Frequency of Specific Immunoglobulin G Antibodies and Immediate Skin Test Reactivity to *Aspergillus fumigatus* Antigen among Adults with Allergic Asthma: Tehran

Agin Kh[1*], Namavary D[2]

[1] Heart and Lung Division, Loqman Hakim Teaching General Hospital Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Traditional Islamic Medical Center, Loqman Hakim Teaching General Hospital Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLEINFO

Article Type:
Original Article

Article History:
Received: 1 March 2012
Revised: 20 March 2012
Accepted: 30 April 2012

Keywords:
Asthma
Allergy
Aspergillus Fumigatus
Skin Test Sensitization

ABSTRACT

Background: The *Aspergillus fumigatus* (AF) has been found the most common etiologic agent in allergic aspergillosis. In addition, AF is responsible for 90% of human infections. Increasing the air pollution in urban area causes the increase in asthma rates. Significant cutaneous sensitization occurs in asthmatic population with AF. In these patients, susceptibility of diseases increases. The aims of this study were to identify frequency of specific IgG antibodies and sensitization to Aspergillus antigens through skin prick-test reactivity (SPT) among adults with allergic asthma and to diagnose atopic phenotype subset.

Method: 201 chronic asthmatic patients were applied in order to instructions based on the increased level of immunoglobulin E antibodies concentrations in allergy, having criteria of the study and specific immunoglobulin G antibodies against AF in serum and SPT reactivity with aspergillus antigen. Thereafter, atopic phenotype was diagnosed.

Results: 42 (21%) subjects had positive skin reactivity to aspergillus antigens, also specific AF IgG antibodies was observed in 122 (61%). 36 (30%) of positive aspergillus skin prick test was found among those who were positive specific AF IgG antibodies of allergic asthma (P=0.001), 81 (40%) subjects of all allergic asthma patients were atopic. 19 (24%) of the atopic subsets had positive aspergillus skin prick test and specific AF IgG antibodies was seen in 41 (50%) of atopic subjects. In addition, significant differences in aspergillus skin prick test was observed between atopic with nonatopic subgroups (P=0.01).

Conclusion: Our finding indicated that significant frequency of specific IgG antibodies seroconvertion against AF in serum and immediate SPT sensitization to AF antigen were detected among chronic bronchial asthma with allergic and also, atopic phenotype subsets.

►*Implication for health policy/practice/research/medical education:* Significant frequency of specific IgG antibodies among chronic bronchial asthma with allergic and also, atopic phenotype subsets.

* *Corresponding author:* Agin Kh, MD. Specialist in Internal Medicine, Pulmonologist, Head of Heart and Lung Division, Loqman Hakim Hospital Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: Agin@sbmu.ac.ir

► *Please cite this paper as:* Agin Kh, Namavary D. Frequency of Specific Immunoglobulin G Antibodies and Immediate Skin Test Reactivity to *Aspergillus Fumigatus* Antigen among Adults with Allergic Asthma: Tehran. *International Journal of Medical Toxicology and Forensic Medicine*. 2012; 2(3):97-102.

1. Introduction:

Aspergillosis is a pulmonary disorder that is caused by Aspergillus species. It is a common ubiquitous saprophytic airborne mold in natural ecology. It enables living in urban and rural areas (1). Despite the most frequency in species, only a few of them can lead to disease in human such as: *Aspergillus fumigatus* (AF), *A. flavus*, *A. terreus*, *A. Niger*, and *A. nidulans*. Current knowledge shows that AF alone is responsible for the initiation of 90% human diseases (2). However, the respiratory tract is the main source of colonization of coincides in human (3). Moreover, increased air pollution in urban area from huge traffic and various industries has been resulted to an increase in atmospheric concentration of AF up to 95%. Additionally, they are induced incidence of allergic diseases (4).

There is a link between AF infection and asthma as follows: Current report showed that chronic continuous inhalation of Aspergillus antigens has been implicated in being causative agents in asthma (5). Moreover, sensitization to fungi in patients with bronchial asthma increases disease severity as a major risk factor (6). The earlier reports indicated the frequency of skin test reactivity to Aspergillus antigens was different among asthmatic patients from 16 to 38% (7). Either frequency of sensitization and specific immunoglobulin G antibodies (IgG-Ab) against AF has not been known in allergic asthma and atopic phenotype.

The purpose of study is to identify frequency of specific IgG-Ab and sensitization to Aspergillus antigens through skin prick-test reactivity (SPT) among adults with allergic asthma and to diagnose atopic phenotype subset.

2. Materials and Methods:

We performed a cross-sectional descriptive and analytic study. It finalized in the Logman Hakim general teaching hospital, pulmonary division of Shahid-Beheshti University of medical sciences (SBMU). Logman hospital is a tertiary referral center for asthma and respiratory disorders. The outpatient visits of respiratory disorders performed in the outpatient clinic. It was estimated to be over 3,500 individuals annually.

The study was designed as follows: In the first stage, sample population randomly enrolled among profile of subjects with chronic persistent asthma and at least 3 years duration. The bronchial asthma was confirmed according to the criteria of the American Thoracic Society (ATS) (8) and questionnaire concerning respiratory symptoms (9). It was completed by looking for additional data (personal and family history, evaluation of the major symptoms of asthma such as: wheeze, chest tightness, shortness of breath, and cough), and topic about atopic phenotype and seasonality of the diseases. However, one physician supported accuracy of all data. Then, total immunoglobulin E concentrations in serum were determined, and the subjects who had associated with allergic level up to 200 Iu/ml (10) were selected as focus population.

Physical examination and pulmonary function test were performed. A total of 201 adults with allergic chronic asthma were completed and followed criteria of study.

In second step, serum specific IgG antibodies against *AF* were determined, and SPT reactivity to AF antigen was evaluated on the subjects who seroconverted.

Subsequently, atopic phenotype was diagnosed on the selected sample population. Validation of the recent model was achieved as follows: The atopia has been defined through the positive reactivity of SPT at least one of the common aeroallergen panels (11). In addition, background of atopic phenotype was obtained from individuals. They included rhinoconjunctivitis 4%, eczema 19%, and urticaria 17%. Clinical manifestations of rhinoconjunctivitis consist of episodic rhinorrhea, nasal stiffness, lacrimation and sneezing to a known allergens in specific seasons with characteristic repetition.

Sensitization to common allergens was measured by SPT reactions to the forearm. 7 allergens included in the panel. It included *Aspergillus fumigatus*, *Alternaria alternate*, Mites (*Dermatophagoides pteronyssinus*), cockroach (*Blatella germanica*), Grasses, Tree (Blossoming), and feather. Standardization of SPT was performed according to position paper of European Academy of Allergy and Clinical Immunology (EAAC) (12). The extracted allergens were applied on the left and right of the volar aspects of the forearm (Allergopharma Joachim Ganzer KG, West Germany). The lancet was introduced vertically into the skin through the allergen solution. Negative control was a buffered saline solution, 0.4% phenol and positive control included a solution containing 1.7 mg histamine, 9 mg NaCl, 4 mg phenol, and 363 mg glycerol. After 15 minutes, the mean diameter of any wheals that was formed by the allergens was compared with reaction to histamine and negative control. If the wheal diameter was half the size of positive controls and/or at least 3 mm or greater than negative control with flare reaction, the reaction of the skin test interpreted as positive (13).

Blood samples were collected for determining total leukocyte and differential count (TLC), blood eosinophil count (BEC), specific IgG-Ab against AF, and serum IgE concentration. Total IgE levels were measured according to Manufacture's protocol through Enzyme-Linked Immunosorbent Assay kit (ELISA) (Padtan Elm, Iran Co Ltd). Specific IgG antibodies against AF were determined by ELISA method in serum (IBL-Hamburg). The cut -off point for specific IgG-Ab was 12 u/ml.

Exclusion criteria included received antihistamines three days prior to undergoing skin tests. Non-smokers were defined as those who had not smoked for as long as one-year. The subjects had the history of smoking, parasitic infestation and in disagreement to be continued study, were omitted. Aside, none of the subjects had ever undergone a skin allergy testing.

All data analyzed using the SPSS version 15. Data presented as mean±SEM. Compare of means performed with Chi-square test and Independent samples-t test. Significant value was set at <0.05.

3. Results:

201 subjects who had chronic persistent allergic asthma completed criteria of the study. The mean (±SD) age was 32.75±12.54 years. It ranged 20–60 years. Sex distribution included males 103 (51%) and females 98 (49%).Table 1 shows demographic characterizations of subjects with allergic asthma and subsets.

Specific AF IgG antibodies in serum observed in 122 (61%) target population. Of those 42 (21%) had positive immediate SPT to Aspergillus antigen. Frequency of sex distribution in specific AF IgG antibodies and Aspergillus SPT was highly significant (female:male ratio was 62:25). No statistically significant differences were detected between gender with frequency of AF IgG antibodies and immediate SPT to Aspergillus antigen ($P=0.28$, $P=0.24$ respectively).

Of the all allergic asthmatic patients, 81 (40%) patients were tested positive to the allergic panel according to defined criteria of atopy. Specific AF IgG antibodies were seen in (41) 50% of atopic subjects. Positive reactivity to SPT Aspergillus antigen was found in 19 (24%) of atopic subjects. Significant differences of

Table 1: Characterization of allergic asthma population and subgroups.

		Atopic Asthma	Non-atopic Asthma	Allergic Asthma
Number of subjects		81(40%)	120(60%)	201
Gender	**Male**	41	57	103
	female	40	63	98
Mean age/ year		31.23±12.34	33.78±12.64	32.75±12.5(20-60)
Immunoglubuline E antibodies		481.88±26.117	567.18±468.33	532.80±388.51
Specific Aspergillus Fumigatus IgG antibodies		33.45+/_52.01	25.34±32.24	28.61±41.43(1-200)
White blood cell count		7274.07±1311.56	7203±1557	7753.73±2168.54
Mean peripheral blood eosinophia percentage		4.03±3.12	3.47±2,61	3.69±2.87
Duration of asthma/year		5.90±2.03	6 ±2.06	5.97±2.05

Aspergillus SPT reaction was observed between atopic and nonatopic subgroups ($P<0.01$). Frequencies of distribution of positive reaction to aeroallergen skin test were 25 (29.8%), 38(45.2%), 19(22.6%).

No significance differences were observed between variables of IgE, Specific AF IgG antibodies, peripheral blood eosinophilia, with atopic and non-atopic allergic asthmatic subsets (Table 1).

No Significant difference of positive specific AF IgG antibodies was observed between subsets of atopic and nonatopic allergic asthma ($P=0.4$).

Mean total IgE levels were recorded 532.80±388.51 Iu/ml in allergic asthma and 481.88±26.117 Iu/ml in atopic subset.

4. Discussion:

Sample population of chronic bronchial asthma showed High prevalence of seroconverting of specific IgG antibodies against AF in serum and immediate SPT sensitization to AF antigen. The current results detected in the both allergic and atopic phenotype subsets.

In order to recent knowledge, it can be deduced that Tehran has all susceptible conditions for asthmatic patients exposed to AF. AF is the most common fungi in ambient air (14). In addition, concentration of AF in cities atmosphere with air pollution was significant up to 95% (15). Furthermore, chronic inhalation of ambient air molds occurs naturally in the respiratory tract of asthmatic subjects. Epidemiologic ground indicated that industrialization causes raising prevalence of asthma and allergic diseases in urban than rural areas between both developed and developing countries (16).

Additionally, atopic phenotype was the more common in the asthmatic population. It was diagnosed up to two- thirds of some reports.

The results indicated 21% frequency of AF that induced sensitization skin test among allergic asthma which in fact agrees with current studies. Henderson *et al* published the first report of immediate skin test reaction to AF since 1968 years. He observed positive test in 23% of chronic lung diseases (17). However, Hendrich *et al* reported primary design of study of SPT sensitization to AF among asthmatic patients (1975). They showed positive reaction 16% SPT to AF in 656 asthmatic patients (18). Latest report was concerned to 500 asthmatic subjects with 26% positivity of SPT against AF antigen (1980) (19). All earlier studies were conducted among asthmatic population but our study was adversely performed in asthmatic patients with allergic background.

In addition, we found 21% SPT positivity to AF in Tehran. The data was improved with previous report of Cleveland and London studies 1975. Scholars demonstrated that frequency of immediate skin reactivity to AF antigens was 28% and 23% in the recent cities, respectively (20). Sensitization rate of SPT positivity to AF antigen was higher in atopic subsets

than allergic asthmatic population. Recent knowledge reveals that at least two thirds of asthmatic patients are atopic with skin reactivity to common allergens (21). Our finding agrees with unique study of Malo *et al* in this context. They found skin sensitization to AF among 21.5% of the (200) atopic asthma.

Significant increasing of immunoglobulin E antibodies in serum as allergic marker of in patient with bronchial asthma may be resulting in followed conditions. Exposure to traffic related air pollution from motor vehicles can enhance allergic inflammation and induced developing immune response (19). In addition, the fungus are causes 20-30% of all allergic respiratory cases (23). Present of 42% of atopic phenotype in the study samples may be another casual factor. Earlier report also demonstrated that AF is a saprophytic mold with many allergic proteins that can be able to implement into tenacious sputum of patients with airway obstruction and progressively realized allergen antigens in human and leads to allergic reaction and increasing of IgE concentration in serum (22).

Development of respiratory allergy is different between Genders. Men are more usually offended by respiratory allergy than women (23). The results are compatible with current study. Our resulting indicated that the atopic phenotype was higher in male (42%) than female (39%). In addition, SPT to AF was remarkable in male (24%).

In conclusion, obtaining results demonstrated that frequency of immediate skin test reactivity to *Aspergillus fumigatus* among allergic group and atopic subgroup supported previous reports in the world. Allergic asthmatic population revealed significant positive seroprevalence of specific IgG antibodies against *Aspergillus fumigatus*. However, the results were highly meaningful in atopic subsets.

Acknowledgments:
The author gives thanks for Miss Mahnaz Soltanpour and all colleagues of chest clinic in Logman Hakim general teaching hospital.

References

1. Soubani AO, Chandrasekar PH. The Clinical Spectrum of Pulmonary Aspergillosis Chest. 2002;121(6):1988-99.
2. Latgé JP. Aspergillus fumigatus and Aspergillosis. Clin Microbiol Rev. 1999;12(2):310-50.
3. Fridkin SK, Jarvis WR. Epidemiology of nosocomial fungal infections. Clin Microbiol Rev. 1996;9(4):499-511.
4. Jang AS, Yeum CH, Son MH. Epidemiologic evidence of a relationship between airway hyperresponsiveness and exposure to polluted air. Allergy. 2003;58(7):585-8.
5. Ross MA, Curtis L, Scheff PA, Hryhorczuk DO, Ramakrishnan V, Wadden RA, Persky VW Association of asthma symptoms and severity with indoor Bioaerosols. Allergy. 2000;55(8):705-11.
6. Zureik M, Neukirch C, Leynaert B, Liard R, Bousquet J, Neukirch F; European Community Respiratory Health Survey.. Sensitization to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. BMJ. 2002 24;325(7361):411-4.
7. Maurya V, Gugnani HC, Sarma PU, Madan T, Shah A. Sensitization to Aspergillus antigens and occurrence of allergic bronchopulmonary aspergillosis in patients with asthma. Chest. 2005;127(4):1252-9
8. National asthma education and prevention program. Expert panel report II: Guidelines for the diagnosis and management of asthma . Bethesda, MD: national heart lung and blood institute, national institutes of health, publication 1997. No.97-4051
9. Bai J, Peat JK, Berry G, Marks GB, Woolcock AJ.. Questionnaire items that predict asthma and other respiratory conditions in adults .Chest. 1998; 114(5):1343-8.

10. Baldacci S, Omenaas E, Oryszczyn MP. Allergy markers in respiratory epidemiology. Eur Respir J. 2001; 17(4):773-90.
11. Dreborg S.Skin testing.The safety of Skin tests and the information obtained From using different methods and concentrations of allergen. Allergy. 1993;48:473–475.
12. Allergen standardization and skin tests Position paper. Allergen standardization and skin tests. The European Academy Of Allergology and Clinical Immunology.Allergy. 1993;48(suppl.14):48–821.
13. Drebrg S, Backman A, Basomba A, Bousquet J, Dieges P, Malling H-J. Skin tests used in Type 1 allergy testing Position paper. Sub-Committee on Skin Tests of the European Academy of Allergology and Clinical Immunology. Allergy. 1989; 44(Suppl. 10): 1–59.
14. National Institute of Health. [Expert panel report 2]. Guidelines for the diagnosis and management of asthma: Clinical practice guidelines. Bethesda (MD): National Heart, Lung, and Blood Institute; 1997. NIH Publication No.: 97-4051.
15. Jones BL, Cookson JT. Natural atmospheric microbial conditions in a typical suburban area. Appl Environ Microbiol. 1983;45(3):919-34.
16. Zeyrek CD, Zeyrek F, Sevinc E, Demir E. Prevalence of asthma and allergic diseases in Sanliurfa, Turkey, and the relation to environmental and socioeconomic factors: is the hygiene hypothesis enough?. J Investig Allergol Clin Immunol. 2006;16(5):290-5.
17. Henderson AH, Englis MP, and Vecht RJ. Pulmonary aspergillosis, a survey of its occurrence in patients with chronic lung disease and a discussion of the significance of diagnostic test. Thorax. 1968;23(5):513-8.
18. Hendrick DJ, Davies R.I, D'Souza MF, Pepys J. An analysis of skin prick test reaction in 656 asthmatic patients. Thorax. 1975;30(1):2-8.
19. Benatar SR, Keen GA, Du Toit Naude W. Aspergillus hypersensitivity in asthmatics in Cape Town. Clin Allergy. 1980;10(3):285-91.
20. Schwartz HJ, Citron KM, Chester EH, Kaimal J, Barlow PB, Baum GL, Schuyler MR.. A comparison of the prevalence of the sensitization to Aspergillus antigen among asthmatics in Cleveland and London. J Allergy Clin Immunol. 1978;62(1):9-14.
21. O'Driscoll BR, Hopkinson LC, Denning DW. Mold sensitization is common amongst patients with severe asthma requiring multiple hospital admissions. BMC Pulm Med. 2005;18;5:4.
22. Kurup VP, Cremeri R: Aspergillus antigens. Available at:[http://www.aspergillus.man.ac.uk/secure/articles]. Posted January 13th 2001
23. Gioulekas D, Damialis A, Papakosta D, Spieksma F, Giouleka P, Patakas D. Allergenic fungi spore records (15 years) and sensitization in patients with respiratory allergy in Thessaloniki-Greece. J Investig Allergol Clin Immunol. 2004;14(3):225-31.