Egyptian Journal of Medical Microbiology Volume 33 / No.3 / July 2024 25-31 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Clinical and Immunological Response to Sublingual Allergen-Specific Immunotherapy: Cumulative Dose-Response Relationship

[1]Heba W. Abdelwahab, [1]Amina Abd El-Maksoud, [1]Mohamed A. Elmoneim, [2]Mona I. Shaaban, [3]Mohammed A. Abu El-Enin, [4]Fathalla Belal, [2]Abeer M. Abd El-Aziz*

[1]Chest Medicine Department, Mansoura University, Egypt
[2]Microbiology & Immunology Department, Faculty of Pharmacy, Mansoura University, Egypt
[3]Department of Medicinal Chemistry, Faculty of Pharmacy, Mansoura University, Egypt; Department of Pharmacy, Basrah University College of Science and Technology, Basra 61004, Iraq
[4]Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Mansoura University, Egypt

Key words:
Sublingual-swallow immunotherapy (SLIT); immunological response; clinical response; dose-response relationship; specific IgG4

Corresponding Author:
Abeer M. Abd El-Aziz
Associate Professor of Microbiology & Immunology, Faculty of Pharmacy, Mansoura University, Egypt
Tel.: 01550232220
dr_abeer@mans.edu.eg
amafm3@yahoo.com

ABSTRACT

Background: *Initial studies on sublingual allergen-specific immunotherapy (SLIT) used low doses, but it soon became clear that cumulative dosages higher than the doses used in subcutaneous immunotherapy (SCIT) were required to guarantee clinical efficacy.* ***Objective:*** *This study aimed to evaluate the cumulative dose-dependent clinical and immunological responses to SLIT.* ***Methodology:*** *Patients with allergic airway diseases were included in the study. Patients underwent a skin prick test (SPT) and then received sublingual allergen-specific immunotherapy. Clinical and immunological response variables were recorded at 0, 3, and 6 months intervals of the SLIT schedule (with progressively increasing doses).* ***Results:*** *The study included 74 patients with allergic airway diseases. Symptom and medication scores significantly decreased from baseline (P <0.0001). Hay-specific IgE levels did not decrease significantly, but mite-specific IgE levels decreased by 146%. Hay-specific IgG4 levels increased by 44. 44% after six months of immunotherapy (P<0.01). Eosinophil cationic protein (ECP) levels decreased significantly in patients who underwent six months of sublingual-swallow immunotherapy.* ***Conclusion:*** *There is a clinical cumulative dose-response relationship after SLIT. However, apart from ECP, the immunological cumulative dose-response relationship varies between the allergens.*

INTRODUCTION

Immunotherapy has been utilized for allergy management since 1911[1]. Sublingual-swallow immunotherapy (SLIT) was created as a solution to the issue of systemic reactions associated with standard subcutaneous immunotherapy (SCIT) [2].

Initial studies on SLIT used small dosages; however, it soon became clear that cumulative doses higher than the doses used in SCIT were required to guarantee clinical efficacy[3]. These doses, up to five hundred times the SCIT dosage, have been proven to be safe in previous studies[4].

Nevertheless, because allergen dosages are usually individualized, there are limited data on the dose-response relationship in immunotherapy [5]. Additionally, the efficiency of immunotherapy may depend on factors that affect bioavailability, such as the volume in which the extract is suspended and the method of administration (drops vs tablets in SLIT). As a result, researchers cannot directly compare their findings when using products from different companies to validate a dose-response relationship for a specific allergen extract[5].

While the mechanisms of SCIT are not completely proven, several types of research suggest that the production of 'blocking IgG antibodies' could be valuable for its effectiveness. IgG antibodies block allergen-induced IgE-dependent histamine release by basophils and inhibit the attachment of IgE allergen complexes to antigen-presenting cells[6].

ECP is primarily deposited in eosinophil granules and secreted when secretory constituents (for instance immunoglobulins and complements) are stimulated. ECP can be detected in most biological fluids as a marker of eosinophil inflammation[7]. This study aimed to investigate the cumulative dose-dependent clinical and immunological responses to SLIT in allergic airway diseases.

METHODOLOGY

This pre/post-interventional study included patients over the age of 12 years old with allergic airway diseases such as bronchial asthma (BA) and allergic rhinitis (AR) who were treated at an allergen immunotherapy clinic, Chest Medicine department, Mansoura University. Smokers and patients with autoimmune disorders were excluded from the study. All methods were performed in compliance with the Institutional Research Board guidelines of the Faculty of Medicine at Mansoura University, Egypt. The present study received approval from Mansoura University Institutional Review Board (approval code: PR.20.08.82). Written informed consent was obtained from all participating patients.

BA was diagnosed according to the 2022 Global Initiative for Asthma (GINA) guideline, while AR was diagnosed based on the 2017 Allergic Rhinitis and its Impact on Asthma (ARIA) guideline[8, 9].

Enrolled patients underwent skin prick test and then received SLIT in suitable bottles with a dropper mechanism (produced at the allergen immunotherapy preparation unit, Mansoura University) [10, 11].

The skin prick test included extracts from common aeroallergens in our environment, as well as positive and negative controls. Results were read 15-20 minutes after the test, with a positive result indicated by a wheal diameter ≥3 mm[11].

The initial dilution in SLIT dosing schedule for most patients was 1:10,000. Additional dilution was done for serious cases. Each concentration lasts for two months with a build-up phase and a maintenance phase[12]. Drops applied under the tongue every 3 days. Gradually increasing both the number of drops and the concentration of SLIT over the course of the treatment plan based on the patient's tolerance. The maximum cumulative dose administered to each patient is restricted by their tolerance levels to minimize the potential for side effects. So, the cumulative dose was different from one patient to another.

Clinical and immunological variables were recorded at 0, 3 and 6-month intervals during the SLIT regimen (with gradually escalating doses). Any side effects were also noted.

The clinical response variables included symptom and medication scores[13]. The overall improvement was categorized as follows: complete improvement, where patients had no symptoms and did not require medication (symptom and medication scores = 0); partial improvement, with a decrease in symptom and/or medication scores but not reaching 0; and no improvement in either symptom or medication scores.

Immunological response measurements:

Determination of allergen-specific IgE and IgG4:

Allergen-specific IgE to the selected allergens: hay and mites were measured using Goat anti-human IgE secondary antibody, HRP (Thermo Fischer Scientific, UK). On the first day, polystyrene micro-titer ELISA plates (Nunc, Maxisorp, Denmark) were coated with 100 µl of the tested allergens in carbonate coating buffer and incubated at 4˚ C overnight. Secondly, the plates were washed with a washing buffer three times with TBE/tween (200 µl) and the plates were tapped to remove any remaining wash solution. Each well was blocked with blocking buffer (TBE 200 µl) for 2 h at 26˚C. The plates were washed with washing buffer (three times with TBE 200 µl). Patient's sera were diluted 1:10 and added to each well (in duplicate) and incubated overnight at 4˚ C. Negative control wells containing only buffer were also included in each plate. On the third day, micro-titer ELISA plates were aspirated and washed thoroughly. Monoclonal Goat anti-human IgE secondary antibodies were diluted 1:1000 and added to each well (100 µl). The plates were then incubated for 2 h at 26°C. The reaction was visualized by TMB substrate solution (ThermoFischer Scientific, UK), 50 µl of TMB (Sigma-Aldrich, USA) was added to each well and incubated in the darkness for 20 min. To cease the reaction, 100 µl 1M HCl solution was introduced to each well. The color development was detected at 405 nm with an ELISA micro-titer plate reader (Bio Tek instruments EI800, USA).

Similarly, allergen specific IgG4 levels to hay and mite were measured using a Mouse anti-human IgG4 FC secondary antibody, HRP (ThermoFischer Scientific, UK) in an Enzyme Linked Immunosorbent Assay following the manufacturer's instructions. The percentage change in IgE and IgG4 levels at three or six months after treatment was calculated for each patient compared to their levels before treatment. The average percentage change (Change %) was then calculated for all patients in each group.

Determination of human serum ECP:

ECP in serum was analyzed as a predictor for short-term efficacy after SLIT. ECP levels were determined at baseline level, 3 months, and 6 months after allergen-specific SLIT [14].

For quantitative determination of ECP levels in blood sera, a commercial sandwich enzyme-linked immune sorbent analysis kit (Cat. No: E1391Hu, Bioassay Technology Laboratory, China) was utilized following the kit's instructions. Briefly, anti-human ECP pre-coated plate was bound with ECP present in the sample serum. Next, biotinylated human ECP antibody was introduced to interact with the sample ECP. Subsequently, streptavidin-horseradish peroxidase conjugate was added to form a complex, and the plate was then incubated at 37 °C for 1 hr. After washing, unbound streptavidin-HRP was washed away, and the bound complex was incubated with two substrate solutions for 10 min at 37 °C. The reaction was halted by adding 50 µl stop solution, and the resulting color

was subsequently measured at 405 nm using a microplate reader (Bio Tek instruments EI800, USA). The absorbance was transformed to concentration levels using a standard calibration curve. The inter-assay and intra-assay coefficients of variation (CV) of the used ECP kit were lower than 10% and 8% respectively while the limit of detection was found to be 0.25 ng/ml.

Statistical analysis:

Variables were analyzed using SPSS v.16. Continuous variables were presented as mean (SD). Categorical variables were presented as numbers and percentages. The Wilcoxon Signed Ranks Test was used for comparing paired ordinal data, while paired t-tests were used for continuous data. A significance level of 0.05 was chosen for the p-value.

RESULTS

Inclusion criteria

This study included seventy-four patients with allergic rhinitis (AR) and bronchial asthma (BA). Clinical response was assessed in 61 patients after 3 months of SLIT due to treatment interruption. Nineteen patients continued SLIT until the study's completion (after 6 months of SLIT). The mean age of enrolled patients was 27 ±12.5 years, with 63.5% being female. BA was diagnosed in 68.9% of the patients, while 74.3% had AR. Pollen sensitization was found in approximately 77% of the patients, mold sensitization in 68.9%, and mite sensitization in 60.8% (Table 1).

The cumulated doses (in µg) administered during the study were 86.38±39.8 after 3 months and 1372.4±631after 6 months of SLIT (Table 2, Table 3).

Table 1: Characteristics of patients.

	n (74)	**%**
Age, years (mean ±SD)	27 ±12.5	
Sex		
Males	27	36.5
Females	47	63.5
Diagnosis *		
Allergic rhinitis	55	74.3
Bronchial asthma	51	68.9
Allergen sensitization pattern*		
Molds	51	68.9
mp1	30	40.5
mp2	27	36.5
Cotton dust	14	18.9
Chicken feather	12	16.2
Straw	24	32.4
Mite	45	60.8
Cat	17	23
Pigeon feather	13	17.6
Wheat	25	33.8
Hay	37	50
Dog hair	9	12.2
goat hair	15	20.3
Baseline symptom score		
0 / 1/ 2 /3	0/3/28/43	0 /4.1/ 37.8 /58.1
Baseline medication score		
0 /1 /2 /3	0/7/52/15	0 /9.5 /70.3 /20.3

**Not mutually exclusive.mp1: mixed pollen 1(Chenopodium album, Conyza, and Tamarix aphylla pollen combined in one bottle. mp2: mixed pollen 2 (Polypogon monspeliensis, Cynodon dactylon, and Arundo donax are mixed in additional bottle)*

Improvement of symptoms and medication scores after SLIT

After 3 months of SLIT, there was a significant reduction in symptoms and medication scores compared to baseline levels (*P*= <0.0001). Of the participants, 72.1% showed partial improvement in symptom scores, while 24.6% showed partial improvement in medication scores. Complete improvement in medication scores was seen in 29.5% of patients. By the 6-month mark, 26.3% had completely improved in symptom scores, a significant increase from 0% at 3 months (*P*= 0.002). Additionally, the percentage of patients with complete improvement in medication scores rose to 63.2% (*P*= 0.02). Throughout the study, no severe adverse effects were reported, with only 4.7% experiencing vomiting or sore throat (Table 2, Table 3).

Table 2: Improvement of symptoms score and medication score after 3 months of sublingual immunotherapy.

	n (61)	(%)	*P* value
Symptom score **0 / 1/ 2 /3**	0/26/26/9	(0/42.6/42.6/14.8)	Z=-6.1* P <0.0001
Medication score **0 /1 /2 /3**	18/14/36/3	(29.5/23/42.6/4.9)	Z=-5.06* *P* <0.0001
Improvement (symptom score) **Complete improvement** **Partial improvement** **No improvement**	 0 44 17	 (0) (72.1) (27.9)	
Improvement (medication score) **Complete improvement** **Partial improvement** **No improvement**	 18 15 28	 (29.5) (24.6) (45.9)	
Cumulated dose (µg) administered after 3 months (mean ±SD)	86.38±39.8		

**Wilcoxon Signed Ranks Test*

Table 3: Improvement of symptoms score and medication score after 6 months of sublingual immunotherapy.

	n (19)	(%)	*P* value
Symptom score **0 / 1/ 2 /3**	5/13/1/0	(26.3/ 68.4/5.3/0)	Z=-3.8* *P* <0.0001
Medication score **0 /1 /2 /3**	12/1/6/0	(63.2/5.3/31.6/0)	Z=-3.4* *P* 0.001
Improvement (symptom score) **Complete improvement** **Partial improvement** **No improvement**	 5 14 0	 (26.3) (73.7) (0)	Z=-3.05* *P* 0.002
Improvement (medication score) **Complete improvement** **Partial improvement** **No improvement**	 12 2 5	 (63.2) (10.5) (26.3)	Z=-2.2* *P* 0.02
Cumulated dose (µg) administered after 6 months (mean ±SD)	1372.4±631		

**Wilcoxon Signed Ranks Test*

Immunological response to SLIT

Regarding the immunological response to SLIT, there was no substantial decrease in hay-specific IgE levels, however, a substantial decrease (146%) in the mite-specific IgE levels was detected. Moreover, a prominent decrease (172%) in the mite specific IgE levels was also found after six months of treatment (*P*<0.01) (Table 4).

Table 4: Levels of Serum-specific IgE after 3 and 6 months of sublingual immunotherapy

IgE levels	OD450 nm		% Change	*P* value	OD450 nm	% Change	*P* value
	Before Immunotherapy	After three months			After six months		
Hay-specific IgE	0.039±0.004	0.0536±0.007	+ 21	<0.05	0.075±0.010	+ 39	<0.01
Mite-specific IgE	0.718±0.065	0.330±0.03	- 146	<0.01	0.416± 0.041	- 172	<0.01

OD450 nm: optical density at 450 nm, mean ±SD, +: percent increase, - : percent decrease

Hay-specific IgG4 levels showed a significant increase (44.44%) after six months of SLIT (*P*<0.01). However, no substantial increases in its values were detected after 3 months of SLIT (*P*>0.05). Also, no substantial differences between the levels of mite specific IgG4 either after 3 or 6 months of SLIT (Table 5).

Table 5: Levels of Serum-specific IgG4 after 3 and 6 months of sublingual immunotherapy

IgG4 levels	OD450 nm		% Change	*P* value	OD450 nm	% Change	*P* value
	Before Immunotherapy	After three months			After Six months		
Hay-specific IgG4	0.04±0.005	0.0416± 0.003	**+ 3**	**> 0.05**	0.07±0.007	**+ 44.44**	**<0.01**
Mite-specific IgG4	0.078±0.010	0.074± 0.006	**- 4**	**> 0.05**	0.076±0.012	**- 3**	**>0.05**

OD450 nm: optical density at 450 nm, mean ±SD, +: percent increase, - : percent decrease

The serum level of ECP decreased significantly in studied patients who had six months of SLIT compared with their basal measurement ($P = 0.042$). However, no significant difference in ECP levels could be found three months after SLIT compared to the baseline level (Figure 1).

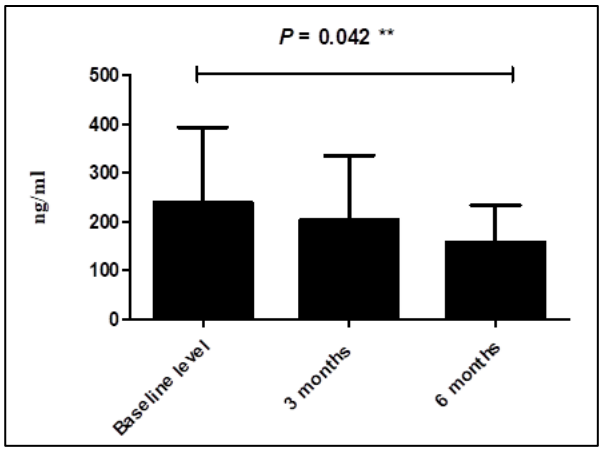

Fig. 1: Serum Eosinophil cationic protein levels after 3 and 6 months of sublingual immunotherapy.
** *Statistical significance*

DISCUSSION

The efficiency of SLIT is a dosage dependent, however, it is also recognized that large doses of allergen extracts are associated with the emergence of systemic reactions. This enhances the requirement of the optimum dose, which was described as, the dose of SLIT causing a clinically significant outcome in most patients without producing intolerable adverse effects[15].

There is a high variability of the liquid SLIT preparations. There was no explanation provided for the dosages used in most of the previous research and the optimum dose with SLIT solutions is not clear[16]. So, our study aimed to detect the cumulative dose-response relationship of SLIT produced in our unit by using our schedule with multiple allergens SLIT. SLIT in the present study included simultaneous administration of more than two non-cross-reacting allergens by SLIT. In Europe, SLIT is mainly used as a monotherapy and the majority of multiple-allergen SLIT had only two allergen extracts[17].

The cumulated doses (in µg) given during the study were 86.38±39.8 after 3 months and 1372.4±631after 6 months of SLIT (Table 2, Table 3).

Despite the study design was not that of a dose-response study design, data analysis powerfully supports a dose-response relationship. The study demonstrated a significant decrease in symptoms and medication scores after 3 months of SLIT. Following 6 months of SLIT with higher cumulative doses, 26.3% of patients showed complete improvement in symptom score compared to 0% at the 3-month follow-up. Additionally, the percentage of patients with complete improvement in medication score increased to 63.2%.

Despite the high cumulative dosages used throughout the study, SLIT was efficient and safe. No severe adverse effects were detected during the study.

Also, the study of *André et al* [1] proposed an association between the cumulative dosage of SLIT (with ragweed pollen extract tablets) and the clinical efficacy in patients with AR. In contrast to our study, *André et al* [1] had the placebo group in addition to the active treatment group.

In our study, the immunological response to SLIT showed no significant decrease in hay-specific IgE levels, but a significant decrease (146%) in mite-specific IgE levels was observed. Additionally, hay-specific IgG4 levels significantly increased after six months of SLIT, with no significant changes detected after 3 months. There were no significant differences in mite-specific IgG4 levels after 3 or 6 months of SLIT.

Marcucci et al [15], concluded that SLIT with a cumulative dose 375 times higher than that of SCIT was more efficient than SLIT with a cumulative dose 85 times higher than SCIT. They also observed a decrease in both IgE and IgG4 levels after seasonal exposure in the group of patients who received higher doses of SLIT.

In *Didier et al* [18], A dose-response relationship was noted as the 100-IR dose was more efficient than the placebo but less efficient than the 300-IR and 500-IR

doses. Also, there was a progressive increase in IgG4 level with raising SLIT dose, which indicates a dose impact for IgG4.

Furthermore, *Durham et al* [19] approve the dose-dependent effectiveness of the grass allergen tablet.

In the study conducted by *Nikolov et al* [20], specific IgE did not show significant differences in patients receiving SCIT. the difference between IgE levels and allergic reactions could be due to differences in the specificity of IgE for tested allergens. Similarly, IgE specific to peanuts was not considered a significant indication of immunotherapy or clinical reactivity[21].

The ragweed-specific IgG4 and mite-specific IgG4 were increased after SLIT in *Piconi et al* [22] and *Virchow et al* [23].

Likewise, specific levels of IgG4 often fail to correlate with tolerance. In some studies, sublingual immunotherapy did not show a significant difference in the levels of IgG4 compared to the placebo groups[24].

Our study demonstrated that a six-month course of SLIT showed a decrease in serum ECP levels as reported in other studies[4,25]. Several studies reported the decline of elevated serum levels of ECP after effective therapy.[26] Thus, controlling circulating eosinophil activation could be one of the significant working mechanisms behind the clinical effect of immunotherapy.

The present study was constrained by a small sample size, in addition to a limited number of patients who completed the entire study.

CONCLUSION

There is a clinical cumulative dose-response relationship after sublingual immunotherapy. However, apart from ECP, the immunological dose-response relationship varies between the allergens.

Author contributions:

H.W.A., M.A.E., M.I.S., M.A.A., and A.M.A. all performed the practical work and analysed the data. H.W.A, M.I.S., and A.M.A. participated in lining out the protocol, troubleshooted, revised the data analysis, wrote the first draft of the manuscript and revised the final format. A.A.E., and F.B. designed and supervised research.

Data Availability:

All study data are included in the article and any other data are available upon reasonable request.

Financial support:

This study was supported by Mansoura University, Egypt.

Competing interests:

All authors declare no conflict of interest.

REFERENCES

1. André C, Perrin-Fayolle M, Grosclaude M, Couturier P, Basset D, Cornillon J, Piperno D, Girodet B, Sanchez R, Vallon C, Bellier P, Nasr M. A Double-Blind Placebo-Controlled Evaluation of Sublingual Immunotherapy with a Standardized Ragweed Extract in Patients with Seasonal Rhinitis: Evidence for a Dose-Response Relationship. Int Arch Allergy Immunol 1 June 2003; 131 (2): 111–118.
2. CSM Update: Desensitising vaccines. Br Med J (Clin Res Ed). 1986 Oct 11;293(6552):948.
3. Bousquet J, Van Cauwenberge P (editors). Allergic rhinitis and its impact on asthma (ARIA). Position paper endorsed by WHO. J Allergy Clin Immunol 2001; 108(Suppl. 5): 147– 334.
4. Wilson DR, Torres-Lima M, Durham S. Sublingual immunotherapy for allergic rhinitis. Cochrane Database Syst Revg 2003; 2: CD002893.
5. Calderón MA, Larenas D, Kleine-Tebbe J, Jacobsen L, Passalacqua G, Eng PA, Varga EM, Valovirta E, Moreno C, Malling HJ, Alvarez-Cuesta E, Durham S, Demoly P. European Academy of Allergy and Clinical Immunology task force report on 'dose-response relationship in allergen-specific immunotherapy'. Allergy. 2011 Oct;66(10):1345-59.
6. Rossi RE, Monasterolo G, Coco G, Silvestro L, Operti D. Evaluation of serum IgG4 antibodies specific to grass pollen allergen components in the follow up of allergic patients undergoing subcutaneous and sublingual immunotherapy. Vaccine. 2007 Jan 15;25(5):957-64.
7. Bystrom J, Patel SY, Amin K, Bishop-Bailey D. Dissecting the role of eosinophil cationic protein in upper airway disease. *Curr Opin Allergy Clin Immunol*. 2012;12(1):18–23. doi:10.1097/ACI.0b013e32834eccaf.
8. Brożek JL, Bousquet J, Agache I, Agarwal A, Bachert C, Bosnic-Anticevich S, Brignardello-Petersen R, *et al* . Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. J Allergy Clin Immunol. 2017;140(4):950-958.
9. Global Initiative for Asthma (GINA) Main Report. *2022 GINA Report, Global Strategy for Asthma Management and Prevention.* https://ginasthma.org/gina-reports.
10. Abu El-Enin MA, Rabie Shehab El-Din EM, Abdelwahab HW, et al. Preparation of chemically stable allergen-specific sublingual immunotherapy

from Egyptian allergens. J Clin Lab Anal 2022;36:e24261.

11. Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, Durham S, *et al*. The skin prick test - European standards. Clin Transl Allergy. 2013 Feb 1;3(1):3.
12. Abdelwahab HW, Abd El-Maksoud A. Nondaily dosing schedule of allergen-specific sublingual immunotherapy: efficacy and safety. Clin Exp Vaccine Res. 2023 Apr;12(2):121-126.
13. Eifan AO, Akkoc T, Yildiz A, Keles S, Ozdemir C, Bahceciler NN, et al. Clinical efficacy and immunological mechanisms of sublingual and subcutaneous immunotherapy in asthmatic/rhinitis children sensitized to house dust mite: an open randomized controlled trial. Clin Exp Allergy 2010; 40:922–932.
14. Marcucci, F., Sensi, L., Frati, F., Senna, G., Canonica, G., Parmiani, S. and Passalacqua, G. (): Sublingual tryptase and ECP in children treated with grass pollen sublingual immunotherapy (SLIT): safety and immunologic implications. Allergy. 2001; 56(11): 1091-1095
15. Marcucci F, Sensi L, Di Cara G, Incorvaia C, Frati F. Dose dependence of immunological response to sublingual immunotherapy. Allergy. 2005 Jul;60(7):952-6.
16. Larenas-Linnemann D. Direct comparison of efficacy of sublingual immunotherapy tablets for rhinoconjunctivitis. Ann Allergy Asthma Immunol. 2016;116(4):274-86.
17. Nelson, H.S. Sublingual Immunotherapy for Aeroallergens: Optimal Patient Dosing, Regimen and Duration. Curr Treat Options Allergy.2014; 1, 79–90.
18. Didier A, Malling HJ, Worm M, Horak F, Jäger S, Montagut A, André C, de Beaumont O, Melac M. Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. J Allergy Clin Immunol. 2007 Dec;120(6):1338-45.
19. Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once-daily grass allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol. 2006 Apr;117(4):802-9.
20. Nikolov G, Todordova Y, Emilova R, Hristova D, Nikolova M, Petrunov B. Allergen-Specific IgE and IgG4 as Biomarkers for Immunologic Changes during Subcutaneous Allergen Immunotherapy. *Antibodies*. 2021; 10(4):49
21. Santos AF, James L. Bahnson HT, Shamji MH, Couto-Francisco NC, Islam S, Houghton S, Clark AT, Stephens A, Turcanu V, Durham SR. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. Journal of Allergy and Clinical Immunology. 2015, 135(5), pp.1249-1256.
22. Piconi S, Trabattoni D, Rainone V, Borgonovo L, Passerini S, Rizzardini G, Frati F, Iemoli E, Clerici M. Immunological effects of sublingual immunotherapy: clinical efficacy is associated with modulation of programmed cell death ligand 1, IL-10, and IgG4. The Journal of Immunology. 2010. 185(12), pp.7723-7730.
23. Virchow JC, Backer V, Kuna P, Prieto L, Nolte H., Villesen HH, Ljørring C, Riis B, de Blay F. Efficacy of a house dust mite sublingual allergen immunotherapy tablet in adults with allergic asthma: a randomized clinical trial. Jama. 2016. 315(16), pp.1715-1725.
24. Pajno GB, Morabito L, Barberio G, Parmiani S. Clinical and immunologic effects of long-term sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, placebo-controlled study. Allergy, 2000; 55(9), pp.842-849.
25. Schamann F, García J. Sublingual immunotherapy with cat epithelial extract. Personal experience. Allergologia et Immunopathologia. 2001; 29(2): 60-65.
26. Kendirli S, Cevit Ö, Inci D, Altıntas D, Yılmaz M, Karakoc G.Effect on serum ECP, NO and MCP-1 level of specific immunotherapy in ashmatic children monosensitized to mite.Journal of Allergy and Clinical Immunology. 2006; 117(3): 722.