Correlation of total IgE, house-dust mite specific IgE and absolute eosinophils in an asthmatic pediatric population

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The severity of asthma is influenced by multiple factors including absolute eosinophil count, total IgE and specific IgE resulting from sensitization to various allergens such as those from the house dust mite species, *Blomia tropicalis* (*Bt*), *Dermatophagoides pteronyssinus* (*Dp*), and *Dermatophagoides farinae* (*Df*). An observational-analytical, case-control method was used to examine 250 age- and sex-matched allergic asthma and non-asthmatic controls. Total serum IgE and HDM-specific IgE were determined using ELISA, while complete blood count using automated Coulter Counter was used to obtain the absolute eosinophil count. Log-transformed data were statistically analyzed for correlation. Significantly elevated levels of total IgE ($p<0.0001$), HDM-specific IgE ($p<0.0001$) and absolute eosinophil count ($p<0.0001$) were observed in allergic asthma cases compared to non-asthmatic controls. Patients with allergy, regardless of atopy status, and patients with allergic asthma concurrent with allergic rhinitis, showed significant correlation between *Df*-specific IgE and absolute eosinophil count ($p=0.03$). Moreover, significant correlation was observed between *Dp*-specific IgE and absolute eosinophil counts in allergic asthmatics concurrently with allergic rhinitis and atopic dermatitis ($p=0.003$). Multiple regression analyses showed that an increase in *Dp*-specific IgE will yield an expected increase in absolute eosinophil count holding other variables constant among allergic asthmatics, concurrently suffering with allergic rhinitis and atopic dermatitis ($p=0.009$). HDM-specific IgE, particularly *Dp*- and *Df*-specific IgE, is significantly correlated with and contributing to the absolute eosinophil count in allergic asthma among selected population of pediatric patients in the Philippines.

KEYWORDS

Asthma, allergic rhinitis, total serum IgE, house-dust mite specific IgE, absolute eosinophil count

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Table 1: Demographic profiles of 125 age- and sex-matched paired cases and controls.

Variable		Allergic Asthma only	Allergic Asthma with Allergic Rhinitis	Allergic Asthma with Atopic Dermatitis	Allergic Asthma with Allergic Rhinitis and Atopic Dermatitis	Non-asthmatic Controls
Mean Age		9.56 (SD 3.84)	8.36 (SD 3.91)	10.5 (SD 3.54)	7.81 (SD 4.21)	8.60 (SD 4.08)
Sex	**Females**	44% (4/9)	32% (28/88)	100% (2/2)	35% (9/26)	34.00% (43/125)
	Males	55% (5/9)	68% (60/88)	0	65% (17/26)	66.00% (82/125)
Pets at home			52% (65/125)			73.60% (92/125)

INTRODUCTION

The global prevalence of asthma and allergies has increased in both developed and developing countries over the last few decades, despite reports of reduced prevalence of asthma symptoms in North America and Western Europe (GINA 2010). According to the Global Asthma Report of 2011, some 235 million people worldwide suffer from this non-communicable immune disorder (The Global Asthma Report 2011). The prevalence of asthma ranges from 1-18% in different populations, and continues to rise in areas where prevalence was previously low (Lee et al. 2008). With the current trend, it is estimated that about 100 million will develop asthma by 2025, which may result to approximately 250,000 premature deaths each year (Holgate et al. 2011). House dust mite (HDM) allergens are the most common cause of sensitization among patients that suffer from asthma and other allergic diseases. Approximately up to 85% of asthmatics in North and South America, Europe, Southeast Asia and Australasia are typically HDM allergic (Thomas et al. 2010). Among the Asian countries, Singapore (Shek et al. 2010), Thailand (Trakultivakorn et al. 2002), Korea (Jeong et al. 2012), and the Philippines (Ramos et al. 2007) have reported increased sensitization due to HDM allergens.

Asthma is the most common chronic disease of the airways closely associated with atopic diseases. Confirmation of allergy and identification of the triggering allergen is an important requirement in asthma management. By conventional practice, detailed medical history and physical examination bring about a diagnostic suspicion, which needs further confirmation by methods that detect specific immune responses such as measurement of total and allergen-specific IgE (Sanchez-Borges et al. 2011). A strong association between acute episodes of asthma measured by emergency room visits or hospital admissions and elevated total IgE (IgE ≥200 IU/mL) has been reported (Erwin et al. 2007). Moreover, HDM sensitization is a major risk factor for asthma (Platts-Mills et al. 2011; Shek et al. 2010; Trakultivakorn et al. 2002). IgE responses to allergens from HDMs can significantly contribute to the total serum IgE which influences asthma pathogenesis and severity (Erwin et al. 2007; Gent et al. 2009]. Increased eosinophils in peripheral blood and airway secretion are vital features of asthma, and its severity. Furthermore, eosinophil degranulation and its associated products such as eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), major basic proteins (MBP), and eosinophil peroxidase are also important determinants of the disease. Airway inflammation and exacerbations have been linked to elevated levels of these products (Kim et al. 2012). Quantification of eosinophils can help in the differential diagnosis of asthma phenotypes and assess the response to anti-inflammatory treatment of asthma. Correlation of these asthma phenotypes will improve diagnosis and subsequently evaluation and treatment of asthma, especially in the pediatric population where allergic asthma is consistently high and is predicted to increase in the next decades.

MATERIALS AND METHODS

Study design and subject recruitment

An observational-analytical, case-control method of research was used to examine a total of 250 age- and sex-matched allergic asthma and non-asthmatic subjects recruited from the Philippine Children Medical Center (PCMC), Philippines. The study was approved by the Institutional Ethics Board Committee of the PCMC.

Subjects were screened using questionnaires adapted from the Childhood and Adult Asthma Diagnosis Guide set by the International Study of Asthma and Allergy in Childhood (ISAAC) and the International Primary Care Airways Group (IPAG). Questionnaires for Allergic Rhinitis, adapted from Allergic Rhinitis and Its Impact on Asthma (ARIA) was also administered to evaluate allergic rhinitis. The questionnaires supplemented the medical history of the subjects and the diagnosis of the pediatric pulmonologists. Mild, moderate to severe asthma cases were included in the study.

Cases were defined as those with physician-diagnosed atopic asthma, with >2 respiratory symptoms, with asthma and concurrent allergic rhinitis, or with allergic dermatitis or both, at the time during which sampling was conducted. Controls were non-asthmatics (1) without physician confirmed diagnosis

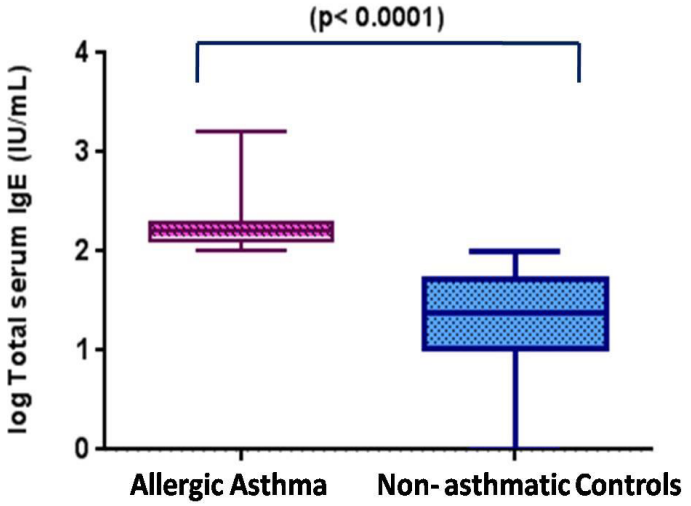


Figure 1. Log total serum IgE concentration of 125 allergic asthma cases and 125 non-asthmatic controls as determined by enzyme-linked immunosorbent assay.

of asthma; (2) those who answered negatively to the screening questionnaire for childhood asthma; and (3) those whose total serum IgE level were below 100 IU/mL. Blood samples (~5 mL) were collected for the determination of total and dust mite specific serum IgE levels, and absolute eosinophil count.

Cases were randomly recruited from Philippine Children's Medical Center (PCMC). Controls were from various sources such as accompanying persons in the hospital, or volunteers from the community. Controls were active participants/volunteers who qualified in the selection criteria set in the study. In order to eliminate other diseases that may present as confounders, all patients (both cases and controls) underwent check-up by qualified physicians.

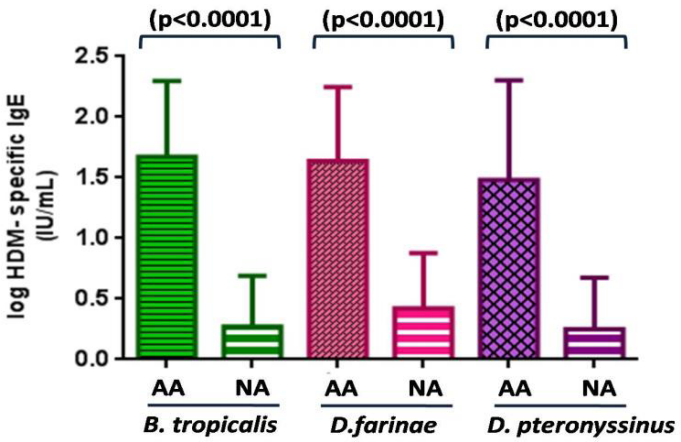
Cases with total serum IgE concentration less than 100 IU/mL were not included in the study. Conversely, prospective controls with physician diagnosed asthma and total serum IgE concentration equal to or greater than 100 IU/mL were also excluded in the study. Prospective cases and controls must not be siblings nor related up to third degree of consanguinity. Those who were diagnosed with infectious pulmonary diseases such as tuberculosis were excluded from the study.

Total serum IgE determination

Enzyme linked immunosorbent assay (ELISA) was used to determine the total serum IgE concentration of asthmatic Filipino paediatric patients and non-asthmatic controls. Purified unlabelled anti-human IgE (BD Pharmingen) was coated overnight onto ELISA plates (Greiner Bio-One, Germany) at 4°C. Fifty microliters of 1% BSA (Sigma) in Phosphate Buffered Saline with 0.05% Tween 20 (PBS-T) was used as a blocking buffer. ELISA plates were incubated overnight with 50 µL of 5x diluted human sera. Bound IgE were detected with biotinylated anti-human IgE (BD Pharmingen, CA, USA) diluted 5000x in blocking buffer. Plates were again washed and incubated with 2000x diluted ExtrAvidin-alkaline phosphatase conjugate (Sigma-Aldrich) for 1 hour. Finally, colorimetric reaction was performed using p-nitrophenyl phosphate (Sigma-Aldrich). Absorbance was read at 405 nm using an ELISA reader (BioTek ELx50). Human IgE (Pharmingen) was used as the standard per plate in the calculation of IgE concentration. Samples from allergic asthma patients with total IgE concentration of ≥100 IU/mL, and non-asthmatic subjects with < 100 IU/mL, were included in the study.

HDM-specific IgE determination

Enzyme linked immunosorbent assay (ELISA) was likewise used to evaluate the sensitization profiles of asthmatic cases and non-atopic controls. Aqueous allergen extracts from the house dust mite species *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus* and *Blomia tropicalis* were coated onto ELISA plates at a concentration of 10 ug/mL. Fifty microliters of 1% bovine serum albumin (BSA) (Sigma) in Phosphate Buffered Saline with 0.05% Tween 20 (PBS-T) was used as a blocking buffer. HDM-specific IgE were detected using biotinylated anti-human IgE (BD Pharmingen) diluted 5000x in blocking buffer. Plates were again washed and incubated with 2000x dilution of ExtrAvidin-alkaline phosphatase conjugate (Sigma-Aldrich) for 1 hour. Finally, colorimetric reaction was performed using p-nitrophenyl phosphate (Sigma-Aldrich). Absorbance was read



Positive Reactivity	83/125	72/125	70/125
% Reactivity	66%	62%	56%
Cut off Value	8.30	10.35	11.50

Figure 2. Log HDM-specific IgE concentration (IU/mL) of 125 allergic asthma (AA) cases and 125 non-asthmatic (NA) controls as determined by enzyme-linked immunosorbent assay.

Table 2. Correlation between log total and HDM-specific serum IgE and absolute eosinophil counts in allergic cases and controls.

Variables	All allergic cases (125)		Allergic Asthma only		Allergic Asthma with AR[1]		Allergic Asthma with AR[1] and AD[2]	
	C	p-value	C	p-value	C	p-value	C	p-value
Total IgE and absolute eosinophil count	0.1584	0.0778	0.1381	0.7231	0.1802	0.055	0.1084	0.5982
HDM- specific IgE and absolute eosinophil count								
Bt-specific IgE and absolute eosinophil count	0.0299	0.7406	0.2411	0.5321	0.0584	0.5371	0.1006	0.6248
Df-specific IgE and absolute eosinophil count	0.1988	0.0263	-0.1693	0.6632	0.2030	0.0303	0.2625	0.1952
Dp-specific IgE and absolute eosinophil count	0.1479	0.0998	-0.0861	0.8257	0.1359	0.1494	0.5497	0.0036

[1]AR, allergic rhinitis; [2]AD, atopic dermatitis

at 405nm using an ELISA reader (BioTek ELx50). Human IgE (Pharmingen) was used as the standard per plate in the calculation of IgE concentration. Asthmatic samples that registered values ≥cut-off values were considered sensitized with the particular HDM allergens used.

Absolute eosinophil count determination

Five hundred microliters of freshly extracted whole blood sample was used for complete blood count (CBC). CBC was obtained using the Beckman Coulter Counter to determine differential white blood cell count and compute for the absolute eosinophil count. Absolute eosinophil count of less than 350 cells/μL of blood was used as the reference value for normal levels of eosinophils.

Statistical analysis

Data were analyzed using statistical packages SAS9.2, STATA, GraphPad Prism 6 and MS Excel (Microsoft Corp., Redmond, WA, USA). Quantitative variables were log transformed and analyzed for normality to determine parametric or nonparametric statistical tools to be used. Differences and association among quantitative data were considered significant at p-value <0.05.

RESULTS

A total of 125 age and sex match-paired allergic asthma cases and non-asthmatic controls were included in the study from among 216 cases and 220 controls that were recruited for the study. Among the qualified asthmatics, 70% (88 out of 125) have concurrent allergic asthma and allergic rhinitis, 2% (2 out of 125) have concurrent allergic asthma and atopic dermatitis, 21% (26 out of 125) have concurrent allergic asthma and allergic rhinitis and atopic dermatitis, and 7% (9 out of 125) have allergic asthma only. Cases and controls were selected based on inclusion and exclusion criteria and doctor-diagnosed allergic conditions. Patients were mostly males in all the allergic groups (Table 1). Interestingly, 74% of the subjects in the control group have pets at home compared to only 55% among the allergic patients.

Significantly elevated total serum IgE concentration was observed in different allergic groups ($\bar{x}$=2.27, SD=0.26, p<0.001) as compared to the controls (Figure 1). The range of total serum IgE concentration was between 100-1700 IU/mL, with 75% (94 out of 125) of the allergic patients registering total IgE concentrations between 100-200 IU/mL, 18% (23 out of 125) between >200-800 IU/mL, and 6% (8 out of 125) with greater than 800 IU/mL. HDM-specific serum IgE against *B. tropicalis* ($\bar{x}$=1.67, SD=0.62, p<0.0001), *D. farinae* ($\bar{x}$=1.63, SD=0.61, p<0.0001), and *D. pteroynissinus* ($\bar{x}$=1.48, SD=0.82, p<0.0001), were significantly increased in the different allergic groups as compared to controls (Figure 2). HDM specific IgE concentration was higher against *B. tropicalis* ($\bar{x}$=1.67, SD=0.62, p<0.0001), indicating higher prevalence of sensitization to allergens from this species of house dust mites as compared with other house dust mite allergen sources that were used in the study. Using the mean allergen-specific IgE plus one SD as cut-off value to determine positive reactivity among the allergic asthma patients, the sensitization rates were computed as 66% for *Bt*, 62% for *Df*, and 56% for *Dp*. HDM-specific IgE concentration of patients was observed to be greater than 100 IU/mL among 31% (39 out of 125) for *Bt,* 30% (37 out of 125) for *Df*, and 26% (32 out of 125) for *Dp*. Specific serum IgE concentrations against the three HDM allergens ranges from 0 to 882 IU/mL (*Bt*), -359 IU/mL (*Df*), and -1588 IU/mL (*Dp*). Specific IgE produced against *Dp* was higher in concentration compared to other allergens used in the study, despite lower sensitization prevalence for this house dust mite species. Significantly high absolute eosinophil counts among all allergic asthma groups (mean 2.667, SD 0.38, p<0.0001) as compared with the controls were observed (Figure 3).

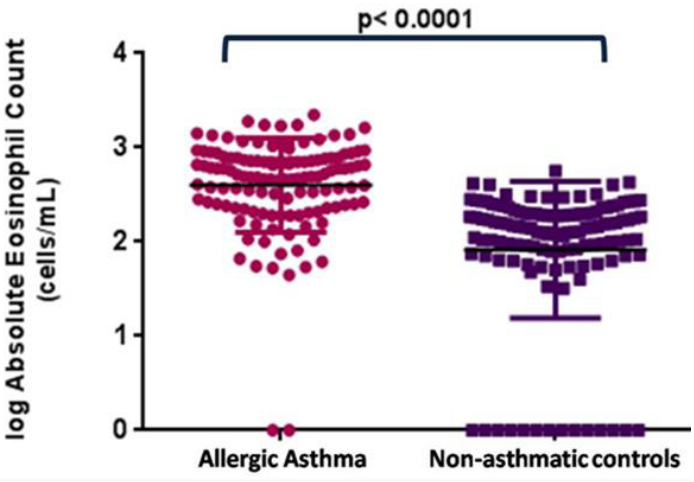


Figure 3. Log absolute eosinophil count (cells/ mm^3) of 125 allergic asthma (AA) cases and 125 non-asthmatic (NA) controls.

Correlation between serum IgE concentration and absolute eosinophil count was likewise determined (Table 2). For all atopic cases and AA with AR cases, *Df*-specific IgE and absolute eosinophil count have significant correlation (p-values=0.03). Allergic asthma with allergic rhinitis and atopic dermatitis patients showed significant relationship between *Dp*-specific IgE and absolute eosinophil count (p-value=0.004). No significant correlation was observed for any of the variables in cases with allergic asthma only.

Multiple regression analysis of total IgE concentration and absolute eosinophil count showed that in all the three groups of cases, the sign of the coefficient estimate of the total IgE concentration is positive signifying that an increase in the total IgE concentration is expected to increase the absolute eosinophil count. However, their p-values are greater than 0.05 which means that total IgE concentration has no significant contribution in explaining the variability in the absolute eosinophil count. Nevertheless, it may be noted that the value of coefficient estimate of the total IgE is largest for allergic asthma with allergic rhinitis cases followed by all allergic cases (Table 3).

For allergic asthma cases with allergic rhinitis and atopic dermatitis, *Dp*-specific IgE levels appeared to be significant. Its estimated parameter is positive which means that an increase in *Dp*-specific IgE concentration will yield an expected increase, on the average, on the absolute eosinophil count, holding other variables constant (Table 3).

DISCUSSION

One of the most important predictors of the presence and persistence of asthma is atopy. Atopy is used to indicate a genetic predisposition to mount an IgE response to low levels of airborne allergens, typically from pollens and HDMs. HDM allergens are the most prevalent triggers of allergic sensitization among asthmatics. Despite varying geographical differences, up to 85% of asthmatics in highly populated centers of North and South America, Europe, South East Asia and Australia are typically HDM allergic (Platts-Mills et al.1989). Total IgE is a reflection of the threshold for the production of relatively large amounts of Th2 cytokines, while specific IgE reflects an allergen-induced immune response. The median level of total IgE is typically 200–400 kU/L in uncomplicated atopic disease. A total IgE of more than 1,000 kU/L or IU/mL suggests a combination of abnormalities (Martinez 1998). Further analysis of data showed that 93.6% of allergic cases have concentration of total serum IgE between 100-800 IU/mL, and only a very small percentage has outlying concentration greater than 800 IU/mL (data not shown). This could be due to multiple sensitization to several allergen sources which could have contributed to the increase in the total serum IgE concentration.

Allergy to house dust mites can be diagnosed using skin-prick test, Enzyme-Linked Immunosorbent Assay (ELISA) or Radioallergosorbent test (RAST) (Milian and Diaz 2004). The most important house dust mites in drier areas are *D. pteronyssinus* and *D. farinae*. *D pteronyssinus* prefers temperate/tropical coastal regions, whereas *D. farinae* is more abundant in continental climates (Thomas et al. 2010). *B. tropicalis*, found in subtropical regions and tropical regions like South America, the Caribbean and Asia, is the most important of the storage mites (Chua 2007) and usually occurs in conjunction with *D. pteronyssinus*. In this study, 66% of the cases were sensitized to *B. tropicalis*, 62% to *D. farinae*, and 56% to *D. pteronyssinus*. The inhalant allergens used was based on a previous study by Ramos and colleagues, which showed high prevalence of HDM sensitization and cross-reactivity between the allergens in these HDM species among Filipino allergic patients (Ramos et al. 2007).

The onset of allergic rhinitis and asthma usually occurs during childhood, adolescence or early adulthood (AAAAI 2000). By adulthood, female subjects more frequently have asthma, and more commonly have the severe form of asthma (Koppelman and Sayers 2011). Moreover, asthma and atopic dermatitis are highly associated with allergic rhinitis and the latter is a risk factor for asthma (Terrehorst et al. 2002). In this study, asthma cases are almost always in conjunction with allergic rhinitis or atopic dermatitis or both. The prevalence of asthma for boys aged 0-17 years is over 30% higher than the rate among girls of the same age. In adults, the prevalence leans toward females who have asthma than in adult males (Nish 2004). Lifestyle factors such as pet exposure during the first years of life have been associated with a reduced occurrence of allergic rhinitis and asthma among school children (Hesselmar et al. 1999). This could explain the result of this study wherein 74% of the control group has pets at home while only 52% of the subjects with allergic states have them. This may either be explained by the induction of allergen-

Table 3. Multiple regression analysis of log total and HDM-specific IgE and absolute eosinophil counts in allergic cases and controls.

Dependent variable: Absolute eosinophil count*	parameter estimate	standard error	test stat	p-value
All allergic cases regardless of atopy status				
Total IgE concentration	0.3026	0.1701	1.78	0.0778
Bt concentration	-0.03	0.0749	-0.37	0.7103
Df concentration	0.18	0.0961	1.92	0.0566
Dp concentration	0.07	0.0597	1.17	0.2461
AA only[1]				
Total IgE concentration	0.14414	0.39074	0.37	0.7231
Bt concentration	0.52280	0.67721	0.77	0.4750
Df concentration	-0.15932	0.22526	-0.71	0.5110
Dp concentration	-0.02273	0.42565	-0.05	0.9595
AA with AR[2]				
Total IgE concentration	0.345	0.1787	1.94	0.0550
Bt concentration	0.1891	0.1062	1.78	0.0777
Df concentration	0.056	0.0627	0.89	0.3756
Dp concentration	0.056	0.063	0.89	0.376
AA with AR and AD[3]				
Total IgE concentration	0.1791	0.3952	0.53	0.5982
Bt concentration	0.144	0.1765	0.82	0.4236
Df concentration	-0.017	0.1804	-0.09	0.9261
Dp concentration	0.2795	0.097	2.88	0.0087

*IU/ml
[1]AA, allergic asthma cases only;
[2]AA with AR, allergic asthma with allergic rhinitis;
[3]AA with AR and AD, allergic asthma with allergic rhinitis and atopic dermatitis

specific tolerance or may be due to exposure to the microbial flora associated with pets (Holger and Renz 2007).

Eosinophils are known to be the main effector cells of allergic process. Increased eosinophil levels have been observed in asthma, which correlates with disease severity. Eosinophils are important both during the initial and later stages of allergic airway diseases (Walsh 2010). Upon activation, eosinophils release extracellular granule proteins, such as eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), MBP, and eosinophil peroxidase (Moqbel et al. 2008), proinflammatory cytokines, chemokines and lipid mediators. Based on results presented herein, there is a significant difference between the absolute eosinophil counts in all allergic cases ($p < 0.0001$) as compared with the controls. These results are similar with the increased level of absolute eosinophil count observed among allergic rhinitis patients with asthma among Indian respiratory allergy patients (Chowdary et al. 2003). Further analysis of the data of this study showed that the mean of the log absolute eosinophil count among allergic asthma cases with allergic rhinitis and atopic dermatitis ($\bar{x}$=2.667, SD=0.38) and allergic asthma cases with allergic rhinitis ($\bar{x}$=2.635, SD=0.06) are higher than in allergic asthma cases only ($\bar{x}$=1.847, SD=0.12).

Clinically, asthma is either allergic or nonallergic. Allergic asthma is characterized by younger, early-onset, male predominance, and independent factors that include smoking and rhinitis. On the other hand, nonallergic asthma is characterized by older, late-onset, higher female/ male ratio among patients, negative familial history of hypersensitivity nor other individual allergic sign, negative allergen test, and poor response to hormone treatment (Pillai et al. 2011; Fan 2006). Allergic asthma, allergic rhinitis, and atopic dermatitis are almost invariably accompanied by elevated levels of IgE. Results of genetic analyses of families have shown that bronchial hyperresponsiveness (BHR) and IgE levels are linked (Postma et al. 1995). IgE-induced mast cell degranulation *in vivo* triggers the release of preformed vasoactive mediators, prostaglandins and leukotrienes synthesis, and the transcription of cytokines, which rapidly induce mucosal edema, mucous production, and smooth muscle constriction, and eventually elicit an inflammatory infiltrate in the bronchial mucosa. A second wave of hypersensitivity responses (late-phase reaction, LPR), dependent upon eosinophils, occur many hours after the acute reaction, manifesting as a second wave of decreased airflow 4-8 hours after the initial allergen contact in allergic asthmatics. This chronic airway symptom results from persistent late-phase inflammatory responses in situations of perennial allergen exposure (Oettgen and Geha 1999). On the other hand, viruses or bacteria and mold, emotional change and gastroesophageal backflow induce nonallergic asthma (Fan 2009).

It has been previously reported that there is a positive correlation among allergen type, total serum IgE, eosinophil and bronchial hyperresponsiveness, suggesting that all three may play a role in the development of bronchial asthma in rhinitis patients (Di Lorenzo et al. 1997). A positive correlation among serum total IgE levels, total blood eosinophil counts and eosinophil cationic protein (ECP) among allergic rhinitis patients has also been reported (Chen et al. 2006; Meijer et al. 2002). Peripheral eosinophil count was reported to be significantly higher only in asthma with rhinitis ($p<0.005$) but not in allergic rhinitis alone (Takahashi et al. 2010). Interestingly, increased eosinophils were observed not only in peripheral blood but also in the airways of atopic asthmatics (Dong-Ho et al. 2000), confirming the roles eosinophils play in the pathogenesis of allergic asthma alone or in combination with other allergic diseases.

D. farinae survive better in drier climates, such as in the Philippines, as compared to *D. pteronyssinus* (Ramos et al. 2007). Potent allergens from *D. pteronyssinus* have been characterized, including *Der p1* which influences allergenicity through protease activity which can disrupt the epithelium, thus allowing access to antigen presenting cells, release inflammatory cytokines and

elicit inflammatory reactions, activate and recruit basophils and stimulate production of Th2-inducing cytokines (Jeong et al. 2012). *Der p*2 is known to activate respiratory epithelial cells and induce secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, IL-8, monocyte-chemotactic protein-1 (MCP-1) and macrophage inflammatory protein-3α (MIP-3α). In addition to enzyme activities, there are also other proteins from *D. pteronyssinus* that may enhance allergenicity such as allergens with affinities to lipids (*Der p* 2), chitin-binding proteins (*Der p* 5,21,23), and allergens from muscle (*Der p* 10,11) (Jeong et al. 2012). Significant correlation and multiple regression analysis between *Dp*-specific IgE with absolute eosinophil count among allergic asthma patients with concurrent allergic rhinitis and atopic dermatitis could be explained by the fact that in an environment where there is abundance of dust mite species individuals with skin allergies are more sensitized to *D. pteronyssinus* as compared to other dust mite species (Shek et al. 2010).

The correlation and multiple regression analyses of total IgE, HDM-specific IgE and absolute eosinophil counts in a population of pediatric patients with allergic asthma have been shown in this study, validating the important roles of these phenotypes in the pathogenesis of allergic asthma in the pediatric population. These results give evidences to the association of serum IgE with bronchial hyperresponsiveness and asthma (Beeh et al. 2000). Eosinophil recruitment into bronchial mucosa where allergic inflammation has occurred contributes to the late asthmatic reaction and mucus hypersecretion reflecting a significant correlation between eosinophil activation and asthma severity (Filipovic and Cekic 2001).

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CONFLICT OF INTEREST

All authors have declared no conflict of interests.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Maricar W. Ching was involved in the conceptualization, subject recruitment, sample collection, data gathering and analysis, and writing of the manuscript.

Jennifer Maries G. Yap was also involved in the subject recruitment, sample collection, data gathering and analysis.

Kevin Carl P. Santos was involved in the statistical analyses and interpretation of the data gathered.

Cesar M. Ong was the pediatric pulmonologist who diagnosed and classified all the subjects that were recruited as being asthmatic or non-asthmatic.

John Donnie A. Ramos was involved in the conceptualization and planning of the study and writing the manuscript.

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