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Correlation of *Blomia tropicalis*-specific immunoglobulin epsilon profiles with family history of atopy in a Filipino population

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ABSTRACT

Background: House dust mites are the major source of indoor allergens in the tropical and subtropical regions with *Blomia tropicalis* (Bt) allergens as one of the leading causative agents of sensitization among patients from the tropics. Despite the clinical importance of Bt in various populations, its allergenicity remains unclear among Filipino allergic patients.

Objective: This study determined the sensitization profiles of allergic Filipinos against Bt allergens and its correlation with atopy.

Methods: Total immunoglobulin epsilon (IgE) (n = 960), Bt-specific IgE (n = 247), and *Blomia tropicalis* 5 (Blo t 5)-specific IgE (n = 87) profiles of allergic and nonallergic subjects were measured through enzyme-linked immunosorbent assay (ELISA). Point-biserial correlation coefficient was used to determine the association between Bt-specific IgE levels and selected demographics. Inhibition ELISA was performed to measure the inhibition capacity of recombinant Blo t 5 (rBlo t 5) against Bt allergen extracts.

Results: Mean total IgE levels of allergic cases (n = 171) were significantly higher ($P < 0.001$) compared to the mean IgE levels of nonallergic controls (n = 76). Among allergic subjects, 58% were sensitized to Blo t extract and 80% of which were sensitized to rBlo t 5 allergen. A positive correlation was observed between Bt-specific IgE and family history of atopic disease ($P = 0.031$). Inhibition assay revealed that 54% mean reactivity of 7 plasma samples was caused by rBlo t 5, validating that rBlo t 5 is a major allergen in Bt.

Conclusions: This study has shown the importance of Bt as an allergen source that sensitizes atopic Filipino subjects. Hence, inclusion of Bt allergen extract and rBlo t 5 in the panel for allergy diagnosis and immunotherapy in Filipino populations is strongly recommended.

Keywords: Allergen; atopy; *Blomia tropicalis*; Blo t 5; IgE

1. Introduction

Over the last few decades, allergic diseases such as allergic asthma (AA), allergic rhinitis (AR), and atopic dermatitis (AD) have dramatically become prevalent worldwide, sometimes referred to as an 'epidemic of the 21st century' [1]. Such debilitating disorders are arbitrated by an abnormal immunoglobulin epsilon (IgE)-mediated immune response known as type I hypersensitivity reactions to an otherwise harmless foreign protein known as allergens [2]. About 20% to 30% of the world's population in both developed and developing countries suffer from allergic disorders, majority of which are triggered by aeroallergens from house dust mites (HDMs) species. HDMs are astigmatid arachnids that have lived in close association with humans from which they obtain their food—dead skin cells. They also feed on pet dander and can live in upholstered furniture, fabrics, and other parts of the house that provide a favorable environment for dust mites to thrive [3].

HDMs are known to be linked with the development and exacerbation of allergic diseases. Continuous exposure to HDMs can lead to chronic bronchial hyperresponsiveness and even increased wheezing [4]. In Southeast Asian countries with tropical climates, they continue to pose a threat as it leads to year-round disease manifestations that worsen over time [5]. Of much clinical relevance, about 33% to 47% of atopic patients are sensitized to HDMs in the Philippines [6]. With this alarming prevalence of HDM-mediated allergic diseases in the country marked by medical and socioeconomic burdens among Filipino individuals, there is a crucial need for a more efficient means of diagnosis and treatment and other efforts to control environmental exposure to HDM allergens [7].

Proper treatment and prevention of HDM-mediated allergic diseases require accurate and reliable identification of allergic triggers [8]. Fortunately, techniques in molecular cloning and recombinant protein production have paved the way for the development of pure and chemically well-defined allergens for both diagnosis and allergen-specific immunotherapy [9]. *Blomia tropicalis* (Bt) is one of the clinically important HDM species identified

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in tropical and subtropical regions and is classified as one of the leading causative agents of sensitization among atopic patients in the tropics [10, 11]. Of the 13 reported allergens from Bt, *Blomia tropicalis 5* (Blo t 5) has been identified as an important trigger of allergic sensitization and a major Blo t allergen [12]. Despite the clinical importance of Bt in various populations, its allergenicity remains unclear among Filipino allergic patients, specifically in rural environments, particularly in Ilocos Norte. In this study, we investigated IgE-mediated sensitization of Bt allergen extract and the recombinant Blo t 5 (rBlo t 5) among the Filipino population from Ilocos Norte, Philippines.

2. Materials and methods

2.1. Ethics

The design, sampling, experimental protocols, questionnaires, and other pertinent documents of this study were approved by the University of Santo Tomas Graduate School Ethics Review Board Committee (Rm 301., Thomas Aquinas Research Complex, Espana Blvd., Manila, Philippines). The acting Chair of the University of Santo Tomas Graduate School Ethics Review Board Committee, Marie Antonette J. Sunga-Vargas, authorized the approval of the study on November 22, 2022, with protocol number GS2019 PNEX004.

2.2. Study design and subjects

Subjects from the Ilocos Region, Philippines were first screened at the Northwestern University through an interview to assess their eligibility. A consent form, patient information sheet, assent form, and a modified International Study of Asthma and Allergies in Childhood-based questionnaire were used as a primary screening process. Subjects included are natural-born Filipinos and have lived, worked, or studied in Ilocos Norte, Philippines for more than 2 years and are not blood related. Subjects currently on medications (eg, antihistamines, corticosteroids, anti-inflammatory medications, and immunosuppressive medications) and pregnant or breastfeeding women were excluded in this study. A total of 960 plasma samples were collected based on the inclusion and exclusion criteria. Cases include individuals with self-reported AA, AR, and/or AD, with a total IgE level of ≥100 IU/mL, which was used as a secondary screening process where 171 cases and 76 controls were identified. Controls include individuals without self-reported allergies having a total IgE of <100 IU/mL. The power of the sample size was computed using G*power softwarev3.1 [13].

2.3. Blood extraction and phenotyping

Blood samples were collected from cases and controls with the assistance of a registered phlebotomist. Approximately 5 mL of blood was extracted via venipuncture. Blood plasma samples were isolated by centrifugation at 10,000 rpm for 10 minutes, which were then stored in aliquots at –20°C until use. Total IgE, Bt-specific IgE, and rBlo t 5-specific IgE were determined using enzyme-linked immunosorbent assay (ELISA).

2.4. Total IgE ELISA

ELISA was used to determine the total IgE concentrations of the allergic cases and nonallergic controls following standard protocols based on the KOMA ELISA kit. All procedures were done at room temperature unless otherwise stated. A 20-µg/vial coating antibody IgE was diluted in a 10 mL of 50-mM carbonate-bicarbonate buffer (pH 9.6). A 100 µL of diluted coating antibody was then coated per well onto ELISA plates (Corning Costar Inc., NY, USA) and incubated overnight at 4°C with constant gentle shaking. Plates were then blocked with 100 µL of 1% bovine serum albumin (BSA) (Fisher BioReagents, MA, USA) diluted in 1× phosphate buffered saline (PBS) for 1 hour. After incubation, 50 µL of human plasma or serial dilution of the protein standards (31.25 ng/mL to 0.49 ng/mL) was added to each well in duplicates followed by incubation for another 2 hours. Afterwards, a 20-µg/vial detection antibody (Labiskoma, KR) was reconstituted in 100-µL sterile water and then further diluted with 1% BSA solution. A 100-µL diluted detection antibody was added per well and plates were incubated for an hour. Lastly, colorimetric reaction was performed by adding 100 µL of tetramethylbenzidine (TMB) (Sigma-Aldrich, Saint Louis, MO, USA) per well. In between steps, ELISA plates were washed 3 times with 1× PBS with 0.05% Tween 20 (Loba Chemie Pvt Ltd, IN) using an ELISA Washer (Lisawash 3000, IN). Absorbance at 605 nm was read 30 minutes after the addition of TMB on the ELISA plate reader (SPECTROstar Nano, USA). Total IgE concentrations were expressed as international units per milliliters (IU/mL; 1 IU/mL = 2.44 ng/mL) [14].

2.5. Preparation of Bt aqueous extract

Frozen Bt weighing 1.0331 g from the Department of Parasitology, Mahidol University, Bangkok, Thailand was mechanically ground using a precooled mortar and pestle for 1 hour. Chemical extraction was performed by adding 10 mL of 1× PBS and 100 µL of phenylmethylsulfonyl fluoride protease inhibitor slowly while grinding the mites. The mite extract suspension was incubated at 4°C for 16 hours with gentle constant shaking prior to centrifugation. The supernatant Bt allergen aqueous extract was isolated by centrifugation at 14,000 rpm for 20 minutes using refrigerated centrifuge (DLAB D3024R, USA). Lastly, Bt allergen aqueous extract was quantified using Bradford assay.

2.6. Bt-specific IgE ELISA

ELISA was used to evaluate the sensitization profiles of cases and controls against Bt allergen extract. All procedures were done at room temperature unless otherwise stated. Bt extract was diluted in a 50-mM carbonate-bicarbonate buffer (pH 9.6) to have a final concentration of 10 µg/mL. A 50-µL diluted coating Bt extract was then added per well onto an ELISA plate (Corning Costar Inc., NY, USA) and was incubated overnight at 4°C with gentle constant shaking. Plates were blocked with 100 µL of 1% BSA (Fisher BioReagents, MA, USA) diluted in 1× PBS for 1 hour. A 5× diluted human plasma (50 µL) in 1% BSA was added to each well in duplicates and the plates were incubated for 2 hours. Afterwards, 100 µL of diluted Horseradish peroxidase conjugated with antihuman IgE was added per well and plates were incubated for an hour. Lastly, colorimetric reaction was performed by adding 100 µL of TMB (Sigma-Aldrich, Saint Louis, MO, USA) per well. In between steps, ELISA plates were washed 3 times with 1× PBS with 0.05% Tween 20 (Loba Chemie Pvt Ltd, IN) using an ELISA Washer (Lisawash 3000, IN). Absorbance at 605 nm was read 30 minutes after the addition of TMB on the ELISA plate reader (SPECTROstar Nano, USA).

2.7. rBlo t 5-specific IgE ELISA

ELISA was used to evaluate the sensitization profiles of selected Bt-positive allergic cases and nonallergic controls against rBlo t 5. rBlo t 5 was obtained from Allergy and Asthma Laboratory of National University of Singapore. All procedures were done at room temperature unless otherwise stated. A 5-µg/mL concentration of rBlo t 5 in a 50-mM carbonate-bicarbonate buffer (pH 9.6) was coated onto an ELISA plate (Corning Costar Inc., NY, USA) and was incubated overnight at 4°C with gentle constant shaking. Plates were blocked with 1% BSA (Fisher BioReagents, MA, USA) diluted in 1× PBS for 1 hour. A 50-µL human plasma diluted 5× in 1% BSA was added to each well in duplicates and the plates were incubated for 2 hours. Afterwards, 100 µL of diluted horseradish peroxidase conjugated with antihuman IgE was added per well and was incubated for an hour. Lastly, colorimetric reaction was performed by adding 100 µL of TMB (Sigma-Aldrich, Saint Louis, MO, USA) per well. In between steps, ELISA plates were washed 3 times with 1× PBS with 0.05% Tween 20 (Loba Chemie Pvt Ltd, IN) using an ELISA Washer (Lisawash 3000, IN). Absorbance at 605 nm was read 30 minutes after the addition of TMB on the ELISA plate reader (SPECTROstar Nano, USA).

2.8. Inhibition assay

Inhibition of IgE reactivity using ELISA was performed to determine the inhibition capacity of rBlo t 5 against Bt allergen extract. Seven Bt extract and rBlo t 5-positive samples were used. All procedures were done at room temperature unless otherwise stated. A 10-µg/mL concentration of Bt extract diluted in a 50-mM carbonate-bicarbonate buffer (pH 9.6) was coated onto ELISA plates (Corning Costar Inc., NY, USA) at 50 µL per well and was incubated overnight at 4°C. Simultaneously, each plasma was preabsorbed with 5 µg/mL of rBlo t 5 and was also incubated overnight at 4°C. The incubated plate was blocked with 1% BSA (Fisher BioReagents, MA, USA) diluted in 1× PBS for 1 hour. The preabsorbed plasma, along with the corresponding unabsorbed plasma in duplicates, was added to the plate and incubated for 2 hours. Afterwards, 100 µL of diluted horseradish peroxidase conjugated with antihuman IgE was added per well and was incubated for an hour. Lastly, colorimetric reaction was performed by adding 100 µL of TMB (Sigma-Aldrich, Saint Louis, MO, USA) per well. In between steps, ELISA plates were washed 3 times with 1× PBS with 0.05% Tween 20 (Loba Chemie Pvt Ltd, IN) using an ELISA Washer (Lisawash 3000, IN). Absorbance at 605 nm was read 30 minutes after the addition of TMB on the ELISA plate reader (SPECTROstar Nano, USA).

2.9. Statistical analysis

Data were analyzed using SPSS v.29, Graphpad Prism 9, and MS Excel. Mean total IgE, relative Bt-specific IgE, and relative rBlo t 5-specific IgE concentrations were determined. Unpaired 2-tailed Student *t* test was also utilized for the analysis of each IgE concentration. Power of the sample size was calculated using G*power software. Point-biserial correlation coefficient was also used to determine association of relative Bt-specific IgE levels with cesarean mode of delivery, family history of atopic diseases, being a smoker, exposure to secondhand smoke, and exposure to household pets. Statistical significance was set at P value <0.05.

3. Results

3.2. Demographic profiles of allergic cases and nonallergic controls

From a total of 690 self-reported cases of allergic diseases and 220 self-reported nonallergic cases using standard questionnaires as primary screening process, a total cohort of 247 subjects (171 allergic cases and 76 nonallergic controls) were included in the study (Table 1). Power of sample size was determined to be at 95%. Patients with multiple allergies (MAs) are reported as a co-occurrence of at least 2 of the allergic diseases mentioned above. Out of 4 classified types of allergies, the majority of the cases exhibited MA (55.56%), with AA concurrent with

Table 1.

Demographic profiles of allergic cases and nonallergic controls

Demographic	Category	Allergic cases (n = 171)	Nonallergic controls (n = 76)
Type of allergy	AA only	18 (10.53%)	NA
	AR only	43 (25.15%)	
	AD only	15 (8.77%)	
	MA	95 (55.56%)	
Family history of allergies	With atopy	54 (31.58%)	15 (19.74%)
	Without atopy	56 (32.75%)	36 (47.37%)
	Unanswered	61 (35.67%)	25 (32.89%)
Presence of pets at home	Yes	141 (82.46%)	65 (85.53%)
	No	30 (17.54%)	11 (14.47%)
Smoker	Yes	18 (10.53%)	2 (2.63%)
	No	151 (88.30%)	74 (97.37%)
	Unanswered	2 (1.17%)	0 (0%)
Exposure to secondhand smoke	Yes	57(33.33%)	24 (31.58%)
	No	114 (66.67%)	52(68.42%)
Type of birth	Normal delivery	128 (74.85%)	64 (84.21%)
	Cesarean section	30 (17.54%)	8 (10.53%)
	Unanswered	13 (7.60%)	4 (5.26%)
Age	≤21 years old	126 (73.68%)	61 (80.26%)
	>21 years old	45 (26.32%)	15 (19.74%)
Sex	Male	31(18.13%)	7 (9.21%)
	Female	140 (81.87%)	69 (90.79%)

AA, allergic asthma only; AD, atopic dermatitis only; AR, allergic rhinitis only; MA, multiple allergies; NA, not applicable.

AR and/or AD. AA and AR were the most common combination (24.56%), followed by co-occurrence of all the 3 disorders (23.98%). Female subjects had higher preponderance than male having a ratio of 11:2 (female:male) in all allergy types identified (Fig. 1). Allergic disorders were also reported to be present among other family members of the allergic subjects. As for other demographic profiles, a substantial number of allergic cases have exposure to pets at home (82.46%), are nonsmokers (88.30%), have no exposure to secondhand smoke (66.67%), and were born via normal delivery (74.85%). Age distribution in both allergic and nonallergic cases ranged from 9 to 63 years old. About 73.68% of the allergic subjects fall within the pediatric age group and 6 of which were from ages 9 to 10 years (Table 1).

3.2. Allergic cases have a significantly higher mean total IgE than nonallergic controls

The mean total IgE levels of the 171 allergic cases were significantly higher (222.27 IU/mL) in comparison to that of the 76 nonallergic controls (38.99 IU/mL) (Fig. 2). The range of the total IgE levels of the allergic cases was also higher (100.32–925.41 IU/mL) compared to the nonallergic controls (2.09–95.65 IU/mL). In addition, 62 out of the 171 allergic cases (36.26%) exhibited elevated levels of total IgE, having higher levels than the mean (222.27 IU/mL) for allergic cases. Interestingly, in 62 cases with elevated total IgE than the mean, 15% are birthed through cesarean, 11% had family history of allergies, 32% are exposed to secondhand smoke, and 60% have pets at home. Similarly, total IgE levels of the 6 allergic cases aged 9 to 10 years were also elevated, ranging from 114.17 to 474.28 IU/mL, with 2 cases exhibiting total IgE levels higher than the mean.

3.3. Allergic cases exhibited 58% Blomia tropicalis-specific IgEs

Bt-specific IgE levels of the 171 allergic cases were significantly higher than nonallergic controls ($P < 0.0001$). Majority of allergic cases (58%) exhibited positive reaction with allergens from Bt aqueous extract using a cutoff value of 0.474, corresponding to mean + 1 standard deviation (SD) of the relative Bt-specific IgE concentration of the 76 nonallergic controls (Fig. 3). Allergic cases have higher relative Bt-specific IgE concentrations, with the highest reaching 1.551, in comparison with nonallergic controls, which reached 0.605. Among the allergic cases, 4 plasma reached relative Bt-specific IgE levels greater than 1. Interestingly, among Bt-positive allergic cases, 19% are birthed through cesarean delivery, 32% have family history of allergic disease, 12% were active smokers, 32% were exposed to secondhand smoke, and 82% were exposed to household pets.

3.4. Majority of Bt-positive allergic cases were sensitized to rBlo t 5

Levels of rBlo t 5-specific IgE were significantly higher in allergic cases than nonallergic controls ($P < 0.0001$). Majority of selected Bt-positive allergic cases (80%) also exhibited a positive reaction with rBlo t 5 allergen using a cutoff value of 0.410, corresponding to mean + 1 SD of the relative rBlo t 5-specific IgE of 42 nonallergic controls (Fig. 4). Majority of the allergic cases have a relative rBlo t 5-specific IgE ranging from 0.400 to 0.600. Highest relative rBlo t 5-specific IgE of allergic cases and nonallergic controls reached 1.297 and 0.603, respectively. In addition, 5 plasma from the allergic cases exhibited relative Bt-specific IgE levels greater than 1. Surprisingly, 28% of Bt- and rBlo t 5-sensitized patients have a family history of allergies, 36% are exposed to secondhand smoke, and 86% have pets at home.

3.5. rBlo t 5 exhibited a 54% inhibition against Bt extract

Percentage inhibition of rBlo t 5 allergen against Bt allergen extract ranged from 37% to 76%. Plasma 6 had the lowest percentage of inhibition while plasma 5 had the highest having 76% inhibition (Fig. 5). The average inhibition of 7 plasma samples (rBlo t 5 against Bt extract) is 54%. These results indicate that rBlo t 5 is the major sensitizing allergen in the Bt allergen extract that triggers IgE production among exposed atopic individuals.

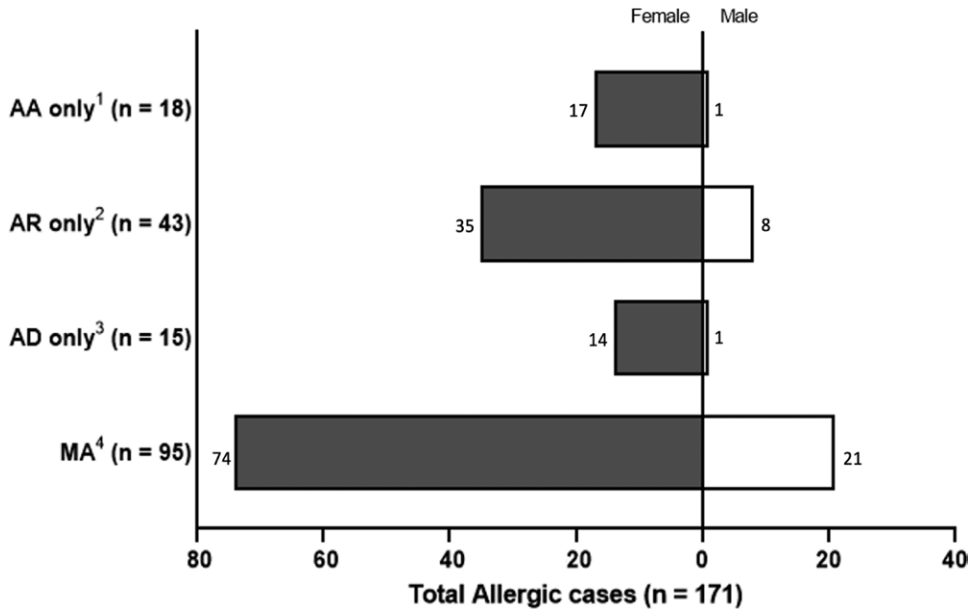

Figure 1. Sex distribution of allergic cases (n = 171) according to their type of allergy indicated as [1]allergic asthma only, [2]allergic rhinitis only, [3]atopic dermatitis only, or [4]multiple allergies.

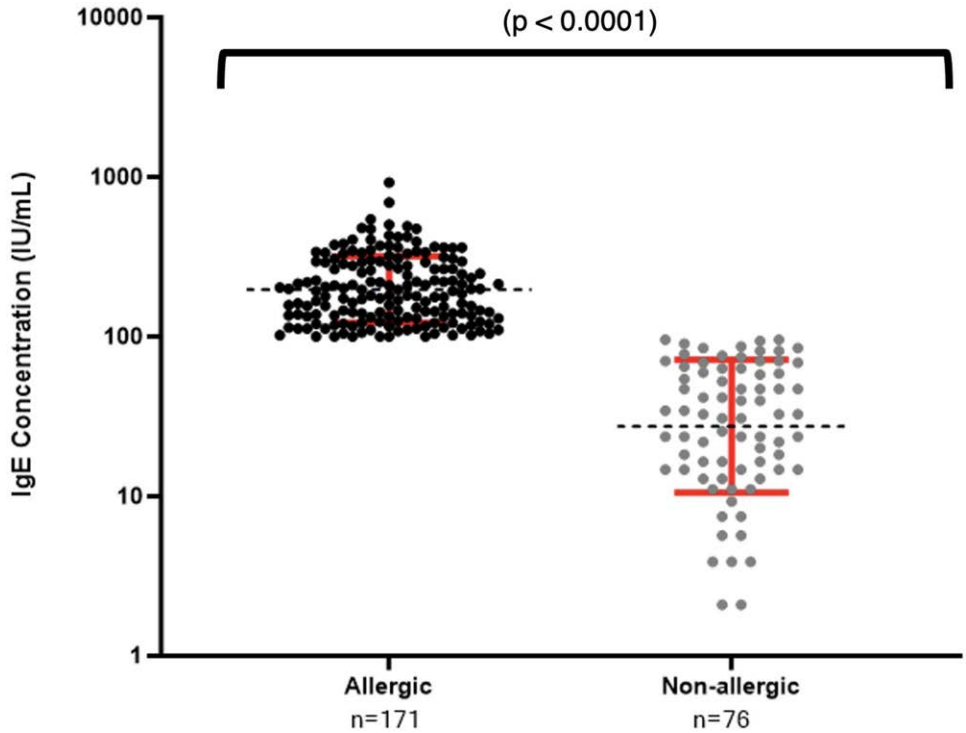

Figure 2. Total IgE concentration (IU/mL) of allergic cases (n = 171) and nonallergic controls (n = 76). Broken lines in each group indicate the geometric mean of the total IgE levels. Red lines represent the standard deviation range of the total IgE levels. IgE, immunoglobulin epsilon.

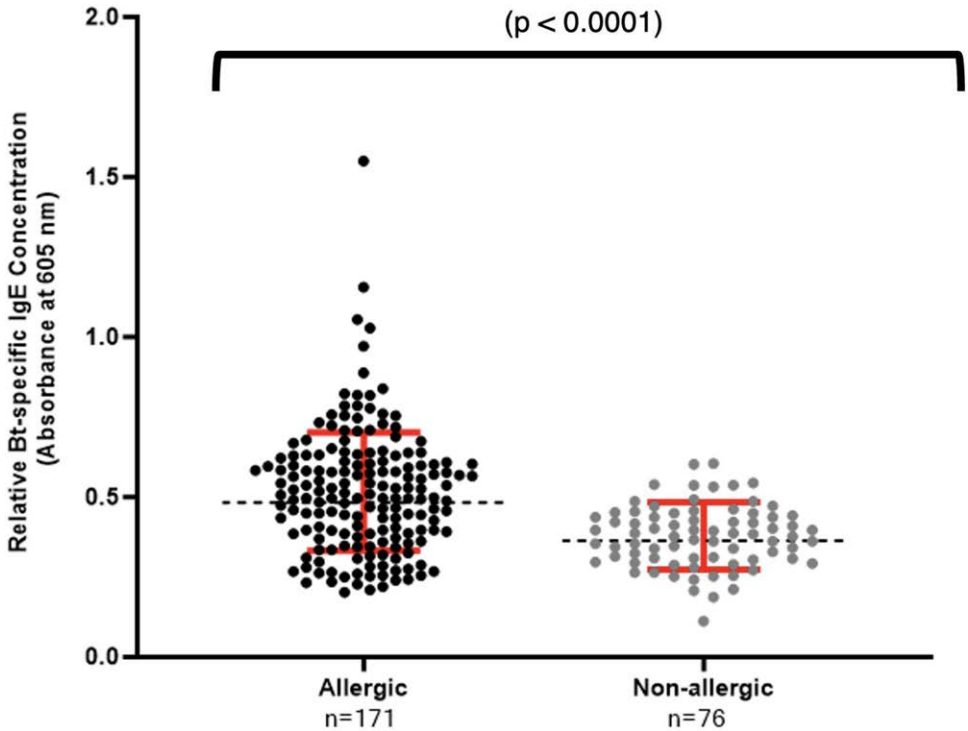

Figure 3. *Blomia tropicalis* (Bt)-specific IgE profiles of allergic cases (n = 171) and nonallergic controls (n = 76) as determined by ELISA. Broken lines in each group indicate the mean of the Bt-specific IgE levels. Red lines represent the standard deviation range of Bt-specific IgE levels. ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin epsilon.

3.6. Family history of atopic disease is a predictive factor for elevated Bt-specific IgE

Point-biserial correlation coefficient analysis was used to identify the strength and association of Bt-specific IgE levels and allergenicity of cases and controls (Table 2). A positive correlation between Bt-specific IgE levels and family history of atopic disease was observed (P = 0.031). Although a weak relationship (CC = 0.171) between IgE levels and family history

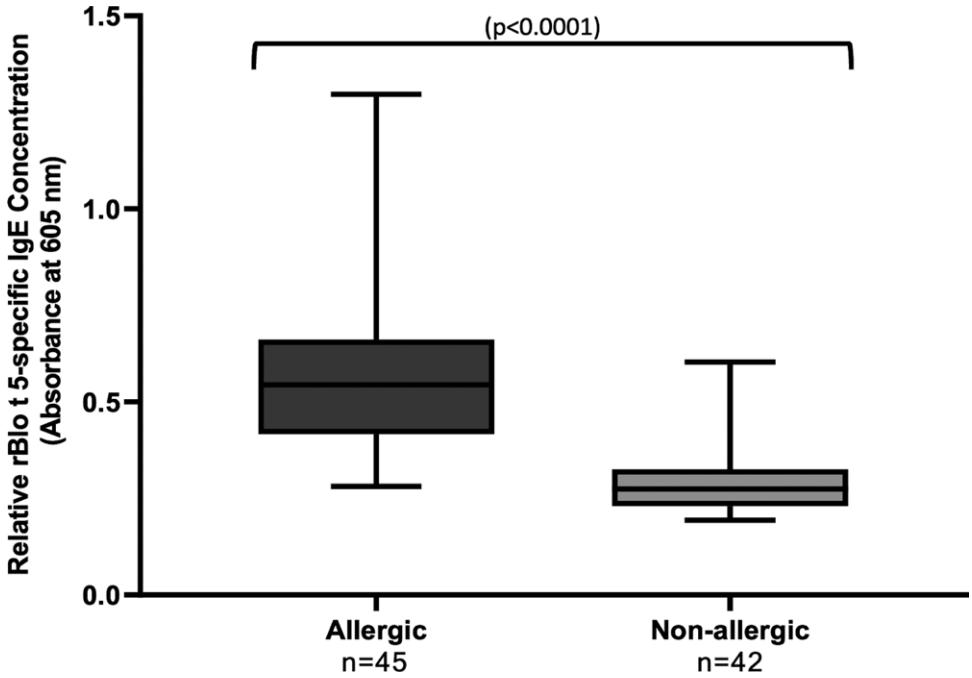

Figure 4. Recombinant Blo t 5-specific IgE profiles of allergic cases (n = 45) and nonallergic controls (n = 42) as determined by ELISA. Blo t 5, *Blomia tropicalis* 5; ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin epsilon.

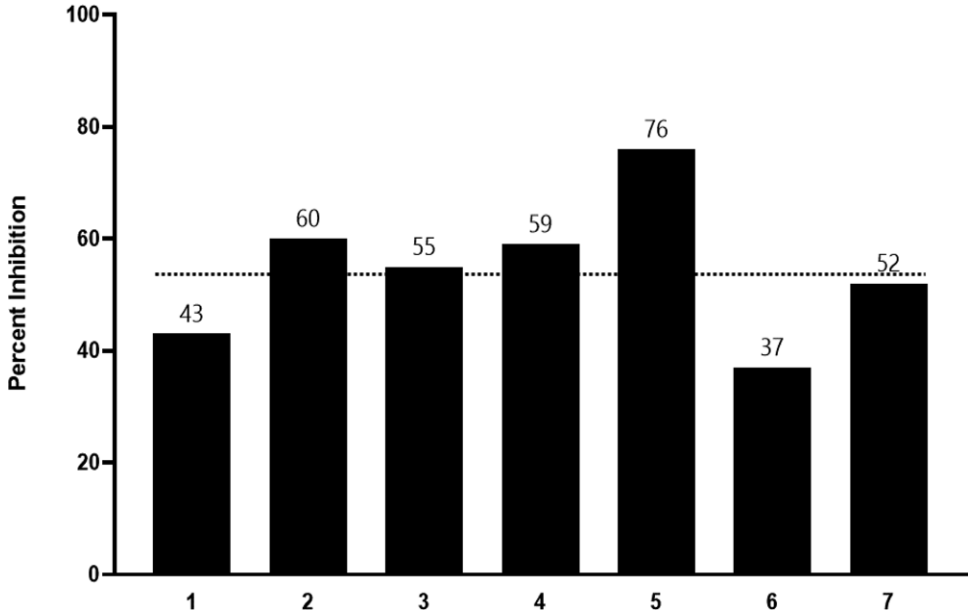

Figure 5. Inhibition assay results of rBlo t 5 on the IgE reactivity of the Bt extract. Average inhibition at 54% is indicated by the broken line. Bt, *Blomia tropicalis*; IgE, immunoglobulin epsilon; rBlo t 5, recombinant *Blomia tropicalis* 5.

was established, the results indicate that family history to allergies could be a predictive factor for a person to exhibit elevated Bt-specific IgE. On the other hand, there is no correlation between Bt-specific IgE and cesarean mode of delivery (CC = 0.030; P = 0.652), smoking (CC = 0.090; P = 0.162), exposure to secondhand smoke (CC = 0.015; P = 0.824), and exposure to household pets (CC = 0.002; P = 0.975). Similarly, using the odds ratio, allergic cases that have a family history of atopic disease have 2.25× the odds of having elevated Bt-specific IgE than controls with a P value of 0.025.

4. Discussion

HDMs are distributed worldwide but their prevalence varies from one region to another, thus the need for an investigation specific to a particular geographic location [17]. In tropical areas such as the Philippines, the ubiquitous domestic mites of Bt remain as the predominant species. To date, however, there is only limited information regarding the sensitization profiles of the Filipino population to Bt allergens based on IgE reactivity. This paper reports the sensitization profiles of Filipino subjects from Ilocos Norte to Bt and its major allergen rBlo t 5.

Table 2.

Point-biserial correlation between Bt-specific IgE and the various demographics of cases and controls

Dependent variable: Bt-specific IgE concentration (n = 247)	Coefficient	*P* value	OR (95% CI)
Cesarean mode of delivery*	0.030	0.652†	1.904 (0.826–4.389)
Family history of atopic disease‡	0.171	0.031§	2.250 (1.105–4.581)
Smoker¶	0.090	0.162†	4.411 (0.997–19.514)
Exposure to secondhand smoke‖	0.015	0.824†	0.874 (0.482–1.583)
Exposure to household pets**	0.002	0.975†	0.795 (0.375–1.685)

As a general rule in interpreting the correlation coefficient: 0–0.25, weak relationship; 0.26–0.5, moderately weak relationship; 0.51–0.75, moderately strong relationship; 0.76–1, strong relationship [15]. General rule in interpreting odds ratio (OR): OR = 1, exposure does not affect odds of outcome; OR > 1, exposure associated with higher odds of outcome; OR < 1, exposure associated with lower odds of outcome [16].

*Cesarean mode of delivery: yes = 1; no = 0.

†Not significant at $P = 0.05$.

‡Family history of atopic diseases: with family history = 1; without family history = 0.

§Statistically significant at $P = 0.05$.

¶Smoker: yes = 1; no = 0.

‖Exposure to secondhand smoke: yes = 1; no = 0.

**Exposure to household pets: with pets = 1; without pets = 0.

In the present work, Bt is shown to be responsible for the sensitization of the majority of allergic Filipino individuals from Ilocos Norte. Results of this study have lower sensitization compared to an earlier study conducted among local allergic patients in Metro Manila, demonstrating an 89% sensitization to Bt [18]. A similar study investigated the sensitization of selected Filipino allergic subjects from inside and outside Metro Manila and has shown that about 91% are reported to be sensitized to Bt allergens [19]. This study confirms that Bt is still of great concern in rural populations in the Philippines other than those in highly urbanized areas such as Metro Manila, although demonstrating lower sensitization. One possible mechanism is the increase in allergen production via urbanization, changing lifestyle, and climate change [20]. The rise in temperature due to climate change has modified human lifestyles to spend more time indoors, which in turn increases their exposure to HDMs. The use of air conditioning systems in urbanized areas contributes to the higher frequency of sensitization to susceptible individuals since humidity is an important determinant of HDM growth and survival [21].

The onset of atopic diseases generally begins early in life and steadily decreases with advancing age [22]. Interestingly, results presented herein are in agreement with previous epidemiological observations regarding pediatric patients (21 age groups) [23]. In an epidemiological study that analyzed the incidence of asthma from childhood to late adulthood, the occurrence of AA was highest in early childhood [24]. The risk of developing respiratory allergy such as asthma and rhinoconjunctivitis during late childhood was higher among children who had persistent allergic dermatitis (persist after age 2) [25]. Early onset of eczema was also revealed to be associated with increased risk of sensitization to inhalant allergens [26]. Meanwhile, conflicting evidence exists with respect to gender in the prevalence of allergic diseases among children and adults. Contrary to our results, one study that focused on the sensitization of different allergens among children by age and sex have revealed that males are more sensitive to several aeroallergens than females [27]. Asthma and rhinoconjunctivitis appear to predominate in males during their early childhood but this gender preferences reverses over time due to the influence of sexual hormones, adherence to treatment, and different lifestyles adopted by men and women as they age among others, leading to the predominance of allergic diseases in females [28-30].

AA, AR, and AD have been implicated as the most prevalent chronic immunological diseases worldwide [31, 32]. A subgroup of people exhibiting MAs has also been identified within a group of atopic individuals. AA, AR, and AD were found to be linked with one another as they share a large number of disease-associated proteins [33]. In a study that evaluated multimorbidity among the Indian population allergic to HDMs, the prevalence of AA in concomitant with AR was found to be 65.24% and was more frequent in males (56%) than in females (44%), which is in contrast with our results. The same paper also reported a strong association among AA-AR coexistence and presence of family history of atopy, presence of pets and animals at home, and passive smoking [34]. A cross-sectional study conducted in a Polish cohort has found that allergic multimorbidity was strongly associated with polysensitization to cat and mite allergens [35]. Hence, potential sensitization to specific allergens other than Bt may be a risk factor in the prevalence of MA since it is worth noting that 42.11% (72/171) of allergic cases were not sensitized to Bt. Genetic predisposition may increase the risk of developing MA and may also be a predictive factor in Bt sensitization [15, 36]. And since a family generally stays in one specific place, the correlation of family history and Bt-specific IgE can also be attributed to the environmental factors that the family is exposed to [15, 20].

Accumulating evidence has suggested that genetic, developmental, and environmental factors interlinked through IgE-associated and non–IgE-associated mechanisms influence the risk of allergic sensitization [37]. At present, only a few studies have investigated the correlation between Bt-specific IgE and other risk factors such as cesarean mode of delivery, household pets, cigarette smoking, and exposure to secondhand smoke [38-40]. While these may be considered by other authors as an important factor in the development and progression of allergic disorders, results of this study showed that there was no significant correlation between these factors and Bt sensitization [15, 41]. The role of birth practices in the incidence of allergic diseases can be stemmed from the 'hygiene hypothesis' by Strachan, an epidemiologist who proposed that overly clean environments may contribute to the development of several diseases. Reduced exposure to vaginal microflora during cesarean mode of delivery could therefore increase the likelihood of having an allergic disease due to dysbiosis of gut microbiota [42]. As the concept was further explored in allergy and immunology, the role of household pets and tobacco smoking has also been investigated. It was deduced that exposure to domestic pets early in life may prevent the development of atopic diseases whereas tobacco smoke was found to have immunosuppressant effects, increasing the risk of allergy development by suppressing T-cell activity and promoting Th2-cell response, thus triggering IgE production [43, 44]. Another study also confirmed the positive association between exposure to smoke and allergic diseases, namely AD and AR, as well as allergic multimorbidity [45]. We, therefore, recommend the investigation of the correlation between Bt-specific IgE and the risk factors aforementioned above in future studies.

Recombinant DNA technology has been used over the years to isolate and produce purified Bt allergens sustainably [46]. The 14 kDa rBlo t 5, the first major allergen isolated from Bt, has been linked to the development of allergic diseases [47]. Though its biological function remains unknown, studies have reported sensitization of Bt allergic patients to rBlo t 5. Among the 45 Bt-positive allergic cases in this study, 36 (80%) were also sensitized to rBlo t 5 allergen compared to 9 (23.4%) Bt-negative cases that were not sensitized to rBlo t 5. In another

paper, 40% to 70% of Bt-sensitized patients were sensitized to rBlo t 5. The current study validates the importance of rBlo t 5 as a major allergen in Bt. Our study showed high inhibition capacity of rBlo t 5 against the IgE reactivity of the Bt extract, with an inhibition capacity ranging from 37% to 76% and an average of 54% inhibition. Results presented herein are a great improvement from the percent inhibition ranging from 5.77% to 38.30%, averaging 21.05% in a previous study [48]. Due to rBlo t 5 sensitizing the majority of Bt-positive allergic cases along with its high reactivity with the Bt allergen extract, rBlo t 5 can be established as a major allergen in the Filipino population from Ilocos Norte.

This study provided an extensive analysis on the allergenicity of Bt among Filipino allergic patients. However, even though this study provided a substantial analysis, there are several limitations. First, the secondary screening process of total IgE levels having <100 IU/mL or ≥100 IU/mL was not conventionally employed to discriminate allergic cases from nonallergic controls. Furthermore, an exclusion criterion that excludes patients who have taken antihistamines, corticosteroids, anti-inflammatory drugs, or immunosuppressive treatments may remove a considerable number of clinical cases with respiratory allergies, as well as those with more severe illness. Moreover, as this study only focuses on allergy symptoms manifestations in patients with allergic asthma, allergic rhinitis, and atopic dermatitis, this study lacks on elucidating disease severity, sensitization distribution or patterns across the different diseases, and even comparisons with different HDM species.

In conclusion, the results of this study revealed that Bt is a significant allergen source that sensitizes allergic Filipino subjects from Ilocos Norte. The study likewise showed that family history of allergic diseases could serve as a predictive factor in elevated Bt-specific IgE of allergic cases. Furthermore, we validate the clinical significance of rBlo t 5 as a major allergen in Bt that causes allergic sensitization among atopic individuals. Thus, this study strongly recommends the inclusion of Bt allergen extract and rBlo t 5 in the panel of reagents for the diagnosis and immunotherapy of HDM allergies in the Filipino population.

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Conflicts of interest

The authors have no financial conflicts of interest.

Author contributions

John Donnie A. Ramos and Maureen B. Sabit designed and supervised the study. Chanie Y. Patanindagat and Jamie Ezra B. Tarun revised the study and the manuscript. Chanie Y. Patanindagat, Jamie Ezra B. Tarun, Ryla Jasmine T. Pajaro, Jhon Jerald D. Pintucan and Patricia Nichole M. Quilang performed the experimental research, data analysis, and manuscript preparation. All authors approved the final manuscript.

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