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Multiple House Dust Mite Allergen- Sensitization Profiles in Children with Allergic Asthma

Jennifer Maries G. Yap[1], **Maricar W. Ching**[2,3*], **Cristan Q. Cabanilla**[4] **and John Donnie A. Ramos**[1,5]

[1]*Biological Sciences Department, College of Science, University of Santo Tomas, Philippines*

[2]*Department of Biological Sciences, College of Science and Technology, Centro Escolar University, Philippines*

[3]*The Graduate School, Centro Escolar University, Philippines*

[4]*Philippine Children's Medical Center, Quezon City, Philippines*

[5]*Research Center for the Natural and Applied Sciences, University of Santo Tomas, Philippines*

[*]**Corresponding author:** Maricar W. Ching, Department of Biological Sciences, College of Science and Technology Centro Escolar University, 1005, Manila, Philippines, Tel: 6327356861; Fax: 6327354451; E-mail: cindywiscoching@gmail.com

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Abstract

Background: Allergic asthma is a common chronic inflammatory disorder which affects populations, with increasing prevalence among children. The risk of developing asthma is uncertain but depends on the interaction of environmental and genetic factors. The role of house dust mite (HDM) allergen exposure in the development of sensitization and asthma remains unclear.

Objective: This study determined the sensitization profiles of common HDM species in a population of asthmatic children, which is essential in the development of highly specific and accurate diagnostic and therapeutic strategies for asthma in children.

Methods: The immunoglobulin E-binding activity of allergens from the HDM species *Blomia tropicalis (Bt), Dermatophagoides farina (Df),* and *Dermatopahagoides pteronyssinus (Dp)* were determined in 250 age- and sex-matched paediatric allergic asthma and non-atopic Filipino subjects using Enzyme-linked immunosorbent assay and western blot analysis.

Results: Majority of the allergic asthma patients tested were sensitized with multiple allergens from different HDM species where 33% exhibited sensitizations to any two HDM species and 26% were sensitized with allergens from three HDMs. HDM allergens of different molecular weights bind to IgE in allergic asthma patients tested. In addition a significant correlation was observed between total IgE and HDM-specific IgE level among allergic asthma patients (Bt p-value=0.038; Df p-value=0.045; Dp p-value=0.003), suggesting a significant contribution of dust mite allergens in the up-regulation of total serum IgE levels of allergic asthma patients.

Conclusion: The results obtained in this study suggest that the HDM species *Bt, Dp,* and *Df* are important sources of allergens that trigger multiple sensitization in children with allergic asthma in the Filipino population. The incorporation of *Bt, Dp,* and *Df* allergens in the panel of diagnostic allergens for HDM allergy and allergic asthma is highly recommended.

Keywords: Allergy; Asthma; Total IgE; House dust mite-specific IgE

Introduction

Allergy is a hypersensitivity reaction initiated by immunological mechanisms characterized by the production of elevated levels of allergen-specific immunoglobulin E (IgE) [1]. Allergic asthma is one of the common chronic respiratory disorders that affect approximately 300 million people of different age worldwide with an increasing prevalence among children [2]. The risks of developing asthma are still uncertain but depend on different complex interaction of hereditary and environmental factors. House dust mite (HDM) allergen exposure is a significant trigger of exacerbations for many asthmatic patients [3]. More than 90% of children with asthma develop specific IgE sensitization against allergens from HDM, hence, dust mite allergy is strongly associated with asthma [4-6]. The three most common species that is associated with the development of asthma and other allergic diseases are *Dermatophagoides pteronyssinus* (Dp), *Dermatophagoides farinae* (Df), and *Blomia tropicalis* (Bt) [7].

IgE cross reactivity is a common feature of mite allergens, especially in those from taxonomically related species. In vitro cross reactivity studies between *Dermatophagoides* species and extracts of other mite species showed that mites share common and species-specific allergens [8]. As a result, multiple sensitizations to dust mite species *Dermatophagoides sp.* and *Blomia sp.* allergens are often observed among asthmatic children living in tropical and subtropical countries including Philippines [9-11].

Understanding the sensitization profiles of common HDM species in a population of asthmatic children is invaluable in understanding the pathogenesis of HDM allergy and is important in the development of highly specific and accurate diagnostic and therapeutic strategies for

asthma in children. Sensitization to multiple allergens from different HDM species among asthmatic children is presented in this paper.

Materials and Methods

Study design and subjects

The study utilized a case-control method employing 125 age- and sex- match paired allergic asthma patients and non-asthmatic control subjects with no history of allergy. Subjects were recruited from the Philippine Children's Medical Center (PCMC). The PCMC Institutional Review Board approved the study design, conduct of sampling, and experimental protocols. In addition, informed consents and assents were obtained from the patients.

An initial screening was conducted utilizing standardized questionnaires of the International Study of Asthma and Allergy in Childhood (ISAAC) and the International Primary Care Airways Group (IPAG) for recruitment of subjects. Cases were defined in the study as physician-diagnosed asthmatic patient (with asthma alone, concurrent with allergic rhinitis or atopic dermatitis or both) with elevated levels of serum total IgE (≥ 100 IU/mL) with at least one type of house dust mite (HDM)-specific IgE (≥ 50 IU/mL) while non-atopic controls were defined as non-asthmatic subjects without any history of allergy and serum total IgE level of <100 IU/mL and HDM-specific IgE level <50 IU/mL. Furthermore both cases and controls must be (1) naturally born Filipino; (2) unrelated to individual already participated in the study; (3) was born and living in Luzon Island; and (4) aged 6 months to 18 years old at time of study.

Clinical protocol and Phenotyping

Five milliliters of blood was extracted from the subjects by venipuncture. Blood serum samples were isolated by centrifugation at 10,000 rpm for 10 minutes. Total serum IgE and HDM-specific IgE were quantitated using Enzyme-Linked Immunosorbent Assay (ELISA). IgE concentration was expressed as IU/mL and was log transformed to normalize distribution.

Enzyme-Linked Immunosorbent Assay

Enzyme Linked Immunosorbent Assay (ELISA) was used to evaluate the profile of sensitization of the allergic asthma patients and non-atopic control sera against allergen extracts from 3 HDM species *D. Farinae, D. Pteronyssinus,* and *B. tropicalis.* Briefly, for sandwich ELISA 10 µg/mL of unlabelled anti-human IgE (Pharmingen, CA, USA) and for indirect ELISA 10 µg/mL of the HDM aqueous extracts were coated onto ELISA plates overnight at 4°C using 50 µL of 0.1 M $NaHCO_3$, pH 8.3. Plates were blocked with 1% BSA (Sigma) in Phosphate Buffered Saline with 0.05% Tween 20 (PBS-T) for 1 hour at room temperature. ELISA plates were incubated overnight with 5x diluted human sera, then for 1 hour at room temperature with biotinylated anti-human IgE (Pharmingen, CA, USA) diluted 1000x in blocking buffer. Plates were incubated with 2000x dilution of ExtrAvidin-Alkaline phosphatase conjugate (Sigma) for 1 hour. Finally, colorimetric reaction was performed using p-nitrophenyl phosphate (Sigma). Absorbance was read at 405 nm an ELISA reader (BioTek). Human IgE (Pharmingen) was used as a standard per plate in the calculation of IgE concentration.

Sodium Docecyl Sulfate- Polyacrylamide Gel Electrophoresis

Aqueous HDM extracts were analyzed by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Protein samples were mixed with 2X loading buffer and were boiled for 5 minutes. Samples were separated on a 15% Tris-Glycine gel using the Mini PROTEAN electrophoresis system (BioRad, Hercules, CA, USA). Gel was run at 110 Volts for 75 minutes. Broad Range Marker (BioRad, Hercules, CA, USA) was used as standard. SDS-PAGE gel was stained with Coomassie brilliant blue R-250 (BDH laboratory Supplies, Poole, England).

Western blot analysis

The IgE reactivity of selected sera samples to specific allergens found in the three HDM extracts was determined by western blot analysis. In brief, Bt, Dp, and Df aqueous extracts were electrophoresed on a 15% Tris-Glycine gel and electroblotted onto Hybond-C Nitrocellulose membrane (Amersham Life Sciences, Buckinghamshire, England) using the MiniProtean 3 cell (BioRad, Hercules, CA, USA) at 120 V for 60 minutes. The membrane was blocked with 5% skimmed milk diluted with PBS-T. The membrane was incubated overnight with selected human sera diluted with blocking buffer (5x dilution) at 4°C. Then the membrane was incubated with biotinylated anti-human IgE (Sigma-Aldrich, Saint Louis, MO, USA) for 1 hour. Finally, the membrane was incubated with peroxidase-conjugated ExtrAvidin (Sigma-Aldrich, Saint Louis, MO, USA) for 1 hour. The membrane was washed three times with PBS-T between steps. Results were detected by incubation with Alkaline Phosphatase Color Development Solution (BioRad).

Statistical analysis

Data were analyzed using statistical packages SAS9.2, STATA, GraphPad Prism 6, SPSSv16 and MS excel. (Microsoft Corp., Redmond, WA, USA). Statistical significance was defined as p-value is <0.05.

Results

One hundred twenty five match-paired study subjects were recruited for the study with a mean age of 8.34 ± 3.90 years ranging from 6 months to 18 years old. Most of the qualified subjects were male and aged 7-10 years old. Most of the allergic asthma children were not exposed to smoking environment and received Bacillus Calmette–Guérin (BCG) immunization. Their family history of asthma reported that most of them had both their parents and sibling suffering from asthma. Parental history of asthma was observed more frequently than maternal history (Table 1).

Total IgE levels in allergic asthma cases are significantly elevated ($t=16.08$, $df=124$, $p<0.0001$) compared to non-atopic controls (For figure refer to Philippine Science Letter, 6, 241-248; 2013). The mean total IgE concentration of the allergic asthma cases (247.52) is notably higher and is almost eight times than the non-atopic controls (32.89). Total IgE concentration of allergic asthma cases ranges from 100-1700 IU/mL, 20% (30/125) had <200 IU/mL. In addition, a significant difference between the two means of the allergic asthma cases and non-atopic control group of the log HDM-specific IgE (IU/mL) was observed in each dust mite species tested (*Blomia tropicalis*, $t=20.20$, $df=124$, $p<0.0001$; *Dermatophagoides pteronyssinus*, $t=16.37$, $df=124$, $p<0.0005$; *Dermatophagoides farinae*, $t=18.48$, $df=124$, $p<0.0001$).

Across three HDM species tested, an evident difference in the means of the allergic asthma cases (*Bt*=91.83, *Df*= 80.81, *Dp*=80.09) and non-atopic controls (*Bt*=2.74, *Df*=2.98, *Dp*=4.19) were observed. HDM-specific IgE means of allergic asthma cases ranges from less than 50 to 1500 IU/mL. Thirty five percent (44/125) of the Bt- specific IgE concentration was greater than 100 IU/mL. Dp-specific IgE concentration registered the highest reading at 1587 IU/mL and 26% (33/125) registered to have <100 IU/mL. Thirty three percent (41/125) of the allergic asthma cases had results of 50-100 IU/mL Df-specific IgE concentration and 30% (37/125) had results higher than 100 IU/mL.

		Allergic Asthma Cases (n=125)	Non-atopic Controls (n=125)
Age	Mean ± SD	8.34 ± 3.90	8.6 ± 4.08
Sex	Female	43 (34.4%)	43 (34.4%)
	Male	82 (65.6%)	82 (65.6%)
Environment Tobacco Smoke Exposure	Yes	31 (24.8%)	60
	No	93 (74.4%)	65
Family History of asthma	Maternal Only	7 (5.6%)	NA[1]
	Paternal Only	14 (11.2%)	
	Both Parents	11 (8.8%)	
	Sibling Only	17 (13.6%)	
	Parental and Sibling	60 (48%)	
	None of the family members	16 (12.8%)	
BCG immunization	Yes	111 (88.8%)	ND[2]
	No	5 (4%)	

Table 1: Demographic profiles of the 125 allergic asthma cases and 125 non-atopic controls

Allergens form HDM species Bt, Df, and Dp triggered elevated IgE production in allergic asthma cases. Using a cut-off value of 8.30, 10.35, 11.50 for Bt, Df, and Dp respectively, which corresponds to the mean+1 SD of NA, more than 60% of the allergic asthma cases reacted positively (For figure refer to Philippine Science Letter, 6, 241-248; 2013).

Detailed analysis of the profile of IgE reactivity of the 125 allergic asthmatic cases showed multiple sensitivity to different allergens of the three HDMs. Reactions to Bt, Df, Dp alone showed that most of the cases were sensitized to Bt (23/125) followed by Dp (16/125) and Df (12/125). On the other hand, highest double positive reactions were observed between Bt and Df (20/125) followed by reactions to Dp and Df (14/125) and Bt and Dp (8/125).

Furthermore, 26% (32/125) of the patients tested showed triple positive IgE- binding positive reactions to Bt, Df, and Dp allergens (Figure 1).

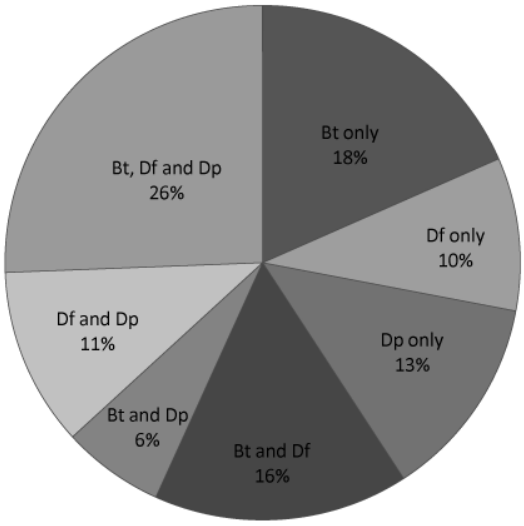

Figure 1: Summary of monosensitization and multisensitization profiles of 125 allergic asthma cases; Legends: Bt: Blomia tropicalis; Df: Dermatophagoides farina; Dp: *Dermatophagoides pteronyssinus*

IgE reactivity of three HDM

Different protein bands were obtained from the three HDM extracts using SDS-PAGE. Protein bands ranging from approximately 20-150 kDa from the three HDM extracts were observed under 15% Tris-glycine gel. Western blot analysis was used to examine the IgE reactivity of the patients' sera that binds to the protein bands observed in SDS-PAGE. Five sera samples that resulted positive reactions to the three HDM species as determined by ELISA were selected for IgE-binding activity. Multiple serum IgE reacted to different proteins in the HDM aqueous extract with molecular weight ranging from 20-100 kDa. Majority of patients' IgE reacted to an approximately 70 kDa protein in the Bt extract. On the other hand patients' IgE reacted to an approximately 60 kDa, 80kDa protein in the Dp and Df extract respectively. It was also observed that there are minor IgE-reactive protein bands of <25 kDa, and approximately 30 kDa, 40 kDa, 50 kDa, 60 kDa, and 70 kDa among the three HDM species (Figure 2). These IgE reactive proteins contribute to the allergic sensitization among local asthmatic children tested.

Correlation between total IgE and HDM-specific IgE

Correlation between total serum IgE and HDM-specific IgE was also tested in the study (Table 2). Significant correlation between total IgE and the three HDM (*Bt* p-value=0.038; *Df* p-value=0.045; *Dp* p-value=0.003) was observed among the allergic asthmatic patients. Furthermore, the study also analyzed the correlation among HDM specific IgE (Table 2). Patients with allergic asthma showed a significant correlation between *Bt* and *Df* (p-value<0.0001) as well as between *Df* and *Dp* (p-value<0.0001). Using multiple regression model *Df* and *Dp* IgE concentration was significantly contributing to the increase of total IgE concentration. This means that for every one unit increase in *Df* (parameter estimate=1.37, test stat=3.11, p=0.0023), on the average, the total IgE concentration is expected to

increase by 1.37 controlling for *Bt* and *Dp*. The coefficient of *Bt* is also positive but not significant (parameter estimate=0.33, test stat=1.33, p-value=0.1857). This means that an increase in *Bt* is expected to increase the average total IgE concentration.

		Correlation value	P-value	Remarks
Total IgE vs. HDM-specific IgE	Total IgE vs Bt- specific IgE	0.185	0.038	S[1]
	Total IgE vs Df- specific IgE	0.18	0.045	S
	Total IgE vs Dp-specific IgE	0.261	0.003	S
HDM-specific IgE	Bt- specific IgE vs Df-specific IgE	0.404	<0.0001	S
	Bt- specific IgE vs Dp-specific IgE	0.146	0.105	NS[2]
	Df- specific IgE vs Dp-specific IgE	0.455	<0.0001	S

Table 2: Correlation between total IgE and HDM-specific IgE in 125 allergic asthma cases

HDM: House Dust Mite; Bt: *Blomia tropicalis;* Df: *Dermatophagoides farinae,* Dp: *Dermatophagoides pteronyssinus.*[1]S: significant; [2]NS: not significant

Discussion

The prevalence of allergic diseases such as allergic asthma, allergic rhinitis, and atopic dermatitis is increasing across all populations especially among children [12]. Hence, it is imperative to evaluate potential risk factors to these diseases. An important factor to be considered in the development of asthma is the influence of exposure to house dust mite allergens [13]. Different studies confirmed that sensitization to indoor allergens such as house dust mites are major risk factors in allergy, especially in asthma [14]. Three dust mite species were used in this study to determine the sensitization profile of the Filipino asthmatic children. Common house dust mites implicated in allergic diseases, such as *Blomia tropicalis* (Bt), *Dermatophagoides farinae* (Df) and *Dermatophagoides pteronyssinus* (Dp), are considered ubiquitous in every household, preferably in areas with high relative humidity (>45%) and warm temperatures, between 65 to 85°F [15]. *B. tropicalis*, which is morphologically different from the other dust mites, is geographically distributed in tropical and subtropical regions, although can also be found in temperate countries. It has been traditionally referred to as "storage mites" because they have been found mainly in stored grain. *Dermatophagoides* species, commonly known as the "house dust mites," are predominantly found in temperate and tropical regions. *D. farinae* and *D. pterynissinus* are the most common species which are widely distributed worldwide. Various studies have reported double sensitization involving *B. tropicalis* and *D. pterynissinus* [10,16-19]. Results of this study showed that *B. tropicalis* is responsible for the sensitization of 66% (83/125) allergic asthmatic Filipino population. This is consistent with the reports of very high sensitivity to *B. tropicalis* among asthmatic population in other countries in Asia such as in Taiwan [18], Singapore and Malaysia [16]. High prevalence and high variability of *B. tropicalis* was also observed among atopic subjects in (40%) India and in (82%) Indonesia [18]. Surprisingly, *D. pteronyssinus* obtained the lowest reactivity at 56% and *D. farinae* at 62%.

Further analysis of the sensitization profile also showed that, patients with allergic asthma (16%) were sensitized to both *B. tropicalis* and *D. farinae*. The result of double sensitization to *B. tropicalis* and *D. farinae* is consistent with the report of Ramos et al, which showed 5% (11/210) double positive reactions to *B. tropicalis* and *D. farinae*, as compared to 3% (7/210) and 2% (4/210) sensitization to *B. tropicalis* and *D. pteronyssinus*, and *D. pteronyssinus* and *D. farinae,* respectively. This is not surprising, considering that the climatic conditions in a tropical country like the Philippines and the fact that *D. farinae* is more able to survive in drier climatic conditions than *D. pteronyssinus*, which prefers consistently damp conditions [11]. A previous work investigating the acrofauna in houses of allergic patients in Metro Manila (Philippines) revealed that *Dermatophagoides* and *Blomia* species are the most abundant HDM species [20]. Our results showed that 26% of the asthmatic patients were sensitized to the three dust mites tested, indicative of moderate prevalence of house dust mite sensitization among Filipino allergic asthmatic patients. Different house dust mite species can possibly co-inhabit homes and dust mites allergens may cause parallel allergic sensitization among susceptible individuals [11].

Our results showed significant correlation between *D. farinae* and *D. pteronyssinus*, and between *D. farinae* and *B. tropicalis*. These results support the double positive reactions between Dp and Df, as well as in Df and Bt which have an average reaction of 13% (Table 2). Significant correlation may indicate that as the level of Df-specific IgE increases, the level of Dp-specific IgE also increases. The observed multiple specific IgE reactivities can be attributed to the presence of cross-reactive allergens from different HDM species.

Sensitization to different house dust mite allergens showed that patients who have asthma and atopic dermatitis have highest sensitization rate to *D. farinae* and *D. pteronyssinus* in 2 out of 3 HDM species tested and to all three HDM species tested (data not shown). This is consistent with the result of a study by Shek et al, wherein patients with eczema were generally more sensitized to the panel of dust mite allergens, indicating susceptibility to HDM. In another study, it was observed that eczema in the first 3 months of life was a risk factor for sensitization to aeroallergens by 5 year of age and subsequent manifestation of respiratory allergies [21]. Moreover, another study showed that children with eczema were significantly more sensitized to a panel of allergens tested than those without skin diseases. The degree of sensitization was also observed to be directly associated with the severity of eczema, especially for HDM allergens [22].

In this study, asthmatic patients showed high sensitivity to Bt. This is similar with the results of a study by Shek et al. which reported that asthmatics are more sensitized to *B. tropicalis,* more specifically to Blot 5 allergen. In Singapore, there is also a higher reactivity observed in *B. tropicalis* among HDM- sensitized patients [10]. Likewise, Manolio [23] reported that *B. tropicalis* sensitization was more prevalent than *D. pteronyssinus* sensitization and that *D. pteronyssinus* sensitization without *B. tropicalis* sensitization was uncommon. In addition, the study found that dual sensitization to *D. pteronyssinus* and *B. tropicalis* resulted in higher risk of asthma.

In previous reports of sensitization involving HDM species, it is notable that patients with skin allergies are found to be more

sensitized to D. pteronyssinus while individuals with respiratory allergies are more sensitized to B. tropicalis. Protease activities of allergens from D. pteronyssinus can influence allergenicity through several mechanisms such as causing disruption of the epithelium, allowing access of allergens to antigen-presenting cells, activation of complement and kallilkrein, and induction of inflammatory reactions, thus possibly aggravating skin allergies upon contact [24]. Molecular weight of Der p 1, which is a major allergen of D. pteronyssinus, is 25 kDa cysteine protease carried by large particles of median size 25 mm [16,25]. Because of their size, these particles do not stay airborne, and deposit rapidly upon skin contact. It may also be the absence of Der p1 protease inhibitor in sweat, distinct to patients with eczema, which increases their susceptibility to D. pteronyssinus sensitization [16]. B. tropicalis allergens are noted for their proteolytic activity, lipid binding activity, chitin- binding activity, muscle proteins, and allergens without autoadjuvant or adjuvant activity. Chitinase is known to play an important role in the pathogenesis of asthma [24]. In addition, that the major allergen of B. tropicalis which is Blo t5 has a molecular weight only of 14 kDa thus can remain airborne longer resulting in airway sensitization [16]. The IgE binding reactivity among the three HDM species tested confirmed that proteins from HDM contribute to the allergic sensitization of asthmatic patients and that exposure to these allergens may contribute to the pathogenesis of asthma.

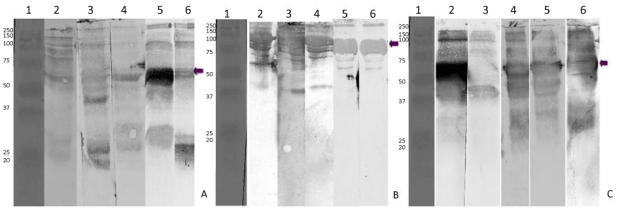

Figure 2: Western blot analysis showing the IgE reactivity of major protein bands from house dust mite aqueous extracts using five allergic patients' sera. A: *Blomia tropicalis* extract; B: *Dermatophagoides farina* extract; and C: *Dermatophagoides pteronyssinus* extract. Lane 1: protein marker (BioRad Pre-stained Standards Broad Range) while lanes 2-6: different sera from selected allergic patients. Arrow A: majority of patient's IgE reacted to ~70kDa protein; Arrow B: majority of patient's IgE reacted to ~80kDa protein; Arrow C: majority of patient's IgE reacted to ~60kDa protein.

The results of this study are consistent with the results of previous studies that showed significantly elevated total and HDM-specific IgE levels among allergic patients as compared with the controls (Figures 1 and 2) [26]. It is well established that specific serum IgE antibody can be quantitatively high, relative to the total serum IgE. It could also be noted that higher serum IgE is related to the prevalence and severity of asthma and other allergic disease [27]. Any assay for specific IgE antibody is inevitably a conservative estimate because it is dependent on the affinity of the antibody for the allergen and that the assay for total IgE uses high affinity antibodies for the isotype-specific epitopes on the heavy chain and is unlikely to underestimate total IgE [28]. Based on our results, the highest correlation value was observed between total IgE and HDM- specific IgE level among allergic asthmatics. Thus it can be said that HDM sensitization could have possibly contributed to the elevation of total IgE level among allergic asthmatic patients. This finding validates the use of specific IgE measurement in the diagnostic and therapeutic strategies for house-dust mite induced asthma.

Results of this study confirm that the total serum IgE and HDM-specific IgE are significantly correlated among allergic asthmatic Filipino paediatric population. Sensitization to *B. tropicalis* and *D. farinae* is more prevalent among allergic asthmatics and those with concurrent allergic rhinitis, while sensitization to *D. farinae* and *D.pteronyssinus* is significant only among asthmatics with atopic dermatitis but not in patients with asthma alone. Incorporation of the three house dust mite allergens in the panel of diagnostic and immunotherapeutic allergens for allergic paediatric population is highly recommended.

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