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COMPARISON OF PROTEIN PROFILES AND ALLERGENICITY OF DIFFERENT BODY PARTS AND GENDERS OF *Scylla paramamosain*

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Abstract: This study aimed to determine the protein profiles and allergenicity of different body parts and genders of *Scylla paramamosain*. Raw extracts of mix and six individual body parts were prepared from the respective crab flesh and then analyzed by SDS-PAGE and immunoblotting using sera from 10 crab-allergic patients. The mix body part extract contains 24 protein fractions, while all individual body parts have almost similar protein profiles with 16 to 21 protein bands between 15 to >250 kDa. Various IgE-binding patterns were detected in immunoblotting of mix and individual body parts. Generally, the female leg was identified as the most allergenic part, while the female abdomen was demonstrated as the least allergenic part based on the number and intensity of IgE-binding proteins. Five major allergens at 95, 50, 42, 38 and 36 kDa were identified as potential major allergens. As a conclusion, this study indicated that all body parts of *S. paramamosain* have numerous and various allergenic proteins, contributing to variable allergenicity between the crab body parts. These findings are useful for the advancement of the diagnosis and management of crab-allergic patients.

Keywords: *Scylla paramamosain*, mud crab, allergy, SDS-PAGE, immunoblotting.

1. Introduction

Mud crab is highly demanded as a protein food source in many countries [1]. Mud crabs belong to Family Portunidae under genus *Scylla* [1]. Among the *Scylla* spp., *S. paramamosain* is one of the most abundant mud crab species inhabiting coastal and mangrove areas throughout Malaysia [1,2]. However, the consumption of mud crab can also cause allergy [3]. Crab allergy is one of the most common IgE-mediated food allergies and is often associated with severe reactions [3]. Tropomyosin, a 34-38 kDa protein has been demonstrated to be the major allergen for crabs [3-7]. Apart from tropomyosin, arginine kinase was also identified as the major allergen in mud crab [8,9].

Almost all body parts of both male and female crabs contribute to local economy and commonly consumed by local peoples [1,10-12]. The variations of protein content in crab body parts and gender might play an important role in crab allergenicity particularly among

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crab consumers, thus should also be addressed in allergy studies. However, so far there is no study reported on protein profiles and allergenicity of individual body parts of male and female crab. Therefore, this study was conducted to determine the protein profiles and allergenicity of different crab parts and genders of *S. paramamosain* by proteomics approach.

2. Materials and Method

Crab Extracts

Live male and female *S. paramamosain* were collected from Sungai Petani, Kedah. Seven extracts were prepared from the crab flesh (mix crab parts, male abdomen, male claws, male legs, female abdomen, female claws and female legs) following the methods described by Rosmilah et al. [4]. Briefly, the crab flesh was homogenized in PBS solution, followed by an overnight extraction at 4 ºC. The crab homogenates were then centrifuged, filtered, dialyzed, lyophilized and stored at -20 ºC until use.

Serum Samples

Sera from 10 crab-allergic patients and a negative control serum from a non-allergic individual were used in this study. This project was approved by Medical Research and Ethics Committee (MREC), Ministry of Health, Malaysia.

SDS PAGE

The protein profile of each crab extracts was determined by sodium dodecyl sulfate-polyacrylamide gelelectrophoresis (SDS-PAGE). Briefly, the extracts were treated with Laemmli buffer and loaded into wells of 12% resolving gel with 5% stacking gel. The crab proteins and a pre-stained molecular weight markers were then separated at 120 mA for 50 minutes using a Mini Tetra Cell System (Bio-Rad, USA). After completed, the gels were stained by Coomassie Brilliant Blue R-250 and analyzed by an imaging densitometer (Bio-Rad, USA).

Immunoblotting

Briefly, the protein bands in SDS-PAGE gel were electro-transferred to a nitrocellulose membrane at 250 mA for 70 minutes using Mini Transblot System (Bio-Rad, USA). After completed, the membrane was stained with Ponceau S, cut into strips, washed with TTBS solution and blocked with 5% low-fat milk in TBS solution. The strips were then incubated with the individual sera overnight at 4˚C. The IgE binding proteins on the strips were identified by a detection system containing of biotinylated goat antihuman IgE (KPL, USA), conjugated streptavidin-alkaline phosphatase and alkaline phosphatase conjugate substrate (BioRad, USA).

3. Results and Discussion

SDS-PAGE

In general, the protein profiles of all extracts from different body parts and genders of *S. paramamosain* are almost identical (Figure 1). Only slight differences could be seen in the intensities of some bands. The mix parts extract revealed the highest bands; approximately 24 bands in the range of >250 to 15 kDa. This finding is in accordance with previous study on local crab species, *Portunus pelagicus* and *Charybdis feriatus* which exhibited 20 protein bands [4].

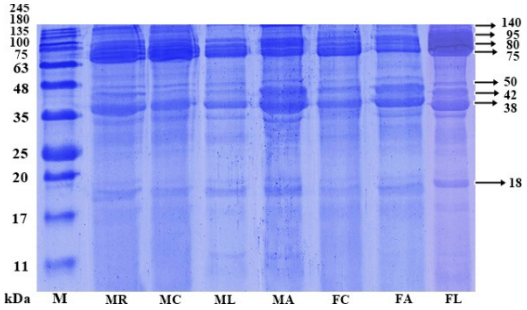

Figure 1: The comparison of protein profiles between mix and individual body parts of *S. paramamosain*. Lane MR, MC, ML, MA, FC, FA and FL are mix parts, male claw, male leg, male abdomen, female claw, female leg and female abdomen, respectively. M is molecular weight markers in kilo Dalton (kDa).

Meanwhile, among the individual body parts and genders of *S. paramamosain*, at least 21 to 16 protein bands were detected. Overall, several prominent bands ranging from 140-75 kDa, 50-38 kDa and 18 kDa were seen in all extracts, but with slight changes in their intensities. Both the female abdomen and female claws have the highest number of bands, while the male claw has the least number of bands. This result indicating that female *S. paramamosain* has higher protein composition than the male crab. According to SDS-PAGE analysis, both female and male mud crab extracts indicated homogenous protein profile patterns. A similar report had also shown no significant difference in the protein band pattern of male and female blue crabs [11,12].

Immunoblotting

Figure 2 displayed the IgE-binding proteins of mix parts of *S. paramamosain* using sera from 10 mud crab-allergic patients. The mix body parts has 18 IgE-binding proteins between 18 to >250 kDa, indicating *S. paramamosain* has numerous allergenic proteins. This finding agrees with Nurul et al. [13] which also detected multiple IgE-binding proteins between 23 and 250 kDa in mud crab *S. serrata.* Five proteins at 36, 38, 42, 50 and 95 kDa were identified as the major allergens for *S. paramamosain* (binding frequencies ≥ 50%). The 36 and 38 kDa bands

might be corresponded to tropomyosin, a well-known shellfish major allergen at 32 to 38 kDa [4-6,13], while the 42 kD might be identical to arginine kinase or actin [7-9].

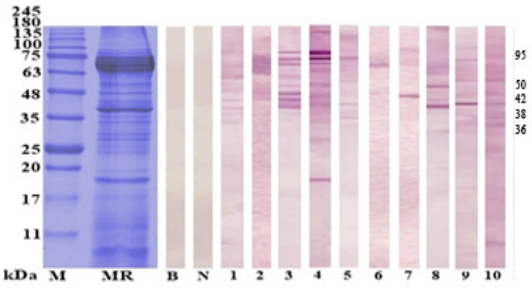

Figure 2: Immunoblotting results of mix parts of *S. paramamosain* using sera from 10 crab-allergic patients (lane 1 to 10). Lane M is molecular mass markers in kiloDalton (kDa); lane MR is mix parts; lane B and N are blank and negative control, respectively. Arrows indicated the major allergens in kDa.

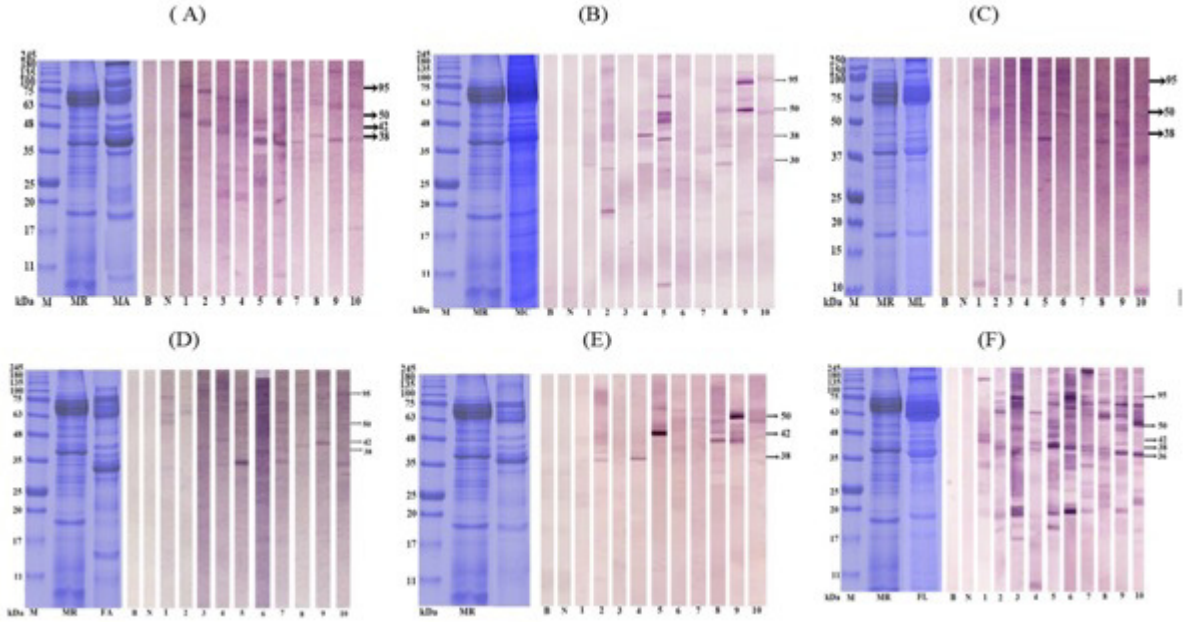

Figure 3: Immunoblotting results of six body parts of *S. paramamosain* using sera from 10 mud crab-allergic patients (lane 1 to 10). A, B, C, D, E and F are male abdomen, male claw, male leg, female abdomen, female claw, female leg, respectivly. Arrows indicated the major allergens in kDa.

In general, similar as the mix parts, immunoblotting results of individual crab parts and genders have also detected multiple IgE-binding proteins with various IgE-binding patterns (Figure 3). Among male crabs, male claws has the highest number of IgE-binding bands (19 bands), followed by male legs (15 bands) and male abdomen (14 bands). Surprisingly, among female crabs, female legs has the highest number of IgE-binding bands (20 bands), followed by female abdomen (14 bands) and female claws (13 bands). Therefore, based on the number of IgE-binding bands, the allergenicity of crab parts are in the following order: female legs >

male claws > male legs > female abdomen = male abdomen > female claws. Immunoblotting of male abdomen, female abdomen, female claws and female legs have identified all five major allergens of *S. paramamosain* at 36, 38, 42, 50 and 95 kDa, whereas immunoblotting of male claws and male legs have recognized only four major allergens at 36, 38, 50 and 95 kDa. Thus, according to the number of major allergens, female legs, female claws, female abdomen and male abdomen can be considered as more allergenic than other crab parts (female legs = female claws = female abdomen = male abdomen > male claws = male legs). However, based on the IgE-binding intensity, the most prominent IgE-binding intensity was seen in immunoblotting of female legs, followed by male claws and female claws. The binding intensities between male abdomen, male legs and female abdomen are almost identical. Therefore, the allergenicity order based on the band intensities are as follows: female legs > male claws > female claws > female abdomen = male abdomen = male legs.

This finding indicated that crab parts have variable allergenicity in terms of the number of IgE-binding bands, number of major allergens and IgE-binding band intensities. Based on these three characteristics results, this study suggested that the female legs can be regarded as the most allergenics part than the other crab parts. In general, among local consumers, crab leg is the least popular parts as it contains very little amount of meat. However, in certain countries such as Japan, the only parts that is marketed an consumed is the crab legs and claws as these parts have been claimed for their health-promoting characteristics as a valuable source of proteins and essential fatty acids [14]. Nevertheless, based on this findings, this study may suggest the crab-allergeic patients to avoid the consumption of female crab legs as it may have high potential to elicit allergic reactions to crabs. However, immunoblotting tests using more sera, supported by an extensive clinical studies among various crab-allergic patient populations are needed to confirm this finding. To the best of our knowledge, this study is the first to report the allergenicity variation between different body parts and genders of crab.

In general, this study revealed that individual sera exhibited a remarkable heterogeneity in detecting *S. paramamosain* allergens in all extracts. Such IgE-reactivity variation among crab-allergic patients might be as a result of genetic and allergen exposures differences among crab-allergic patients, which might reflect their allergy symptom variations [4].

5. Conclusion

Overall, all body parts and genders of *S. paramamosain* have various allergenicity. This finding is important for clinician and patients to improve the diagnostic and management

approach of crab allergenic patients in this country. It should be noted that these findings are new discoveries in allergy studies as this study is the first to report the allergenicity variation between different crab parts and genders.

Acknowledgements

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