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Determining the Prevalence of Allergy to *Scylla olivacea* among Local Atopic Populations

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ABSTRACT

Crab allergy is one of the most prevalent food allergies that can trigger severe allergic reactions. Following the consumption of crab, reports of potentially lethal reactions, including anaphylactic shock, are prevalent. In numerous Asian countries and other nations to which mud crabs were brought, the species *Scylla olivacea* is commonly consumed. This study aimed to determine the prevalence of allergy to Scylla olivacea among local atopic populations using the skin prick test (SPT). Proteins were isolated from the flesh of the mud crab (*S. olivacea*). A total of 500 individuals, comprising 234 males and 266 females with atopic diseases, were examined. Among them, 112 individuals (22%) were sensitized to Mud crab (*S. olivacea*), 162 individuals (32%) were sensitized to *Portunus pelagicus*, and 106 individuals (21%) were sensitized to *Charybdis feriatus*. The study concluded that the prevalence of *S. olivacea* sensitization among people was 22%. In patients sensitized to *S. olivacea*, house dust mites elicited the highest incidence of positive skin prick test (SPT) reactivity at approximately 98%, followed by aeroallergens and other seafood allergens at 96%.

Keywords: allergy, mud crab, *Scylla olivacea*, 2-DE, immunoblotting, mass spectrometry

INTRODUCTION

Crab allergy is prevalent and can result in severe allergic reactions. Reactions, including anaphylactic shock, resulting in death have been documented following the intake of crab meals (Lopata et al., 2016). This study aimed to ascertain the prevalence of allergy to *S. olivacea* among local atopic populations by skin prick testing (SPT). Immediate hypersensitivity to food constitutes a significant global health issue. (Kamath et al., 2014).

Significant discrepancies between Asian and other communities were revealed by the food allergy data. The prevalence of crab allergy was reported to be higher among some Asian communities compared to American and European populations, as crab intake varies throughout these regions (Lopata et al., 2016; Azemi et al., 2021). In Malaysia, crab is regarded as the most prevalent food allergen among local patients with allergic rhinitis and asthma, with prevalence rates of 78% and 16%, respectively (Zailatul et al. 2015), as it constitutes a significant component of the local diet (Kim et al., 2017).

MATERIALS AND METHODS

Preparation of Mud Crab (*S. olivacea*)

The mud crab samples were collected from three location sites in Merbok River. Crabs were dissected completely and the whole body tissues removed and washed in deionized water. Based on a study by Rosmilah et al. (2013), the first step was a homogenization of 5g muscle tissue samples with 100 ml of phosphate buffer saline reagent (PBS, pH 7.2), then extact by using an incubator shaker at 4°C under continuous shaking (50 rpm) overnight. After that, the homogenates were then centrifuged at 14.000 rpm for 15 min at 4°C and supernatants then filtered using filter papers, then by using sterile syringe filters 0.22 µm. The extracts then

lyophilized (freezedrying) using a freeze dryer. The lyophilized extracts then were stored at -20°C til next use.

Individuals for Prevalence Analysis

The present study analyzed data from 500 patient cases with specific conditions reported to the Allergy Clinic at Hospital Kuala Lumpur from January 2015 to December 2018 to ascertain the prevalence of allergy to *S. olivacea*. The patients' allergy conditions were validated by the skin prick test (SPT).

Data Analysis

Demographic data, clinical history, allergy symptoms, and all skin prick test (SPT) results were documented. Frequencies and simple associations were computed using Fisher's exact test with version 23 of SPSS software. Results would be statistically significant if the P-value is less than 0.05.

RESULTS AND DISCUSSION

Demographic Data

This study investigated five hundred individuals with specific conditions referred to the Allergy Clinic at Hospital Kuala Lumpur from January 2015 to December 2018. A total of 500 individuals, comprising 234 males and 266 females, were researched about disease topics. Malays comprised the majority at 65%, while Indians, Chinese, and others contributed 16%, 15%, and 5%, respectively. The data indicated that sensitivity to *S. olivacea* was more common among Malays than among other ethnic groups. Nevertheless, this rationale remains ambiguous as no research on the racial distribution of allergy illnesses has been undertaken in Malaysia (Zailatul et al., 2015; Ateshan et al., 2020). The ages of all people ranged from 5 to 55 years or older, with a mean age of 34.7 years. Meanwhile, the majority of those sensitized to *S. olivacea* were aged between 5 and 17 years. Fewer persons allergic to *S. olivacea* were present in the age groups of 55 years and older.

Table 1 illustrated that females exhibit a higher sensitivity (53%) to *S. olivacea* than males, who show a sensitivity of 47%. Notably, our findings align with earlier research that similarly demonstrated a female majority in allergy investigations (Asha'ari et al., 2010; Ibekwe et al., 2016).

Table 1. Demographic features of the individuals

Demographic data		All individuals (n = 500) (n) %	Individuals sensitized to Mud crab (*S. olivacea*) (n = 112) (n) %
Gender	Male	(234) 47	(59) 53
	Female	(266) 53	(53) 47
Ethnicity	Malay	(320) 64	(73) 65
	Chinese	(76) 15	(24) 22
	Indian	(80) 16	(7) 6
	Others	(24) 5	(8) 7
Age	5 - 17	(185) 37	(32) 29
	18 - 24	(84) 17	(26) 23
	25 - 34	(82) 16	(22) 20
	35 - 44	(58) 12	(16) 14
	45 - 54	(62) 12	(9) 8
	≥ 55	(29) 6	(7) 6

Clinical History and Symptoms of Allergic Reactions

The clinical histories of the individuals are presented in Table 2. A significant proportion of the individuals have a history of allergic rhinitis (84%). In individuals sensitized to *S. olivacea*, 88% were diagnosed with allergic rhinitis, 22% with allergic conjunctivitis, and 45% had food allergies. Additionally, 19% were affected by asthma, 22% by contact dermatitis, and 9% by sinusitis. A patient with sinusitis exhibited sensitization to *S. olivacea.*

Table 2. Clinical history of the individuals

Clinical History	All individuals (n = 500) (n) %	Individuals sensitized to Mud crab (*S. olivacea*) (n = 112) (n) %
Rhinitis	(421) 84	(99) 88
Conjunctivitis	(110) 22	(25) 22
Eczema	(89) 18	(16) 14
Asthma	(97) 19	(21) 19
Sinusitis	(36) 7	(10) 9
Contact Dermatitis	(29) 6	(25) 22
Food allergy	(211) 42	(50) 45

Allergic rhinitis presents as the predominant clinical history among individuals. Allergic rhinitis has been reported to affect approximately 20% of the population across various regions (Zailatul et al., 2015; Ateshan et al., 2019; Ha et al., 2019). It may not pose a direct threat to life, yet it is associated with a decline in quality of life and work productivity, as well as heightened healthcare costs (Pefura-Yone et al. 2015). Allergic rhinitis can be triggered by various factors, including genetic predisposition, hormonal changes, smoking, obesity, lifestyle choices, and numerous environmental influences, such as exposure to inhalant allergens and air pollution (Pefura-Yone et al., 2015; Zhang et al., 2014).

A total of 500 individuals, 234 males and 266 females, with a topic diseases were studied, individuals were sensitized to Mud crab (*S. olivacea*) is 112 with precentage 22%, individuals were sensitized to *Portunus pelagicus* is 162 with precentage of 32% and individuals were sensitized to *Charybdis feriatus* is 106 with precentage of 21% showed in Table 4.12. Most of crab species, provoked an allergy reactions including the skin, respiratory, gastrointestinal and cardiovascular system (Lopsta & Lehrar 2009; Ateshan & Saxena 2015; Jasim et al., 2021).

Table 3. The prevalence of allergy to crab among individuals

Species	All individuals (n) % (n = 500)
(*Portunus pelagicus*) Blue crab	(162) 32
(*S. olivacea*) Mud crab	(112) 22
(*Charybdis feriatus*) Red crab	(106) 21

Skin Prick Test Reactivity

In this investigation, all participants underwent skin prick testing (SPT) using *S. olivacea* extract combined with various allergen extracts utilized in the typical allergy diagnostic panel at the clinic, encompassing other seafood, aeroallergens, additional food allergens, and house dust mites. The findings were documented in Table 4.

Table 4. Frequency of SPT positivity to other allergens tested among individuals

Allergen	All individuals (n) % (n = 500)	Individuals sensitized to Mud crab *(S. olivacea)* (n) % (n = 112)
House Dust Mites	(467) 93	(110) 98
Aeroallergens	(437) 87	(108) 96
Other seafood allergens	(417) 83	(108) 96
Portunus pelagicus	(162) 32	(88) 79
Charybdis feriatus	(106) 21	(77) 69

The majority of individuals exhibit SPT positive to sea-food and aero-allergens. House Dust Mites (HDM) were identified as the most significant allergens, eliciting strong sensitivity in 93% of participants, followed by aero-allergens at 87% and other seafood allergens at 83%. This finding is consistent with recent studies that revealed a higher prevalence of shellfish allergy, particularly to prawns and crabs, in Asia compared to Western countries. This may be influenced by the geographic intake of shellfish (Zailatul et al., 2015).

Among the *S. olivacea*-sensitized individuals, House Dust Mites triggered a highest frequency of positive SPT reactivity at 98%, followed other seafood allergens 96%, Aeroallergens 96%, *Portunus pelagicus* (79%) and *Charybdis feriatus* (69%). This was not surprising as *Portunus pelagicus* and *Charybdis feriatus* are grouped under same phylum (Amaral et al., 2018) and highly consumed by local people (Zailatul et al., 2015).

In this investigation, House-Dust Mites (HDM) exhibited the highest SPT positivity among all participants (93%) and among *S. olivacea*-sensitized people (98%). Malaysia experiences a consistently warm and humid environment year-round. This tropical temperature provided an optimal growing habitat for many species of house-dust mites (HDM). Consequently, the high frequency of house-dust mite (HDM) allergy shown in this investigation was not unexpected, aligning with findings from other Asian nations with similar climates (Lim et al. 2015). House dust mites are well established as a primary source of allergens that may trigger rhinitis, asthma, and atopic dermatitis (Mahram et al., 2013).

Elevated ambient temperatures coupled with high humidity create conducive conditions for house dust mites to proliferate in Malaysia (Lim et al., 2015) and in other Asian nations with comparable climates, including Thailand, Vietnam, Hong Kong, and Indonesia (Andiappan et al., 2014). In Asia, house dust mite (HDM) is the predominant allergenic trigger, succeeded by pollen, insects, and fungi (Pawankar et al., 2009).

The Association of SPT Positivity to *S. olivacea* and Other Allergens

The incidence of allergy illnesses was greater in urbanized and developed cities compared to rural areas (Andiappan et al., 2014; Zailatul et al., 2015). Malaysia, especially its capital Kuala Lumpur, was experiencing urbanization and westernization, accompanied by a significant rise in the number of motor vehicles in use. Such conditions were accompanied by later rises in air pollution. Recent research demonstrated substantial correlations between elevated air pollution levels and an increased risk of allergy sensitization and prevalence of allergic rhinitis (Zhang et al., 2014). Among the *S. olivacea* -sensitized individuals, House Dust Mites triggered a highest frequency of positive SPT reactivity at 98%, followed other seafood allergens 96%, Aeroallergens 96%, *Portunus pelagicus* (79%) and *Charybdis feriatus* (69%). This was not surprising as *Portunus pelagicus* and *Charybdis feriatus* are grouped under same phylum (Amaral et al., 2018) and highly consumed by local people (Zailatul et al., 2015).

The study unexpectedly reveals strong cross-sensitization with a moderate to weak positive correlation between *S. olivacea* and the majority of seafood allergens examined. It was demonstrated that individuals exposed to *S. olivacea* may exhibit sensitivity to the seafood

species *Portunus pelagicus, Charybdis feriatus*, and aeroallergens. This may pertain to IgE cross-reactivity among analogous epitopes in crab allergens and other allergens from various organisms (Bessot et al., 2010; Lim et al., 2015). A notable positive correlation was seen between *S. olivacea* sensitization and the seafood species *Portunus pelagicus, Charybdis feriatus*, and aeroallergens.

Table 5. Association of SPT positivity between Mud crab (*S. olivacea*) and other allergens

Allergens	P-value	r-value
Other seafood	0.000**	0.504
Blue crab (*Portunus pelagicus*)	0.006**	0.257
Red crab (*Charybdis feriatus*)	0.000**	0.504
Aeroallergens	0.000**	0.504
House Dust Mites	0.055	0.563
Other food allergens	0.000**	0.031

Note. ***P*-values less than 0.01

Correlation of SPT positivity between Mud crab (*S. olivacea*) and other allergens

The correlation between total IgE and SPT positivity crab (*S. olivacea, Portunus pelagicus, Charybdis feriatus)* and allergen of other seafood, aeroallergens, house dust mites, other food. Pearson correlation was carried out as showen in Table 6. The results indicated that there is a significant and weak positive correlation between total IgE with SPT positivity to red crab (*Charybdis feriatus*) ($p = 0.024$, $q = 0.219$), aeroallergens ($p = 0.001$, $r = 0.153$), house dust mites ($p = 0.000$, $r = 0.214$). Many studies have been conducted on the cross-reactivity between *S. olivacea* and seafood, *Portunus pelagicus*, *Charybdis feriatus*, and aeroallergens. Clinical evidence of poly-sensitization and co-sensitization to allergens in seafood-allergic individuals has been thoroughly documented (Bessot et al., 2010; Rubaba et al., 2012). Cross-reactivity may arise when particular antibodies, initially heightened in response to one allergen, interact with analogous epitopes from additional allergens of distinct origins (Rubaba et al., 2012). The root cause of these reactions may be the presence of significant structural homology between allergenic proteins from unrelated sources (Rubaba et al., 2012). The primary allergens causing acute hypersensitivity reactions in shellfish, such as crab tropomyosin and arginine kinase, have been documented in previous research (Lopata & Lehrer, 2009; Abdel Rahman et al., 2011). Tropomyosin is fundamental to muscle functioning across all organisms and is regarded as a pan-allergen (Wong et al., 2016), having been extensively reported as the principal cause of IgE cross-reactivity between *S. olivacea* and other invertebrates, such as the house dust mite (Bessot et al., 2010). Nonetheless, this outcome does not indicate any substantial correlation between *S. olivacea* and mite sensitizations, perhaps owing to the limited number of individual samples examined. In epidemiological investigations, a limited sample size would not yield precise estimations or credible answers to a research hypothesis (Hajian-Tilaki, 2011).

Table 6. Correlation of SPT positivity between Mud crab (*S. olivacea)* and other allergens

Allergens	*P*-value	r-value
Sea food	0.235	0.051
Portunus pelagicus	0.555	0.047
Charybdis feriatus	0.024*	0.219
Aeroallergens	0.001**	0.153
House Dust Mites	0.000**	0.214
Other seafood allergens	0.594	0.027

Note: * Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

CONCLUSION

In this study, it appears that majority of the individuals were allergic rhinitis patients (84%), followed by food allergy (45%), conjunctivitis (22%), asthma (19%), eczema (18%), sinusitis (7%) and contact dermatitis (6%). The prevalence of *S. olivacea* sensitization among the individuals was 22%. Among the *S. olivacea*-sensitized individuals, house dust mites triggered the highest frequent of (positive SPT) reactivity at 98%, followed by aeroallergens and other seafood allergens (96%). A total of 79 and 69% of the *S. olivacea*-sensitized individuals were also have SPT positivity to *Portunus pelagicus* and *Charybdis feriatus*, respectively. The study found significant cross sensitizations but moderate to weak positive associations between *S. olivacea* and all tested allergen except house dust mites.

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