Association between house dust mite (HDM) sensitisation and asthma control using skin prick test and HDM antigen specific IgE levels in saliva of Malaysian children

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Background: Sensitisation to house dust mite (HDM) has been regarded as a major risk factor for development of asthma. This study was carried out to investigate the profiles of HDM sensitisation among Malaysian children with asthma.

Material and Methods: The association between HDM sensitisation and control and severity of asthma was investigated. The salivary HDM specific IgE levels were quantified in different grades of control and severity of asthma in 125 unselected asthmatic children aged 5-12 years old attending the asthma follow-up clinic in Hospital Tuanku Ja'afar Seremban. An additional 29 non-asthmatic patients were selected as control. The skin prick test to assess sensitisation to *Dermatophagoides pteronyssinus* (DP) and *Dermatophagoides farinae* (DF) was performed on all the participants. A questionnaire regarding the control and severity of asthmatic symptoms of the subject was administered. Saliva was collected by voluntary spitting and ELISA was used to quantify the IgE specific to HDM antigen.

Results: There was a significant association between sensitisation to DP and DF and the control of asthma. The association between DP sensitisation and severity of asthma just failed to reach a significant level although there is a clear trend for this. Significant association was found between DF sensitisation and severity. The HDM specific IgE in the saliva was significantly higher in asthmatic patients compared to non-asthmatic patients. There was no significant difference between the specific IgE levels in patients with different severity status of asthma.

Conclusion: Salivary IgE levels may not be an appropriate indicator of the patients' asthmatic condition in this study. However, it can be concluded that there is significant association between the sensitisation of HDM and the control and severity of asthma.

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Introduction

Asthma is defined as a chronic inflammatory disorder characterised by reversible obstruction of the airways.[1] Evidence suggests that it has been documented by ancient Egyptians.[2] Currently, approximately 235 million people worldwide have been diagnosed with asthma. It is the most common chronic disease among children and its prevalence is increasing by an astounding fifty percent every decade. Asthma is a disease that has a high prevalence in both developing and developed countries, thus causing a heavy socio-economic burden.[3, 4] In the United States, there were almost half a million hospitalisation due to asthma in 2002. The total annual cost of the management of asthma was estimated to be $16.1 billion in the same year.[5] Besides that, the "disability adjusted life years" (DALY), representing the loss of one year of healthy life due to asthma worldwide is 15.0 x 10^6 years, representing 1% of the total disease burden.[6]

Studies on the causation of this under-diagnosed and under-treated disease have found that genetic and environmental factors play an important role in its development.[3, 7-8] The Phase One of International Study of Asthma and Allergies in Childhood (ISAAC) found that there is a marked variation in the prevalence of asthma symptoms between different countries. This variation can be up to 15-fold and is likely due to environmental factors.[9]

The house dust mite (HDM) is the most potent indoor source of environmental allergen. Its relationship with asthma has been established for decades. The most important species of HDM that have been widely studied are the *Dermatophagoides spp*.[10-12] They play an important role in the onset and development of asthma.[13]

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The European house dust mite, *Dermatophagoides pteronyssinus* (DP) and American house dust mite, *Dermatophagoides farinae* (DF) can be found easily in the household setting. They find comfort in warm, dark and damp environment; are abundant in bedroom mattresses, living room upholstered sofas, bedroom floors and living room carpets.[14-16]

There are sixteen known allergens of the HDM with the major allergens being the enzymes in faecal matter.[17-18] The allergens can cause airway hyper-responsiveness and increase frequency of nocturnal wheezing in asthmatic patients.[10, 19-20] A study in England found that the clinical activity and severity of asthma in mite-sensitive patients were related to the level of exposure to mite allergen, which further emphasise the importance of HDM as a causative agent for asthma.[21]

To date, there are only a few studies on the sensitisation profiles of HDM in childhood asthma in South East Asia.

The general objective of this study was to determine the profiles of some Malaysian asthmatic children who were sensitised to the HDM (DP and DF). The association of HDM sensitisation on the control and severity of childhood asthma was also investigated.

Another objective of this study was to test the suitability of using saliva in immunoassay to quantify levels of Immunoglobulin (Ig)E and its association with the severity and control of asthmatic patients. The comparison between the result of skin prick test and IgE titre in the saliva was also studied.

Materials and Methods

Based on previous studies, the proportion of asthmatic patients sensitised to HDM is not less than 80% but that associated with severe asthma is not known. The minimum recommended number of asthmatics to be studied if the estimate is to fall within 10% points of the true proportion with 95% confidence, is a sample size of at least 61. However, as the asthmatic children recruited for the study were not randomized, a larger number (125) was included in the study.

The subjects were unselected children attending an asthma follow-up clinic or admitted in the ward in Hospital Tuanku Ja'afar Seremban. They were patients diagnosed to be suffering from asthma. A total of 29 non-asthmatic children attending the general clinic in Hospital Tuanku Ja'afar Seremban were also recruited as the control group.

Skin prick test was performed on all the participants. Their saliva was collected. A questionnaire regarding the control and severity of asthmatic symptoms of the participant was administered. The control and severity of asthma were assessed in accordance to the Global Initiative for Asthma (GINA) guideline 2008.[22]

The saliva collected was selected randomly and the IgE specific to both DP and DF was quantified by the enzyme linked immunosorbent assay (ELISA).

Results

The majority of the patients with mite sensitisation are sensitised to both species of HDM (91 out of 125 or 72.8%). Only a small percentage of patients were sensitised to either DP (10 or 8.0%) or DF (11 or 8.8%) alone.

The skin prick test showed differences between patient groups and their DP and DF sensitisation rates (Table 1). Asthmatic patients had a significantly higher percentage of sensitisation when compared to the control group. Asthmatic patients in the older age groups had a significantly higher percentage of sensitisation to both DP and DF. A higher percentage of Malays (79.9%) was positive to DP than Chinese and Indians (53.3 % and 53.6% respectively). There are no significant association between gender and the rate of sensitisation of asthmatic children.

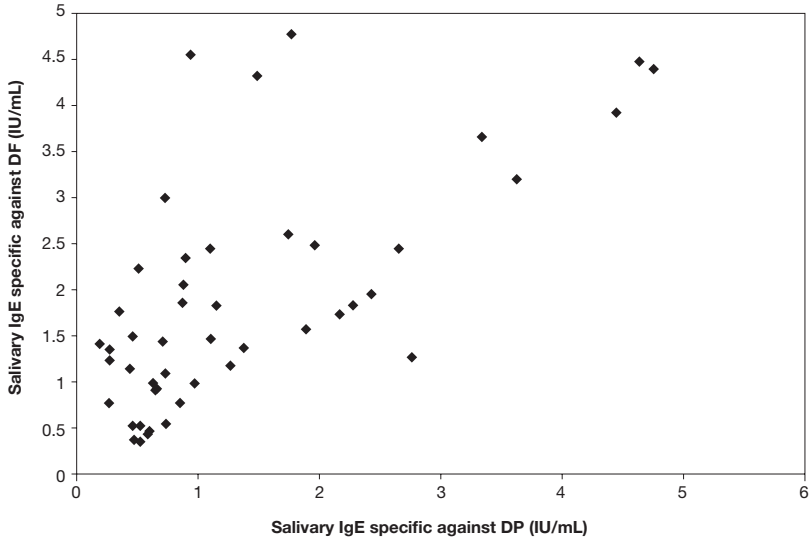
Table 1: Skin prick test results against *Dermatophagoides pteronyssinus* (DP) and *Dermatophagoides farina* (DF) antigens in asthmatic and non-asthmatic children

	DP		P VALUE	DF		P VALUE
	POSITIVE (WHEAL >= 4 MM)	NEGATIVE (WHEAL < 4MM)		POSITIVE (WHEAL >= 4 MM)	NEGATIVE (WHEAL < 4MM)	
Asthmatic (n=125)	71.2%	28.8%	0.002	72.8%	27.2%	0.001
Non-asthmatic (n=29)	41.4%	58.6%		37.9%	62.1%	
Age group (asthmatics)						
5-8 years old	61.0%	39.0%	0.017	61.0%	39.0%	0.005
9-12 years old	80.3%	19.7%		83.3%	16.7%	
Ethnic group (asthmatic)						
Malay	79.9%	20.1%	0.014	75.9%	24.1%	0.310
Chinese	53.3%	46.7%		73.3%	26.7%	
Indian	53.6%	46.4%		60.7%	39.3%	
Gender (asthmatic)						
Male	68.5%	31.5%	0.428	68.5%	31.5%	0.200
Female	75.0%	25.0%		78.8%	21.2%	

Salivary IgE Specific against HDM

There was significant positive correlation between salivary IgE specific titre against DP and DF (Spearman's rank coefficient = 0.644, P value < 0.001; Figure 1).

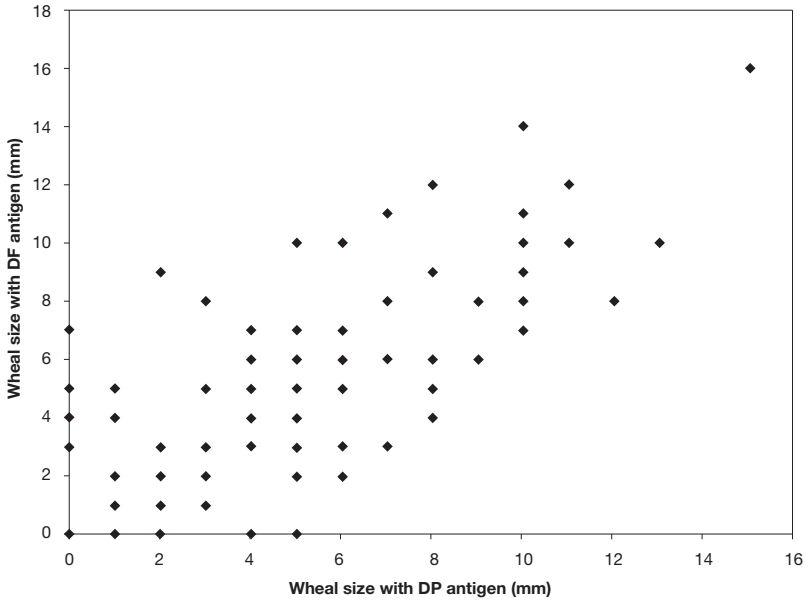
Figure 1: Scatter plot of salivary specific IgE levels for DP against that of DF (R value = 0.644; P<0.001)



Wheal size of skin prick test

The wheal size of the skin prick test with DP and DF antigen was also highly correlated (Spearman's rank coefficient = 0.749, P value < 0.001; Fig. 2)

Figure 2: Scatter Plot of wheal size of skin prick test with DP antigen against that of DF antigen (R value = 0.749; P< 0.001)



Mite sensitisation and the control and severity of asthma

Table 2: Shows the comparison between DP sensitisation and control and severity of asthma. There was a significant association between DP sensitisation and the control of asthma. Patients with poorer control have a higher percentage of DP sensitisation when compared to patients with better control. However, there is no significant association between DP sensitisation and the severity of asthma.

Table 2: DP sensitisation and the control and severity of asthma

DP SENSITIZATION	CONTROL OF ASTHMA		SEVERITY OF ASTHMA	
	CONTROLLED	PARTLY CONTROLLED & UNCONTROLLED	MILD	MODERATE & SEVERE
Positive (wheal >= 4 mm)	55.6%	75.5%	57.7%	74.7%
Negative (wheal < 4 mm)	44.4%	24.5%	42.3%	25.3%
P value	P = 0.043		P = 0.083	

Table 3 shows the comparison between DF sensitisation and control and severity of asthma. There was a significant association between DF sensitisation and the control of asthma. Patients with poorer control have a higher percentage of DF sensitisation as compared to patients with better control. There was also a significant association between DF sensitisation and the severity of asthma. Patients with worse asthma exacerbation had a higher percentage of DF sensitisation when compared to patients with milder exacerbation.

Table 3: DF sensitisation and the control and severity of asthma

DF SENSITIZATION	CONTROL OF ASTHMA			SEVERITY OF ASTHMA		
	CONTROLLED	PARTLY CONTROLLED	UNCONTROLLED	MILD	MODERATE	SEVERE
Positive (wheal >= 4 mm)	55.6%	73.8%	83.8%	50.0%	78.2%	79.5%
Negative (wheal < 4 mm)	44.4%	26.2%	16.2%	50.0%	21.8%	20.5%
P value	P = 0.042			P = 0.013		

Salivary IgE and the control and the severity of asthma

The mean titre (OD values) of salivary IgE specific against HDM in asthmatic patients was significantly higher than that of non-asthmatic patients for DP (OD = 1.37 ± 0.17 vs. 0.6 ± 0.19; P = 0.006) and DF (OD = 1.85 ± 0.19 vs. 0.23 ± 0.06; P < 0.001) respectively.

The mean salivary IgE specific titre against HDM in subjects with controlled asthma was lower than that of subjects with partly controlled and uncontrolled asthma. This was seen for both IgE mean specific titres against DP and DF. The lower OD values in controlled, compared to partly controlled or uncontrolled asthma for DP were however, not statistically significant (1.15 ± 0.35 vs. 1.35 ± 0.19; P = 0.566). Similarly, the OD values with DF antigen in controlled compared to partly controlled and uncontrolled asthma were also not statistically significant (1.58 ± 0.31 vs. 1.85 ± 0.19; P = 0.401).

The mean IgE titre against DP and DF antigens in subjects with mild compared to moderate and severe asthma was lower. However, the difference was not statistically significant (2.36 ± 0.69 vs. 1.22 ± 0.19; P = 0.140 and 2.56 ± 0.69 vs. 1.78 ± 0.69; P = 0.286)

Discussion

In this study, we found that more boys than girls had asthma. A study conducted in Sweden concluded that being a girl increased the likelihood of having asthma.[23] A similar trend was reported in studies conducted in Indonesia by Sheikh *et. al.*[24] and in a study conducted in a Caucasian population.[25] This might be due to geographical differences, and the relationship between asthma and gender should be further investigated.

The percentage of mite sensitive asthma patients was significantly more than that of the control group. DP and DF sensitisation were significantly associated with the asthmatic status of the subject. Sensitisation to HDM has been established as a causative factor for development of asthma.[10, 19] The high prevalence of sensitisation in our study indicates that most of the Malaysian asthmatic children might develop asthma due to HDM sensitisation.

In our study, we found that 71.2% and 72.8% of asthmatics were sensitised to DP and DF respectively. Liam *et. al.* did a similar study in the Malaysian asthmatic population. They skin prick tested patients with eight aeroallergens and found 68% sensitised to at least 1 type of allergen, 93.6% to DP and 81.4% to DF.[26] Chew *et. al.* skin prick tested asthmatic and/or allergic rhinitis patients in Singapore and found that the sensitisation rates for DP and DF were 93.4% and 92.3% respectively.[27] A considerable difference could therefore be seen in these studies. Natural causes which would affect the HDM allergen load such as temperature and relative humidity are unlikely to be responsible for these differences as the climate around the region is stable and similar, if not the same. Therefore, these differences are due to experimental design. Socioeconomic difference between these study areas might be responsible for these discrepancies. The prevalence of allergic asthma has been increasing as communities adopt a more urbanised lifestyle.[6] This could explain the high prevalence rate of HDM sensitisation among Singaporean subjects, while our study was in a community perceived as relatively less urbanised compared to Singapore.

The differences seen in above studies can also be explained by the 'hygiene theory'. It states that individuals living in urban area lack early immune maturation. Their under-stimulated immune system allows a pro-allergic immune development.[28] Murton and Madden found that wooden houses have a higher amount of HDM compared to brick houses.[14] Individuals living in brick houses were not exposed to heavy loads of HDM antigen as compared to individuals living in wooden houses. Therefore, individuals living in wooden houses in rural areas are less likely to develop allergic reaction towards HDM. This explains the high prevalence rate of mite sensitive asthmatic patients in Singapore as compared to our study which was carried out in Seremban, where patients come from a less urbanised area.

Yeoh *et. al.* conducted a similar study in both Malaysian and Singaporean subjects. They found that 57% of asthmatic children are sensitised to Der p 1, the most potent allergen of DP. In our study, 71.2% of asthmatic children are sensitised to DP. A difference of almost 14% could be seen. This difference could be due to the diagnostic method. Our diagnostic method was skin prick test whereas they used ELISA to detect IgE specific to HDM antigen in subject's serum.[29]

There is a significant association between DP and DF sensitisation and the age group of patients. The older age group has a higher sensitisation percentage to both DP and DF than that of younger age group. Similar results were obtained by Miraglia del Giudice *et. al.*, who did a similar study in southern Italy.[30]

The findings above may be due to several reasons. Allergic sensitivity would only manifest in skin prick test after prolong and continuous exposure to high doses of allergen. Therefore, the period of exposure might not be sufficient enough for it to manifests in the skin prick test for younger children. The low prevalence of sensitisation to DP and DF in the younger age group can also be due to the fact that the development of asthma before the seventh year of age is mainly due to factors other than atopy.[30]

Our study shows that there is significant association between DP sensitisation and ethnic group among asthmatic subjects. Malays were reported to have higher prevalence of sensitisation as compared to Chinese and Indians (79.9%). A study in Indonesia, with a mainly Malay population, by Baratawidjaja *et. al.* reported a similar result (77.3%).[31]

Leung *et. al.* conducted a study in Hong Kong and found that more than 85% of Chinese asthmatic children were sensitised to HDM.[32] In our study, only 73.3% of Chinese subjects are mite sensitised. This could be due to the insufficient sample size of our Chinese population. As Hong Kong is mainly urbanised, the prevalence of atopy is expected to be higher than that of our study as our sample collection was from a relatively less urbanised community.

The level of specific IgE against DP and DF is highly correlated. Chew *et. al.* reported similar results in a study in Singapore.[27] A study conducted in Klang Valley found that DP and DF are present in all houses investigated.[33] Therefore, the exposure to DP and DF allergen is common in the Malaysian household. This could also be due to cross reactivity between these two species.[34] Mild cross reactivity with other storage mites present in the house could be a possibility as well.[35]

The correlation between wheal size of skin prick test with DP and DF antigen is high. The reason for this observation is as explained above. However, there is no correlation between the wheal size of DP and DF and their respective specific IgE levels. Teoh *et. al.* who used a semi-quantitative method reported similar results.[36] Therefore, consistent findings were obtained despite difference in methodology.

We found that there is a significant association between the control of asthma and sensitisation to DP and DF or to either one of them. Studies done in other regions reported similar findings. Nelson suggested that HDM allergens play an important role in the development and persistence of asthma symptoms.[37] A study conducted in the United States by Henderson *et. al.* examined factors linked to recurrent wheezing and found that most patients were sensitised to HDM.[38] Cookson *et. al.* found that the risk of bronchial hyper-responsiveness, which is the main contributor of asthmatic symptoms, increased up to 4-fold in young patients skin prick tested positive for HDM.[39]

Several studies have determined the relationship of HDM sensitisation and the frequency of wheezing. Sears *et. al.* studied a group of children in New Zealand and demonstrated that sensitivity to HDM is associated with increased episodes of wheezing.[40] Peat *et. al.* studied a group of unselected children in Australia and determined that wheezing, recent asthma medication, and urgent medical visits for the treatment of asthma symptoms correlated with a positive skin test result to HDM.[41] Warner and Price found that nocturnal wheezing is significantly more common in the children with skin hypersensitivity to the mite than in those without this allergy.[10]

Besides that, exposure to HDM allergens in sensitised individuals could cause exacerbations.[42] Sporik *et. al.* concluded that majority of children admitted to hospital for exacerbations of asthma were HDM sensitive and were exposed to HDM allergens.[43]

There were a high percentage of mite sensitive patients with severe asthma. The association between DP sensitisation and severity of asthma just failed to reach a significant level although there is a clear trend for this. Significant association is found between DF sensitisation and severity. Gent *et. al.* reported similar findings. They found that children with atopy were more likely to have severe asthma compared to children without atopy.[44] Several epidemiological findings also suggested that exposure to HDM antigen was an important environment factor in the severity of asthma.[43, 45]

Nitschke *et. al.* studied the mite concentration in beds of mite sensitive patients and its relation to severity of asthma. It was observed that presence of high levels of mite allergen is associated with wheeze at night, cough during the day, and daytime asthma attacks.[46] Zock *et al.* found that exposure to dust mite allergen is associated with increased shortness of breath.[45] Clinical activity and severity of asthma in mite-sensitive patients is related to exposure to mite allergens. The severity measured by the peak expiratory flow rate (PEFR) variability, and forced expiratory volume (FEV1),[21] by Gent *et. al.* suggests that disease severity could be reduced in all asthmatic children by reduction of dust mite levels.[44] However, discrepancies have been reported in another study.[47]

There is no available evidence that environmental interventions would, or would not benefit difficult asthmatics. However, there is nothing to lose from making reasonable efforts to reduce allergen exposure. Therefore, basic interventions should be introduced to families of children with difficult asthma.

We found that the wheal size of skin prick test is not associated with the control and severity of asthma. A similar result was reported by Simpson *et. al.* They performed skin prick test with a few allergens (dust mite, cats, dogs etc.) and none of them are associated with current wheeze.[48]

In our study, we found that asthmatic patients had a higher titre of specific IgE against both DP and DF when compared to non-asthmatic patients. Similar results were shown by a study carried out in Finland by Tuula Hyypia.[49] The subjects with poorer controls have a higher specific IgE titre. Simpson *et. al.* studied a cohort of children and found that the levels of allergen specific IgE were associated with current wheeze.[48]

Subjects with moderate and severe asthma have lower IgE levels than that of subjects with mild asthma. The severity of acute asthma assessed was the severity of the most recent exacerbation. For severe exacerbation, oral corticosteroid was prescribed on top of the inhaled corticosteroid. Therefore, the medication prescribed could have an effect on the levels of allergen specific salivary IgE.

There are several limitations of the present study. The study was conducted by convenience sampling. Therefore, there may be differences in terms of urban versus rural populations, the effects of pollution and the effects of Westernization on the prevalence of atopic diseases.

There could have been some bias in the results since the study was conducted over a limited 4-month period as seasonal change might affect the presentation of the signs and symptoms of asthmatic patients.[50] The severity of acute asthma that was being assessed was the most recent exacerbation. Therefore, it could be altered by the medication given over the long course of management of chronic asthma.

In conclusion, there is a high prevalence of sensitisation to HDM among childhood asthmatic patients in Malaysia. The sensitisation of HDM tested by skin prick method is associated with poorer control and severity of patient's asthmatic condition.

In our study, although subjects with poorer control of asthma had higher specific IgE levels, the difference seen was not significant. Subjects with more severe asthma have a lower specific IgE levels although it is not statistically significant.

Therefore, it can be concluded that salivary specific IgE levels may not be an appropriate indicator of the patients asthmatic condition in this study as compared to that of skin prick test.

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