รายงานวิจัยฉบับสมบูรณ์

โครงการ

การศึกษาหาสารก่อภูมิแพ้ที่จำเพาะของกุ้งก้ามกรามและกุ้งกุลาดำ ที่เป็นสาเหตุของการแพ้กุ้งในคนไทย

Proteomics and immunological analysis of novel *Macrobrachium rosenbergii* and *Penaeus monodon* allergens

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สัญญาเลขที่ MRG4880065

รายงานวิจัยฉบับสมบูรณ์

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Proteomics and immunological analysis of novel *Macrobrachium rosenbergii* and *Penaeus monodon* allergens

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สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษา และสำนักงานกองทุนสนับสนุนการวิจัย
(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกอ. และ สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

บทคัดย่อ

ที่มาของปัญหา

การแพ้กุ้งถือเป็นปัญหาที่สำคัญของการแพ้อาหารในเด็กไทย โดยทั่วไปผู้ป่วยที่แพ้กุ้งมักจะแพ้ทั้งกุ้งน้ำจืดและกุ้งน้ำเค็ม อย่างไรก็ดียังมีผู้ป่วยบางรายที่รายงานว่าแพ้กุ้งชนิดใดชนิดหนึ่ง ยังไม่เคยการศึกษาเพื่อหาสารก่อภูมิแพ้ที่จำเพาะของกุ้งกุลาดำและกุ้งก้ามกรามที่ไม่ข้ามกลุ่มกันมาก่อน ยิ่งไปกว่านั้น ยังไม่เคยมีการเปรียบเทียบผลของการทดสอบทางผิวหนังที่ได้จากสารสกัดจากกุ้งที่นำเข้าจากต่างประเทศ เทียบกับสารสกัดจากกุ้งกุลาดำ (กุ้งน้ำเค็ม) และกุ้งก้ามกราม (กุ้งน้ำจืด) และการทดสอบผิวหนังแบบ prick to prick test (PTP, เอาเข็มทดสอบสะกิดอาหารแล้วมาสะกิดผิวหนังของผู้ป่วย)

วัตถุประสงค์

1. เพื่อรายงานผู้ป่วยที่แพ้กุ้งกุลาดำ (*Penaeus monodon, Pm*) หรือกุ้งก้ามกราม (*Macrobrachium rosenbergii*, *Mr*) อย่างใดอย่างหนึ่งหรือทั้งสองอย่าง
2. เพื่อเปรียบเทียบผลการทดสอบทางผิวหนังที่ได้จากการทดสอบด้วยสารสกัดจากกุ้งที่นำเข้าจากต่างประเทศ (ComSPT) เทียบกับสารสกัดจากกุ้งกุลาดำ (*Pm*SPT) และกุ้งก้ามกราม (*Mr*SPT) และการทดสอบทางผิวหนังแบบ PTP (*Pm*PTP, *Mr*PTP)
3. เพื่อหากลุ่มสารก่อภูมิแพ้ที่จำเพาะต่อกุ้งกุลาดำหรือกุ้งก้ามกราม

วิธีการศึกษา

การศึกษาทำในผู้ป่วยเด็กไทย อายุมากกว่า 5 ปี ที่มีประวัติของการแพ้กุ้ง ผู้ป่วยจะได้รับการทดสอบผิวหนังโดยใช้สารสกัดจากกุ้งกุลาดำและกุ้งก้ามกราม และ PTP เปรียบเทียบกับสารสกัดจากกุ้งที่นำเข้าจากต่างประเทศ หลังจากนั้นผู้ป่วยจะได้รับการทดสอบโดยการรับประทานกุ้งกุลาดำหรือกุ้งก้ามกราม โดยจะทำห่างกัน 2-4 สัปดาห์ ซีรั่มของผู้ป่วยจะได้รับการวัด Specific IgE ต่อกุ้งกุลาดำและกุ้งก้ามกราม โดยการทำ Immunoblot, 2D-immunoelectrophoresis และกลุ่มสารก่อภูมิแพ้ของกุ้งทั้งสองชนิดจะได้รับการตรวจโดย mass fingerprint เพื่อหาสารก่อภูมิแพ้ที่เป็นไปได้

ผลการศึกษา

ผู้ป่วยเด็ก 68 รายสามารถถูกแยกได้เป็น 4 กลุ่ม จากผลของการทดสอบอาหาร ผู้ป่วยที่แพ้กุ้งกุลาดำอย่างเดียวพบร้อยละ 17.65 แพ้กุ้งก้ามกรามอย่างเดียวร้อยละ 23.53 แพ้กุ้งทั้งสองอย่างร้อยละ 47 ไม่แพ้ทั้งสองอย่างร้อยละ 11.76 จากการทดสอบทางผิวหนังพบความสัมพันธ์ระหว่าง ComPTP-*Pm*SPT, ComSPT-*Pm*PTP, ComSPT-*Mr*PTP, *Pm*SPT-*Pm*PTP และ *Mr*SPT-*Mr*PTP ในผู้ป่วยที่แพ้กุ้งกุลาดำ ขนาดของ *Pm*SPT ที่ $\geq$30 mm จะทำนายผลการทดสอบอาหารที่เป็นผลบวกได้ร้อยละ 80 ส่วนขนาดของ *Pm*PTP และ ComSPT ที่ $\geq$22.5 mm และ $\geq$20 mm ตามลำดับ จะทำนายผลการทดสอบอาหารที่เป็นบวกได้ร้อยละ 95 ในผู้ป่วยที่แพ้กุ้งก้ามกรามขนาดของ *Mr*SPT ที่ $\geq$30 mm จะทำนายผลการทดสอบอาหารที่เป็นบวกได้ร้อยละ 95 การทดสอบโดยการทำ

Immunoblot, 2D-immunoelectrophatsis และ Mass fingerprint พบสารก่อภูมิแพ้ที่จำเพาะของกุ้งกุลาดำที่อาจเป็นไปได้ ได้แก่ Lipid binding protein, Chemosensory protein, Nonmuscle myosin heavy chain-B, Oxygen transporter, Cdk inhibitor, และ Zinc ion binding. ส่วนสารก่อภูมิแพ้ของกุ้งก้ามกรามที่อาจเป็นไปได้ ได้แก่ Calcium ion binding protein, Heme binding protein, Amalyse inhibitor, Protein domain specific binding, Protein kinase activity, และ Chaperone protein. ทั้งนี้จำเป็นจะต้องได้รับการทดสอบเพิ่มเติมต่อไป

สรุป

ผู้ป่วยที่มีประวัติแพ้กุ้งอาจแพ้กุ้งน้ำเค็มหรือกุ้งน้ำจืด อย่างใดอย่างหนึ่งได้ ขนาดของการทดสอบทางผิวหนังโดยใช้สารสกัดจากกุ้งแต่ละชนิดหรือ PTP อาจช่วยทำนายผลการทดสอบอาหารที่เป็นบวก การทราบกลุ่มของสารก่อภูมิแพ้ที่จำเพาะในกุ้งแต่ละชนิดจะมีประโยชน์ที่จะผลิตชุดทดสอบการแพ้กุ้งหรือทำวัคซีนเพื่อรักษาโรคแพ้กุ้งในอนาคต

Abstract

Background: Allergy to specific shrimp species has not been systematically studied by oral challenges. Identification of the unique, non cross-reacting allergens among different shrimp species has never been reported. The concordance between skin test reactivity from commercial and crude shrimp extracts as well as from prick to prick (PTP) test has never been studied.

Objective: 1) To report cases of *Penaeus monodon (Pm*, seawater shrimp) or *Macrobrachium rosenbergii (Mr, freshwater shrimp)*-specific allergy among shrimp-allergic children. 2) To identify unique allergens from both *M. rosenbergii* and *P. monodon.* 3) To compare skin tests using commercial and crude shrimp extracts plus PTP method.

Methods: Children $\geq$ 5 years of age with history of shrimp allergy were recruited. Skin prick tests (SPT) using *Pm* (*Pm*SPT*)* and *Mr (Mr*SPT) extracts as well as PTP method (*Pm*PTP*, Mr*PTP) were done compared to commercial shrimp extract (ComSPT). Open challenges to both shrimp species were performed. Patients' serum was used to identify specific IgE to shrimp allergens by immunoblot and 2D-immunoelectrophoresis. Groups of potential allergens were studied by mass fingerprint analysis.

Results: Sixty-eight patients were divided into 4 groups by food challenges. Specific allergy to *Pm* and *Mr* were identified in 17.65% and 23.53% of subjects, respectively. Positive and negative challenges to both shrimp species were found in 47.06% and 11.76% of subjects, respectively. Correlations between mean wheal diameter (MWD) from ComSPT-*Pm*SPT*,* ComSPT-*Pm*PTP*,* ComSPT-*Mr*PTP*, Pm*SPT-*Pm*PTP and *Mr*SPT-*Mr*PTP were observed. In patients with *Pm* allergy, *Pm*SPT with MWD of 30 mm provided 80% positive predictive probability. *Pm*PTP and ComSPT MWD of 22.5 and 20 mm provided 95% positive predictive probability, respectively. In patients with *Mr* allergy, *Mr*SPT with MWD of 30 mm provided 95% positive predictive probability. After IgE-immunoblot, 2-D electrophoresis and mass fingerprint analysis, the candidate unique allergens from *P. monodon* were Lipid binding protein, Chemosensory protein, Nonmuscle myosin heavy chain-B, Oxygen transporter, Cdk inhibitor, and Zinc ion binding. The candidate unique allergens from *M. rosenbergii* were Calcium ion binding protein, Heme binding protein, Amalyse inhibitor, Protein domain specific binding, Protein kinase activity, and Chaperone protein. However, further experiments are needed to confirm that these allergens are specific to shrimp allergy.

Conclusion: Specific allergy to *Pm* or *Mr* confirmed by food challenges was demonstrated. Predictive probability of SPT may be helpful in the setting where food challenge is not feasible.

Groups of candidate unique allergens from both kinds of shrimp were identified. However, further experiments are needed to confirm that these allergens are specific to shrimp allergy.

หน้าสรุปโครงการ (Executive Summary)
ทุนพัฒนาศักยภาพในการทำงานวิจัยของอาจารย์รุ่นใหม่

1. **ชื่อโครงการ** (ภาษาไทย) การศึกษาหาสารก่อภูมิแพ้ที่จำเพาะของกุ้งก้ามกรามและกุ้งกุลาดำที่เป็นสาเหตุของการแพ้กุ้งในคนไทย

   (ภาษาอังกฤษ) Proteomics and immunological analysis of novel *Macrobrachium rosenbergii* and *Penaeus monodon* allergens

2. **ชื่อหัวหน้าโครงการ** หน่วยงานที่สังกัด ที่อยู่ หมายเลขโทรศัพท์ โทรสาร และ e-mail

   พ.ญ. อรทัย พิบูลโภคานันท์

   ภาควิชา กุมารเวชศาสตร์ คณะแพทย์ศาสตร์ศิริราชพยาบาล

   ถนน พรานนก เขตบางกอกน้อย จังหวัด กรุงเทพ รหัสไปรษณีย์ 10700

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3. **สาขาวิชาที่ทำการวิจัย** Molecular Biology for Medicine
4. **งบประมาณรวมทั้งโครงการ** 480,000 บาท
5. **ระยะเวลาดำเนินงาน** 2 ปี
6. **ชื่อนักวิจัยที่ปรึกษา** ศาสตราจารย์ น.พ. ปกิต วิชยานนท์
7. **ปัญหาที่ทำการวิจัย และความสำคัญของปัญหา**

Seafood is recognized as the common cause of food hypersensitivity reactions. The prevalence of seafood allergy in Thai adult and pediatric population is 65% and 66% respectively of all food allergic cases. Furthermore, shrimp has been identified as a major cause of allergic reaction (69%) among seafood allergic children referred to allergy clinic at Siriraj Hospital. Symptoms of shrimp allergy can develop in multiple organs such as skin, respiratory system, and gastrointestinal system. Severity of shrimp allergic reactions could cause dead. The shrimp allergic reactions also appear to be life-long despite shrimp avoidance. Also from an observation at the allergy clinic at Siriraj hospital, there are 2 subpopulations of shrimp allergic patients that are 1) allergic to only giant freshwater shrimp, *Macrobrachium rosenbergii* (กุ้งก้ามกราม, *M. rosenbergii*) and 2) allergic to only seawater shrimps, *Penaeus monodon* (กุ้งกุลาดำ, *P. monodon*) and *Fenneropenaeus merguiensis* (กุ้งแชบ๊วย, *F. merguiensis*). Because these three species of shrimp are the favorite consumed shrimps around the world including Thailand, it is important to identify unique novel antigens which are specifically found in either freshwater or seawater

shrimps. The finding of unique antigens would create a new knowledge and an awareness of freshwater shrimp allergy to allergists since there is no reports of freshwater shrimp allergy to date. Also information from this study could be further used to clone and produce recombinant antigens that could be a starting material in diagnostic kits as well as in future study of B and T cell epitopes of shrimp allergens. Shrimp allergy diagnostic kits could help to identify shrimp allergic patients faster and without anaphylaxis risk. The knowledge from the epitope study could be used in generating a safety and effective recombinant allergens, which is lack of IgE-binding capacity but retains T cell epitopes intact to allow tolerance, in future shrimp allergic immunotherapy.

8. วัตถุประสงค์

8.1 To report cases of species-specific allergy *to M. rosenbergii* (กุ้งก้ามกราม) or *P. monodon* (กุ้งกุลาดำ)

8.2 To compare skin tests reactions from commercial shrimp extract, crude extract of *M. rosenbergii* or *P. monodon*, or prick to prick test

8.3 To identify unique allergens from both *M. rosenbergii* and *P. monodon*

9. ระเบียบวิธีวิจัย

Shrimp allergic patients were skin prick tested using commercial shrimp extract (ComSPT), crude extract of *M. rosenbergii* (*Mr*SPT) and *P. monodon* (*Pm*SPT), as well as prick to prick test to *M. rosenbergii* (*Mr*PTP) and *P. monodon* (*Pm*PTP). Open-food challenges were performed before serum collection. The crude extracts were separated by SDS-PAGE gel before electroblotted onto a nitrocellulose membrane. The blotted extracts were incubated with the shrimp-allergic serum. Bound IgE Abs were detected using labeled antibody against human IgE. The crude extracts were analyzed by 2-D immunoblotting with pooled sera from shrimp allergic patients which showed high IgE binding to the specific band of allergen on Western blots, then bound IgE was detected using labeled antibody against human IgE. After identifying antigens, 2-D electrophoresis and immunoblotting were repeated. Blotted proteins were visualized by Ponceau S staining. The protein spots (antigens) were excised and sent for N-terminal sequence analysis.

10. แผนการดำเนินการ

Month	**1-6**	**7-12**	**13-18**	**19-24**
Protocol set up, preparation of equipments and lyophilized shrimp	X			
Get approval from the ethic committee	X			
Patient identification, skin testings and food challenges, serum collection	X	X		
Serum collection from control subjects	X	X		
Preparation of shrimp extract for immunoblot		X	X	
SDS-PAGE and immunoblotting with IgE			X	X
2-D immunoblotting			X	X
N-terminal microsequencing			X	X
Prepare for publications				X

11. **ผลงานที่คาดว่าจะตีพิมพ์ในวารสารวิชาการระดับนานาชาติ**

ชื่อเรื่องที่คาดว่าจะตีพิมพ์:

1. Specific allergy to *Penaeus monodon* (seawater-shrimp) or *Macrobrachium rosenbergii* (freshwater-shrimp) in shrimp allergic children
2. Proteomics and immunological analysis of the novel unique *M. rosenbergii* and *P Monodon* allergens (this paper will need more experiments to be able to publish in the high impact journal)

ชื่อวารสารที่คาดว่าจะตีพิมพ์: (อันใดอันหนึ่งเท่าที่จะทำได้)

- Journal of Allergy and Clinical Immunology, impact factor 7.6
- Allergy, impact factor 4.1
- Clinical and Experimental Allergy, impact factor 3.5
- International Archives of Allergy and Immunology, impact factor 2.2
- Annals of Allergy Asthma & Immunology, impact factor 1.9

13. **ผลการวิจัย**

Sixty-eight patients were divided into 4 groups by food challenges. Specific allergy to *P. monodon* and *M. rosenbergii* were 17.65% and 23.53%, respectively. Positive and negative challenges to both kinds of shrimp were 47.06% and 11.76%, respectively. Correlations between mean wheal diameter (MWD) from ComSPT-*Pm*SPT, ComSPT-*Pm*PTP, ComSPT-*Mr*PTP, *Pm*SPT-*Pm*PTP and *Mr*SPT-*Mr*PTP were observed. In patients with *P. monodon* allergy, *Pm*SPT provided 80% positive predictive probability at 30 mm. *Pm*PTP and ComSPT provided 95% positive predictive probability at 22.5 and 20 mm, respectively. In patients with *M. rosenbergii* allergy, *Mr*SPT provided 95% positive predictive probability at 30 mm. The immunoblotting of *P. monodon* extract with serum IgE from patients who had specific allergy to *P. monodon*, showed that 9/10 patients had specific IgE against 2-7 bands of *P. monodon* proteins. The immunoblotting of *M. rosenbergii* extract with serum IgE from patients who had specific allergy to *M. rosenbergii*, showed that 8/9 patients had specific IgE against more than 3 bands of *M. rosenbergii* proteins. After 2-D electrophoresis and mass fingerprint analysis, the candidate unique allergens from *P. monodon* were Lipid binding protein, Chemosensory protein, Nonmuscle myosin heavy chain-B, Oxygen transporter, Cdk inhibitor, and Zinc ion binding. The candidate unique allergens from *M. rosenbergii* were Calcium ion binding protein, Heme binding protein, Amalyse inhibitor, Protein domain specific binding, Protein kinase activity, and Chaperone protein.

14. **สรุปผลการวิจัย**

Specific allergy to *P. monodon* or *M. rosenbergii* was demonstrated. SPT using crude extracts and PTP are useful tools for screening shrimp sensitization. Predictive probability of SPT is helpful where food challenge is not feasible. Groups of candidate unique allergens from both kinds of shrimp were identified. However, further experiments are needed to confirm that these allergens are specific to shrimp allergy.

เนื้อหางานวิจัย

Introduction

Shellfish are recognized as a common cause of food hypersensitivity and the leading cause of food-induced anaphylaxis.[1, 2] The prevalence of seafood allergy in Thai adult and pediatric population is 65% and 66% respectively of all food allergic cases. Among shellfish allergies, shrimp is the most frequent culprit.[2, 3] Symptoms of shrimp allergy develop in multiple organs such as skin (52-90%), respiratory (42%), gastrointestinal (35%), and shock (10%).[4] The major shrimp allergen, tropomyosin, has been identified as a pan-allergen commonly found in other invertebrates such as lobster, crab, mollusk, squid, mussel, cockroach, and house dust mite.[5-8] Cross-reactivity between shrimp and other crustaceans has been demonstrated.[9] However, there was also a report of isolated allergy to specific shrimp species in patients with shrimp allergy.[10]

Two popular shrimp species, *Penaeus monodon (*กุ้งกุลาดำ, black tiger shrimp) and *Macrobrachium rosenbergii* (กุ้งก้ามกราม, giant fresh water prawn) are harvested in Thailand (Figure 1). *P. monodon* which is cultivated in saltwater, is the most widely distributed and marketed shrimp in the world. *M. rosenbergii* which is cultivated in freshwater, is increasingly recognized in the world-market due to its texture and palatability. Surprisingly, there is no report of *M. rosenbergii* allergy in any medical literature. From an observation at the allergy clinic, Siriraj hospital, Thailand, there are subpopulations of shrimp allergic patients who are allergic to only *M rosenbergii* but can tolerate *P. monodon* or vice versa. It is important to identify unique novel antigens which are specifically found in either freshwater or seawater shrimps. The finding of unique antigens would create a new knowledge and an awareness of freshwater shrimp allergy to allergists since there is no reports of freshwater shrimp allergy to date. Also information from this study could be further used to clone and produce recombinant antigens that could be a starting material in diagnostic kits as well as in future study of B and T cell epitopes of shrimp allergens. Shrimp allergy diagnostic kits could help to identify shrimp allergic patients faster and without anaphylaxis risk. The knowledge from the epitope study could be used in generating a safety and effective recombinant allergens, which is lack of IgE-binding capacity but retains T cell epitopes intact to allow tolerance, in future shrimp allergic immunotherapy.

Objectives

The objectives of this study were:

1. To report cases of species-specific allergy *to M. rosenbergii* (กุ้งก้ามกราม) or *P. monodon* (กุ้งกุลาดำ)
2. To compare skin tests reactions from commercial shrimp extract, crude extract of *M. rosenbergii* or *P. monodon*, or prick to prick test
3. To identify unique allergens from both *M. rosenbergii* and *P. monodon*

Materials and Methods

Subjects

Patients ≥ 5 years of age who came to the pediatric allergy clinic, Siriraj Hospital, Thailand, from June 1st, 2005 to December 31th, 2006, with a history of shrimp allergy were asked to participate in this study. The study was approved by the Ethic Committee, Siriraj Hospital. Informed consent was obtained from the patients (or parents if patients were <18 years of age). Patients with a history of severe anaphylactic reaction from seafood, pregnancy, underlying diseases such as cardiovascular (CVS), hepatobiliary, and renal diseases were excluded. None of the patients was on systemic corticosteroid or ß-blocking agent. Patients with allergic diseases were asymptomatic on the days of skin tests and food challenges.

Skin-test procedure11

The skin prick tests (SPT) using *P. monodon* and *M rosenbergii* extracts (*Pm*SPT and *Mr*SPT) as well as PTP method using cooked *P. monodon* and *M rosenbergii* (*Pm*PTP and *Mr*PTP, pricking the food and then pricking the skin of the patients) were performed with 10 mg/ml of histamine phosphate and glycerinated saline as positive and negative control. The *P. ionodon* and *M rosenbergii* extracts were freshly prepared by making the dilution of 1:10 (wt/volume) of lyophilized shrimp in glycerinated saline. This concentration was proved to be a non-irritating concentration in 10 non-atopic subjects (data not shown). SPT to commercial extracts (Center Laboratory, Port Washington, NY) of shrimp (ComSPT), *Dermatophagoides pteronyssinus (Dp)*; *Dermatophagoides farinae (Df)* and *Periplaneta americana* (American cockroach, *Pa*) were also done. Antihistamines were discontinued for ≥7 days prior to skin testing. The size of wheal and flare reactions was recorded in millimeters (mm). The mean of the largest and midpoint orthogonal diameters was designated as mean wheal diameter (MWD) and considered positive if it was ≥3 mm compared to negative control.

Food-challenged procedure[11-12]

Food challenges to *P. monodon and M rosenbergii* were done in patients with skin tests positive to ComSPT, *Pm*SPT, *Mr*SPT, *Pm*PTP or *Mr*PTP. Shrimp was eliminated from the diet for 2 weeks before challenge. The starting dose was 500 mg of lyophilized shrimp in capsule. The dose was doubled every 15 min. Cumulative provocation dose (PD) schedule of lyophilized shrimp was as follows: 500 mg, 1.5 g, 3.5 g, 7.5 g, and 15.5 g. The reason to use capsule was to avoid oral-mucosal reaction at the initial challenge. Young patients who could not swallow capsule were allowed to skip this process. To identify oral-mucosal reactions, 2 g of cooked

shrimp was wiped on inner lips and placed in the mouth for 5 min. Lips swelling/itching or throat itching were recorded as positive reactions. Fifteen minutes later, open-feeding to cooked shrimp were initiated at 1 gram and the doses were doubled every 15 minutes until reactions were observed or maximal dose was reached. Cumulative PD schedule of cooked shrimp was as follows: 1 g, 3 g, 7 g, 15 g, 31 g and 63 g. Vital signs as well as patient's symptoms and signs were recorded every 15 minutes. Emergency resuscitation equipments and drugs were available in case of emergency. Challenge to the other species of shrimp was conducted 2-4 weeks after the 1st challenge.

Preparation of shrimp extract for immunoblot

Extracts of *M. rosenbergii* and *P. monodon* were prepared separately. A piece of shrimp muscle was excised and ground in a mortar filled with liquid nitrogen, then was extracted in 50 mM PBS, pH 7.0, containing 0.2 mM DTT and 1 mM PMSF for 16 hr at 4°C with constant stirring. The extract was centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant was collected and dialyzed against 10 mM sodium phosphate buffer, pH 7.0, with several changes for 48 hr at 4°C and was stored at -20°C.

SDS-PAGE and immunoblotting with IgE

Three µl of crude extract were separated by a 15% SDS-PAGE gel. For immunodetection of IgE-binding proteins, the separated proteins were electroblotted onto a nitrocellulose membrane, which was then blocked with PBS-3% skimmed milk and incubated for 16 hr at 4°C with a 1/10 diluted shrimp-allergic serum in PBS-3% skimmed milk. Bound IgE Abs were detected using HRP-labeled goat anti-human IgE Abs (KPL, MD, USA). The chemiluminescence signal provided by ECL plus western blotting detection kit (GE Healthcare, UK) was detected by exposure to X-ray film.

2-D immunoblotting

Crude extracts were analyzed by 2-D immunoblotting. In brief, for the first dimension, 0.5 mg of shrimp extract was applied to an immobilized pH gradient gel strip containing pH range of 3–10 ampholytes, and isoelectric focusing was performed in a Multiphor II horizontal electrophoresis system (GE Healthcare, UK). After isoelectric focusing, the strip was subjected to SDS-PAGE on 12.5% gels. For specific IgE immunodetection, proteins on the 2-D gel were blotted onto a PVDF membrane, and incubated with pooled sera from shrimp allergic patients which showed high IgE binding to the specific band of allergen on Western blots. Bound IgE Abs were detected using HRP-labeled rabbit anti-human IgE Abs (KPL, MD, USA). The

chemiluminescence signal provided by ECL plus western blotting detection kit (GE Healthcare, UK) was detected by exposure to X-ray film.

Mass Fingerprint analysis

Two 2-D PAGE gels were performed in parallel. The spots indicating bound IgE-protein on the x-ray film was aligned with the identical 2-D PAGE gel stained by Coomassie Blue G-250. Matched spots, containing presumed allergens, on the stained 2-D PAGE gel were excised and subjected for in-gel digestion with trypsin. The digested peptides were analyzed by MALDI-TOF MS. The reported mass to charge ratio (m/z) of peptides were searched for matched m/z ratio of proteins deposited at www.ExPaSy.org.

Data collection and analysis

Data were expressed as individual values or the mean ± SD for group. Median and range were used for non-normally distributed data. Spearman's rho correlations were used to compare MWD from the different methods of skin tests in all patients. Comparison of MWD from skin tests between groups of challenge positive and negative patients was made using Mann-Whitney U test. Differences between groups were considered significant at a p value of ≤ 0.05. The logistic regression analysis was used to predict probabilities of the outcome of food challenges by MWD of different skin tests

Results

Demographic data

Seventy-two patients, who had a history of shrimp allergy and with positive skin test to either ComSPT, *Pm*SPT, *Pm*PTP, *Mr*SPT *Mr*PTP, were recruited. Four patients developed anaphylaxis on the 1st challenge and were excluded from the study. Three of these patients developed anaphylaxis to *P. monodon and* one to *M. rosenbergii,* Demographic data, clinical history, results of skin tests, cumulative PD of shrimp and symptoms upon challenges were shown in Table I-IV. Overall, the 68 patients who completed both challenges were divided into 4 groups; Group I consisted of patients who had positive food challenges to *P. monodon* only (n=12, 17.65%), Group II patients who had positive food challenges to *M. rosenbergii* only (n=16, 23.53%), Group III patients who had positive food challenges to both shrimp species (n=32, 47.06%), and Group IV patients who had negative food challenges to both shrimp species (n=8, 11.76%). Patient characteristics were shown as follows for sex and mean (±SD) age: 11 males, 1 females; age 11.67±2.96 years for group I, 10 males, 6 females; age 10.00±2.80 years for group II, 19 males, 13 females; age 9.78±2.42 years for group III, 4 males, 4 females; age 10.63±2.93 years for group IV.

Underlying allergic diseases and clinical history of shrimp allergy

All patients had underlying allergic diseases with 94.12% respiratory allergies (asthma, allergic rhinitis, or allergic rhinoconjunctivitis), 13.24% urticaria, 7.35% atopic dermatitis and 2.94% vernal keratoconjunctivitis. Clinical histories of shrimp allergy were reported mainly in the skin-mucosal system (92.65%), followed by respiratory (29.41%), GI (14.71%) and cardiovascular system (CVS, 1.47%).

Results of skin tests

Most patients had positive reactions to all skin tests including ComSPT, *Pm*SPT, *Mr*SPT, *Pm*PTP and *Mr*PTP. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of different skin tests were as follows: 88.33%, 37.50%, 91.38% and 30% for ComSPT; 97.73%, 8.33%, 66.15%, and 66.67% for *Pm*SPT, 100%, 4.17%, 65.67% and 100% for *Pm*PTP, 97.92%, 5%, 71.21% and 50% for *Mr*SPT, 100%, 0%, 70.59% and not applicable NPV for *Mr*PTP because no patient had negative *Mr*PTP. Correlations of MWD from different skin tests in all patients were shown in Figure 2. There were fair correlations between MWD from ComSPT-*Pm*SPT (Figure 2a), ComSPT -*Pm*PTP (Figure 2b), and ComSPT -*Mr*PTP (Figure 2d), with the correlation coefficient of 0.415, 0.459, and 0.335, respectively. However, there was no

correlation between MWD from ComSPT-*Mr*SPT (Figure 2c, r=0.221, p=0.07). A moderate correlation between MWD from *Pm*SPT-*Pm*PTP (Figure 2e, r=0.560) and a fair correlation between MWD from *Mr*SPT-*Mr*PTP (Figure 2f, r=0.484) were observed.

MWD from different skin tests were further compared in groups of patients with positive or negative challenges to *P. monodon* or *M. rosenbergii* (Figure 3). Overall, median MWD from all of the skin tests were larger in the groups with positive than negative challenges to *P. monodon* (Figure 3a). However, only MWD from *Pm*SPT and *Pm*PTP showed statistically significant (p<0.05). Figure 3b shows MWD from different skin tests in patients with positive and negative challenges to *M rosenbergii.* There were no statistically differences between any skin tests in both groups although MWD from *Mr*SPT were larger in the group with positive than the group with negative challenges to *M rosenbergii* (median 10.75 vs 6.75 mm, p=0.058). We calculated the predictive probability of MWD to determine the outcome of food challenges and logistic regression was used to establish the reasonable cut-off level (Figure 4). In patients with positive challenges to *P. monodon* (group I and III), *Pm*SPT provided 80% positive predictive probability at MWD of 30 mm. *Pm*PTP and ComSPT provided 95% positive predictive probability at MWD of 22.5 and 20 mm, respectively (Figure 4a). In patients with positive challenges to *M. rosenbergii* (group II and III), *Mr*SPT provided 95% predictive probability at MWD of 30 mm. Predictive probability could not be determined for *Mr*PTP and ComSPT (Figure 4b).

Results of SPT to *D. pteronyssinus*; *D. farinae* and *P. americana* extracts were shown in Table I-IV. SPT positive to *D. pteronyssinus*; *D. farinae* and *P. americana* extracts were 94.12%, 95.59% and 80.88%, respectively.

Results of food challenges

Results of food challenges were shown in Table I-III. In group I (Table I), 3/9 patients (no. 4, 5, 7) developed symptoms upon challenges with *P. monodon* capsules. Patient no. 4 had anaphylaxis[13] when he took 7.5 g of *P. monodon* capsules. Patient no. 8 refused to swallow capsule after he took 3.5 g of *P. monodon* capsules. Four of 9 patients developed oral-mucosal reactions. The cumulative PD of cooked *P. monodon* ranged from 3-31 g. Patient no. 9 developed anaphylaxis with the cumulative PD of only 3 g. In group II (Table II), all patients who could swallow capsules had no reaction with maximum dose of *M rosenbergii* capsules. Thirteen of 16 patients developed oral-mucosal reactions. Nine of these patients had significant symptoms that they refused to ingest cooked *M rosenbergii*. The cumulative PD of *cooked M rosenbergii* ranged

from 1-63 g. Patient no. 9 developed anaphylaxis with the cumulative PD of only 1 g. In group III (Table III), patients developed allergic reaction to both shrimp species. Upon challenges to *P. monodon*, 3/32 patients (no. 3, 11, 25) developed symptoms after they ingested *P. monodon* capsules. Patient no. 25 developed anaphylaxis after ingested 3.5 g of *P. monodon* capsules. Twenty of 29 patients developed oral-mucosal reactions. Thirteen of these patients had significant symptoms that they refused to ingest cooked *P. monodon*. The cumulative PD of cooked *P. monodon* ranged from 1-31 g with anaphylaxis occurred in patients no. 7 and 24. Upon challenges with *M. rosenbergii*, 2/32 patients (no. 8, 11) developed symptoms after they ingested *M. rosenbergii* capsules. Twenty-two of 30 patients developed oral-mucosal reactions. Fourteen of these patients had significant symptoms that they denied to ingest cooked *M. rosenbergii*. Patients no. 14, 23, and 30, developed anaphylaxis in addition to oral-mucosal reaction. The cumulative PD of cooked *M. rosenbergii* ranged from 1-31 g with anaphylaxis occurred in patients no. 2 and 18.

The most common symptom upon challenges was in the skin-mucosal system (95.65%), followed by respiratory (23.91%), GI (16.3%) and CVS (3.26%). Anaphylaxis was found in 11.92 %. Symptoms from *P. monodon* or *M. rosenbergii* were not significantly different (data not shown).

SDS-PAGE and immunoblotting with serum IgE

Results from immunoblotting of *P. monodon* extract with serum IgE from group I patients showed that 9/10 patients had specific IgE against two to seven bands of proteins in the *P. monodon* extract (Figure 5). Of these 9 patients, 7 patients had specific IgE against >45 kDa proteins while 6 patients had specific IgE against ~38-40 kDa protein and 4 patients had specific IgE against <25 kDa protein. In contrast, results from immunoblotting of *M. rosenbergii* extract with serum IgE from group II patients showed that 8/9 patients had specific IgE against more than 3 bands of proteins in the shrimp extract (Figure 6). Eight patients had specific IgE against >45 kDa proteins while 7 patients had specific IgE against <25 kDa proteins and interestingly, only 4 patients had specific IgE against ~38-40 kDa protein.

2-D SDS-PAGE and immunoblotting with serum IgE

Results from immunoblotting of PVDF membrane blotted from 2D SDS-PAGE gel (Figure 7A) of *P. monodon* extract using a pool serum IgE from group I patients showed that specific IgE bound to multiple spots of proteins with pI 3.5-7, (Fig 7B). The matched spots as indicated in a white circle were excised, in-gel digested by trypsin, and analysed by MALDI-TOF

MS. Interestingly, results from immunoblotting of PVDF membrane blotted from 2D SDS-PAGE gel (Fig 8A) of shrimp *M. rosenbergii* extract using a pool serum IgE from group II patients showed that specific IgE bound to multiple spots of proteins with much narrow pI 4-5.5 (Fig 4B). The matched spots as indicated in a white circle were excised, in-gel digested by trypsin, and analysed by MALDI-TOF MS.

Mass Fingerprint analysis by MALDI-TOF MS

Results of mass fingerprint analysis for *P. monodon* <25 kDa proteins were mass-to-charge (m/z) ratio of these peptides (Fig. 9). After searching for possible matched m/z of all proteins deposited at ExPaSy protein database, candidate proteins were Lipid binding protein, Chemosensory protein, Nonmuscle myosin heavy chain-B, Oxygen transporter, Cdk inhibitor, and Zinc ion binding. Results of mass fingerprint analysis for *M. rosenbergii* <25 kDa proteins were mass-to-charge (m/z) ratio of these peptides (Fig. 10). After searching for possible matched m/z of all proteins deposited at ExPaSy protein database, candidate proteins were Calcium ion binding protein, Heme binding protein, Amalyse inhibitor, Protein domain specific binding, Protein kinase activity, and Chaperone protein. Currently, cloning of cDNA coding for some of these proteins is in progress and beyond the scope of this study. Recombinant protein will be expressed and tested for serum IgE binding.

Discussion

Shrimp is one of the common foods causing mild to life-threatening allergic reactions. It was previously thought that patients with shrimp allergy would develop hypersensitivity reaction to all kinds of shrimp due to the common major allergen, tropomyosin. However, species-specific shrimp allergy was reported in one study, which showed specific shrimp allergens in two shrimp species, white *(Penaeus setifecus*) and brown *(Penaeus aztecus)* shrimp based on RAST and RAST-inhibition assays.[10] Nevertheless, species-specific shrimp allergy has never been confirmed by food challenge in the medical literature.

Our study showed that shrimp allergic children recruited by clinical history and skin tests to shrimp, could be divided into 4 groups based on reactions to *P. monodon* and *M. rosenbergii* challenges. Almost half of the patients (47%) developed symptoms to both kinds of shrimp probably due to the cross-reactivity between common allergens. Twelve percent of the patients had no symptoms to both kinds of shrimp. This was supported by the previous study which showed some discrepancy of self-reported food-induced symptoms and food challenges.[14] Almost 1/5 of patients developed isolated allergy to *P. monodon* (23%) or *M. rosenbergii* (18%). Although these 2 kinds of shrimp are in the same order *Decapoda*, *P. monodon* is in the suborder *Dendrobranchiata* and the family *Penaeidae*, but *M. rosenbergii* is in the suborder *Pleocyemata* and the family *Palaemonidae*.[15] The difference in suborder level may explain why these 2 species contain unique allergens which do not cross-react to each other. Further study to identify these unique allergens is ongoing.

Our food-challenged protocol contained 3 steps of challenges, 15 min apart. The ingestion of capsule bypassed oral-mucosal reaction. It took 2 separate days; 2-4 weeks spaced out to completely challenge both kinds of shrimp. A double blind, placebo controlled food challenge was difficult to perform since most patients refused to participate in the study if it took longer than one day for each challenge. Most reactions were objective symptoms and the frequency of organ involvement went along well with clinical history. The most common symptom upon challenges was in the skin-mucosal system, followed by respiratory, GI and CVS which was the same as food challenges to milk, egg, peanut and wheat.[16] Although the patients with anaphylaxis to shrimp were excluded, our challenges elicited 12% of anaphylaxis which responded well to emergency treatment. No fatality occurred in this study.

All of our patients had other underlying allergic diseases with predominant respiratory allergy due to the high number of respiratory allergy in our center. The high rate of sensitization

to *D. pteronyssinus* (94%), *D. farinae* (96%) and *P. americana* (81%) in shrimp allergic children from this study was certainly higher than the sensitization found in children with asthma (44-67%) and allergic rhinitis (37-88%) from our previous reports.[17, 18] This could be explained by cross-reactivity between tropomyosin in shrimp (group I allergen), house dust mites (Der p 10/Der f 10) and cockroach (Per a 7) or poly-sensitization in these patients. In the study by Ayuso et al, shrimp-allergic sera recognize 7/8 peptides homologous to Pen a 1 epitopes in Der p 10/Der f 10 and 6/8 epitopes in Per a 7, indicating highly cross-reactivity between tropomyosin from shrimp, house dust mites and cockroach.[7]

We observed a moderate correlation between MWD from *Pm*SPT and *Pm*PTP and a fair correlation between MWD from *Mr*SPT and *Mr*PTP. This may extrapolate that SPT using crude extract is comparable to PTP method. Our study demonstrated a fair correlation between skin test reactions from ComSPT versus *Pm*SPT, *Pm*PTP and *Mr*PTP, but not *Mr*SPT. This was not surprising since the company used Mexican brown shrimp, *Penaeus aztecus* to make commercial extract. Therefore, shrimp in other genus such as *M. rosenbergii* may contain different minor allergens. In general, allergy skin prick tests to food extracts are considered sensitive but not specific. The NPV is high although the PPV is rarely higher than 50%.[19] Our study found that ComSPT had the lowest sensitivity and NPV. This finding was supported by other 2 reports which found that accuracy of SPT using crude extracts (milk, egg and soy) [20] or PTP method using fresh foods (fruits and vegetables) [21] was significantly higher than that using commercial food extracts. The comparison between SPT to commercial and crude shrimp extracts or PTP test has never been reported. In the cases of shrimp allergy, the NPV of 30% from ComSPT may be unacceptable since a number of patients may be missed. This is considered dangerous in cases of anaphylaxis if patients are allowed po take shrimp without further challenge.

Figure 3a showed significant larger MWD from *Pm*SPT and *Pm*PTP but not ComSPT in patients with positive than negative challenges to *P. monodon*. In contrast, MWD from all of the 5 skin tests could not showed statistical significant between the patients with positive and negative challenges to *M. rosenbergii* (Figure 3b). However, MWD from *Mr*SPT had the potential to be larger in the patients with positive than negative challenges to *M. rosenbergii*.

SPT to food is a useful tool for identification of food sensitization. However, confirmation of food allergy requires oral food challenges. In practice, some allergy centers consider food challenges to be time consuming, expensive and may cause serious outcome including life-threatening anaphylaxis. Therefore, recent studies tried to identify the wheal size

from SPT of specific foods to predict the outcome of oral food challenges.[22, 23] In these studies, wheal size of SPT to cow's milk, hen's egg and peanut were identified to predicted probabilities of positive food challenges. In our study, predictive probabilities of SPT to shrimp were calculated to determine positive challenges in patients with shrimp allergy. These cut-off levels may be useful in the clinical setting where food challenge test is not feasible. However, a large study population with different ethnicity may be needed to set up a standard cut-off value.

Serum from group I and group II patients were further studies by IgE immunoblotting to detect potential novel antigens. In group I patients, IgE immunoblotting revealed that 6 patients had specific IgE to ~38-40 kDa allergens (Figure 5). These proteins are reported to be the molecular weight of tropomyosin and arginine kinase (group I and group II shrimp allergens, respectively). Interesting, 7 and 4 of group I patients had specific IgE to >45 kDa and <25 kDa allergens, respectively. We were interested in low molecular weight protein since it could cross mucosal barrier and trigger mucosal immune response. Therefore, further analysis of this protein was done by 2-D electrophoresis and immunoblot analysis of *P. monodon* allergens as shown in Figure 7. The spot of allergen <25 kDa was analysed by mass fingerprint analysis (Figure 9) and the candidate allergens were identified. In the same way, in group II patients, IgE immunoblotting revealed that 4 patients had specific IgE to ~38-40 kDa allergens (Figure 6). Eight and 7 of group II patients had specific IgE to >45 kDa and <25 kDa allergens, respectively. The low molecular weight protein <25 kDa was studied by 2-D electrophoresis and immunoblot analysis as well as mass fingerprint analysis (Figure 8, 10). After searching for possible matched m/z of all proteins deposited at ExPaSy protein database, numbers of candidate proteins were returned. Further study to prove that these proteins are novel shrimp allergens are beyond the scope of our study but are ongoing.

In conclusion, we demonstrated patients with specific allergy to *P. monodon* or *M. rosenbergii* confirmed by food challenges. Furthermore, the correlations of SPT using crude extracts and PTP of both shrimp species as well as commercial shrimp extract were studied. The importance of these skin tests to differentiate between the group with positive and negative challenges as well as the predictive probability of MWD to identified positive challenge was determined. To our knowledge, these are new data on shrimp allergy. Identification of the candidate proteins which may have unique allergenic property from these 2 shrimp species, is the beginning step to understand the discrepancy between the results of shrimp challenges. It will also be useful for the development of diagnostic kit as well as allergen immunotherapy in the future.

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Figure legend

Figure 1. Pictures of *Penaeus monodon* and *Macrobrachium rosenbergii.*

Penaeus monodon (1a) are characterized by the dark stripes that encircle the shell of the shrimp. *Macrobrachium rosenbergii* (1b) have relatively big head compared to the body and the bluish shell.

Figure 2. The correlations between mean wheal diameters (MWD) from different skin tests.

MWD from different skin tests were tested for correlation coefficient. Dots represent individual value. Regression line, r value for correlation coefficient and p value are shown.

Figure 3. Mean wheal diameter (MWD) from different skin tests in groups of patients with positive or negative challenges to *Penaeus monodon* or *Macrobrachium rosenbergii.*

Box plots of MWD from different skin tests in patients with positive (group I and III) and negative (group II and IV) challenges to *Penaeus monodon* are shown in Figure 3a. Figure 3b shows MWD from different skin tests in patients with positive (group II and III) and negative (group I and IV) challenges to *Macrobrachium rosenbergii*.

Figure 4. Predictive probabilities for mean wheal diameter (MWD) from different skin tests to determine the positive food challenges.

Predictive probabilities for MWD (mm) from different skin tests to determine positive food challenges are shown. Figure 4a and 4b demonstrates predictive probabilities of skin tests in the group with positive challenge to *Penaeus monodon* and *Macrobrachium rosenbergii*, respectively. Thin lines represent predicted probability and MWD.

Figure 5. Immunoblotting of *P. monodon* extract with serum IgE from group I patients who had positive food challenges to *P monodon* only.

Specific IgE Abs were detected using HRP-labeled goat anti-human IgE antibodies. The chemiluminescence signal provided by ECL plus western blotting detection kit. Lane M=MW marker for ECL; lanes 1-10=immunoblots showing binding of serum IgE of individual group I-patients; lane N=immunoblot using serum from a normal subject.

Figure 6. Immunoblotting of *M. rosenbergii* extract with serum IgE from group II patients who had positive food challenges to *M. rosenbergii* only.

Specific IgE Abs were detected using HRP-labeled goat anti-human IgE antibodies. The chemiluminescence signal provided by ECL plus western blotting detection kit. Lane M=MW

marker for ECL; lanes 1-9= immunoblots showing binding of serum IgE of individual group II-patients; lane N=immunoblot using serum from a normal subject.

Figure 7. 2-D electrophoresis and immunoblot analysis of *P monodon* allergens.

A) 2D SDS-PAGE gel of *P. monodon* extract and B) immunoblotting of PVDF membrane blotted from 2D SDS-PAGE gel with pooled serum IgE from group I patients who had positive food challenges to *P. monodon* but negative food challenges to *M. rosenbergii*. Specific IgE Abs were detected using HRP-labeled coat anti-human IgE antibodies. The chemiluminescence signal provided by ECL plus western blotting detection kit. The white circle was matched spots that were subjected for MALDI-TOF MS.

Figure 8. 2-D electrophoresis and immunoblot analysis of *M. rosenbergii* allergens.

A) 2D SDS-PAGE gel of *M. rosenbergii* extract and B) immunoblotting of PVDF membrane blotted from 2D SDS-PAGE gel with pooled serum IgE from group II patients who had positive food challenges to *M. rosenbergii* but negative food challenges to *P. monodon*. Specific IgE Abs were detected using HRP-labeled goat anti-human IgE antibodies. The chemiluminescence signal provided by ECL plus western blotting detection kit. The white circle was matched spots that were subjected for MALDI-TOF MS.

Figure 9. MALDI-TOF MS profile of trypsin digested-peptides of spot in Figure 7.

Mass-to-charge (m/z) ratio of trypsin digested-peptides of *P. monodon* <25 kDa proteins was analyzed by MALDI-TOF MS. The prominent mass peaks were chosen for database searches.

Figure 10. MALDI-TOF MS profile of trypsin digested-peptides of spot in figure 8.

Mass-to-charge (m/z) ratio of trypsin digested-peptides of *M. rosenbergii* <25 kDa proteins was analyzed by MALDI-TOF MS. The prominent mass peaks were chosen for database searches.

Output

Manuscript: Specific allergy to *Penaeus monodon* (seawater shrimp) or *Macrobrachium rosenbergii* (freshwater shrimp) in shrimp allergic children

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2) บทความสำหรับการเผยแพร่

Specific allergy to *Penaeus monodon* (seawater-shrimp) or *Macrobrachium rosenbergii* (freshwater-shrimp) in shrimp allergic children

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Abstract

Background: Allergy to specific shrimp species has never been confirmed by oral challenges. The association between commercial and crude shrimp extracts as well as prick to prick (PTP) test has never been reported.

Objective: To identify cases of *Penaeus monodon (Pm)* or *Macrobrachium rosenbergii (Mr)*-specific allergy in shrimp-allergic children. Comparison of skin tests using commercial and crude shrimp extracts plus PTP method was investigated.

Methods: Children ≥5 years of age with history of shrimp allergy were recruited. Skin prick tests (SPT) using *Pm* (*Pm*SPT*)* and *Mr (Mr*SPT) extracts as well as PTP method (*Pm*PTP*, Mr*PTP) were done compared to commercial shrimp extract (ComSPT). Open challenges to both shrimp species were done.

Results: Sixty-eight patients were divided into 4 groups by food challenges. Specific allergy to *Pm* and *Mr* were 17.65% and 23.53%, respectively. Positive and negative challenges to both kinds of shrimp were 47.06% and 11.76%, respectively. Correlations between mean wheal diameter (MWD) from ComSPT-*Pm*SPT*,* ComSPT-*Pm*PTP*,* ComSPT-*Mr*PTP*, Pm*SPT-*Pm*PTP and *Mr*SPT-*Mr*PTP were observed. In patients with *Pm* allergy, *Pm*SPT provided 80% predictive probability at 30 mm. *Pm*PTP and ComSPT provided 95% predictive probability at 22.5 and 20 mm, respectively. In patients with *Mr* allergy, *Mr*SPT provided 95% predictive probability at 30 mm.

Conclusion: Specific allergy to *Pm* or *Mr* confirmed by food challenges was demonstrated. Predictive probability of SPT may be helpful in the setting where food challenge is not feasible.

Clinical Implication: SPT using crude extracts and PTP test are useful tools for screening shrimp sensitization prior to food challenge.

Capsule summary

Specific allergy to *Penaeus monodon* or *Macrobrachium rosenbergii* was demonstrated. SPT using crude extracts and PTP are useful tools for screening shrimp sensitization. Predictive probability of SPT is helpful where food challenge is not feasible.

Key words: food allergy, food challenge*, Macrobrachium rosenbergii*, *Penaeus monodon*, predictive probability, prick to prick test, shrimp allergy, skin prick test, shrimp extract

Abbreviation

ComSPT = skin prick test to commercial shrimp extract

CVS = cardiovascular

Df = Dermatophagoides farinae

Dp = Dermatophagoides pteronyssinus

GI = gastrointestinal

Mr = Macrobrachium rosenbergii

*Mr*PTP = prick to prick test using cooked *M rosenbergii*

*Mr*SPT = skin prick tests using *M rosenbergii* extracts

MWD = mean wheal size diameter

NPV = negative predictive value

Pa = Periplaneta Americana

PD = provocation dose

Pm = Penaeus monodon

*Pm*PTP = prick to prick test using cooked *P. monodon*

*Pm*SPT = skin prick tests using *P. monodon* extracts

PPV = positive predictive value

PTP = prick to prick

SPT = skin prick test

Introduction

Shellfish is recognized as a common cause of food hypersensitivity and the leading cause of food-induced anaphylaxis.[1, 2] Among shellfish allergy, shrimp is the most frequent culprit.[2, 3] Symptoms of shrimp allergy develop in multiple organs such as skin (52-90%), respiratory (42%), gastrointestinal (GI, 35%), and shock (10%).[4] The major shrimp allergen, tropomyosin, is identified as a pan-allergen commonly found in other invertebrates such as lobster, crab, mollusk, squid, mussel, cockroach, and house dust mite.[5-8] Cross-reactivity between shrimp and other crustaceans was demonstrated.[9] However, there was also report of isolated allergy to specific shrimp species in patients with shrimp allergy.[10]

Two popular shrimp species, *Penaeus monodon (*black tiger shrimp) and *Macrobrachium rosenbergii* (giant fresh water prawn) are harvested in Thailand (Figure 1). *P. monodon* is cultivated in saltwater but *M rosenbergii* is cultivated in freshwater. *P. monodon* is the most widely distributed and marketed shrimp in the world. *M. rosenbergii* is increasingly recognized in the world-market due to its texture and palatability. Surprisingly, there is no report of *M. rosenbergii* allergy in any medical literature. From an observation at the allergy clinic, Siriraj hospital, Thailand, there are subpopulations of shrimp allergic patients who are allergic to only *M rosenbergii* but can tolerate *P. monodon* or vice versa.

The objective of this study was to identify cases of *P. monodon* or *M. rosenbergii*-specific allergy in shrimp allergic children. The importance of skin tests using *P. monodon* or *M. rosenbergii* extracts and prick to prick (PTP) method using cooked *P. monodon* or *M. rosenbergii* compared to commercial shrimp extract was also investigated.

Materials and Methods

Subjects

Patients ≥5 years of age who came to the pediatric allergy clinic, Siriraj Hospital, Thailand, from June 1st, 2005 to December 31th, 2006, with history of shrimp allergy were asked to participate in this study. The study was approved by the Ethic Committee, Siriraj Hospital. Informed consent was obtained from the patients (or parents if patients were <18 years of age). Patients with history of severe anaphylactic reaction from seafood, pregnancy, underlying diseases such as cardiovascular (CVS), hepatobiliary, and renal diseases were excluded. None of the patients was on systemic corticosteroid or β-blocking agent. Patients with allergic diseases were asymptomatic on the days of skin tests and food challenges.

Skin-test procedure[11]

The skin prick tests (SPT) using *P. monodon* and *M rosenbergii* extracts (*Pm*SPT and *Mr*SPT) as well as PTP method using cooked *P. monodon* and *M rosenbergii* (*Pm*PTP and *Mr*PTP, pricking the food and then pricking the skin of the patients) were performed compared to 10 mg/ml of histamine phosphate (positive control) and glycerinated saline (negative control). The *P. monodon* and *M rosenbergii* extracts were freshly prepared by making the dilution of 1:10 (wt/volume) of lyophilized shrimp in glycerinated saline. This concentration was proved to be a non-irritating concentration in 10 non-atopic subjects (data not shown). SPT to commercial extracts (Center Laboratory, Port Washington, NY) of shrimp (ComSPT), *Dermatophagoides pteronyssinus (Dp)*; *Dermatophagoides farinae (Df)* and *Periplaneta americana* (American cockroach, *Pa*) were also done. Antihistamine was discontinued for ≥7 days prior to skin testing. The size of wheal and flare reactions was recorded in millimeters (mm). The mean of the largest and midpoint orthogonal

diameters was designated as mean wheal diameter (MWD) and considered positive if it was ≥3 mm compared to negative control.

***Food-challenged procedure*[11-12]**

Food challenges to *P. ionodon and M rosenbergii* were done in patients with skin tests positive to ComSPT, *Pm*SPT, *Mr*SPT, *Pm*PTP or *Mr*PTP. Shrimp was eliminated from the diet for 2 weeks before challenge. The starting dose was 500 mg of lyophilized shrimp in capsule. The dose was doubled every 15 min. Cumulative provocation dose (PD) schedule of lyophilized shrimp was as follows: 500 mg, 1.5 g, 3.5 g, 7.5 g, and 15.5 g. The reason to use capsule was to avoid oral-mucosal reaction at the initial challenge. Young patients who could not swallow capsule were allowed to skip this process. To identify oral-mucosal reactions, 2 g of cooked shrimp was wiped on inner lips and placed in the mouth for 5 min. Lips swelling/itching or throat itching were recorded as positive reactions. Fifteen min later, open-feeding to cooked shrimp started at 1 g and doubled the dose every 15 min was performed. Cumulative PD schedule of cooked shrimp was as follows: 1 g, 3 g, 7 g, 15 g, 31 g and 63 g. Vital signs as well as patient's symptoms and signs were recorded every 15 min. Emergency resuscitation equipments and medicines were prepared. Challenge to another kind of shrimp was conducted 2-4 weeks after the 1st challenge.

Data collection and analysis

Data were expressed as individual values or the mean ± SD for group. Median and range were used in non-normally distributed data. Spearman's rho correlations were used to compare MWD from the different methods of skin tests in all patients. Comparison of MWD from skin tests between groups of challenge positive and negative patients was made using Mann-Whitney U test. Differences between groups were considered significant at a p value of ≤0.05. The logistic regression analysis was

used to predict probabilities of the outcome of food challenges by MWD of different skin tests

Results

Demographic data

Seventy-two patients, who had history of shrimp allergy and skin tests positive to ComSPT, *Pm*SPT*, Pm*PTP*, Mr*SPT or *Mr*PTP, were recruited. Four patients developed anaphylaxis on the 1st challenge and could not complete the 2nd one. Three and one of these patients developed anaphylaxis to *P. monodon and M. rosenbergii,* respectively. Demographic data, clinical history, results of skin tests, cumulative PD of shrimp and symptoms upon challenges were shown in Table I-IV. Overall, 68 patients were divided into 4 groups. Group I referred to patients who had positive food challenges to *P. monodon* only (n=12, 17.65%). Group II referred to patients who had positive food challenges to *M. rosenbergii* only (n=16, 23.53%). Group III referred to patients who had positive food challenges to both shrimp species (n=32, 47.06%). Group IV referred to patients who had negative food challenges to both shrimp species (n=8, 11.76%). Patient characteristics were shown as follows for sex and mean (±SD) age: 11 males, 1 females; age 11.67±2.96 years for group I, 10 males, 6 females; age 10.00±2.80 years for group II, 19 males, 13 females; age 9.78±2.42 years for group III, 4 males, 4 females; age 10.63±2.93 years for group IV.

Underlying allergic diseases and clinical history of shrimp allergy

All patients had underlying allergic diseases with 94.12% of respiratory allergies (asthma, allergic rhinitis, or allergic rhinoconjunctivitis), 13.24% of urticaria, 7.35% of atopic dermatitis and 2.94% of vernal keratoconjunctivitis. Clinical histories of shrimp allergy were reported mainly in the skin-mucosal system (92.65%), followed by respiratory (29.41%), GI (14.71%) and cardiovascular system (CVS, 1.47%).

Results of skin tests

Most patients had positive reactions to all skin tests including ComSPT, *Pm*SPT, *Mr*SPT, *Pm*PTP and *Mr*PTP. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of different skin tests were as follows: 88.33%, 37.50%, 91.38% and 30% for ComSPT; 97.73%, 8.33%, 66.15%, and 66.67% for *Pm*SPT, 100%, 4.17%, 65.67% and 100% for *Pm*PTP, 97.92%, 5%, 71.21% and 50% for *Mr*SPT, 100%, 0%, 70.59% and not applicable NPV for *Mr*PTP because no patient had negative *Mr*PTP. Correlations of MWD from different skin tests in all patients were shown in Figure 2. There were fair correlations between MWD from ComSPT versus *Pm*SPT (Figure 2a), *Pm*PTP (Figure 2b), and *Mr*PTP (Figure 2d), with the correlation coefficient of 0.415, 0.459, and 0.335, respectively. However, there was no correlation between MWD from ComSPT and *Mr*SPT (Figure 2c, r=0.221, p=0.07). A moderate correlation between MWD from *Pm*SPT and *Pm*PTP (Figure 2e, r=0.560) and a fair correlation between MWD from *Mr*SPT and *Mr*PTP (Figure 2f, r=0.484) were observed.

MWD from different skin tests were further compared in groups of patients with positive or negative challenges to *P. ionodon* or *M. rosenbergii* (Figure 3). Overall, median MWD from all of the skin tests were bigger in the groups with positive than negative challenges to *P. monodon* (Figure 3a). However, only MWD from *Pm*SPT and *Pm*PTP showed statistically significant (p<0.05). Figure 3b shows MWD from different skin tests in patients with positive and negative challenges to *M rosenbergii.* There were no statistically differences between any skin tests in both groups although MWD from *Mr*SPT were bigger in the group with positive than the group with negative challenges to *M rosenbergii* (median 10.75 vs 6.75 mm, p=0.058). We calculated the predictive probability of MWD to determine the outcome of food challenges and logistic regression was used to establish the

reasonable cut-off level (Figure 4). In patients with positive challenges to *P. monodon* (group I and III), *Pm*SPT provided 80% predictive probability at MWD of 30 mm. *Pm*PTP and ComSPT provided 95% predictive probability at MWD of 22.5 and 20 mm, respectively (Figure 4a). In patients with positive challenges to *M. rosenbergii* (group II and III), *Mr*SPT provided 95% predictive probability at MWD of 30 mm. Predictive probability could not be determined for *Mr*PTP and ComSPT (Figure 4b).

Results of SPT to *D. pteronyssinus*; *D. farinae* and *P. americana* extracts were shown in Table I-IV. SPT positive to *D. pteronyssinus*; *D. farinae* and *P. americana* extracts were 94.12%, 95.59% and 80.88%, respectively.

Results of food challenges

Results of food challenges were shown in Table I-III. In group I (Table I), 3/9 patients (no. 4, 5, 7) developed symptoms upon challenges with *P. monodon* capsules. Patient no. 4 had anaphylaxis[13] when he took 7.5 g of *P. monodon* capsules. Patient no. 8 denied po swallow capsule after he took 3.5 g of *P. monodon* capsules. Four of 9 patients developed oral-mucosal reactions. The cumulative PD of cooked *P. monodon* ranged from 3-31 g. Patient no. 9 developed anaphylaxis with the cumulative PD of only 3 g. In group II (Table II), all patients who could swallow capsules had no reaction with maximum dose of *M rosenbergii* capsules. Thirteen of 16 patients developed oral-mucosal reactions. Nine of these patients had significant symptoms that they denied to ingest cooked *M rosenbergii*. The cumulative PD of cooked *M rosenbergii* ranged from 1-63 g. Patient no. 9 developed anaphylaxis with the cumulative PD of only 1 g. In group III (Table III), patients developed allergic reaction to both shrimp species. Upon challenges to *P. monodon*, 3/32 patients (no. 3, 11, 25) developed symptoms after they ingested *P. monodon* capsules. Patient no. 25 developed anaphylaxis after ingested 3.5 g of *P. monodon* capsules. Twenty of 29

patients developed oral-mucosal reactions. Thirteen of these patients had significant symptoms that they denied to ingest cooked *P. monodon*. The cumulative PD of cooked *P. ionodon* ranged from 1-31 g with anaphylaxis occurred in patients no. 7 and 24. Upon challenges with *M. rosenbergii*, 2/32 patients (no. 8, 11) developed symptoms after they ingested *M. rosenbergii* capsules. Twenty-two of 30 patients developed oral-mucosal reactions. Fourteen of these patients had significant symptoms that they denied to ingest cooked *M. rosenbergii*. Patients no. 14, 23, and 30, developed anaphylaxis in addition to oral-mucosal reaction. The cumulative PD of cooked *M. rosenbergii* ranged from 1-31 g with anaphylaxis occurred in patients no. 2 and 18.

The most common symptom upon challenges was in the skin-mucosal system (95.65%), followed by respiratory (23.91%), GI (16.3%) and CVS (3.26%). Anaphylaxis was found in 11.92 %. Symptoms from *P. monodon* or *M. rosenbergii* were not significantly different (data not shown).

Discussion

Shrimp is one of the common foods causing mild to life-threatening allergic reactions. It was previously though that patients with shrimp allergy would develop hypersensitivity reaction to all kinds of shrimp due to the common major allergen, tropomyosin. However, species-specific shrimp allergy was reported in one study, which showed specific shrimp allergens in two shrimp species, white *(Penaeus setifecus*) and brown *(Penaeus aztecus)* shrimp based on RAST and RAST-inhibition assays.[10] Nevertheless, species-specific shrimp allergy has never been confirmed by food challenge in the medical literature.

Our study showed that shrimp allergic children recruited by clinical history and skin tests to shrimp, could be divided into 4 groups based on reactions to *P. monodon* and *M. rosenbergii* challenges. Almost half of the patients (47%) developed symptoms to both kinds of shrimp probably due to the cross-reactivity between common allergens. Twelve percent of the patients had no symptoms to both kinds of shrimp. This was supported by the previous study which showed some discrepancy of self-reported food-induced symptoms and food challenges.[14] Almost 1/5 of patients developed isolated allergy to *P. monodon* (23%) or *M. rosenbergii* (18%). Although these 2 kinds of shrimp are in the same order *Decapoda*, *P. monodon* is in the suborder *Dendrobranchiata* and the family *Penaeidae*, but *M. rosenbergii* is in the suborder *Pleocyemata* and the family *Palaemonidae*.[15] The difference in suborder level may explain why these 2 species contain unique allergens which do not cross-react to each other. Further study to identify these unique allergens is ongoing.

Our food-challenged protocol contained 3 steps of challenges, 15 min apart. The ingestion of capsule bypassed oral-mucosal reaction. It took 2 separate days; 2-4 weeks spaced out to completely challenge both kinds of shrimp. A double blind,

placebo controlled food challenge was difficult to perform since most patients denied participation in the study if it took longer than one day for each challenge. Most reactions were objective symptoms and the frequency of organ involvement went along well with clinical history. The most common symptom upon challenges was in the skin-mucosal system, followed by respiratory, GI and CVS which was the same as food challenges to milk, egg, peanut and wheat.[16] Although the patients with anaphylaxis to shrimp were excluded, our challenges elicited 12% of anaphylaxis which responded well to emergency treatment. No fatality occurred in this study.

All of our patients had other underlying allergic diseases with predominant respiratory allergy due to the high number of respiratory allergy in our center The high rate of sensitization to *D. pteronyssinus* (94%), *D. farinae* (96%) and *P. americana* (81%) in shrimp allergic children from this study was certainly higher than the sensitization found in children with asthma (44-67%) and allergic rhinitis (37-88%) from our previous reports.[17, 18] This could be explained by cross-reactivity between tropomyosin in shrimp (group I allergen), house dust mites (Der p 10/Der f 10) and cockroach (Per a 7) or poly-sensitization in these patients. In the study by Ayuso et al, shrimp-allergic sera recognize 7/8 peptides homologous to Pen a 1 epitopes in Der p 10/Der f 10and 6/8 epitopes in Per a 7, indicating highly cross-reactivity between tropomyosin from shrimp, house dust mites and cockroach.[7]

We observed a moderate correlation between MWD from *Pm*SPT and *Pm*PTP and a fair correlation between MWD from *Mr*SPT and *Mr*PTP. This may extrapolate that SPT using crude extract is comparable to PTP method. Our study demonstrated a fair correlation between skin test reactions from ComSPT versus *Pm*SPT, *Pm*PTP and *Mr*PTP, but not *Mr*SPT. This was not surprising since the company used Mexican brown shrimp, *Penaeus aztecus* to make commercial extract. Therefore, shrimp in

other genus such as *M. rosenbergii* may contain different minor allergens. In general, allergy skin prick tests to food extracts are considered sensitive but not specific. The NPV is high although the PPV is rarely higher than 50%.[19] Our study found that ComSPT had the lowest sensitivity and NPV. This finding was supported by other 2 reports which found that accuracy of SPT using crude extracts (milk, egg and soy) [20] or PTP method using fresh foods (fruits and vegetables) [21] was significantly higher than that using commercial food extracts. The comparison between SPT to commercial and crude shrimp extract or PTP test has never been reported. In the cases of shrimp allergy, the NPV of 30% from ComSPT may be unacceptable since a number of patients may be missed. This is considered dangerous in cases of anaphylaxis if patients are allowed to take shrimp without further challenge.

Figure 3a showed significant higher MWD from PmSPT and PmPTP but not ComSPT in patients with positive than negative challenges to *P. monodon*. In contrast, MWD from all of the 5 skin tests could not showed statistical significant between the patients with positive and negative challenges to *M. rosenbergii* (Figure 3b). However, MWD from *Mr*SPT had the potential to be bigger in the patients with positive than negative challenges to *M. rosenbergii*.

SPT to food is a useful tool for identification of food sensitization. However, confirmation of food allergy requires oral food challenges. In practice, some allergy centers consider food challenges to be time consuming, expensive and may cause serious outcome including life-threatening anaphylaxis. Therefore, recent studies tried to identify the wheal size from SPT of specific foods to predict the outcome of oral food challenges. [22, 23] In these studies, wheal size of SPT to cow's milk, hen's egg and peanut were identified to predicted probabilities of positive food challenges. In our study, predictive probabilities of SPT to shrimp was calculated to determine positive

challenges in patients with shrimp allergy. These cut-off levels may be useful in the clinical setting where food challenge test is not feasible. However, a large study population with different ethnicity may be needed to set up a standard cut-off value.

In conclusion, we demonstrated patients with specific allergy to *P. monodon* or *M. rosenbergii* confirmed by food challenges. Furthermore, the correlations of SPT using crude extracts and PTP of both shrimp species as well as commercial shrimp extract were studied. The importance of these skin tests to differentiate between the group with positive and negative challenges as well as the predictive probability of MWD to identified positive challenge was determined. To our knowledge, these are new data on shrimp allergy.

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Table I. Demographic data, clinical history, SPT, cumulative provocation doses of *Penaeus monodon* and symptoms upon challenges in group I patients who had positive food challenges to *P. monodon* but negative food challenges to *Macrobrachium rosenbergii*.

N	Sex	Age (y)	Underlying allergic diseases	History of reactions to shrimp	SPT positive to *Dp, Df,* or *Pa*	*P. monodon* cumulative PD			Symptoms upon challenges
						Shrimp capsules (g)	Oral-mucosal reaction	Cooked shrimp (g)	
1	M	12	AR	LSI, NV	*Dp, Df, Pa*	15.5	No	7	TI
2	M	14	ARC	LSI, U	*Dp, Df, Pa*	ND	No	31	LSI, U
3	M	15	AS, ARC	AG, R	*Dp, Df, Pa*	ND	No	31	TI
4	M	10	AS, AR	LI, R, SP	*Dp, Df, Pa*	7.5	ND	ND	W, R, CP, NV, A
5	M	15	AR	F, P	*Dp, Df, Pa*	15.5	ND	ND	F, P
6	M	13	AS, AR	LSI, U, W	*Dp, Df, Pa*	15.5	No	31	TI, CP
7	M	12	AS, ARC	U, R	*Dp, Df, Pa*	15.5	ND	ND	U
8	M	9	AS, AR	LSI, U	*Dp, Df, Pa*	3.5	Yes	31	LSI, U
9	M	8	AR	LSI, NV	*Dp, Df, Pa*	15.5	Yes	3	LSI, AP, A
10	M	11	AR	LSI, R	*Dp, Df*	15.5	Yes	15	LSI, R
11	F	6	AS, AR	LI, U	*Dp, Df, Pa*	ND	Yes	31	LSI, U, AG
12	M	15	AR	LI, F, U	*Dp, Df, Pa*	15.5	No	31	F

A, anaphylaxis; AG, angioedema; AP, abdominal pain; AR, allergic rhinitis; ARC, allergic rhinoconjunctivitis; AS, asthma; CP, chest pain; *Dp*, *Dermatophagoides pteronyssinus*; *Df, Dermatophagoides farinae*; F, flushing; LI, lip itching; LSI, lip swelling/itching; ND, not done; TI, throat

itching; NV, nausea/vomiting; P, skin pruritus without rash; *Pa*, *Periplaneta americana*; PD, provocative dose; R, rhinitis; SP, substernal pain; SPT, skin prick tests; U, urticaria; W, wheezing.

Table II. Demographic data, clinical history, SPT, cumulative provocation doses of *Macrobrachium rosenbergii* and symptoms upon challenges in group II patients who had positive food challenges to *M. rosenbergii* but negative food challenges to *Penaeus monodon*.

N	Sex	Age (y)	Underlying allergic diseases	History of reactions to shrimp	SPT positive to *Dp, Df,* or *Pa*	*M. rosenbergii* cumulative PD			Symptoms upon challenges
						Shrimp capsules (g)	Oral-mucosal reaction	Cooked shrimp (g)	
1	M	10	ARC	LI, U	*Dp, Df, Pa*	15.5	Yes	3	LI, U
2	M	10	AS, AR	LSI, U	*Dp, Df, Pa*	15.5	Yes	ND	LSI, U
3	F	9	AS, AR, U	LI, U	*Dp, Df, Pa*	15.5	No	63	LSI, U
4	F	14	U	U	*Dp, Df*	15.5	Yes	ND	LI, U
5	F	9	AS	LSI, U	*Dp, Df*	15.5	Yes	7	LSI, U
6	M	6	AR	LSI, U, AG	*Dp, Df, Pa*	15.5	Yes	ND	LSI, AG
7	M	14	AS, AR, U	U, W, SP	*Dp, Df, Pa*	ND	No	31	LI, R
8	M	8	AS	LSI, U	*Pa*	15.5	Yes	31	LSI
9	M	9	AS, AR	LSI	*Df, Pa*	15.5	No	1	LSI, U, AP, NV, A
10	M	8	AR	LSI	*Dp, Df, Pa*	15.5	Yes	ND	LSI
11	F	14	AS, AR	TI	*Dp, Df*	15.5	Yes	31	LSI
12	F	5	AS, AR	LSI	neg	ND	Yes	ND	LSI, U
13	M	14	AS, AR	LSI, U, AG	*Dp, Df, Pa*	15.5	Yes	ND	LSI, U, AG
14	M	11	AR	LSI, U	*Dp, Df*	15.5	Yes	ND	LSI, U, TI
15	M	9	AR	LI, R, Di	*Dp, Df, Pa*	15.5	Yes	ND	LSI, U
16	F	10	AR	LI, R, Di	*Dp, Df, Pa*	15.5	Yes	ND	LSI, U

A, anaphylaxis; AG, angioedema; AP, abdominal pain; AR, allergic rhinitis; ARC, allergic rhinoconjunctivitis; AS, asthma; Di, diarrhea; *Dp*, *Dermatophagoides pteronyssinus*; *Df, Dermatophagoides farinae*; F, flushing; LI, lip itching; LSI, lip swelling/itching; ND, not done; TI, throat itching; NV, nausea/vomiting; P, skin pruritus without rash; *Pa, Periplaneta americana*; R, rhinitis; SP, substernal pain; SPT, skin prick tests; U, urticaria; W, wheezing.

Table III. Demographic data, clinical history, SPT, cumulative provocation doses of *Macrobrachium rosenbergii* and *Penaeus monodon* and symptoms upon challenges in group III patients who had positive food challenges to both *M. rosenbergii* and *P. monodon*.

N	Sex	Age (y)	Underlying allergic diseases	History of reactions to shrimp	SPT positive to *Dp, Df,* or *Pa*	*P. monodon* cumulative PD			Symptoms upon challenges *Pm*	*M. rosenbergii* cumulative PD			Symptoms upon challenges *Mr*
						Shrimp capsules (g)	Oral-mucosal reaction	Cooked shrimp (g)		Shrimp capsules (g)	Oral-mucosal reaction	Cooked shrimp (g))	
1	M	10	AR	LI, U, R, CP	neg	15.5	No	3	LI, CP, R	ND	No	15	LI, U, R
2	M	13	AS, AR	LSI, U, R	*Dp, Df, Pa*	15.5	No	31	LI, U, R, EI	15.5	Yes	3	LSI, AP, A
3	F	8	AS, AR	LSI, U, AG, R	*Dp, Df, Pa*	7.5	ND	ND	LSI, U, R, EI	ND	Yes	1	LSI, AG, R, EI
4	M	6	AR, U	LI, U	*Dp, Df, Pa*	ND	No	15	LI, U, P	ND	No	31	LI, U
5	F	11	AR	U	*Dp, Df, Pa*	15.5	Yes	31	LI, R	7.5	No	3	LI, R
6	F	12	AR, AD	LI, U, R	*Dp, Df, Pa*	15.5	Yes	3	LI, U, R	ND	No	15	LSI
7	F	12	AR, AD	LSI, U	*Dp, Df*	15.5	Yes	31	LI, U, AG, R, AP, A	ND	Yes	7	LSI
8	F	12	AR, U	LSI, U, R	*Dp, Df, Pa*	15.5	No	31	LI, AG, R	3.5	ND	ND	LSI
9	M	12	AS, ARC	R, NV	*Dp, Df, Pa*	15.5	No	3	R, NV	15.5	No	3	R, NV
10	M	12	AR	LI, R	*Dp, Df, Pa*	15.5	No	31	R, AP, EI	15.5	No	3	LI, R
11	M	8	AR	U	*Dp, Df, Pa*	15.5	ND	ND	U	3.5	ND	ND	U
12	F	9	AS, AR, AD	LSI	*Dp, Df, Pa*	15.5	No	31	LSI	ND	Yes	ND	LSI
13	M	5	AS	LSI	*Dp, Df*	ND	Yes	ND	LSI	ND	Yes	ND	LSI
14	F	8	AS, AR, AD	LSI, U	*Dp, Df, Pa*	ND	Yes	1	LSI, U	15.5	Yes	ND	LSI, NV, AP, A
15	M	8	AR, VKC	LSI	*Dp, Df, Pa*	ND	Yes	ND	LSI	ND	Yes	31	LSI
16	M	9	AR, AD	LSI	*Dp, Df*	ND	Yes	1	LSI, TI	ND	Yes	ND	LSI, U
				LSI, U,									

17	M	10	AS, ARC	W, R	*Dp, Df, Pa*	15.5	Yes	15	LSI, U	3.5	Yes	1	LSI, U
18	F	13	AR	LSI, NV	*Dp, Df, Pa*	15.5	No	1	NV	15.5	Yes	3	LSI, NV, Dy, A
19	M	5	AS, AR	LSI	*Dp, Df, Pa*	ND	Yes	1	LSI, U	ND	Yes	ND	LSI, U
20	F	9	AR	LSI, NV	*Dp, Df, Pa*	15.5	Yes	ND	LSI, NV	15.5	Yes	ND	LSI
21	M	11	AS, AR	LSI, U, R	*Dp, Df, Pa*	ND	Yes	ND	U	15.5	Yes	31	LSI, U
22	M	8	VKC	LSI, U, R	*Dp, Df, Pa*	ND	Yes	ND	LSI, U	ND	Yes	3	LSI, U, R
23	M	10	ARC	LSI, U	*Dp, Df, Pa*	15.5	Yes	ND	LSI, U, R	15.5	Yes	ND	LSI, U, NV, R, A
24	F	13	AR	LSI, U	*Dp, Df, Pa*	15.5	No	31	U, W, A	15.5	Yes	ND	LSI, U
25	M	12	AS	W, NV	*Dp, Df, Pa*	3.5	ND	ND	U, AP, LSI, NV, A	ND	Yes	ND	LSI, U
26	M	13	AR	LSI	*Dp, Df, Pa*	15.5	Yes	ND	LI, U	15.5	No	3	LI, U
27	M	7	ARC	LSI	*Dp, Df, Pa*	3.5	Yes	ND	LSI, U	3.5	Yes	ND	LSI, U
28	M	11	AS, AR	LSI	*Dp, Df, Pa*	15.5	Yes	ND	LSI, U	15.5	Yes	ND	LSI, U
29	F	11	AR	LSI, U	*Dp, Df, Pa*	15.5	Yes	ND	LSI, U	15.5	Yes	ND	LSI, U
30	F	6	AR	LSI, U, AG	*Dp, Df*	3.5	Yes	ND	LSI, U, TI	7.5	Yes	ND	LSI, U, AP, A
31	M	8	AS, AR	NV	*Dp, Df, Pa*	ND	Yes	ND	LSI, U	ND	Yes	ND	LSI, U, TI
32	F	11	AS, AR	LSI	*Dp, Df, Pa*	15.5	Yes	ND	LSI, U	15.5	No	1	LSI, U

A, anaphylaxis; AD, atopic dermatitis; AG, angioedema; AP, abdominal pain; AR, allergic rhinitis; ARC, allergic rhinoconjunctivitis; AS, asthma; CP, chest pain; *Dp*, *Dermatophagoides pteronyssinus; Df, Dermatophagoides farinae*; Dy, dyspnea; EI, eye itching; LI, lip itching; LSI,

lip swelling/itching; ND, not done; TI, throat itching; NV, nausea/vomiting; P, skin pruritus without rash; *Pa, Periplaneta americana*; R, rhinitis; SPT, skin prick tests; U, urticaria; VKC, vernal keratoconjunctivitis; W, wheezing.

Table IV. Demographic data, clinical history, and SPT, in group IV patients who had negative food challenges to both *Penaeus monodon* and *Macrobrachium rosenbergii*.

N	Sex	Age (y)	Underlying allergic diseases	History of reactions to shrimp	SPT positive to *Dp, Df,* or *Pa*
1	M	6	AS	LSI	*Dp, Df*
2	M	14	AS, AR	LI	*Dp, Df, Pa*
3	F	11	AR, U	U	*Dp, Df, Pa*
4	F	8	U	U	*Dp, Df*
5	F	13	AR	LSI	*Dp, Df, Pa*
6	M	13	U	LSI, U, AG	*Dp, Df, Pa*
7	F	12	ARC, U	U	*Dp, Df, Pa*
8	M	8	AS, ARC	LSI, U, W, R	*Dp, Df, Pa*

AG, angioedema; AR, allergic rhinitis; ARC, allergic rhinoconjunctivitis; AS, asthma; *Dp*, *Dermatophagoides pteronyssinus*; Df, *Dermatophagoides farinae*; LI, lip itching; LSI, lip swelling/itching*; Pa, Periplaneta americana*; R, rhinitis; SPT, skin prick tests; U, urticaria; W, wheezing.

Figure legend

Figure 1. Pictures of *Penaeus monodon* and *Macrobrachium rosenbergii.*

Penaeus monodon (1a) are characterized by the dark stripes that encircle the shell of the shrimp. *Macrobrachium rosenbergii* (1b) have relatively big head compared to the body and the bluish shell.

Figure 2. The correlations between mean wheal diameters (MWD) from different skin tests.

MWD from different skin tests were tested for correlation coefficient. Dots represent individual value. Regression line, r value for correlation coefficient and p value are shown.

Figure 3. Mean wheal diameter (MWD) from different skin tests in groups of patients with positive or negative challenges to *Penaeus monodon* or *Macrobrachium rosenbergii.*

Box plots of MWD from different skin tests in patients with positive (group I and III) and negative (group II and IV) challenges to *Penaeus monodon* are shown in Figure 3a. Figure 3b shows MWD from different skin tests in patients with positive (group II and III) and negative (group I and IV) challenges to *Macrobrachium rosenbergii***.**

Figure 4. Predictive probabilities for mean wheal diameter (MWD) from different skin tests to determine the positive food challenges.

Predictive probabilities for MWD (mm) from different skin tests to determine positive food challenges are shown. Figure 4a and 4b demonstrates predictive probabilities of skin tests in the group with positive challenge to *Penaeus monodon* and *Macrobrachium rosenbergii*, respectively. Thin lines represent predicted probability and MWD.

บทความสำหรับการเผยแพร่

ผู้ป่วยที่แพ้กุ้งมักจะเข้าใจว่าไม่สามารถรับประทานกุ้งทุกชนิด แต่ในทางคลินิกมีผู้ป่วยบางรายที่รายงานว่าแพ้เพียงกุ้งชนิดใดชนิดหนึ่ง น่าจะเป็นไปได้ว่ากุ้งแต่ละชนิด เช่น กุ้งน้ำจืด (กุ้งก้ามกราม, *Mr*) หรือกุ้งน้ำเค็ม (กุ้งกุลาดำ, *Pm*) อาจมีกลุ่มสารก่อภูมิแพ้ที่จำเพาะโดยไม่ข้ามกุลุ่มกัน การแยกสารก่อภูมิแพ้กลุ่มนี้จะมีประโยชน์ในการใช้ทดสอบการแพ้อาหารจากกุ้ง หรือการผลิตวัคซีนจากกุ้งในอนาคต ในปัจจุบันการทดสอบภูมิแพ้ทางผิวหนังทำโดยใช้สารสกัดจากกุ้งที่มาจากต่างประเทศ ซึ่งกุ้งที่นำมาทำสารสกัดก็ไม่ใช่กุ้งชนิดที่มีในประเทศไทย จึงเป็นที่น่าสนใจว่าการทดสอบภูมิแพ้โดยใช้สารสกัดจากกุ้งในประเทศไทย เช่น กุ้งกุลาดำ (*Pm*SPT) และกุ้งก้ามกราม (*Mr*SPT) หรือการทดสอบโดยใช้การสะกิดเนื้อกุ้งแล้วไปสะกิดผิวหนังผู้ป่วย (Prick to prick test, PTP) โดยใช้กุ้งทั้ง 2 ชนิด (*Pm*PTP, *Mr*PTP) จะให้ผลการทดสอบเป็นอย่างไรเมื่อเทียบกับสารสกัดกุ้งจากต่างประเทศ

การศึกษานี้จึงมีวัตถุประสงค์เพื่อรายงานผู้ป่วยที่แพ้กุ้งน้ำจืด (กุ้งก้ามกราม, *Mr*) หรือกุ้งน้ำเค็ม (กุ้งกุลาดำ, *Pm*) อย่างใดอย่างหนึ่ง หรือทั้งสองอย่าง นอกจากนี้การศึกษานี้ยังได้พยายามหากลุ่มของสารก่อภูมิแพ้ที่จำเพาะในกุ้งทั้งสองชนิด และได้เปรียบเทียบการทดสอบผิวหนังที่ใช้สารสกัดจากกุ้งทั้งสองชนิดหรือวิธี PTP เทียบกับการใช้สารสกัดกุ้งจากต่างประเทศ

วิธีการศึกษา ทำโดยการชักชวนผู้ป่วยเด็กไทยที่มีอายุมากกว่า 5 ปี ที่มีประวัติแพ้กุ้งมาเข้าร่วมการศึกษา ผู้ป่วยจะได้รับการทดสอบทางผิวหนังโดยใช้สารสกัดจากกุ้ง และการทดสอบการแพ้กุ้งโดยการรับประทาน เลือดของผู้ป่วยจะถูกนำไปทดสอบเพื่อหาภูมิแพ้ (IgE) ต่อสารก่อภูมิแพ้ที่จำเพาะในกุ้งทั้งสองชนิด

ผลการศึกษา ได้มีผู้ป่วยเด็กเข้าร่วมโครงการ 68 ราย สามารถแบ่งออกได้เป็น 4 กลุ่ม จากผลของการทดสอบอาหาร โดยพบผู้ป่วยที่แพ้กุ้งกุลาดำอย่างเดียวร้อยละ 17.65 แพ้กุ้งก้ามกรามอย่างเดียวร้อยละ 23.53 แพ้กุ้งทั้งสองอย่างร้อยละ 47 ไม่แพ้ทั้งสองอย่างร้อยละ 11.76 จากการทดสอบทางผิวหนัง พบว่าสารสกัดจากกุ้งกุลาดำและกุ้งก้ามกรามให้ผลการทดสอบที่ใกล้เคียงกับสารสกัดจากกุ้งที่มาจากต่างประเทศ นอกจากนี้ยังสามารถพบกลุ่มสารก่อภูมิแพ้ที่มีความจำเพาะจากกุ้งกุลาดำและกุ้งก้ามกราม ซึ่งจำเป็นจะต้องได้รับการทดสอบเพิ่มเติมต่อไป

สรุป ผู้ป่วยที่มีประวัติแพ้กุ้งอาจแพ้กุ้งเพียงกลุ่มใดกลุ่มหนึ่ง และสามารถรับประทานกุ้งชนิดอื่นได้ อย่างไรก็ดีควรได้รับการตรวจโดยการทดสอบผิวหนัง และการทดสอบโดยการรับประทานกุ้งก่อนที่จะไปรับประทานเองที่บ้าน การทดสอบโดยใช้สารสกัดจากกุ้งกุลาดำและกุ้งก้ามกรามสามารถให้ผลดีเทียบเท่ากับสารสกัดจากกุ้งที่มาจากต่างประเทศ