

Development of ELISA for Diagnosis of Allergy to *Dermatophagoides farinae*

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Summary

An indirect enzyme linked immunosorbent assay (ELISA) was developed for the diagnosis of allergy to a house dust mite, *Dermatophagoides farinae*. The efficacy of the ELISA was then evaluated against a prick test using a commercial allergen. Eighty five suspected allergic rhinitis patients from the Otorhinolaryngology Department, Kuala Lumpur General Hospital, were tested with the ELISA and prick test. Prick test and ELISA results were positive in 84.7% and 80.0% of the patients respectively. The ELISA was found to have 87.5% sensitivity, 61.5% specificity, 92.6% positive predictive value, 47.1% negative predictive value, 7.4% false positive and 52.9% false negative. There was total agreement between the prick test and ELISA for prick test grades of 3+ and 4+. It is concluded that the ELISA is a useful assay for detection of individuals who are highly sensitive to *D. farinae*.

Key words: *Dermatophagoides farinae*, ELISA, prick test, allergy.

Introduction

Dermatophagoides farinae is one of the few species of house dust mites which have been implicated as producers of allergens affecting man. The mite is very common in human habitations in Peninsular Malaysia; it has been found on mattresses, pillows, floors, mats and carpets¹. It has been reported that 56% of asthmatic and 65% of rhinitis patients are allergic to *D. farinae*².

The most common and easy means of diagnosing allergies is through the use of various forms of skin tests³. Skin prick test is cheap, quick and safe. Results, however, can be modified by antihistamine therapy, and may be misleading in skin disorders such as dermatographism and generalised eczema⁴. For patients in whom skin tests are unreliable, *in vitro* tests can be used for diagnosis of atopies through detection of elevated levels of specific IgE. Commercial test kits are available, but their high costs have limited their use. Less costly in-house assays can be developed for use in the country.

The purpose of this study was to develop an in-house enzyme linked immunosorbent assay (ELISA) for detection of IgE specific to *D. farinae*. The usefulness of the ELISA was then evaluated against a prick test using commercial *D. farinae* extract.

Materials and Methods

Mite extracts

D. farinae colonies kept in the Division of Acarology, Institute for Medical Research, were used to prepare whole mite culture extract. The extract was prepared by elution of whole mite cultures in a phosphate

buffered saline solution (pH 7.2) for 24 hrs at 4°C. The suspension was next centrifuged at 3,000 rpm for 10 mins to remove waste particles. The supernatant was then used for the ELISA.

Subjects

During the period March to June 1992, a total of 85 clinically suspected allergic rhinitis patients from the Otorhinolaryngology Department, Kuala Lumpur General Hospital, were included in the study. Informed consent was obtained from all the patients. These patients were examined first by prick test and then had 5 mls of venous blood withdrawn. The sera obtained were stored at -20°C until analysed.

Sera of 8 volunteers with no history of allergy to *D. farinae* and with negative prick test to the mite were pooled to form a negative control for the ELISA.

Prick test

Prick tests were conducted using a commercial *D. farinae* allergenic preparation (Bencard, UK). Drops (approximately 6 µl per drop) of the allergenic solution, histamine and diluent controls were placed on the volar part of the arm of each patient. A new sterile lancet was then used to make a superficial prick through each drop of solution; care was taken not to draw blood. The largest diameter of the resulting wheal was measured after 15 mins. A positive result was recorded if the wheal produced by the allergen was larger than that produced by the diluent control. The wheals were graded according to the manufacturer's instructions.

ELISA

The technique of indirect ELISA⁵ was used. Mite extract was diluted in sodium carbonate bicarbonate buffer, pH 9.6, to a protein concentration of 8 µg/ml. A 200 µl aliquot of the diluted extract was placed into each well of a 96-well polystyrene microtiter plate (Dynatech Laboratories, Virginia, USA). The plate was stored overnight at 4°C. The plate was next washed with phosphate buffered saline plus 0.05% Tween 20, pH 7.4 (PBST), for 3 mins. Washing was repeated 5 times. Following the washing step, 250 µl of 1% bovine serum albumin (BSA) solution was added to each well. The plate was incubated in a humid box at room temperature. After an hour, the BSA solution was removed and washing repeated as before. Two hundred µl of serum were added; no serum was added to wells designated as blanks. Each serum sample was examined in triplicate. The plate was incubated for 2 hrs in the humid box at room temperature. The plate was next washed as above and 200 µl of a suitably diluted goat anti-human IgE peroxidase conjugate (KPL, USA) was added into each well. The plate was incubated for 3 hrs, washed as before and tapped dry. Two hundred µl of 0.04% orthophenylenediamine in phosphate citrate buffer pH 5.0 was added. After incubation for 30 mins, the enzymatic reaction was stopped by the addition of 50 µl of 2.5M sulfuric acid. The optical density (OD) of each well at a wavelength of 492 nm was measured in an ELISA reader (Dynatech Laboratories, Virginia, USA). The mean OD of each test sample was determined. Samples with ODs greater than the mean of the negative control were considered positive.

Statistical analyses

The ELISA readings were compared with prick test results by construction of a 2x2 table. The sensitivity, specificity and other related parameters of the ELISA were then determined.

The mean of the OD readings for each prick test grade were compared by Analysis of Variance (ANOVA) at 95% level of significance.

Results

Of the 85 patients tested, 84.7% and 80.0% were positive to the prick test and the ELISA respectively (Table I). The majority of patients who were prick test positive were also ELISA positive. In comparison with the prick test as a standard, the ELISA was more sensitive than specific. The assay had a higher positive than negative predictive value; the percent of false negative was also high.

Table I
Comparison of ELISA with prick test for diagnosis of allergy to *D. farinae*

ELISA	Prick test	
	Positive	Negative
Positive	63	5
Negative	9	8
<i>Sensitivity</i>	=	87.5%
<i>Specificity</i>	=	61.5%
<i>Positive predictive value</i>	=	92.6%
<i>Negative predictive value</i>	=	47.1%
<i>False positive percent</i>	=	7.4%
<i>False negative percent</i>	=	52.9%

Table II
Comparison of ELISA with prick test for diagnosis of allergy to *D. farinae*

ELISA	Negative	Prick test			
		Positive			
		1+	2+	3+	4+
Negative	8	1	9	0	0
Positive	5	8	16	12	26
Total	13	9	25	12	26
% positive	38.5	88.9	64.0	100.0	100.0

The percent of false negative was highest for prick test grade of 2+ (Table II). All patients with prick test grades of 3+ and 4+ had positive ELISA results as well; ELISA readings for this group of patients were significantly higher than those with other positive and negative prick test grades ($p < 0.005$) (Table III).

It was observed that as prick test grades increased, so did the variation in ELISA readings. There was a positive correlation between positive prick test grades and ELISA readings ($r = 0.51$; $p < 0.001$).

Discussion

The low specificity of the ELISA is not unique to *D. farinae*. A similar ELISA to detect IgE specific to another house dust mite, *Dermatophagoides pteronyssinus*, had a specificity of 38.9% only⁶. It would appear that a low specificity is inherent in the ELISA when compared with the prick test.

Although both the prick test and ELISA detect allergy to *D. farinae*, the basis of their modes of action differ. A positive prick test is indicative of the presence of specific IgE bound to mast cells present in the skin, whereas the ELISA detects specific IgE in the serum. This difference is probably one of the reasons for the low specificity of the ELISA when compared with the prick test.

Table III
Mean of ELISA readings according to prick test (PT) grades

PT grade*	No of patients examined	ELISA reading* Mean±SD
Negative	13	0.04 ± 0.03
1+	9	0.06 ± 0.03
2+	25	0.09 ± 0.15
3+	12	0.15 ± 0.18**
4+	26	0.33 ± 0.28**

Mean ELISA reading for negative control is 0.05.

* $r=0.51$; $p<0.001$.

** differences significant; $p<0.005$.

Another difference between the prick test and the ELISA, which can account for the low specificity, is the source of allergens used. It is not certain what the exact nature of the allergenic solution used in the prick test is, i.e., whether the solution is extract of mites alone or of mite cultures. It has been reported that there are differences in the types of allergens present in the 2 different extracts mentioned above⁴.

Conclusion

It is concluded that the ELISA described above is a useful alternative for the prick test. In view of its low specificity and high false negative percent, it should be used only when it is not possible to conduct the prick test. Even then, patients with highly apparent clinical signs of an allergy but with negative ELISA results should be encouraged to undergo prick testing for confirmation.

The ELISAs for *D. farinae* and *D. pteronyssinus* are now available in the Institute for Medical Research, Kuala Lumpur, for the diagnosis of allergy to these mites. Interested clinicians should contact the senior author for further details.

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