

Clinical Relevance of Shrimp Sensitization in Patients with Allergic Rhinitis: Anti-Der p 10 IgE as Predictor

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Keywords

Allergy · Atopy · Challenge test · House dust mite · IgE · Rhinitis · Shrimp

Abstract

Introduction: Cross-reactivity between shrimp and house dust mite (HDM) proteins has been widely documented. In tropical region, shrimp (5–15%) and mite sensitization (80–95%) is prevalent in allergic patients. However, the clinical relevance of shrimp sensitization in patients with allergic rhinitis (AR) has been poorly studied. The aim of this study was to determine the prevalence and the clinical relevance shrimp IgE sensitization in AR patients sensitized to *Dermaphagoides pteronyssinus*. **Methods:** The study was conducted in Medellín (Colombia). A cross-sectional study in patients with AR sensitized to HDM was performed in 3 steps: (i) assessment of IgE sensitization frequency to shrimp *Penaeus azteca*, *Litopenaeus vannamei*, and tropomyosin homologous allergens rDer p 10, rPen a 1, and rLit v 1, (ii) evaluation of the clinical relevance of shrimp sensitization using oral challenge test (OCT) and (iii) identification of possible risk factors for positive-OCT results. Ethical committee approval was obtained. **Results:** From 443 patients with AR, 86 (19.4%) were sensitized to shrimp and 23 of them (26.7%)

had shrimp allergy diagnosis. Thirty-six of the patients sensitized to shrimp (41.2%) reported not previously consumed this food and eleven of them had a positive-OCT (30.5%). There was not statistically significant difference in total IgE or sIgE (*D. pteronyssinus*, *P. azteca*, *L. vannamei*, rPen a 1, and rLit v 1) between OCT groups (positive vs. negative results). Anti-Der p 10 IgE was associated with risk for a positive-OCT in different multivariable scenarios. **Discussion/Conclusion:** Our results suggest that in patients with HDM-associated AR and shrimp IgE sensitization is necessary to evaluate the clinical relevance of shrimp IgE even if the patient has never consumed shrimp because of cross-reactivity. Anti-Der p 10 could be a possible biomarker of clinical relevance to shrimp sensitization and could reduce the need for OCTs.

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Introduction

House dust mite (HDM) sensitization is a major risk factor for the development of respiratory allergies such as asthma and rhinitis [1] in the Tropics [2]. On the other hand, shrimp is one of the most important sources of

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food allergy which is frequently associated with IgE-mediated hypersensitivity reactions [3, 4]. There are some allergens which share structural homology and belong to the same group of proteins such as tropomyosin, which is the main allergen of shrimp in frequency of sensitization, but in HDM sensitization, it represents a minor allergen with a frequency of sensitization of 5–15% [5]. The prevalence of shrimp sensitization differs among different populations, influenced by different factors such as frequency of consumption and method of preparation [6].

Study in orthodox Jews who, due to their social habits, do not consume shrimp or any crustacean, have shown IgE reactivity to shrimp proteins in that population [7]. Sensitization to this crustacean is also present after starting allergen-specific immunotherapy with HDM in patients previously not sensitized [8, 9]. In a study conducted among patients with different allergic diseases, it was observed that the sensitization to shrimp was around 20% and half of these patients had not previously consumed shellfish [10]. These results suggest that in some populations especially in tropical regions the intensity of exposure to HDM may be an important factor for shrimp sensitization even more than shrimp consumption habits.

The frequency in which IgE sensitization (atopy) is associated with the development of symptoms (allergy) varies according to the allergen. Pascal et al. [11] reported that 67% of patients sensitized to shrimp had an allergic reaction during an oral challenge test (OCT). Nevertheless, other studies suggest that least than 25% of patients sensitized to shrimp had an allergic reaction [12, 13]. These results suggest that a significant percentage of subjects may be sensitized to shrimp without having clinical relevance, and in this case, they do not require restrictions on shrimp consumption.

Considering the cross-reactivity of HDM with shrimp, a question that arises is How often patients with allergic rhinitis (AR) associated with HDM sensitization have a shrimp allergy? By responding to this question, we will be able to better understand the relationship of HDM/Shrimp co-sensitization, and the clinical impact that it can have on the patient with AR.

Materials and Methods

Study Design and Participant Characteristics

A cross-sectional study with patients diagnosed with AR [14, 15], sensitized to *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) who attended the allergology service of the “IPS Universita-

ria” clinic or the “Unidad Alergológica” clinic in Medellín, Colombia.

We selected patients over 14 years old with chronic AR according to ARIA guideline for >1 year and sensitization to *D. pteronyssinus* according to skin prick test (SPT) and sIgE with clinical relevance according to the medical history. Sensitization to other HDM (*Blomia tropicalis* and *Dermatophagoides farinae*) or insects (*Periplaneta americana*, *Blatella germanica*, and *Solenopsis invicta*) was evaluated by SPT and/or sIgE also documented. Evaluation of asthma and other atopic diseases were done according international guidelines (GINA 2020 <https://ginasthma.org/>, EPOS 2020 <https://epos2020.com/>). Patients with other causes of rhinitis (e.g., mechanical and vasomotor) were discharged. Age was based on Ethical Committee recommendation. Among the exclusion criteria was having a comorbidity or systemic drugs that could affect the interpretation of the results (e.g., irritable bowel syndrome, immunodeficiencies, cyclosporine, and omalizumab). All the patients were asked about their seafood consumption habits.

To reach the objective of the study, we carried out the following steps:

1. We selected a group of patients who attended between 2017 and 2019 in the centers based on the selection criteria.
2. Among the patients who agreed to participate, the frequency of shrimp sensitization was evaluated by SPT and specific IgE. (Nine patients refused to participate).
3. Among shrimp-sensitized patients, the frequency of shrimp allergy was assessed using OCT.
4. We performed exploratory analyzes of the characteristics of the patients according to the OCT results.

Skin Sensitization

The SPT was performed using allergen extract, histamine solution, and diluent according to GA2LEN recommendations [16]. A positive result was considered when the allergen extract tested had a wheal of 3 mm or greater compared to the negative control. *D. pteronyssinus* and *P. azteca* extracts were supplied by Immunotek Lab (Madrid, Spain). *L. vannamei* extract was prepared at the Institute for Immunological Research of the University of Cartagena (Cartagena, Colombia), as indicated in the online suppl. material (see www.karger.com/doi/10.1159/000516005 for all online suppl. material).

Total IgE and Specific IgE

Serum levels of total IgE and specific IgE (*D. pteronyssinus*, rDer p 10, Pen a, and rPen a 1) were measured using a fluoroenzyme immunoassay (Phadia ImmunoCap System, Uppsala, Sweden). Values over 0.35 KU_A/mL were considered positive. Sera yielding specific IgE levels above 100 IU/mL were diluted (1: 5) to maintain the test within the dynamic range and calculate the exact value of the measurement.

IgE Level to boiled *L. vannamei* extract and rLit v 1 were measured using the ELISA technique and IgE levels expressed in optical density: the cutoff points for each tested extract tested with ELISA was calculated from the results obtained from 5 healthy nonallergic, nonatopic subjects, using 2 standard deviations as an evaluation measure (shrimp extract 0.125, *L. vannamei* 1 0.123). Preparation details of *L. vannamei* extract and rLit v 1 are present in online suppl. material. *P. azteca* was noncooked and *L. vannamei* was cooked.

Table 1. General characteristics of patients with AR: Out of 443 patients with HDM sensitization, 86 patients have sensitization to shrimp

General characteristics	Total (n = 443)	Shrimp (n = 86)	p value
Age, years, median (range)	20 (52)	23.2 (42)	>0.1
Sex: female, n (%)	223 (50.3)	46 (53.4)	>0.1
Asthma, n (%)	112 (25.2)	31 (36)	>0.1
Der p sensitization, n (%)	443 (100)	86 (100)	>0.1
Der f sensitization, n (%)	440 (99.3)	86 (100)	>0.1
Cockroach sensitization to Bla g or Per a, n (%)	120 (27)	20 (24)	>0.1
<i>Solenopsis invicta</i> sensitization, n (%)	76 (17.1)	14 (16.2)	>0.1
Total IgE, IU/mL, mean $\pm$ SD ⁺	223 $\pm$ 111	247 $\pm$ 150	>0.1
Shrimp consumption, n (%)	na	42 (48.8)	na
Consumption in the 6 months before the atopy test, n (%)	na	20 (23.5)	na
Shrimp reactions, n (%)	na	7 (8.1)	na
Shrimp anaphylaxis, n (%)	na	3 (3.4)	na

“Atopy test” refers to the evaluation of the presence of IgE against allergens by SPT or specific IgE. na, no applicable; HDM, house dust mite; AR, allergic rhinitis; SPT, skin prick test. ⁺ Total IgE was measured in 245 patients.

Oral Challenge Test

For shrimp OCT, a single-blind placebo-controlled food challenge protocol was followed. A total of 90 g of shrimp (*P. azteca*) was administrated in a protocol of 4 doses: First, placebo was supplied (chicken croquettes). We asked the patients about possible food allergies before the challenge test to foods other than shrimp, so the risk of a positive challenge by the poultry meat and not by the shrimp is very low. Then shrimp croquettes in 3 doses (9, 27, and 54 g) were supplied. This rush protocol was done considering that the patients had no history of reaction with shrimp. Previous studies refer to multiple doses for shrimp-challenge test [17]. We calculated the doses according to the average consumption of a dish with shrimp. Between each dose, there was an interval of 30 min under medical supervision. After the last administration, the subjects were under inspection for 2 h. Shrimp and chicken croquettes were prepared with the same additional ingredients and cooking recipe.

A positive challenge (positive-OCT) was considered when objective symptoms such as hives, angioedema, 30% decrease in the peak expiratory flow, vomiting, or diarrhea were presented. In any of these cases, the challenge was stopped. In case of nonspecific symptoms, challenge test was continued. The OCT was performed in patients with shrimp IgE sensitization, except in those patients with a clear history of shrimp anaphylaxis in the 12 months previous to date of the test.

Statistical Analysis

For the descriptive analysis of the sociodemographic and clinical aspects of the study population, absolute and relative distributions and summary indicators such as arithmetic mean, median, standard deviation, quartiles, interquartile range, minimum values, and maximum values were used, as appropriate for its distribution.

The normality criterion of some variables was established using the Kolmogorov-Smirnov test. We evaluated the relationship be-

tween various variables using the Mann-Whitney U test, Pearson's χ^2 , and Fisher's exact test. A multivariate logistic regression was applied as an exploratory and adjustment method to evaluate the different clinical and sociodemographic characteristics between the groups of patients with positive-OCT and negative-OCT tests. A <0.05 p value was considered statistically significant.

Results

Patients Characteristics

A total of 443 patients with AR and Der p IgE sensitization were evaluated for shrimp sensitization. Eighty-six (19.4%) had sIgE to shrimp (Table 1), 98% to Der f, 35% to Blo t, and 23% to cockroaches spp. No differences were found in the sociodemographic characteristics between the shrimp-sensitized and the shrimp-nonsensitized patients.

Three patients had clinical history of anaphylaxis with shrimp in the last year, so they were considered positive for shrimp allergy without confirmatory OCT. Patients with history of possible minor shrimp reaction but not anaphylaxis underwent confirmatory OCT.

Positive-OCT to Shrimp Was Associated with High IgE to rDer p 10

We performed a total of 83 OCT. The positive-OCT group consisted of 20 patients with a positive challenge and 3 patients with a history of anaphylaxis to shrimp in the last year, and therefore, 23 patients had allergy

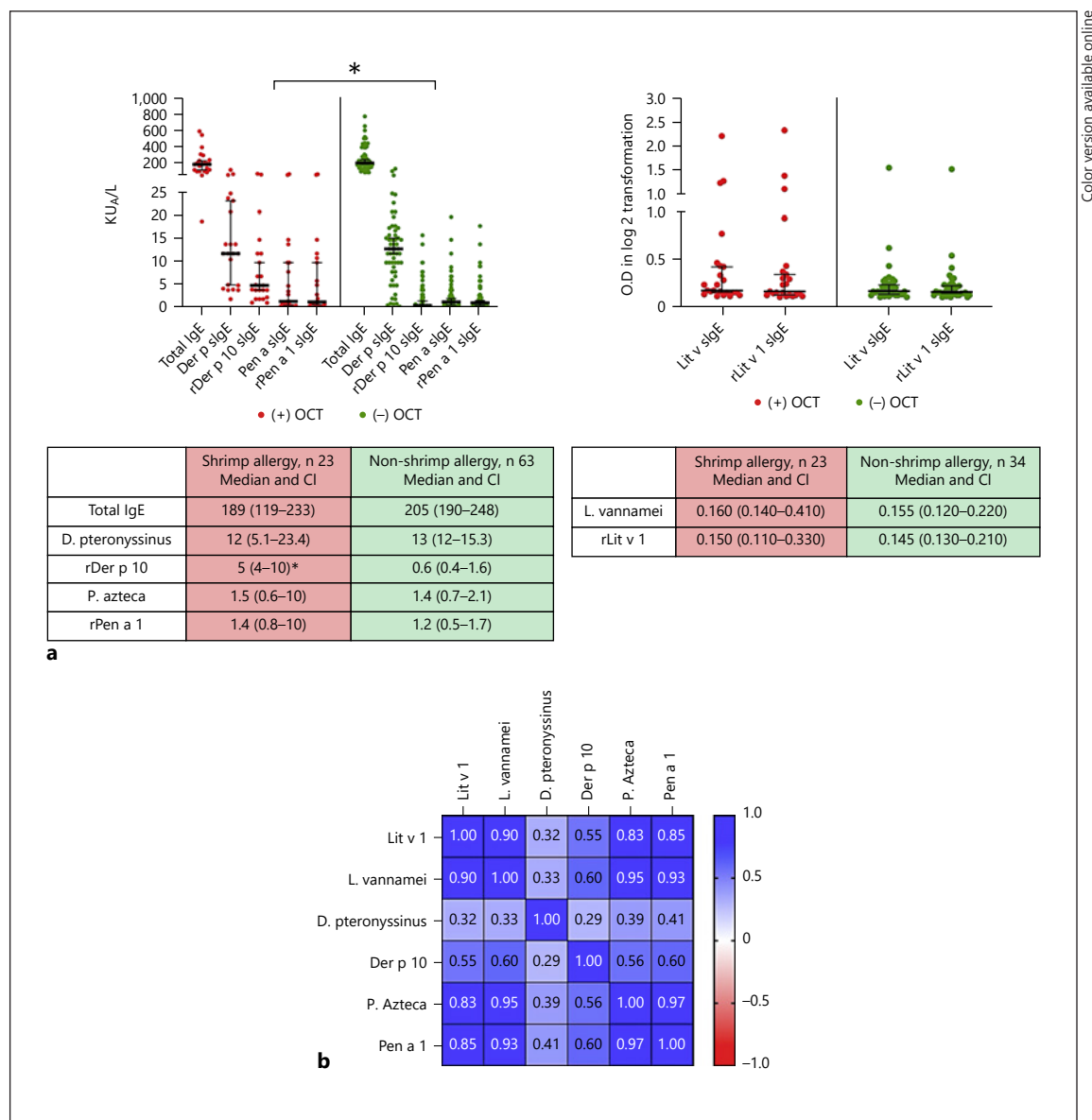


Fig. 1. a IgE sensitization according to results of OCT; sIgE against *L. vannamei* and Lit v 1 were measure with ELISA and expressed in OD in Log 2 transformation. **b** Correlation among IgE levels. CI, confidence interval; OD, optical density; OCT, oral challenge test. * $p < 0.01$. Lit v = *L. vannamei*.

to shrimp. When we compared patients according to challenge test results, there was not a different between total IgE or sIgE levels against *D. pteronyssinus*, *L. vannamei*, *P. azteca*, rLit v 1, and rPen a 1, and however, positive-OCT patients had significantly higher anti-Der p 10 IgE levels ($p = 0.009$) (Fig. 1a). There was a high correlation ($r > 0.7$) between IgE levels against shrimp extracts, rLit v 1, and rPen a 1. IgE levels against rDer p 10 had a moderate correlation with rLit v 1 ($r = 0.4$)

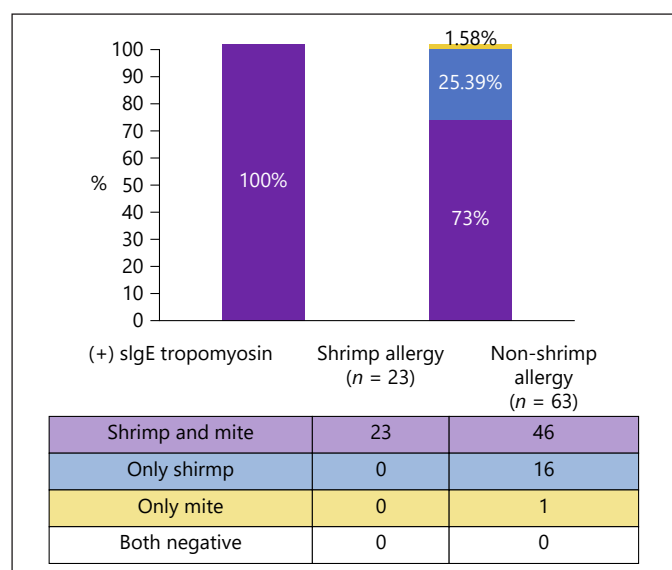
and rPen a1 ($r = 0.6$) the tropomyosin of the two-shrimp species.

All patients of the positive-OCT group had IgE against the tropomyosin from mites and shrimp (Fig. 2). The most common symptoms during the provocation test were skin reactions ($n = 18$, 78.2%), gastrointestinal symptoms ($n = 8$, 34.7%), and respiratory symptoms ($n = 6$, 26%). Three of them required adrenaline.

Table 2. General characteristics of patients according OCT results

General characteristics	Shrimp allergy (n = 23)	Nonshrimp allergy (n = 63)	p value
Age median, (range)	22.3 (14–54)	23.6 (15–45)	>0.1
Sex: female, n (%)	9 (39.1)	37 (58.7)	<0.001
Asthma, n (%)	5 (21.7)	26 (41.2)	>0.1
Total IgE, IU/mL (mean ± SD)	209±145	261±153	0.09
Shrimp consumption, n (%)	11 (47.8)	31 (49.2)	>0.1
Consumption in the 6 months before the atopy test, n (%)	5 (21.7)	15 (23.8)	>0.1
Shrimp reactions, n (%)	2 (8.6)	5 (7.9)	>0.1
Shrimp anaphylaxis, n (%)	3 (3.4)	0	>0.1

“Atopy test” refers to the evaluation of the presence of IgE against allergens by SPT or specific IgE. na, no applicable; OCT, oral challenge test; SPT, skin prick test.

**Fig. 2.** Co-sensitization to tropomyosin from HMD and shrimp among patients with positive IgE to shrimp extract. HDM, house dust mite.

Exploration of Variables Associated with OCT Results

Eleven of 23 patients with allergy to shrimp (47.8%) reported not having consumed this food or any other shellfish, which suggests that shrimp sensitization was by cross-reactivity to HDM or any other allergenic source (Table 2). There was no difference between OCT groups according to the frequency of shrimp consumption. Total IgE levels and frequency of asthma were higher in negative-OCT group but not statistically significant ($p = 0.09$ and 0.24 , respectively). Anti-Der p 10 IgE and female gen-

der were the only variables with a significant difference between OCT groups ($p < 0.001$).

Variables that presented some difference in OCT groups or have biological plausibility to be associated with one OCT results were included in logistic regression analysis to explore their possible usefulness as predictor variables (Table 3). To avoid linearity or separation bias, variables with a high correlation were evaluated in different analyzes (sensitivity analysis in 3 scenarios changing the number of variables and the type of shrimp extract to be included in the regression model). Anti-rDer p 10 IgE levels were only variable that presented a significant association to OCT-positive group after adjusting for the other variables. Female association disappeared after adjusting. The optimal cut-off of anti-rDer p 10 IgE levels to discriminate patients with a positive-OCT was over $5 \text{ KU}_A/\text{mL}$ according to the Youden index.

Discussion/Conclusion

Our study yields some interesting results: (i) the frequency of shrimp sensitization was 19.4% among patients with AR with IgE sensitization to HDM; (ii) 26.7% of them have shrimp allergy; (iii) 52.2% of them never consumed shrimp; and (iv) anti-rDer p 10 IgE levels could be a biomarker to predict clinical relevance of IgE sensitization to shrimp.

In the Tropics, there is a high frequency of exposure to HDM [1, 2] and several studies indicate that tropomyosin from different invertebrates shares a high identity in amino acid sequence [10, 18, 19]. Because about half of the

Table 3. Multiple regression models with different scenarios according to the included variables

Dependent variable	Scenario #1 OR (CI)	Scenario #2 OR (CI)	Scenario #3 OR (CI)
Gender	na	0.3 (0.08–1.3)	0.3 (0.08–1)
Age	na	0.9 (0.9–1.05)	0.9 (0.9–1)
Asthma	na	0.3 (0.06–1.6)	0.3 (0.0–1.7)
Total IgE	na	1 (0.99–1)	0.9 (0.9–1)
Shrimp consumption	na	1.1 (0.2–4.8)	0.6 (0.1–2.3)
<i>D. pteronyssinus</i>	na	na	1 (0.9–1)
<i>P. azteca</i>	na	na	0.8 (0.6–1.2)
<i>L. vannamei</i>	na	na	1.2 (0.2–5.0)
rDer p 10	1.22 (1.08–1.4)*	1.39 (1.1–1.8)*	1.32 (1–1.7)*
rLit v 1	na	1.3 (0.02–6.6)	na
rPen a 1	na	0.8 (0.5–1)	na

na, no applicable; OR, odds ratio; CI, confidence interval 95%. * $p < 0.01$. Each scenario has different variables included in the regression model.

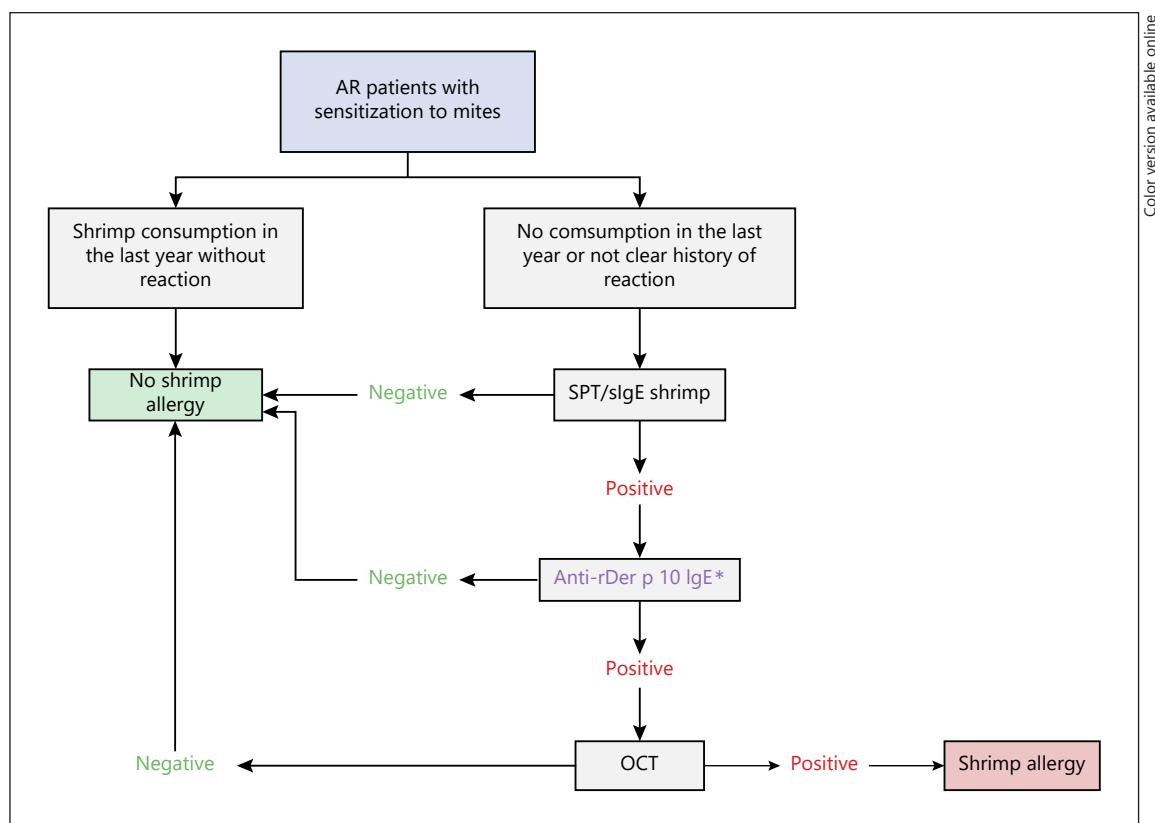


Fig. 3. Suggested flowchart according to the results of the study for the evaluation of shrimp allergy in patients with AR associated with HDM sensitization. *Anti-rDer p 10 IgE is a possible biomarker but has to be validated in new studies; if unavailable go to. AR, allergic rhinitis HDM, house dust mite.

patients in this study denied having previously consumed shellfish, shrimp sensitization is highly likely to be secondary to cross-reactivity [11]. Considering the low sensitization to cockroach, mites appear to be the main cause of this cross-reactivity. The high correlation found between IgE against rDer p 10, rPen a 1, and rLit v 1 supports this hypothesis. These results are in line with a previous study where shrimp sensitization was observed in people who do not consume shrimp due to their religious practices [7] and with studies where sensitization to shrimp appears after start mite immunotherapy [8, 9, 20, 21].

We focused on the population of patients with AR with HDM sensitization because this population is quite frequent and not routinely asked about the consumption of shellfish unless the patient reports a previous food reaction. Our study shows that 26% of AR patients with shrimp sensitization have an allergic reaction to shrimps, but this represents only 5% of the population of patients with rhinitis and HDM sensitization (23/443 patients). From these results, several questions arise which have to be considered in clinical guidelines: (a) should SPT with shrimp be performed on all patients with AR associated with HDM sensitization? and (b) in case of positive SPT to shrimp, should the OCT routinely be performed? The result of OCT shows that a not insignificant percentage of the patients can present reactions with the consumption of shrimp, but on the other hand, despite the fact that shrimp sensitization was high, 2 of 3 patients had a negative challenge. It is necessary to identify new biomarkers that allow us to select patients who require (or not) a shrimp oral challenge test. Several studies have proposed different factors as possible biomarkers to predict a reaction to shrimp, including having asthma, levels of IgE to shrimp extract, anti-rDer p 10 IgE, and sensitization to shrimp tropomyosin [11, 12, 22]. However, despite we use shrimp extract with different preparations (*P. azteca* was crude and *L. vannamei* was boiled) and tropomyosin from 2 shrimps' species, the only factor that seems to be associated with a positive shrimp-challenge was the presence of HDM tropomyosin sensitization. According to our results, it seems that a high proportion of shrimp sensitization is due to a cross-reactivity with HDM. This hypothesis is supported by the fact that in some tropical regions consumption of shrimp is occasional, while exposure to mites is constant and sensitization to HDM is more frequent than sensitization to shrimp or cockroach [3, 4]. Therefore, anti-Der p 10 IgE could be a specific predictor to define the clinical relevance of shrimp sensitization. Based on these results, we propose a manage-

ment algorithm to define which patients require evaluation of IgE sensitization to shrimp and OCT (Fig. 3). This approach could help to respond unmet needs in the diagnosis and treatment of patients with shrimp allergy [23]. Additionally, several allergens other than tropomyosin have been found to cross-react to shrimps and HDM [11, 24] and should be included in future studies. Moreover, at least 50% of patients sensitized to shrimp are not sensitized to HDM and tropomyosin, but the commercial extracts used for SPT to diagnose HDM and shrimp sensitization are largely unsatisfactory and only a few allergens are available for a molecular diagnosis.

Further analysis is necessary for a large number of patients to define the role of anti-Der p 10 IgE and some studies suggest that clinical relevance of IgE sensitization is dependent upon the level of specific IgE to tropomyosin [11, 22, 25]. Nevertheless, based on our results, 5 KU_A/mL seems to be an optimal cutoff point to predict positive-OCT.

There was a similar prevalence rate of shrimp sensitization among patients with asthma in total group versus shrimp-sensitized group. We did not observe a higher risk of shrimp allergy in asthmatic group; however, this was an exploratory observation because the size of sample data was focus in rhinitis. Shrimp allergy has generally been considered permanent, but recent studies suggest that remission may occur in a group of patients [26]. A better understanding of the sensitization patterns and their clinical impact could make it possible to identify which patients could have a remission of the disease.

Our study has some weaknesses; we did not perform inhibition analyzes to confirm cross-reactivity between different tropomyosin. Although inhibition analyzes would provide more information about the primary sensitizer, this was not the principal objective of the study and previous results support that HDM sensitization is an important risk factor for shrimp sensitization [10, 12, 27]. Another weakness of the study is that we did not evaluate the clinical relevance of sensitization to HDM, however, previous studies in this population show that 80% of patients with AR and HDM sensitization had a positive nasal challenge test [28], and most of clinical guidelines indicated that nasal challenge test is indicated only when there are doubts in the diagnostic, for example, when the patient is sensitized to multiple different sources. Although we started from a large group of patients with rhinitis ($n = 443$), the shrimp-challenge tests were performed only in those sensitized to this crustacean ($n = 86$), which reduced the sample size. Despite this, the results represent the population of interest, and the challenge tests

were performed on a large sample size compared to other related studies [11–13].

In conclusion, in patients with AR and sensitization to HDM, it is necessary to question for consumption of shrimp and consider carrying out additional tests to evaluate the risk of reactions with this food. Further studies are needed to assess the role of anti-Der p 10 IgE as a biomarker and the possible development of a predictive model.

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Statement of Ethics

The project was approved by the Ethics Committee of the University of Antioquia and the “IPS University” clinic (Code 2017-106 and INR52-2016, respectively). All patients who underwent sensitization or challenge tests signed an informed consent. This project was supported by an interinstitutional grant from Univer-

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Conflict of Interest Statement

The authors have no conflicts of interest. This project (Code 2017-16193) was supported by an interinstitutional grant (Code IN52-2016) of “IPS Universitaria” Clinic and the University of Antioquia for their technical support.

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Author Contributions

All the authors contributed equally to the writing and review of this article. Susana Diez contributed the central idea. Leonardo Puerta, Dalgys Martinez, and Jorge Sanchez performed the experimental part.

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Crude shrimp extract preparation

Peeled shrimps without shell and veins were boiled at 60 °C in 100 mL in 0.1M phosphate-buffered saline (PBS), pH 7.4 for 15 min. Afterward, the content was homogenized by a blender for 3 minutes in 0.01M (1:10 wt/vol) PBS, and shaking for 16 hours at 4 °C, then centrifuged at 6,500 r.p.m g for 30 minutes at 4°C. The final solution was dialyzed overnight (ON) against deionized water using 3.5 kDa cut-off dialysis membrane (Spectra/Dialysis membranes, Houston, TX,USA) and filtered through a 0.45-µm membrane. Protein content was measured by Bradford (Bio/Rad, Laboratories, San Francisco, CA, US).

Preparation of rLit v 1

Lit v 1 was produced in the Institute for Immunological Research by expression of the recombinant using expression vector pET45b+/Lit v1 (Genscript, Nanjing, China) in transformed *Escherichia coli* strain BL21 (DE3). A single fresh colony was grown in LBA culture medium ON at 37 °C, then an aliquot was diluted 1:20 in LBA and incubated until an optical density (OD) of 0.5 was reached. Protein expression was induced by the addition of 1 mM isopropyl-β-D-thio-galacto-pyranoside (IPTG), and incubation for 4 h at 37 °C. Then, cells were then harvested by centrifugation at 6500 rpm at 4 °C for 15 min. The cell pellets were solubilized in lysis buffer (8 M urea, 100 mM NaH₂PO₄, and 10 mM Tris, pH 8.0) at room temperature (RT) and sonication on ice using an ultrasonic homogenizer. Insoluble material was removed by centrifugation. The supernatant containing the recombinant protein was loaded to a Ni-NTA affinity column (Invitrogen, Carlsbad, CA, USA), and purified in denaturing conditions, following the instructions from the manufacturer. Fractions containing eluted proteins were pooled and dialyzed against 10 mM Tris, pH 8.0. The protein concentration was determined by Bradford assay (Bio-Rad Laboratories, San Francisco, CA, US). The purity and integrity of all proteins were assessed by SDS-PAGE.

Determination of serum IgE reactivity to *L. vanamei* extract and Lit v 1

Serum IgE level to *L. vannamei* extract ant Lit v1 were determined by ELISA as follows: microtiter plate wells (Immulon-4 Dynatech, Chantilly, VA, USA) were coated with 0.5 µg of Lit v 1 or 5 ug of shrimp extract in sodium carbonate/bicarbonate buffer at 4 °C ON. After three washes with PBS-Tween 20 (PBS-T), the wells were incubated with 100 µL of blocking buffer (PBS-T, 1% BSA, 0.02% sodium azide) for 3 hours at RT in a wet chamber. Then wells were incubated with 100 µL of human serum diluted 1:5 for IgE, ON at RT and after washing, the wells were incubated with 100 µL of alkaline phosphatase-conjugate anti human IgE (Sigma A3525) diluted 1:500 for 2 h at RT. After a final washing the wells were incubated with 100 µL of substrate p-nitrophenyl phosphate (Sigma)diluted in 10% diethanolamine, 0.5mM MgCl₂ at RT for 30 min. Absorbance at 405 nm wavelength was determined using a spectrophotometer (Spectra MAX 250, Molecular Device, Sunnyvale, CA, USA). All experiments were performed in duplicate.