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Pre-seasonal immunotherapy is effective in both monosensitized and polysensitized patients with allergic rhinitis

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KEY WORDS

Pre-seasonal immunotherapy; monosensitized; polysensitized; specific IgE; specific IgG4.

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IMPACT STATEMENT

Pre-seasonal allergoid immunotherapy is clinically and immunologically effective in pollen allergic polysensitized patients.

Summary

Background. The effectiveness of pre-seasonal allergoid immunotherapy in polysensitized patients is not well-known. The aim of this study was to compare the clinical efficacy and immunological changes of pre-seasonal allergoid immunotherapy in mono and polysensitized patients with grass pollen allergy.

Methods. 46 patients with seasonal allergic rhinitis undergoing pre-seasonal grass pollen immunotherapy and 28 cases followed by conventional drug treatment were included. These groups were divided into monosensitized and polysensitized ones. All patients were followed between March-September with symptom-medication scores, and visual analogue scale. The quality of life was assessed using the Mini-RQLQ questionnaire. Phleum pratense specific IgE and IgG4 measurements were performed before and after 7 weeks of immunotherapy. **Results.** In the immunotherapy group, 15th weekly symptom-medication scores and VAS scores between May and August were found to be significantly lower than those in the control group ($p < 0.05$). Phl p specific IgE and IgG4 levels were significantly higher after immunotherapy compared to those before immunotherapy ($p = 0.001$). Furthermore, Phl p specific IgG4 levels after immunotherapy were also significantly higher than in the control group ($p = 0.001$). Improvements in activities-practical problems and non-nose/eye symptoms quality of life scores were significantly different between two groups ($p < 0.05$). There was no difference in terms of clinical and immunological parameters in mono and polysensitized patients ($p > 0.05$). **Conclusions.** Clinical improvement with pre-seasonal grass pollen immunotherapy is accompanied by important increase in specific IgG4 blocking antibodies. A single-allergen immunotherapy can lead to similar clinical efficacy and immunological changes in polysensitized as well as monosensitized patients with grass pollen allergy.

Introduction

Allergen-specific immunotherapy represents an effective treatment for allergic rhinitis caused by pollen allergy. Pre-seasonal short-term immunotherapy is a different immunotherapy program than the conventional immunotherapy protocols. The use of allergoids as immunotherapy compounds is expected to result in earlier immunological and clinical effects (1-4).

The prevalence of polysensitization is greater than monosensitization in allergic population, and it is reported to account for more than 50% of patients with respiratory allergies (4). Polysensitization is defined as the co-sensitization to two or more non-cross-reacting allergens from diverse sources evaluated either by skin prick testing (SPT) or serum-specific IgE assays. However, polysensitized patients may not always be polyallergic. Due to the absence of general recommendations by guidelines, the clinical management approach to polysensitized patients is not standardized (5, 6). Although large-scale clinical trials of grass pollen sublingual tablets showed that polysensitized patients benefited at least as much from allergen immunotherapy as monosensitized patients, the effects of pre-seasonal allergoid immunotherapy by injection route are not known on the clinical efficacy and immunologic response in polysensitized patients (7).

The aim of the present study was to compare the clinical efficacy and immunological changes of pre-seasonal allergoid immunotherapy in monosensitized and polysensitized patients with seasonal allergic rhinitis with grass pollen allergy.

Materials and methods

Study design

A total of 74 patients aged between 18-60 years old with seasonal allergic rhinitis were included in the study. Their inclusion criteria were: IgE-mediated moderate to severe persistent seasonal allergic rhinitis with symptoms during the pollen seasons (between March and September), symptoms of allergic rhinoconjunctivitis requiring medication during the last season and the presence of positive skin-prick test reactivity to grass pollen. The study was designed as an experimental study with two arms: one arm being the pre-seasonal immunotherapy group treated with 7 injections before the pollen season, and the second arm being the control group who were prescribed oral antihistamines and/or nasal corticosteroids when needed during the pollen season. The immunotherapy group consisted of 46 patients who had moderate to severe persistent seasonal allergic rhinitis receiving grass pollen allergoid immunotherapy. As the control group, 28 cases with moderate to severe persistent seasonal allergic rhinitis were included in the study and treated with medical treatment. The subjects were divided into two groups as monosensitized and polysensitized according to their skin prick test sensitivity in both the immunotherapy and control groups. Patients sensitized to only

grass pollens were categorized as monosensitized patients. In addition to grass pollen sensitivity, patients who showed sensitivity to other non-cross-reactive allergens from diverse sources (house dust mites and/or cat and/or dog dander and/or mold spores and or *Blattella germanica*) were categorized as polysensitized ones. This group had no history of clinically allergy to other allergens except grass pollen (presence of polysensitization but clinically monoallergic).

The time course of the study along with the clinical and laboratory investigations performed are outlined in **figure 1**. All subjects gave their written informed consent, and the Local Ethics Committee of Ankara University (Turkey) approved the protocol. The study was performed in accordance with the 1964 Helsinki declaration.

Skin prick tests

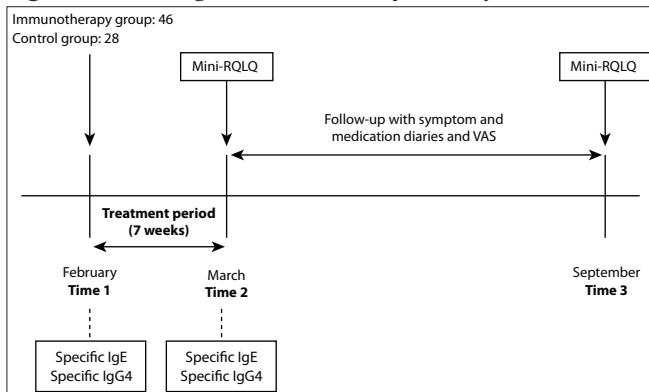
Skin prick tests were carried out with standard panel consisting of grass mix (*Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis* and *Fescuca pratensis*), cereal mix (*Hordeum vulgare*, *Avena sativa* and *Triticum sativa*), *Secale cereale*, weed mix (*Artemisia vulgaris*, *Urtica dioica*, *Taraxacum vulgare*, *Plantago lanceolata* and *Chenopodium album*), trees mix 1 (*Salix caprea*, *Populus alba*, *Ulmus scabra*, *Alnus glutinosa*, *Coryllus avellana*), trees mix 2 (*Betula verrucosa*, *Fagus sylvatica*, *Quercus robur* and *Planatus orientalis*) mold mix 1 (*Alternaria alternata*, *Cladosporium herbarum*, *Botrytis cinerea*, *Curvularia lunata*, *Fusarium moniliforme* and *Helminthosporium halodes*), mold mix 2 (*Aspergillus fumigatus*, *Mucor mucedo*, *Penicillium notatum*, *Pullularia pullulans*, *Rhizopus nigricans* and *Serpula lacrymans*), feather mix, cat and dog dander, house-dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), cockroach (*Blattella germanica*) and Latex (Allergopharma, Reinbek, Germany). Wheal (edema with erythema) of at least 3 mm or greater in diameter than the negative control after 20 minutes was considered positive reaction. Histamine dihydrochloride (10 mg/ml) was used for the positive control and physiologic saline was used for the negative control.

Determination of specific IgE and IgG4 levels

Phleum pratense (Phl p) specific IgE (sIgE) and specific IgG4 (sIgG4) (UNI-CAP 100, Phadia) antibody measurements were performed at baseline (Time 1) and after immunotherapy (Time 2) in the immunotherapy and control groups. The levels of sIgE and sIgG4 were quantified using the CAP fluoroenzyme immunoassay system according to the recommendations of the manufacturer's (Phadia, Uppsala, Sweden). For Phl p sIgE, the reference value was taken as > 0.35 kUA/L, and > 0.17 mgA/L for sIgG4.

Immunotherapy protocol

The immunotherapy product was a preparation of extracts of grasses treated with formaldehyde to produce an allergoid and then adsorbed on to aluminum hydroxide (Allergopharma, GmbH&Co, Germany). It was supplied in two concentrations, 1,000 therapeutic units TU/mL (vial A) and 10,000 TU/mL (vial B). Pre-seasonal immunotherapy treatment protocol was

Figure 1 - The design and time course of the study.

Time 1: Before immunotherapy (Baseline); Time 2: After immunotherapy; Time 3: After pollen season.

administered by injection weekly for seven weeks before starting pollen season. Subcutaneous injections commenced with 0.1 ml of strength-A in February followed by an approximate doubling of the dose weekly up to 0.6 ml of strength-B. Dose adjustments were made according to the individual tolerance.

Assessment of clinical efficacy

All patients were followed between 1st March to 1st September with symptom and medication scores, and visual analogue scale (VAS). Nasal (itching, sneezing, rhinorrhea and obstruction) and ocular (itching or watery-eyes) symptoms were recorded daily on a scale of 4: 0 – no symptoms, 1 – mild symptoms, 2 – moderate symptoms, and 3 – severe symptoms. The rhinoconjunctivitis symptom score (SS) was calculated as the mean of the daily symptom score (8). For rescue medication, patients were instructed to use a stepwise regimen (step 1: 5 mg of oral desloratadine, step 2: fluticasone furoate nasal spray and step 3: 4 mg of oral methylprednisolone). Medication scores (MS) were assigned as follows: 0 – no medication, 1 – desloratadine, 2 – nasal fluticasone furoate and 3 – oral methylprednisolone. The highest score for a given day was recorded as the MS (8). Weekly scores were obtained by adding up and averaging daily scores for each given week. Every month, patients assessed the severity of allergic symptoms on a 10-cm visual analogue scale (VAS) (with 0 cm indicating no symptoms and 10 cm indicating the highest level of symptoms). The QoL was evaluated using the Turkish version of the Mini Rhinoconjunctivitis Quality of Life Questionnaire (Mini-RQLQ) (9). Mini-RQLQ questionnaire was administered twice, once before pollen season (beginning of March – Time 2) and once after pollen season (beginning of September – Time 3).

Pollen counts

Airborne pollen measurements were carried out in Ankara, during the pollen season from 1st March to 1st September with a

Burkard volumetric 7-day spore trap. A Burkard spore trap was used for 7-day sampling onto Melinex tape coated with a thin film of Lubriscal (Thomas Scientific, Swedesboro, NJ). Tapes were changed weekly, cut into 48 mm segments, and mounted on microscope slides. Slides were colored with glycerin jelly containing basic fuchsin and examined microscopically at 400x magnification using a single longitudinal traverse lens. Microscope counts were converted into atmospheric concentrations and expressed as pollen grains/m³.

Statistical analysis

Statistical analysis was performed using SPSS software version 15 (SPSS, Chicago, Ill., USA). Normality of distribution was analyzed with the Shapiro-Wilk test. Comparisons between groups were performed using the Mann-Whitney U test. Intragroup comparisons were made Friedman test or the Wilcoxon signed-rank test. A value of $p < 0.05$ was considered statistically significant. The primary end point was a difference between monosensitized and polysensitized patients with regard to the SS and MS, VAS and quality of life scores and serum levels of sIgE and sIgG4 during pollen season. The secondary end point was a difference between immunotherapy and control groups with regard to the SS and MS, VAS and quality of life scores and serum levels of sIgE and sIgG4 during pollen season.

Results

A total of 46 patients with seasonal allergic rhinitis undergoing pre-seasonal grass pollen immunotherapy and 28 control cases followed by conventional drug treatment were included in the present study. There was no difference between two groups in terms of demographic characteristics (**table I**). The number of monosensitized/polysensitized patients were 37/9 and 20/8 in immunotherapy and control groups, respectively. Distribution of sensitization profile against other inhalant allergens except grass pollen in polysensitized group was shown in **figure 2**. Skin prick test reactivity was observed mostly against house dust mites and cat as perennial allergens in this group.

Clinical assessment in immunotherapy and control groups

Symptom-medication score and VAS

In the immunotherapy group 11th, 13th, 14th, 15th, 16th, 17th, 18th, 19th, 21th and 22th weekly SS at the peak of the grass pollen period were found to be significantly lower than those in the control group ($p = 0.02$, $p = 0.004$, $p = 0.006$, $p = 0.002$, $p = 0.01$, $p = 0.01$, $p = 0.008$, $p = 0.003$, $p = 0.03$, $p = 0.01$, respectively) (**figure 3a**). MS recorded in 15th week were found to be lower in the immunotherapy group in the peak pollen time ($p = 0.02$) (**figure 3b**). VAS scores were also decreased in May-June-July-August in the immunotherapy group ($p = 0.003$, $p < 0.001$, $p = 0.007$, $p = 0.002$) (**figure 4a**).

Quality of life

There was no difference between the immunotherapy and control groups with regard to overall score and domains of Mini-RQLQ questionnaire before the pollen season (Time 2) ($p = 0.17$, $p = 0.18$, $p = 0.44$, $p = 0.33$, $p = 0.46$, $p = 0.57$, respectively) (figure 5a). However, improvements in activities and practical problems and non-nose/eye symptoms quality of life domain scores were significantly better in the immunotherapy group after the pollen season (Time 3) ($p = 0.001$, $p = 0.03$, $p = 0.01$, respectively) (figure 5b).

Clinical assessment in monosensitized and polysensitized patients

Symptom-medication scores and VAS

No difference was found between monosensitized and polysensitized patients with respect to weekly SS and MS in the immunotherapy group ($p > 0.05$) (figure 3c,d). VAS scores of the polysensitized group during March, April and June was significantly lower than monosensitized patients in the immunotherapy group ($p = 0.04$, $p = 0.02$, $p = 0.04$) (figure 4b).

Quality of life

All domains of Mini-RQLQ quality of life were significantly higher in the monosensitized group compared with the polysensitized group before the pollen season (Time 2) ($p = 0.002$, $p = 0.01$, $p = 0.005$, $p < 0.001$, $p = 0.01$, $p = 0.002$, respectively) (figure 5c). After the pollen season (Time 3), there was no difference between the monosensitized and polysensitized patients with regard to overall score and domains of Mini-RQLQ questionnaire ($p = 0.31$, $p = 0.37$, $p = 0.24$, $p = 0.19$, $p = 0.11$, $p = 0.23$, respectively) (figure 5d).

Allergen-specific IgE and IgG4 levels in the immunotherapy and control groups

Phl p sIgE values just after immunotherapy were higher than baseline levels in the immunotherapy group ($p < 0.001$). There were no significant differences in terms of IgE values between the immunotherapy and control groups after immunotherapy ($p = 0.1$). In the immunotherapy group, Phl p sIgG4 values after immunotherapy were found to be significantly higher than baseline

levels ($p < 0.001$). Phl p sIgG4 levels after immunotherapy were significantly higher in the immunotherapy group than in the control group ($p < 0.001$) (figure 6a).

Allergen-specific IgE and IgG4 levels in monosensitized and polysensitized patients

There was no difference in Phl p sIgE levels between mono and polysensitized patients in the immunotherapy group at two time points ($p = 0.38$, $p = 0.42$, respectively).

Furthermore, Phl p sIgG4 values did not differ between monosensitized and polysensitized patients at baseline, and just after immunotherapy ($p = 0.999$, $p = 0.5$, respectively) (figure 6b).

Correlations

A weak negative correlation was observed between the baseline activities-practical problems-nasal symptoms-overall QoL domain scores, 5th week SS and sIgG4 values after immunotherapy

Figure 2 - Distribution of sensitivity to inhalant allergens other than grass pollen in polysensitized patients in the immunotherapy and control groups.

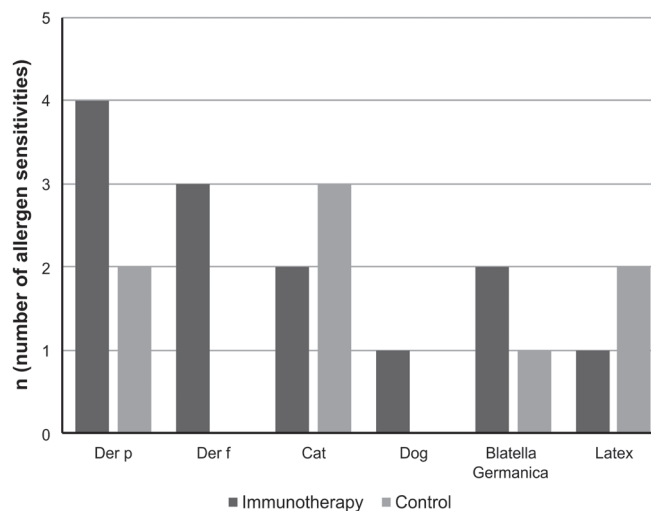
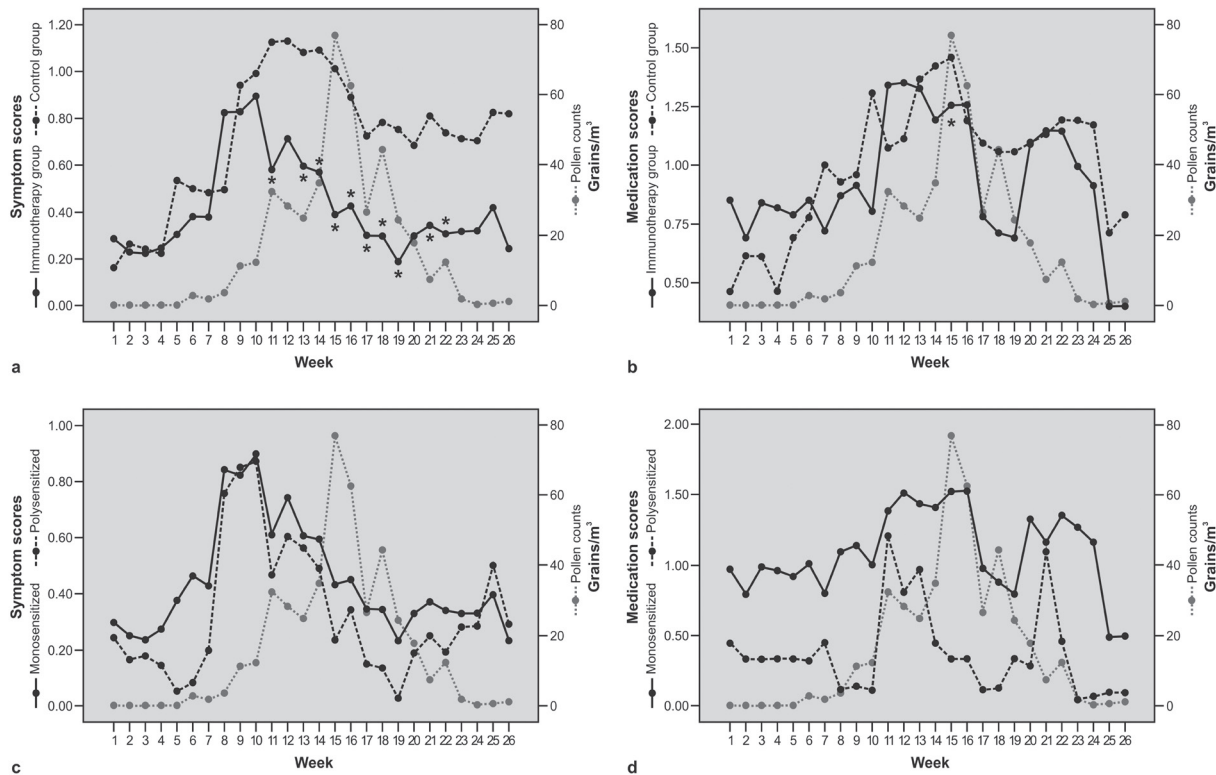


Table I - Characteristics of the study population.

	Immunotherapy group	Control group	P-value
Sex (F/M)	29/17	17/11	0.8
Age, years (Mean \pm SD)	34.9 \pm 10.6	34.2 \pm 12	0.5
Median duration of rhinitis, years (min-max)	1-30	1-25	0.3
Monosensitized/Polisensitized	37/9	20/8	0.3
Results of skin-prick testing, mm*	7.9 \pm 3.8	7.7 \pm 3.4	0.7

*Values are the mean \pm SD wheal diameter (to a mixture of six grasses).

Figure 3 - Weekly symptom-medication scores and pollen counts: (a,b) immunotherapy and control groups; (c,d) monosensitized and polysensitized patients in the immunotherapy group.



* $p < 0.05$.

($r = -0.36$, $p = 0.01$; $r = -0.32$, $p = 0.03$; $r = -0.37$, $p = 0.01$; $r = -0.33$, $p = 0.02$; $r = -0.34$, $p = 0.02$, respectively). There was no significant correlation between sIgG4 levels and MS or VAS scores in the immunotherapy group.

Discussion

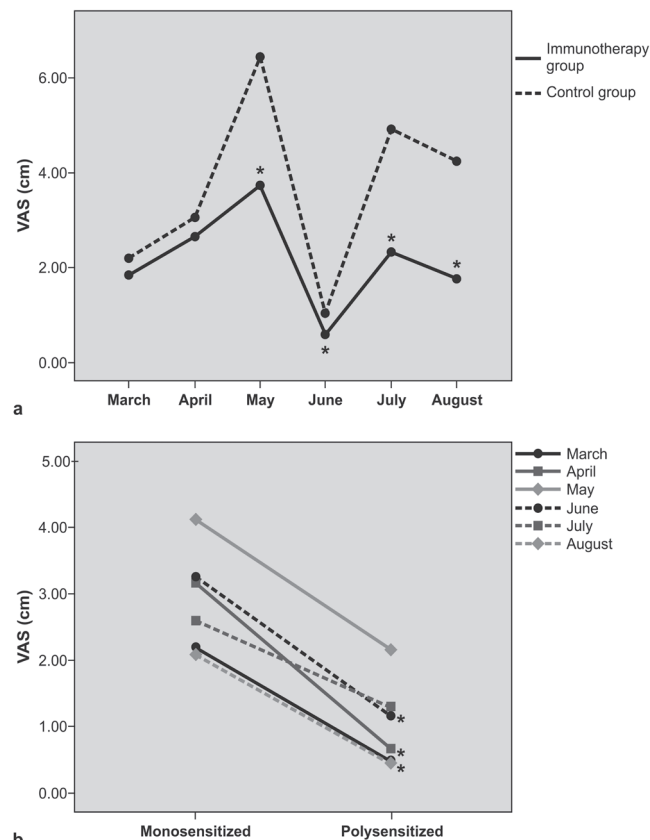
As an important finding, this study demonstrated that clinical improvement with pre-seasonal allergoid immunotherapy is accompanied by a significant increase in sIgG4 blocking antibodies despite short-term injections. Furthermore, this effect was comparable between polysensitized but monoallergic and monosensitized patients. To our knowledge this is the first study to show the clinical and immunological efficacy of pre-seasonal allergoid immunotherapy in monosensitized and polysensitized patients with seasonal allergic rhinitis.

Allergen-specific immunotherapy is the only immunomodulatory treatment modality that leads to the development of long-term tolerance to allergens. The formation of peripheral T cell tolerance to allergens with immunotherapy plays a critical role (10).

In this study we measured specific IgG4 to assess the immunological effect of pre-seasonal allergoid immunotherapy. However, it should be emphasized that the production of IgG4 blocking antibodies is also associated with a number of other immunological mechanisms. Mast cell and basophil desensitization are responsible for the early effects after initiation of therapy. Then, modulation of T and B cell responses, induction of peripheral T regulatory (Treg) cells, increase in IL-10 and TGF- β levels, changes in allergen-specific antibody responses (decrease in IgE, increase in blocking antibodies such as IgG4 and IgA) occur. In the late response, the production of mast cells, eosinophils and their mediators is reduced in the target tissue (11). IgG4-related immunological effects are also responsible for clinical effects following reduction of allergic inflammation (12).

Although there are studies demonstrating that pre-seasonal allergoid immunotherapy is clinically effective, there is limited data regarding its immunological effects. It is expected that immunological and clinical effects of allergoid immunotherapy emerge earlier and become more marked in contrast to conventional immunotherapy (3, 4). In the placebo-controlled study of Pas-

Figure 4 - Monthly VAS scores: (a) immunotherapy and control groups; (b) monosensitized and polysensitized patients in the immunotherapy group.



* $p < 0.05$.

torella *et al.*, it was reported that symptom-medication scores in May were significantly lower than placebo group and there was a significant increase in sIgE, sIgG1 and sIgG4 levels in the early period in active groups with seasonal allergic rhinitis. However, higher sIgG4/sIgG1 ratio was found to be associated with high symptom-medication scores (13). In the placebo-controlled study of Corrigan *et al.* in 154 patients with seasonal allergic rhinoconjunctivitis responsible from grass pollen, it was demonstrated that symptom and medication scores were significantly lower and sIgG1 vs sIgG4 levels were higher than placebo group in the pre-seasonal immunotherapy group (2). In a study comparing perennial and pre-seasonal immunotherapy, the increase in sIgG4 levels at the end of 2nd year was found to be higher in the perennial group, however the difference between two groups was not significant indicating that pre-seasonal immunotherapy had also an early immunological effect (11, 14). Additionally, an early improvement in clinical outcomes and quality of life accompa-

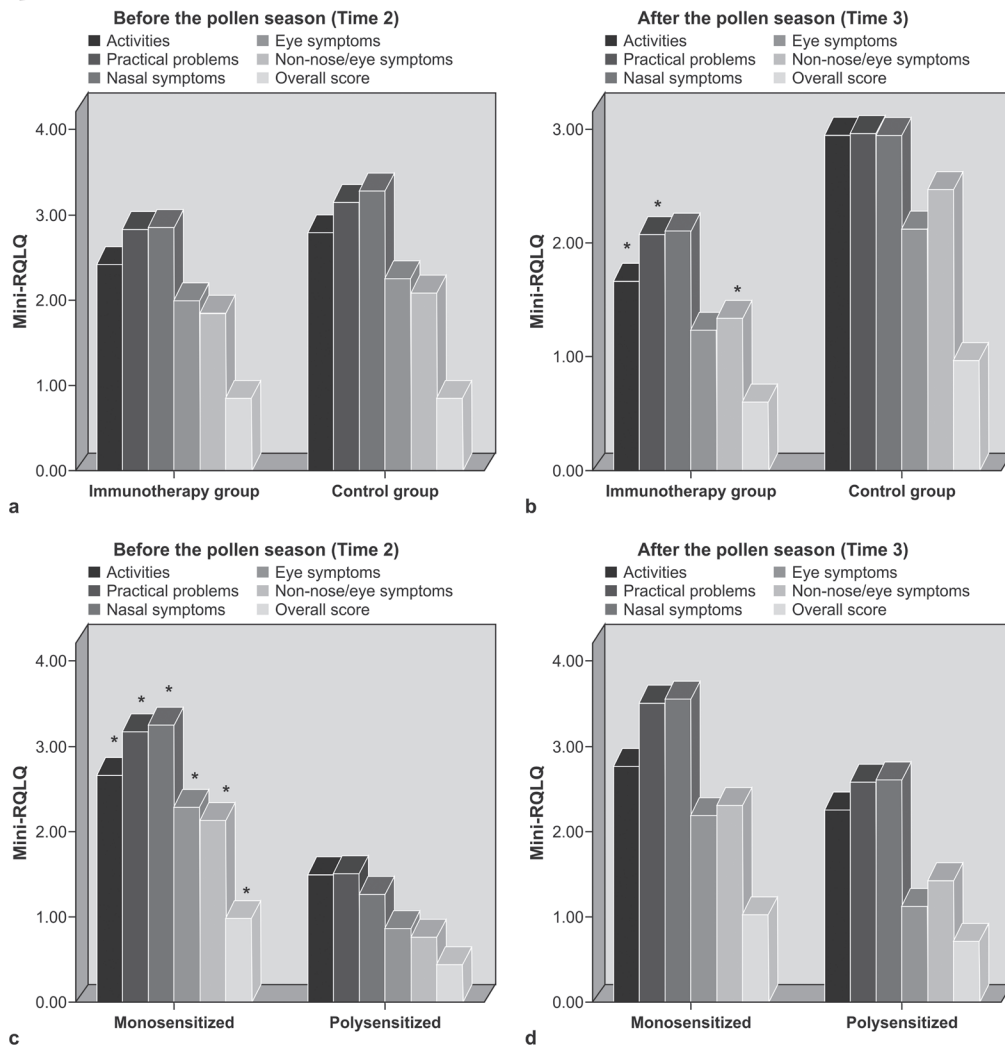
nied by sIgG4 increase was also demonstrated with pre-seasonal immunotherapy (15, 16). The findings of this study were in accordance with previous studies in which significant clinical improvement was shown with allergoid immunotherapy.

We know that conventional immunotherapy induces increase in the allergen-specific IgG4 antibody production within a few weeks. In our study, we also looked for the answer to question on how allergoid immunotherapy affects specific IgG4 levels in early period. Phl p sIgG4 levels were found to be increased after 7 weeks of allergoid immunotherapy in the active group compared to control subjects as well as it was accompanied by significant improvement in symptom and drug scores in this study. Increase in sIgG4 with allergoid immunotherapy is important as it indicates the emergence of humoral immune response in B lymphocytes in early period of allergoid immunotherapy. In our opinion the sustained immunological benefit achieved after short term allergoid immunotherapy is also a notable finding of this study. In support of our findings, in another study performed in our clinic, we found that sIgG4 levels were significantly higher after pre-seasonal allergoid immunotherapy in patients with grass pollen allergy (17). It is expected that antigenic stimulation induces specific IgE production during the early period generally first 6 months of immunotherapy and then starts to decrease synthesis of IgE antibodies. In accordance with this findings, we detected increase in specific IgE antibody levels in patients receiving immunotherapy. Nevertheless, there was no difference between active and control groups in terms of sIgE levels after 7 weeks of immunotherapy.

According to epidemiological and clinical studies, it was established that 50-80% of cases with allergic rhinitis diagnosed polysensitization. Polysensitized patients display a different clinical profile than monosensitized ones since their condition is associated with more severe disease that influences quality of life more markedly (18). It was usually believed that immunotherapy was less effective in polysensitized than monosensitized patients in previous years, however it has been recently demonstrated that immunotherapy was efficacious in polysensitized patients as well (1, 6). In contrast to previous studies, we observed that single allergen immunotherapy with grass pollen extract in which it was most relevant allergen responsible for the most bothersome symptoms, can lead to both clinical improvement and also humoral changes such as increase in blocking antibody production in polysensitized but clinically monoallergic patients.

In the literature, most of the studies has been reported about the effectiveness of sublingual and tablet forms for grass pollen extract. It is seen that previous comparison studies between monosensitized and polysensitized patients were focused on sublingual route (19, 20). However, Passalacqua *et al.* highlighted that the optimal regimen is pre-seasonal immunotherapy in patients with seasonal allergic rhinitis (21). In a single study, conventional subcutaneous immunotherapy performed with a

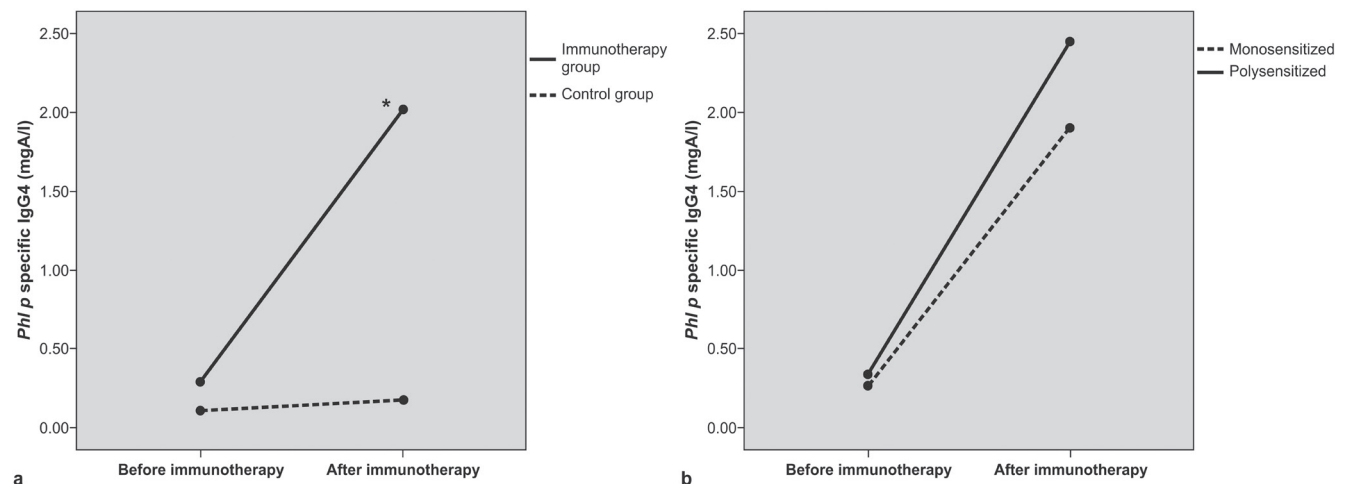
Figure 5 - Mini-RQLQ domains: (a,b) immunotherapy and control groups; (c,d) monosensitized and polysensitized patients in the immunotherapy group.



* $p < 0.05$.

single grass pollen in patients with seasonal allergic rhinitis, was found to be effective and safe, and no difference was found between monosensitized and polysensitized patients with respect to symptom scores and quality of life (22). Additionally, authors recently consider that in case of polysensitized patients, if they have no seasonal symptoms related to grass pollens and most relevant perennial allergen responsible for clinical symptoms, it may be recommended single-AIT (23). In another study carried out in our clinic, we found that increase in sIgG4, sIgE and total IgE antibodies after cluster immunotherapy performed with single Der p allergen was more marked in polysensitized patients than that in monosensitized patients (24). In addition to these

data, in this study we demonstrated that the clinical effectiveness of single (Der p) allergen immunotherapy was comparable between monosensitized and polysensitized patients who had clinically monoallergy to most relevant house dust mite allergen. In our study, sensitization profile in polysensitized patients was shown in **figure 2**. House dust mites and cat allergen sensitization was found as predominant perennial allergens. However, all patients had described only seasonal allergic symptoms due to grass pollen sensitization suggesting clinically relevant monoallergy. Main limitation of the present study is that it is not a double-blind placebo-controlled study. However, we used a group receiving only drug treatment as control group who have similar

Figure 6 - *Phl p* specific IgG4. (a) Immunotherapy and control groups; (b) monosensitized and polysensitized patients in the immunotherapy group.* $p < 0.05$.

clinical characteristics with the active treatment group. We believe that using such control groups is also valuable in immunotherapy studies, as we compared both groups with objective parameters. Another limitation is that the number of polysensitized patients is lower than the number of monosensitized patients. Based upon the data obtained in this preliminary study, it may be suggested that in patients with seasonal allergic rhinitis, pre-seasonal short-term allergoid immunotherapy leads also to production of protective type sIgG4 blocking antibodies during early period despite the increase in sIgE as well in conventional immunotherapy. In addition, clinical improvement in the patients is quite promising for the early period of pre-seasonal immunotherapy. Importantly, improvement in symptom-medication scores and quality of life after allergoid immunotherapy was found to be similar between monosensitized and polysensitized groups despite lower number of polysensitized patients in contrast to false beliefs. This finding was accompanied by the increases in sIgG4 after immunotherapy both in monosensitized and also polysensitized patients. In conclusion, although our study suggests that in early period of allergoid grass pollen immunotherapy polysensitized monoallergic patients may benefit as much as monosensitized patients do, these results need to be further supported by clinical and immunological effectiveness of immunotherapy in large-scale studies.

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Contributions

DM: conceptualization. DM, ŞS: study design, funding. ZM, BAS: supervision. ŞS, DS, CD: materials. ŞS, DS: data collection and/or processing. DG: statistical analysis and/or data interpretation. ŞS: literature review, manuscript preparation. ŞS, DM, ZM, BAS: critical review.

Conflict of interests

The authors declare that they have no conflict of interests.

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