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Immediate Hypersensitivity to Marine Snail (*Cerithidea obtusa*) Allergen among Local Patients with Atopic Diseases

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Abstract

Snail allergy is rare, but it can lead to severe allergic reactions. Life-threatening reactions including anaphylactic shock have been often reported after snail consumption. *Cerithidea obtusa* is a commonly consumed sea snail in Malaysia and other Asian countries. The purpose of this study was to determine the frequency of immediate hypersensitivity reactions to this species of snail by skin prick test (SPT). Snail proteins were extracted from the snail flesh. A total of 131 patients with atopic diseases were skin prick tested with the snail extract. Out of 131 subjects, 10 (8%) were sensitized to *C. obtusa* extract. Most of these subjects are allergic rhinitis (70%), allergic conjunctivitis (60%), asthma (20%) and food allergic (20%) patients. As a conclusion, this study showed that the immediate hypersensitivity to marine snail, *C. obtusa* exists among local atopic patients at a low frequency of 8%. It seems like a patient with *C. obtusa* sensitization is also probably to be sensitized to other shellfish.

Keywords allergy, snail, *cerithidea obtusa,* skin prick test, cross-reactivity

INTRODUCTION

Immediate hypersensitivity to food is a major health problem in many parts of the world [1, 2]. The food allergy data demonstrated significant differences between Asian and Western populations. It was reported that the prevalence of shellfish allergy is higher among Asian populations than Western populations, as shellfish consumption varies between countries [3]. In Malaysia, shellfish were described as the most common food allergens among local allergic rhinitis and asthma patients, with the prevalence of 78% and 16% respectively [4], as this shellfish is one of the major components of the local diet [5].

To date, molluscan shellfish allergy is increasingly recognized [2]. Shellfish was included in the list of most commonly allergenic foods in Europe [2]. Among molluscs, snail is a popular delicacy among Asian, European, North American and Australian populations [2, 5]. However, snail was also considered as an allergen which can cause life-threatening allergic reactions. Ingestion of snail developed severe respiratory symptoms primarily asthma and rhinitis in hypersensitive individuals [6]. Severe systemic symptoms such as anaphylactic shock can also occur after consumption of snails, and several death cases were documented [7].

To date, the most often caused of snail allergies in American and European countries are *Helix aspersa, Helix terrestre, Helix pomatia, Bolinus brandaris, Theba pisana* and *Cernuella virgate* [2, 7]. However, none of the snails are commonly consumed in Malaysia. Instead, *Cerithidea obtusa*, locally known as obtuse horn shell or 'siput sedut' is an edible snail in coastal areas of Asian countries including Malaysia [5]. Thus, this study aimed to determine the prevalence of snail allergy in Malaysian atopic population by skin prick test (SPT).

MATERIALS AND METHODS

Preparation of Snail Extract

Live *Cerithidea obtusa* samples were collected from a local supplier in Tanjong Malim, Perak. Snails were dissected and their whole body tissues were removed and washed in deionised water. In brief, the snail body tissues were homogenized in 0.1M phosphate buffered solution (PBS), pH 7.2 (1:10 weight/volume). The extraction was carried out overnight for 8 hours at 4 °C with continuous stirring. After centrifugation for 15 minutes at 14,000 rpm at 4 °C, the supernatant was passed through a sterile 0.22 µm syringe filter. The filtrate was freeze-dried and stored at -20 °C [2].

Subjects

131 patients with atopic diseases referred to Allergy Clinic, Hospital Kuala Lumpur were selected as the subjects in this study. The total number of subjects was determined based on the latest prevalence data of seafood allergy at 5.2% in the nearest Asian country, Singapore [8]. The precision (d = 0.04) was used, following the method of Pourhoseingholi *et al.* [9]. Purposive sampling (non-probability sampling) technique was used to recruit the subjects. Patients who meet the predetermined inclusion and exclusion criteria (Table 1) were selected as subjects.

Table 1 Inclusion and exclusion criteria of subjects

Inclusion criteria	Exclusion criteria
Aged ≥ 18 years old	Pregnant women
Had a clinical history of atopic diseases such as allergic rhinitis, allergic conjunctivitis, asthma and sinusitis	Taking anti-histamine medications within 72 hours of consultation
Have positive Skin prick test (SPT) results to at least one type of allergen tested	Have chronic skin disease (dermographism)

The atopic status of the subjects was screened by a detailed questionnaire and confirmed by skin prick test (SPT). Allergic responses to *C. obtusa* allergens were then determined by SPT to the raw *C. obtusa* extract. The clinical history and clinical symptoms of the subjects were recorded. A full verbal explanation of the research was given and consent form was collected from all subjects. Ethical approval has been obtained from Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia prior to conducting this study. This study was performed for 20 months from Mei 2014 to Dec 2015.

Skin Prick Test

All subjects were skin prick tested with a panel of 19 food allergens and 9 common aeroallergens. Briefly, SPT was performed by placing a drop (5-10 µl) of allergen extracts on the forearm of the subjects, followed by pricking the skin with a sterile lancet. Histamine solution (1 mg/mL) as a positive control and Coca solutions (PBS with 50% glycerol) as a negative control were included in the test. After 20 minutes, the SPT reaction was measured according to the international guidelines [4]. A mean wheal diameter of 3 mm or more compared with the negative control was regarded as a positive reaction.

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 Statistical Package for Social Sciences (SPSS) software version 20. The results were declared to be statistically significant if the *P*-values is less than 0.05.

RESULTS AND DISCUSSION

Demographic Data

A total of 131 subjects, 54 males and 77 females, with atopic diseases were studied. Malay constituted the majority (69%), followed by Chinese at 14%, Indian (13%) and others (4%). 8% of the Malay subjects (8/91) and 11% of the Chinese subjects (2/18) were sensitized to *C. obtusa*. This value showed that *C. obtusa* sensitization appeared to be slightly more prevalent among Chinese 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 [4].

The age of all subjects were ranging between 19 to 63 years with the mean age of 34.7 years. Meanwhile, subjects sensitized to *C. obtusa* were in the age group of 18 to 44 years. No *C.obtusa*-allergic subjects were in the 45-54 and more than 55 years age groups.

Table 2 demonstrated that females are more sensitized (60%) to *C. obtusa* compared to males (40%). Interestingly, this finding is in accordance with other studies that also showed female predominance in their allergy studies [10, 11].

Table 2 Demographic features of the subjects

	Demographic data	All subjects	Subjects sensitized to *Cerithidea obtusa*
		% (n) (n=131)	% (n) (n=10)
Gender	Male	41 (54)	40 (4)
	Female	59 (77)	60 (6)
Ethnicity	Malay	69 (91)	80 (8)
	Chinese	14 (18)	20 (2)
	Indian	13 (17)	0 (0)
	Others	4 (5)	0 (0)
Age	18-24	34 (45)	50 (5)
	25-34	40 (52)	40 (4)
	35-44	13 (17)	10 (1)
	45-54	7 (9)	0 (0)
	≥55	6 (8)	0 (0)

Clinical History and Symptoms of Allergy

Clinical histories of the subjects were shown in Table 3. Majority of the subjects have histories of allergic rhinitis (74%). Among *C. obtusa*-sensitized subjects, 70% were patients with allergic rhinitis, 60% were allergic conjunctivitis patients, followed by asthma (20%) and urticaria (10%). No patient with eczema and sinusitis was sensitized to *C. obtusa*.

Table 3 Clinical history of the subjects

Clinical History	All subjects	Subjects sensitized to *Cerithidea obtusa*
	% (n) (n=131)	% (n) (n=10)
Allergic rhinitis	74 (97)	70 (7)
Allergic conjunctivitis	40 (51)	60 (6)
Eczema	10 (13)	0 (0)
Asthma	15 (20)	20 (2)
Sinusitis	8 (11)	0 (0)
Urticaria	7 (9)	10 (1)

Allergic rhinitis appeared as the majority clinical history of our subjects. It was reported that allergic rhinitis affecting about 20% of the population across regions [4]. 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 [12]. 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 air pollution [12, 13].

The clinical symptoms of subjects were presented in Table 4. Most of the *C. obtusa*-sensitized subjects (80%) have clinical symptoms of running nose and eye itching. Blocked nose showed the second higher of symptoms (70%).

Table 4 Clinical symptoms of the subjects

	Clinical Symptoms	**All subjects**	**Subjects sensitized to *Cerithidea obtusa***
		% (n) (n=131)	**% (n) (n=10)**
Nasal	Running nose	62 (81)	80 (8)
	Blocked nose	61 (80)	70 (7)
	Sneezing	57 (75)	50 (5)
	Itchy nose	40 (53)	50 (5)
	Nose bleeds	1 (1)	10 (1)
	Loss/ decrease of sense of smell	5 (7)	20 (2)
	Mouth breathing/ snoring	2 (3)	20 (2)
	Sniffling	1 (1)	10 (1)
Eye	Redness	31 (40)	60 (6)
	Itching	44 (58)	80 (8)
	Watery	30 (39)	50 (5)
	Dark circles	3 (4)	0 (0)
	Puffiness	5 (6)	0 (0)
	Eye discharge	7 (9)	0 (0)
Sinus	Headaches	12 (16)	20 (2)
	Sore throats	12 (3)	10 (1)
	Post nasal drip	8 (10)	0 (0)

	Clinical Symptoms	All subjects	Subjects sensitized to *Cerithidea obtusa*
		% (n) (n=131)	% (n) (n=10)
	Bad breath	5 (7)	10 (1)
	Hoarseness	1 (1)	0 (0)
	Throats clearing	1 (1)	0 (0)
	Throat itchiness	2 (2)	10 (1)
Skin	Rash	5 (6)	10 (1)
	Hives	6 (8)	10 (1)
	Eczema	8 (11)	0 (0)
	Swelling	2 (2)	0 (0)
	Pruritus	6 (8)	0 (0)
	Blisters	2 (2)	0 (0)
Ear	Ear discharge	3 (4)	0 (0)
	Painful	2 (2)	0 (0)
	Ringing	3 (3)	10 (1)
	Hearing loss	4 (5)	0 (0)
	Itching	8 (10)	20 (2)
Chest	Wheezing	4 (5)	0 (0)
	Coughing	12 (3)	0 (0)
	Tightness	1 (1)	0 (0)
	Shortness of breath	2 (2)	0 (0)
	Bronchitis	0 (0)	0 (0)
	Palpitation	0 (0)	0 (0)

Skin Prick Test Reactivity

Skin Prick Test (SPT) is considered as the primary mode of skin testing to diagnose IgE-mediated allergic diseases in patients. SPT is inexpensive, sensitive and produces immediate result [14]. In this study, all subjects were skin prick tested to *C. obtusa* extract along with other allergen extracts used in the standard panel for allergy diagnosis at the clinic, including seafood, foods, aeroallergens and microorganisms. Results were recorded in Table 5.

Table 5 Frequency of SPT positivity to various allergens among subjects

Allergen groups	Allergen	All Subjects	*C. obtusa*-sensitized subjects
		% (n) (n=131)	% (n) (n=10)
Seafood	Marine snail (*Cerithidea obtusa*)	8 (10)	100 (10)
	Freshwater snail	9 (12)	60 (6)
	Malaysian cockle	15 (19)	90 (9)
	Carpet clam	13 (17)	70 (7)
	Tropical oyster	6 (8)	40 (4)
	Asian clam	9 (12)	40 (4)
	Asian green mussel	5 (7)	30 (3)

	Crab	21 (27)	50 (5)
	Prawns	37 (48)	70 (7)
	Squid	9 (12)	40 (4)
	Fish	14 (18)	50 (5)
Foods	Egg	3 (4)	10 (1)
	Fruits	8 (10)	20 (2)
	Meats	5 (7)	0 (0)
	Nuts	7 (9)	20 (2)
	Cereals	13 (17)	40 (4)
	Honey	7 (9)	20 (2)
	Royal jelly	11 (14)	20 (2)
	Black sesame	9 (12)	30 (3)
Aeroallergens	Latex	0 (1)	10 (1)
	Grass	3 (4)	0 (0)
	Pollen	2 (2)	0 (0)
	Cockroach	18 (23)	30 (3)
	Cat dander	21 (28)	40 (4)
	Mites	85 (111)	80 (8)
Microorganisms	Yeast	3 (4)	10 (1)
	Fungal	11 (14)	20 (2)
	Anisakis simplex	4 (5)	20 (2)

Most of the subjects have SPT positivity to seafood and aeroallergens. Prawn was among the seafood allergens that caused the highest sensitivity in 37% of the subjects, followed by crab (21%), cockle (15%) and fish (14%). 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 [4].

Among the *C.obtusa*-sensitized subjects, cockle triggered the highest frequency of positive SPT reactivity at 90%, followed by carpet clam and prawns (70%). This is not surprising as snails, cockles and clams are grouped under same phylum, Mollusca [2], and highly consumed by local people [4].

In this study, mites showed the highest SPT positivity among all subjects (85%) as well as among *C. obtusa*-sensitized subjects (80%). Malaysia has a tropical climate with warm and humid 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 study, in accordance with other reports in other Asian countries with similar climate [15]. HDM is well-demonstrated as a main allergen source that induced rhinitis, asthma and atopic dermatitis [3, 16]. Ambient temperatures and high humidity offer favourable conditions for HDM to thrive in Malaysia [15] and in other Asian countries with similar climates such as Thailand, Vietnam, Hong Kong and Indonesia [17]. In Asia, HDM is the major triggering allergen followed by pollens, insects and fungi [3].

In addition, it was reported that the prevalence of allergic diseases was higher in westernized and developed cities than in rural areas [4, 17]. 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 [13].

Amongst food allergens tested, cereal was found to be the most prevalent allergen in 13% of all subjects and 40% among *C. obtusa*-allergic subjects. It was interesting to note that none of the *C. obtusa*-sensitized subjects showed positive SPT reactions to meat, grass and pollen, while only one subject demonstrated positive SPT toward egg, latex and yeast allergens.

Table 6 Association of SPT positivity between *C. obtusa* and other allergens

Allergens		***P-value***	***R-value***
Seafood	Freshwater snail	0.00*	0.507
	Malaysian cockle	0.00*	0.616
	Carpet clam	0.00*	0.488
	Tropical oyster	0.00*	0.407
	Asian clam	0.00*	0.303
	Asian green mussel	0.01*	0.315
	Crab	0.03*	0.209
	Prawns	0.04*	0.199
	Squid	0.00*	0.307
	Fish	0.00*	0.303
Foods	Egg	0.27	
	Fruits	0.17	
	Meats	1.00	
	Nuts	0.14	
	Cereals	0.04*	0.231
	Honey	0.14	
	Royal jelly	0.29	
	Black sesame	0.05*	0.021
Aeroallergens	Latex	0.08	
	Grass	1.00	
	Pollen	1.00	
	Cockroach	0.38	
	Cat dander	0.21	
	Mites	0.68	
Microorganisms	Yeast	0.28	
	Fungal	0.29	
	Anisakis simplex	0.05*	0.243

Note. **P*-values less than 0.05

Table 6 shows the association of SPT positivity to *C. obtusa* and other allergens. Surprisingly, our study found significant cross-sensitizations but with moderate to weak positive association between *C. obtusa* and all seafood allergens tested. Thus, it was shown that a *C. obtusa*-sensitized individual has possibility to sensitive to crustacean and other molluscan shellfish. These may be related to IgE-cross-reactivity between similar epitopes in snail allergens and other allergens from different organisms [2, 7, 18, 19]. A significant with weak positive association was also seen between *C. obtusa* sensitization and an arthropod, *Anisakis simplex*, a fish parasite.

The cross-reactivity between molluscs, crustaceans and certain invertebrates has been extensively studied. Clinical evidence of polysensitization and co-sensitization to these allergens in seafood-allergic patients were well-documented [7, 18, 20]. Cross-reactivity could occur when a specific antibody, initially elevated against an allergen, reacts to a similar epitope in other allergen from different sources [20]. The presence of high structural homology between allergenic proteins from unrelated sources could be the underlying cause of this reaction [20]. The major allergen responsible for the immediate-hypersensitivity reactions in molluscs including snails is tropomyosin [1]. Tropomyosin played primary role in muscle function of all organisms and thus was considered as a pan-allergen [21], which was well-documented as the major cause of IgE-cross-reactivity between snails and other invertebrates mainly HDM [6, 7, 19]. However, our result did not suggest a significant association between snail and mite sensitizations, possibly due to a small number of subjects studied. In epidemiologic studies, small sample size will not yield a precise estimate and reliable answers to a study hypothesis [22].

CONCLUSION

This study showed that, among atopic population studied, the frequency of marine snail sensitization was found at the frequency of 8%. It appears that the snail-allergic individual has also possibility of being allergic to other shellfish allergens. Nevertheless, this insight is revealed from a relatively small number of subjects and additional study with a larger sample size is needed to confirm this finding.

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