



THE ROLE OF SPECIFIC IgE ANTIBODIES FOR *ASPERGILLUS FUMIGATUS* ALLERGENS IN PATIENTS WITH SEVERE ASTHMA

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ABSTRACT

One hundred patients with severe asthma of age more than 16 years were included; 26% were men and 74% were women. The mean age of the patients was 43 years. Blood samples were collected from patients and control subjects for assessment of total serum IgE by using (ELISA) method while, assessment of specific IgE antibodies to *Aspergillus fumigatus* were performed using allergosorbent test (EAST). More than half of patients 51 (51%) had positive results with wide variation for *A. fumigatus* allergens specific IgE levels. Both of serum total and *A. fumigatus* specific IgE levels the difference was highly significant ($P < 0.001$) among patient groups as well, when compared to control group. Immediate skin reactivity to *A. fumigatus* allergens were observed in 20% of patients, most of them had elevated levels of specific IgE to this fungus, and very high statistical significance ($P < 0.001$) correlation was observed between skin prick test and *A. fumigatus* specific IgE level.

KEYWORDS: severe asthma, IgE, *Aspergillus fumigatus*, EAST.

INTRODUCTION

Asthma affects about 8% of the adult population; most of them have mild to moderate asthma symptoms that can be relieved with appropriate treatment. However, it is estimated, that only 5% - 10% of patients with asthma have severe disease and remain to experience uncontrolled asthma even after receiving of *aggressive* treatments including corticosteroids (Chanez, 1999). Many environmental factors have been associated with many aspects of asthma development and exacerbation including fungal allergens, air pollutants, and other environmental chemicals (Kelly and Fussell, 2011). Greater than 80% of asthmatic children are sensitized to a variety of stimuli including microbial allergens due to the ubiquity in the environment or microbial colonization in respiratory tracts and the high rate of sensitization in asthma (Singh and Busse, 2006).

MATERIALS & METHODS

Patients and controls

A total of 100 patients (74 females, and 26 male) their age range from 16-80 years with severe asthma attending the respiratory clinic or admitted as an inpatient on the respiratory wards of Al- yarmouk Teaching Hospital-Baghdad, with age range 16-83 years (mean age 43 years) and 50 apparently healthy controls without asthma were eligible for inclusion in this study, the collection period was between July 2014 and March 2015.

Patients selection

Inclusion criteria

All patients aged 16 years or older with severe asthma without comorbidity.

Exclusion criteria

Those that had comorbid advance disease such as heart failure, liver disease and pulmonary tuberculosis

Blood samples

About 5 ml of peripheral blood samples were withdrawn from patients and control group to estimate the levels of total serum immunoglobulin E (IgE) and specific IgE antibodies to *A. fumigatus*

Estimation of total IgE

Total IgE levels were measured according to the manufacturer's instructions (Dr fooke) using Enzyme Immuno Assay for quantitative measurement of total IgE levels in human serum is represented as IU/ml.

Estimation of specific IgE (sIgE) antibodies for *A. fumigatus* allergens

Enzyme Allergo Sorbent Test (EAST) was used for quantitative estimation of *A. fumigatus* allergen specific IgE in patients serum according to manufacturer protocol (Dr Fooke).

RESULTS

Total serum IgE level

The total serum IgE level of patients ranged from 15 to 2807 IU/ ml (mean, 220.87 IU/ml). In control subjects, the level ranged from 12 to 105 IU/ ml and the mean of IgE levels was found to be 31 IU/ml. According to manufacturer protocol the upper normal range limit has been set at 124 IU/ml. The asthmatic patients were classified into three groups (A-B-C) were illustrated in table (1) based on total serum IgE level titers. Group A includes patients with normal IgE levels ranged from 15-122 IU/ ml with a mean (82 IU/ml), whereas group B patients had serum IgE level more than this limit, within less than 1000 IU/ml ranged from 155-875 IU/ml (Mean 295 IU/ml). Furthermore, 4 patients in group C had serum IgE level more than 1000 IU/L with a range from 1250-2807 (Mean1661.25 IU/ ml).

TABLE 1: Serum IgE levels in controls and asthmatic cases

Subjects	Number of subjects	IgE range (IU / ml)	IgE Mean (IU / ml)
Control	50	12-105	31±17
Patients with normal IgE level (IgE 124 IU/L)	67	15-122	82±27.9
Patients with high level (IgE 1000 IU/L)	29	155-875	295±183.35
Patients with very high level (IgE 1000 IU/L)	4	1250-2807	1661.25±764.1

The statistical tests were done including (ANOVA) to reveal a significant difference of IgE level between groups at the P< 0.05 level of significance of the four groups Post hoc comparisons using the Scheffe test indicated that the control group (mean ± SD = 31.0 ± 17.0) was highly significantly (< 0.001) differ from group B; group A (mean ± SD = 82.0 ± 27.9) was highly significantly (< 0.001) differ from group A and B; group B (mean ± SD= 295.0± 183.35) was highly significantly (< 0.001) differ from the other three groups; group C (mean ± SD = 1661± 764) was highly significantly (< 0.001) differ from the other three groups.

Detection of serum specific IgE (sIgE) to the *Aspergillus fumigatus* allergens

Aspergillus fumigatus allergen serum sIgE levels were detected according to specifications, the results then converted into standardized classes from 0 to 4, in accordance with the instructions of the manufacturer. More than half of patients 51 (51%) showed positive results with wide variation of sIgE levels for *A. fumigatus* allergens and 49 patients (49%) exhibited negative results (Table 2).

TABLE 2: Classification of results of allergen specific IgE assay

Class	k UA/l	Specific IgE level	No. of subjects			
			Controls		Patients	
			No.	%	No.	%
0	Below 0.35	Negative	42	(84%)	9	(%)
1	0.35-0.69	Low	8	(16%)	12	(12%)
2	0.7-3.49	Moderate	0	(0%)	18	(18%)
3	3.5-16.99	High	0	(0%)	4	(14%)
4	>17	Very high	0	(0%)	7	(7%)
			Total = 50		Total = 100	

Chi- square test; x² (41.3), P<0.0001 (very high statistical significance)

Most members of the control group (84%) showed negative results while, (16%) exhibited low levels of sIgE antibodies against *A. fumigatus* allergens.

Skin prick testing (SPT) with *Aspergillus fumigatus* allergens

Skin prick test to *A. fumigatus* allergens was done for all patients and controls according to (Stallergenes)

instructions. Skin reactivity was observed in 20 (20%) of asthmatic patients, which showed immediate cutaneous reactivity to *A. fumigatus* allergens in patients with different levels of serum specific IgE antibodies (sIgE). only one of controls member (2%) showed positive skin reactivity (Table 3).

TABLE 3: Relationship between *A. fumigatus* sIgE antibodies and skin test reactivity in patients with severe asthma

Allergen specific IgE levels	Subjects							
	Patients				Control			
	No.	%	Skin reactivity	%	No.	%	Skin reactivity	%
Negative	49	49 %	0/49	0%	42	84%	0/42	0%
Low	12	12%	2/12	16.6%	8	16%	1/8	12.5%
Moderate	18	18%	6/18	33.3%	0	0%	0	0%
High	14	14%	7/14	50%	0	0%	0	0%
Very high	7	7%	5/7	71.4%	0	0%	0	0%
Total	100	100%	20/100	20%	50	100	1/50	2%

Fisher exact test; Exact Contingency (9.9), P<0.001 (very high statistical significance)

Association between specific IgE levels and SPT positivity

Aspergillus fumigatus sIgE antibodies was detected in 51% of patients while, 49 patients (49%) were negative. From total of 50 control subjects 42(84%) of them revealed negative results for both *A. fumgatus* serum sIgE and SPT while 8 (16%) demonstrated low levels of sIgE

antibodies, only one (1/8) revealed positive reaction in the SPT (Table 3). Overall, there was a positive correlation between sIgE level and skin test.

DISCUSSION

Serum total and specific IgE levels

The normal IgE levels was observed in about two third of patients and more than half of patients (51%) had variant

levels of *IgE* specific for *A. fumigatus* allergens, this result was in agreement with that of Ramey *et al.* (2005) who indicated that sensitization to *A. fumigatus* is more common in severe form of asthma and about one third of patients had high serum total IgE and 60% of all studied patients with asthma had IgE specific to *A. fumigatus* allergens

In current study the mean of serum total IgE Levels were more than twice as high in allergic subjects as in controls. The difference was highly significant ($P < 0.001$) in all three groups of patients as compared to each other and controls this finding was compatible with Agha *et al.* (1997) and Korn *et al.* (2012). Out of patients within normal total IgE group about one third of them had IgE specific for *A. fumigatus* but higher rates were recorded among patients within high level (IgE 1000 IU) and very high level (IgE 1000 IU/L) groups which was detected in 79% and 75% respectively. Statistically the differences among three groups very highly significant, this finding coincided with that of Tanaka *et al.* (2014), who also abstracted that more severe form of asthma was observed in patients with elevated levels than in those with normal or slightly elevated IgE levels and the rates of *A. fumigatus* sIgE levels was much higher in asthmatics with increased IgE levels than in those with normal levels. In this study Profound elevation of total IgE in patients could not be absolutely summation from accumulation of sIgE immunoglobulin, but mainly resulted from the gathering of specific and non-specific pools. The specific IgE could be used as crucial evidence for allergy diagnosis, the total IgE may play a vital role for screening allergic disease.

Serum IgE specific to the *Aspergillus fumigatus* allergens and skin reactivity

A more recent study from Japan published by Fukutomi *et al.* (2016) conclude the diagnosis of *A. fumigatus* can be markedly validated by the confirmation of this fungal sensitization post measuring IgE to specific allergen components, as well as skin reactivity to *A. fumigatus* specific allergens. Skin prick testing (SPT) is necessary test to confirm sensitization in IgE-mediated allergic diseases (type I allergy) in patients with severe asthma and in this study it was found that 20 % of asthmatic patients had positive skin reactivity to *A. fumigatus* antigens, and showed very high statistical significance ($P < 0.001$) of correlation between the frequency of positive skin prick test and the concentration of sIgE to *A. fumigatus* allergens, this result consistent with the finding of Heinzerling *et al.* (2013) who published that concordance between SPT and sIgE antibody levels results was between 85% - 95% but the relevance of such sensitivity to allergens both clinical symptoms and familial history must be taken into account. In a study from, India, published by Podder *et al.* (2010) skin-prick test was applied on 1079 asthmatic patients using a variety of sixteen common aero-allergens; *A. fumigatus* was found to be a most common of which 22% had positive skin

reactivity. Similarly Maurya *et al.* (2005) found that 28% of patient showed positive skin reactivity to *A. fumigatus* allergens. In present study 2% of controls showed a positive skin test results in spite of absence of high concentration of sIgE to *A. fumigatus* allergens, this finding coincide with that of Fairs *et al.* (2010) and Woolnough *et al.* (2015) who proved that low concentration of specific IgE of doubtful clinical relevance due to cross-reactivity exists between fungal allergens even among distantly related genera.

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