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Specific IgE in the Identification of Allergens in Allergic Rhinitis Malaysian Patients

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SUMMARY **The specific serum IgE levels to 20 allergens were determined by enzyme immunosssay in 90 Malaysian patients with allergic rhinitis. Ninety-two percent of patients had elevated IgE to at least 1 of the allergens. The housedust mites *D. pteronyssinus* and *D. farinae* were the major allergens, elevated IgE to either allergen being present in 86% of the patients. Prick skin tests were carried out in some of the patients, housedust mites, cat fur, dog hair and shrimp were the allergens used. Close correspondence was found between IgE and prick skin tests to the mites.**

Allergic rhinitis is an irritating and persistent allergy which is not life-threatening but severely affects the quality of life of those suffering from it. It is a common problem and has been claimed to account for about 35% of the patients seeking treatment in otorhinolaryngologic clinics in Malaysia,[1] and in neighbouring Singapore.[2] The main allergens in Malaysia which precipitate the symptoms of allergic rhinitis are housedust mites *(Dermatophagoides pteronyssinus and Dermatophagoides farinae)*.[1,3] Gan and Rajagopalan,[4] using radioallergosorbent assay found elevated serum IgE in 70% of patients with atopic allergy symptoms, and housedust mites were the main antigens. Other major local allergens identified are cat fur,[3] shrimp, milk and weed.[4] As far as we are aware, there is no documented work on serum IgE correlated with skin tests reactivities in Malaysian allergic rhinitis patients. Here we report the measurement by enzyme immunoassay of specific serum IgE in 90 patients with allergic rhinitis, and correlation of specific IgE with skin test reactivities in those on whom skin tests were carried out.

MATERIALS AND METHODS

Blood collection

Venous blood was obtained from 90 patients with clinical symptoms of allergic rhinitis seeking treatment at the Allergy Clinic of the Kuala Lumpur University Hospital. Clinical symptoms included lacrimation/red eye, itch (ear, nose, throat, palate), sneezing, rhinorrhea and nasal obstruction. There were approximately equal males and females, their ages ranging from 6 to 50 years (Table 1). Samples were also obtained from 25 individuals without any symptom of allergy, between ages 20 to 35, from the Institute of Advanced Studies in the University of Malaya.

Table 1. Age groups of allergic rhinitis patients.

Age group (years)	Number
1 to 10	10
11 to 20	31
21 to 30	31
31 to 40	13
41 to 50	5

Enzyme immunoassay (EIA)

Twenty allergens were tested (Table 2). EIA was performed according to the protocol given in the VENTRAX specific IgE kit (Ventrax Lab Inc., Portland, ME, USA). Briefly, allergen discs were placed in tubes and each incubated with 100 μl of the test serum

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Table 2. Allergens used in the enzyme immunassay.

Dust mites:		
D1	-	*Dermatophagoides pteronyssinus*
D2	-	*Dermatophagoides farinae*
Epithelia:		
E1	-	Cat epithelium
E2	-	Dog epithelium
Foods:		
F1	-	Chicken egg white
F9	-	Rice
F14	-	Soybean
F24	-	Shrimp
Grasses:		
G2	-	Bermuda grass
G6	-	Timothy grass
G17	-	Bahia grass
Cockroach - I6		
Molds:		
M1	-	*Penicilium notatum*
M2	-	*Cladosporium herbarum*
M3	-	*Aspergillus fumigatus*
M4	-	*Mucor racemosus*
M5	-	*Candida albicans*
M6	-	*Alternaria tenuis*
Weeds:		
W4	-	False weed
W9	-	English plantain

overnight. After washing 3 times with saline triton-X, each disc was incubated with 50 μl anti-human IgE conjugated to alkaline phosphatase overnight. The discs were washed 3 times before 200 μl of freshly-prepared 1 mg/ml p-nitrophenyl -phosphate was added for 90 minutes. One ml 1N NaOH was added as the stop solution. The colour was read spectrophotometrically as absorbance at 405 nm. The optical density (OD) readings were converted to adjusted 'counts' according to the formula provided:

$$\frac{\text{OD of patient sample} \times 25.000}{\text{mean OD of 25 U/ml calibrator}}$$

The counts were then classified according to the scoring system in Table 3. IgE readings that are 'high' and 'very high' are taken to be elevated.

Table 3. Classification of adjusted optical density readings.

Class	Specific IgE concentration	Range of adjusted 'counts'
0	undetectable	<500
0/I	very low	501-750
I	Low	751-1600
II	moderate	1601-3600
III	high	3601-8000
IV	very high	>8000

Due to a shortage of allergen discs, only 43 allergic rhinitis sera were tested against I6 (cockroach), in addition D1 and M4 were not tested against the non-allergic sera.

Prick skin test

Prick skin tests were carried out as in Lee.[4] Five antigens were used, the housedust mites *D. pteronyssinus,* housedust extract, cat fur, dog hair and shrimp.

The prick skin test readings are as follows:

Negative		No wheal or erythema (diluent control)
1+	:	No wheal. Erythema not more than 3 mm
2+	:	Wheal up to 3 mm diameter with associated erythema
3+	:	Wheal between 3 mm and 5 mm diameter with erythema
4+	:	Any larger reaction or one with pseudopodia. Readings of 4+ are taken as significant reactions.

RESULTS

Ninety-two percent of 90 allergic rhinitis patients had elevated IgE titres to at least 1 of 20 allergens, while 83% had elevated titres to 2 or more allergens (Table 4). The housedust mites *D. pteronyssinus* and *D. farinae* were the major allergens, elevated IgE were found in 79% and 78% of the patients, respectively (Table 5). Taken together, 86% of the patients had elevated titres to either of the mites. The grass family (G2, G6, G17) is the second group of main allergens, IgE being elevated in 18 to 24% of the patients. Cockroach (I6) is the least important, only 1% of the patients had elevated titres.

Of the 25 non-allergic adults, 1 had elevated IgE to 2 allergens, 9 persons had elevated titres to only 1 allergen (Table 4). In the person with elevated IgE to 2 allergens, IgE were to *D. farinae* D2 and dog epithelium E2. Three others were elevated to D2 alone, 5 to E2 alone and 1 to M6 alone (Table 6).

Correspondence between prick skin reactivities and IgE titres (Table 7)

Forty-one allergic rhinitis patients had significant prick skin reac-

Table 4. Number of allergens with elevated IgE levels.

No. of allergens	0	1	2	3	4	5	6	7	8	9
No. of rhinitis patients	7	8	27	21	6	9	4	1	5	2
No. of non-allergic persons	15	9	1	0	0	0	0	0	0	0

Table 5. IgE in allergic rhinitis patients.

Allergen	Very high	High	Moderate	Low	Very low	Undetectable	% Elevated
D1	65	6	12	5	0	2	79
D2	57	13	4	14	0	2	78
E1	3	6	28	38	7	8	10
E2	0	8	29	33	10	10	9
F1	0	3	10	44	9	24	3
F9	1	2	24	39	10	14	3
F14	4	8	19	34	8	17	13
F24	4	10	20	34	5	17	16
G2	7	15	10	33	11	14	24
G6	8	11	13	35	11	12	21
G17	5	11	14	43	10	7	18
M1	2	2	28	50	4	4	4
M2	3	1	19	39	13	15	4
M3	0	4	13	56	8	9	4
M4	1	2	22	41	13	11	3
M5	1	2	14	48	11	14	3
M6	0	2	35	43	4	6	2
W4	3	6	23	42	8	8	10
W9	2	5	18	50	11	4	8
I6	1	0	14	21	2	5	1

tions to housedust extract and also elevated serum IgE to housedust mites *(D. pteronyssinus* or *D. farinae)*, while 2 did not have significant skin test reactivity or elevated IgE. Two patients with significant skin test reactivities did not have elevated IgE, while 4 without significant skin test reactivity had elevated IgE. These give a correspondence of 88% between IgE to housedust mites and skin test reactivities to housedust extract.

Similarly, an 83% correspondence was observed between skin test reactivities and IgE to *D. pteronyssinus*, 64% between skin test reactivities to dog hair and IgE to dog epithelium E2, 50% between skin test reactivities and IgE to shrimp E24 and 38% between skin test reactivities to cat fur and IgE to cat epithelium E1.

DISCUSSION

The IgE class of immunoglobulins has long been known to be important in the mediation of the allergic response.[5] Many allergic conditions such as asthma, rhinitis, eczema and dermatitis lead to increased IgE levels.[6,7] The benchmark for allergy testing is skin testing, and a definitive diagnosis is by measurement of allergen-specific serum IgE.[8] It must also be borne in mind that some allergies are not entirely IgE-mediated, such as in pathogenic fungal hypersensitivity and delayed food sensitivity.[9,10] The testing of IgE has traditionally been by the radioallergosorbent test (RAST).[11] Modifications of RAST have also been used.[12] Detections of antibodies are often hampered by the limitation of suitable antigens, particularly antigens from local allergens. Such a limitation is also encountered when using the prick skin test, currently used in many allergy clinics for the determination of specific allergens.

Our results from prick skin tests and IgE measurement from using the VENTRAX EIA kit are in agreement with results of earlier reports from this region, in which housedust mites were consistently found to be the major allergens of rhinitis.[1,2,3,13] Ninety two percent of the allergic rhinitis patients had elevated IgE to at least 1 of 20 allergens, 79% had elevated IgE to *D. pteronyssinus* and 78% to *D. farinae*. 86% had elevated IgE to either of the mites. In a non-age matched control group, 16% had elevated IgE to *D. farinae*, thus based on IgE in a seroepidemiological survey, many false positives will be included, but specific IgE in allergic rhinitis patients will identify specific allergens.

Cat epithelium E1 is not an important IgE-inducing antigen, although cat fur is an important allergen in skin tests. In contrast to atopic allergy patients,[4] shrimp was not an important IgE-inducing antigen in allergic rhinitis patients.

Table. 6. IgE in allergic rhinitis individuals.

Allergen	Very High	High	Moderate	Low	Very Low	Undetectable
D2	2	2	7	8	2	4
E1	0	0	5	13	3	4
E2	0	6	4	10	4	1
F1	0	0	1	11	4	9
F9	0	0	4	13	2	6
F14	0	0	9	7	4	5
F24	0	0	3	10	7	5
G2	0	0	4	11	6	4
G6	0	0	0	16	1	8
G17	0	0	1	10	7	7
M1	0	0	1	19	3	2
M2	0	0	0	14	6	5
M3	0	0	2	16	3	4
M5	0	0	0	12	8	5
M6	0	1	1	12	4	7
W4	0	0	2	12	5	6
W9	0	0	0	14	1	10
I6	0	0	3	7	8	7

Table 7. Correlation between prick skin tests and serum IgE.

Skin test \ IgE	Moderate and less	Elevated
Housedust extract	*D. pteronyssinus* and *D. farinae* (D1 + D2)	
1+ to 3+	2	4
4+	2	41
D. pteronyssinus	*D. pteronyssinus* (D1)	
1+ to 3+	3	3
4+	5	37
Dog hair	Dog epithelium (E2)	
1+ to 3+	14	0
4+	10	4
Shrimp	Shrimp (F24)	
1+ to 3+	10	2
4+	10	2
Cat fur	Cat epithelium (E1)	
1+ to 3+	16	1
4+	38	8

Moulds have been found to be important allergens in America,[14] but are not important IgE-inducing allergens in Malaysia. This is consistent with previous local reports, although it remains surprising because the humid environment in Malaysia is conductive to fungal growth. The grasses are important IgE-inducing allergens, with elevated IgE being present in 18 to 24% of the patients.

A screening panel consisting of D1, D2 and G2 as in the VENTRAX EIA kit will be able to determine the major allergens in 89% of allergic rhinitis patients.

An 88% correspondence was observed between skin test reactivities to housedust extract and IgE to housedust mites *(D. pteronyssinus* or *D. farinae)* , which is not surprising since housedust extracts contain high percentages of mites.[15] The correspondence between skin test reactivities and serum IgE to housedust mites *D. pteronyssinus* was 83%. The less empirical *in vitro* EIA is therefore an effective alternative method to skin tests in the determination of allergy to housedust mites. An *in vitro* test has the advantage of being more convenient, more specific than the skin test, which is occasionally affected by the physical condition of the patient and the medication the patient is taking.

The correspondence between skin test reactions to cat fur and IgE to cat epithelium was only 38%, indicating that the allergens used in the skin tests and the EIA, being different preparations, differed significantly antigenically. The correspondence between skin reactions to dog hair and IgE to dog epithelium was however a closer 64%. The correspondence between skin test reactions and IgE to prawns was 50%, which may be a reflection of food allergies being both IgE and non-IgE induced. It is obvious that antigens must be comparable before valid comparisons can be done. Furthermore, allergens from inhalant allergens should be distinguished from food allergies.

Specific IgE are markers of allergens and the EIA kit used in this study was particularly useful in the detection of allergies to housedust mites. In addition, quantitation of allergic reactions is important in the effective application

of desensitisation treatment using allergen extracts.[1 6] The semi-quantitative EIA can provide essential information on the starting dosages for such immunotherapy. Hence serum IgE measurement, by EIA in this study, can be a useful adjunct to clinical judgement in the assessment and practical management of allergic rhinitis patients.

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