

DO *IN VITRO* IgE TESTS HAVE A ROLE IN IDENTIFYING ATOPIC ASTHMA?

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ABSTRACT

Background: Optimal management of asthma mandates the identification of an IgE-mediated sensitisation to allergen (atopy).

Objective: To explore the role of *in vitro* IgE tests in the identification of atopic asthma (AA).

Setting: Allergy Clinic, King Abdulaziz University Hospital (KAUH), Jeddah, Saudi Arabia.

Methods: This was a prospective study of 191 adult asthmatics. A positive reaction to common inhalant allergens (sensitisation) using skin-prick tests (SPTs) identified AA versus non-atopic asthma (NAA) patients. Patients also underwent *in vitro* IgE tests: total serum IgE, and specific multi-allergen IgE test (Phadiatop) using the Immuno-CAP system.

Results: A total of 171 adult asthmatics were included, ranging in age from 12 to 64 years old (mean=32 years \pm 13 SD); 64% were females. Moderate-persistent or mild-persistent asthma occurred in 50% and 38.3% respectively. Atopy, identified by positive SPTs, was present in 86%. Mean total IgE was higher in AA than in NAA patients: 722 \pm 888 SD versus 43 \pm 49 SD (df=1, $p < 0.05$). Total IgE was elevated (>190 IU/ml) in 70% of the AA group versus none of the NNA group. A significant linear trend was detected for means of total IgE levels of asthma severity ($F=8.2$, $p < 0.01$). Phadiatop was positive in 88% of the AA group versus 2% of the NAA; there was a significant correlation between positive SPT and Phadiatop (df=1, $p < 0.001$). Positive Phadiatop had a sensitivity of 88% and specificity of 87.5% in detecting allergen sensitisation.

Conclusion: Sensitisation to common inhalant allergens by SPT or Phadiatop was high in asthmatics at KAUH. Based on sensitivity and specificity, *in vitro* allergy tests (Phadiatop more than total IgE) can be utilised as complementary tools in the identification of AA. Strategies to modulate allergen sensitisation may improve the care of patients with AA.

INTRODUCTION

Asthma, the commonest recurrent respiratory disease, is characterised by chronic inflammation of the airways, which is frequently aggravated by exposure to inhalant allergens.^{1,2} These can activate an IgE-antibody-mediated allergic reaction (type 1 hypersensitivity or atopy).^{1,3}

Recent asthma guidelines have made advances in clinical and laboratory assessment, management and prevention of asthma.^{4,5} In conjunction with the clinical evaluation, optimal assessment of asthma includes recommendations for allergy testing to identify allergen sensitisation, particularly in uncontrolled asthma.^{4,6}

The skin-prick test (SPT) is considered the golden standard in the identification of allergen sensitisation and can help in establishing the diagnosis of atopic asthma (AA) versus non-atopic asthma (NAA).^{3,7} Unfortunately, although SPT is the most accurate, sensitive and cost-effective technique in the identification of allergen sensitisation, it needs a specialised medical care set-up (e.g. allergy clinic).^{3,8} When such a facility is not available, *in vitro* allergy testing may be an alternative to SPT in the identification of allergen sensitisation and AA.

An increase in the total serum IgE has been documented in approximately two-thirds of adults with asthma.⁹⁻¹¹ Although measuring total IgE is not recommended routinely, increased levels support the diagnosis of asthma rather than other respiratory illnesses, and suggest the possible role of an allergen-induced immunological reaction.^{10,12}

Specific multi-allergen *in vitro* tests of IgE antibodies to a mixture of common inhalant allergens have been developed to screen patients with atopic illnesses.^{13,14} Further identification of sensitisation to each calibrated allergen requires a more explicit allergy work-up, such as SPT or specific IgE antibodies to each allergen.^{3,6,12} The increased awareness of asthma in the Arabian peninsula and the availability of advanced laboratory techniques stimulated research of the possible role of allergy testing in the assessment of asthma.^{4,15} Among health care providers, there is an underestimation of the role of *in vitro* allergy tests in the identification of allergen sensitisation and in differentiating between AA and NAA. Hence, the purpose of this study was to explore the characteristics of *in vitro* IgE tests in the identification of AA at King Abdulaziz University Hospital (KAUH).

METHODS

Subjects

This was a prospective cross-sectional study of 191 chronic asthmatics, older than 12 years old. Subjects with the diagnosis of bronchial asthma based on history and physical examination were sequentially selected from patients visiting the allergy outpatient clinic at KAUH. The clinical assessment of asthma severity was conducted according to asthma guidelines.^{15,16} Subjects were classified in terms of asthma severity into four groups: mild intermittent, mild persistent, moderate persistent, and severe persistent. Patients with other medical illnesses identified by history or physical examination or those on any chronic oral medication were excluded. Verbal consent was obtained by explaining the study purpose to the candidates. This study was carried out during the period January 1997 to April 2000.

In vivo allergy tests

The diagnosis of AA was identified by the presence of a positive reaction to one or more common inhalant allergens using the *in vivo* standard SPT on the patient's forearm.^{3,10} The allergen extracts were obtained from Greer Laboratories, United States of America.

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The inhalant allergen panel included: tree pollen: *Acacia*, *Atriplex canescens*, *Cupressus arizonica*, *Eucalyptus globulus*, *Prosopis juliflora*; weed pollen: *Artemisia tridentata*, *Amaranthus hybridus*, *Ambrosia trifida*, *Chenopodium alba*, *Plantago lanceolata*, *Salsola pestifer*; grass pollen: *Cynodon dactylon*, *Phelum pratense*; moulds: *Alternaria alternata*, *Aspergillus mix*, *Candida albicans*, *Cladosporium herbarum*, *Fusarium moniliforme*, *Penicillium notatum*, *Rhizopus nigricans*; cat hair; mixed feathers (chicken, duck, goose); house-dust mites (HDMs): *Dermatophagoides pteronyssinus* (Dp), *Dermatophagoides farinae* (Df); cockroach mix (*Periplaneta americanus*, *Blatella germanica*).

A positive SPT was any reaction showing ≥ 3 mm wheal with erythema to one or more allergens. A solution of 0.1% histamine solution was used as a positive control, and a wheal size reaction of ≥ 5 mm was considered adequate. Antihistamines or other drugs that might inhibit the SPT were stopped for the appropriate duration in relation to the effect of each medication.

In vitro allergy tests

Venous blood samples were drawn for measurement of the total serum IgE level and specific multi-allergen IgE antibodies. The original method for obtaining an IgE count, the radio-allergosorbent test (RAST), has evolved from a radio-immunoassay to a test that involves enzymatic or fluorometric processes.¹⁷ Total serum IgE, and specific IgE antibodies to common inhalant allergens were measured by the radio-immunofluorescent CAP system, Phadiatop (Phadia, Sweden) which is available in the clinical immunology laboratory at KAUH. The normal range of total serum IgE is 10-190 IU/ml serum; an elevated IgE >190 kU/l. The Phadiatop test is a multi-allergen *in vitro* test that contains a mixture of several allergens bound in the matrix to detect the most common specific IgE types present in serum.¹³ It is reported as either a positive or negative result for a statistically significant level of specific IgE, but does not detect the presence of a particular specific IgE type. Additionally, in order to explore the correlation between SPT and *in vitro* specific IgE antibodies, the Phadiatop test was also performed on rhinitis cases with negative SPT.

Data analysis

Frequency tables, correlation analyses by chi-square test, and test of linearity by ANOVA were carried out using an SPSS statistical program (Version 12).

RESULTS

Twenty asthmatics were excluded from the study because of incomplete data; 171 cases fulfilling the clinical diagnosis of asthma completed the study. Their ages ranged from 12 to 64 years (mean = 32 ± 13 SD); 110 (64.3%) were female. Levels of clinical severity were moderate persistent in 86 asthmatics (50.3%) followed by mild persistent in 65 asthmatics (38%) (Fig.1). AA identified by positive SPT (sensitisation) was detected in 147 asthmatics (86%).

The different characteristics of *in vitro* allergy tests among all asthmatics are demonstrated in Table I. Using the ANOVA test, a significant difference was detected between the mean total IgE among AA versus NAA ($df=1$, $p<0.05$). Total serum IgE was elevated (>190 IU/ml) in 102 of AA cases (69.4%), and none in NAA. Additionally, using the ANOVA test, a significant linear trend was detected between means of total serum IgE and different groups of asthma severity ($F=8.2$, $p=0.003$) (Fig. 2).

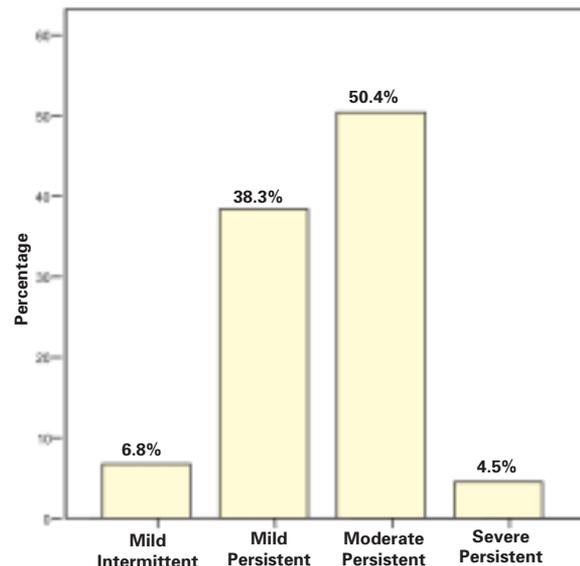


Fig. 1. Distribution of different levels of asthma severity.

Phadiatop was positive in 130 cases with AA (88.4%) and in only 3 NAA (2%), reflecting a significant correlation between positive SPT and Phadiatop ($df=1$, $p<0.001$). Positive Phadiatop tests in patients with asthma had a sensitivity of 88% and specificity of 87.5% in the detection of sensitisation to common inhalant allergens (atopy).

DISCUSSION

Although a remarkable scientific effort has been invested to improve understanding of the pathophysiology, diagnosis and treatment of asthma, less effort has been given to the clinical utilisation of laboratory tests. A presumptive diagnosis of asthma based on clinical assessment and empirical management with beta-agonist (bronchodilators) and/or inhaled corticosteroids is a reasonable and effective approach in many patients. However, in patients with significant symptoms that are not controlled with these standard measures, specific allergy testing may be warranted to establish the correct diagnosis of AA and identify the offending allergens.^{3,6}

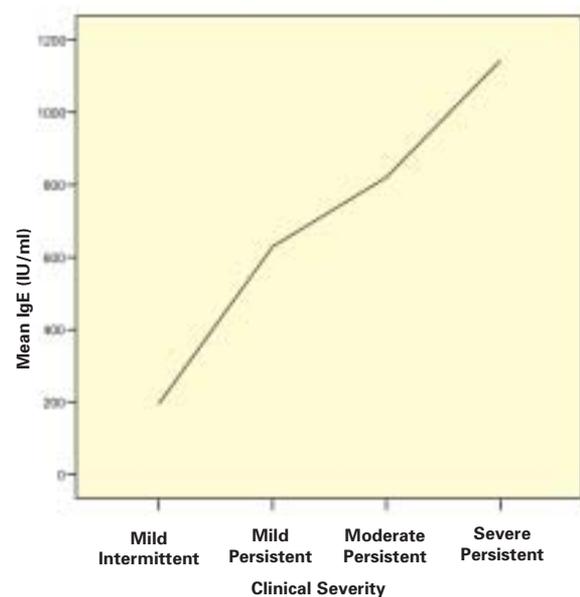


Fig. 2. Total serum IgE and levels of asthma severity.

Table 1. Characteristics of *in vitro* allergy tests in atopic asthma versus non-atopic asthma

Character	All asthma cases	Atopic asthma (AA) Positive SPT	Non-atopic asthma (NAA) Negative SPT
Total number	171 (100%)	147 (86%)	24 (14%)
Age range (years)	10 - 64	10 - 64	11 - 55
(Mean \pm SD)	(32 \pm 13)	(32 \pm 13)	(33 \pm 12)
Female	108 (63%)	93 (64%)	15 (63%)
Range of total IgE (IU/ml)	0 - 5000	1 - 5000	0 - 162
Mean \pm SD	650 \pm 864SD	722* \pm 888SD	43 \pm 49SD
Total IgE			
Normal IgE	69 (40%)	45 (31%)	24 (100%)
Elevated IgE	102 (60%)	102 (69%)	0
Negative Phadiatop	38 (22%)	17 (12%)	21 (88%)
Positive Phadiatop	133 (78%)	130** (88%)	3 (2%)
* df=1, p<0.05	Significant in AA versus NAA		
** df=1, p<000.1	Significant in AA versus NAA		

SPT was positive in 85% of the adult asthmatic cases tested in the allergy clinic at KAUH, which is higher than the range (29%-77%) in internationally reported asthma population studies.¹⁸ SPT reports from other cities in Saudi Arabia showed positive reactions in more than half the patients with a history of respiratory allergy and asthma.^{19,20} The higher rates of sensitisation to inhalant allergens at KAUH needs future exploration, and may be attributed to different environmental, social and genetic factors of the study population.

In this study, total IgE was elevated in more than two-thirds of the identified AA cases, which is in the higher range of reports in international literature.^{10,11,21} Although elevated total IgE is neither sensitive nor specific to AA, it can be helpful when combined with other findings in differentiating between AA and NAA, and also in epidemiological studies.^{3,12} Elevated total IgE can also present in patients with allergic rhinitis, atopic dermatitis, other atopic diseases, parasitic infection, allergic bronchopulmonary aspergillosis (ABPA), allergic fungal sinusitis, and also some non-allergic diseases.^{3,10}

On the other hand, normal levels of total IgE do not preclude full allergy work-up to identify sensitisation to allergens.^{10,11} If there is a small increase in IgE antibodies against one or a few allergens, this may not alter the total IgE level in the serum, which will be reported in the normal range.^{10,11} Type of sensitising allergens, their concentration levels, seasonal variation, and the prolonged duration of exposure to allergens indoors have been correlated with higher IgE levels. Hence, if allergy is suspected, specific IgE antibody level to specific allergens should be measured. This can be achieved by using specific *in vitro* IgE tests or SPTs, which can be positive in the face of a normal IgE level.^{3,12,22}

There was a significant correlation between higher total IgE level and increased levels of asthma severity. Measuring total serum IgE may correlate with clinical asthma severity, and it is a helpful tool to support the clinical assessment and the subsequent therapeutic management.^{3,5}

This study also revealed a very high correlation between positive *in vitro* Phadiatop inhalant allergen

mixture and *in vivo* SPT results, which is compatible with the literature.^{7,24,25} The Phadiatop test has been shown to be highly sensitive and specific in differentiating individuals who are sensitised to common inhalant allergens from those who are not. The *in vitro* multi-allergen IgE test is recommended as an aid in diagnostic and referral decisions, and in mass-screening programmes for patients suspected of having an inhalant allergic diathesis.^{14,26} A positive Phadiatop test requires further investigations in order to explore each possible offending specific allergen by either SPT or antigen-specific IgE antibodies test. *In vitro* specific IgE antibodies to inhalant allergens are not affected by skin reactivity or medications, have no risk of systemic reaction, are less traumatic and better tolerated.^{27,28} However, *in vitro* testing is less sensitive than SPT, the results are not available immediately, and it becomes more costly when a large panel of allergens is tested.^{27,28} A positive Phadiatop test in the face of a negative SPT can be

due to exposure to hidden or unknown inhalant allergens, intake of medication that might have interfered with SPT reactivity, and weak reactions to SPT (erythema only or a wheal <3 mm) that require the use of more sensitive tools like the intradermal skin test.^{8,12}

A positive SPT or *in vitro* IgE test suggests mainly sensitisation to the tested allergen, which may or may not have any clinical correlation.^{3,12,22} Occasionally, in order to confirm the clinical impact of an allergen in exacerbating asthma symptoms, further *in vivo* allergy testing, such as bronchoprovocation with the suspected allergen, may be conducted.²⁹ Once a clinically relevant allergen is identified, applying targeted avoidance strategies can lead to better symptom control and should be an integral part of management and prevention plans of asthma.^{3,5,30} Furthermore, specific allergy testing provides guidance about which allergens to include in allergen immunotherapy, which is a therapeutic option in AA especially when avoidance and medications no longer control the patient's symptoms.^{31,32} A total IgE level is indicated in the evaluation of asthmatic patients being considered for therapy with monoclonal antibody to IgE.^{33,34,35} Early appropriate clinical staging of asthmatic patients and assessment of suitable allergy parameters will help in choosing the optimal therapy in conjunction with the appropriate patient education to prevent further morbidity and mortality.

In conclusion, the prevalence of allergic sensitisation to common inhalant allergens using either SPT or multi-allergen IgE antibodies was a common feature in asthmatics at KAUH. Based on sensitivity and specificity, *in vitro* IgE tests (multi-allergen IgE antibodies more than elevated total IgE) can be utilised as a complementary allergy test to *in vivo* SPT in the search for allergen sensitisation in patients with asthma. The higher the total serum IgE, the more likely the presence of atopy. Additionally, the Phadiatop test can be used as an alternative allergy test in helping to identify patients with AA, particularly when SPT is not readily available. Strategies directed to modulate allergen sensitisation, as identified by appropriate allergy tests, may improve the care of patients with AA.

Declaration of conflict of interest

The author has no conflict of interest.

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