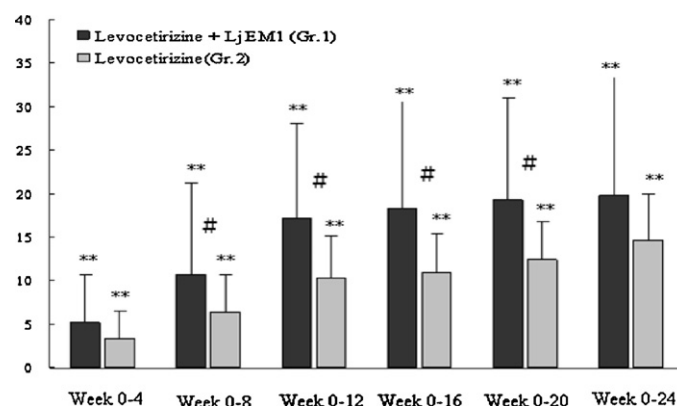


Fig. 3. Change from baseline in nPEFR. Significant change compared with baseline in both groups (* $P < 0.05$, ** $P < 0.001$). Gr.1 significantly improved nPEFR value compared with Gr.2 at weeks 8, 12, 16, and 20 (# $P < 0.05$).

In laboratory tests, the total eosinophil count in peripheral blood, ECP, total serum IgE, and resistin levels were not significantly different compared with the run-in period in either group after the 12- and 24-week treatments. Both groups showed a significant increase in IFN- γ and IL-10 levels and a significant decrease in IL-4 levels at 12 and 24 weeks, but group 1 had a greater increase in IFN- γ than did group 2 after the first 12-week period. We observed a significant increase in serum TGF- β in group 1 at 12 weeks ($P < 0.001$), which persisted through the second 12-week period. Group 2 had an increase in TGF- β levels only during the second 12-week period (Table 2 and Fig. 4). No serious adverse events were recorded in either group.

4. Discussion

Oral antihistamines are the mainstay treatment for allergies in adults and children. Classic and non-sedating antihistamines are effective for relieving most nasal symptoms, including itching, sneezing, and rhinorrhea, because they effectively block the histamine H₁-receptors that trigger plasma exudation and edema formation. However, oral H₁-receptor antagonists have only minor effects on nasal obstruction [29,30]. Our previously published studies [28] and most clinical trials show that levocetirizine is a potent antihistamine that can relieve the symptoms of PAR [31,32].

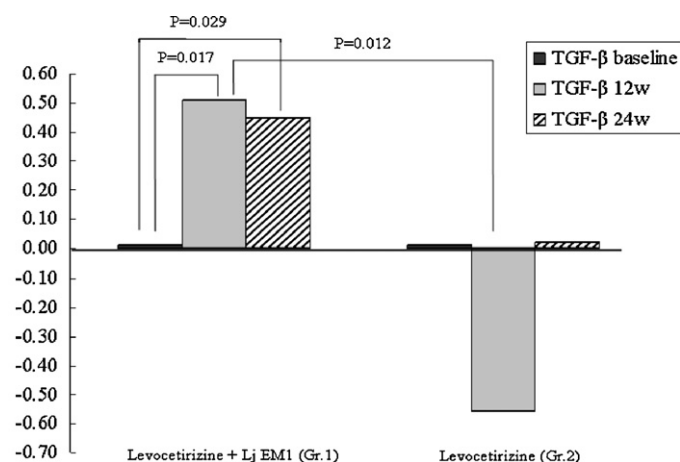


Fig. 4. Concentration of TGF- β changed from baseline in both groups. Gr.1 showed significantly increased concentration of TGF- β compared with baseline at weeks 12 and 24 ($P < 0.05$) and also showed significantly increased concentration of TGF- β compared with Gr. 2 at week 12 ($P < 0.05$).

Probiotics, which improve the intestinal microbial balance, may modulate immune responses that are beneficial for the prevention of atopic eczema in infants [33,34]. The results of recent studies support the benefits of probiotics in both the prevention and treatment [35] of early allergic disease by modulating the inflammatory response and immunity [36]. However, whether these bacteria have clinical applications in the prevention or treatment of established allergic airway diseases remains controversial and requires more comprehensive clinical trials.

Only a few earlier clinical trials have explored the clinical effects of probiotic administration on AR in children [15,16,23]. In this study, our results suggest that supplementary consumption of *Lj* EM1 with levocetirizine could effectively mitigate the symptoms of AR, when compared with levocetirizine alone. We found that TSS in both groups improved progressively compared with that during the run-in period at weeks 4, 8, and 12 ($P < 0.05$), and treatment for group 1 was more efficacious than treatment for group 2 at week 4 ($P = 0.014$), 8 ($P = 0.011$), and 12 ($P < 0.009$). During the second 12-week period, group 2 improved continually and progressively, while group 1 did not (Table 2 and Fig. 1). In terms of most individual symptom scores, group 1 showed more improvement than group 2 during the first 12 weeks, especially in nasal congestion and conjunctival itching, but this difference disappeared gradually during the second 12-week period (Table 3). We further determined that nasal and non-nasal symptom scores and sleep quality had the same trend in both groups (Table 4).

The results of the pulmonary function FEV₁ and FVC tests indicated that probiotic *Lj* EM1 did not inhibit the effect of levocetirizine and may have partially improved pulmonary function (Table 2). This claim is strengthened by the results of the nPEFR.

IgE and eosinophils play important roles in the etiology of allergic diseases. IgE is the effector molecule and eosinophils are effector cells in IgE-associated allergic disorders. Both IgE and eosinophils are increased in patients with AR. Current evidence suggests that allergen-induced alterations in the composition and/or size of the gastrointestinal microflora [37] are due to modulation of the IgE response [38]. In this respect, probiotics have been shown to stabilize and repair the defense barriers of the intestinal mucosa by normalizing the effects of infection or allergic inflammation on intestinal permeability and the balance of gastrointestinal microflora [37].

Patients with AR have been reported to have increased numbers of mast cells, basophils, T cells, and especially eosinophils, in nasal

smears and biopsies. The increase in eosinophils was accompanied by an increase in the levels of eosinophilic granular proteins such as ECP, eosinophil peroxidase (EPO), and the major basic protein (MBP), which may cause damage and desquamation of airway epithelial cells, alter airway hypersensitivity and cilia function, and elicit local edema. Consumption of probiotics has been shown to reduce the level of eosinophils in allergic patients [39]. In our present study, we saw no significant difference in serum IgE levels between the 2 groups. We noted that serum eosinophils, ECP, and nasal eosinophils were lower in the levocetirizine plus *Lj* EM1 group than in the levocetirizine group, but this difference was not significant. Hseuh et al. [40] reported that patients with PAR had higher serum levels of resistin that increased with disease severity. However, in this study, we saw no significant difference in serum resistin before or after treatment in either group.

Cytokines are immunoregulatory molecules that balance Th1- and Th2-mediated immune responses. Cytokines secreted by Th2 cells can help recruit and activate eosinophils in the nose. IL-4 is a Th2 cytokine that plays a role in recruiting eosinophils and promoting IgE production by inducing an isotype switch in B cells; these functions of IL-4 are inhibited by the Th1 cytokine IFN- γ . Several studies have suggested that diet supplementation with probiotics alters the allergic response in several ways. They may shift the Th2 cytokine profile by promoting the production of Th1 cytokines [41,42], downregulate IgE production by inhibiting IL-4 and IL-5 [43], or interact with professional antigen-presenting cells such as dendritic cells (DCs) in the gastrointestinal mucosa to induce systemic immunological tolerance [44,45]. IL-10 and TGF- β are anti-inflammatory Tr-related cytokines that attenuate the release of pro-inflammatory cytokines and reduce allergen-induced airway inflammatory reactions and non-specific airway responsiveness [46]. Probiotics can induce the development of a population of CD4⁺ T cells that produce TGF- β and IL-10 [47], which then suppress the allergen-induced inflammatory response [44,48,49]. In the present study, we found a significant increase in Th1 cytokines and decrease in Th2 cytokines, but there was no difference between the 2 treatment groups. These results appear to rule out the possibility that probiotics function as Th1 adjuvants to counterbalance the predominant Th2 cytokines in atopic children, as was found in the study by Chen et al. [50]. On the other hand, in our study, levels of IL-10 and TGF- β increased for at least 12 weeks following probiotic treatment, especially for TGF- β (Table 2 and Fig. 4). Therefore, it appears that Tr may be induced in the probiotic-treated subjects, which then suppresses allergen-induced AR.

The increase in TGF- β in the levocetirizine plus *Lj* EM1 group is rather interesting. TGF- β induces an isotype switch to IgA in the GALT, and the molecule is secreted from DC in Peyer's patches [46]. TGF- β also induces oral tolerance through induction of Tr cells [51,52]. Based on these findings Akbari et al. [52] have suggested that intestinal DCs may be responsible for the induction of Tr cells, which may be induced directly or indirectly to produce TGF- β [52]. This is consistent with the hypothesis that cell wall components of *Lactobacillus* could induce maturation and activation of DCs via toll-like receptor 2, as well as by producing various proinflammatory cytokines and chemokines [53,54].

Our results demonstrate that levocetirizine plus *Lj* EM1 is efficacious in the treatment of AR. However, there are several limitations to this clinical trial. The open-label design makes it difficult to compare the clinical response between the 2 treatment groups, but any bias may have been decreased by using the crossover method. Another issue that remains unresolved is whether probiotics provide long-term benefit (more than 12 weeks) in reducing AR and conferring immunologic tolerance to allergens after the supplements have been discontinued. Peltola et al. [55] and Pohjavuori et al. [56] have confirmed that the innate

immune response can be modulated by a *Lactobacillus* probiotic. Until now in the worldwide literature there are only few studies demonstrating the efficacy of such an association on a clinical basis [16,23,50,57]. There is also no metaanalysis and no general agreement to propose to the majority of patients suffering from an allergic rhinitis such an association.

In conclusion, supplementary treatment of *Lj* EM1 with levocetirizine was found to effectively alleviate the symptoms of allergic rhinitis. The treatment might also induce Tr cells, which could modulate the balance of Th-1/Th-2 responses, and this effect could persist at least 3 months after discontinuation of *Lj* EM1 treatment. No serious adverse events were recorded in either study group. Further studies particularly randomized control test and multicenter studies are necessary to define the place of probiotics in the treatment of AR in children.

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