Species-specific evaluation of patients with suspected crab allergy

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ABSTRACT

Rationale: Conserved sequence identity between shellfish allergens has been described but clinical significance is poorly understood. We present two patients with history of clinical reaction to single crab species with negative commercial skin prick testing (SPT) and serum specific IgE (sIgE) testing to crab but symptoms during oral food challenge (OFC) despite sustained oral tolerance to other crab and shellfish species.

Methods: SPT was preformed to house dust mite (HDM), fresh crabs and commercial crab extract, as well as sIgE to crab. Western blot analysis was conducted using patient serum on crude protein extracts isolated from different crab species, SPT reagents and tropomyosin.

Results: As shown in Table 1, SPT was positive to HDM but negative to shellfish for both patients, including crab (Atlantic blue crab, *Callinectes sapidus)*. Crab sIgE (snow crab, *Chionoecetes opilio*) <0.10 kU/L and total IgE 19 and 33IU/mL for patients 1 and 2, respectively. SPT to fresh, steamed, unseasoned crabmeat was performed on patient 1, resulted 3mm to Dungeness and King crabs and 1mm to snow crab. During OFC, patient 1 developed urticaria, nausea and severe abdominal pain after the second dose (less than 3g snow crab protein), while patient 2 developed mild throat pruritus but completed the challenge (>8g blue crab protein). In western blot analysis (Figure 1), the IgE profile was unremarkable when patient sera were compared to sera obtained from two controls (control 1 non shellfish-eating/non allergic; control 2 shellfish-eating/non-allergic). In the same analysis, while both patients sera were positive to HDM, they were negative for IgE to tropomyosin.

Conclusions: Our studies demonstrate limitations of current diagnostic approaches in capturing allergy to specific crab species. Additional studies with inclusion of more patients, extract lots, and expanded individual shellfish should provide more detailed insights into shellfish allergy and understanding of diagnostic extract and variability.

RESULTS

Table 1 (below): Summary of clinical and laboratory evaluation. Both patients had negative SPT and sIgE to crab, but symptoms during OFC; notably with positive testing to HDM on SPT and western blot analysis but negative IgE to crab and tropomyosin.

Allergy Test		Patient 1 Results	Patient 2 Results
SPT	HDM	Positive	Positive
	Commercial crab extract (Greer®, *Callinectes* spp.)	Negative	Negative
	Fresh cooked crab	• 3mm Dungeness & King • 1mm Snow crab	Not applicable
sIgE	Crab sIgE (LabCorp™, snow crab, *Chionoecetes* spp.)	<0.1 kU/L	<0.1 kU/L
	Total IgE	19 IU/mL	33 IU/mL
OFC	Crab species	Snow crab (*Chionoecetes opilio)*	Blue crab (*Callinectes sapidus)*
	Results	Urticaria, nausea, severe abdominal pain (<3g protein)	Mild throat pruritus (>8g blue crab protein)
Western Blot (IgE)	HDM	Positive	Positive
	Tropomyosin and crab	Negative (similar to control)	Negative (similar to control)

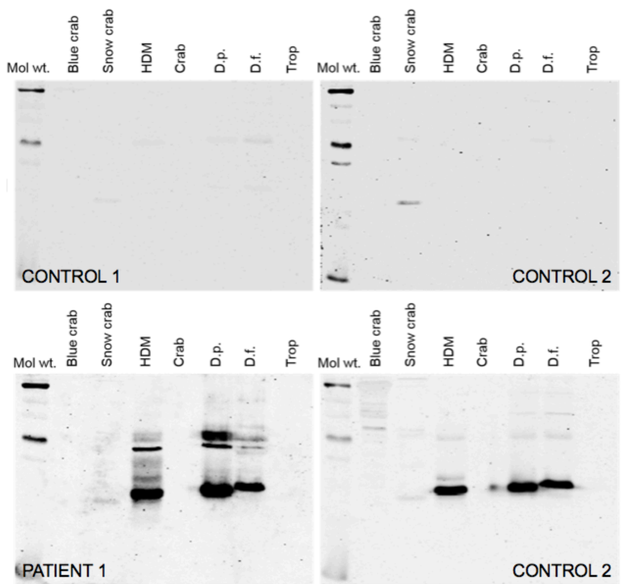

Figure 1 (left): Western blot analysis. Patients compared to two study member controls: control 1 non shellfish-eating/non allergic, control 2 shellfish eating/non allergic. Both patients +IgE to HDM and *D.p./D.f.* extracts, but -IgE to crab (fresh and extract) and tropomyosin.

Crab claw meat (20 µg protein) (Atlantic blue crab, *Callinectes sapidus*; snow crab, *Chionoecetes opilio*). HDM (Greer®) (20 µg protein). Crab (Greer®, F12 *Callinectes* spp.), D.p. (*Dermatophagoides pteronyssinus*), D.f. (*Dermatophagoides farinae*) (Greer®, SPT extract). Trop: tropomyosin (8 µg protein). Mol.wt.: molecular weight marker.

DISCUSSION

- Current standards of testing for shellfish allergy lack refinements and do not account for species variations, thus failing to cover the entire spectrum of shellfish allergies.
- The commercial crab allergen extract commonly used for allergy diagnostics is derived from a single crab species, Atlantic blue crab *Callinectes sapidus* (Greer®), while sIgE ImmunoCAP® testing by LabCorp™ targets snow crab, *Chionoecetes* spp.
- Tropomyosin, the major invertebrate pan-allergen found in all edible crustacean species, shares highly conserved amino acid sequences among crustaceans, as well as high sequence identity with HDM[1].
- The use of western blot analysis allowed for evaluation of IgE to specific crab species outside commercially available testing, as well as direct comparison of possible allergenic proteins between different crab species, dust mites, and tropomyosin compared to non-allergic controls.
- Patient 1 had clinical reactions to snow crab, patient 2 to blue swimmer crab (*Portunus pelagicus*), despite negative SPT and sIgE and known tolerance of other crab and shellfish species in the context of confirmed dust mite allergy.
- Our studies suggest that allergic sensitization to crab species and HDM are not molecularly linked by shared allergenic determinants.

CONCLUSIONS

- Our studies demonstrate the that currently available tools for diagnostics of shellfish allergies are limited in their ability to distinguish between species.
- Further, our molecular level analyses reveal the lack of patient IgE binding to any crab proteins, suggesting that the potential allergen is likely conformational.

REFERENCES

1. Ruethers, T, et al. *Mol. Immunol.* 100 (2018): 28-57.