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A real-life study of the efficacy of sublingual immunotherapy against weed allergies in the Serbian population

Étude en vraie vie de l'efficacité de la désensibilisation par voie sublinguale pour les pollens d'herbacées dans la population serbe

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ABSTRACT

Introduction. – In the Serbian population allergy to weed pollen is the most common type of pollen-associated allergy, ahead of grass and tree pollens. Besides causing discomfort, allergy to pollen is often associated with rhinitis and asthma. Allergen-specific immunotherapy (ASIT) is the only treatment that can lead to potential long-term immune modification while reducing development of new sensitization and halting disease progression. The aim of the present study is to evaluate the efficacy of sublingual immunotherapy (SLIT) to weeds in the adult patient population using vaccine produced by the local Serbian Torlak Institute for virology, vaccines and serum.

Methods. – Adult patients with a clinical history of allergic rhinitis with and without asthma were included in the study. IgE-mediated sensitization to grass, tree and weed pollens was confirmed by skin prick testing and/or positive specific IgE. Patients were divided into two groups: patients with allergy to tree and grass pollen and patients with allergy to weeds. All patients received SLIT for three years, either with or without additional symptomatic therapy.

Results. – Three-year SLIT therapy led to significant improvement in several parameters, including skin-prick reactivity, decrease in specific IgE and use of symptomatic therapy, with mild adverse effects and high patient satisfaction concerning therapy.

Conclusion. – Three-year SLIT is a safe and efficient treatment option for respiratory allergy to weeds. Further observations in a larger number of patients could provide a better epidemiological evaluation of SLIT, but the positive effects we observed in our study may be considered representative despite the small number of patients.

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R É S U M É

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Désensibilisation par voie sublinguale

Introduction. – Dans la population serbe, l'allergie aux pollens d'herbacées est la pollinose la plus fréquente, devant les allergies aux pollens d'arbres et de graminées. En dehors d'une altération de la qualité de vie, l'allergie aux pollens est souvent associée à une rhinite ou un asthme. L'immunothérapie spécifique aux allergènes (ITA) est le seul traitement pouvant entraîner une modification immunitaire à long terme en réduisant le risque de nouvelles sensibilisations et en interrompant la progression de la maladie. Le but de cette étude est d'apprécier l'efficacité d'une immunothérapie par voie sublinguale (SLIT) pour les herbacées dans une population adulte en utilisant des vaccins produits par l'institut serbe Torlak pour la virologie, les vaccins et les sérums.

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Méthodes. – Des patients adultes avec une histoire clinique de rhinite allergique avec ou sans asthme ont été inclus dans cette étude. Une sensibilisation IgE médiée aux pollens de graminées, d'arbres ou d'herbacées a été confirmée par prick-tests et/ou IgE spécifiques élevées. Les patients ont été divisés en 2 groupes : patients allergiques aux arbres et aux graminées et patients allergiques aux herbacées. Tous les patients ont reçu la SLIT pendant trois ans avec ou sans adjonction d'un traitement symptomatique.

Résultats. – Le traitement par SLIT a apporté une amélioration significative de plusieurs paramètres dont la positivité des tests cutanés, la diminution des taux d'IgE spécifiques et l'usage de médicaments symptomatiques, avec des effets indésirables légers et un indice de satisfaction des patients élevé pour le traitement.

Conclusion. – Un traitement par SLIT de trois ans est une option thérapeutique bien tolérée et efficace dans l'allergie respiratoire aux pollens d'herbacées. Des observations complémentaires portant sur un plus grand nombre de patients pourraient apporter une meilleure évaluation épidémiologique de la SLIT, mais les résultats positifs observés dans notre série doivent être considérés comme significatifs en dépit du faible nombre de patients inclus.

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1. Introduction

Weeds are an unrelated group of plant species of no commercial value. These plants reproduce invasively and are considered undesirable in human-controlled environments. Weeds possess a high ability to adapt, allowing them to thrive in environments in which natural vegetation has been damaged. Besides economic and agricultural harm, they represent a threat to human health as their pollen constitutes an important source of seasonal allergens.

Allergenic weed pollen triggers type I hypersensitivity reactions in patients. These weeds belong to the *Asteraceae*, *Amaranthaceae*, *Plantaginaceae*, *Urticaceae* and *Euphorbiaceae* plant families. To date, 34 weed pollen allergens are listed in the IUIS allergen nomenclature database, and they have been physicochemically and immunologically characterized to varying degrees [1].

The *Asteraceae* is a large family of flowering plants that includes some 20,000 species. Important allergenic members are ragweed (*Ambrosia*), mugwort (*Artemisia*), feverfew (*Parthenium*), and sunflower (*Helianthus*) [1]. In the Serbian population, allergy to weed pollen is the most common type of pollen-associated allergy, ahead of grass and tree pollen allergies [2]. Besides causing discomfort, allergy to pollen is often associated with rhinitis and asthma [3]. Pharmacological treatments, which include antihistamines, corticosteroids, and leukotriene inhibitors, can control the symptoms but do not modify the natural cause of disease. Allergen-specific immunotherapy (ASIT) is the only treatment that can lead to potential long-term immune modification, reduce the development of new sensitization and halt disease progression [4]. Immunotherapy can be administered subcutaneously or sublingually. Subcutaneous immunotherapy (SCIT) has been shown to be effective in paediatric patients [5] but the inconvenience of injection and the risk of serious side-effects has led to the emergence of newer therapeutic concepts. Sublingual immunotherapy (SLIT) is associated with a lower incidence of systemic reactions and long-term administration leads to a decrease in symptoms and improved disease course [6,7].

Weeds, especially ragweed, are thought to be spreading through southern and central Europe [8], and the Pannonian Plain is one of the areas with the highest pollen counts [9]. The allergenic impact of *Ambrosia* spp. is very high owing to its tendency to induce asthma about twice as often as other pollens, and to the notable cross-reactivity between major allergens within the *Ambrosia* genus, as well as between *Ambrosia* and *Artemisia* [10–12]. The aim of present study is to compare the efficacy of SLIT against weeds using vaccine produced by the local Serbian Institute for virology, vaccines and serum (Torlak) in an adult patient population. This was a

single-centre randomized, controlled study prospectively followed for three years.

2. Material and methods

2.1. Study design

The study was conducted at the KBC “Bežanijska kosa” in Belgrade, Serbia, between January 2001 and September 2009. It included 34 patients of both sexes with clinical manifestations of allergic rhinitis, with or without asthma symptoms. Inclusion criteria were met if participants presented symptoms during the pollination season coupled with IgE-mediated allergy to pollen as confirmed by a positive skin-prick test and positive specific IgE (CAP-FEIA, Pharmacia-Upjohn, Uppsala, Sweden). All patients included in the study provided written informed consent in accordance with the Helsinki Declaration. The study did not require Ethics Committee/IRB authorisation and conformed to the legal standards.

2.2. Patients

Thirty-four patients with a clinical history of allergic rhinitis, with or without asthma, were included in the study. IgE-mediated sensitization to grass, tree and weed pollen was confirmed by skin prick testing (SPT) and/or positive specific IgE (CAP-FEIA, Pharmacia-Upjohn, Uppsala, Sweden). Twelve patients had allergic rhinitis, sixteen had rhinitis with conjunctivitis, four had rhinitis, conjunctivitis and asthma, and two patients had rhinitis and asthma. Twenty patients (80%) had polysensitization. They were treated with the allergenic extract to which they displayed the strongest sensitization. Nineteen patients were treated with weed pollen, ten with grass pollen, and five with tree pollen.

Diagnosis of asthma was confirmed on the basis of patient's history and a positive bronchoprovocation test with acetyl choline or clinical findings indicating pulmonary obstruction and a positive bronchoprovocation test with acetyl choline. Demographic and clinical data are shown in Table 1.

2.3. Skin testing

After detailed history-taking, all subjects underwent SPTs with a standard commercial panel of inhalant, food allergens and latex (Torlak, Belgrade Serbia): grass mix, olive, parietaria, mugwort, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, dog, cat, peanut, walnut, hazelnut, milk, egg, fish, shrimp, peach, apple, lentil, banana and latex.

Skin testing was performed on the volar aspect of the forearm using histamine (1%) and saline as the positive and negative controls, respectively. Tests were considered positive if the wheal produced had a mean diameter of at least 3 mm [13].

2.4. Bronchoprovocation test

Non-specific bronchodilatation testing was performed using acetyl choline dissolved in distilled water at a concentration of 8 mg/mL. Patients inhaled increasing doses of acetyl choline solution in series of 10 inhalations up to 60 inhalations in total, or until FEV decreased from the starting value by 20%. Tests were considered positive where a 20% decrease in FEV was observed.

2.5. Protocol for sublingual immunotherapy (SLIT)

Vaccine for SLIT produced by the local Serbian Institute for virology, vaccines and serum Torlak, Belgrade, Serbia, was used in the study. Sublingual-swallow “vaccines” were made from tree pollen (*Betula papyrifera*), grass pollen (*Dactylis glomerata* and *Phleum pratense*) and weed pollen (*Ambrosia elatior* and *Ambrosia trifida*). The allergen extracts for each type of vaccine were prepared in phosphate-buffered saline with 50% glycerol. The quality of allergen extract was tested using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and a western blot technique. The potency of the solution was expressed as protein nitrogen units (PNU)/mL and the solution was prepared in three strengths: 16, 125 and 1000 PNU/mL. According to the manufacturer's recommendations, in the build-up phase (45 days), patients received gradually increasing concentrations of the extract, starting with one drop of 16 PNU/mL and rising to 15 drops over 15 days. The daily dose was taken on a sugar cube sublingually in the morning, half an hour before breakfast. This process was also repeated for the 125 and 1000 PNU/mL doses. Finally, patients were switched to a maintenance phase regimen comprising 15 drops at 1000 PNU/mL twice a week for the following 36 months. The study timeline is summarized in Fig. 1.

2.6. Clinical evaluation

Follow-up for all patients consisted of:

- regular clinical visits at 15-day intervals and then once monthly;
- additional clinical visits, telephone calls;
- diary of symptoms, drug intake and adverse reactions.

Before the introduction of SLIT and after one year, during the same time of year (pollination season, i.e. the highest level of

exposure to allergen), patients were asked to complete their symptom and medication score diary on a daily basis over a one-month period. Adverse reactions during SLIT were recorded separately by means of questionnaires and clinical visits. At the end of the study, patients were asked to complete a self-assessment diary card giving details of symptoms, medication intake and therapeutic efficacy.

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism v.7 for Windows (San Diego, California, USA). A significance level of $P \leq 0.05$ was used for analysis of variance by means of the Kruskal-Wallis test followed by Tukey's post-hoc test ($P \leq 0.05$). Correlation between different parameters was analysed at a significance level of $P \leq 0.05$.

3. Results

Thirty-four patients were included in the study, 17 men and 17 women (mean age 43.7 ± 9.9 ; range 18–52 years). The patients were divided into two groups: a tree and grass group consisting of patients with allergies to tree and grass pollen (15 patients: 6 men and 9 women), and a grass group consisting of patients with weed allergies (19 patients: 11 men and 8 women). All patients received SLIT with and without additional symptomatic therapy. Three-year SLIT therapy led to significant improvement in several parameters, including skin-prick reactivity (wheal and flare reaction), decrease in specific IgE, and use of symptomatic therapy.

A statistically significant ($P=0.05$) decrease in mean wheal diameter was observed after three years of SLIT in the weed group, while the tree and grass group did not show a significant decrease. The mean pre-therapy score in the tree and grass group was 10.23 ± 3.44 mm while mean post-therapy diameter was 7.84 ± 3.79 mm. In the weed group, the pre-therapy diameter was 9.74 ± 3.93 mm, while the post-therapy value decreased to 3.16 ± 1.02 mm. On the other hand, flare reaction also showed a statistically significant ($P=0.05$) decrease post-SLIT. Mean pre-SLIT flare in the tree and grass group was 14.88 ± 14.16 mm vs. 3.25 ± 5.05 mm post-SLIT. In the weed treatment group mean pre-SLIT flare was 14.78 ± 17.00 mm vs. 5.42 ± 8.91 mm post-SLIT (Fig. 2).

The inclusion criteria for SLIT were the presence of symptoms during the pollination season, specific IgE level, and skin reactivity. Nineteen patients had a decrease in specific IgE level while the remaining patients maintained the same IgE class as prior to SLIT. Of these 19 patients, 7 were in the tree and grass group vs. 12 in the

Table 1
Demographic and clinical characteristics of patients from the study.

Demographic and clinical characteristics of patients from the study			
Variable	Tree and grass group	Weed group	P
M:F ratio	6:9	11:8	>0.05
Age (in years)	38.0 ± 10.5	49.4 ± 9.3	>0.05
Rhinitis	4	8	>0.05
Rhinitis and conjunctivitis	9	7	>0.05
Rhinitis, conjunctivitis and bronchial asthma	2	2	>0.05
Rhinitis and bronchial asthma	1	1	>0.05
Polly sensitization			
Yes	11	15	>0.05
No	4	4	>0.05
Bronchoprovocation test			
Yes	2	2	>0.05
No	13	17	>0.05

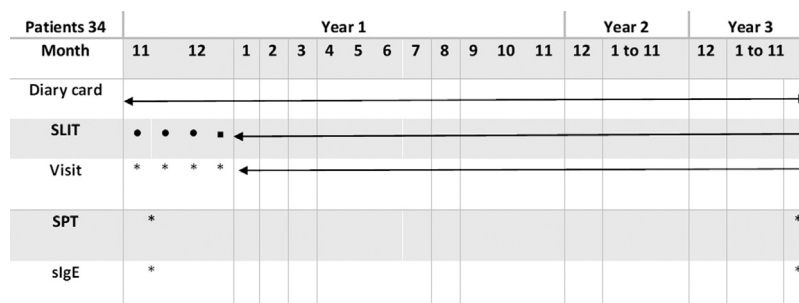


Fig. 1. Timeline of the study: •: SLIT build-up phase; ■: SLIT maintenance phase; * only once; ↔ Continue Abbreviations: sIgE, specific Immunoglobulin E; SLIT, sublingual immunotherapy.

weed group. None of the patients had a higher measured specific IgE value after three years of SLIT (Fig. 3).

Symptomatic therapy used before, during and after slit included antihistamines, nasal glucocorticoids, inhaled glucocorticoids and leukotriene antagonists. In the tree and grass group, most patients (66.67%) used two medications as symptomatic therapy prior to SLIT (Table 2), while after SLIT, patients used either no medication (26.67%), one medication occasionally (antihistamines: 26.67%) or one medication regularly (antihistamines or nasal steroids: 40%). In the weed group, most patients (78.95%) used two medications (antihistamines and nasal steroids) prior to the study treatment (Table 2), while post-treatment most patients used either no medication (21.00%) or one medication occasionally (antihistamine: 47.37%).

During our study, only mild adverse reactions were observed (Fig. 4) However, the majority of patients did not exhibit any adverse effects during SLIT: 71.4% in tree and grass group and 78.9% in the weed group.

After a three-year course of SLIT, patients were asked to complete a self-assessment diary for symptoms and

therapeutic efficacy. They were asked to answer two questions: whether they deemed therapy to be effective (yes/no question) and overall therapeutic efficacy graded on a scale of 1 to 10. Of all the study subjects, only one considered therapy unsatisfactory (3.3%), while the remainder of the study population considered the therapy effective (96.7%).

The subjective grade assigned to SLIT efficacy ranged from 4 to 10 (Fig. 5). None of the study subjects graded SLIT in the range of 1–3, with most considering SLIT to be in the 7–9 range, and one third of the patients graded SLIT as 8 (Fig. 5). The mean grade for all study subjects was 7.70 ± 1.49 . The subjective grade in the tree and grass group was 7.57 ± 1.73 vs. 7.71 ± 1.36 in the weed group.

4. Discussion

This randomized controlled study is the first to evaluate the safety, efficacy and patient satisfaction of SLIT to weed allergies in a Serbian population. In the study we found:

- a statistically significant decrease in specific IgE and skin flare reactivity post SLIT;

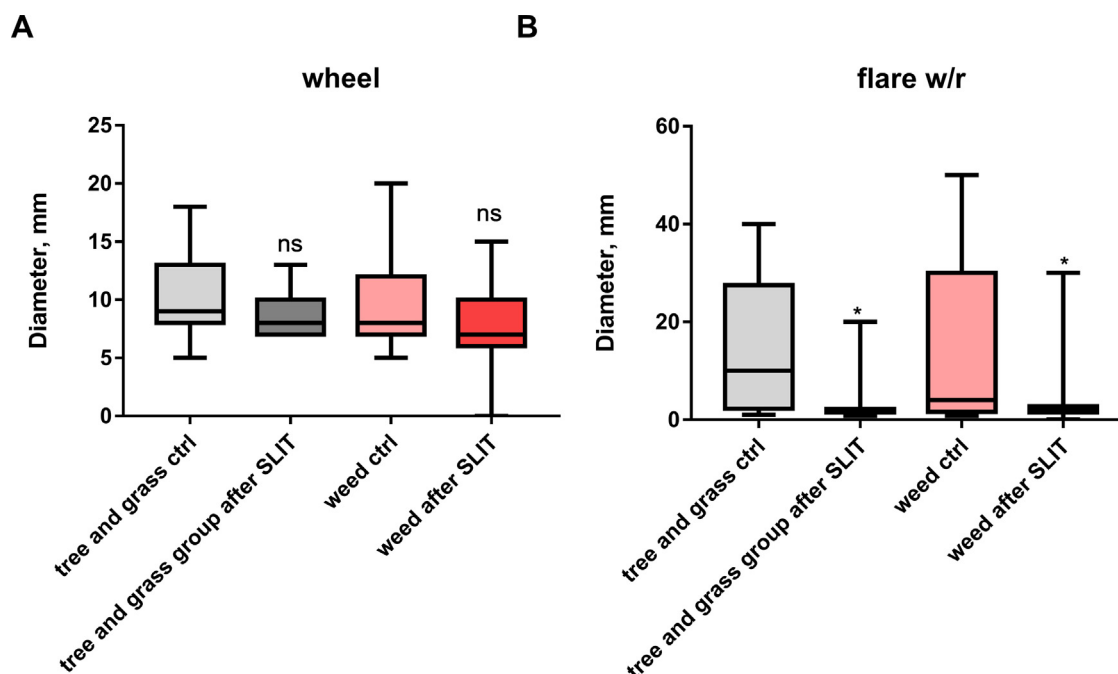


Fig. 2. Skin reactivity before and after SLIT in tree and grass group (grey) and weed group (pink): (A) wheal reaction and (B) flare reaction before and after SLIT. Statistical significance between groups was set at $P < 0.05$. NS indicates nonsignificant.

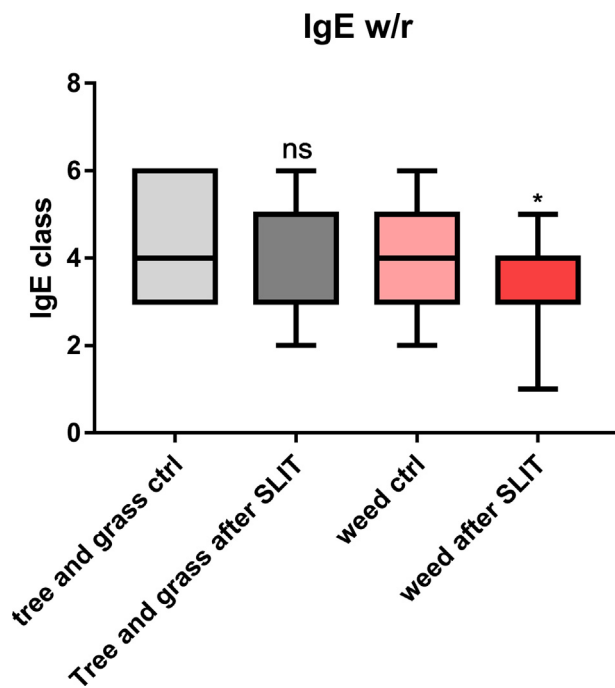


Fig. 3. Level of specific IgE in tree and grass group (grey) and weed group (pink) before.

- mild adverse effects reported by 26.6% of patients vs. 40% in the tree and grass group;
- patients in the weed group considered therapy satisfactory, with an average score of 7.71 ± 1.36 vs. 7.57 ± 1.73 in the tree and grass group.

SLIT was first introduced as a therapeutic approach in the late 1980s and many studies have confirmed its efficacy and safety as an alternative to SCIT [14–16]. A recent meta-analysis confirmed the efficacy of SLIT against various seasonal allergens, including weeds, by demonstrating a significant decrease in symptom scores in both adults and children [17]. The long-term beneficial effects of SLIT in addition to prevention of further sensitisation were observed long after completion of treatment [18]. Furthermore, a better safety profile coupled with a more convenient route of administration render SLIT was judged more favourable among both patients and certain physicians [16].

Our study included thirty-four patients treated at KBC “Bezanijska kosa”. Most patients had allergic rhinitis or rhinitis with conjunctivitis, while a smaller number presented rhinitis, conjunctivitis and bronchial asthma or rhinitis and bronchial asthma. SPT demonstrated sensitization to pollen in all patients, while 70.3% were poly-sensitized. Desensitization was performed using SLIT vaccine to pollen produced by the Serbian Institute for virology, vaccines and serum

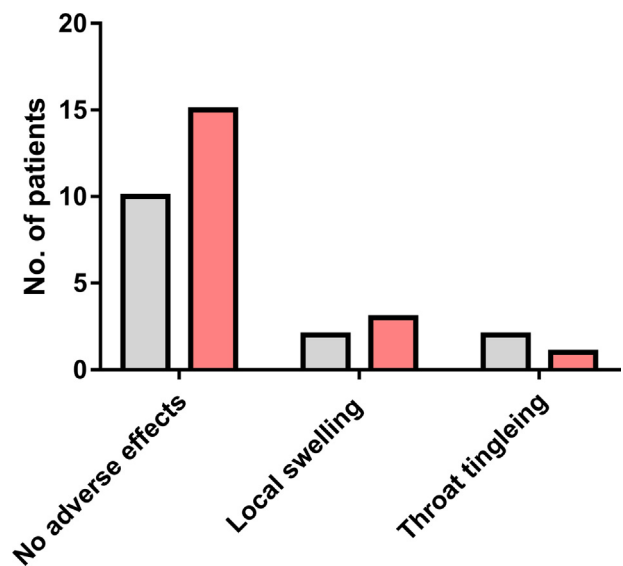


Fig. 4. Adverse effects during SLIT in tree and grass group (grey) and weed group (pink).

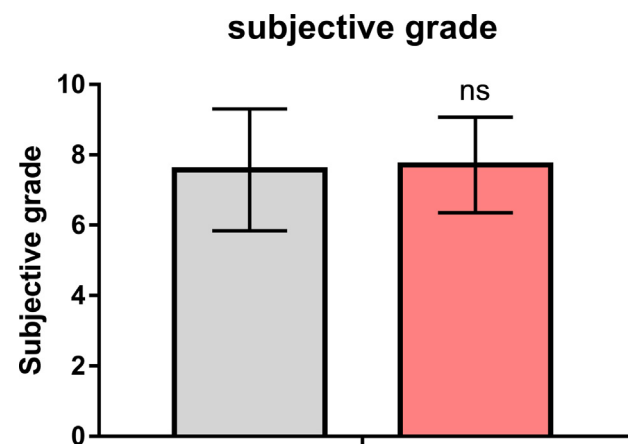


Fig. 5. SLIT subjective grade in tree and grass group (grey) and weed group (pink). Statistical significance between groups was set at $P < 0.05$. NS-indicates non-significant.

in Torlak. The efficacy of SLIT was assessed by comparing symptoms and use of symptomatic therapy, change in SPT reactivity and levels of specific IgE before and after 3 years of therapy.

SLIT reactivity was assessed by measuring both wheal and flare reactivity. Although wheal diameter did not show a statistically significant reduction in either group, flare reaction decreased significantly. Decreased skin reactivity after SLIT is consistent with previous findings in other studies using a similar SLIT formulation (i.e. drops) [19].

Table 2
Symptomatic therapy before and after SLIT.

	Tree and grass before SLIT	Tree and grass after SLIT	Weed before SLIT	Weed after SLIT
None	1	4	1	4
Antihistaminic occasionally	0	4	0	9
Antihistaminic	2	3	2	1
Nasal glucocorticoid	0	3	0	1
Antihistaminic, nasal glucocorticoid	10	1	15	3
Antihistaminic, nasal glucocorticoid, inhalatory glucocorticoid	1	0	1	1
Antihistaminic, nasal glucocorticoid, leukotriene antagonist	1	0	0	0

The decrease in the level of specific IgE post-SLIT was more pronounced in the weed group. Twenty study subjects showed a decrease in specific IgE: 12 in the weed group and 8 in the tree and grass group. The remaining 14 patients (7 in the weed group and 7 in the tree and grass group) showed no decrease in specific IgE levels upon completion of therapy. While some studies noted a rise in specific IgE levels post SLIT, most were shorter in duration [20]. ASIT may lead to an initial rise in specific IgE levels before downregulation over the subsequent months [21]. Some authors describe a decrease in specific IgE at a later stage of SLIT or after therapy, and a drop in specific IgE does not necessarily correlate with clinical improvement [22].

Besides a decrease in skin reactivity and level of specific IgE, a marked improvement in symptoms coupled with reduced use of symptomatic medication was noted in both groups after SLIT. Dahl et al. conducted a double-blind, placebo-controlled study using a pill formulation of grass allergen extracts for SLIT and noted a reduction in rhinoconjunctivitis symptom score and in rhinoconjunctivitis medication score of 30% and 38% respectively [23]. A similar but more moderate improvement was observed by Durham et al. in a multinational, multicentre, randomized, placebo-controlled trial of SLIT using grass-allergen tablets [24].

Several studies have confirmed SLIT as a safe and effective therapeutic option since the adverse reactions observed during SLIT were fewer and milder than with other routes of administration [14,15]. It should be emphasized that during our trial not a single case of systemic side effects was recorded, and all side effects were local and mild. Side effects during SLIT are usually present in the initial phase; they are limited to the oral mucosa in 40% to 75% of cases and do not usually require dose reduction or therapy withdrawal [25]. Dahl et al. also noted very mild symptoms localised mainly to the oral cavity and well tolerated by patients, with drops in only 4% [23]. All 34 patients enrolled in our study completed their SLIT and there were no drop-outs.

After 3 years of SLIT patients were asked to complete a satisfaction survey about symptoms and symptomatic therapy during and after therapy. All subjects interviewed except one declared themselves to be satisfied with the therapy. Subjects were further asked to grade SLIT on a scale of 1 to 10. The average grade in both groups was around 8, with no statistically significant difference between the groups.

Although the positive impact of SLIT against most allergens is well known, our study provided fresh insight into the efficiency and safety of SLIT to weeds, and into patient satisfaction regarding clinical improvement. This is the first study conducted in a Serbian population to evaluate a non-standardized allergenic extract in a real-life setting. We may conclude that this type of SLIT constitutes a safe and efficient treatment for respiratory allergies. A major drawback of our study is the small number of patients included. Conversely, one of the main strengths of the study is its length. While most study protocols involved treatment periods ranging from 16 weeks to 12 months, in our study patients were treated for three years. Further observations in a larger number of patients could provide a better epidemiological assessment of SLIT against weed allergens, but the positive effects we observed in our study may be considered representative despite the small patient population.

Disclosure of interest

The authors declare that they have no competing interest.

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