3.1 Responses to questionnaire

House dust mite (HDM) allergenicity in patients and healthy subjects was determined from clinical history, SPT and subsequently, through serology by using the enzyme-linked immunosorbent assay, ELISA. The SPT as described in **section 2.2**, was used among adult patients and normal healthy subjects. However, among the healthy subjects, getting more potential donors to undergo the SPT voluntarily was difficult due to the invasive nature of the SPT. This reluctance resulted in the inevitable low number of controls in the whole study.

Background information obtained from the patients (Group I and II) attending the UMMC Chest and ENT clinics and the controls, is as shown in **Table 3.1**. The asthmatic patients showed higher average age (44 yr old) than the allergic rhinitis patients (32 yrs). The average age of the control group was 25 yrs old while the average age of asthmatic children was 8. More female patients sought treatment for their allergy in all symptomatic groups especially among asthmatics (almost 70%). Three main races show almost equivalent percentage in each group. Nearly twice as many Malay children visited the asthma clinic compared to the other two races. A quarter of the allergic rhinitis patients also had asthma while 22% and 10% also had eczema and urticaria respectively. In the adult asthmatic group, 30% reported that they also suffered from rhinitis, 28% of them had eczema and 11% had urticaria too. Among the asthmatic children, 7 (6%) had both parents with allergy and 58 (42%) of these patients had at least one parent with allergy; 35 (60%) of them with maternal history. Only 56 (39%) of the 150 children had sibling(s) with allergy and 77 or 54% of them had parents without history of allergy. This relatively large percentage of patients without parental history of allergy reflects the independent nature of their sensitivity. This is not surprising as there have been increasing reports of external triggers in the environment (cigarette smoke, exhaust fumes, etc.), which can influence the susceptibility of the immune system of children to develop allergy.

Table 3.1: Demographic data for the allergic rhinitis patients (Group I), asthmatics (Group II), asthmatic children (Group III), and controls (Group IV) subjects involved in this study.

	(Group I) Allergic rhinitis patients (n=291)	(Group II) Adult asthmatics (n=298)	(Group III) Asthmatic children (n=150)	(Group IV) Normal subjects (n=30)
Age (mean±SD)	32 ± 13	44 ± 14	8 ± 4	25 ± 2
Sex				
Female	169 (58%)	206 (69%)	86 (57%)	15 (50%)
Male	122 (42%)	92(31%)	64 (43%)	15 (50%)
Ethnic background				
Malay	120 (41%)	112 (38%)	72 (48%)	15 (50%)
Chinese	96 (33%)	79 (27%)	37 (25%)	11 (37%)
Indian	71 (24%)	99 (33%)	39 (26%)	4 (13%)
Others	4 (1%)	8 (3%)	2(1%)	-
Family history				
Maternal affected	-	-	42 (28%)	NA
Paternal affected	-	-	30 (20%)	NA
Siblings affected	-	-	56 (37%)	NA
Children affected	-	-	-	NA
Associated allergies				
Asthma	72 (25%)	-	-	NA
Rhinitis	-	89 (30%)	115 (77%)	NA
Eczema	63 (22%)	82 (28%)	55 (37%)	NA
Urticaria	28 (10%)	33 (11%)	-	NA

NA: not available

In this group of children too, 11 (8%) had no other allergic symptoms. The rest had at least one other persistent allergic manifestation; 115 (77%) had rhinitic symptoms (such as runny nose, sneezing or itchy nose); 55 (39%) had eczema and 45 (32%) had allergy related-GIT symptoms such as vomiting, nausea or diarrhoea (**Table 3.1**). The number of children in the study affected by asthma *per se* is considered very low. However it is alarming to note that the majority had rhinitis, adding to their asthmatic problem. It was therefore clear that these children suffered mostly respiratory-related symptoms suggesting their susceptibility to the onslaught of foreign agents like aeroallergens.

While food can be ascertained by asking the patients about food intake information, house dust mite allergy was found to be difficult to elucidate based only on the question "Do you develop allergic reactions during or after some dusting or cleaning activities?" Most often the presence of aeroallergens in suspension in the still air is sufficient to enter the important portals of entry such as the eyes, nose and throat; to further elicit or trigger the allergic symptoms in these patients.

3.2 Results of Skin Prick Tests

In both groups, most patients showed reactivity to at least one of the allergens challenged but almost 10% of each patient group did not react to any of the allergens. Reactivity of the allergic rhinitis and asthmatic patients against the panel of allergens are as summarised below in **Table 3.2**. The panel of allergens used in the SPT for allergic rhinitis patients consisted of a mixture of aeroallergens (i.e. two HDMs) and food allergens. The asthmatics were tested with aeroallergens since food allergens did not elicit significant responses in the subjects. A positive SPT on the arm of a patient is shown in **Fig. 3.1**.

Table 3.2: Percentage of reactivity of allergic rhinitis (AR) and asthmatic (AA) patients as shown by SPT

Number of allergen(s)	No. of AR patients (Total tested= 291)	No. of AA patients (Total tested= 298)
0 *	30 (10.3%)	52 (16.7%)
1	33 (11.4%)	24 (8.2%)
2	42 (14.4%)	26 (8.6%)
2-5	91(31.3%)	114 (38.2%)
> 5	95 (32.6%)	84 (28.3%)

[N.B. * with the exception of histamine, the positive control]

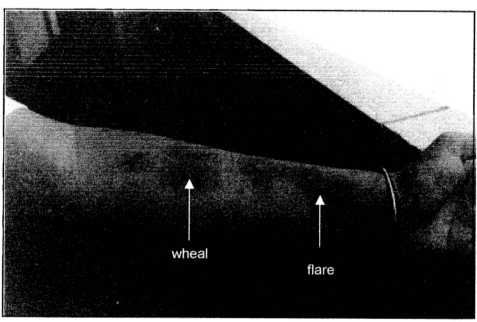


Fig. 3.1: An example of a SPT results on the arm of a patient. The wheal is seen as a small bump on the skin; and flare (erythema) is the red coloration near the area of prick, distinct from the rest of the skin. The diameter of the wheal is taken when it has a round shape; and for an oval shaped wheal; the mean of perpendicular diameters across is taken.

3.2.1 SPT results of allergic rhinitis patients

The SPT showed that most of these patients reacted positively to house dust mite allergens (75.5% to *D. pteronyssinus*; 71.7% to *D. farinae* and 65.5% to *B. tropicalis*) when compared to the rest of the aeroallergens (**Fig. 3.2**). This proved that most patients had sensitization to house dust mite allergens and indirectly implies the underlying role of HDM in triggering allergic symptoms among most of these allergic rhinitis patients. Cockroach (*Periplaneta americana*), a common domiciliary organism in Malaysia, also proved to be a significant source of allergens, affecting more than 50% of the patients.

House dust preparation containing main substance in the domestic dust such as dead skin, food debris etc. also produced positive SPT results in 40% of these patients. Other airborne allergens or aeroallergens did not elicit similarly strong responses in these patients, producing positive results in less than 20% of the patients i.e. epithelial mix, 16.6%; cat hair, 15.9%; *Acacia* 12%; mold mix, 11%; grass mix 10% and fungus *Aspergillus fumigatus*, 6.7%. Seafood extracts of crab, oyster, shrimp induced positive SPT responses in nearly 30% of the allergic rhinitis patients, followed by chilli pepper and egg white (both 25.8%), soybean (22%), chicken (15.1%) and beef (12.1%) extracts. These food extracts however did not match the high responses elicited by aeroallergens especially the mite extracts, which affected more than a two-fold percentage of the response to food.

3.2.2 Skin prick test results of adult asthmatic patients

Among the asthmatic patients too, SPT showed that HDM allergenicity was highest, affecting more than 70% of the patients (**Fig. 3.3**). The difference in percentage suggests that asthmatic patients were more affected by HDM than the allergic rhinitis patients. Out of these adult asthma patients, 78.9% of the patients were affected by *D. pteronyssinus* and 73.1 % were affected by *D. farinae*. Of interest here is the slightly higher

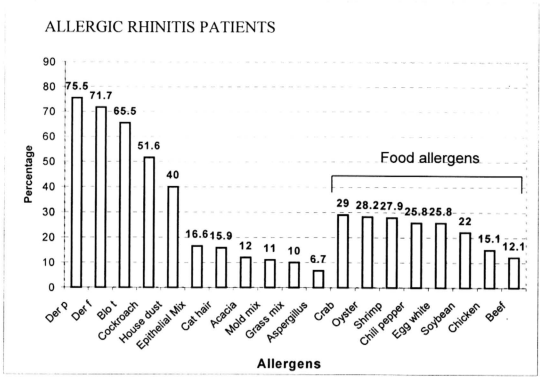


Fig. 3.2: Results of the skin prick tests, showing the percentage of allergic rhinitis patients (N=291) reacting to various allergens including aeroallergens and food extracts.

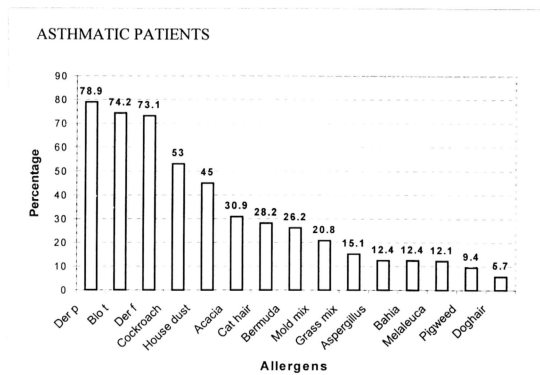


Fig. 3.3: Results of the skin prick tests, showing the percentage of asthmatic patients (N=298) reacting to various allergens including aeroallergens and plant allergens.

percentage of asthma patients (74.2%) affected by *B. tropicalis*, compared to the percentage of allergic rhinitis group (65.5%). This gave an overall picture that dust mites contribute airborne allergy triggers to asthmatics more than other aeroallergens. Domestic allergens such as cockroach and house dust each produced 53% and 45% positive results in the SPT. Together with the 51.6% of the allergic rhinitis patients in this study, cockroach allergens also affected 53% of asthmatics. Therefore, cockroach should be recognised as an allergy trigger in addition to the unhygienic connotation it brings to a household.

Both domestic allergens, cat hair and dog hair were found to have different effects on the asthma patients, with cat hair producing positive SPT in 28.2% of the 298 patients while dog hair affected only 5.7%. This wide difference stems from different practice of pet keeping in Malaysian homes. While cat lovers do not mind having cat in-doors, dogs are seldom allowed in the house. Moreover, in the Muslims' household, rearing dogs is forbidden. Therefore, the lower percentage of response was noticed. Indeed as found in many studies (Pomés *et al.*, 2001), cat hair allergens (e.g. Fel d 1) have been found to be important allergy triggers especially among asthmatics. Allergens of plant or fungal origin yielding pollen or spores produced positive SPT results in about 30% or less. Indeed, hay fever is not a common occurrence in Malaysia as most of the airborne allergens are perennial and tend to be present in the air, fortunately at low concentrations; unlike the dreaded seasonal pollen such as Lol p 1 and Lol p 5 found in temperate countries. Interestingly, although high humidity in Malaysia makes it a fertile ground for fungal growth, fungal spores do not seem to evoke a significant percentage of positive SPT among the patients. The mixed mould extract and the *A. fumigatus* extract were found to produce low responses in SPT, suggesting as if the patients in general have developed a tolerance to the fungal allergens.

3.2.3 Comparison of frequency of SPT results

Both groups of adult patients showed high reactivity (>60%) to all three species of HDMs tested in the SPT (**Table 3.3**). *D. pteronyssinus* produced highest positive SPT results (75.5% in allergic rhinitis patients and 79.5% in adult asthmatic patients) while *D. farinae* elicited positive SPT responses in 71.1% in allergic rhinitis patients and 73.5% in adult asthmatic patients. *B. tropicalis* was later included in the study, and showed equally high positive results; 65.5% of the 160 allergic rhinitis and 74.2% of the 140 adult asthmatics patients.

Cockroach was also found to be a major contributor of potent allergens in the SPT eliciting positive results in 51.6% of the allergic rhinitis patients and 53.4% of the adult asthmatic patients. House dust showed 40% and 46% positivity in allergic rhinitis and adult asthmatic group respectively. This perhaps was not a good candidate to be included in a SPT, due to a few reasons. First, this preparation was not standardised as the other allergens, and the contents were not precisely known. Had the contents been known, the extract could still be deemed unsuitable as the origin of this house dust extract would be of great difference coming from "house(s)" in a temperate country (i.e. USA) where climate, food (therefore, food residues) and even the HDM species might differ from our tropical settings. Furthermore, the HDM allergenic contents might be incorrect proportionally.

Cat hair, dog hair and epithelial mix extracts (constituting extracts of allergens that contained fur, hair, dander or dead skin, and feathers), were found to affect only a small percentage of the allergic patients. Cat hair extract produced wheals in about 16% in allergic rhinitis patients and about 27% of the adult asthmatic patients. Dog hair was initially included in the SPT panel of allergens for adult asthmatic patients but was excluded in the allergic rhinitis panel when found to elicit a low response from the patients (5.7%). Therefore, dog hair was found to be a mild, if not unimportant allergen among the asthmatic patients in contrast with cat hair allergens.

Table 3.3: Comparison of allergy prevalence of 291allergic rhinitis patient and 298 adult asthmatic patients in skin prick tests

Allergens	Allergic rhinitis patients (N=291) (%)	Adult asthmatic patients (N=298) (%)	Normal healthy subjects (N=30) (%)
HDM-*D. pteronyssinus*	75.5	79.5	74.2
HDM-*D. farinae*	71.7	73.5	61.3
* HDM-*B. tropicalis*	65.5	73.3	58.0
House dust	40.0	46.0	29.0
Cat hair	15.9	26.8	25.8
Cockroach	51.6	53.4	48.0
Acacia sp.	12.0	30.5	16.1
Grass mix	10.0	15.1	6.5
Epithelial mix	16.6	*NT*	*NT*
Mold mix	11.0	20.8	12.9
Aspergillus sp.	6.7	12.4	9.7
Bermuda grass.	*NT*	25.8	12.9
Rough Pigweed	*NT*	9.4	6.5
Melaleuca sp.	*NT*	12.1	9.7
Bahia grass	*NT*	12.4	25.8
**Dog hair	*NT*	5.7	3.2
Crab	29.0	*NT*	*NT*
Oyster	28.2	*NT*	*NT*
Shrimp	27.9	*NT*	*NT*
Chili pepper	25.8	*NT*	*NT*
Egg white	25.8	*NT*	*NT*
Soybean	22.0	*NT*	*NT*
Chicken	15.1	*NT*	*NT*
Beef	12.1	*NT*	*NT*

[*NT* : not tested
* : *Blomia tropicalis* was included later in the study, only on 160 allergic rhinitis patients and 140 adult asthmatic patients.
** : Dog hair was only tested on non-Muslim asthmatics, n=183 and 15 normal subjects]

Food allergens were tested against allergic rhinitis patients only. Seafood induced the highest reactions with crab (29%), oyster (28.2%) and shrimp (27.9%) while the others, egg white, soybean, chili pepper gave positive SPT results in 25.8%, 22 % and 25.8% respectively. Meat such as chicken and beef gave less frequent SPT responses of not more than 20%. These foods were included in our panel based on some feedback from patients in our preliminary study and because they are common in the Malaysian dishes. In the case of food allergy it is difficult to ascertain if these foods were in fact triggers of allergy in these patients, as most of the patients denied having any adverse reactions to a particular food, tested positive in the SPT. However, there were some patients who appreciated the revelation that food was related to their allergy plight, through the SPT results.

Upon finding out that food had little or ambiguous effect on the allergic manifestations in the allergic rhinitis patients, the panel of allergens were then modified by including more aeroallergens especially for the asthmatic patients in a following study. Most patients did not show much reaction to these allergens; grass mix, *Acacia* sp., mold mix and the fungus, *A. fumigatus* induced positive results in 10%, 12%, 11% and 6.7% respectively. However in the asthmatic group, these same allergens could elicit a significant number of positive results with *Acacia* sp. (30.5%); grass mix (15.1%); mold mix (20.8%); and *A. fumigatus* (12.4%). Other aeroallergens included in the SPT for the asthmatics were grasses of Bermuda, rough pigweed and *Bahia;* each showed 25.8%, 9.4% and 12.4% positive results. Pollen extract from the *Melaleuca* sp. induced 12.1% positive SPT results. Between these two tree species (*Acacia* and *Melaleuca*), *Acacia* elicits more response from the patients with more than a two-fold percentage. This interesting finding is not surprising since the *Acacia* such as the *Acacia mangium* is a common tree grown along our main roads in the city of Kuala Lumpur.

The results of the SPTs of the two groups of patients showed that the allergens induced a frequency that was significantly different ($p<0.05$); clearly seen when comparing the reactions when patients were challenged with aeroallergens. The adult asthmatics gave higher percentage of response. Results also showed that the vast majority (>70%) of patients visiting the hospital with allergic rhinitis and asthma had reacted positively to extracts of HDM. Both *Dermatophagoides* sp. and *Blomia* sp. seemed to elicit almost equivalent number of reactions in the patients and therefore justified the emphasis given to house dust mite allergenicity in this study.

HDM allergy was also found to be predominant among the volunteers who underwent SPT as normal healthy subjects (**Fig. 3.4**). The first three high responses in the SPT of these subjects showed that the three HDMs species tested were recognised by them, with *D. pteronyssinus* affecting 74.2% followed by *D. farinae* (61.3%) and *B. tropicalis* (58%). Cockroach also affected almost half of these subjects (15/31) while house dust allergy were traced in almost a third (9/31) of them. This pattern of allergenicity to HDMs, cockroach and house dust was observed to be similar in the three groups of allergic rhinitis, asthma and normal subjects. This may not be an unusual phenomenon, which only serves to show common allergy affecting the Malaysian population, but it also indirectly shows the predominance of **domestic aeroallergens** in the Malaysian household.

When comparing SPT responses of both groups (patients and NHS) to the same allergens such as HDM and cockroach, adult asthmatics showed higher percentage in most cases. However because food was not tested against asthmatics, a true picture of food sensitization could not be drawn.

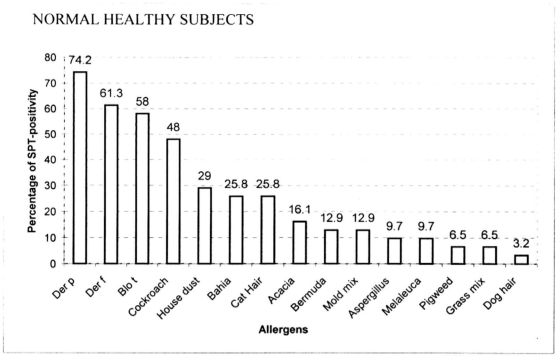


Fig. 3.4: Percentage of SPT reactivity to the some allergens tested on 30 normal healthy subjects.

3.3 *In vitro* investigation of HDM allergy

The sera of subgroups comprising 160 allergic rhinitis patients, 140 asthmatics, 150 asthmatic children and 30 normal healthy subjects were used in the ELISA for the mite specific IgE detection as described in section **2.8.1**. Only the last 160 allergic rhinitis patients and the last 140 adult asthmatics were pre-selected for this *in vitro* investigation because they had undergone the SPT with *B. tropicalis* extract, included later in this study. Extracts of the three HDM were used as antigens and HRP-conjugated anti-human IgE antibodies served as the secondary antibody. The use of OPD as the colorimetric reagent required the absorbance to be taken at 450 nm. Test on each serum was carried out in duplicates and the nett absorbance was obtained from the mean of negative control wells, giving the dOD value. An arbitrary dOD of 0.2 served as the cut-off value based on the reading of the majority of the normal healthy subjects. The overall results of the ELISA showing HDM allergy prevalence are shown in **Table 3.4**, followed by further description of the results of each group.

Table 3.4: HDM allergy prevalence as shown by positive IgE binding reactivities (dOD> 0.2) in ELISA

	N	Der p	Der f	Blo t
Allergic rhinitis patients	160	72 (46%)	68 (43%)	76 (48%)
Adult asthmatic patients	140	98 (71%)	47 (34%)	90 (65%)
Asthmatic children	150	113 (75%)	128 (85%)	111 (74%)
Normal healthy subjects	30	9 (30%)	6 (20%)	8 (26%)

3.3.1 HDM allergy in allergic rhinitis patients

The profiles of HDM sensitivity as detected by ELISA on sera of 160 allergic rhinitis patients is shown in **Fig. 3.5.** The IgE binding reactivity to the crude extracts showed absorbance that ranged from 0 to as high as 3.8. Blo t-IgE antibodies were detected more frequently than antibodies to the *Dermatophagoides* spp., as reflected by the even distribution of absorbance. Mean values of dOD were almost equivalent for both *Dermatophagoides* spp., but Blo t showed the highest mean value of nearly 0.8. This was a result of most sera having stronger binding capacity to the allergen. Therefore, in terms of frequency and strength of binding, *B. tropicalis* allergy seemed more pronounced than *Dermatophogoides* allergy in allergic rhinitis patients.

Corresponding absorbance values were used to construct graphs of paired IgE-binding reactivities of the patients to the HDM, with conversion to logarithmic values, resulting in **Fig. 3.6.** The correlation value of each graph was also obtained. Such depiction of paired specific IgE binding reactivities cannot be taken to verify cross-reactivities of two HDM species, as would an inhibition assay. However, such presentation is able to reflect on the pattern of sensitisation of patients to these HDMs, and in terms of exposure to these allergens in the environment leading up to the immunogenic responses in these patients.

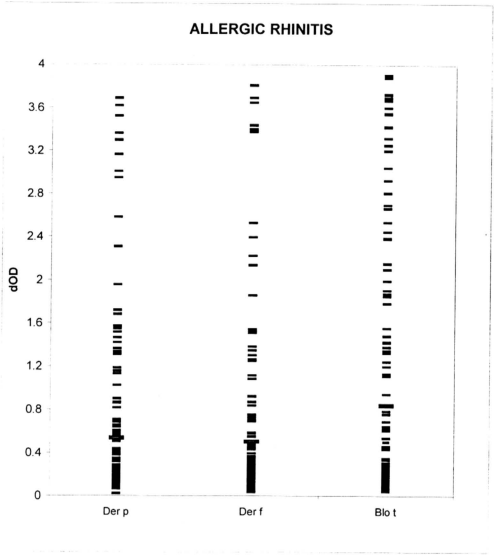


Fig. 3.5: HDMs sensitivity as detected by ELISA on sera of 160 allergic rhinitis patients. HDM species: *D. pteronyssinus* (Der p), *D. farinae* (Der f) and *B. tropicalis* (Blo t). The longer lines are mean values of each group.

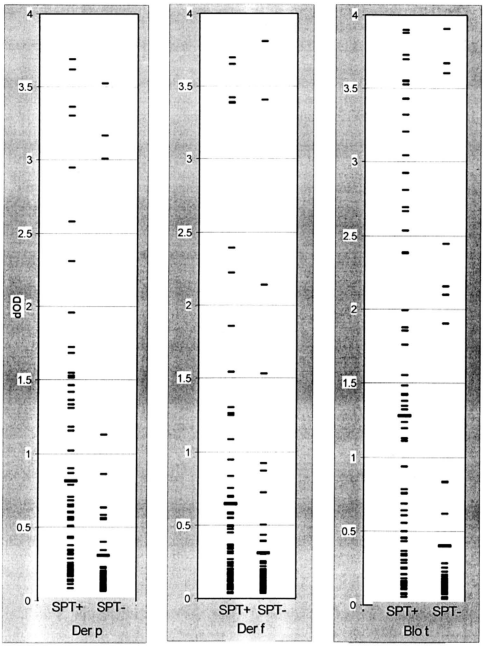
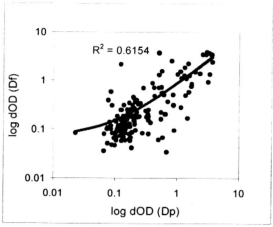


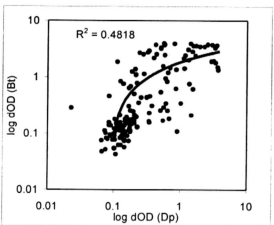
Fig. 3.6: IgE binding reactivities of SPT-positive (SPT+)/ (n=79) and SPT-negative (SPT-)/(n=81) allergic rhinitis patients to the three HDM species, *D. pteronyssinus* (Der p). *D. farinae* (Der f) and *B. tropicalis* (Blo t). The longer lines are mean values of each group.

In the first graph of **Fig. 3.7**, allergens from the same species of HDM, Der p and Der f, appropriately showed higher correlation value (R^2=0.6154) of the OD, reflecting the high concomitant response to Der p and Der f, but the confluence of points nearer to the x-axis suggests that these allergic patients were more affected by Der p allergens than Der f allergens. Similarly, in the second graph (R^2=0.4818), the confluence of points at the upper part of the graph, nearer to the y-axis, again reflects the higher response to Blo t compared to Der f. Finally, when put up together, the paired points suggest an almost equivalent response to Der p and Blo t allergens in these patients' sera, yielding the correlation coefficient (R^2=0.5011). This value is second to that of the comparison of Der p and Der f, thereby confirming the dominance of Der p and Blo t allergenicity among these allergic rhinitis patients.

3.3.2 HDM allergy in adult asthmatic patients

The same analysis performed on the IgE reactivities of the 140 asthmatic patients (**Fig. 3.8**) however showed slight differences in the pattern of binding. These graphs for the asthmatic patients showed more dispersed absorbance; therefore producing correlation coefficients that were lower when compared to those of the allergic rhinitis patients (**Fig. 3.9**). *D. pteronyssinus* and *B. tropicalis* remained the two species among the three which produced greater IgE binding capacity, each 71% and 65% of the population tested by ELISA. Surprisingly, Der f allergens were only recognized by 34% of the 140 sera tested. As in the profiles of HDM sensitivity of allergic rhinitis patients, ELISA of these 140 asthmatics showed IgE binding reactivity to the crude extracts showed absorbance that ranged from 0 to as high as 3.8 (**Fig. 3.8**). Similarly, Blo t-IgE antibodies were detected more frequently than antibodies to the *D. farinae*. Mean values of dOD were highest for *B. tropicalis* and lowest in *D. farinae*. Therefore, in terms of frequency and strength of binding of specific IgE antibodies, *B. tropicalis* allergy seemed more pronounced than *Dermatophogoides* spp. in asthmatic patients too.





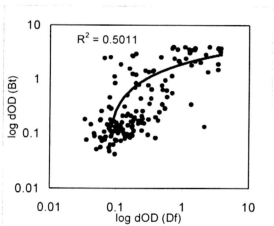


Fig. 3.7: IgE reactivities against 3 HDM extracts in 160 allergic rhinitis patients (Dp –*Dermatophagoides pteronyssinus*); Df – *D. farinae*; Bt-*Blomia tropicalis*) Absorbance was taken at dual wavelengths of 490/630 nm and dOD values wer in logarithmic values. The correlation coefficient (R^2) was obtained for eac group.

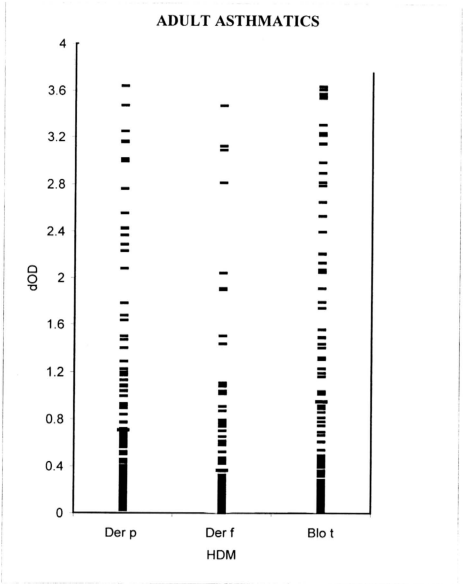


Fig. 3.8: HDMs sensitivity as detected by ELISA on sera of 140 asthmatic patients. The longer line represents the mean values. HDM species: *Dermatophagoides pteronyssinus* (Der p), *D. farinae* (Der f) and *B. tropicalis* (Blo t).

Corresponding absorbance values of paired IgE binding reactivities (**Fig. 3.9**) conversion to logarithmic values, as in **Fig. 3.10** gave correlation values that were lower than that obtained from the analysis of allergic rhinitis patients (**Fig. 3.7**). This reflects that the degree and pattern of HDM sensitisation of asthmatic patients differ from that of the allergic rhinitis patients. In the first graph of **Fig. 3.10**, paired IgE binding to Der p and Der f when compared still gave the highest values (R^2=0.483, p<0.005) while the paired IgE binding of Der p and Blo t also gave a significant correlation value of (R^2=0.3568, p<0.005); as there were some patients who had very low binding capacity. With regard to Blo t and Der f allergens, these asthmatic patients showed an interesting picture of IgE binding capacity as a result of a diffused cluster of points at the upper part of the graph, away from the x-axis, again reflecting the higher response to Blo t compared to Der f. Not only was the correlation coefficient very low (R^2=0.0786, p<0.005) the logarithmic values of the absorbance appeared skewed in favour of Blo t. The higher frequency of *B. tropicalis* sensitization compared to *D. farinae*, therefore suggests a higher exposure to it. Finally, when put up together, the paired points suggest an almost equivalent response to Der p and Blo t allergens in these asthmatics' sera, yielding the correlation coefficient, R^2=0.3568. Indirectly too, the highly dispersed pattern of absorbance and the low correlation coefficient (R^2=0.0786) obtained when comparing the responses to Der f and Blo t suggests the lack of concomitant allergy to these two species of HDMs in the population studied. *B. tropicalis* proved to be the more dominant species between the two mites (*B. tropicalis* and *D. farinae*), therefore showing more points on the upper left of the graph. This reflects the order of prevalence of HDM species in affecting allergic rhinitis patients to be *D. pteronyssinus*, *B. tropicalis* and *D. farinae*. In addition, lower reactivity to Der f allergen presented in **Table 3.5** indirectly suggests its lower prevalence in the household in the tropics, as reported frequently; while the higher responses to both Der p and Blo t allergens agree with their higher prevalence in the patients' environment. In conclusion, most of these allergic rhinitis patients showed more responses to Der p and Blo t allergens.

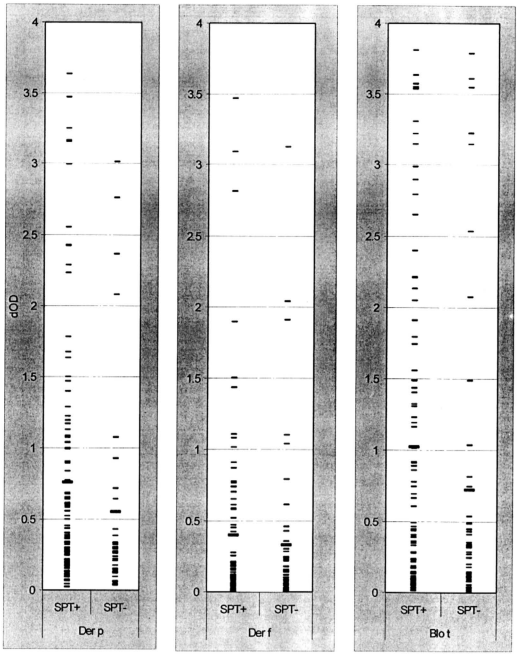
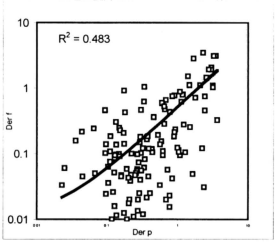
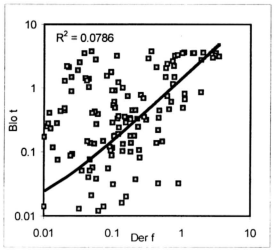


Fig. 3.9: IgE binding reactivities of SPT-positive (SPT+)/ (n=70) and SPT-negative (SPT-)/(n=70) asthmatic patients to the three HDM species, *D. pteronyssinus* (Der p), *D. farinae* (Der f) and *B. tropicalis* (Blo t). The longer lines are mean values of each group.





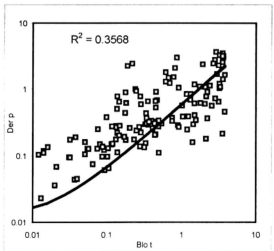


Fig. 3.10: IgE reactivities against 3 HDM extracts in 140 asthmatic patients. Absorbance was at dual wavelengths of 490/630 nm and dOD values were in logarithmic values. The correlation coefficient (R^2) was obtained for each graph.

3.3.3 HDM allergy in asthmatic children

All three crude extracts of the species of HDMs were tested by using the indirect ELISA against the sera of 150 young patients presenting with asthmatic symptoms. The results were further analysed by comparison of the titres of specific IgE thereby classifying these patients according to magnitude of sensitisation to these allergens. Of these 150 children, 90% had sensitisation to at least one of the three HDM allergens in this study, in which 98 (65.3%) had specific-IgE to all three HDMs. *D. pteronyssinus*-specific IgE was found in 75.3% of these children; *D. farinae*-specific IgE in 85.3% and 74.3% had IgE against *B. tropicalis* **(Fig. 3.11)**. Eighteen (12%) children showed monosensitization; mostly to *D. farinae* (8.7%) followed by *B. tropicalis* (2%) and *D. pteronyssinus* (0.7%). Eleven (8.7%) of these children had IgE to both species of *Dermatophagoides* mites tested but not to *B. tropicalis*. Of these 150 asthmatic children, specific IgE to the HDM could not be detected in 15 (10%) of them. It was interesting to find out that *D. farinae* affected the children more than *D. pteronyssinus*. The almost equivalent percentage of sensitisation to both *D. pteronyssinus* and *B. tropicalis* also showed the significance of *B. tropicalis* in triggering allergy. Indeed, this species was found to be an important HDM in Malaysian homes (Mariana *et al.*, 2000), besides the commonly reported *Dermatophagoides* spp.

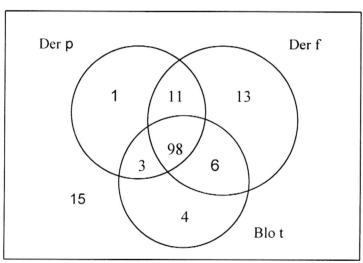


Fig. 3.11: House dust mite allergenicity of 150 children. Der p = *Dermatophagoides pteronyssinus*, Der f = *D. farinae*; Blo t = *Blomia tropicalis*.

3.4 Dust sampling in houses of 10 year-old children

One of the objectives of this survey of indoor allergen levels in the Asia/Pacific region was to measure the mean levels of exposure to three aeroallergens (Der p 1, Der f 1, Fel d 1) for a representative sample of children from geographically dispersed study centres. However, emphasis was given on the presence of house dust mite allergens particularly, Der p 1 and Der f 1.

3.4.1 Analysis of the questionnaire

The number of occupants in the 36 houses ranged from 3 to 9 with the number of children in the houses ranging from 1 to 7. Means for number of occupants and children in house were 6 and 3 respectively. There was no correlation between number of occupants and the presence of mite allergens in the houses ($r<0.01$).

Duration of their occupancy ranged from more than a year to 27 years (mean: 10 yrs). Out of the 36 houses visited; 50% had carpets with age of carpets ranging from few months to 10 years (mean 1.5 yrs). Half of the children slept of spring mattresses while another half slept on foam and/or rubber mattresses. Of these children, 8 (22%) experienced wheezing, 15 (42%) had runny nose in the morning and 11 (31%) had eczema on part(s) of their body. Pets were found in 8 (22%) of the houses and six of the owners showed at least one allergy symptom.

3.4.2 Analysis of the dust samples

Mite allergens of *D. pteronyssinus* (Der p 1) and *D. farinae* (Der f 1) were detected in 27 of 36 (75 %) children's homes selected for this house dust study. Due to the small amount of dust samples obtained from a few of the houses, most levels were considered to be below the detection limit. Levels of detectable mite allergens ranged from 0.26 μg/g to 103.97 μg/g of Der p 1 and 0.19 μg/g to 84.23 μg/g of Der f 1 allergens; mean values being

6.33 μg/g and 2.26 μg/g respectively. Both these mite allergens were detected in 11 (31%) of the children's homes. Der f 1 allergen was also found only on the floors of 3 (8%) houses and in mattress samples of 12 (33%) houses. **Table 3.6** shows the maximal mite allergen load in the houses involved in the study, with stratified levels of the two mite allergens.

Out of the 17 children who had allergic rhinitis, 9 (53%) of them had detectable Der p 1 and/or Der f 1 in their houses. Three children (43%) of 7 who had asthma had detectable Der p 1 and/or Der f 1 allergens in their houses. In the case of 9 who had allergic dermatitis, 7 (78%) had detectable Der p 1 and/or Der f 1 allergens. Twelve of the 18 (67%) inner sprung mattresses and 7 of the 18 (39%) foam or rubber mattresses harboured mite allergens. Der p 1 was still found on the only mattress which had an anti-mite cover. .

Table 3.6: The maximal allergen load of Group 1 allergens of *D. pteronyssinus* (Der p 1) and *D. farinae* (Der f 1) in houses of 36 schoolchildren.

	≤ 0.01 μg/g	0.011-9.99 μg/g	10 -19.99 μg/g	>20 μg/g
Der p 1				
Floor	19 (53%)	9 (25%)	4 (11%)	4 (11%)
Mattress	14 (42%)	15 (42%)	2 (6%)	3 (6%)
Der f 1				
Floor	33 (92%)	3 (8%)	-	-
Mattress	23 (64%)	9 (25%)	1(3%)	2(6%)

Der p 1 allergen was detected more frequently in the dust samples from the floor and mattress of the houses whereas Der f 1 was more prevalent in the floor dust samples, as seen in **Fig. 3.12**. In fact, the incidence of Der f 1 allergen was almost equivalent in the dust and almost 50% had detectable levels of Der p 1 in the dust samples and more than half of the number of mattresses studied (56%) had Der p 1 compared to 33% for Der f 1. Similarly, Der f 1 was not so prevalent in the dust samples from the floors, detectable only in 3 (8%) of the samples. Dust samples from mattresses were found to harbour more allergens than the floor. While most house shoed concomitant presence of both allergens, some of the houses showed presence of only Der p 1 while single incidence of Der f 1 was detected in 3 houses, all occurring at high concentrations (**Fig. 3.13**).

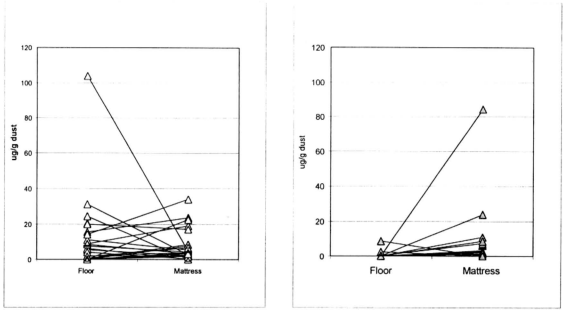


Fig. 3.12: The maximal allergen load of Der p 1 (left) and Der f 1 (right) on the floors and mattresses in the homes of 36 schoolchildren.

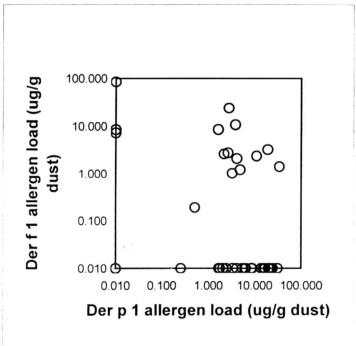


Fig. 3.13: The Der p 1 and Der f 1 prevalence in the floor and mattress dust samples (in logarithmic values of the dOD) in the homes of 36 schoolchildren, represented by open circles.

3.5 Detection of IgA and IgG antibodies in allergy patients and controls

The ELISA method as described in **section 2.7.3** was used to detect the antibody levels in the sera collected. For IgG detection, serum was used at 1:50 dilution and the anti-human IgG at 1:3000. As for both IgE and IgA, which are found in low concentrations in the serum, serum and saliva were tested at 1:10 dilution, and the anti-human immunoglobulins used were at 1:1000. In this semi-quantitative assay, the reading of absorbance or optical density (OD) at dual wavelengths of 492/540 nm was taken as proportional to the antibody titre bound to the antigens (allergenic molecules). A dOD reading of above or equal to 0.200 was considered a positive result.

3.5.1 Antibody levels in sera of negative controls

The preliminary assay, involving ten sera of the SPT-negative controls to measure the levels of mite-specific IgE (anti-Der p and anti-Der f IgE) antibody, using ELISA yielded results shown in **Fig. 3.14**; that is consistent with the fact that specific-IgE antibody is low in a non-atopic individual. In this OPD-substrate system, the dOD obtained were all below the value 0.200. This value was thus taken arbitrarily to act as reference for positivity in most ELISA assays involving OPD as the colorimetric reagent in this study.

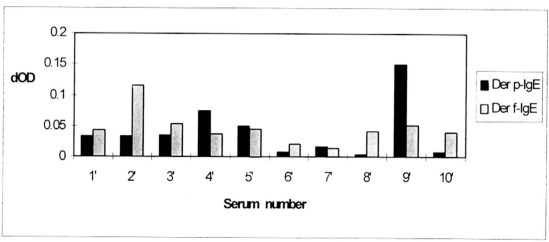


Fig. 3.14: Specific-IgE titres of ten SPT-negative controls. [N.B.: Der p and Der f denote the allergenic extracts of HDM-*D. pteronyssinus* and *D. farinae*] A dOD ≥ 0.200 was considered a positive result.

3.5.2 Antibody levels in the sera of atopic patients

Although IgE antibody is the key feature in allergy, other antibodies such as anti-mite IgG and IgA in the serum were investigated too. Sera from 38 allergic rhinitis patients were simultaneously examined for various anti-Der p antibody levels (IgE, IgG and IgA). **Fig. 3.15** shows the antibody profile of the 38 allergic rhinitis patients. IgE remained least detected, showing low dOD and less frequency among the patients. The low absorbance reflects its presence in lower quantity in the serum. However anti-mite IgG was detected more frequently and at higher titres, showing that most of these patients have been sensitized by Der p allergens. IgA antibodies in the serum and those in the saliva showed almost equivalently with anti-IgG. Looking at this trend of binding of various antibodies in each allergic subject suggests that other than IgE antibodies, salivary IgA might play a role in allergy determination, making it a basis for the study as reported in **section 3.6.**

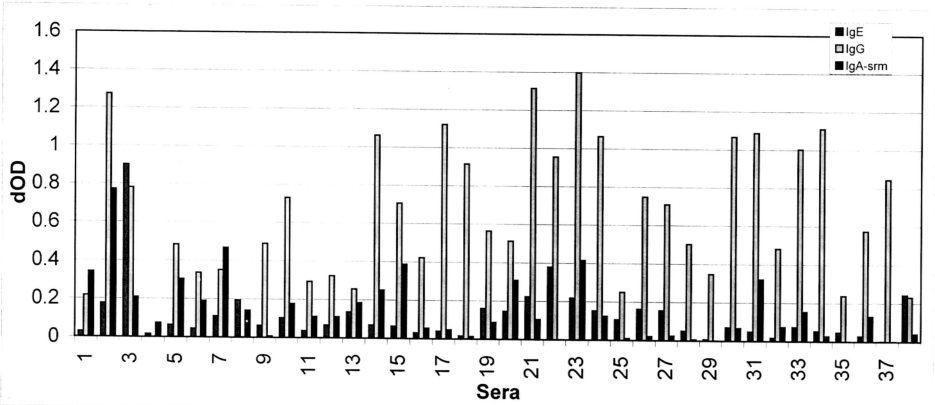


Fig. 3.15: Antibody profile of 38 allergic rhinitis patients. Serum antibodies to Der p allergen (IgE, IgG and IgA) in each patient were detected by ELISA.

3.6 Mite specific salivary IgA antibodies of allergic rhinitis patients to *D. pteronyssinus* extract

Of interest here was the presence of the anti-mite IgA antibodies in the saliva. Not only did they show more frequently in the antibody array, the titres of these salivary IgA were also much higher. This preliminary examination of the antibodies in allergic patients became the basis for further studying salivary anti-IgA in trying to find out if it could serve as an indicator for allergy, comparing it with the established IgE.

Serum and saliva samples were taken from fifty-two subjects with a history of allergic rhinitis (mean age: 29.1 ± 12.5 yrs; age range: 10 - 57 yrs) following SPT. Another group comprising 13 normal healthy subjects (mean age: 37.9 ± 12.0 yrs; age range: 23-58 yrs) with neither perennial rhinitis nor any clinical symptoms of allergy served as negative controls. SPT was carried out as described previously in section **2.2.3** while indirect ELISA as described in section **2.2.4** was then used to measure mite-specific antibodies; IgE in the serum and IgA in the saliva.

Three subgroups were compared in this analysis. The first group (n=42) was patients with clinical history of allergic rhinitis and positive SPT. Eleven patients (26%) had positive IgE levels and 21 patients (50%) were IgA-positive (**Table 3.7**, **Fig. 3.16**). The second group comprised of patients (n=10) with clinical history but was SPT-negative. None in this group had IgE antibodies but 3 patients (30%) had salivary IgA antibodies to Der p (**Table 3.7**). When groups were combined (n=52) and individual patients were examined, 7 (13%) had both serum IgE and salivary IgA antibodies and IgE alone was positive in four patients (8%). Twenty-four patients (46%) were negative for both serum IgE and salivary IgA antibodies to Der p. Interestingly; there were 17 patients (33%), negative for serum IgE who had salivary IgA antibodies to Der p. In the control group of

healthy normals (n=13) all were IgE-negative and one gave a weak salivary IgA reactivity to Der p (**Fig. 3.16**).

Out of the 52 allergic rhinitis patients, 42/52 (81%) reacted to Der p. Among these SPT-positive patients, the *in vitro* ELISA test for IgE detected specific antibodies to Der p I in only 11 (26%) of the patients sera. There was a greater frequency of IgA positivity in the saliva compared with mite-specific serum IgE. This trend of antibody presence was similarly observed for SPT-negative rhinitic patients. Interestingly, in individual samples, there was 17/52 (33%) IgE-negative rhinitic patients who had anti-Der p IgA antibodies in their saliva.

Table 3.7: Positivity of Der p-specific serum IgE and salivary IgA in allergic rhinitis patients and controls

	Allergic rhinitis patients		Negative controls (SPT-negative) **(n=13)**
	SPT-positive **(n=42)**	SPT-negative **(n=10)**	
+IgE (serum)	11 (26%)	0 (0%)	0 (0%)
+IgA (saliva)	21 (50%)	3 (30%)	1 (8%)

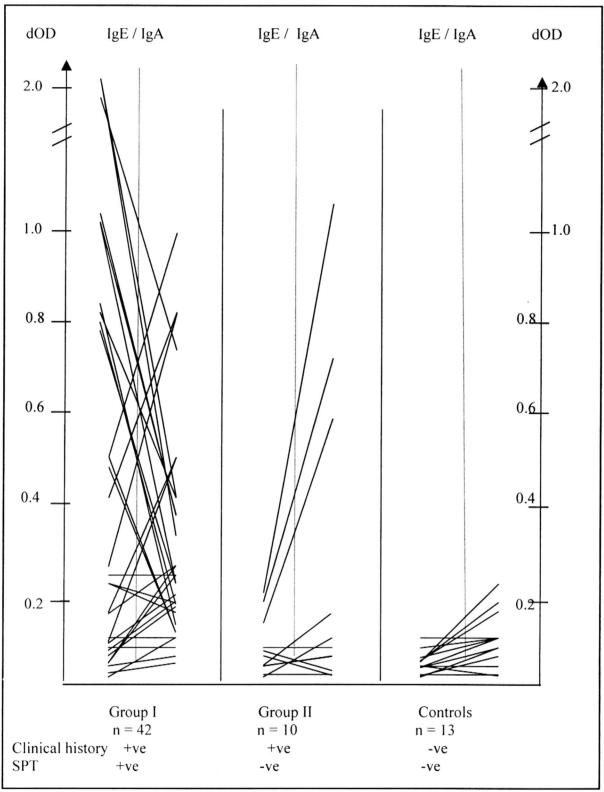


Fig. 3.16: The dOD representing binding reactivities of antibodies in the sera (IgE) and saliva (IgA) from allergic rhinitis patients and a control group to house dust mite *D. pteronyssinus*. A positive anti-mite reaction was considered when dOD ≥ 0.2.

3.7 House dust mite sensitisation and severity of asthma

From the adult asthmatic patients in this study, a group of 54 patients were observed (**Table 3.8**), ages ranging from 18 to 77 years (mean: 46 ± 12) to study the relationship of the duration of asthma and the age of onset with the severity of this disease. The severity status of these asthmatics (mild, moderate and severe) was ascertained based on the International Consensus Report on the Management of Asthma (Anon., 1992). Group I (n=42) consisted of adult asthmatic patients with clinical history of asthma and a positive SPT response to Der p allergens. Group II (n=12) included patients with clinical history but did not respond in SPT. The third group was the controls (n=13). The optical density (OD) of equal or above 0.2 was taken as a positive antibody binding reactivity. From these 54 asthmatics, SPT was able to distinguish 42 (78%) Der p-positives and 12 (22%) negatives. In contrast, the ELISA detected a lower frequency of anti-mite IgE positivity i.e. only 25 (46%) of the patients' sera were Der p-specific IgE-positive; with only one SPT-negative case showing the presence of IgE.

A closer look at the severity of asthma with that of their SPT responses revealed that most patients reacted to Der p (above 70%) within their groups (**Table 3.9**). There were 8 out of 10 (80%) patients who reacted to Der p in the mild group, 23 out of 30 (77%) in the moderate and 11 out of 14 (78%) in the severe group, all representing a percentage of nearly 80% of the study population of 54 patients. An almost equivalent percentage of patients (20-23%) in each group did not react to either of the mite allergens during SPT. Serum IgE titres against *D. pteronyssinus* mites in relation to the severity of asthma shows a bigger percentage in the mild (50%) and the moderate group (60%) (**Table 3.10**). Therefore, ELISA failed to show elevated anti-mite IgE titres in almost 50% of the mild and severe group respectively and 37% of the patients with moderate asthma. There was no proportional increase of percentage of patients having elevated anti-Der p IgE, with that of severity.

Table 3.8: The mean age, age of onset and the duration of asthma of three status groups of adult asthmatics

SEVERITY	N	Mean age (± SD) yrs	Mean age of onset (± SD) yrs	Mean duration of asthma (± SD) yrs
MILD	10	41.2 ± 15.9	27.0 ± 19.0	14.4 ± 8.0
MODERATE	30	46.4 ± 11.5	25.2 ± 16.0	21.8 ± 12.5
SEVERE	14	48.0 ± 7.2	26.1 ± 16.4	21.9 ± 15.0

Table 3.9: SPT results against Der p allergen of the 54 patients in comparison to their asthma severity

	N	**SPT**	**n**	**n/N (%)**	**n/54 (%)**
MILD	**10**	+	8	80	15
		-	2	20	4
MODERATE	**30**	+	23	77	43
		-	7	23	13
SEVERE	**14**	+	11	78	20
		-	3	21	6

Table 3.10: ELISA results of serum anti-mite IgE of the 54 patients in comparison to their asthma severity

	N	**ELISA**	**n**	**n/N (%)**	**[n/54] %**
MILD	**10**	+	5	50	9
		-	5	50	9
MODERATE	**30**	+	18	60	33
		-	12	40	22
SEVERE	**14**	+	3	21	6
		-	11	79	20

In fact, the severe group of patients showed an equivalent percentage of anti-mite IgE positivity with the mild sufferers. Among patients with moderate asthma the anti-mite IgE positivity to Der p was found in 60% of the patients, representing 33% of the total patients tested. Still, 28/54 (52%) patients failed to exhibit elevated IgE titres in the ELISA. There appears to be no direct association between frequency of elevated serum IgE and severity of asthma, although high anti-Der p SPT-positive results were detected in those groups. The percentage of anti-mite IgE positivity within the moderate asthma sufferers was almost three times the percentage of severe sufferers.

In this study group, patients with mild asthma have suffered the disease for as long as 4 years to 25 years while in the moderate group of patients, all the 30 patients suffer the disease for as long as 4 to 56 years. The 14 severe patients have experienced asthma for as short as only 2 years to as long as 54 years. The severe and the moderate asthmatics suffer nearly twice the disease duration of the mild asthmatic patients. This also proves that disease duration is neither an indication nor the determining factor of the severity of disease. Determination of cross-reactivity is another important part of the evaluation of serodiagnostic assays since cross-reactivity is an unwanted phenomenon when the aim is specific diagnostic tests.

In summary, of the 54 patients who were involved in this part of the study, 10 of them were classified as mild asthmatics, 30 as moderate asthmatics, while 14 were classified as severe asthmatics. The SPT showed almost equivalent percentage of positive incidence (~80%) and of negative (~20%) incidence in all the three groups of asthmatics. Therefore, most of the patients in each group showed positive SPT reaction to Der p allergen. In the group of mild asthmatics, equivalent percentage of positive and negative IgE reactivity was observed, whereas in the moderate group, there was more positive IgE binding reactivity (60%) than the IgE-negative. However in the severe group, a lower percentage of IgE reactivity was found in comparison to the high absence of specific Der p-IgE antibodies in the sera (~80%).

3.8 Discussion

Determination of HDM antigenicity through clinical history and SPT

Currently, the diagnosis of sensitisation to HDMs is based on clinical history and on SPT utilizing extracts prepared from HDM cultures. However, the reliability of the diagnosis largely depends on the quality of extract used, which is directly influenced by several factors including the raw material used, protease content and storage conditions. Therefore, the use of commercial extracts from different sources in *in vitro* and *in vivo* tests may show some degree of discrepancy (Nikolaizik *et al.*, 1996). Even standardised extracts are heterogeneous mixtures of proteins subjected to batch variation with unknown allergen contents.

Clinical history of allergy can sometimes be misleading, especially in patients who have strong aeroallergen sensitivity and chronic low dose exposure to the allergens, e.g., house dust mite or cat hair. Often, patients will notice symptoms from irritants (due to a nonspecific nasal hyperactivity) like smoke, cold air and perfumes but will not notice immediate symptoms at home. The patient will wrongly assume these irritants as allergens, when they are actually mere secondary irritant triggers. Therefore case history is considered not sufficiently informative with regards to house dust mite allergy, especially in a population that is not aware or well informed of its existence.

Results from SPT on allergic rhinitis patients (N=291) and asthmatic patients (N=298) showed that almost 76% of allergic rhinitis patients and 80% of the asthmatics reacted to the *D. pteronyssinus* allergen while 72% and 74% of the respective groups showed positive results to *D. farinae*. Interestingly *B. tropicalis,* previously known as a storage mite, has begun to be noticed as an important contributor of allergens in our local household too. Using ELISA for IgE reactivity, among the subgroup of the allergic rhinitis patients (N=160), only 72 (46%) and 68 (43%) reacted to the Der p and Der f allergens

while 76 (48%) reacted to Blo t allergen. On the other hand, asthma patients (N=140) showed a higher percentage of positive reactions to Der p (71%) and Blo t (65%) but only 34% to Der f.

Most asthmatics react to environmental aeroallergens with acute broncho-constriction response. The mechanism involves IgE-mediated reactions but not all asthmatic symptoms are caused by IgE immune response (Poulter *et al.*, 1994). This is seen in this study where patients with positive SPT did not have elevated IgE. In comparison to their asthma severity, all three groups showed high responses in SPT, resulting in more than 70% of patients within each severity group (mild: 80%; moderate: 80% and severe: 78%) (**Table 3.9**). However, through ELISA using the sera of the patients, anti-Der p IgE antibodies were only detected in 50% of the mild asthmatics, 60% of the moderate asthmatics and 21% of the severe asthmatics (**Table 3.10**).

Thirty percent of children in the world are now allergic to HDM, a figure that has increased over the last two decades along with the incidence of asthma (Thomas *et al.*, 1998). Within the paediatrics population in this study, the complete picture of HDM allergy could not be ascertained due to the lack of a SPT profile of the subjects. As such, HDM allergy could only be based on ELISA. In these 150 child asthmatics, 75% had specific IgE to Der p; 85% to Der f and 74% to Blo t. Although these high responses to Der p and Blo t were not surprising, matching those of the adult asthmatics; the percentage of response to Der f was rather unusual, as adult asthmatics had shown a much lower percentage of Der f sensitisation. This result gives rise to speculations such as the possibility of "tethering" allergy to Der f as an atopic subject grows older or the possibility that adult asthmatics are not affected by *D. farinae* as much as *D. pteronyssinus*. However too, this result is not surprising as *D. farinae* was among the species that accounted for only 1% of mites identified in this region (Chew *et al.*, 1999).

In the SPT-negative control group, albeit a small number, they showed response in the SPT, indicating the state of non-sensitisation. In the normal healthy subject group, subjects often also showed lower or no response in SPT; when compared to their counterparts who were allergic. Nevertheless, although they did not complain of allergic symptoms; this group reacted to the allergens in the SPT. This study suggests that a negative-SPT for mite and/or IgE-negative serology cannot necessarily rule out an involvement of mite immunity in asthma patients. This is true from our data **(Fig. 3.16)** that showed a considerable number of asthma patients having antibodies to mites in their saliva although they did not respond positively in SPT or in the IgE mite ELISA.

Seroprevalence of HDM allergenicity through ELISA

This seroprevalence study through ELISA showed high frequency of HDM sensitization among the patients, reflecting the presence of the mite allergens in Malaysian houses, with *D. pteronyssinus* and *B. tropicalis* among the predominant species. The percentage of sensitisation showed allergy to *D. pteronyssinus* was more prevalent but the mean of values of the absorbance against *B. tropicalis* was higher than against *D. pteronyssinus,* suggesting the relatively higher magnitude of IgE recognition to *B. tropicalis.* Responses to these mites also differ at state levels as mite specific IgE traced in the sera of donors from 6 different sites in Malaysia showed notably that more subjects in the East Malaysia are exposed and sensitised than subjects from the Peninsular Malaysia.

Dust sampling for Der p 1 and Der f 1

The dust samples study for the detection of Der p 1 and Der f 1 traced these allergens more frequently in mattresses. Therefore, an exposure-response relationship would indicate that the bed is where mites thrive the most. The higher frequency of Der p 1 incidence in the household indicates higher frequency of *D. pteronyssinus* prevalence

in Malaysian homes. As it is well established that Der p 1 is found in the fecal particles of the mites, this is indication that a frequent change of the mattress cover is needed to control Der p 1 exposure.

Is mite- specific IgA in the saliva a useful marker?

Saliva, with its lysozyme, has been used in assays involving bacterial and food antigens to measure salivary IgA, IgG and IgM by microbiologists (Russel *et al.*, 1989). However, scarce literature reviews on saliva as a useful biological fluid for allergy immunoassay, together with the fact that it is easily acquired from the patients, justifies further study on saliva in allergy diagnosis. Perennial manifestations of persistent runny nose, sneezing, and itchy or blocked nose often force rhinitis patients to resort to mouth breathing (King, 1990), therefore exposing the oral cavity to more aeroallergens such as the dust, pollen and HDMs (Platts-Mills *et al.*, 1990). Since there is contact between oral mucosa and aeroallergens, the presence of mite-specific IgE and IgA in the salivary samples was suspected and therefore investigated. In this study, through ELISA, Der p-specific IgA was detectable in the saliva. Despite many trials, IgE antibody in the saliva failed to be detected, perhaps due to its inherent scarcity.

However, the detection of IgA to HDM in saliva is novel and has not been vastly reported previously although this study found an unclear inverse correlation of salivary IgA with serum IgE. Since the IgA anti-mite reactivity is infrequent in normal healthy controls, the elevated IgA levels detected in patients' saliva may be part of the immunological disturbances in asthma. This indeed reflected the exposure to mite allergens in the environment and the activation of local mucosal immune system (Platts-Mills *et al.*, 1990). The precise involvement of IgA in mite allergy-related asthma remains to be determined. Others have however described certain association between IgA and atopy as well as

idiotypic interactions of both IgG and IgA anti-IgE antibodies in sera (Salvaggio *et al.*, 1973; Magnusson, 1994)

As seen in this study, detection of mite-specific salivary IgA can only be used as an additional indicator to complement the serum HDM-IgE level for mite allergy determination in rhinitic patients. To say that saliva is useful marker would need a more refined study eliminating possibilities of cross-reactivity to food allergens, i.e. with more definite specificity. Although saliva is easily obtained from patients and non-invasive, recommendations for any future attempts to collect salivary samples should include being free of food residues; preferably tested immediately and not kept for a considerable period as was done in this study. Perhaps, with the recombinant allergens, a multi-allergen assay could be constructed, preferably with the allergens adsorbed onto membranes and tested as 'dip-sticks" with the saliva collected.