

The Relevance of Specific Serum IgG, IgG₄ and IgE in the Determination of Shrimp and Crab Allergies in Malaysian Allergic Rhinitis Patients

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The diagnosis of food allergy is often difficult as symptoms vary widely and the current confirmatory diagnostic method remains the double-blind placebo-controlled food challenge, which is complex and time consuming. In Malaysia, diagnosis of food allergy is largely dependent on clinical history but interestingly specific immunoglobulin G₄ (IgG₄) titer is used in some clinics as a diagnostic aid for food allergy although the significance of IgG₄ in food allergy remains contradictory.¹ In this study, we set out to verify the relevance of three food-specific immunoglobulins, namely IgG, the subclass IgG₄ and IgE to two common food allergens in Malaysia, shrimp and crab.

MATERIALS AND METHODS

Subjects

Allergic rhinitis patients attending the allergic rhinitis clinic in University Hospital, Kuala Lumpur, Malaysia, were interviewed according to a questionnaire, which

SUMMARY The significance of food specific serum IgG₄ antibody in food allergy is unclear and this led us to investigate the relevance of specific IgG₄, along with IgG and IgE antibodies to two common food allergens in Malaysia. Enzyme-linked immunosorbent assay (ELISA) was used to measure the serum antibodies in 143 allergic rhinitis patients' sera, of which 47 were from patients with clinical indication of shrimp allergy, 46 with clinical indication of crab allergy and 50 without indication to either allergy. Clinical indication of allergy was based on answers to a questionnaire or results of the skin prick test. We found that the elevation of specific IgE or IgG₄ is associated with shrimp and crab allergies but elevation of specific IgG is not associated with either allergy. However, the clinical utility of elevated specific IgG and IgG₄ levels is pending further investigation.

included personal details, symptoms of food allergy (respiratory, gastrointestinal, and others), family history of allergy, accommodation history and the causes of allergic symptoms. Based on this questionnaire, the patients were classified as positive (Q+) or negative (Q-) to shrimp and crab allergy. They were also subjected to skin prick test² and classified positive (SPT+) or negative (SPT-) to shrimp and crab. Control sera were taken from individuals who were both Q- and SPT-.

Forty seven sera (patients between 4 to 57 years old, mean age = 27 years) from patients aller-

gic to shrimp based on results of the questionnaire (Q) and skin prick test (SPT) were used as test sera for shrimp allergy.

Forty six sera (patients between 4 to 57 years old, mean age = 28 years) from patients allergic to crab based on the same selection criteria were used as test sera for crab allergy.

Fifty sera from allergic rhinitis patients (patients between 15

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to 54 years old, mean age = 35 years) who claimed not to have allergy either to shrimp or to crab (Q-) and also did not react positively by the skin prick test (SPT -) were used as negative control sera.

Enzyme-linked immunosorbent assay (ELISA)

ELISA for specific IgG

Antigens (Bencard) were used at a concentration of 2 μ g/ μ l of phosphate buffered saline (PBS) and 50 μ l was used per well. ELISA procedures were followed as described in Sam *et al.*² with minor modifications. PBS/2% bovine serum albumin (BSA) was used as blocking solution. Sera were diluted 1:50 in assay diluent (PBS/2% BSA/0.05% Tween 20). After 2 hours of incubation, the plate was washed and rabbit anti-human IgG horse radish peroxidase (HRP) conjugate (Dako) was used at a dilution of 1:3,000 and allowed to react with the antigens for 1 hour before 1 mg/ml *o*-phenylenediamine (OPD added with 1 μ l/ml of 30% H₂O₂) was added for colorimetric reaction. The reaction was stopped by addition of 4 M H₂SO₄ after 15 minutes. Optical density was measured in an MRX ELISA reader at 490/630 nm.

ELISA for specific IgG₄

The steps were the same as in the ELISA for IgG except that biotinylated anti-human IgG₄ and the avidin-HRP conjugate were used at 1:1,000. After a 1-hour incubation, the plate was washed and colorimetric reaction was carried out in the same manner as in ELISA for IgG.

ELISA for specific IgE

The steps for this ELISA were similar to those of the ELISA used for IgG except that peroxidase conjugated anti-human IgE was used at 1:1,000, the serum incubation time was overnight at 4°C and the sera were tested at higher concentrations (1:5) due to the low levels of IgE.

χ^2 analysis

Two by two contingency χ^2 analysis was carried out. The three categories of test sera, namely Q-, SPT+; Q+, SPT- and Q+, SPT+, were grouped as "allergic".

RESULTS

The patterns of elevation in

immunoglobulins against shrimp and crab were similar in that the number of subjects with elevated IgG were the least, followed by IgG₄ and IgE. The net OD between a well with antigen and one without is the Δ OD and a Δ OD higher than the cut-off value of (mean + 2 standard deviations) of the negative control group was taken as elevated.

In the case of shrimp allergy, the elevation in specific IgG₄ and IgE were significantly associated (IgG₄, $\chi^2 = 4$, $0.050 > p > 0.025$; IgE, $\chi^2 = 9$, $p < 0.001$) with the subjects' status of allergy.

For crab allergy, the same trend appeared: both elevation in IgG₄ and IgE were significantly associated with the subjects' status of allergy (IgG₄ $\chi^2 = 7$, $0.010 > p > 0.005$; IgE $\chi^2 = 11$, $p < 0.001$)

It was found that the elevation of IgG, IgG₄ and IgE did not correspond to each other (Table 1). In the shrimp allergy group, only one individual who showed elevated IgG also had elevated IgG₄. Among the 12 subjects detected with elevated IgE against shrimp, only 3 (25.0%) showed elevated IgG₄ as well. Two of them

Table 1 Correspondence between specific serum Ig elevation, clinical history and skin-prick results

	Allergic status of subjects with elevated Ig			
	Test sera			Negative control
	Q+ SPT+	Q+ SPT-	Q- SPT+	Q- SPT-
IgG against shrimp	0/8	0/6	2/33 (6.1%)	3/50 (6.0%)
IgG ₄ against shrimp	1/8 (12.5%)	0/6	5/33 (15.2%)	1/50 (2.0%)
IgE against shrimp	1/8 (12.5%)	2/6 (33.3%)	9/33 (27.3%)	2/50 (4.0%)
IgG against crab	0/9	Nil	2/37 (5.4%)	1/50 (2.0%)
IgG ₄ against crab	4/9 (44.4%)	Nil	4/37 (10.8%)	1/50 (2.0%)
IgE against crab	2/9 (22.2%)	Nil	8/37 (21.6%)	1/50 (2.0%)

were from the Q-, SPT+ category whereas the remaining one was from the Q+, SPT+ group.

For the crab allergy group, none of those who showed elevated IgG showed elevation in their IgG₄. On the other hand, among the 10 subjects with elevated IgE against crab, 4 (40.0%) also showed elevated IgG₄. Two were from the Q-, SPT+ category while the other two were from the Q+, SPT+ category.

DISCUSSION

Our data demonstrated that elevation in specific serum IgE or IgG₄ is significantly associated with allergy to shrimp or crab. The elevation of specific IgG is not an indicator of either shrimp or crab allergy. The role of IgE in food allergy is well established,^{3,4} and it is not surprising that many allergic patients have elevated IgE.

The role of IgG in food allergy is unclear. IgG₄ is immunopathogenic and induces Type III hypersensitivity but it has also been proposed to have a blocking effect on IgE-mediated responses. This may be due to IgG₄ being heterogeneous- the majority being protective antibodies while a minority is immunopathogenic.¹

To assess the usefulness of serum IgG, IgG₄ or IgE for clinical diagnosis of shrimp or crab allergy, the antibody levels were analyzed with reference to the allergic status obtained through a questionnaire.

For shrimp allergy, in none of the 14 subjects with positive questionnaire outcome could specific IgG be detected, only in one of the 14 subjects (7.1%) with Q+ was IgG₄ detected while in 3 IgE was detected.

For crab allergy, in none of the 9 subjects with positive questionnaire outcome were IgG detected, while in 4 were IgG₄ and in 2 IgE were detected.

Not surprisingly, some of the patients with negative questionnaire outcome but with positive skin prick test result were found to have elevated Igs. For shrimp allergy, IgG was elevated in 2/33, IgG₄ in 5/33 and IgE in 9/33 patients. For crab allergy, IgG was elevated in 2/37, IgG₄ in 4/37 and IgE in 8/37 of the patients.

Whether data from a questionnaire are reliable depends on the patients' interpretation of allergic responses, some of which might be caused by food intolerance and not true food allergy, but assuming the clinical history is reliable, our data demonstrated a lack of correlation between clinical history and serum antibodies.

The skin prick test is relatively easy to perform, however, its reliability has been questioned.⁵ According to Sampson and Albergo,⁵ skin prick test has excellent negative predictive accuracy (index 82% to 100%) but poor and variable positive predictive accuracies (25% to 75%) for food allergy. The skin prick test can add confusion, since many positive responses lack correlating clinical symptoms and sometimes negative skin prick tests occur despite the presence of obvious allergic symptoms.⁵ In the present study, we found 12.8% (6 out of 27) of patients who claimed to be allergic to shrimp had negative skin prick test results to shrimp but none who claimed to be allergic to crab showed negative skin prick test results to crab. Thirty three patients who said they were not allergic to

shrimp were SPT+ while 37 who said they were not allergic to crab were SPT+.

We further considered the wheal size obtained in SPT in relation to their elevation in specific Igs. We found that in both allergies to shrimp and crab, wheal size shown by a subject did not correlate with the presence of elevated Igs. The correlation values between the wheal size and high titer values of Igs were insignificant (coefficient of correlation less than 0.5).

One of the Igs tested in this project, IgG₄, is a marker of allergy of certain but not all foods. Shakib⁶ reported that specific IgG₄ was a good indicator for milk allergy causing eczema in adults but was not a good indicator of egg allergy. However, it must be noted as well that the two food items studied by Shakib⁶ were quite different in nature and kind whereas the two food items studied in this work were similar. The existence of common allergens between shrimp and crab is highly probable.⁷

It was observed that for both shrimp and crab allergies, individuals showing elevation in specific IgG₄ level were not necessarily the ones showing elevation in specific IgE. For example, for shrimp allergy, only 25.0% of the ones with elevated IgE showed elevation in their specific IgG₄ titers while in the case of crab allergy, 40.0% had simultaneously elevated IgE and IgG₄ titers. This may be due to the fact that IgE is mainly involved in type I hypersensitivity whereas IgG₄ is promoting Type III hypersensitivity. This finding is in agreement with other reports, such as that of Bellanti *et al.*⁸ who found that many food allergic children

were found to have no detectable level of specific IgE antibodies.⁸

We found from this study that if shrimp and crab allergy is determined by either clinical or skin-prick test results, the elevation of specific IgG in sera is not a marker of either shrimp or crab allergy. On the other hand, the elevation of specific IgG₄ and the elevation of specific IgE are associated with crab allergy and shrimp allergy, and the elevation of specific IgE is a better indicator for both shrimp and crab allergy. However, the clinical relevance of elevated specific IgG₄ and IgE in shrimp and crab allergies should not be overstated. They can at best be used as complementary tests since the percentage of patients with detectable serum IgE and IgG₄ was too low for these *in vitro* tests to have important clinical relevance.

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