Journal of Allergy & Therapy
ISSN: 2155-6121

Yadzir et.al, J Allergy Ther 2015, 6:4
DOI: 10.4172/2155-6121.1000216

Research Article Open Access

Production of Allergen Extracts for Bivalve Allergy Diagnosis Using Skin Prick Test

Zailatul Hani Mohamad Yadzir[1,2*], Rosmilah Misnan[2], Faizal Bakhtiar[1], Noormalin Abdullah[1], Hanisom Abdullah[2] and Shahnaz Murad[1]
[1]Allergy and Immunology Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia
[2]Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia

[*]**Corresponding author:** Yadzir ZHM, Allergy and Immunology Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia, Tel: +603-26162785, Fax: +603-26912019; E-mail: zailatul@imr.gov.my

Received date: June 23, 2015; **Accepted date:** July 27, 2015; **Published date:** August 03, 2015

Abstract

Background: Whilst the consumption of bivalves is reasonably high in Malaysia, the frequency of allergy to this group of shellfish in the local population is largely unknown. The aim of this preliminary study was to produce bivalve allergen extracts and to investigate the frequency of bivalve sensitization among the local atopic population.

Methods: Raw allergen extracts were prepared from 5 different species of bivalves. Their protein profiles were studied using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Results: In SDS-PAGE, the bivalve extracts demonstrated 10 to 23 protein bands. The five protein profiles varied considerably but most visible protein bands lay within 25-100 kDa. At the same time, fifty patients with a history of atopy were skin prick tested (SPT) with these raw extracts of bivalves. Of the 50 subjects, 13 (26%) had a positive SPT to at least one of the 5 bivalve extracts tested, 8 (61%) reacted to one bivalve extract while only one (8%) reacted to all 5 bivalve extracts. The frequency of skin test reactivity to Malaysian cockle was the highest at 22%, followed by Carpet clam, 12% and 4% to the other 3 bivalve extracts; Tropical oyster, Asian clam and Asian green mussel.

Conclusion: This study showed that of the five different bivalve species, cockle was found to have the highest frequency in skin test reactivity. It appears that an individual, who is sensitized to a mite, is also likely to be sensitized to bivalves.

Keywords: Mollusc; Bivalve; Allergy; Atopy; Skin prick testing; Allergen extracts; Mites

Introduction

Molluscs are frequently grouped together with crustaceans under the term shellfish. However, molluscs represent a completely separate phylum (Mollusca), whilst crustacean shellfish are classified under the phylum Arthropoda. The most important divisions of the phylum Mollusca are the classes Bivalvia (clam, cockle, mussel, scallop and oyster), Cephalopods (octopus, cuttlefish and squid) and Gastropods (abalone and snail) [1].

Molluscs play an ever-increasing role in the human diet and the world economy [2]. Molluscan shellfish meat is recommended in several dietary regimes for their high protein content, low fat/cholesterol profile, the presence of good lipids, significant amounts of omega-3-fatty acids, dietary essential amino acids, vitamin B12 and several important minerals such as zinc, iron and copper [3,4]. However, molluscan shellfish allergy is receiving attention [5]. Molluscan shellfish including bivalves are considered to be among a group of allergenic foods. The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) [6] have identified shellfish, including molluscs, as one of the eight major sources of food allergens. The European Union has also added molluscan shellfish to the list of most commonly allergenic foods in Europe [7].

The prevalence of allergy to bivalves varies throughout the world. It is likely to parallel consumption patterns being more frequent in locales where consumption is high [8]. IgE-mediated bivalve allergy has been described in several countries including France [9], Spain [10], South Africa [11], Japan [12,13], Canada [14], Italy [15,16], Germany [17], Sweden, Denmark, Estonia Lithuania and Russia [18].

Symptoms of bivalve's allergy are largely similar to those of allergic reactions reported for other foods. Common symptoms include mild oral allergy (itching of the lips, mouth and throat and swelling of the lips, tongue, throat and palate), cutaneous reactions (urticaria, eczema), respiratory symptoms (asthma, rhinitis), gastrointestinal symptoms (diarrhoes, vomiting) and systemic reactions (anaphylactic shock) [8,19]. Allergies to bivalves have also been documented in work places that handle bivalves. These have been found to occur primarily through inhalation of aerosols generated during cutting, scrubbing or cleaning, cooking or boiling and drying [20-22]. Reactions can also occur through the skin as a result of directly handling the bivalves [23,24].

Allergies to bivalves are diagnosed similarly to other food allergies. Double-blind, placebo-controlled food challenge (DBPCFC) is the gold standard to establish a food allergy but is a time-consuming and expensive technique not widely practised outside research centers.

Skin prick tests (SPTs) used in conjunction with history and physical plus clinical judgment is acceptable practice for diagnosing IgE-mediated food allergy [25].

In Malaysia, bivalves have long been part of the diet of the local population [26]. The major edible bivalves that are frequently consumed include clams, cockles, mussels and oysters. Although bivalves are likely the most frequently ingested class of molluscan shellfish, local data on allergy to bivalves is not available. In this preliminary study, we investigated the frequency of bivalve sensitization among the local atopic population by skin prick tests (SPT) using in house allergen extracts prepared from local bivalves.

Materials and Methods

Bivalve species

Five commercially important and commonly consumed bivalve species were used in this study: *Anadara granosa* (Malaysian cockle/Kerang), *Crassostrea belcheri* (Tropical oyster/Tiram tropika), *Corbicula fluminea* (Asian clam/Kepah Asia), *Perna viridis* (Asian green mussel/Kupang hijau Asia) and *Paphia textile* (Carpet clam/Lala). All bivalve samples were purchased directly from the local market. The bivalve samples were stored in the laboratory freezer at -20°C to reduce biological deterioration prior to analysis.

Subjects

Study subjects include 50 out-patients and in-patients with atopic disease referred to the Allergy Clinic, Hospital Kuala Lumpur (HKL). Subjects were enrolled according to the predetermined inclusion and exclusion criteria as stated:

Inclusion criteria: Subjects aged ≥ 18 years old.

Subjects had a history of allergy-related diseases such as allergic rhinitis, asthma, sinusitis or allergic conjunctivitis.

Skin prick test (SPT) results showed positive reactions to at least one type of allergen on the allergen panels tested.

Exclusion criteria: Pregnant women.

Received anti-histamine within 72 hours of consultation.

Subjects have chronic skin disease (dermographism).

This study received ethics approval from the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia and patients gave their informed consent before participating in the study.

Preparation of bivalve allergen extracts

In the preparation of the bivalve extracts, the shell was split open and the inner muscle tissue was used for protein extraction. About 20 g of the muscle mass was homogenized in 200 ml of 0.1 M phosphate buffered saline (PBS), pH 7.2 for 10 min using a Waring blender. This homogenate was then agitated overnight at 4°C, followed by centrifugation at 4,500 and 14,000 rpm, for 30 and 15 min, respectively. The recovered, clear supernatant was sterilized by passage through a 0.22 µm syringe filter, frozen and lyophilized. The lyophilized extracts were stored at -20°C until further use. The protein content of the extracts was estimated using the Total Protein Kit (Sigma, USA).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out under denaturing conditions with the stacking and separating gels containing 5% and 12% of acrylamide, respectively. Bivalve extracts were dissolved in Laemmli sample buffer in the presence of 5% 2-mercaptoethanol and heated at 97°C for 4 min before being loaded onto the gel. Each lane was loaded with 10 µl of sample, containing 10 µg of protein or protein standards before the proteins were separated for 45 min at 120 mA and 200 V using the Mini-Protean 3 system (Biorad, USA). The separated proteins were then visualized by staining with Coomassie brilliant blue R-250. Molecular weights of the protein bands were determined by comparison with molecular weight markers using an Imaging Densitometer GS800 and Quantity One Software (Biorad, USA).

Skin prick test (SPT)

The SPTs were carried out on the forearm using sterile lancets. A drop of the allergen extract was placed on the forearm after which the skin under the drop was pricked with a sterile lancet. The size of the wheal was recorded after 15 minutes: a wheal 3 mm or greater than the negative control was regarded as positive. Saline solution and histamine hydrochloride 1% served as negative and positive controls, respectively (Alk Abello, Spain).

Data analysis

Data recorded included demographic data, clinical history and symptoms of allergy and SPT results. All data were analyzed using the Statistical Package for Social Sciences (SPSS) software. Simple associations were assessed with frequency tables and Fisher's exact test for two independent proportions. P-values less than 0.05 were considered statistically significant.

Results

Total protein content of bivalve extracts

Table 1 shows the total protein content of bivalve allergen extracts. Overall, the total protein content of the five species of bivalves fell in the range between 3.5 mg/ml to 5.2 mg/ml. *Corbicula fluminea* (Asian clam/Kepah Asia) showed the highest protein content at 5.2 mg/ml while *Paphia textile* (Carpet clam/Lala) was found to have the lowest at 3.5 mg/ml.

Species	Total Protein (mg/ml)
Anadara granosa (Malaysian cockle/Kerang)	4
Crassostrea belcheri (Tropical oyster/Tiram tropika)	3.8
Corbicula fluminea (Asian clam/Kepah Asia)	5.2
Perna viridis (Asian green mussel/Kupang hijau Asia)	4.8
Paphia textile (Carpet clam/Lala)	3.5

Table 1: Total protein content of bivalve allergen extracts.

Protein profile of bivalve extracts

The SDS-PAGE of bivalve extracts demonstrated 10 to 23 protein bands (Figure 1). The protein bands were in the wide molecular weight range of 13-250 kDa. The protein profiles of the 5 bivalve extracts varied considerably, although majority of the visible protein bands ranged between within 25-100 kDa.

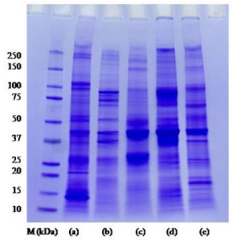

Figure 1: SDS-PAGE profiles of (a) *A. granosa*, (b) *P. textile*, (c) *C. belcheri*, (d) *C. fluminea* and (e) *P. Viridis* extracts. Lane M, molecular mass markers.

Demographic data

	Demographic Data	Subjects	Subjects sensitized to bivalves
		(n=50), % (n)	(n=13), % (n)
Gender	Male	40 (20)	77 (10)
	Female	60 (30)	23 (3)
Ethnicity	Malay	68 (34)	76 (10)
	Chinese	8 (4)	8 (1)
	Indian	18 (9)	8 (1)
	Others	6 (3)	8 (1)
Age	18-24	46 (23)	54 (7)
	25-34	32 (16)	38 (5)
	35-44	10 (5)	8 (1)
	45-54	2 (1)	0 (0)
	≥55	10 (5)	0 (0)

Table 2: Demographic data of the subjects.

Fifty subjects, 20 males, 30 females, with a history of atopy were studied. Table 2 shows the demographic data of these subjects. The mean age was 29.9 years with ages ranging between 18 to 58 years. Of the 50 subjects, 13 (26%) had a positive SPT to at least one of the 5 bivalve extracts tested.

In the group of subjects that were found to be sensitized to bivalves, males appeared to be more prevalent than females. Most subjects were in the 18-24 year age group. In terms of the subject's ethnicity, Malays comprised the majority.

Clinical history and symptoms of allergy

Table 3 summarizes the clinical histories of these subjects. Most of the subjects were allergic rhinitis patients, 78%, followed by allergic conjuctivitis patients, 46%. Clinical symptoms were shown in Table 4. The most common presenting symptom was rhinorrhoea, 64%.

Clinical History	Subjects	Subjects sensitized to bivalves
	(n=50), % (n)	(n=13), % (n)
Allergic rhinitis	78 (39)	85 (11)
Allergic conjuctivitis	46 (23)	46 (6)
Eczema	18 (9)	23 (3)
Asthma	16 (8)	23 (3)
Sinusitis	12 (6)	8 (1)
Urticaria	2 (1)	0 (0)

Table 3: Clinical history of the subjects.

	Clinical Symptoms	Subjects	Subjects sensitized to bivalves
		(n=50), % (n)	(n=13), % (n)
Nasal	Running nose	64 (32)	77 (10)
	Blocked nose	58 (29)	77 (10)
	Sneezing	50 (25)	62 (8)
	Itchy nose	34 (17)	54 (7)
	Loss/decrease of sense of smell	2 (1)	8 (1)
	Mouth breathing/snoring	2 (1)	8 (1)
	Sniffling	2 (1)	8 (1)
Eye	Redness	40 (20)	62 (8)
	Itching	52 (26)	62 (8)
	Watery	26 (13)	23 (3)
	Dark circles	2 (1)	0 (0)
	Puffiness	8 (4)	15 (2)
	Eye discharge	12 (6)	8 (1)
Sinus	Headaches	6 (3)	8 (1)
	Sore throats	2 (1)	8 (1)

	Post nasal drip	2 (1)	0 (0)
	Bad breath	4 (2)	8 (1)
	Hoarseness	2 (1)	0 (0)
	Throat itchiness	2 (1)	8 (1)
Skin	Rash	8 (4)	15 (2)
	Hives	10 (5)	8 (1)
	Eczema	12 (6)	15 (2)
	Pruritus	6 (3)	0 (0)
	Blisters	2 (1)	0 (0)
Ear	Ear discharge	2 (1)	0 (0)
	Painful	2 (1)	0 (0)
	Ringing	2 (1)	0 (0)
	Itching	2 (1)	0 (0)
Chest	Wheezing	2 (1)	0 (0)
	Coughing	6 (3)	8 (1)
	Tightness	2 (1)	0 (0)
	Shortness of breath	2 (1)	8 (1)

Table 4: Clinical symptoms of the subjects.

Skin prick test (SPT) reactivity

Table 5 shows the SPT reactivity to various allergens tested. Most of the subjects showed positive reactions to aeroallergens and seafood allergens. Mites were among the aeroallergens that elicited the highest sensitivity in 76% (n=38) of the subjects while amongst the seafood allergens, prawn was found to be the most common allergen in 48% (=24) subjects.

Allergens		Subjects
		(n=50), % (n)
Seafood	Malaysian cockle	22 (11)
	Carpet clam	12 (6)
	Tropical oyster	4 (2)
	Asian clam	4 (2)
	Asian green mussel	4 (2)
	Crab	30 (15)
	Prawns	48 (24)
	Squid	10 (5)
	Snails	6 (3)
	Fish	14 (7)
Foods	Egg	2 (1)
	Fruits	4 (2)
	Meats	6 (3)
	Nuts	10 (5)
	Cereals	16 (8)
	Honey	12 (6)
	Royal jelly	12 (6)
	Black sesame	12 (6)
Aeroallergens	Latex	4 (2)
	Grass	8 (4)
	Pollen	8 (4)
	Cockroach	28 (14)
	Cat dander	30 (15)
	Mites	76 (38)
Microorganisms	Yeast	10 (5)
	Fungal	10 (5)
	Anisakis simplex	8 (4)

Table 5: SPT positivity to various allergens.

Amongst the bivalve extracts tested, Malaysian cockle elicited the highest frequency of positive skin test reactivity in 22% (n=11) of the subjects. Carpet clam was the second most common bivalve to elicit a positive reaction in the SPT, 12%, followed by Asian clam, 4%, Tropical oyster, 4% and Asian green mussel, 4%.

In the group of subjects that were sensitized to bivalves, more than half of the subjects, 61% (n=8) displayed a positive skin reaction to a single bivalve extract (monosensitive) and 23% (n=3) were in the oligosensitive group with positive skin reactions to two bivalve extracts. Only one patient had positive skin reaction to all the bivalve allergens tested (Table 6).

Number of bivalve allergens	Number of subjects positive SPT
	(n=13), % (n)
1	61 (8)
2	23 (3)
3	0 (0)
4	8 (1)
5	8 (1)

Table 6: Distribution of positive SPT in the group of subjects that were sensitized to bivalves.

Among subjects positive to bivalves, significant cross sensitization was found between bivalves and different types of allergens; specifically crustacean shellfish (prawn and crab), arachnids (mites), insects (cockroach) and other molluscan shellfish (squid) (Table 7).

Discussion

SPT is a safe, sensitive and rapid method for screening patients with suspected allergy to shellfish [27,28]. However, the limited availability of commercial SPT reagents for this group [29] indicates a need for the production of in-house bivalve allergen extracts for SPT.

Allergens		P-value
Seafoods	Crab	< 0.001*
	Prawns	0.003*
	Squid	0.0006*
	Snails	0.16
	Fish	0.06
	Egg	0.26
	Fruits	0.06
	Meats	0.16
Foods	Nuts	0.1
	Cereals	0.66
	Honey	0.32
	Royal jelly	0.32
	Black sesame	0.32
	Latex	0.06
	Grass	0.05
Aeroallergens	Pollen	0.05
	Cockroach	0.00002 *
	Cat dander	0.17
	Mites	0.022 *
	Yeast	0.1
Microorganisms	Fungal	0.1
	Anisakis simplex	0.05

Table 7: Association of bivalve allergens SPT results with other allergens.

The SDS-PAGE of the 5 bivalve extracts revealed individual protein profiles that varied considerably although the majority of visible protein bands lie within the 25-100 kDa range. These differences might be influenced by endogenous and exogenous factors [4,30]. The endogenous factors consist of genetic differences, physiological status, reproductive cycle and feeding habits. The exogenous factors include habitat, abundance of food available, temperature, size, dissolved organic matter/debris, soil composition, starvation and time available for feeding [4,30].

Allergic reactions to bivalves occur not only after ingestion of cooked bivalves, but they may also be triggered by raw bivalves, particularly after direct contact [23,24] or inhalation of bivalve odours or fumes [20-22]. Therefore, in this study, the raw extract was used in skin prick tests, predominantly to avoid missing IgE-binding proteins. Additionally, raw extract contains both thermostable and thermolabile proteins, whereas the cooked extract comprises only thermostable proteins.

In our study, it appears that the majority of our subjects were allergic rhinitis patients. Allergic rhinitis is a highly prevalent disease affecting 20% of the general population in developed countries [31]. The most common cause of allergic rhinitis is allergic sensitization to aeroallergens with most persistent aeroallergens being found indoors [31]. Mites are the best described persistent indoor allergens and the warm and humid tropical climates offer favourable conditions for them to thrive. In our skin prick test, house dust mites elicited the highest positive reactions of all allergens studied.

Furthermore, the present study was carried out in the Allergy Clinic, Hospital Kuala Lumpur, which caters for patients who are mainly urban dwellers. Recent epidemiological studies suggest that urban living and exposure to air pollution at home and work are risk factors for allergic rhinitis [32,33]. This may be due to the exposure to ozone, nitrogen dioxide and especially particulate matter related to traffic and industrial activities [32]. In contrast, those who live in rural areas showed a considerably lower prevalence of allergic rhinitis [33,34].

In this study, amongst the bivalve extracts tested, Malaysian cockle was found to elicit the highest frequency of skin test reactivity. The reason for this is not clear. However, it could be that this is due to the low price of the Malaysian cockle in the local markets and its consequent higher consumption as compared to other bivalve species.

It is apparent that an individual sensitized to a bivalve, is also likely to be sensitized to crustacean shellfish (prawn and crab), arachnids (mites), insects (cockroach) and other molluscan shellfish (squid). This is possibly due to the IgE cross-reactivity of proteins which could be derived amongst the crustaceans itself, molluscs itself, between crustaceans and molluscs, and between crustaceans and terrestrial arthropods, such as cockroaches and mites [35]. Cross-reactivity is caused by homologous proteins containing conserved sequence motifs, which are IgE-binding epitopes [35].

The principal allergen in many molluscs is the protein tropomyosin, which is also the major allergen in many crustaceans [8,36-42]. Tropomyosin is also considered to be responsible for cross-reactivity between other arthropods such as mites or cockroach [43,44]. Tropomyosin showed very high homologies of up to 98% among crustacean species, including crawfish, crab and lobster [1]. Crustacean allergic subjects also often react to species of the mollusc group. Leung et al. [45] demonstrated *in vitro* that sera from nine crustacean allergic patients had IgE binding to antigens from all ten mollusc species tested. Furthermore, Reese et al. [46] reported that the amino acid sequence identity between mollusc tropomyosins varies from 68 to 88% and between crustaceans and molluscs is 56-68%. Reese et al. [46] also demonstrated that tropomyosin from house dust mite has 75-80% homology to shrimp and fruitfly tropomyosin and 65% homology to mollusc tropomyosin. Tropomyosins from house dust mite and cockroach have high sequence identities to shellfish tropomyosin of around 80%.

In conclusion, this study showed that of the five different bivalve species, cockle was found to have the highest frequency in skin test reactivity. It appears that an individual who is sensitized to a mite, is also likely to be sensitized to bivalves. However, these preliminary

findings are derived from a relatively small group of subjects and extension of this study with a larger study group is clearly warranted.

Acknowledgements

The authors thank the Director General of Health, Malaysia for permission to publish this paper. This work was supported by a grant UPSI 2013-0197-102-01.

Conflicts of Interest

We declare that we have no conflicts of interest.

References

1. Woo CK, Bahna SL (2011) Not all shellfish "allergy" is allergy! Clin Transl Allergy 1: 3.
2. Wild LG, Lehrer SB (2005) Fish and shellfish allergy. Curr Allergy Asthma Rep 5: 74-79.
3. Laxmilatha P (2009) Proximate composition of the surf clam Mactra violacea (Gmelin 1791). Indian J Fish 56: 147-150.
4. Li G, Li J, Li D (2010) Seasonal variation in nutrient composition of Mytilus coruscus from China. J Agric Food Chem 58: 7831-7837.
5. Lee AJ, Gerez I, Shek LP, Lee BW (2012) Shellfish allergy--an Asia-Pacific perspective. Asian Pac J Allergy Immunol 30: 3-10.
6. Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) (2001) Evaluation of allergenicity of genetically modified foods. Report of a joint FAO/WHO expert consultation of allergenicity of foods derived from biotechnology.
7. European Food Safety Authority (EFSA) (2006) Opinion of the scientific panel on dietetic products, nutrition and allergies on a request from the Commission related to the evaluation of mollusks for labeling purposes. EFSA J 327: 1-25.
8. Taylor SL (2008) Molluscan shellfish allergy. Adv Food Nutr Res 54: 139-177.
9. Rancé F, Grandmottet X, Grandjean H (2005) Prevalence and main characteristics of schoolchildren diagnosed with food allergies in France. Clin Exp Allergy 35: 167-172.
10. Castillo R, Delgado J, Quiralte J, Blanco C, Carrillo T (1996) Food hypersensitivity among adult patients: epidemiological and clinical aspects. Allergol Immunopathol (Madr) 24: 93-97.
11. Zinn C, Lopata A, Visser M, Potter PC (1997) The spectrum of allergy to South African bony fish (Teleosti). Evaluation by double-blind, placebo-controlled challenge. S Afr Med J 87: 146-152.
12. Arai Y, Sano Y, Ito K, Iwasaki E, Mukouyama T, et al. (1998) [Food and food additives hypersensitivity in adult asthmatics. I. Skin scratch test with food allergens and food challenge in adult asthmatics]. Arerugi 47: 658-666.
13. Tomikawa M, Suzuki N, Urisu A, Tsuburai T, Ito S, et al. (2006) [Characteristics of shrimp allergy from childhood to adulthood in Japan]. Arerugi 55: 1536-1542.
14. Parker SL, Leznoff A, Sussman GL, Tarlo SM, Krondl M (1990) Characteristics of patients with food-related complaints. J Allergy Clin Immunol 86: 503-511.
15. Novembre E, Cianferoni A, Bernardini R, Mugnaini L, Caffarelli C, et al. (1998) Anaphylaxis in children: clinical and allergologic features. Pediatrics 101: E8.
16. Nettis E, Pannofino A, Dambra P, Loria MP, Di Maggio G, et al. (2001) IgE-mediated urticaria/angioedema after ingestion of mussels. Acta Derm Venereol 81: 62.
17. Zuberbier T, Edenharter G, Worm M, Ehlers I, Reimann S, et al. (2004) Prevalence of adverse reactions to food in Germany - a population study. Allergy 59: 338-345.
18. Eriksson NE, Möller C, Werner S, Magnusson J, Bengtsson U, et al. (2004) Self-reported food hypersensitivity in Sweden, Denmark, Estonia, Lithuania, and Russia. J Investig Allergol Clin Immunol 14: 70-79.
19. Vickery BP, Chin S, Burks AW (2011) Pathophysiology of food allergy. Pediatr Clin North Am 58: 363-376.
20. Nakashima T (1969) Studies on bronchial asthma observed in cultured oyster workers. Hiroshima J Med Sci 18: 141-184.
21. Desjardins A, Malo JL, L'Archevêque J, Cartier A, McCants M, et al. (1995) Occupational IgE-mediated sensitization and asthma caused by clam and shrimp. J Allergy Clin Immunol 96: 608-617.
22. Jeebhay MF, Robins TG, Lehrer SB, Lopata AL (2001) Occupational seafood allergy: a review. Occup Environ Med 58: 553-562.
23. Freeman S, Rosen RH (1991) Urticarial contact dermatitis in food handlers. Med J Aust 155: 91-94.
24. Glass WI, Power P, Burt R, Fishwick D, Bradshaw LM, et al. (1998) Work-related respiratory symptoms and lung function in New Zealand mussel openers. Am J Ind Med 34: 163-168.
25. Gendeh BS, Murad S, Razi AM, Abdullah N, Mohamed AS, et al. (2000) Skin prick test reactivity to foods in adult Malaysians with rhinitis. Otolaryngol Head Neck Surg 122: 758-762.
26. Hamli H, Idris MH, Kamal ABM, Wong SK (2012) Diversity of Edible Mollusc (Gastropoda and Bivalvia) at Selected Divison of Sarawak, Malaysia. International Journal on Advanced Science, Engineering and Information Technology 2: 5-7.
27. Wu AY, Williams GA (2004) Clinical characteristics and pattern of skin test reactivities in shellfish allergy patients in Hong Kong. Allergy Asthma Proc 25: 237-242.
28. Lopata AL, Kamath S (2012) Shellfish allergy diagnosis- gaps and needs. Curr Allergy Clin Immunol 25: 60-66.
29. Pedrosa M, Boyano-Martínez T, García-Ara C, Quirce S (2014) Shellfish Allergy: a Comprehensive Review. Clin Rev Allergy Immunol .
30. Singh YT, Krishnamoorthy M, Thippeswamy S (2012) Seasonal changes in the biochemical composition of wedge clam, Donaxscortum from the Padukere beach, Karnataka. Recent Res Sci Technol 4: 12-17.
31. Asha'ari ZA, Yusof S, Ismail R, Che Hussin CM (2010) Clinical features of allergic rhinitis and skin prick test analysis based on the ARIA classification: a preliminary study in Malaysia. Ann Acad Med Singapore 39: 619-624.
32. Hedlund U, Rönmark E, Eriksson K, Lundbäck B, Järvholm B (2008) Occupational exposure to dust, gases and fumes, a family history of asthma and impaired respiratory health. Scand J Work Environ Health 34: 381-386.
33. Lám HT, Ván T Tng N, Ekerljung L, Rönmark E, Lundbäck B (2011) Allergic rhinitis in northern vietnam: increased risk of urban living according to a large population survey. Clin Transl Allergy 1: 7.
34. Eriksson J, Ekerljung L, Lötvall J, Pullerits T, Wennergren G, et al. (2010) Growing up on a farm leads to lifelong protection against allergic rhinitis. Allergy 65: 1397-1403.
35. Liu GM, Cheng H, Nesbit JB, Su WJ, Cao MJ, et al. (2010) Effects of boiling on the IgE-binding properties of tropomyosin of shrimp (Litopenaeus vannamei). J Food Sci 75: T1-5.
36. Emoto A, IshizakiS, Shiomi K (2009) Tropomyosins in gastropods and bivalves: Identification as major allergens and amino acid sequence features. Food Chem 114: 634-641.
37. Abdel Rahman AM, Lopata AL, O'Hehir RE, Robinson JJ, Banoub JH, et al. (2010) Characterization and de novo sequencing of snow crab tropomyosin enzymatic peptides by both electrospray ionization and matrix-assisted laser desorption ionization QqToF tandem mass spectrometry. J Mass Spectrom 45: 372-381.
38. Rosmilah M, Shahnaz M, Zailatul HM, Noormalin A, Normilah I (2012) Identification of tropomyosin and arginine kinase as major allergens of Portunus pelagicus (blue swimming crab). Trop Biomed 29: 467-478.
39. Misnan R, Murad S, Yadzir ZH, Abdullah N (2012) Identification of the major allergens of Charybdis feriatus (red crab) and its cross-reactivity

with Portunus pelagicus (blue crab). Asian Pac J Allergy Immunol 30: 285-293.

40. Yadzir ZH, Misnan R, Abdullah N, Bakhtiar F, Arip M, et al. (2012) Identification of the major allergen of Macrobrachium rosenbergii (giant freshwater prawn). Asian Pac J Trop Biomed 2: 50-54.
41. Yadzir ZH, Misnan R, Murad S (2012) Identification of tropomyosin as major allergen of white squid (Loligo edulis) by two-dimensional immunoblotting and mass spectrometry. Southeast Asian J Trop Med Public Health 43: 185-191.
42. Abramovitch JB, Kamath S, Varese N, Zubrinich C, Lopata AL, et al. (2013) IgE Reactivity of Blue Swimmer Crab (Portunus pelagicus) Tropomyosin, Por p 1, and Other Allergens; Cross-Reactivity with Black Tiger Prawn and Effects of Heating. PLoS One 8: e67487.
43. Ayuso R, Reese G, Leong-Kee S, Plante M, Lehrer SB (2002) Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. Int Arch Allergy Immunol 129: 38-48.
44. DeWitt AM, Mattsson L, Lauer I, Reese G, Lidholm J (2004) Recombinant tropomyosin from Penaeus aztecus (rPen a 1) for measurement of specific immunoglobulin IgE antibodies relevant in food allergy to crustaceans and other invertebrates. Mol Nutr Food Res 48: 370-379.
45. Leung PS, Chow WK, Duffey S, Kwan HS, Gershwin ME, et al. (1996) IgE reactivity against a cross-reactive allergen in crustacea and mollusca: evidence for tropomyosin as the common allergen. J Allergy Clin Immunol 98: 954-961.
46. Reese G, Ayuso R, Lehrer SB (1999) Tropomyosin: an invertebrate pan-allergen. Int Arch Allergy Immunol 119: 247-258.