Serum Level of IL 10 is Significantly Increased in Allergic Rhinitis Patients on Subcutaneous Immunotherapy and Vitamin D Supplementation

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For allergic rhinitis (AR), subcutaneous immunotherapy (SCIT) is proved to be effective in improving symptoms and outcome. It increases IL-10 production which helps in inducing peripheral tolerance to allergens. The role of vitamin D supplementation as an adjuvant to SCIT in patients with allergic rhinitis should be investigated. Objective of this study was to assess role of Vitamin D supplementation with SCIT in inducing tolerance to pollen, increasing IL10 and improving symptoms in AR patients. 48 AR patients were included. Skin prick test was done then baseline and final nasal symptoms scores rating, serum level of IL 10 and specific IgE were measured in two groups of patients; group 1 on SCIT and group 2 on SCIT and vitamin D supplementation. A statistically significant decrease of total nasal symptoms scores in group 2 when compared with group 1 (*P* < 0.001). IL 10 was increased in group 2 than group 1 with a statistically significant difference (*P* < 0.001) while, specific IgE was decreased in group 2 than group 1 with a statistically significant difference (*P* =0.01).There was a significant negative correlation between serum level of IL 10 and both nasal symptoms scoring and specific IgE (p < 0.001 and 0.028, respectively). In conclusion, serum level of IL 10 is significantly increased in AR patients on SCIT and Vitamin D supplementation.

Allergic rhinitis is an IgE-mediated inflammation of mucous membranes of the upper airways involving nose, sinuses, eyes, middle ear, Eustachian tubes, and pharynx [1]. In 2010, it affected up to 10-30% of population wide world resulting in nasal congestion, itching, sneezing, and clear rhinorrhea. Severe AR is usually associated with impairments in quality of life, disrupting sleep and work performance [2].

Specific immunotherapy (SIT), known as desensitization or hypo-sensitization, is a main line of therapy for allergic diseases; attenuating allergic symptoms, inducing blocking antibodies and generating tolerant immune cells. It has been used for several years in allergic diseases management with documented therapeutic effects. However, further improvement of SIT is still needed [3, 4].

New hypothesis links AR to subnormal level of vitamin D (1α,25- dihydroxyvitamin D_3) (1,25 D_3). Vitamin D has several effects on the innate and adaptive immune systems, which might be relevant in the attenuation or protection against allergic symptoms, modulation of the severity of exacerbations and reduction of morbidity [5]. Vitamin D also modulates regulatory T-cell (Treg cells) function and interleukin-10 (IL-10) production which regulates the effector response associated with allergic diseases, including inhibition of cytokine production by T-helper-2 cells (Th2) as well as eosinophil and mast cell function [6].

This study aimed to evaluate the role of vitamin D supplementation as an adjuvant to SCIT for treatment of AR patients.

Subjects and Methods

Subjects

This study is a non-randomized controlled trial including 48 patients suffering from vitamin D deficiency and AR to date palm pollen. They were recruited from the Allergy and Immunology Unit, Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University from December 2018 to May 2019.The study was reviewed and approved by the Institutional Review Board (IRB), Faculty of Medicine, Zagazig University [IRB number: 5570, 2018]. Informed consents were obtained from all the study participants.

Inclusion criteria: Patients suffer from AR with allergy to date palm pollen and moderate vitamin D deficiency.

Exclusion criteria: Patients that were allergic to allergens rather than date palm pollen, severe persistent asthma, associated broncho-pulmonary disorders, infectious diseases, other respiratory or systemic diseases, steroid-dependent, non-cooperative patients, pregnant females, smokers, and patients on immunotherapy before the start of the study.

Patients were divided into 2 groups: group 1; patients on subcutaneous immunotherapy for date palm pollen and group 2; patients on subcutaneous immunotherapy for date palm pollen and vitamin D supplementation.

Patients were subjected to: Full detailed allergy history and vitamin D level assay, Nasal symptoms scoring, Skin prick test, Specific IgE test for date palm pollen, IL 10 assay, Symptoms scoring, IL 10 and specific IgE were assessed at baseline and after 6 months of Immunotherapy, Nasal symptoms scoring: Nasal symptoms were ranked in AR patients as in Table 1 according to Modh *et al.*, 2014 [7]:

Table 1.Description of nasal symptoms and their scores

Nasal symptom	Scoring*
Rhinorrhea	0-3
Nasal blockage	0-3
Sneezing	0-3
Nasal itching	0-3
Anosmia	0-3
Total nasal symptoms scoring	*0-15

*0 = No symptom evident, 1 = symptom present but not bothersome, 2 = definite symptom that is bothersome but tolerable, 3 = symptoms that is hard to tolerate. Total scoring was detected by summation of the nasal symptoms.

Skin prick test: Forearm was disinfected by 70% ethyl alcohol then using sterile prick lancet, small prick was made through drop of each allergen solution, negative and positive controls which were formerly placed coded with a marker pen corresponding to the allergens and controls being tested. Different Coca's extracted antigens were used from the Allergy and Immunology Unit, Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University. They were; house dust mites, Smoke, Wool, Cotton, Mixed fungi, Hay dust and Date palm pollen. Saline was used as a negative control and histamine dihydrochloride as a positive control. After 15-20 minutes, the drops were wiped off and each wheal was carefully outlined with a pen. Positive reaction to an allergen showed that, skin becomes itchy within a few minutes and then becomes red and swollen with a wheal in the center.

Specific IgE for pollen: 3 ml blood were collected by venipuncture under complete aseptic conditions. Samples were allowed to clot then centrifuged at 1000 x g for 15 minutes. Sera were collected and stored at -20 °C until tested. Enzyme linked immunosorbent assay (ELISA) was used for the quantitative determination of specific IgE in human serum according to manufacturer's instructions (RIDASCREENR Spec. IgE, Germany). Briefly, cellulose discs were placed in the wells of a microwell plate and allergens were attached to them. 50 µl of samples, standard dilutions and controls were added to each well then, the plate was incubated for 1 h at 37°C after covering it. Wash was done using supplied diluted wash buffer, each well was washed 6 times. 50 µl conjugate was loaded into each well then, the plate was incubated for 1 h at 37°C after covering it. Wash was repeated.100 µl of Substrate was added to each well. The plate was incubated at room temperature for 15 min then 50 µl of stop reagent was added to each well. The absorbance was read at 405 nm.

IL 10 assay: IL 10 human ELISA kit was used for quantitative determination of IL 10 in human serum according to manufacturer's instructions (Thermo Fisher Scientific, Austria).Briefly, after preparation of reagents, samples, standard dilutions,100 µL of each standard curve dilution, background control and

diluted sample were added to wells. The plate was incubated for 2 hrs. at room temperature after covering. 300 µL of wash buffer was used to wash each well 5 times. 100 µL of diluted detection antibody was added to each well. The plate was incubated at room temperature after covering it for 1 hour. Wash step was repeated. 100 µ of diluted SA-HRP was added to each well. The plate was incubated at room temperature after covering it for 1 hour. Wash step was repeated. 100 µl of TMB Substrate was added to each well. The plate was incubated at room temperature for 15 mins. 100 µL of Stop Solution was added to wells. The absorbance was read at 450 nm.

Vitamin D supplementation: Subjects in group 2 received 50000 IU weekly for 3 months then, 2000 IU daily of vitamin D supplement for further 3 months.

Statistical Analysis

All data were collected, tabulated and statistically analyzed using SPSS 20.0 software for windows (SPSS Inc., Chicago, IL, USA, 2011). Quantitative variables were described using their means and standard deviations. Chi-square test ($\chi 2$) was used to compare proportions as appropriate. Mann-Whitney test was used to compare quantitative data as appropriate. The strength of the relationship between two sets of data was assessed using Spearman's rank correlation coefficient. Independent t- test was used for quantitative analysis of normally distributed data for detection of difference between two different groups. *P* values for calculated statistics tests were obtained. A *P* value <0.05 was considered statistically significant

Results

This study enrolled 48 AR patients, divided into two groups; group 1 included 24 patients (11 males and 13 females), their age ranged (18-56 years), and group 2 included 24 patients (10 males and 14 females) aged between 19 and 58 years (Table 2).

Table 2. Demographic data of both patient groups.

Variable	Group 1 (n=24) SCI	Group 2 (n=24) SCI+VIT D	*P* value
Age			
Mean ±SD	(35.21±11.11)	34.5±12.12)	NS
Range	18-56	19-58	
Sex			
Male (No%)	11(45.8%)	10(41.7%)	NS
Female (No%)	13(54.2%)	14(58.3%)	

P>0.05 is not significant (NS).

Nasal symptoms scores were measured in both groups at the start and end of study, a statistically significant decrease of total nasal symptoms scores was observed in group 2 when compared to group 1 with a range of 2-6 and *P* <0.001 (Table 3).

At the end of the study, the mean serum level of IL 10 in group 2 was significantly higher than in group 1 (Mean ±SD; 11.98±4.52 versus 4.562±2.467) *P* <0.001 (Table 4).

However, serum level of specific IgE at the end of the study was significantly higher in group 1 as compared to group 2, *P* = 0.01 (Table 5).

Table 3. Nasal symptoms in both groups at the start and at the end of study.

Variable	Group 1	Group 2	*P* value
Baseline nasal symptoms			
Mean ±SD	11±2.13	11.16±2.08	NS
Range	8-15	8-15	
Final nasal symptoms			
Mean ±SD	7.58±2.15	4.04±1.33	<0.001
Range	4-13	2-6	

Independent T test. *P*>0.05 is not significant (NS).

Table 4.Serum level of IL 10 in both groups at the start and at the end of the study:

Variable	Group 1	Group 2	*P* value
Baseline IL 10 (pg/ml)			
Mean ± SD	3.66±2.44	4.03±2.44	
Median	2.8	3.5	*NS*
Range	1.2-8.3	1.3-8.5	
Final IL 10 (pg/ml)			
Mean ±SD	4.56±2.47	11.98±4.52	
Median	4.4	11.95	<0.001
Range	1.4-8.5	1.5-19	

Mann-Whitney test. *P*>0.05 is not significant (NS).

Table 5. Serum level of specific IgE in both groups at the start and at the end of the study:

Variable	Group 1	Group 2	*P* value
Baseline Specific IgE (pg/ml)			
Mean ±SD	25.28±13.83	21.61±16.63	
Median	22.5	19.25	*NS*
Range	0.7-55	0.4-51	
Final Specific IgE (pg/ml)			
Mean ±SD	15.112±10.181	8.4±9.785	
Median	12	6.3	0.01
Range	0.1-32	0-39	

Mann-Whitney test. *P*>0.05 is not significant (NS).

There was a significant negative correlation between serum level of IL 10 and Specific IgE at end of the study (P = 0.028) as demonstrated in Figure 1.

There was a significant negative correlation between serum level of IL 10 and nasal symptoms scores at the end of the study (P <0.001) as demonstrated in Figure 2.

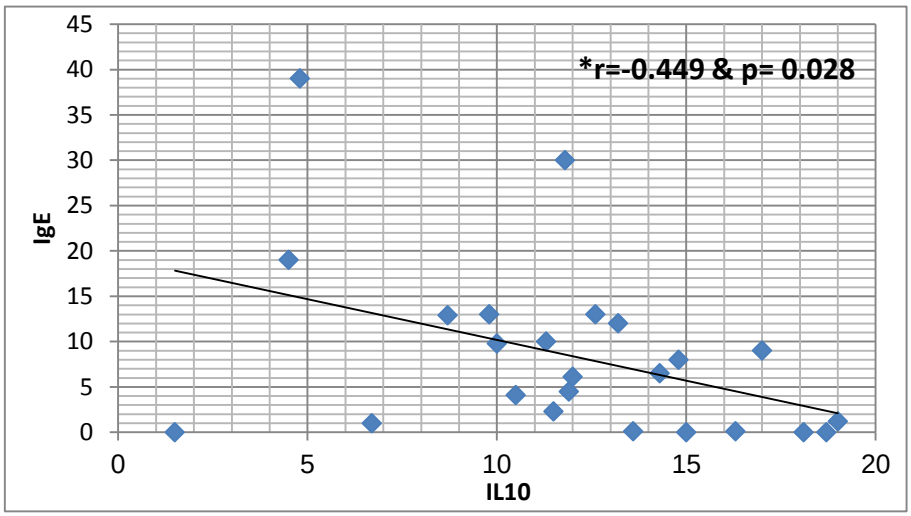

Figure 1. Correlation between serum level of IL 10 and Specific IgE at the end of the study (*Spearman's correlation, P<0.05 significant).

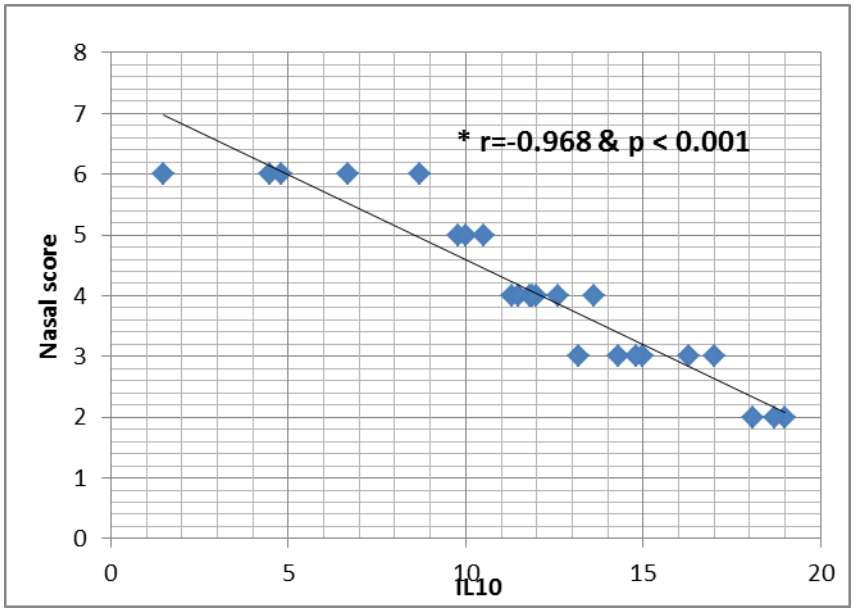

Figure 2. Correlation between serum level of IL 10 and nasal symptoms scores at the end of the study (*Spearman's correlation P< 0.0.1 highly significant).

Discussion

Because more comparative clinical trials are needed to evaluate the safety, clinical efficacy, and cost effectiveness of various adjuvants, we aimed, in the present study, to investigate the efficacy and cytokine response of vitamin D as an adjuvant to conventional SCIT in AR patients by using specific IgE, IL 10 and clinical response score as parameters for evaluation.

In this non-randomized clinical trial, 48 AR patients were divided into two groups. Both groups were homogenous regarding age and sex.

When we assessed the levels of specific IgE to date palm pollen for our patient groups at the start of the study and after 6 months of treatment, we found a statistically significant lower level in group 2 than in group 1. This finding supports the immunomodulator effects of vitamin D on the immune system as reported in other studies that vitamin D modifies and regulates immune cell functions, macrophage differentiation, dendritic cell antigen presentation, increasing the regulatory T cell numbers and activity, and also inhibit Th17 differentiation [8, 9].

Our finding goes hand in hand with the results of Fernandez De Cordova [10] and Grant *et al.* [11] which indicate that vitamin D supplementation reduces the aeroallergen sensitization. Also, the study by Hypponen *et al.* [11] found that serum vitamin D levels had a significantly negative correlation with IgE. Another research by Yip *et al.* [12] in Australia using mice samples suggested the suppression of degranulation of IgE-mediated mast cells after vitamin D administration.

In the current study, we assessed the level of IL 10 for both groups before and after treatment, a statistically significant difference between both groups, after 6 months treatment, was detected. Also, our finding that there was a negative correlation between specific IgE and IL10 after 6 months of treatment goes in line with data of Ashour *et al.* [13] They reported a direct significant relationship between vitamin D levels, IL-10 in asthmatic patients. Also Yip *et al*. [12] declared that IL-10 could be produced by mast cells due to induction by Vitamin D_3. Hypponen *et al*. [14] added that the anti-inflammatory IL-10 can be produced by APC, Th2 cells and mast cells.

Furthermore, our observation of a significant reduction in the total nasal symptoms scores after vitamin D supplementation and SCIT in group 2 of patients, with a significant negative correlation between specific IL10 and nasal symptom scores proved that SCIT with vitamin D supplementation induces clinical improvement of AR patients. This agrees with Malik *et al*. [15] and Modh *et al*. [7] who found similar results. Also Jerzynska *et al*. [16] found improvement of nasal symptoms in children, occurred more with SLIT (sublingual immunotherapy) plus vitamin D group therapy.

The action mechanism of vitamin D could be explained by its ability to control Th2-mediated cell regulation, enhancing tolerance of the plasma cells [17]. Also, inhibition of Interleukin 5 by vitamin D leads to resistance to the process of differentiation, maturation, migration, and infiltration of eosinophils into nasal mucosa [12].

In addition, vitamin D also, has a role in Th1-mediated cell regulation, such as NK cells, TGF-β and IL-10. In addition, it also may decrease IFN-γ which inhibit MHC

class II activation and inhibit TLR causing suppression of inflammatory responses [12].

In conclusion, using vitamin D as an adjuvant to SCIT was effective in decreasing specific IgE, increasing IL10 and improving nasal symptoms in AR patients.

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