Specific IgE Immunoassay for House Dust Mites by Using Multiple Allergen Simultaneous Test (MAST) in Asthmatic Children

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ABSTRACT

Background: house dust mites are considered as a major cause of bronchial asthma in young children. Inhalation of dust mite allergens by hypersensitive individuals can result in acute attacks of bronchial asthma. Multiple allergen simultaneous test (MAST) for the detection of specific IgE of different allergens is one of the important diagnostic tools for bronchial asthma. **Patients and Methods:** the study group consisted of forty children suffering from bronchial asthma selected from the outpatient clinic of Pediatrics, Faculty of Medicine, Al-Azhar University, New Damietta, Egypt as well as another normal forty healthy volunteer children were selected as controls. Total IgE immunoassay and quantitative measurement of allergen specific IgE (Biocheck GmbH, Germany) were estimated for all of them. **Results:** it was found that, out of 27 positive IgE subjects, 20 were positive by poly-check test providing sensitivity of 74.1%; and out of 53 negative IgE subjects, 50 were negative, providing specificity of 94.3%. **Conclusion:** Poly-check (MAST), in vitro tests can be used in screening for allergens in asthmatic children with high specificity.

Keywords:house dust mites, bronchial asthma, allergy, specific IgE, multiple allergen simultaneous test (MAST)

INTRODUCTION

Immunoglobulin E (IgE) has an essential role in type I hypersensitivity reaction in many allergic diseases, such as bronchial asthma, sinusitis, food allergies, urticaria and atopic dermatitis as well as allergic rhinitis [1]. During the allergic response, the plasma cells produce IgE antibodies, which are capable of binding a specific allergen via its Fab portion. Different allergens stimulate the production of corresponding allergen-specific IgE antibodies [2].

The determination of specific allergens is one of the most important tests for the diagnosis and treatment of allergy [3] where Multiple Antigen Simultaneous Test (MAST) is a common tool for identifying and measuring of specific IgE for indoor allergens e.g. house dust mite's species (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) [4].

AIM OF THE WORK:

The present study was designed to detect specific IgE for house dust mites by using Multiple Allergen Simultaneous Test in asthmatic children.

PATIENTS AND METHODS:

1. Study population

The study group consisted of forty children suffering from bronchial asthma. They were selected from the outpatient clinic of Pediatrics, Faculty of Medicine, Al-Azhar University, New Damietta, Egypt during follow up. A detailed history including name, age, sex and factors aggravating symptoms e.g. dust, cold, smoking, exercise and insecticides was taken. They were examined for symptoms of bronchial asthma and other allergic conditions e.g. allergic rhinitis, dermatitis, sinusitis, conjunctivitis and urticaria as well as clinical presentation and frequency of attacks per year. Another normal forty healthy volunteer children of the same age and sex were selected as a control group. Exclusion criteria included smoking, parasitic infections, TB, and other lung diseases.

2. Specific IgE immunoassay using MAST panel

The Polycheck® (Biocheck GmbH, Germany) is an enzyme immune assay for the quantitative measurement of allergen specific IgE in serum

Received:24/4/2017 accepted:3/5/2017 DOI: 10.12816/0038196

(Fig 1). Twenty lines of relevant allergens are coated together with five lines of calibrators on a carrier membrane, which is located in the well of the Polycheck cassette. The Twenty lines represent the following allergens : Birch pollen, Alder pollen, Hazel pollen, White Oak pollen ,Timothy Grass pollen, Rye pollen, Mugwort pollen, Plantain pollen, *D. pteronyssinus*, *D. farinae*, Dog epithelia, Cat epithelia, Horse epithelia, Guinea Pig epithelia, Hamster epithelia, Rabbit epithelia, *Aspergillus fumigatus*, *Cladosporium herbarum* , *Penicillium notatum* and *Alternaria alternate*.

During incubation of the patient's serum, the allergen specific IgE binds to the corresponding allergens. Non bound serum components are removed by washing. Monoclonal ligand-labelled anti IgE antibodies bind to allergen bound IgE. Unbound antibodies are removed by washing. Enzyme labelled anti ligand bind to the immune complexes, surplus of enzyme conjugate is removed by washing. The substrate solution is added and specifically bound enzymes convert the colourless substrate to a dark precipitate. The colour intensity of the lines is proportional to the respective allergen specific IgE concentration in the patient's serum. With the help of Polycheck imaging software (BIS), a PC and a scanner, the Polycheck cassettes are interpreted. Each single allergen will be identified and according to the calibrator curve present in each cassette the concentration of each allergen specific IgE is quantified. The class scores of the IgE concentration are shown in **Table (1)**.

3. Total IgE immunoassay levels were estimated in all the subjects of the study by ELISA using kits from RADIM diagnostics (Italy) according to the manufacturer's procedure. The study was done after approval of ethical board of Al-Azhar university and an informed written consent was taken from each participant in the study.

4. Statistical analysis

Statistical package for social sciences package (SPSS Inc., USA), version 22 was used for data analysis. Comparison between groups was done by independent samples student (t) test, Mann-Whitney test and Chi square tests. Correlation between variables was calculated by spearman's correlation coefficient. P value < 0.05 was considered statistically significant.

RESULTS:

In the present work, both study and control groups were compared as regard to age and sex distribution. The mean age in the study group was 7.23±2.65 compared to 6.88±2.46 in the control group. In addition, females represented 65.0% of study group and 47.5% of the control group. Both study and control groups were comparable regarding to sinusitis and conjunctivitis and no one had urticaria. In the study group, there was positive history of exposure to dust (80.0%), cold (80.0%), exercise (55.0%), smoking (50.0%), and insecticides (30.0%) as aggravating factors. The most common clinical findings were asthma in all subjects of study group, allergic rhinitis in 40% and dermatitis (22.5%). The frequency of attack in study group was less than 1 month in 10.0%, 1-2 months in 40.0%, 2-3 months in 32.5%, 3-4 months in 7.5% and 4-5 months in 10.0% **(Table 2)**.

Total IgE was positive in 67.5% of the study group compared to none in the control group. The median value of IgE in the study group was 320 compared to 16.50 in the control group. The individual poly-check test returned no positivity in the control group, while in the study group, birch, hazel, timothy, mugwort, plantain, dog, pig, hamster, asp and pen allergens, each were positive in one subject (2.5%); while alder, oak and cat were positive in 2 subjects (5.0%); while rye, horse, rabbit, cladosp, and alt were negative. Finally, the most common positive allergen was *D. pteronyssinus* in 50% of patients while *D. farinae* was positive in 25%. When considering all positive poly-check tests (one or more allergen is positive), it was positive in 23 children (57.5%) **(Table 3)**. When comparing polycheck test with measured levels of total IgE, it was found that, out of 27 positive total IgE children, 20 were positive by poly-check test (specific IgE) providing sensitivity of 74.1%, and out of 53 negative IgE children, 50 were negative, providing specificity of 94.3% **(Table 4)**.

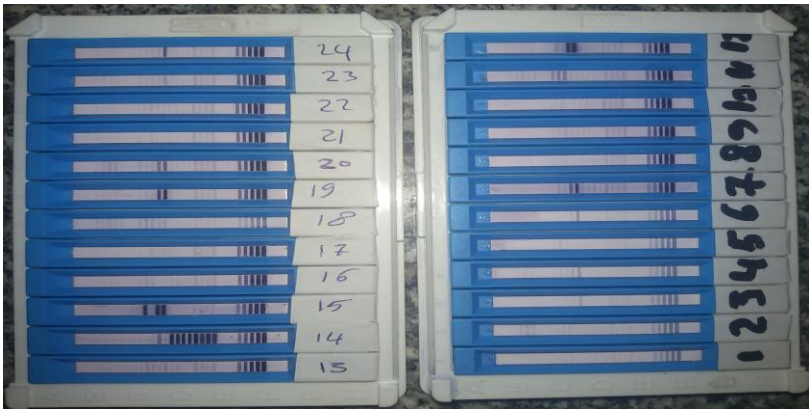

Figure (1): The Polycheck® specific IgE immunoassay MAST panel

Table (1): Class scores of the specific IgE concentration.

Class	Concentr. [kU/l]	Explanation
0	<0.35	No specific antibody detection
1	0.35 - <0.7	Very weak antibody concentration
2	0.7 - <3.5	Weak antibody concentration
3	3.5 - <17.5	Clear antibody concentration
4	17.5 - <50	Strong antibody concentration
5	50 - <100	Very strong antibody concentration
6	> 100	Extremely high antibody concentration

Table (2): Demographic data (history of exposure and clinical data of studied children**)**

		Study	Control	test	p
Age		7.23±2.65	6.88±2.46	0.61	0.54(ns)
Sex	Male	14(35.0%)	21(52.5%)	2.48	0.12(ns)
	Female	26(65.0%)	19(47.5%)		
Positive history of exposure to aggravating factors	Dust	32(80.0%)	0(0.0%)	***52.66***	***< 0.001****
	Cold	32(80.0%)	0(0.0%)	***52.66***	***< 0.001****
	Smoking	20(50.0%)	0(0.0%)	***26.33***	***<0.001****
	Exercise	22(55.0%)	0(0.0%)	***29.96***	***<0.001****
	Insecticides	12(30.0%)	0(0.0%)	***13.94***	***<0.001****
Clinical Data	Bronchial asthma	40(100.0%)	0(0.0%)	***80.0***	***<0.001****
	Allergic rhinitis	16(40.0%)	0(0.0%)	***19.75***	***<0.001****
	Dermatitis	9(22.5%)	0(0.0%)	***10.01***	***0.002****
	Sinusitis	1(2.5%)	0(0.0%)	1.01	0.50(ns)
	Conjunctivitis	2(5.1%)	0(0.0%)	2.11	0.24(ns)
	Urticaria	0(0.0%)	0(0.0%)	-	-
Frequency	0.00	0(0.0%)	40(100.0%)	***80.0***	***<0.001****
	< 1 month	4(10.0%)	0(0.0%)		
	1month s– 2 months	16(40.0%)	0(0.0%)		
	> 2 months - 3 months	13(32.5%)	0(0.0%)		
	>3 months - 4 months	3(7.5%)	0(0.0%)		
	> 4 months - 5 months	4(10.0%)	0(0.0%)		

Table (3): Total IgE and poly-check in studied children

		Study		Control		Statistics	
		n	%	n	%	Test	P value
IgE	Positive	27	67.5%	0	0.0%	***40.75***	***<0.001****
	Negative	13	32.5%	40	100.0%		
Total (mean±SD); median		(508.20±621.95); 320		(18.85±12.96); 16.50		***4.97***	***<0.001****
Individual Allergens Of polycheck	Birch	1(2.5%)		0(0.0%)		1.03	0.50(ns)
	Alder	2(5.0%)		0(0.0%)		2.05	0.24(ns)
	Hazel	1(2.5%)		0(0.0%)		1.03	0.50(ns)
	Oak	2(5.0%)		0(0.0%)		2.05	0.24(ns)
	Timothy	1(2.5%)		0(0.0%)		1.03	0.50(ns)
	Rye	0(0.0%)		0(0.0%)			
	Mugwort	1(2.5%)		0(0.0%)		1.03	0.50(ns)
	Plantain	1(2.5%)		0(0.0%)		1.03	0.50(ns)
	pteronyssinus	20(50.0%)		0(0.0%)		***26.66***	***<0.001****
	Farina	10(25.0%)		0(0.0%)		***11.42***	***0.001****
	Dog	1(2.5%)		0(0.0%)		1.03	0.50(ns)
	Cat	2(5.0%)		0(0.0%)		2.05	0.24(ns)
	Horse	0(0.0%)		0(0.0%)			
	Pig	1(2.5%)		0(0.0%)		1.03	0.50(ns)
	Hamster	1(2.5%)		0(0.0%)		1.03	0.50(ns)
	Rabbit	0(0.0%)		0(0.0%)			
	Asp	1(2.5%)		0(0.0%)		1.03	0.50(ns)
	Cladosp	0(0.0%)		0(0.0%)			
	Pen	1(2.5%)		0(0.0%)		1.03	0.50(ns)
	Alt	0(0.0%)		0(0.0%)			
All	Positive	23(57.5%)		0(0.0%)		***32.28***	***<0.001****
	Negative	17(42.5%)		40(40.0%)			

Table (4): Relation between polycheck and total IgE results.

		IgE				Statistics	
		Positive (27)		Negative(53)			
		n	%	n	%	Test	p
Poly-check	Positive	20	74.1%	3	5.7%	40.87	<0.001*
	Negative	7	25.9%	50	94.3%		
Sensitivity		74.1%					
Specificity		94.3%					
PPV		86.95%					
NPV		87.72%					
Overall accuracy		87.5%					

NB: Sensitivity, specificity, PPV and NPV were calculating using total IgE as reference standard.

DISCUSSION

Bronchial asthma is a common worldwide health problem. It was estimated that, about 300 million people having asthma [5], associated with atopy or other allergic diseases [6].

The diagnosis of asthma usually starts with a detailed clinical history. When this history suggests a link between allergic symptoms and suspected allergen exposure, both in vitro and in

vivo strategies for the detection of specific IgE antibodies are advocated to reveal sensitization [7].

Any patient may be sensitized to a single allergen (monosensitized), or to many allergens (oligosensitized), or to a huge number of different biological allergens (polysensitized). The characterization of the allergic profile is very important, because sensitization to multiple allergens is usually linked to a high prevalence and severity of asthma [8].

The progress in biochemistry and molecular biology allowed the description, cloning, recombinant manufacture, and purification of allergen proteins permitting the discovery and quantification of IgE antibodies to these proteins [9]. These allergens allowed the development of in vitro assays for the allergen-specific IgE and the development of purified allergen standards for different allergen exposure assessments as well [10].

Here, it was intended to evaluate multiple allergen simultaneous tests (MAST) in detection specific IgE for house dust mites in asthmatic children.

The panel of allergens included in the poly-check test seems to be comprehensive although it included only 20 allergens. From previous literature, it was reported that, the most widely distributed sources of allergens are the pyroglyphid *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* mites [11], temperate grass pollens [12] and cat epithelia [13]. Other important allergens with less global distributions are birch [14], olive [15], ragweed and mugwort pollens [16]. Cockroach allergy is important for inner-city dwellers in America [17]. Dog allergy has been more evident in regions with low exposure to other allergens but is also a frequent source of sensitization elsewhere [18]. This explains why this panel was chosen for the present work.

In the present work, 67.5% of the asthmatic children were IgE sensitised, while none of the control group were IgE sensitized. These results are partially comparable to those reported by **Patelis *et al.*** [6] who found that, 70.0% of asthmatic patients were IgE sensitized. However, they found IgE sensitization in about 40.0% of non-asthmatic subjects. This can be explained in the light of different inclusion criteria and the fact that, their study patients were adults, while ours are children. In addition, the different panel of allergen was different.

In the present work, house dust mites, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* were the most common detected allergens (50.0% and 25.0% respectively). Other detected allergens were just in one or two subjects. These results support the previous literature, where it was reported that, house dust mite (HDM) allergy is strongly implicated in the pathogenesis of respiratory allergic disease [19, 20], and a large proportion of patients with allergic rhinitis (AR), allergic asthma (AA), or both are sensitized to HDM, predominantly *D. pteronyssinus* and *D. farinae* [21, 22, 23].

In addition, the results of the present study are comparable to those reported by **Jung *et al.*** [4] who reported that, high total IgE group showed significantly higher positive rates and number of positive allergen specific IgE test kits used compared to low total IgE group. They added, only two of the allergens, *D. farinae* and *D.pteronyssinus* had positive concordance rates. Allergen specific IgE to these two allergens showed good correlation with total IgE.

The results of the present study revealed that, the poly-check test has a sensitivity of 74.1% for detection of specific IgE. These results are within that reported by **Ahlgrim *et al.*** [24] who reported that, a comparison of the multiplex ISAC 112 and singleplex analysis of specific IgE to grass pollen allergens revealed statistically significant correlations of the results with all the allergens. However such comparison must be explained cautiously due to different sets of allergens used in each study. In addition, the allergen binding to the test surface, differs between the two technologies, could influence the sensitivity.

In conclusion, poly-check, in vitro tests can be used in screening for allergens in asthmatic children with high specificity.

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