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Development of an Enzyme-Linked Immunosorbent Assay for Detection of IgE Antibodies Specific to *Dermatophagoides pteronyssinus*

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SUMMARY **Allergy to *Dermatophagoides pteronyssinus* was determined in 61 rhinitis patients using prick test (PT), enzyme-immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA). A total of 43 patients tested positive with PT. Forty six patients were positive when tested with EIA and ELISA. With PT as standard test, EIA was found to have 83.7% sensitivity and 44.4% specificity; ELISA had 81.4% sensitivity and 38.9% specificity. There was a linear relationship between absorbance values obtained by EIA and ELISA. The performance time was 8 hours, 24 hours and 30 minutes for ELISA, EIA and PT respectively. The cost per test for ELISA, EIA and PT was US$ 0.20, US$ 5.20 and US$ 0.14 respectively. It was concluded that ELISA was more cost-effective than EIA and should be used to supplement PT for a more complete diagnosis of allergy.**

The importance of mites as a causative agent of house dust allergy has been clearly demonstrated.[1] Among the multitude of mites recovered from dust of houses in Peninsular Malaysia, the most common and abundant was the species *Dermatophagoides pteronyssinus*.[2-4] This species has been shown to be an important producer of allergens affecting man in many countries. The presence of allergy to this species has been detected in the local population in Malaysia.[5,6]

Skin tests are the most common *in vivo* techniques for the diagnosis of allergy. These tests include intracutaneous, scratch and prick tests.[7] In 1967, the radioallergosorbent test (RAST) was introduced for the measurement of IgE antibodies to specific allergens.[8] The RAST utilizes radiolabelled conjugates to detect IgE antibodies. Presently commercial kits which use enzyme conjugates are available. In Malaysia, these kits are not widely used because of their prohibitive costs.

The use of an enzyme-linked immunosorbent assay (ELISA) for detection of IgE in atopics is relatively new. It has, however, been used for diagnosis of parasitic infections.[9] A study was conducted to develop an *in vitro* test for allergy to *D. pteronyssinus* based on the ELISA. This report discusses the design of the assay and comparison of the cost-effectiveness of the ELISA with the prick test (PT) and a commercial enzyme-immunoassay (EIA).

MATERIALS AND METHODS

Preparation of mite extracts

Mites were obtained from colonies kept in the Institute for Medical Research, Kuala Lumpur. These mites were maintained on a diet of yeast and cereals. Whole mites and fecal pellets were separated from the substrate by suspending batches of 50 gm of substrate and mite materials in 500 ml of saturated NaCl solution. The suspension was stirred and allowed to stand for 15 min. The layer of materials floating on the suspension was transferred to another 500 ml of saturated NaCl solution. The process of flotation and removal of top layer was repeated until the layer was free of substrate when examined under a dissecting microscope. The final layer consisted of mites and fecal pellets.

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Isolated mites and fecal pellets were resuspended in one volume of 0.9% NaCl solution. The suspension was homogenised using a tissue grinder and drive assembly. After homogenisation for 5 min in an ice-bath, the homogenate was kept overnight at 4° C. The homogenate was then centrifuged at 20,000 × g for 15 min at 4° C. Supernatant obtained was passed through a 0.2 μm filter. The protein content of the extract was determined using a protein assay kit (Bio-Rad Loboratories, California, USA) and was found to be 2.7 ± 0.1 mg/ml. The extract was lyophilized and stored at -20° C until required.

Subjects

Patients with rhinitis attending the Otorhinolaryngology Clinic in the Universiti Kebangsaan Malaysia, Kuala Lumpur, were included in the study. A total of 61 patients was tested with PT. Sera of these patients were tested with EIA and ELISA.

PT

D. pteronyssinus extract was obtained from Allergologist Laboratorium Kobonhavns (ALK, Connecticut, USA) A 1 mg/ml histamine hydrochloride solution and glycerinated diluent (ALK) were used as positive and negative controls, respectively. A volume of 0.01 ml reagent was placed on the flexor aspect of a patient's forearm. A sterile lancet was introduced into the skin through the drop of reagent; care was taken not to draw blood. The diameters of the wheals were recorded after 15 min. Reactions with wheals larger than that produced by the negative control were considered positive.

EIA

A Phadezym RAST® kit (Pharmacia, Uppsala, Sweden) was used. Fifty μl of sera were placed over reference paper discs coated with *D. pteronyssinus* extract in test tubes. Tubes were incubated for 3 hours at 30° C. After incubation, sera were removed and washing solution added. Tubes were stood for 10 min and the washing repeated twice. After complete removal of the washing solution, 50 μl of enzyme-anti-IgE solution were added to each tube and incubated overnight at 30° C. Tubes were washed thrice as above and 200 μl of development solution added to all tubes as well as to two empty tubes which served as blanks. Tubes were incubated at 37° C. After 2 hours, 1000 μl of stop solution were pipetted into all tubes. The absorbance of the products was measured at 420 nm.

ELISA

The technique of indirect ELISA was used. Mite extract was diluted with sodium carbonate-bicarbonate buffer pH 9.6, to 2 μg protein/ml and 200 μl were transferred into each well in a polystyrene microtiter plate (Dynatech Laboratories Ltd., Virginia, USA). The plate was incubated overnight at 4° C and washed with phosphate-buffered saline plus 0.05% Tween 20 pH 7.4 (PBST). Following that, 250 μl of 1% BSA were added to each well and incubated at 30° C. After an hour, the plate was washed with PBST and 200 μl of sera added. No serum was added to wells designated as blanks. After incubation for 2 hours at 30° C, the plate was washed with PBST. Next 200 μl of goat anti-human IgE peroxidase conjugate (Kirkegaard & Perry Laboratories, Inc. Maryland, USA) were added and incubated for 3 hours at 30° C. After incubation, the plate was washed with PBST and 200 μl of 0.04% ortho-phenylenediamine in phosphatecitrate buffer pH 5.0, was added to each well. After 30 min, 50 μl of 2.5M sulfuric acid were added to stop the reaction. The absorbance of each well was read at 492 nm and corrected against the absorbance of the blanks.

Sera from 15 individuals with no known allergy to *D. pteronyssinus* and who gave negative EIA results were pooled together and designated as negative control serum. Human sera which had absorbance values greater than the mean plus three times the standard deviation of the absorbance values of three replicate wells of negative control sera were considered positive.

RESULTS

Test results are shown in Table 1. It was found that 43 cases were positive when tested with PT; however, 46 cases tested positive with EIA and ELISA.

Using PT as the standard test, the sensitivity and specificity of

Table 1. Prick test (PT), Enzyme–immunoassay (EIA) and enzyme–linked immunosorbent assay (ELISA) analyses of patients with rhinitis.

Results	No. of cases
PT +ve, EIA +ve, ELISA +ve	32
PT +ve, EIA +ve, ELISA –ve	4
PT +ve, EIA –ve, ELISA +ve	3
PT +ve, EIA –ve, ELISA –ve	4
PT –ve, EIA +ve, ELISA +ve	10
PT –ve, EIA +ve, ELISA –ve	0
PT –ve, EIA –ve, ELISA +ve	1
PT –ve, EIA –ve, ELISA –ve	7
Total	61

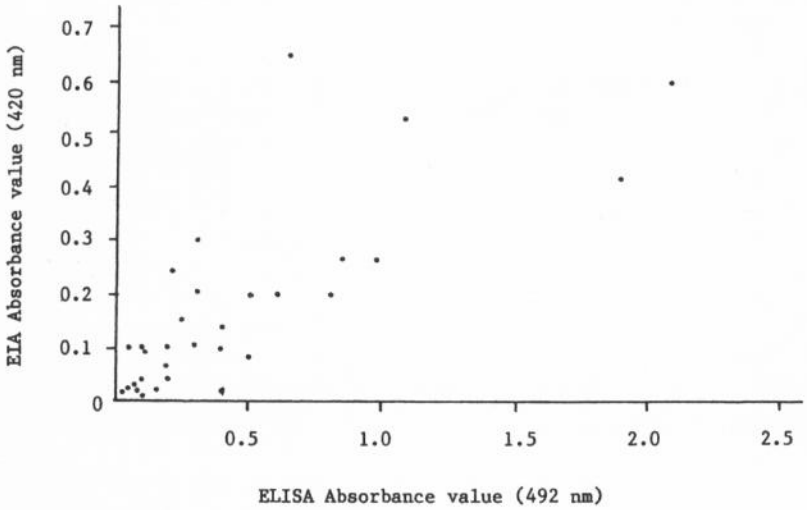

Fig. 1 Scatter diagram of enzyme–immunoassay (EIA) and enzyme–linked immunosorbent assay (ELISA) analyses of pateints with rhinitis.

ELISA and EIA were determined.[10] EIA had 83.7% sensitivity and 44.4% specificity. ELISA had 81.4% sensitivity and 38.9% specificity.

The absorbance readings for EIA and ELISA are displayed in a scatter diagram in Fig. 1. It was found that there was a positive linear relation ($p < 0.05$) with a regression line of :

$Y = 0.033 + 0.282X$ where
X = absorbance of ELISA
Y = absorbance of EIA

The EIA and ELISA required 50 and 4 μl of test sera respectively. The EIA thus required 12 times as much sera as the ELISA.

The performance time was determined for each test. For PT, this was calculated from the introduction of extract on the skin of the patient to the sizing of the wheal and flare. For EIA and ELISA, the time was recorded from the blocking step to determination of absorbance values. The PT was the fastest; wheals and flares could be observed within 30 min after introduction of extract. EIA results were available only after 24 hours; ELISA results were obtained in a shorter time of 8 hours.

Cost per test inclusive of cost of materials, chemicals and manpower, was calculated, EIA and ELISA cost approximately US$5.20 and US$0.20 per test respectively. ELISA was thus 26 times less costly than EIA. PT cost even less, at US$0.14 per test.

DISCUSSION

It was apparent that while ELISA had similar sensitivity as EIA, it was less specific. That might be due to differences in potency between extracts used in the two tests. The composition of extract used in EIA was not defined. In ELISA, crude extracts were used. The specificity of the ELISA can be improved with the use of purified allergens such as *Der p* I.[11] It has been demonstrated that as much as 95% of human IgE antibodies are directed against epitopes on *Der p* I. The different types of conjugates used could be another reason for the differences in specificity. EIA used a beta-galactosidase conjugate whereas peroxidase conjugate was used in the ELISA.

The low specificity of ELISA and EIA as compared to PT was most probably due to the different sites where IgE antibodies were detected. PT detects IgE bound to skin mast cells whereas ELISA and EIA measure IgE in serum. In earlier studies, a significant proportion of asymptomatic individuals had IgE antibodies to mites.[12] The presence of specific IgE is thus more a risk factor than a direct cause of symptoms.

The lower volume of serum required to perform ELISA compared to EIA is an advantage when a limited volume of serum is available; a larger panel of allergens can be tested with ELISA. This character also makes ELISA an ideal seroepidemiological tool in mass surveys where finger-prick blood is sufficient.

The PT was the most convenient, most inexpensive and had the shortest performance time among the three tests examined. The PT has certain limitations. Reactions can be inhibited by antihistamines and other medications.[13] The volume of allergen that actually enters the skin of the patient is not consistent; this presents a problem when the severity of the reaction is to be rated. There is always the danger of severe systemic reaction when highly sensitive patients are tested. For a more complete diagnosis of allergy, the PT should be carried out and supplemented by an *in vitro* test.

ELISA results can be obtained faster than EIA results and it is also less costly. Thus in spite of being less specific, the ELISA is more cost-effective than EIA. The ELISA will be a good choice as an *in vitro* test to supplement PT.

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