ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (1998) 16:69-74

The Value of a Single Skin Prick Testing for Specific IgE *Dermatophagoides pteronyssinus* to Distinguish Atopy from Non-atopic Asthmatic Children in the Tropics

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SUMMARY In a tropical setting, where the prevalence of house dust mites (*Dermatophagoides pteronyssinus*) is high, we examined the advantage of a single battery of skin prick testing (SPT) for mite as a diagnostic tool by comparing the results of radio-allergo-sorbent-test (RAST) to distinguish allergic from non-allergic asthma in children. Fifty asthmatic children were enrolled in this study. After questioning the parents, SPT were carried out using house dust mite (*D. pteronyssinus*) and other 9 common aero-allergens and blood were taken for measuring the total IgE (PRIST) and specific IgE for mite (RAST). Dust was obtained from 14 asthmatic children's houses and mite counting was done under a high power microscope. With a daily temperature of 27.0 ± 0.5°C and a relative humidity of 80 ± 1%, house dust mites were found in all samples; and 81% of the allergic asthmatic children had positive SPT for *D. pteronyssinus*. SPT for *D. pteronyssinus* had a sensitivity of 95% and specificity of 52% using RAST as gold standard and there was a moderate positive correlation between the size of SPT wheals and RAST scores for *D. pteronyssinus* (r = 0.67 and p = 0.001). The findings of this study suggest that SPT for mites should be used as a screening test and positive SPT should be confirmed by RAST.

It is generally accepted that there are both allergic and non-allergic forms of asthma and that the distinction would be important in relation to the management of this disease (avoidance and immunotherapy). The distinction is usually based on the presence or absence of allergy. Skin test reactivity to one or more allergens is considered capable of distinguishing between these two forms of asthma.[1] Measurement of serum IgE has become a routine procedure in many countries, and the level of this immunoglobulin is sometimes used to distinguish allergic from non-allergic individuals.[2] In tropical settings, however, where the prevalence of intestinal helminths is high, serum IgE levels appear to be much higher than in subtropical countries, which might present a problem in the interpretation of total IgE levels.[3] Nevertheless, an unfortunately increasing number of medical practitioners prefer RAST for screening, rather than devoting time to gathering the best information from the patient's history and skin test results.[4] The major criticisms concerning the use of RAST testing is the lack of sensitivity and its high cost, which have limited the use of this test as a screening procedure in allergy.[5] Various attempts have been proposed to lower the costs of a screening battery based on the importance and frequency of allergens encountered in a given region.[6] Others have reduced the number of tests needed by fixing different antigens on the same disc,[4] the so called "multidisc", which is a more rapid and less expensive procedure than conventional RAST screening. Unfortunately, this screening test produces only qualita-

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tive results (positive or negative), and testing with multidisc appears to be less sensitive than testing with single allergen coated disc.

It has been known for a long time that most asthmatic attacks are caused by aeroallergens, but not until the mid's 60s, Voorhorst *et al.*[7] had proven that the main cause of house dust allergy is the house dust mite (*Dermatophagoides pteronyssinus*). The population of house dust mites depends on the room temperature, humidity and available food.[7,8] Old and humid homes, kapok mattresses and pillows are the most important habitats for mites, with optimal room temperatures between 25°C-30°C and a relative humidity between 70-85%.[8]

In the present study we attempt to assess the advantage of a single skin prick test for *D. pteronyssinus* as a diagnostic tool by comparing it with the results of *in vitro* testing (RAST) for mite, to distinguish allergic from non-allergic asthma in children in an urban setting in the tropics.

MATERIALS AND METHODS

Denpasar, the capital city of Bali, was the site for this study. It is a coastal city with a tropical climate (latitude 9°S) and is situated on a flat plain. The room temperature is between 24°C-32°C and the outdoor relative humidity is between 75%-80%. The city is free from heavy industries, since Bali has been planned and developed for tourism.

Fifty children with asthmatic attacks aged between 1.5 and 13 years, 27 males and 23 females coming for treatment at the outpatient clinic, intensive care unit, or admitted to the Central General Hospital of Denpasar were referred to the Division of Allergy and Immunology, Department of Child Health. These were the subjects of our study. The parents were questioned about cough, wheeze, breathlessness, nasal symptoms, skin diseases and any previous history of asthma, allergic rhinitis and skin atopy. The skin test was then performed and a blood sample taken. In children 8 years and older, a lung function test was carried out and in children whose lung function test appeared to be normal, a histamine inhalation test was done.

The following criteria were used to validate the diagnosis of asthma: one or more episodes of heavy breathing and/or wheezing, which was responsive to a bronchodilator.

Measurement of relative humidity

Data on outdoor relative humidity and daily temperature were obtained from the local climatological service, stationed at the Ngurah Rai International Airport of Denpasar.

House dust extraction and mite count

Specimens of dust from houses of fourteen asthmatic children were collected by the use of a hand held vacuum cleaner fitted with a filter holder. The samples were stored in sealed bags at 4°C before extraction and mite counting. Collection of bedding samples by vacuuming pillows, bedclothes, mattresses and sofas usually required 4 to 5 minutes. Mites were recovered from a 100 mg portion and identified under high power microscope.

Skin prick tests

Prick tests were carried out according to the method of Pepys[9,10] on the forearm using 10 common aeroallergens plus a positive control (histamine diphosphate 1%) as well as a negative control (normal saline). The allergens used included house dust mites (*D. pteronyssinus*), house dust, cockroach, feathers, hairs of cat and dog, wool, kapok, mixed grass pollens, and mould (*Aspergillus fumigatus*). These allergens were supplied by Hollister-Stier (U.S.A.) except *D. pteronyssinus* and house dust which were supplied by the Pharmacy Department, University of Airlangga, Surabaya, Indonesia. The size of the resulting wheals were recorded 15 minutes after the prick test and were considered positive if it was greater than 2 mm after subtraction by the control. Blood was taken from each child and the serum was collected and stored at -20°C and were sent to Perth, Australia and analysed. Serum IgE levels were determined with PRIST method (Pharmacia), and the value was expressed in international units (IU) per milliliter. The normal range in children is up to 200 IU/l.[11] Measurement of specific IgE to house dust mite (*D. pteronyssinus*) was carried out by the RAST method as described by Wide *et al.*[12] Paper disc-coupled allergen from *D. pteronyssinus* was obtained from Pharmacia. The activity count scores representing specific IgE were graded as follows: 4 very strongly positive; 3 strongly positive; 2 clearly positive; 1 borderline and 0 negative.

Statistical analysis

A comparison was made between the daily air temperature and relative humidity in dry and wet season, using the paired student t-test. Statistical significance was considered as p less than 0.01.

RESULTS

Daily temperature and outdoor relative humidity

Relative humidity recorded outdoor in the dry season between April and September (77.3 ± 1.6%) was not significantly different from that measured in the wet season between October and March (79.6 ± 1.34%) and the average temperature recorded in the dry season (27.0 ± 0.5^{0}C) was not significantly different from that measured in the wet season (28.0 ± 0.2°C) (Table 1).

Mite count

Dust was obtained from 14 asthmatic patients' houses and house dust mites were found in all samples. *Dermatophagoides* species were found in 12 of the samples with counts ranging between 20 and 104 mites/g and *D. pteronyssinus* was identified in 10 house dust samples.

Skin Tests

Table 2 shows the percentage of children who had positive reactions to one or more aeroallergens tested. In this Table, all the asthmatic children studied are included. Of all the aeroallergens tested, 8 (16%) children gave a negative result to all aeroallergens and is considered as intrinsic or nonatopic asthma. Forty-two (84%) of the remainder were considered to have atopic asthma; the house dust

Table 1 Relative humidity and daily temperature in wet and dry seasons

Season	Mean daily temperature (°C)	Relative humidity (%)
Wet (October-March)	28.0 ± 0.2	79.16 ± 1.34
Dry (April-September)	27.1 ± 0.47	77.3 ± 1.60
p	> 0.05	> 0.05

Table 2 Skin test results in 50 asthmatic children, a positive reaction is defined as being a wheal ≥ 2 mm in diameter

Skin test positive (n=42)	Number of patients	
	N	%
D. pteronyssinus	34	81.0
House dust	20	47.6
Cockroach	10	24.0
Dog hair	10	24.0
Kapok	11	26.2
Feathers	6	14.2
Cat hair	4	9.5
Wool	4	9.5
Grass pollen	2	4.8
Mould	1	2.4

Table 3 Sensitivity and specificity of the skin prick test using RAST for *Dermatophagoides pteronyssinus* as gold standard

	RAST positive	RAST negative
SPT positive	20	14
SPT negative	1	15
Total	21	29

SPT sensitivity = 20/20+1 x 100 = 95.2%
SPT specificity = 15/15+14 x 100 = 51.7%
Kappa coefficient = 0.36 (Mild association between SPT and RAST for *D. pteronyssinus*)

mite (*D. pteronyssinus*) gave the largest percentage of positive results (81.0 %). House dust was the next most common cause (47.6%), after the mites. Grass pollen and moulds gave the lowest incidence, being 4.8% and 2.4% respectively.

The mean size (± SD) of wheals for *D. pteronyssinus* was 3.8 ± 1.4 mm, for house dust 3.0 ± 1.2 , kapok 2.5 ± 0.8 and cockroach 3.4 ± 0.9 mm.

Specific IgE

In our assessment of the accuracy of the skin prick test, if the results of RAST for mite (*D. pteronyssinus*) were taken as the "gold standard", 20 positive SPT out of 21 positive RAST for *D. pteronyssinus* asthmatic children had positive RAST, and 15 out of 29 asthmatic children with negative SPT for *D. pteronyssinus* had negative RAST, making the sensitivity of the skin tests using RAST for mites as gold standard 95.2% and the specificity 51.7% (Table 3).

Fig. 1 shows that there was a moderate positive correlation between the size of skin test wheals and RAST scores for *D. pteronyssinus* ($r = 0.67$ and $p < 0.001$).

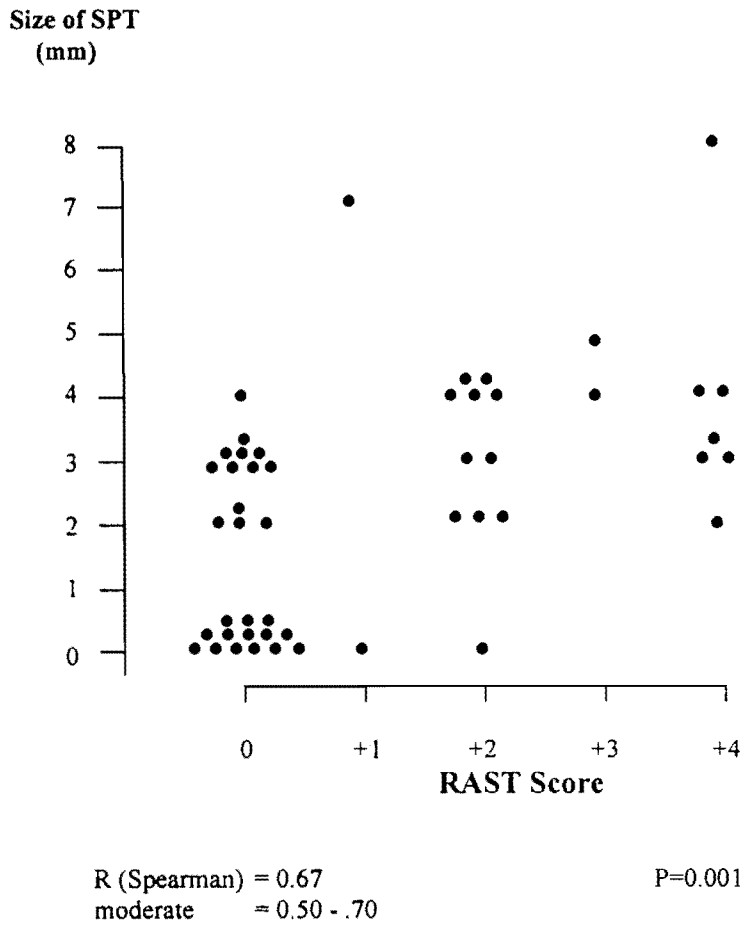

Fig. 1 Correlation between size of skin wheals and RAST score for *D. pteronyssinus* in asthmatic children.

DISCUSSION

Immediate hypersensitivity to house dust mite allergens in the tropics such as Bali is common among patients with allergic rhinitis and asthma. Most of these cases are regarded as perennial, because they do not exhibit the strict seasonal exacerbation typical of pollinosis.[13] Mites were first demonstrated to be a major source of house dust allergens, and it was reported from the Netherlands that the seasonal increase in mite numbers correlate both with increased humidity and increased symptoms.[7] Exposure to mite allergens can be assessed by counting the number of mite bodies in house dust samples, but there are two problems with this method. First, the procedure requires microscopic identification and the number is consequently relatively low; and second, the number of mite bodies is only indirectly related to the total mite allergen levels, because much of the mite allergen is fecal.

The population of house dust mites depends on air outdoor and indoor temperature, humidity and food available.[7,8] Old and humid homes, kapok mattresses and pillows are the important habitats for mites, with an optimal room temperature between 25°C and 30°C, and a relative humidity between 70 and 85%.[14] At 20°C, mites will grow at only half the rate they do at 25°C.[15] This will explain the result of our study that house dust mites were found in all dust obtained from fourteen asthmatic patients' houses and 81% of the asthmatic children had positive SPT for *D. Pteronyssinus*. Mites avoid dry conditions and will move away from the surface of

furniture as drying occurs. Live mites will only present themselves on the surface during high relative humidity, and the number of mites found in surface dust may not relate to the number of mites living in bedding, and may certainly not relate to the total quantity of allergens in dust.[13] After mites, house dust was the second most common cause (47.6%) in our patients. All house dust in the fourteen asthmatic patients' houses in our study contained mites and in 10 out of 14 (71.5%) samples *D. pteronyssinus* was identified. A small number of children gave positive reactions to a number of grass pollens to which they had not been exposed. Ragweed and timothy grass do not grow in this region, and positive reactions probably represent cross reactions to similar aeroallergens from pollen in the local environment. The diagnosis of allergic disease is made primarily by performing a detailed history and physical examination. It is almost always necessary to confirm the presence of specific IgE antibodies directed toward inhalants or foods implicated in the history. Although there is a variety of *in vivo* and *in vitro* methods for assessing the presence of specific IgE antibodies, skin testing (Prick) is preferred for initial testing. With the advent of immunoassay for allergen-specific IgE, it was predicted that the clinical usefulness of skin testing would rapidly be surpassed by the immunoassay.[16] Over the last 20 years, however, skin prick tests are still the major method for detecting allergen-specific IgE used in clinical practice for a number of reasons, eg. the high specificity and moderate sensitivity as shown in Table 3, ease of performance, rapid results, and low cost per test. Skin tests may yield false-negative or false-positive results, and the quality of the allergen extracts appears to be one of the most important variables in determining the value of the skin test.[17] Highly potent and standardized extracts may produce skin tests with fewer false-positive and false-negative reactions, and all diagnostic extracts should be obtained from reputable commercial manufacturers with excellent quality control records. Besides quality, knowledge of the person performing the tests, consistency of procedures, and consistent recording of test results should be enumerated. When properly performed, skin prick testing (SPT) is the most convenient and cost-effective method for detecting allergen-specific IgE, and in our study it appears to have a sensitivity of 95% and specificity of 51.7%, and had a moderate positive correlation between the size of SPT wheals and RAST scores for *D. pteronyssinus* ($r = 0.67$ and $p = 0.001$). SPT should not be used as a diagnostic tool, but is probably more useful as a screening procedure to distinguish allergic from non-allergic asthma. Positive results of SPT should be confirmed with the RAST for mites.

The findings of the present study suggest that, in relation to the high prevalence of mites (mostly *D. pteronyssinus*) in this region, and because a single skin prick test (SPT) for *D. pteronyssinus* had a high sensitivity and moderate specificity, SPT for *D. pteronyssinus* should not be used as a diagnostic tool, but is probably more useful as a screening procedure to distinguish allergic from non-allergic asthmatic children; and positive SPT should be confirmed with RAST for mite.

ACKNOWLEDGEMENT

I thank Prof. Keven Turner for technical support and Prof. Michael Gracey for reviewing the manuscript.

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