

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

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### ABSTRACT

One of the most common food allergies that can cause serious allergic reactions is crab allergy. After eating crab, reports of potentially fatal responses, such as anaphylactic shock, are common. In many Asian nations and other countries to which mud crabs were exported, mud crabs of the species *Scylla olivacea* are frequently consumed.

The aim of this study was to estimate the prevalence of allergy to *S. olivacea* among local atopic populations by skin prick test (SPT). Crab proteins were extracted from the mud crab (*S. olivacea*) flesh. A total of 500 individuals, 234 males and 266 females, with atopic diseases were studied; the number of individuals sensitized to mud crab (*S. olivacea*) was 112 with percentage of 22%, individuals sensitized to *Portunus pelagicus* – 162 with percentage of 32%, and individuals sensitized to *Charybdis feriatus* – 106 with percentage of 21%. As a conclusion, this study showed that the prevalence of *S. olivacea* sensitization among the individuals was 22%. Among the *S. olivacea* -sensitized individuals, house dust mites triggered the highest frequency of positive SPT reactivity at 98%, followed by aeroallergens and other seafood allergens (96%).

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

### INTRODUCTION

Crab allergy is common, so it can lead to severe allergic reactions. Death-led reactions such as anaphylactic shock have been often reported after crab consumption (Lopata et al., 2016). The aim behind this study was to determine the prevalence of allergy to *S. olivacea* among local atopic populations by skin prick test (SPT). Immediate hypersensitivity to food is a vast health problem globally (Kamath et al., 2014).

The food allergy data demonstrated significant differences between Asian and other communities. It was reported that the prevalence of crab allergy is higher among Asian communities than American and European populations, as crab consumption varies between those countries (Lopata et al., 2016; Azemi et al., 2021). For instance, in Malaysia, crab was described as the most common food allergens among local allergic rhinitis and asthma patients, with the prevalence of 78% and 16% respectively (Zailatul et al., 2015), since this crab is one of the major components 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 and their whole-body tissues were 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 phosphate buffer saline (PBS, pH 7.2), then extract by using an incubator shaker at 4°C under continuous shaking (50 rpm) overnight. After that, the homogenates were centrifuged at 14,000 rpm at 4°C for 15 min where supernatants were filtered first using filter papers, then by using sterile syringe filters 0.22 µm. The extracts then

lyophilized (freeze-dried) using a freeze dryer. The lyophilized extracts were stored at -20°C until next use.

### Individuals for Prevalence Analysis

Data of 500 patients with atopic diseases who were referred to the Allergy Clinic, Hospital Kuala Lumpur from January 2015 to December 2018 were analyzed in present study to determine the prevalence of allergy to *S. olivacea*. The allergic conditions of the patients were confirmed using the skin prick test (SPT).

### Data Analysis

Data of demographic figures, clinical history, symptoms of allergy and all SPT results were recorded. Frequencies and simple associations by Fisher's exact test were calculated using the version 23 of (SPSS) software. The results were declared to be statistically significant if the P-values is less than 0.05.

## RESULTS AND DISCUSSION

### Demographic Data

500 patients with atopic diseases referred to Allergy Clinic, Hospital Kuala Lumpur from January 2015 to December 2018 were analyzed in this study. A total of 500 individuals, 234 males and 266 females, with atopic diseases were studied. Malay constituted the majority (65%), while Indian, Chinese and other constitute were (16, 15, and 5%, respectively).

Sixty-five percentage of the Malay individuals, 6% of Indian individuals, 22% of Chinese individuals and 7% other individuals were sensitized to Mud crab (*S. olivacea*). This value showed that *S. olivacea* sensitization appeared to be more prevalent among Malay than other ethnics. However, this reason is not clear as there is no study on the racial distribution of allergic diseases has been conducted in Malaysia (Zailatul et al., 2015; Ateshan et al., 2020). The age of all individuals was ranging between 5 to  $\geq 55$  years with the mean age of 34.7 years. Meanwhile, majority of the individuals sensitized to *S. olivacea* were in the age group of 5 to 17 years. Less *S. olivacea* -allergic individuals were in the  $\geq 55$  and more than 55 years age groups.

Table 1 demonstrated that females are more sensitized (53%) to *S. olivacea* compared to males (47%). Interestingly, this finding is in accordance with other studies that also showed female predominance in their allergy studies (Asha'ari et al., 2010; Ibekwe et al., 2016).

**Table 1. Demographic features of the individuals**

| Demographic data |           | All individuals<br>(n = 500) (n) % | Individuals sensitized to<br>Mud crab ( <i>S. olivacea</i> )<br>(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   |
|                  | $\geq 55$ | (29) 6                             | (7) 6   |

### Clinical History and Symptoms of Allergy

Clinical histories of the individuals were shown in Table 2. Majority of the individuals have histories of allergic rhinitis (84%). Among *S. olivacea* -sensitized individuals, 88% were patients with allergic rhinitis, 22% were allergic conjunctivitis patients and food allergy (45%) followed by asthma (19%), contact dermatitis (22%) and sinusitis (9%). less patient with sinusitis was sensitized to *S. olivacea*.

**Table 2. Clinical history of the individuals**

| Clinical History   | All individuals | Individuals sensitized to<br>Mud crab ( <i>S. olivacea</i> ) |
|--------------------|-----------------|--|
|                    | (n = 500) (n) % | (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 appeared as the majority clinical history of our individuals. It was reported that allergic rhinitis affecting about 20% of the population across regions (Zailatul et al., 2015; Ateshan et al., 2019; Ha et al., 2019). Allergic rhinitis is not a life-threatening disease, but it is related with the decreased quality of life and work productivity and increased the financial costs of health (Pefura-Yone et al., 2015). Allergic rhinitis is triggered by many causes such as genetic hereditary, hormonal changes, smoking, obesity, lifestyle and other environmental factors including exposure to inhalant allergens and gas pollution (Pefura-Yone et al., 2015; Zhang et al., 2014).

A total of 500 individuals, 234 males and 266 females, with atopic diseases were studied, individuals were sensitized to Mud crab (*S. olivacea*) is 112 with percentage 22%, individuals were sensitized to *Portunus pelagicus* is 162 with percentage of 32% and individuals were sensitized to *Charybdis feriatus* is 106 with percentage of 21% showed in Table 3. Most crab species, provoking allergic reaction including the skin, gastrointestinal, respiratory and cardiovascular systems (Lopata & Lehrer 2009; Ateshan & Saxena 2015; Jasim et al., 2021).

**Table 3. The prevalence of allergy to crab among individuals**

| Species                                 | All individuals (n) % |
|---|-----------------------|
|   | (n = 500)             |
| ( <i>Portunus pelagicus</i> ) Blue crab | (162) 32              |
| ( <i>S. olivacea</i> ) Mud crab         | (112) 22              |
| ( <i>Charybdis feriatus</i> ) Red crab  | (106) 21              |

### Skin Prick Test Reactivity

The Skin Prick Test (SPT) is the main method used to detect IgE-mediated allergy disorders in individuals. SPT is non-costly, sensitive and produces immediate result (Lopata et al., 2012; Al Sailawi et al., 2020). In this study, all individuals were skin prick tested to *S. olivacea* extract along with other allergen extracts used in the *standard panel for* allergy diagnosis at the clinic, including other seafoods, aeroallergens, other food allergens and house dust mites. Results were recorded in Table 4.

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

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

Most of the individuals have SPT positivity to seafood and aeroallergens. House Dust Mites was among the various allergens that caused the highest sensitivity in 93% of the individuals, followed by aeroallergens (87%), other seafood allergens (83%). This data is consistent with other study which reported the prevalence of shellfish allergy mainly prawn and crab is more frequent in Asia compared with the western countries. This may be affected by the geographic consumption of shellfish (Zailatul et al., 2015).

Among the *S. olivacea* -sensitized individuals, House Dust Mites triggered the highest frequency of positive SPT reactivity at 98%, followed other seafood allergens 96%, Aeroallergens 96%, *Portunus pelagicus* (79%) and *Charybdis feriatus* (69%). This is 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 study, House Dust Mites showed the highest SPT positivity among all individuals (93%) as well as among *S. olivacea* -sensitized individuals (98%). Malaysia has a warm and humid climate throughout the year. This tropical climate facilitates a suitable growth environment for many types of house dust mite (HDM). Therefore, it was not surprising to reveal the high prevalence of HDM allergy in this present study, in accordance with other reports in other Asian countries with same climate (Lim et al., 2015). House dust mite is well-demonstrated as a main allergen source that induced rhinitis, asthma and atopic dermatitis (Mahram et al., 2013). Ambient temperatures and high humidity offer favorable conditions for house dust mite to thrive in Malaysia (Lim et al., 2015) and in other Asian countries with similar climates such as Thailand, Vietnam, Hong Kong and Indonesia (Andiappan et al., 2014). In Asia, house dust mite is the major triggering allergen followed by pollens, insects and fungi (Pawankar et al., 2009).

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

The prevalence of allergic diseases was higher in westernized and developed cities than in rural areas (Andiappan et al., 2014; Zailatul et al., 2015). Malaysia, particularly its capital city Kuala Lumpur, is undergoing urbanization and westernization, with massive increase in the number of motor vehicle used. These conditions have been accompanied by a subsequent increase of air pollution. Recent studies showed a significant connection between high level of air pollution and an increased risk of allergic sensitization and prevalence of allergic rhinitis (Zhang et al., 2014).

Table 5 shows the association of SPT positivity to *S. olivacea* and other allergens. Surprisingly, this study found significant cross-sensitizations but with moderate to weak positive association between *S. olivacea* and most of all seafood allergens tested. Thus, it was shown that a *S. olivacea* -sensitized individual has possibility to sensitive to a seafood, *Portunus pelagicus*, *Charybdis feriatus* and Aeroallergens. These may be related to IgE-cross-reactivity between similar epitopes in crab allergens and other allergens from different organisms (Bessot et al., 2010; Lim et al., 2015). A significant with positive association was

seen between *S. olivacea* sensitization and a seafood, *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 ( <i>Portunus pelagicus</i> ) | 0.006** | 0.257   |
| Red crab ( <i>Charybdis feriatus</i> )  | 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 shown 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 ( $p = 0.000$ ,  $r = 0.214$ ).

The cross-reactivity between *S. olivacea* and seafood, *Portunus pelagicus*, *Charybdis feriatus*, Aeroallergens has been extensively studied. Clinical evidence of polysensitization and co-sensitization to these allergens in seafood-allergic patients were well-documented (Bessot et al., 2010; Rubaba et al., 2012). Cross-reactivity could occur when a specific antibody, initially elevated against an allergen, reacts to a similar epitope in other allergen from different sources (Rubaba et al., 2012). The presence of high structural homology between allergenic proteins from unrelated sources could be the underlying cause of this reaction (Rubaba et al., 2012). The major allergen responsible for the immediate-hypersensitivity reactions in shellfish including crab is tropomyosin and arginine kinase as reported by other studies (Lopata & Lehrer, 2009; Abdel Rahman et al., 2011). Tropomyosin played primary role in muscle function of all organisms and thus was considered as a pan-allergen (Wong et al., 2016), which was well-documented as the major cause of IgE-cross-reactivity between *S. olivacea* and other invertebrates mainly house dust mite (Bessot et al., 2010). However, our result did not suggest a significant association between *S. olivacea* and mite sensitizations, possibly due to a small number of individuals studied. In epidemiologic studies, small sample size will not yield a precise estimate and reliable answers to a study 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   |
| <i>Portunus pelagicus</i> | 0.555   | 0.047   |
| <i>Charybdis feriatus</i> | 0.024*  | 0.219   |
| Aeroallergens             | 0.001** | 0.153   |
| House Dust Mites          | 0.000** | 0.214   |
| Other seafood allergens   | 0.594   | 0.027   |

\*. 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 frequency 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. This study found significant cross-sensitizations but with moderate to weak positive association between *S. olivacea* and all allergens tested except house dust mites.

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