

**CROSS-REACTIVITY OF COCKROACHES TO HOUSE DUST
MITES AND SEAFOOD IN ALLERGIC RHINITIS PATIENTS IN
HOSPITAL UNIVERSITI SAINS MALAYSIA**

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ABSTRAK (BAHASA MALAYSIA)

Pengenalan: ‘Allergic rhinitis’ (AR) merupakan penyakit yang biasa dihadapi di seluruh dunia, disebabkan oleh tindak balas hipersensitiviti jenis 1, yang memberi implikasi terhadap kualiti hidup dan beban ekonomi. Lipas merupakan aeroalergen yang penting dan menunjukkan kereaktifan silang (KS) dengan aeroalergen yang lain dan juga alergen makanan, disamping menyumbang kepada keterukan AR.

Objektif: Untuk menentukan prevalen pesakit AR yang mempunyai alahan kepada lipas, KS lipas terhadap hama habuk rumah (HHR) dan makanan laut (ML), dan membuat perbandingan keterukan AR dikalangan pesakit yang mempunyai alahan kepada lipas.

Kaedah: Ini adalah kajian keratan rentas yang melibatkan 110 subjek dewasa yang mempunyai penyakit AR. Ujian ‘skin prick’, dan seterusnya ujian spesifik imunoglobulin E dilakukan dengan menggunakan kaedah ‘direct enzyme-linked immunosorbent assay (ELISA)’, manakala ujian KS dilakukan dengan menggunakan kaedah ‘indirect inhibition ELISA’.

Keputusan: Majoriti adalah Melayu (95.5%), perempuan (60.9%) dengan median umur 36.8 tahun. Melalui ujian ‘skin prick’, HHR (93.6%) mencatatkan prevalen tertinggi, diikuti lipas (51.8%) dan ML (40%). Peratusan yang lebih rendah dicatatkan melalui ujian ‘direct ELISA’, tetapi menunjukkan taburan peratusan yang lebih kurang sama (masing-masing 64.5%, 41.8% dan 19.1%). KS antara lipas dan HHR adalah tertinggi, diikuti oleh lipas berlainan spesies dan lipas dengan ML. Alahan terhadap lipas menunjukkan gejala AR yang lebih teruk ($p < 0.05$). tetapi tiada perbezaan apabila perbandingan dilakukan diantara spesies lipas yang berlainan ($p < 0.05$).

Kesimpulan: Lipas adalah sejenis aeroalergen yang penting dan menyumbang kepada keterukan AR dan mempunyai KS yang tinggi dengan HHR, tetapi lebih rendah KS dengan alergen makan (ML) di dalam kajian keratan rentas kami.

ABSTRACT (ENGLISH)

Background: Allergic rhinitis (AR) is a common disease globally, mediated by type 1 immediate hypersensitivity response, with implication on quality of life and economic burden. Cockroach is an important aeroallergen that shows cross-reactivity with other inhalant and food allergens and contributes to the severity of AR.

Objective: This study aimed to determine the prevalence of cockroach sensitization in AR patients, its cross-reactivity with house dust mite (HDM) and seafood, and to compare the severity of AR in response to cockroaches.

Methods: This is a cross sectional study with involvement of 110 adult AR subjects. Skin prick test (SPT), and subsequently specific immunoglobulin E test were performed using direct enzyme-linked immunosorbent assay (ELISA) and cross-reactivity profile was obtained using indirect inhibition ELISA.

Results: Majority of patients were Malay (95.5%), female (60.9%) with median age of 36.8 years. On SPT, HDM (93.6%) showed highest prevalence, followed by cockroach (51.8%) and seafood (40%). Lower percentage seen on direct ELISA but shared similar pattern of distribution (64.5%, 41.8% and 19.1% respectively). Cross-reactivity between cockroach and HDM was the most common, followed by between different species of cockroach and the least was between cockroach and seafood. Sensitization to cockroach demonstrated significant association with increase severity of AR ($p < 0.05$), however no difference in severity comparing the two different types of cockroaches ($p > 0.05$).

Conclusion: Cockroach is an important aeroallergen with major contribution to severity of AR and has high cross-reactivity with HDM but lower cross-reactivity with food allergen (seafood) in our cross-sectional population.

Chapter 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

Allergic rhinitis (AR) is defined as inflammation of nasal mucosa caused by exposure to inhaled allergens leading to immunoglobulin E (IgE) mediated reaction. It is a disease of type 1 immediate hypersensitivity and among the most common disease globally. The prevalence of self-reported AR has been estimated 2% to 25% in children and 1% to greater than 40% in adults (1). The prevalence of confirmed AR in adults in Europe ranged from 17% to 28.5% and usually persists throughout life (1). The global prevalence is almost the same with this region according to a study done among school children in Kelantan, Malaysia which showed prevalence of 27% (2).

AR is characterised into four main categories, namely mild intermittent, mild persistent, moderate/ severe intermittent and moderate/ severe persistent based on duration and frequency as well as severity of symptoms affecting daily activity (1). A study amongst patients newly diagnosed with AR in Malaysia revealed 10% and 21.1% had mild and moderate/ severe intermittent AR respectively, while 20% and 48.9% patients had mild and moderate/ severe persistent AR respectively (3). According to this data, the majority of people belong to the moderate/severe persistent group, which has a substantial impact on their quality of life. AR has been shown to cause significant impairments in sleep, performance at work and school, and social interactions (4). In children, sleep disturbance and fatigue directly impact their learning abilities, and have also been implicated in behavioural disturbances (5). The economic burden is also high, not just from the costs involved in treatment of AR but indirect costs due to absenteeism from school and work and decreased productivity of patients with AR (6).

Most common allergen is from the group of indoor aeroallergen and cockroach being the second most common identified allergen after house dust mite (HDM) (7, 8). There are more than 3500 cockroach species but not many of them live in human dwelling (9). These include *Periplaneta americana* (PA), *Blattella germanica* (BG), *Blatta orientalis*, *Supella longipalpa* and *Periplaneta fulliginosa*. World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature database reports only allergens from German (BG) and American (PA) cockroaches have been approved for systematic allergen nomenclature (10). The German cockroach is light brown in colour, about 16mm length and more common in cool, dry climates, like Europe and United States (9). The American cockroach prefers hot and humid conditions, like Brazil, Taiwan and Thailand, brownish red colour and much bigger with length about 38mm (9). In 2004, Tungtrongchitr *et al.* made an intensive survey of cockroach species in human dwellings in Bangkok and found PA is the most common (72.15%) (11). Cockroach allergens are antigenic proteins, can be from any anatomical part, body fluid, dead body debris and developmental stage of the insect (9). The allergenic particles are found on the surfaces like floors, lamps or tables, but easily disturbed and disseminated by wind.

Specific allergen sensitisation can be detected either using *in vivo* test, such as skin prick test (SPT) or *in vitro* test, by measuring allergen specific IgE using sensitive immunoassays such as radioimmunoassay, enzyme immunoassay or fluorescent immunoassay. Many studies comparing their sensitivity and specificity which showed variable in results and recommendations which may complement each other. Historically, Bernton and Brown confirmed the existence of cockroach allergy by SPT 56 years ago (12). Positive SPT to more than one allergen or high IgE titres particularly

to cockroaches, house dust mites and seafood could be due to co-sensitization or cross-reactivity of these allergens. Sun *et al.* reported a very high degree of IgE-mediated cross-reactivity between the cockroach allergens (61%) and also between cockroach and HDM in 22% of the sera (13). In shrimp sensitized rural children in Southern China, it was found that cockroach being a source of major cross-reactivity allergen (14).

Cross-reactivity is an IgE immune mediated phenomenon in which IgE antibody induces immune response to similar allergenic molecules as they share epitope specific antibody. Thus, this cross-reaction is usually occurred between closely related species or belonged to same protein family (14,15). For example, tropomyosin is believed to be responsible for cross-reactivity between cockroach, HDM and shrimp due to its high sequence homology (14,15). Exposure, host immune response and allergen itself are among key factors for its clinical relevance (16). Co-sensitisation is different from cross-reactivity and it simply mean a person sensitized to more than one allergen, not mediated by shared epitope specific antibody. Cross-reactivity plays important role in diagnosis as well as management especially in patient with initial allergic test showed positive result to multi-allergens and those who may require immunotherapy.

Sensitizations to different types of cockroach allergens are not well studied in Malaysia especially in AR patients. The most prevalent cockroach in Malaysia is still an open question due to inadequate data. Back to 1998, Sam *et al.* identified cockroach being the second most common allergen, 44% in Malaysians diagnosed with asthma, using SPT (17). Another study in 2002 also using SPT showed cockroach being an

important aeroallergen in asthmatic patients with rhinitis (8). Both studies only involved one type of cockroach which is PA. Due to lack of this data in our country, the severity of allergic symptoms in response to different type of cockroaches and sIgE cross-reactivity of cockroaches to other allergens particularly to HDM and seafood cannot be determined.

Our study was conducted with the aimed to determine the prevalence of cockroach sensitization in AR patients, its sIgE cross-reactivity with HDM and seafood, and its contribution to severity of AR.

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Chapter 2

OBJECTIVES OF THE STUDY

CHAPTER 2: OBJECTIVES OF THE STUDY

2.1 GENERAL OBJECTIVES

To determine the cross reactivity of cockroaches to house dust mites and seafood in allergic rhinitis (AR) patients in Hospital Universiti Sains Malaysia (HUSM)

2.2 SPECIFIC OBJECTIVES

The specific objectives for this study are as the following:

- i) To determine prevalence of sensitization to two different types cockroaches in patients with AR
- ii) To determine immunoglobulin E cross reactivity between cockroach allergens and house dust mite and seafood allergens
- iii) To compare the severity of AR in response to two different types of cockroaches

Chapter 3

MANUSCRIPT

3.1 TITLE PAGE

Specific IgE Cross-Reactivity of Cockroaches to House Dust Mites and Seafood in Allergic Rhinitis Patients in a Cross-Sectional Study in a Tropical Tertiary Setting (Hospital Universiti Sains Malaysia)

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3.2 ABSTRACT

Background: Allergic rhinitis (AR) is a common disease globally, mediated by immunoglobulin E (IgE) or type 1 immediate hypersensitivity in response to inhaled allergens, with implication on quality of life and economic burden. Cockroach is an important aeroallergen that shows specific IgE (sIgE) cross-reactivity with other inhalant and food allergens and contributes to the severity of AR.

Objective: This study is aimed to determine the prevalence of cockroach sensitization in AR patients, its sIgE cross-reactivity with house dust mite (HDM) and seafood, and its contribution to severity of AR.

Methods: This is a cross sectional study which include a total of 110 adult AR subjects. Skin prick test (SPT) was performed, and all patients were positive to at least one out of eight allergens [*Periplaneta americana* (PA), *Blattella germanica* (BG), *Dermatophagoides pteronyssinus* (DP), *Dermatophagoides farinae* (DF), *Blomia tropicalis* (BT), shrimp (*Litopenaeus/ Farfantepenaeus*), crab (*Callinectes* spp.) and squid (*Loligo* spp.)] tested. Subsequently specific immunoglobulin E (sIgE) test was performed using direct enzyme-linked immunosorbent assay (ELISA) method and sIgE cross-reactivity profile was obtained using indirect inhibition ELISA method. The results of the direct ELISA were also compared with ImmunoCAP sIgE tests.

Results: Majority of patients were Malay (95.5%), female (60.9%) with median age of 36.8 years. HDM [leaded by BT (90%) followed by DP (50.9%) and DF (49.1%)] was the highest prevalence on SPT, followed by cockroach (PA and BG presented the same prevalence, 39.1%) and seafood [shrimp (30.9%), crab (25.5%) and squid (24.5%)]. sIgE test showed lower percentage but shared almost similar pattern of distribution

(55.5%, 49.1%, 45.5%, 28.2%, 31.8%, 12.7%, 9.1%, and 8.2% respectively). There was strong concordance between the sIgE measured by our direct ELISA method and the ImmunoCAP sIgE test results. The most common sIgE cross-reactivity was between cockroach and HDM (predominantly DP), followed by between different species of cockroach and the least was between cockroach and seafood. Sensitization to cockroach demonstrated significant association to increase severity of AR ($p < 0.05$).

Conclusion: Cockroach is an important aeroallergen with major contribution to severity of AR and has high sIgE cross-reactivity with HDM but lower sIgE cross-reactivity with food allergen (seafood) in our cross-sectional population.

Keywords: Aeroallergen, allergic rhinitis, cockroach, house dust mite, food allergen, skin prick test, enzyme-linked immunosorbent assay, specific immunoglobulin E cross-reactivity

3.3 INTRODUCTION

Allergic rhinitis (AR) is defined as inflammation of nasal mucosa caused by exposure to inhaled allergens leading to immunoglobulin E (IgE) mediated reaction. It is a disease of type 1 immediate hypersensitivity and among the most common disease globally. The prevalence of self-reported AR has been estimated 2% to 25% in children and 1% to greater than 40% in adults (1). The prevalence of confirmed AR in adults in Europe ranged from 17% to 28.5% and usually persists throughout life (1). The global prevalence is almost the same with this region according to a study done among school children in Kelantan, Malaysia which showed prevalence of 27% (2).

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Most common allergen is from the group of indoor aeroallergen and cockroach being the second most common identified allergen after house dust mite (HDM) (7, 8).

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Specific allergen sensitisation can be detected either using *in vivo* test, such as skin prick test (SPT) or *in vitro* test, by measuring allergen specific IgE using sensitive immunoassays such as radioimmunoassay, enzyme immunoassay or fluorescent immunoassay. Many studies comparing their sensitivity and specificity which showed variable in results and recommendations which may complement each other. Historically, Bernton and Brown confirmed the existence of cockroach allergy by SPT 56 years ago (12). Positive SPT to more than one allergen or high IgE titres particularly to cockroaches, house dust mites and seafood could be due to co-sensitization or cross-reactivity of these allergens. Sun *et al.* reported a very high degree of IgE-mediated

cross-reactivity between the cockroach allergens (61%) and also between cockroach and HDM in 22% of the sera (13). In shrimp sensitized rural children in Southern China, it was found that cockroach being a source of major cross-reactivity allergen (14).

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the severity of allergic symptoms in response to different type of cockroaches and sIgE cross-reactivity of cockroaches to other allergens particularly to HDM and seafood cannot be determined.

Our study was conducted with the aimed to determine the prevalence of cockroach sensitization in AR patients, its sIgE cross-reactivity with HDM and seafood, and its contribution to severity of AR.

3.4 MATERIAL AND METHODS

Ethical approval

The study involved recruitment of human subjects. The ethical approval was reviewed and approved by the Human Research Ethics Committee of Universiti Sains Malaysia (study protocol code USM/JEPeM/19120925). The patients/participants had provided their written informed consent prior to participation in this study.

Sample size calculation

Sample size calculation was determined by using sample size calculator Statulator^{beta} (<http://statulator.com/SampleSize/ss1P.html>) for single sample proportion based on previous literatures by Sun *et al.* (13) and Chiou *et al.* (18), for specific objective I (to determine prevalence of sensitization to two different types cockroaches in patients with AR) and II (to determine sIgE cross-reactivity between cockroach allergens and HDM and seafood allergens). Level of confidence (Z) was set at 95% (5% error) for both objectives, while precision of study (Δ) was set at 0.09 and 0.1 for objective I and II respectively. No sample size was calculated for objective III (to compare the severity of AR in response to two different types of cockroaches) as no similar study was performed before. A 20% drop out was added to the largest sample size calculated making it 110 subjects in total.

Subjects

A cross sectional study was conducted with a total of 110 subjects age more than 18 years old. The subjects were recruited from the Otorhinolaryngology – Head and Neck Surgery (ORL-HNS) clinic, Hospital Universiti Sains Malaysia (HUSM), Malaysia. The diagnosis of AR was based on typical allergic symptoms which consist of two or more symptoms of either nasal discharge, nasal blockage, sneezing or nasal itchiness that last more than one hour on most days and confirmed by positive SPT. This was based on Allergic Rhinitis and its Impact on Asthma (ARIA) criteria 2001 (19). Pregnant and breastfeeding women, patient with dermographism, generalised skin disorder, history of nasal surgery, autoimmune disease, immunocompromised or immunosuppressed such as Human Immunodeficiency Virus (HIV) positive patients and diabetes mellitus and patients who consumed steroid or anti-allergy agents within 7 days or less were excluded from the study.

A standardised questionnaire comprised of demographic characteristics (e.g. age, gender, ethnicity, occupation, area of residence and smoking history), nasal symptoms (including duration and severity) based on ARIA guideline, severity of symptoms based on visual analogue scale (VAS), sino-nasal outcome test (SNOTT 22) and total nasal symptom score (TNSS), other relevant medical, allergic and family histories, history of nasal surgery, pet at home, drug history (including compliance and response) and nasal examination (including nasoendoscopy) findings were obtained from all subjects. Symptom severity score was classified into mild, moderate and severe as follows; VAS: mild (score 1-3), moderate (score 4-7), severe (score 8-10); SNOTT 22: mild (score 8-20), moderate (score 21-50), severe (score 51-110); TNSS: mild (score 1-5), moderate (score 7-9), severe (score 10-12).

All participants were subjected to general otorhinolaryngological examination, which included nasoendoscopy using a rigid zero endoscope to look for signs that suggestive of AR such as allergic shiner, Dennie-Morgan line, transverse nasal crease, allergic salute, watery nasal discharge, pale/ bluish boggy inferior turbinate, adenoid hypertrophy, cobble stone pharynx and nasal polyp. The history and examination lasted about 30 minutes.

Skin Prick Test (SPT)

SPT was performed to all subjects after confirmation they were free from systemic corticosteroid or anti-allergy agents for at least one week prior to the test day. The total of 10 allergens were included in this test namely PA, BG, *Dermatophagoides pteronyssinus* (DP), *Dermatophagoides farina* (DF), *Blomia tropicalis* (BT), shrimp, crab, squid, histamine dihydrochloride 0.1% (as positive control) and saline (as negative control). These three types of seafood (shrimp, crab and squid) were chosen as local data showed these were the most prevalence seafood allergens causing allergy in Malaysia (20).

Numbered strip with 2 cm apart were applied onto forearm and sites of skin prick were cleaned using alcohol swab (70% isopropyl alcohol). A drop of each allergen was placed on the forearm skin next to the numbered strip. A sterile lancet used to prick the skin gently through the drop of allergen and changed for every allergen. Excess allergen was then wiped off using sterile absorbent gauze. The subject was reminded not to scratch the site of examination, and the test was read after 15 min. Diameter of the wheal of at least 3 mm or larger than negative control was considered

positive. Subjects with positive SPT were considered confirm to have AR and were included in this study.

Direct enzyme-linked immunosorbent assay (ELISA)

Direct ELISA was used to assess specific immunoglobulin E (sIgE) antibody reactivity to cockroach, HDM and seafood allergens. Three ml of venous blood was withdrawn from the subject and collected in the serum separating tube. The blood sample was kept in the ice box and transported to the laboratory within 2 hours. The blood sample was centrifuged immediately at 2500rpm for 10 minutes using Hettich Zentrifugen Centrifuge Universal 32R machine and serum was collected in the microtube 1.5ml and stored at -20°C.

Extracted allergens namely PA, BG, DP, DF, BT, shrimp (*Litopenaeus/Farfantepenaeus*), crab (*Callinectes* spp.) and squid (*Loligo* spp.) were obtained from Greer Laboratories, Inc. North Carolina, United States.

96-well ELISA plates (Wuxi Nest Biotechnology Co., LTD, Jiangsu, China) were coated overnight at 4°C for 16 to 18 hours with different individual antigens (100 µl/well of antigen at 1 µg/ml) diluted with 1x phosphate buffered saline (PBS). After washing with 1x PBS + 0.05% Tween-20 (Sigma-Aldrich, Missouri, United States) (PBS-T) for 3 times to remove unbound antigen, the plates were blocked with 100 µl/well 1% bovine serum albumin (BSA) (Sigma-Aldrich, Missouri, United States) prepared in 1x PBS for 1 hour at room temperature (RT). The plates were subsequently washed with PBS-T and incubated with 100 µl/well subject's sera diluted at 1:5 in 1x PBS for 2 hours at RT. 1:5 dilution was used based on the optimisation with 1:5, 1:50

and 1:500 dilution, in which 1:5 dilution showed the best detection result consistently. The plates were washed again with PBS-T and incubated with 100 µl/well polyclonal goat anti-human IgE antibody (Bio-Rad Laboratories, Inc., California, United States) diluted at 1:1000 in 1x PBS for 2 hours at RT. 1:1000 dilution was used based on the optimisation with 1:1000 and 1:10000, in which 1:1000 dilution showed better detection result consistently. After washing, the plates were incubated with 100 µl/well Avidin – Horseradish Peroxidase (HRP) (Sigma-Aldrich, Missouri, United States) for 30 minutes at RT. Then, the plates were washed again and developed with 100 µl/well of 3,3',5,5'-tetramethylbenzidine (TMB) substrate reagent (Sigma-Aldrich, Missouri, United States) and incubated for another 30 minutes at RT. Finally, the reaction was stopped with 100µl of 2M sulfuric acid (H₂SO₄), and the absorbance was measured at the wavelength of 450 nm using Thermo Scientific Varioskan Flash microplate reader. To evaluate the specificity of the test, negative controls were used in each plate including: no antigen immobilised on the plate, no serum (primary antibody) applied and no anti-IgE (secondary antibody) added. All samples were assayed in triplicate and the mean value was used for analysis. The ELISA results were presented as optical density (OD) in optical units (OU). The mean absorbance level, plus 2 standard deviations of the negative control [15 AR subjects that SPT negative to all allergens and only weakly positive (wheal ≤4mm) to BT], was used as the cut-off value for all allergens except BT. For BT, the cut off value was based on mean absorbance level, plus 2 standard deviations of 4 AR subjects that SPT negative to HDM allergens (DP, DF, BT). The calculation technique was modified based on the methods described by Lee *et al.* (21) and Sookrung *et al.* (22).

The results of this direct ELISA were subsequently compared with ImmunoCAP specific IgE test (Phadia 100, Thermo Fisher Scientific, MI, USA). Fifty serum samples based on the availability of the remaining volume were used in this test according to the manufacturer's instructions. Specific IgE values ≥ 0.35 kU/L to different allergens was considered positive. A total of 91.3% of samples showed concordant result and strong correlation coefficient, $R^2 = 0.65$ (data not shown). Almost all of the discordant samples (6 out of 7 samples) were from seafood allergens, which could be due to less standardization of these food allergens as compared to the well characterized and standardized cockroach and HDM inhalant allergens.

Indirect Inhibition ELISA

Indirect Inhibition ELISA was used to determine sIgE cross-reactivity between cockroach allergens and HDM and seafood allergens.

96-well ELISA plates (Wuxi Nest Biotechnology Co., LTD, Jiangsu, China) were coated overnight at 4°C for 16 to 18 hours with different cockroach antigens (100 μ l/well of antigen at 1ng/ μ l) diluted with 1x PBS. After washed with PBS-T for 3 times, the plates were blocked with 100 μ l/well 1% BSA + 1x PBS for 1 hour at room temperature (RT). The plates were subsequently washed with PBS-T. Subject's sera (1:5 dilution with 1x PBS) were pre-absorbed with 2 μ g/ml of antigens (diluted in 1x PBS) overnight at 4°C for 14 to 16 hours. The antigen concentration at 2 μ g/ml was used as it showed maximum inhibition rate (plateau) during optimisation with serial two-fold dilution (0.015625, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4 and 8 ng/ μ l). The samples were then loaded on the prepared plates (100 μ l/well) and incubated for 2

hours at RT. The plates were washed again with PBS-T and incubated with 100 µl/well polyclonal goat anti-human IgE antibody diluted at 1:1000 in 1x PBS for 2 hours at RT. After washing, the plates were incubated with 100 µl/well Avidin –HRP for 30 minutes at RT. Then, the plates were washed again and developed with 100 µl/well of TMB substrate reagent and incubated for another 30 minutes at RT. Finally, the reaction was stopped using 100µl of 2M H₂SO₄ and the absorbance measured at a wavelength of 450 nm using Thermo Scientific Varioskan Flash microplate reader. To evaluate the specificity of the test, negative controls were used in each plate including: no antigen immobilised on the plate, no serum pre-absorbed with antigen (primary antibody) applied and no anti-IgE (secondary antibody) added. All samples were assayed in triplicate and the mean value was used for analysis. The ELISA results were presented as OD in optical units (OU). The percentage of inhibition was calculated using formula as follow: $[1 - (\text{absorbance with inhibitor} / \text{absorbance without inhibitor})] \times 100$.

Statistical analysis

The statistical calculation and evaluation were performed with IBM SPSS version 24.0 (IBM, Armonk, NY, USA). The data analysis was derived descriptively, and the inferential statistics mainly used *Pearson chi square test* and *Fisher Exact test*. A p value of < 0.05 is considered significant.

3.5 RESULTS

Demographic characteristics of AR participants are presented in Table 1. A total of 110 participants (43 males and 67 females) were recruited for this study. The data obtained was expressed as mean with standard deviation for numerical variables. The categorical variables were expressed as frequency (n) and percentage (%). The mean age of the subjects was 36.8 years with standard deviation (SD) of 14.7. Majority of the subjects were Malay (n=105, 95.5%), corresponded to the most common ethnic group in our study area, followed by Chinese (n=4, 3.6%), and Siamese (n=1, 0.9%). All subjects were those who attended our clinics or visited HUSM. Predominantly, they are categorized into mild intermittent (n=39, 35.5%), followed by moderate/ severe persistent (n=30, 27.3%), mild persistent (n=28, 25.5%) and moderate/ severe intermittent AR (n=13, 11.8%). The participants involved wide range of occupations with student being the most common group (n=25, 22.7%), followed by housewife (n=19, 17.3%), teacher (n=13, 11.8%), and others.

Number of subjects with SPT and direct ELISA positive toward different types of allergens are illustrated in Figure 1. SPT and direct ELISA results showed high number of subjects were allergic to cockroaches, n=57 (51.8%) and n=46 (41.8%) respectively (Table 2). Most of the subjects were found to be positive to both cockroaches (n=29, 26.4% on SPT, n=20, 18.2% on direct ELISA) compared to individual cockroach (refer supplement). Individually, PA and BG were equally positive on SPT (n=14, 12.7%), while the former slightly lower on direct ELISA (n=11, 10% and n=15, 13.6% respectively)

In total, 103 (93.6%) of subjects were positive to HDM on SPT, predominantly BT (n=99, 90%) followed by DP (n=56, 50.9%) and DF (n=54, 49.1%) (Figure 1 and

Table 2). The percentage was lower on direct ELISA but shared similar pattern of distribution. A total of 71 (64.5%) subjects were positive to HDM on direct ELISA, with BT being the highest (n=61, 55.5%), followed by DP (n=54, 49.1%) and DF (n=50, 45.5%). For seafood, 44 (40%) and 21 (19.1%) subjects were positive on SPT and direct ELISA respectively; with shrimp being the most common (n= 34, 30.9% on SPT; n=14, 12.7% on direct ELISA), followed by crab (n=28, 25.5% on SPT; n=10, 9.1% on direct ELISA) and squid (n=27, 24.5% on SPT; n=9, 8.2% on direct ELISA).

Co-sensitisation and/ or cross-reactivity of both cockroach allergens with other allergens were higher on SPT compared to direct ELISA (Table 3). Both cockroaches showed higher co-sensitization and/or cross-reactivity with HDM compared to seafood. SPT results showed patients sensitised to PA also had high positive result toward BT (90.7%), DP (76.7%), DF (60.5%), shrimp (51.2%), crab (39.5%) and squid (39.5%). This phenomenon also seen in BG sensitised patients (88.4%, 69.8%, 62.8%, 48.8%, 44.2% and 39.5% respectively). Although the percentage of concomitant positive result of PA on direct ELISA with other allergens was lower than SPT but showed almost similar distribution, 71%, 64.5%, 58.1%, 19.4%, 9.7% and 12.9% respectively. BG also shared the same pattern, 77.1%, 71.4%, 68.6%, 28.6%, 14.3% and 20.0% respectively.

Cross-reactivity profiles showed the highest sIgE cross-reactivity were detected between cockroaches and HDM, followed by between the different cockroach species and lastly between cockroaches and seafood, as shown in Table 4. BG had higher sIgE cross-reactivity with HDM [44.5% (49/110)] than PA [40.9% (45/110)]. DP was the most common HDM which cross-reactive with both cockroaches. Small number of subjects had equally sIgE cross-reactivity between both cockroaches and seafood,

[7.3% (8/110)]. There were more sIgE cross-reactivity detected when subject's sera pre-absorbed with BG (as inhibitor) were added to the coated PA plate [13.6% (15/110)], as compared to the reversed [6.4% (7/110)]. Among all co-sensitizations seen on direct ELISA, only PA and DP allergens showed cross-reactivity in all samples.

There was significant different in severity of AR symptoms when comparing group of subjects with and without sensitization to PA or BG, as illustrated in Table 5a and 5b. Subjects that were allergic to PA or BG demonstrated more severe symptoms in all severity scoring systems ($p < 0.05$). However, there was no significant different in AR severity score comparing both cockroach species ($p > 0.05$) (refer supplement). When comparing the negative and positive to either one or both cockroaches' groups, there was significant more severe AR symptoms in later group ($p < 0.05$) (Table 5c).