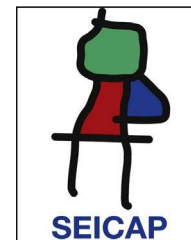




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## ORIGINAL ARTICLE

# Laboratorial characteristics of patients with diarrhoea suffering from egg white allergy

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Egg white protein

### Abstract

**Background:** Egg allergy is associated with diarrhoeal symptoms. However, the mechanism underlying allergic diarrhoea remains unclear.

**Objective:** To determine whether egg white-specific IgE antibodies coexist with egg white-specific IgG antibodies in patients with egg allergy featuring diarrhoeal symptoms, and whether there is any relationship between these two antibody types.

**Methods:** A total of 89 patients with egg allergy featuring diarrhoeal symptoms (average age, 23.2 years; range, 1–78 years), all of whom tested positive for egg white-specific IgG, were enrolled in this study. The concentration of total IgE, egg white-specific IgE and number of eosinophils in the serum were determined.

**Results:** Among the 89 egg white allergic patients tested, 49 (55.1%) patients showed high reactivity to egg white-specific IgG, 48 (53.9%) patients had elevated serum total IgE levels, and 25 (28.1%) patients had elevated absolute eosinophil numbers. Out of the 89 egg white allergic patients, 25 showed elevated egg white-specific IgE antibody levels. Of the 25 patients who were positive for egg white-specific IgE antibody, 21 presented high sensitive reaction to egg white-specific IgG, three presented moderate sensitive reaction to egg white-specific IgG, and one presented mild sensitive reaction to egg white-specific IgG. A moderate correlation between egg white-specific IgG and egg white-specific IgE, egg white-specific IgG and absolute eosinophil number was found in the egg white allergic patients ( $r=0.438$ ,  $P=0.000$ ;  $r=0.322$ ,  $P=0.002$ ). Egg white-specific IgE levels varied in different age groups; the egg white-specific IgE concentration of younger patients (age  $\leq 18$  years, mean rank 54.29) was significantly higher than that of the adult patients (age  $> 18$  years, mean rank 34.61) ( $Z=-3.629$ ,  $P=0.000$ ).

**Conclusion:** Egg white-specific IgE antibody could coexist with egg white-specific IgG antibody in patients suffering from egg white allergy. Aberrant changes in the concentration of egg white-specific IgE antibody were associated with the presence of egg white-specific IgG antibody.

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## Introduction

Food allergies affect approximately 1–10.8% of the general population, and their prevalence seems to be increasing.<sup>1</sup> Egg is one of the most common food allergens. Egg white is the most important source of allergens in the egg, and it contains 23 different proteins.<sup>2</sup> Ovomucoid, ovalbumin, ovomucoid, and lysozyme have been defined as the major allergens in egg white.<sup>3</sup> A high concentration of ovomucoid-specific IgE has been associated with persistent egg white allergy.<sup>4</sup> Another study reported that allergy was associated with more intense IgA and IgG responses to ovalbumin.<sup>5</sup> Previous work in our laboratory showed that cow's milk-specific IgE antibody could coexist with cow's milk-specific IgG antibody in patients suffering from cow's milk allergy. Aberrant changes in the concentration of cow's milk-specific IgE antibody were associated with the presence of cow's milk-specific IgG antibody.<sup>6</sup> We wished to determine whether this phenomenon also occurred in egg white allergic patients with diarrhoeal symptoms. Here, we studied the concentration changes in total IgE, egg white-specific IgE antibody, egg white-specific IgG antibody and eosinophil number in the peripheral blood of egg white allergic patients with diarrhoeal symptoms in the Zhongshan Hospital of the Medical College of Xiamen from November 2009 to September 2010. We further investigated whether there were any relationships between the two antibody types.

## Materials and methods

### Patients

A total of 89 egg white allergic patients (average age, 23.2 years; range, 1–78 years; 53 males and 36 females) who sought medical advice for diarrhoea from November 2009 to September 2010 were diagnosed at the Department of Digestive Health of Xiamen Zhongshan Hospital. The clinical diagnosis of egg white allergy was made following international guidelines.<sup>7,8</sup> The detailed characteristics of the investigated study groups are shown in Table 1. All patients tested positive for specific IgG against egg white. A group of 45 healthy subjects (average age, 26.1 years; range, 1–70 years; 24 males and 21 females) was also recruited. All subjects completed questionnaires regarding gastrointestinal symptoms and egg consumption. The study was approved by the local Research Ethics Committee, and informed consent was obtained from all participants. It was in compliance with the national legislation and Declaration of Helsinki guidelines.

### Measurement of egg white-specific IgG antibody in the serum

Blood samples (4 mL) were drawn from all subjects. The blood samples were allowed to stand for 20–30 min before centrifugation at 3000 rpm for 15 min. The serum was aspirated and frozen at  $-80^{\circ}\text{C}$  for subsequent analysis. Samples were processed in the central laboratory (Xiamen Zhongshan Hospital, China) using a commercially available enzyme-linked immunosorbent KIT (ELISA, Biomerica, Inc. Newport

**Table 1** Clinical characterisation of the study groups.

<i>Number of patients with egg white allergy</i>	89
Average age (years)	23.2 (1–78)
Male/Female	53/36
Mean introduction time of egg white (days)	1–8
Specific IgG to egg white (range, U/mL), positive rate (%)	51.0–948.4 (100%) <sup>a</sup>
Specific IgE to egg white (range, U/mL), positive rate (%)	0–35.6 (28.1%) <sup>a</sup>
Total IgE (range, ng/mL), positive rate (%)	5.8–7896.0 (53.9%) <sup>a</sup>
<i>Number of controls</i>	45
Average age (years)	26.1 (1–70)
Male/Female	24/21
Mean introduction time of egg white (days)	–
Specific IgG to egg white (range, U/mL), positive rate (%)	0–55.0 (4.4%)
Specific IgE to egg white (range, U/mL), positive rate (%)	0–0.4 (2.2%)
Total IgE (range, ng/mL), positive rate (%)	6.27–152 (2.2%)

<sup>a</sup> The proportion of specific IgG to egg white, specific IgE to egg white, and total IgE were significantly higher than the control ( $P=0.000$ ). Fisher's Exact Test was used to determine the significant differences across groups.

Beach, CA, USA). The protocol was performed according to the instructions provided with the ELISA kit. Egg white-specific IgG  $< 50$  U/mL was considered negative; IgG concentrations between 50 U/mL and 100 U/mL were considered to indicate mild sensitivity; IgG concentrations between 100 and 200 U/mL were considered to indicate moderate sensitivity; and IgG concentrations  $> 200$  U/mL were considered to indicate high sensitivity.

### Measurement of egg white-specific IgE antibody in the serum

The egg white-specific IgE antibody in the serum was measured using an ELISA kit (DR. FOOKE Laboratorien GmbH, Germany). Egg white-specific IgE  $< 0.35$  U/mL was considered negative; IgE concentrations between 0.35 and 0.7 U/mL were considered to indicate a weak reaction; IgE concentrations between 0.7 and 3.5 U/mL were considered to indicate a moderate reaction; and IgE concentrations  $> 3.5$  U/mL were considered to indicate a strong reaction.

### Total IgE and eosinophils determination

Total IgE and eosinophils were measured as described in our earlier study.<sup>6</sup>

### Statistical analysis

All statistical analyses were conducted using SPSS for Windows version 17. The data were expressed as the

mean  $\pm$  standard deviation. The means of two independent groups were compared using the unpaired Student's *t*-test. Chi-square tests were used to determine the significant differences across groups. The correlations between egg white-specific IgG and egg white-specific IgE were assessed using Pearson's correlation test.  $P < 0.05$  (2-tailed) was considered to indicate a statistical difference.

## Results

### Serum egg white-specific IgG level

Sera from 89 egg white allergic patients suffering from diarrhoea were subjected to ELISA to measure egg white-specific IgG. The egg white-specific IgG concentration in the 89 egg white allergic patients was  $273.7 \pm 207.4$  U/mL. All patients tested positive for IgG specific to egg white, while only 4.4% of control patients tested positive ( $P = 0.000$ ). Among the 89 egg white allergic patients tested, 19 (21.3%) showed mild reactivity, 21 (23.6%) showed moderate reactivity, and 49 (55.1%) showed high reactivity. The concentrations of egg white-specific IgG in each of these patient groups were  $70.15 \pm 14.37$ ,  $153.6 \pm 23.30$ , and  $404.2 \pm 196.2$  U/mL, respectively.

### Serum total IgE and egg white-specific IgE levels

Among the 89 egg white allergic patients suffering from diarrhoeal symptom, serum total IgE levels increased in 48 patients. The total IgE level and egg white-specific IgE levels in egg white allergic patients was significantly higher than that in controls ( $Z = -5.927$ ,  $P = 0.000$ ;  $Z = -5.292$ ,  $P = 0.000$ ). Of the 89 egg white allergic patients tested, 25 (28.1%) showed elevated levels of egg white-specific IgE. There was no significant difference in the egg white-specific IgE concentrations between male and female patients (data not shown). Egg white-specific IgE levels varied in different age groups; the egg white-specific IgE concentration of younger patients (age  $\leq 18$  years, mean rank 54.29) was significantly higher than that of the adult patients (age  $> 18$  years, mean rank 34.61) ( $Z = -3.629$ ,  $P = 0.000$ , Table 2). Of the 25 egg white allergic patients who had positive for egg white-specific IgE, seven (28.0%) had weak reactions to egg white-specific IgE, with a concentration of  $0.4657 \pm 0.0591$  U/mL; 13 (52.0%) had moderate

reactions to egg white-specific IgE, with a concentration of  $1.236 \pm 0.6440$  U/mL; and five (20.0%) had strong reactions to egg white-specific IgE, with a concentration of  $15.27 \pm 11.97$  U/mL. The levels of both serum total IgE antibody and egg white-specific IgE antibody were elevated simultaneously in 17 patients (19.1%). However, total IgE in egg white allergic patients was not correlated with egg white-specific IgE ( $r = 0.104$ ,  $P = 0.330$ ).

### Serum eosinophil levels

Among the 89 egg white allergic patients, 25 (28.1%) patients had an elevated absolute number of eosinophils. The absolute number of eosinophils in egg white allergic patients was not significantly higher than that in controls ( $Z = -1.026$ ,  $P = 0.305$ ). The absolute number of eosinophil was not correlated with egg white-specific IgE level ( $r = 0.128$ ,  $P = 0.232$ ).

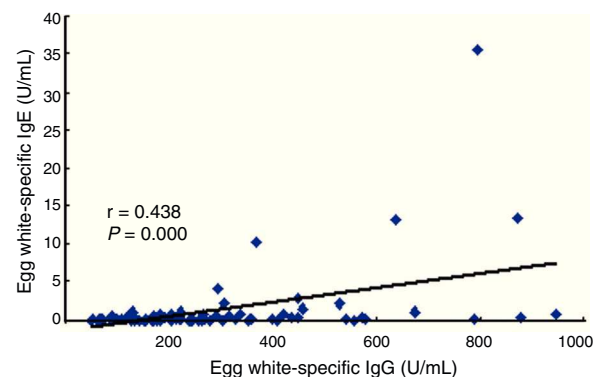
Association of egg white-specific IgG and total IgG, total IgE, egg white-specific IgE, and absolute eosinophil number in egg white allergic patients. Pearson correlation analysis was used to analyse the relationships between egg white-specific IgG and total IgG, total IgE, egg white-specific IgE, and absolute eosinophil number in egg white allergic patients. Egg white-specific IgG was not correlated with total IgG ( $r = 0.026$ ,  $P = 0.810$ ) or total IgE ( $r = 0.156$ ,  $P = 0.143$ ). However, egg white-specific IgG was positively correlated with egg white-specific IgE ( $r = 0.438$ ,  $P = 0.000$ , Fig. 1) and the absolute number of eosinophils ( $r = 0.322$ ,  $P = 0.002$ , Fig. 2) in these patients.

Among the 25 egg white allergic patients with elevated egg white IgE antibody levels, 21 (84.0%) presented high reactivity to egg white IgG antibody, three (12%) presented moderate reactivity, and one (4.0%) presented mild reactivity (Table 3). Five out of the 21 egg white allergic patients positive for egg white-specific IgE antibody and high reactivity to egg white-specific IgG antibody showed high egg white-specific IgE concentrations, 11 had moderate egg white-specific IgE concentrations, and five had low egg white-specific IgE concentrations (Table 3).

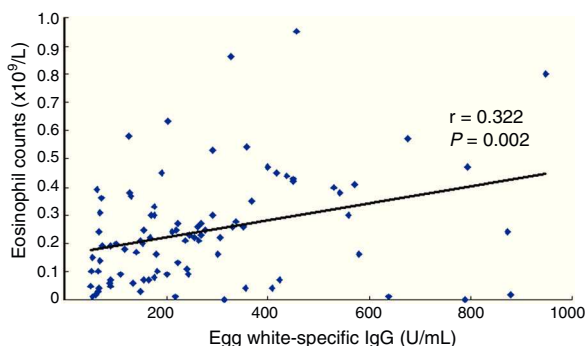
**Table 2** Egg white-specific IgE levels in different age groups.

Study group age (years)	Egg white-specific IgE cases (n)	Change in median value of egg white-specific IgE (U/mL)
$\leq 18$	47	0.240
$> 18$	42	0.015 <sup>a</sup>
Total	89	

<sup>a</sup> The egg white-specific IgE concentration of younger patients (age  $\leq 18$  years) was higher than that of adult patients (Mann-Whitney  $U = 550.5$ ,  $P = 0.000$ ). The difference was statistically significant.



**Figure 1** The relationship between egg white-specific IgG and egg white-specific IgE in egg white allergic patients. Sera from 89 egg white allergic patients were analysed to evaluate the correlation between egg white-specific IgG and egg white-specific IgE. There was a significant quantitative correlation between the levels of egg white-specific IgG and egg white-specific IgE ( $r = 0.438$ ,  $P = 0.000$ ).



**Figure 2** The relationship between egg white-specific IgG and absolute eosinophil number in egg white allergic patients. Sera from 89 egg white allergic patients were analysed to evaluate the correlation between egg white-specific IgG concentration and absolute eosinophil number. There was a significant quantitative correlation between the levels of egg white-specific IgG and the absolute eosinophil number ( $r = 0.322$ ,  $P = 0.002$ ).

## Discussion

Gastrointestinal symptoms (mostly in conjunction with other immediate symptoms) occur in 40–50% of egg white allergy cases following egg ingestion.<sup>2</sup> Immunological changes associated with the ingestion of extensively heated egg in children with egg white allergy were recently published by Lemon-Mule et al.<sup>9</sup> Egg white allergy can be caused by the proteins in the egg white or the egg yolk. The proteins found in egg white are more common triggers of allergic reactions than are yolk proteins. In our study, all patients presented diarrhoea, which is usually observed as a delayed symptom but can also be immediate. Therefore, a reliable laboratory test for egg allergy featuring diarrhoeal symptoms is required. In our study, 89 patients along with diarrhoea were positive for egg white-specific IgG. Among the 89 patients, 55.1% of patients' results exhibited high sensitivity, 23.6% exhibited moderate sensitivity, and 21.3% exhibited mild sensitivity. These results showed that egg white allergic patients with diarrhoea as a predominant symptom were highly sensitive to egg white protein. This was consistent

with a report by Kukkonen et al. They found that allergy was associated with more intense IgA and IgG responses to ovalbumin.<sup>5</sup> Ahrens also showed that egg-allergic patients produce significantly more egg-specific IgG antibodies than controls even when patients are on an allergen-free diet.<sup>10</sup>

It has previously been shown that continuous egg intake boosts systemic IgE responses in allergic patients.<sup>11</sup> Therefore, total IgE and egg white-specific IgE in egg white allergic patients were analysed simultaneously. Of the 89 egg white allergic patients in our study, 53.9% had elevated serum total IgE antibody levels, and 28.1% patients had elevated egg white-specific IgE antibody levels. No correlation was observed between the total IgE and egg white-specific IgE values. Boyano-Martinez et al. found that children with severe or moderate reactions had higher specific IgE levels to egg white and lower serum total IgE than children with mild or no reactions.<sup>12</sup> Fewer patients were positive for egg white-specific IgE, however more patients were positive for egg white-specific IgG. This may be because egg-specific IgG can counteract egg-specific IgE activity through direct competition for epitope binding or via inhibitory FcRIIB-dependent signals.<sup>13</sup> Our experimental results showed that most egg white allergic patients had moderate egg white-specific IgE reactions, followed by low and high reactions. The distribution of reaction intensity to egg white-specific IgE antibody is similar to that in our earlier study of cow's milk-specific IgE antibody.<sup>6</sup> The positive rate of egg white-specific IgE antibody was lower than the positive rate of cow milk-specific IgE (28.1% vs. 40.0%). Of the allergic patients with elevated egg white IgE antibody levels, 21 (84.0%) presented high reactivity to egg white IgG antibody. This result was similar to the result in 40 cow's milk allergic patients with lifted cow's milk IgE antibody level. Among them, 28 (70.0%) present highly sensitive reactivity to cow's milk IgG antibody.<sup>6</sup> These results showed that the egg white-specific IgE could be presented in egg white allergic patients with delayed symptoms. In the majority of cases, the antibody typically responsible for an egg allergic reaction was moderate. Benhamou et al. showed significant differences in egg-specific IgE levels between patients with severe, moderate or absent reactions at challenge; these levels were highest for patients with severe reactions and decreased

**Table 3** Serum egg white-specific IgE levels in egg white allergic patients with different egg white-specific IgG concentrations.

	Egg white allergic patients (n)	Spec. IgE-negative cases (n)	Spec. IgE-positive cases (n)			Rate of spec. IgE positivity (%)	
			Total	Low reaction	Moderate reaction		High reaction
Egg white-specific IgG mild reactivity	19	18	1	1	0	0	5.3%
Egg white-specific IgG moderate reactivity	21	18	3	1	2	0	14.3%
Egg white specific IgG high reactivity	49	28	21	5	11	5	42.9%
Total	89	64	25	7	13	5	28.1%

There were significant differences in the rates of egg white-specific IgE positivity among the groups with mild egg white-specific IgG reactivity, moderate reactivity and high reactivity ( $\chi^2 = 48.10$ ,  $P = 0.000$ ).



with the severity of the reaction.<sup>14</sup> Therefore, the level of egg-specific IgE might be helpful to assess the potential risk of allergic reaction to egg. Jaervinen et al.<sup>15</sup> demonstrated that IgE antibodies against sequential ovomucoid epitopes were found more often in patients with persistent rather than resolved egg allergy.

Eosinophils, along with basophils and mast cells, are important mediators of allergic response and asthma pathogenesis, and their levels are associated with disease severity. Here, the eosinophil number in egg allergic patients was calculated. The findings showed that the number of eosinophils in egg white allergic patients was moderately correlated with egg white-specific IgE level. This result was consistent with those of previous studies.<sup>16</sup> During the initial 4–8 h of the reaction, the site of response is primarily invaded by eosinophils. These infiltrating cells are activated and release mediators such as platelet-activating factor, eosinophil major basic protein, eosinophil cationic protein and cytokines. In the subsequent 24–48 h, lymphocytes and monocytes infiltrate the area and establish a more chronic inflammatory milieu.<sup>17</sup> Therefore, eosinophils contribute to the pathogenesis of the allergic reaction.

We also found that the egg white-specific IgG concentration changes in egg white allergic patients correspond to the changes in egg white-specific IgE concentration and eosinophil number. The egg white-specific IgG concentration in egg white allergic patients was positively correlated with egg white-specific IgE concentration and eosinophil number, respectively. The present observation revealed a close correlation between the production of IgE and the levels of IgG antibody against egg white protein, which may imply a close relationship between the synthesis of IgE and IgG, as previously suggested by Lilja et al.<sup>18</sup> This correlation indicates that egg white-specific IgE antibody in egg white allergic patients with diarrhoea coexists with egg white-specific IgG antibody. The egg white-specific IgE and IgG antibodies associated with the clinical course of egg allergy along with diarrhoea should be studied further.

## Ethical disclosures

**Protection of human and animal subjects.** The procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

**Confidentiality of data.** We have followed the protocols of their work centre on the publication of patient data and that all the patients included in the study have received sufficient information and have given their informed consent in writing to participate in that study.

**Right to privacy and informed consent.** We have obtained the informed consent of the patients and subjects mentioned in the article. Dr. Tian-Ci Yang is in possession of this document.

## Conflict of interest statement

The authors have no conflicts of interest to disclose.

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