Egyptian Journal of Medical Microbiology Volume 27 / No.4 / October 2018133-139 Online ISSN: 2537-0979

ORIGINAL ARTICLE

The Role of Interleukin-9 in Patients with Allergic Rhinitis

[1]Ayman A. Allam, [1]Shymaa A. Mansour*, [2]Tarek A. Emara, [1]Fedaa N. Mohamed, [1]Emad A. Morad

[1]Medical Microbiology and Immunology, Faculty of Medicine, Zagazig University
[2]ENT, Head and Neck Surgery Faculty of Medicine, Zagazig University

Key words:
Allergic Rhinitis, Cytokines, IL-9

Corresponding Author:
Shymaa A. Mansour
Medical Microbiology and Immunology, Faculty of Medicine, Zagazig University
Tel.: 01026919619
shymaa_abdelazim@yahoo.com

ABSTRACT

Background: *Allergic rhinitis (AR) is a common allergic disease. It has a marked effect on quality of life. Various populations of effector and regulatory T cells and their cytokines play a crucial role in allergic inflammation.* ***Objectives:*** *This study was designed to evaluate serum levels of IL-9 in subjects with allergic rhinitis and to investigate the association of its serum level with the severity of the disease, and its clinical parameters.* ***Methodology:*** *This study included 19 AR cases and 19 healthy controls. All patients and controls were subjected to the followings: full medical history, allergy skin test, nasal smear to detect eosinophilia, and measurement of serum levels of IL-9 by enzyme linked immune-sorbent assay (ELISA). Cases were classified according to Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines into mild, moderate and severe.* ***Results:*** *The median values of serum levels of IL-9 in AR cases were higher than in the controls (8.5 vs 6.85 pg/ml) with statistically significant difference (p =0.02). Roc curve analysis, also showed significant association between the serum level of IL-9 in AR patients than controls (P value < 0.0001), the cut point was >7.5 pg/ml in AR patients with sensitivity and specificity of 77.78 and 83.33 % respectively. In addition, there was a significant association between the serum levels of IL-9 and degree of disease severity (P=0.01), and AR symptoms (nasal obstruction and irritative symptoms). There was a significant association between sensitization to multiplicity of allergens and the mean level of IL-9 in AR cases (P< 0.05).* ***Conclusion****: The elevated serum level of IL-9 in AR cases may provide new insight into the patho-physiology of the disease and into development of novel therapeutic targets.*

INTRODUCTION

Allergic rhinitis (AR) is a common allergic disease. Its clinical manifestations include sneezing, rhinorrhea and nasal blockage. [1]. An interaction between both environmental and genetic factors leads to development of AR. IgE-mediated type I hypersensitivity immune response against allergens is the main mechanism for AR[2].

Diverse populations of effector and regulatory T cells play a crucial role in allergic inflammation. Th9 cells lack suppressive function and constitute a distinct population of effector T cells that promote tissue inflammation and mucous production. Th9 produces Interleukin-9 (IL-9) in large quantities and shares uniquely to immune response. Interleukin-9 is a pleiotropic cytokine that is active on many cell types participating in the allergic immune response.[3,4]

It has been claimed that IL-9 increases in the nasal mucosa during the pollen season and significantly decreases after successful allergen-specific immunotherapy[5].Treatment with anti-IL-9 antibodies markedly reduced nasal symptoms as rubbing, sneezing. It also decreased eosinophil infiltration, and responses of Th2, Th9, and Th17, and increased the Treg response[6].

The study was done to evaluate the role of serum level of IL-9 in pathogenesis of AR and to determine its relation to the severity of disease.

METHODOLOGY

The study included 19 cases of AR (who were diagnosed clinically and by intra-dermal skin test) and 19healthy controls who had no history of AR. They were negative by skin allergy tests. Cases were classified according to Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines into mild, moderate and severe AR cases [7].

Full medical history, allergy skin test, and nasal smear to detect nasal eosinophils were done to all patients. Blood samples from patients and controls were withdrawn and tested for measurement of serum levels of IL-9 by ELISA technique.

Intradermal skin tests:

Coca's extracted allergens were used; house dust, smoke, wool, cotton, mixed fungus (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus*), mixed pollens and hay dust. l/1000 diluted allergen extract was used. Saline (0.9% Nacl) was used as a negative control. The intradermal skin testwas done. Results are determined according to the scoring system of the intradermal skin test as follows.[8]

Grade	Erythema	Wheal
Negative	< 5 mm	< 5 mm
0:1+	5-10 mm	5-10 mm
1+	11-20 mm	5-10 mm
2+	21-30 mm	5-10 mm
3+	31-40 mm	5-10 mm or with pseudopods
4+	>40 mm	>15 mm or with many pseudopods

Nasal smear to detect eosinophilia:

Cotton-tipped swab was passed gently through the surface of the middle and inferior concha as far back as possible and rotated to obtain sufficient mucus. The swab is then gently rolled along the surface of a glass microscope slide, air-dried, stained with leishman stain and observed under oil immersion lens. The number of eosinophils was recorded using the criteria for the quantificationas follows:[9]

0 = No cell in any high power field.
+ = 1 to 3 cells some high power.
++ = Some cells in most of the high power field.
+++ = Many cells in all the field.

Serum level of IL-9: this was done by ELISA technique

RESULTS

Table 1 shows the demographic characteristics of the study population. The Patients were aged 10 – 55 years old (mean 30.47 ± 15.01), while the controls were aged (16 – 40) mean (26.72±6.76).

Table 1: Demographic data of the two studied groups:

	Cases (*n*=19)		**Control (*n*=19)**		**T**	***p***
Age (years):						
Mean ± SD	30.47 ± 15.01		26.72 ± 6.76		0.97	0.34
Range	10 – 55		16 – 40			N.S
	No	**%**	**No**	**%**	χ^2	***p***
Sex:					0.23	
Male	8	42.1	9	47.4		0.63
Female	11	57.9	10	52.6		N.S

SD: standard deviation, χ^2**:**Chi-square test; **T:** independent Student t-test

As shown in table (2), AR cases were more common in rural (57.9%) than urban areas (42.1%). 42.1% of patients were students, 68.4 % were nonsmokers. 73.7 % of patients had positive family history, 84.2 % had certain factors worsen the condition, 57.9% had history of operations, 100 % received medical treatment for rhinitis and 42.1 % underwent surgical treatment for rhinitis.

The most common symptom among AR patients was nasal obstruction (73.7%). AR was persistent in 89.5 % and intermittent in 10.5 % of the patients of the study. The association with other allergic symptoms was present in 31.6 %. Diurnal variation of symptoms was present in 57.8 % of AR cases. Seasonal variation of symptoms was present in 42.2 % of AR cases.

Table 2: Risk factors, history and other clinical data of the cases group:

	(n=19)	
	No	**%**
Residence:		
Rural	11	57.9
Urban	8	42.1
Occupation:		
Not working	2	10.5
Farmers	2	10.5
Students	8	42.1
Skilled	4	21.1
Professional	3	15.8
Smoking:		
No	13	68.4
Yes	6	31.6
Family history:		
No	5	26.3
Yes	14	73.7
Certain factors worsen the condition (eg.perfumes, polish …etc:		
No	3	15.8
Yes	16	84.2
Previous treatment for rhinitis:		
1) Medical		
No	0	0
Yes	19	100
2) Surgical		
No	11	57.9
Yes	8	42.1
Symptoms:		
Nasal Obstruction	14	73.7
Rhinorrhea	13	68.4
Sneezing	12	63.2
Nasal itching	5	26.3
Continuinity of symptoms:		
Intermittent	2	10.5
Persistent	17	89.5
Diurnal variation:		
No	8	42.2
Yes	11	57.8
Seasonal variation:		
No	11	57.8
Yes	8	42.2
Association with other allergic symptoms:		
No	13	68.4
Yes	6	31.6
Conjunctival	2	
Asthmatics	4	
Duration of symptoms:		
Mean ± SD	4.24 ± 4.63	
Range	1 – 20	

As shown in table (3), pollens were the commonest positive allergens among AR cases (100%) by allergy skin test. 84.2 % of AR cases were sensitive to multiple allergens while 15.8 % of AR cases were sensitive to a single allergen.

Table 3: Skin test results of the cases group:

	(n=19)	
	No	**%**
Home dust: *+ve*	16	84.2
Smoke: *+ve*	11	57.9
Wood: *+ve*	1	5.3
Fungus: *+ve*	1	5.3
Pollen: *+ve*	19	100
Rice hay: *+ve*	11	57.9
Cotton: *+ve*	0	0
Single allergen	3	15.8
Multiple allergen	16	84.2

As shown in table (4), the serum level of IL-9 was significantly higher in AR cases than in the control group as (p =0.02). Roc curve analysis, (figure 1), also showed significant association between the serum level of IL-9 in AR patients (P value < 0.0001), the cut point was >7.5 in patients with sensitivity and specificity of 77.78 and 83.33 % respectively.

There was no correlation between age of AR cases and IL-9 level. There also was no correlation between the duration of the disease and IL-9 level in AR cases (not shown in tables)

Table 4: Serum level of IL-9 of the two studied groups:

	Cases (*n*=19)	**Control (*n*=19)**	**T**	***P***
IL-9:				
Mean ± SD	7.87 ± 2.85	5.94 ± 2.15	**2.13**	**0.02***
Range	0.7 – 11.1	1.2 – 8.7		
Median	8.5	6.85		

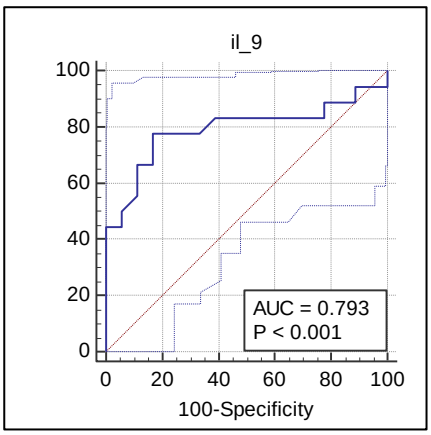

Fig 1: ROC curve analysis of IL-9 of the two studied groups

As shown in table (5), there was a significant relation between the serum level of IL-9 and both nasal obstruction and irritative symptoms and no association between IL-9 levels with other allergic symptoms. There was significant relation between serum level of IL-9 and continuinity of symptoms (p<0.05).

There was no association between serum level of IL-9with diurnal, seasonal variation and presence of other allergic symptoms.

Table 5:Relation between symptoms and IL- 9 level of the case group:

	Nasal obstruction		**T**	***P***
	No (*n*=5)	**Yes (*n*=14)**		
IL-9: *Mean ± SD* *Range*	5.70 ± 2.79 1.9 – 8.3	8.64 ± 2.53 0.7 – 11.1	**2.18**	**0.04***
	Irritative symptoms		**T**	***P***
	No (*n*=2)	**Yes (*n*=17)**		
IL-9: *Mean ± SD* *Range*	5.4 ± 4.66 0.7 – 10.1	8.16 ± 2.35 1.9 – 11.1	**2.16**	**0.04***
	Other Allergic symptoms		**T**	***P***
	No (*n*=13)	**Yes (*n*=6)**		
IL-9: *Mean ± SD* *Range*	7.78 ± 3.17 0.7 – 10.7	8.1 ± 2.67 3.6 – 11.1	0.22	0.83 N.S
	Continuinity of symptoms		**T**	***P***
	Intermittent (*n*=2)	**Persistent (*n*17)**		
IL-9: *Mean ± SD* *Range*	2.75 ± 1.2 1.9 – 3.6	7.9 ± 3.02 0.7 – 11.1	**4.15**	**0.01***
	Diurnal variation		**T**	***P***
	No (*n*=8)	**Yes (*n*=11)**		
IL-9: *Mean ± SD* *Range*	7.45 ± 3.04 1.9 – 10.1	8.17 ± 2.82 0.7 – 11.1	0.53	0.60 N.S
	Seasonal variation		**T**	***P***
	No (*n*=11)	**Yes (*n*=8)**		
IL-9: *Mean ± SD* *Range*	7.29 ± 3.31 0.7 – 10.7	8.63 ± 2.38 3.6 – 11.1	0.96	0.35 N.S

As shown in table (6) and figure (2), there was a significant association between the mean level of IL–9 and degree of disease severity (P<0.05) in AR cases.

Table 6:Relation between severity of symptoms and IL- 9 level of the cases group:

	Severity			**F**	***P***
	Mild (*n*=2)	**Moderate (*n*=8)**	**Severe (n=9)**		
IL-9: *Mean ± SD* *Range*	2.75 ± 1.2 1.9 – 3.6	7.94 ± 3.1 0.7 – 10.7	7.78 ± 3.14 7.1 – 11.1	**6.01**	**0.01***

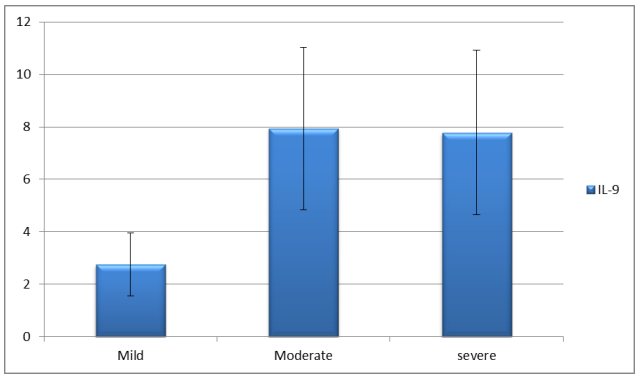

Fig. 2: Relation between severity of symptoms and IL-9 level of the cases group.

As shown in table (7), there was a significant association between sensitization to multiplicity of allergens and the mean level of IL-9 in AR cases (*P*< 0.05).

Table 7: Relation between multiplicity of allergens and IL-9 levels of the two studied groups:

	Single allergen (*n*=3)	**Multiple allergens (*n*=16)**	**T**	**p**
IL-9:				
Mean ± SD	7.2 ± 0.69	9.5 ± 1.8	**2.14**	**0.04***
Range	6.7 – 8.8	0.7 – 11.1		

DISCUSSION

Allergic rhinitis (AR) is a respiratory disease affects people of all ages characterized by inflammation of the nasal cavity [8]. Although not a serious disease, AR has a marked burden on quality of life, and is able to alter patient social life[11].

Current symptoms among our patients were nasal obstruction (73.7%), irritative symptoms; rhinorhea (68.4%), sneezing (63.2%), nasal itching (26.3%) and eye itching (10.53 %). However, a survey done by Amizadeh*et al.*[12]on Kerman high school students to assess prevalence of AR, revealed that symptoms among these patients were nasal obstruction (64.3%), rhinorrhea (76.6%), nasal itching (54.3%) and the rate of eye itching and epiphora was (76.3%).This difference may be explained by the fact that most of our patients had persistant AR, also, due to different locality, genetic susceptibility and different age group.

IL-9 stimulates tissue infiltration by mast cells. T cells, eosinophils, neutrophils, and mast cells are important sources of IL-9. It has been claimed that IL-9 level increases in the nasal mucosa during the pollen season. This IL-9 increase corresponds with tissue infiltration by eosinophils[13]. IL-9 promotes allergic inflammation through effects on hematopoietic cells that then produce IL-13 that affects directly the airway epithelium. However, IL-9 affects directly human primary airway epithelial cells and cells lines as it directly induces mucus genes. Thus, IL-9 may have both direct and indirect effects in the airways [14].

In our study serum level of Il-9 was higher in AR cases (8.5pg/mL) than the control group (6.58pg/mL) with statistically significant difference (*p*=0.02). These results match with Ciprandi[3] who found significant increase in the serum level of IL-9 of the cases.

Ciprandi*et al*,[5]found that median serum IL-9 level in patients with Parietaria allergy was (12.6 pg/ml) and to birch allergy was (4.83 pg/ml). The difference between the cases and control groups was statistically significant (p<0.05).Erpenbeck *et al.*[15] and Fujisawa *et al.*[16] attributed the elevation in IL-9 serum level in AR to allergen exposure which leads to IL-9 induction. So, elevation of IL-9 levels in AR cases showed that IL-9 plays a important role in AR. In addition, in a study on nasal IL-9 expression, the number of IL-9 mRNA–expressing cells in the mucus membrane of the nose of allergic patients increases during the grass pollens season in comparison with baseline values [17].Alsoin a study of Hauber *et al.*[18], they found that exposure to specific allergen significantly increased IL-9-positive cells in nasal mucosa of AR patients whereas saline challenge had no significant effect. IL-9 is the key cytokine to drive mastocytosis[19] and mast cell is one of the key cells in Allergic rhinitis and other allergic diseases.

Other studies claimed an IL-9 association with other allergic diseases. Concentrations of IL-9, IL-4, IL-17A and IL-22 were positively correlated with blood eosinophil numbers in infants with cow milk allergy. Elimination of cow milk decreased plasma concentrations of these cytokines. These data confirm the role of Th2 response in allergic disease and gives a clear evidence for the involvement of Th9 and Th22 cells in allergic disease.[20] In experimental murine asthma, Th9 cells were detected in the respiratory tract and its draining lymph nodes, especially during early phases of the disease [21]. Furthermore, transgenic expression of IL9 is sufficient in itself to cause bronchial hyper-responsiveness through its effect on the respiratory epithelium and the increased release of Th2 cytokines. Transfer of Th9 cells to recipient mice leads to allergic airway disease after treatment with ovalbumin. Furthermore, when these recipient mice were additionally treated with an anti-IL9 antibody, many of the inflammatory features were blocked [22].

In the current study there was a significant correlation between the mean value of IL-9 and the degree of disease severity (*p*=0.01). This result agrees

with Ciprandi[3]who reported that serum IL-9 levels are correlated with symptom severity in pollens-induced AR.

In the present study there was significantassociation between the mean value of IL-9 with the nasal obstruction and also with irritative symptoms. However Ciprandi[3] found that there was a moderate positive relationship between IL-9 and itching, sneezing, and rhinorrhea and a weak negative relationship with obstruction. This difference may be explained by the fact that most of our patients had persistent AR.

In the current study, the skin test revealed that 15.8% of our patient were positive to only one type of allergen while 84.2% of them had multi-allergen sensitization. These results are relatively more or less in consistent with Moitra*et al.*[23]who found that most of SPT positive patients 96.1% were reactive to two or more allergens. In another study, Yuen *et al.*[24] found that 51% of AR patients were sensitive to a single allergen, while 49% were positive to multiple allergens.

Several factors lead to sensitization to multiple allergens. These factors include cross-reactivity between allergens. It is due to the presence of nearly similar allergenic epitopes in botanically close different plant species. Other factors are long-term exposures to close phylogenic source of allergens and interactions of genetic and environmental factors [25].

In the present study, the most dominant allergen causing positive skin test among AR patients was pollen followed by house dust. Also, Bauchau and Durham[26] found that pollen was the most prevalent allergen among AR patients consulting primary care. House dust mite allergens, *Der* and *Der*[27], were the most important sensitizing aeroallergens in china**.**

In Vietnam, Singapore, Thailand and Malaysia, house dust mite and cockroach were the predominant allergens in asthmatics especially adults. House dust mite is the prdominant allergen in the middle and southern part of Europe, while in Finland and Sweden, furry animals such as cat and dog, together with pollen are the main sensitizers.[28]

However other studies claimed that house dust mite was the most prevalent allergen causing positive skin test among clinically suspected AR patients [23,24,29,30]. This difference may be explained by change climate, geography and life style.

Our study also reported that there was a significant association between sensitization to multiplicity of allergens and serum level of IL-9 ($P < 0.05$). This association can explain the association of severe AR with IL-9 serum level as Severity of symptoms was higher in poly-sensitized patients than in mono-sensitized as proved previously by other investigators[31].

CONCLUSION

Serum level of IL-9 in patients with AR was elevated and was correlated with the disease severity. These results suggest that IL-9 contributes to the pathogenesis and pathophysiology of AR. So serum IL-9, an easy, rapid test can help in the diagnosis of AR. The result of this study can also help in giving support to the use of anti IL-9 therapeutics for the management of AR in the future.

REFERENCES

1. Bousquet J, Khaltaev N, Cruz AA, et al.: Allergic rhinitis and its impact on asthma (ARIA). Allergy 2008; 63(suppl s86):8-160.
2. Small P and Kim H:Allergic rhinitis. Allergy, Asthma & Clinical Immunology, 2011; 7(Suppl 1):S3. https://doi.org/10.1186/1710-1492-7-S1-S3
3. Ciprandi G: Serum interleukin in allergicrhinitis. Ann Allergy Asthma Immuno, 2010; 104: 180-181
4. Goswami R and Kaplan MH: A brief history of IL-9. J Immunol, 2011; 186: 3283–3288.
5. Ciprandi G, Amici M, Castellazzi AM et al.: Serum IL-9 Levels Depend on Allergen Exposure: Preliminary Study. Int Arch Allergy Immunol, 2011; 154: 246–248.
6. GuZW, Wang YXW and Cao ZW: Neutralization of interleukin-9 ameliorates symptoms of allergic rhinitis by reducing Th2, Th9, and Th17 responses and increasing the Treg response in a murine model. Oncotarget. 2017 February, 28; 8(9): 14314–14324. doi: 10.18632/oncotarget.15177
7. Brożek JL, Bousquet J, Agache I, et al.: Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. J Allergy ClinImmunol. 2017 Oct;140(4):950-958. doi: 10.1016/j.jaci.2017.03.050. Epub 2017 Jun 8.
8. Norman P (1992): Skin testing in: Rose, NR, DeMaccario, EC and Fahey, JL (eds). Manual of clinical laboratory immunology 4th ed. Washington, American Society for Microbiology.
9. Ojala K, Sipilä P, Sorri M, et al., (1982): Role of atopic allergy in chronic otitis media. Evaluation based on serum IgE and nasal/aural cytologic findings in patients with operated chronic ears. Acta Otolaryngol; 93: 55-60.
10. Holguin F: The atopic march: IgE is not the only road,The Lancet Respiratory Medicine, 2014; 2(2): 88 – 90.

11. Dávila I, Mullol J, Ferrer M: Genetic Aspects of Allergic Rhinitis,J InvestigAllergolClinImmunol, 2009; 19(1): 25-31.
12. Amizadeh M, Safizadeh H, Bazargan N, et al.: Survey on the Prevalence of Allergic Rhinitis and its Effect on the Quality of High School Students' Life, Iran J of Otorhinolaryngol, 2013; 2(25):79-84.
13. Egger M, Mutschlechner S, Wopfner N, et al.: Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. Allergy, 2006; 61:461–476
14. Steenwinckel V, Louahed J, Lemaire M M, et al.: IL-9 pro- motes IL-13-dependent paneth cell hyperplasia and up-regulation of innate im- munity mediators in intestinal mucosa. J. Immunol, 2009; 182: 4737–4743.
15. Erpenbeck VJ, Hohlfeld JM, Volkmann B, et al.: Segmental allergen challenge in patients with atopic asthma leads to increased IL-9 expression in bronchoalveolar lavage f luid lymphocytes. J Allergy Clin Immunol, 2003; 111: 1319–1327.
16. Fujisawa T, Katsumata H and Kato Y: House dust mite extract induces interleukin-9 expression in human eosinophils. AllergolInt, 2008; 57: 141–146.
17. Nouri-Aria K T, Pilette C, Jacobson M R, et al.: IL-9 and c-Kit+ mast cells in allergic rhinitis during seasonal allergen exposure: effect of immunotherapy. J Allergy ClinImmunol, 2005; 116:73–9.
18. Hauber H P, Bergeron C, Toda M, et al.: Up-Regulation of Interleukin-9 and the Interleukin-9-Associated Calcium-Activated Chloride Channel hCLCA1 in Nasal Mucosa Following In Vivo Allergen Challenge, Allergy, Asthma, and Clinical Immunology, 2007; 3(1): (Spring), pp 19–23.
19. Forbes EE, Groschwitz K, Abonia JP, Brandt EB, Cohen E, Blanchard C, Hogan SP: IL-9– and mast cell–mediated intestinal permeability predisposes to oral antigen hypersensitivity. The Journal of Experimental Medicine, 2008; 205(4), 897–913.
20. Barros KV, FlorSilveira VL, Laranjeira MS, Wandalsen NF, Passeti S, de Oliveira R, Calder PC: Evidence for Involvement of IL-9 and IL-22 in Cows' Milk Allergy in Infants. Nutrients, 2017; 9(10), 1048.
21. Kerzerho J, Maazi H, Speak AO, Szely N, Lombardi V, Khoo B, Geryak S, Lam J, Soroosh P, Van Snick J, Akbari O: Programmed cell death ligand 2 regulates TH9 differentiation and induction of chronic airway hyperreactivity. The Journal of allergy and clinical immunology, 2013; 131(4):1048–1057.
22. Koch S, Sopel N &Finotto S: Th9 and other IL-9-producing cells in allergic asthmaSeminImmunopathol, 2017; 39:55.
23. Moitra S, Sen S, Datta A, et al.: Study of Allergenicity Spectrum to Aero Allergens by Skin Prick Testing, Austin J Allergy, 2014; 1(1): 4.
24. Yuen Ap, Cheung S , Tang KC, et al.: The skin prick test results of 977 patients suffering from chronic rhinitis in Hong Kong ,Hong Kong Med J, 2007; 13(2):131-136.
25. Assarehzadegan MA, Shakurnia A and Amini A: The most common aeroallergens in a tropical region in Southwestern Iran. World Allergy Organ J, 2013; 6(1): 7.
26. Bauchau V and Durham SR: Epidemiological characterization of the intermittent and persistent types of allergic rhinitis, Allergy, 2005; 60: 350-353.
27. Lou H, Ma, S, Yan Zhao, Cao F, He F, Liu Z, Bousquet J, Wang C, Zhang L and Bachert C: Sensitization patterns and minimum screening panels for aeroallergens in self-reported allergic rhinitis in China. Scientific Reports, 2017; volume 7, Article number: 9286 (2017)
28. Thi Lâm H, Ekerljung L, Bjerg A, Tuong N V, Lundbäck B and Rönmark E: Sensitization to airborne allergens among adults and its impact on allergic symptoms: a population survey in northern Vietnam. Clinical and Translational Allergy,2014; 4:6. https://doi.org/10.1186/2045-7022-4-6
29. Chaiyasate S , Roongrotwattanasiri K, Fooanant S , et al.: Key Nasal Symptoms Predicting a Positive Skin Test in Allergic Rhinitis and Patient Characteristics According to ARIA Classification, J Med Assoc Thai, 2009; 92 (3): 377-81.
30. Elgendy AM: Prevelance of food allergy in patients with allergic rhinitis, MD Thesis, Faculty of Medicine, Ain Shams University, 2010.
31. Ciprandi G, and Cirillo I.:Monosensitization and polysensitization in allergic rhinitis. Eur J Intern Med., 2011; Dec;22(6):e75-9. doi: 10.1016/j.ejim.2011.05.009. Epub 2011 Jun 8.