Acta Biomed 2021; Vol. 92, N. 3: e2021206 DOI: 10.23750/abm.v92i3.10544

Original article

Interleukin 9 serum level and single nucleotide polymorphism in patients with asthma

Seyed Alireza Mahdaviani[1], Mahsa Eskian[2,3], MirHojjat Khorasanizadeh[2,3], Bahram Bashardoost[4], Sabereh Tashayoie Nejad[1], Hamidreza Jamaati[5], Arezou Rezaei[2], Maryam Sadr[2,6], Zahra Aryan[2,6], Nima Rezaei[2,3,7,8,9]

[1]Pediatric Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran; [2]Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran; [3]Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran; [4]Pediatric department of Bou-Ali Hospital, Ardebil University of Medical Sciences, Ardebil, Iran; [5]Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran; [6]Cancer Immunology Project (CIP), Universal Scientific Education and Research Network (USERN), Tehran, Iran; [7]Pediatric Respiratory Diseases Education and Research Network (PRDERN), Universal Scientific Education and Research Network (USERN), Tehran, Iran; [8]Molecular Immunology Research Center, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran; [9]Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract. *Background:* Asthma is a chronic inflammatory disease of airways which accounts for a huge economic, morbidity, and mortality burden. There are different cytokines that contribute to asthma pathophysiology. Learning about these cytokines leads to attaining novel anti-inflammatory treatments for asthma control. **Objectives:** The objective of this study is to investigate the association between interleukin-9 serum level and gene polymorphism with asthma susceptibility. *Methods:* This was a case-control study of 70 asthmatic patients and 77 healthy control adults aged 18-60. Asthma diagnosis and severity were based on physician diagnosis, pulmonary function test (PFT) and 2016 guild line of Global Initiative for Asthma (GINA). Interleukin 9(IL -9) serum level was measured using sandwich enzyme linked immunosorbent assay. IL9 promoter single nucleotide polymorphism (SNP) (rs2069882) was also assessed using Real-Time PCR System. *Results:* There was no significant association between IL-9 SNP polymorphism and asthma. IL-9 serum level was significantly associated with asthma susceptibility (p value= 0.016) and absolute eosinophil count (AEC) (P value=0.033) however, its correlation with atopic asthma type, asthma severity and Immunoglubin E serum level were not statistically significant. *Conclusion:* Although there was no association between IL-9 SNP and asthma, but IL-9 serum level was significantly correlated with asthma susceptibility and AEC. (www.actabiomedica.it)

Introduction

Asthma is a complex heritable chronic inflammatory disease associated with hyperresponsiveness, inflammation and intermittent reversible obstruction of airways. (1, 2). Asthma is the most common chronic disease among young adults, and about 300 million people worldwide suffer from asthma. WHO estimates a 100 millionincrease in the global prevalence of asthma in the next decade (3, 4). Although current available treatment modalities, such as inhaled corticosteroids, biologic therapies and other anti-inflammatory therapies decreased asthma mortality rates in recent decades (5-7), but incidence of asthma and its

economic burden is still increasing (8) and approximately 15% of patients with asthma do not respond to maximal conventional therapies (9).

Several studies have strongly showed the involvement of T-helper 2(Th2) lymphocytes and their cytokines products in the pathogenesis of asthma (10). Th9 cell is a Th2 subpopulation that produces Interleukin-9(IL9). Genetic and experimental analyses have showed several effects of IL-9 on the pathogenesis of asthma.

Different immune cell types including mast cells, eosinophils and neutrophils can produce IL-9 but the major source is T-helper cells (11). IL-9 can induce IgG and IgE production form B cell lymphocytes, survival and maturation of Eosinophils, T calls, and neutrophils chemotaxis (12, 13). IL-9 inhibits IFN-γ secretion form CD4+T helpers and promotes proliferation of cytotoxic CD8+T cells (14, 15). IL_9 increases airways smooth muscles proliferation which is a key point in chronic asthma progression (16). Moreover, IL-9 has a significant role in the development of asthma by increasing mast cells protease secretion and IgE receptor (FcεRIα) expression which is a significant step in the development of allergic asthma (17).

IL-9 can also rescue Th2 cells from apoptosis, increase survival and proliferation of mast cells, increasing immunoglobulin E serum level, enhance eosinophil development, exhibit epithelial cell hypertrophy, accumulation of mucus within secretory cells, and subepithelial deposition of extracellular matrix proteins. All these features are strongly associated with asthma pathophysiologic mechanism (18-22).

To the best of our knowledge, no study has investigated the association of IL9 cytokine serum level with asthma in adult patients. This study was conducted to assess the association of IL9 serum level and SNP with asthma susceptibility and its severity.

Methods

Study Population

This case-control study was conducted on asthmatic patients and non-asthmatic healthy controls. Asthmatic patients were referred to "Masih Daneshvari Hospital" in Tehran, Iran were included. Age and sex matched healthy individuals were collected from general population. Asthma diagnosis and severity were defended based on history, physical examination, pulmonary function test (PFT) and following the guild line of Global Initiative for Asthma (GINA) 2016. Asthmatic subjects were classified into 3 groups of mild persistent, moderate persistent, and severe persistent (23). Spirometry test was defended according to American Thoracic Society (ATS) standards (24). Atopic asthma type was characterized by positive skin prick test (SPT), with the wheal size of greater than 3mm than saline control as the positive test result (25). Control group had no history of asthma or other respiratory diseases and showed normal PFT. Patients with other pulmonary disease, presence of parasitic infection, liver, cardiovascular, Endocrine or Hematologic disease, malignancy, hypertension, and organ transplantation were excluded from the study in order to prevent false positive high serum level of cytokines. An approval was obtained from the Local Ethics Committee of the Tehran University of Medical Sciences. Accordingly informed written consent was taken from all subjects.

DNA Extraction and Single Nucleotide polymorphism Genotyping

Five millimeters of venous blood were collected from the participants and stored in Ethylene diamante traacetic acid (EDTA) tube at -20°C. DNA was extracted using the standard phenol chloroform method. One promoter polymorphism (315 + 76T>C; rs2069882) was selected for genotyping using TaqMan SNP Genotyping Assay by 96-well 7300 ABI Real-Time PCR System. Polymerase chain reaction products underwent electrophoresis on a 2% agarose gel containing ethidium bromide and were visualized under ultraviolet illumination.

Immunoglobulin E and absolute eosinophil count

Total serum IgE was measured using Enzyme-Linked Fluorescent Assay (ELFA) and Absolute Eosinophils Count (AEC) were measured by an automatic complete blood count (CBC) analyzer

(MEK-7300K, Nihon Kohden Corporation, Tokyo, Japan). Serum IgE value of 120 kUA/l and AEC of 440 Cells/mm3 were considered as upper limits of normal (26-28).

IL9 serum level

Five millimeters of venous blood were obtained from all participants. Samples could clot, and serum was separated by centrifuging at 1500 rpm for 10 min and stored at – 80°C. Serum level of interleukin 9 was measured using sandwich enzyme linked immunosorbent assay (Commercial enzyme-linked immunosorbent) (R&D Cat. No. BMS2081). The assay was performed using the protocols recommended by the manufacturers. The minimum detectable level of IL9 was 0.62 ng/ml.

Statistical Analysis

Data were analyzed using SPSS version 18. Pearson's Chi-square test or Fisher's test(when appropriate) were used to check the association between IL9 SNP allele frequencies and asthma. The One-way ANOVA test was used to check the association between IL9 serum level and asthma susceptibility, severity and type. In all statistical tests, Statistical significance level was defined as P value of <0.05.

Results

This study included 70 patients with asthma diagnosis and 77 healthy control adults aged 18-60. Demographic characteristics are summarized in table-1.

IL9 promoter rs2069882 polymorphisms were genotyped in all cases and control group. All the SNPs were in accordance with Hardy–Weinberg equilibrium in both groups. Although C-allele frequency of rs2069882 SNP was slightly less prevalent among the cases goup compared with control (20% vs 23%), but assessment of genotype and allele distributions showed no statistically significant difference (Table-2).

Mean IL9 serum level was 0.502 mg/dl in asthmatic group and 0.391 mg/dl in control group and the difference was statistically significant (p value= 0.016). However, neither asthma severity nor asthma type (atopic or non-atopic) was significantly associated with IL9 serum level (p value of 0.52 and 0.32, respectively). Further evaluation showed that asthmatic patients with higher serum IL9 level had significantly higher AEC (Figure-1). However, no significant correlation was found between IL9 serum level and IgE serum level among asthmatic patients (P value=0.367).

Discussion

This case-control study reports on the comparison of IL9 serum level and SNP between asthmatic patients and control healthy adults. In this study, we found that asthmatic patients had significantly higher Interleukin-9 serum level in comparison with control group. However, IL9 serum level was not significantly correlated with asthma severity or atopic type. Moreover, IL9 rs2069882 SNPs were not significantly correlated with asthma susceptibility. Further evaluations showed asthmatic patients with higher IL9 serum level had significantly higher AEC, but this relationship was not statistically significant for Ig E serum level.

Table 1. Demographic characteristics

	Control(N=77)	Case(N=70)
Age		
≤40	56(73%)	49(70%)
>40	21(27%)	21(30%)
Sex		
Male	28(36%)	24(34%)
Female	49(64%)	46(66%)
Smoking status		
Smoker	0(0%)	8(11.4%)
Non-smoker	77(100%)	62(88.6%)
Severity		
Mild persistent	-	24(34.3%)
Moderate persistent	-	36(51.4%)
Severe persistent	-	10(14.3%)
Asthma type		
Atopic	-	54(77%)
Non-Atopic	-	16(23%)

Table 2. Allele and Genotype Frequencies of rs2069882

SNP	Allele	Genotype	Asthema (N= 70) n(%)	Controls (N=77) n(%)	P-value	Odds Ratio (OR)	95% Confidence Interval
rs 2069882	C		28(20%)	35(23%)	0.55	0.85	0.48-1.48
	T		112(80%)	119(77%)	ref	ref	ref
		CC	4(5.7%)	3(3.9%)	0.99	1.49	0.32-6.92
		CT	20(28.6%)	29(37.7%)	0.24	0.66	0.33-1.32
		TT	46(65.7%)	45(58.4%)	ref	ref	ref

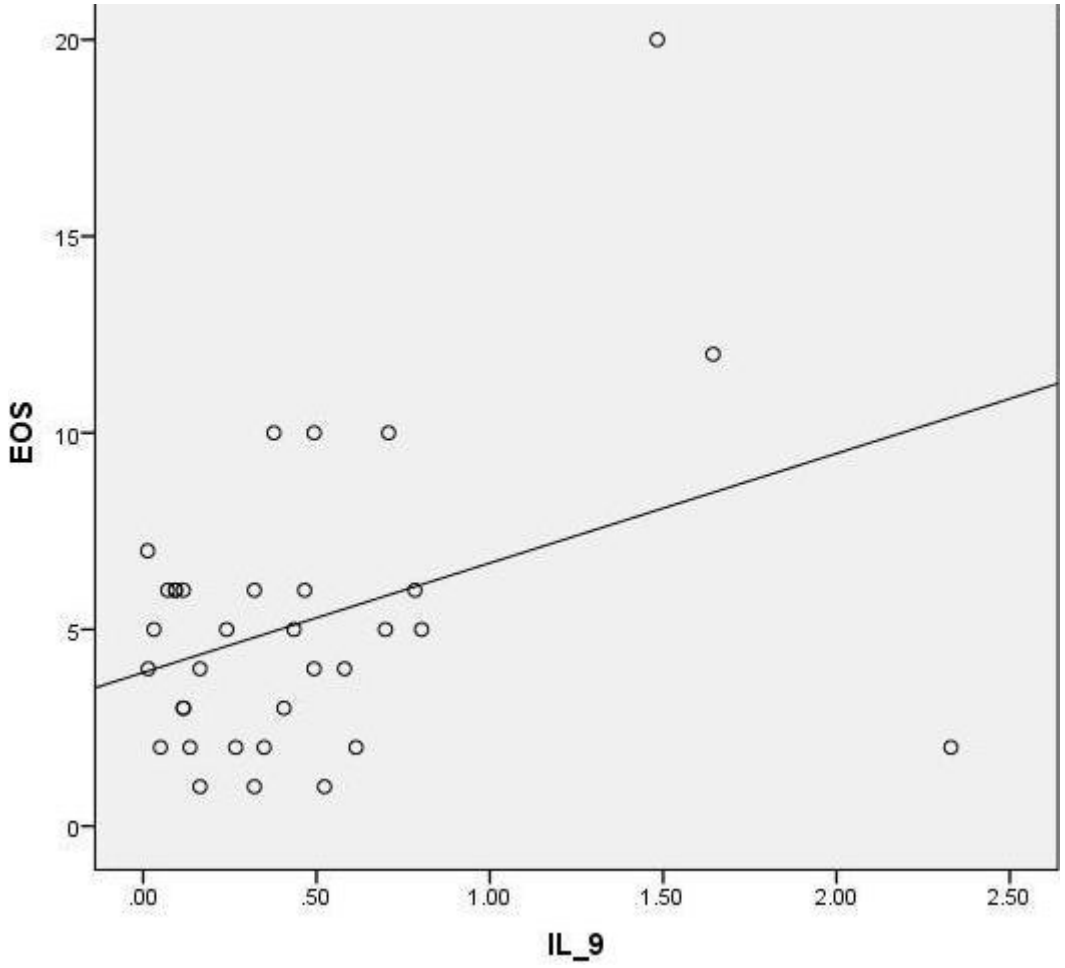

Figure 1. Correlation between IL9 serum level and AEC(P value=0.033)

There are some studies investigated the role of IL9 SNP in asthma. Waldman et al have demonstrated in a meta-analysis of sib pair studies that there is little evidence of IL-9 gene role in asthma pathogenesis(29). Wang et al also showed that allele 122 of the IL-9 gene is slightly associated with asthma (P = 0.047) (30).Some other studies showed that polymorphism on chromosome 5q31–33 which contains the IL-9 gene, is significantly associated with atopy and asthma (31, 32). However, some other studies were consistent with our study, showing no relation between asthma and IL9 SNPs (33). These controversies may

be due to the different environmental exposure that is responsible for allergic response in genetically predisposed individuals (34).

Based on our knowledge, our study is the first clinical study that investigated association between IL9 serum level and asthma susceptibility in adults. However, several sub-clinical studies showed that IL9 plays an important role in asthma pathophysiology; as it has several effects on numerous hematopoietic cells involved in asthma pathogenesis (19-21). IL-9 stimulates the proliferation of activated T cells, promotes the proliferation and differentiation of mast cells and increases production of immunoglobulins by B cells. It also is associated with susceptibility to develop AHR (35, 36). IL-9 has a critical role in regulating number of mast cells which contribute to bronchoconstriction, mucus secretion, mucosal edema, angiogenesis and tissue remodeling (37).

Finding SNPs and diseases associations can be useful for identification of at-risk patients (38). Our study showed that there is no significant association between rs2069882 SNP of IL9 and asthma susceptibility. However, there are some other inconsistent studies. These inconsistent results emphasize on the role of environmental exposure in developing asthma in genetically susceptible patients. Finding these environmental factors may be useful in asthma prevention.

The increased knowledge on cytokines involved in asthma pathophysiology can lead to generating new therapeutic strategies by applying anti-cytokines antibody (39-41). In our study, IL9 serum level was significantly associated with asthma and AEC. Although, this study was a case-control and more randomized clinical trials are needed in this area and anti-IL9 antibody therapy in asthma.

Conclusion

In conclusion rs2069882 SNP of IL9 was not significantly associated with susceptibility to asthma. However, IL9 serum level was significantly higher in asthmatic patients in comparison with healthy individuals and IL9 serum level was significantly correlated with AEC. Further studies are needed toinvestigate environmental exposures which are responsible for asthma in some individuals with IL9 SNP and the role of anti-IL9 antibody in asthma control.

Conflicts of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

References

1. Gauthier M, Ray A, Wenzel SE. Evolving Concepts of Asthma. Am J Respir Crit Care Med. 2015;192(6):660-8.
2. Guidelines for the diagnosis and management of asthma. National Heart, Lung, and Blood Institute. National Asthma Education Program. Expert Panel Report. The Journal of allergy and clinical immunology. 1991;88(3 Pt 2):425-534.
3. To T, Stanojevic S, Moores G, Gershon AS, Bateman ED, Cruz AA, et al. Global asthma prevalence in adults: findings from the cross-sectional world health survey. BMC public health. 2012;12:204.
4. Prevention USCfDCa. National Surveillance of Asthma: United States, 2001–2010 [Available from: http://www.cdc.gov/nchs/data/series/sr_03/sr03_035.pdf.
5. Wechsler ME. Getting control of uncontrolled asthma. The American journal of medicine. 2014;127(11):1049-59.
6. Khorasanizadeh M, Eskian M, Assa'ad AH, Camargo CA, Jr., Rezaei N. Efficacy and Safety of Benralizumab, a Monoclonal Antibody against IL-5Ralpha, in Uncontrolled Eosinophilic Asthma. International reviews of immunology. 2016;35(4):294-311.
7. Khorasanizadeh M, Eskian M, Gelfand EW, Rezaei N. Mitogen-activated protein kinases as therapeutic targets for asthma. Pharmacology & therapeutics. 2017.
8. Loftus PA, Wise SK. Epidemiology and economic burden of asthma. International forum of allergy & rhinology. 2015;5 Suppl 1:S7-10.
9. Hambly N, Nair P. Monoclonal antibodies for the treatment of refractory asthma. Current opinion in pulmonary medicine. 2014;20(1):87-94.
10. Yssel H, Groux H. Characterization of T cell subpopulations involved in the pathogenesis of asthma and allergic diseases. International archives of allergy and immunology. 2000;121(1):10-8.
11. Shimbara A, Christodoulopoulos P, Soussi-Gounni A, Olivenstein R, Nakamura Y, Levitt RC, et al. IL-9 and its receptor in allergic and nonallergic lung disease: increased expression in asthma. J Allergy Clin Immunol. 2000;105(1 Pt 1):108-15.
12. Hültner L, Druez C, Moeller J, Uyttenhove C, Schmitt E, Rüde E, et al. Mast cell growth-enhancing activity (MEA) is structurally related and functionally identical to the novel mouse T cell growth factor P40/TCGFIII (interleukin 9). Eur J Immunol. 1990;20(6):1413-6.

13. Mims JW. Asthma: definitions and pathophysiology. Int Forum Allergy Rhinol. 2015;5 Suppl 1:S2-6.
14. Aron JL, Akbari O. Regulatory T cells and type 2 innate lymphoid cell-dependent asthma. Allergy. 2017;72(8):1148-55.
15. Seumois G, Ramírez-Suástegui C, Schmiedel BJ, Liang S, Peters B, Sette A, et al. Single-cell transcriptomic analysis of allergen-specific T cells in allergy and asthma. Sci Immunol. 2020;5(48).
16. Hauber H-P, Hamid Q. The Role of Interleukin-9 in Asthma. Allergology International. 2005;54(1):71-8.
17. Robinson DS. The role of the mast cell in asthma: induction of airway hyperresponsiveness by interaction with smooth muscle? Journal of allergy and clinical immunology. 2004;114(1):58-65.
18. Soussi-Gounni A, Kontolemos M, Hamid Q. Role of IL-9 in the pathophysiology of allergic diseases. The Journal of allergy and clinical immunology. 2001;107(4):575-82.
19. Zhou Y, McLane M, Levitt RC. Th2 cytokines and asthma. Interleukin-9 as a therapeutic target for asthma. Respir Res. 2001;2(2):80-4.
20. Xing J, Wu Y, Ni B. Th9: a new player in asthma pathogenesis? The Journal of asthma : official journal of the Association for the Care of Asthma. 2011;48(2):115-25.
21. Farahani R, Sherkat R, Hakemi MG, Eskandari N, Yazdani R. Cytokines (interleukin-9, IL-17, IL-22, IL-25 and IL-33) and asthma. Advanced biomedical research. 2014;3:127.
22. Eskian M, Khorasanizadeh M, Assa'ad AH, Rezaei N. Monoclonal Antibodies for Treatment of Eosinophilic Esophagitis. Clinical Reviews in Allergy & Immunology. 2018;55(1):88-98.
23. Global Initiative for Asthma (GINA). 2012 revision [Available from: www.ginasthma.com.
24. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. The European respiratory journal. 2005;26(2):319-38.
25. Antunes J, Borrego L, Romeira A, Pinto P. Skin prick tests and allergy diagnosis. Allergologia et immunopathologia. 2009;37(3):155-64.
26. Chapman MD. Allergen nomenclature. Clin Allergy Immunol. 2008;21:47-58.
27. Heyworth-Smith D CP. Laboratory Diagnosis of Allergy. . QML pathology newsletter, Brisbane. 2017.
28. Sheldon J ML. Allergy diagnosis reference guide. . Clinical Biochemistry, East Kent University Hospitals, NHS P-6. October 2014. .
29. Waldman ID, Robinson BF. Meta-analysis of sib pair linkage studies of asthma and the interleukin-9 gene (IL9). Genet Epidemiol. 2001;21 Suppl 1:S109-14.
30. Wang TN, Chen WY, Huang YF, Shih NH, Feng WW, Tseng HI, et al. The synergistic effects of the IL-9 gene and environmental exposures on asthmatic Taiwanese families as determined by the transmission/disequilibrium test. International journal of immunogenetics. 2006;33(2):105-10.
31. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. Science (New York, NY). 1994;264(5162):1152-6.
32. Noguchi E, Shibasaki M, Arinami T, Takeda K, Maki T, Miyamoto T, et al. Evidence for linkage between asthma/atopy in childhood and chromosome 5q31-q33 in a Japanese population. Am J Respir Crit Care Med. 1997;156(5):1390-3.
33. Laitinen T, Kauppi P, Ignatius J, Ruotsalainen T, Daly MJ, Kaariainen H, et al. Genetic control of serum IgE levels and asthma: linkage and linkage disequilibrium studies in an isolated population. Human molecular genetics. 1997;6(12):2069-76.
34. Hizawa N, Freidhoff LR, Ehrlich E, Chiu YF, Duffy DL, Schou C, et al. Genetic influences of chromosomes 5q31-q33 and 11q13 on specific IgE responsiveness to common inhaled allergens among African American families. Collaborative Study on the Genetics of Asthma (CSGA). The Journal of allergy and clinical immunology. 1998;102(3):449-53.
35. Louahed J, Kermouni A, Van Snick J, Renauld JC. IL-9 induces expression of granzymes and high-affinity IgE receptor in murine T helper clones. Journal of immunology (Baltimore, Md : 1950). 1995;154(10):5061-70.
36. Dugas B, Renauld JC, Pene J, Bonnefoy JY, Peti-Frere C, Braquet P, et al. Interleukin-9 potentiates the interleukin-4-induced immunoglobulin (IgG, IgM and IgE) production by normal human B lymphocytes. Eur J Immunol. 1993;23(7):1687-92.
37. Kearley J, Erjefalt JS, Andersson C, Benjamin E, Jones CP, Robichaud A, et al. IL-9 governs allergen-induced mast cell numbers in the lung and chronic remodeling of the airways. Am J Respir Crit Care Med. 2011;183(7):865-75.
38. Raeiszadeh Jahromi S, Mahesh PA, Jayaraj BS, Holla AD, Vishweswaraiah S, Ramachandra NB. IL-10 and IL-17F Promoter Single Nucleotide Polymorphism and Asthma: A Case-Control Study in South India. Lung. 2015;193(5):739-47.
39. Desai D, Brightling C. Cytokine and anti-cytokine therapy in asthma: ready for the clinic? Clin Exp Immunol. 2009;158(1):10-9.
40. Finiasz M, Otero C, Bezrodnik L, Fink S. The role of cytokines in atopic asthma. Current medicinal chemistry. 2011;18(10):1476-87.
41. Desai D, Brightling C. Cytokines and cytokine-specific therapy in asthma. Advances in clinical chemistry. 2012;57:57-97.

Correspondence:
Received: 26 August 2020
Accepted: 19 November 2020
Nima Rezaei, MD, PhD
Children's Medical Center Hospital, Dr Qarib St, Keshavarz Blvd, Tehran 14194, Iran
Tel: +9821-6692-9234; Fax: +9821-6692-9235
E-mail: rezaei_nima@tums.ac.ir