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ORIGINAL ARTICLE

Component-resolved diagnostics in Thai children with cow's milk and egg allergy

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Abstract

Background and Objectives: Component-resolved diagnostics (CRD) have garnered a lot of attention in recent years in the diagnosis of food allergies. We aimed to investigate sensitization against cow's milk and egg white components, and to study the clinical usefulness of serum food-specific immunoglobulin E (sIgE) to cow's milk and egg white components in Thai children with cow's milk and egg allergies.

Methods: Children with IgE-mediated cow's milk and/or egg allergy were enrolled. Clinical reactions were determined. Specific IgE against cow's milk, egg white, alpha-lactalbumin (ALA), beta-lactoglobulin (BLG), casein, ovomucoid, ovalbumin and conalbumin were measured.

Results: Thirteen cow's milk allergic subjects and 32 egg allergic subjects were identified. The sensitization rate to BLG and casein was 91.7%, followed by ALA (66.7%) for cow's milk, and the sensitization rate to ovalbumin was 93.8%, followed by ovomucoid (81.3%) and conalbumin (37.5%) for egg. Patients in the urticaria group had a higher level of casein sIgE than the atopic dermatitis (AD) group but this difference was not significant (9.8 kU_A/L vs. 0.9 kU_A/L, $p = 0.11$). The level of ovomucoid sIgE was significantly higher in the non-AD group than in the AD group (3.8 kU_A/L vs. 1.3 kU_A/L, $p = 0.048$).

Conclusion: BLG and casein for cow's milk and ovomucoid and ovalbumin for egg were the common components causing sensitization in cow's milk and egg allergic patients. Among the patients with cow's milk allergy, the level of casein sIgE in the urticaria group tended to be higher than the AD group, and in egg allergic patients, the non-AD group had a significantly higher ovomucoid sIgE level compared with the AD group.

Keywords: component-resolved diagnostics, cow's milk allergy, egg allergy, food allergy, Thai children

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Introduction

Food allergies, described as adverse immune responses to food, are responsible for a variety of symptoms and disorders involving the skin and gastrointestinal and respiratory tracts and can be attributed to immunoglobulin E (IgE)-mediated and non-IgE-mediated mechanisms. Adverse immune responses to foods affect approximately 5% of young children and 3% to 4% of adults in Western countries, and appear to have increased in prevalence.[1] Milk, egg, wheat, soy, peanut, tree nuts, fish and shellfish are considered common causes of adverse food reactions.[1] The prevalence of adverse food reactions using a food allergy questionnaire among Thai children was 6.25% and the prevalence of IgE-mediated food allergies was estimated to be approximately 0.45%.[2] The most common trigger foods among younger children (6 months to 3 years) included cow's milk and egg.

Food allergies can cause life-threatening reactions and greatly influence quality of life. Accurate diagnosis of food allergy is important in order to avoid serious allergic reactions and prevent unnecessary dietary restrictions, but it can be difficult. Skin prick testing (SPT) and serum food-specific IgE (sIgE) levels are extremely sensitive testing options, but positive test results for tolerated foods are not uncommon.[3]

Double-blind placebo-controlled oral food challenge (OFC) is currently the gold standard test for diagnosing food allergies.

Due to the time and labor-intensive nature of this test, open food challenge is typically performed in clinical settings. Even open OFC can be time consuming though, and there is a risk of immediate allergic reaction and anaphylaxis.[3] Lieberman et al.[4] reported that 132 (18.8 %) of 701 open OFCs to a variety of foods elicited a reaction in their university-based, outpatient practices over about a 2-year period. Of those reactions, 9.1 % were treated with epinephrine.

There is clearly room for improvement in testing to differentiate asymptomatic sensitization from true clinical allergies prior to OFC. Allergen component-resolved diagnostics (CRD) have garnered a lot of attention in recent years in the diagnosis of food allergies, offering the possibility of a more accurate assessment. Instead of using crude allergen extracts consisting of a mixture of allergenic and non-allergenic components, CRD uses pure allergen proteins, produced by the purification of natural allergen sources or recombinant expression of allergen-encoding complementary DNA. Recently, a number of studies have demonstrated that CRD may improve the specificity of allergy testing in a variety of foods, including cow's milk and egg.[3]

The most important allergens in egg white are the very thermostable ovomucoid (Gal d 1) and the less heat-stable allergens ovalbumin (Gal d 2), ovotransferrin or conalbumin (Gal d 3) and lysozyme (Gal d 4). Patients with a persistent egg allergy have higher sIgE levels to ovomucoid and ovalbumin than children who develop tolerance. Cow's milk contains numerous proteins including alpha-lactalbumin (Bos d 4), beta-lactoglobulin (Bos d 5), bovine serum albumin (Bos d 6) and casein (Bos d 8).[5] Of 104 children with suspected cow's milk or egg allergy, the microarray components Bos d 8 for cow's milk and Gal d 1 and Gal d 2 for egg were the most frequently recognized allergens that had a good ability to predict the food challenge test results.[6]

The objectives of this study were to investigate sensitization patterns against cow's milk and egg white allergen components, and to study the clinical usefulness of sIgE to cow's milk and egg white allergen components in Thai children with cow's milk and egg allergy.

Methods

Study design and participants

This study was a cross-sectional study. Thirty-four participants with IgE-mediated reaction or mixed type reaction to cow's milk and/or eggs had undergone evaluation at the Pediatric Allergy Clinic of Songklanagarind Hospital between March 2014 and March 2015. Clinical information was collected either through a questionnaire or chart review. Written informed consent was obtained. Cow's milk and egg allergies were documented when a patient reported a convincing history of acute reaction after food ingestion in the previous 12 months, had detectable sIgE (≥ 0.35 kU_A/L), had a positive SPT or a positive OFC result. The study was approved by the Institutional Ethics Committee of the Faculty of Medicine, Prince of Songkla University and was registered as a Thai Clinical Trial with ID TCTR20150107002.

SPT and serum sIgE

The SPT was performed on the upper back with a plastic Duotip® lancet (Lincoln diagnostic, Decatur, IL, US). A positive SPT was defined as a mean wheal diameter ≥ 3 mm compared to the negative control. Serum sIgE against cow's milk, egg white and allergen components including alpha-lactalbumin (ALA), beta-lactoglobulin (BLG), casein, ovomucoid, ovalbumin and conalbumin were performed by the ImmunoCAP® system (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer's instructions. The lower and upper detection limit was 0.0 and 100 kU_A/L, respectively. Serum sIgE values of 0.35 kU_A/L or greater were considered indicative of sensitization.

Statistical analysis

The data were analyzed using R statistical software. Variables are displayed as median and interquartile range (IQR). The Wilcoxon rank-sum test was used for comparing the median of sIgE with cow's milk, egg white and allergen components. P-values of less than 0.05 were regarded as significant.

Results

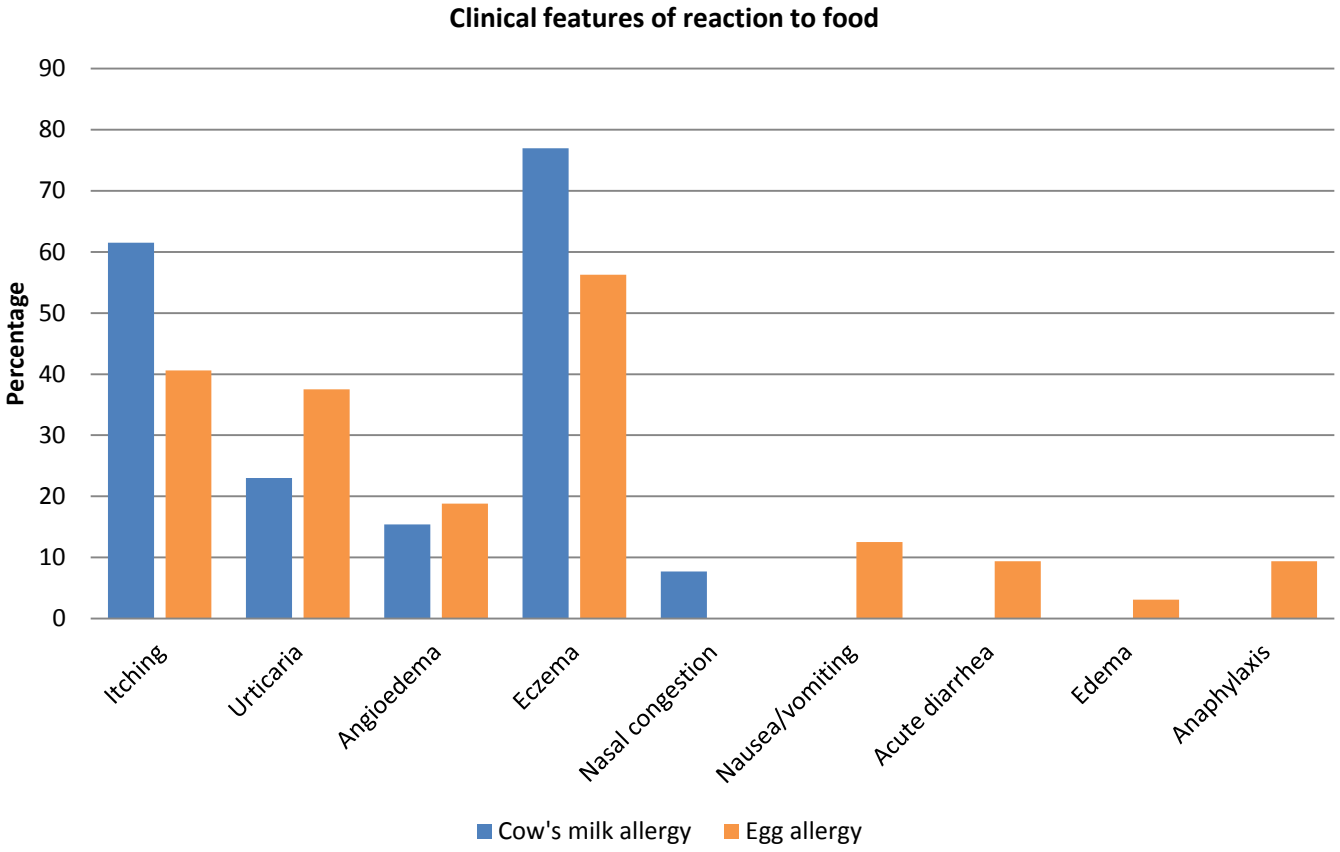
Participants' characteristics

Thirteen cow's milk allergic subjects and 32 egg allergic subjects were enrolled. The baseline characteristics of the patients with cow's milk and egg allergy are presented in **Table 1**. The median age of the onset of cow's milk and egg allergy was 3 months and 6 months, respectively. Eczema was the most common presentation of both cow's milk allergy and egg allergy (**Figure 1a**) and the most common diagnosis of food allergy was atopic dermatitis (AD) (**Figure 1b**). Characteristics and allergologic testing of patients with cow's milk and egg allergy are shown in **Tables 2 and 3**.

Table 1. Demographic characteristics

Characteristics	Cow's milk allergy (n=13)	Egg allergy (n=32)
Current age (mo), median (range)	28 (6-99)	23 (7-99)
Age of food introduction (mo), median (range)	0 (0-9)	6 (0-17)
Age of food reaction (mo), median (range)	3 (1-12)	6 (1-62)
Sex, male, number (%)	9 (69.2)	15 (46.9)
Co-morbidity (allergic disease), number (%)		
• Asthma	2 (15.4)	2 (6.2)
• Allergic rhinitis	1 (7.1)	3 (9.4)
• Atopic dermatitis	11 (84.6)	24 (75)
• Drug allergy	3 (23.1)	3 (9.4)
Skin prick test wheal size (mm), median (range)	5 (0-12) (n=5)	2.5 (0-13) (n=18)
Skin prick to prick test wheal size (mm), median (range)	0 (0-9) (n=5)	13 (5-38) (n=14)
Specific IgE (kU_A/L), median (range)	7.2 (1.27-79.5)	6.21 (0.58-100)

(a)



(b)

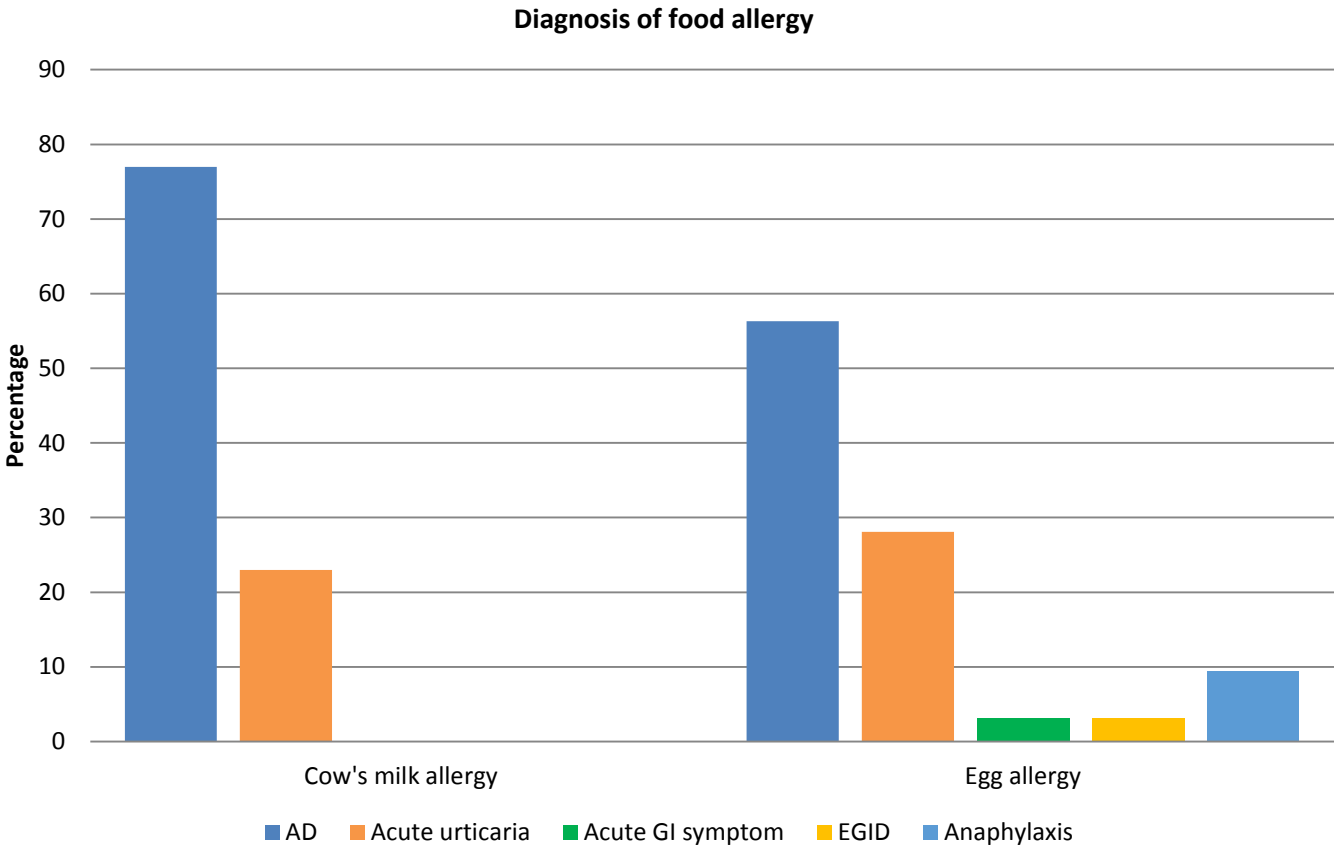


Figure 1. Clinical features of reaction to foods (a) and diagnosis of food allergy (b) in patients with cow's milk and egg allergy

Table 2. Characteristics and allergologic testing in patients with cow's milk allergy

Patient	Sex/age (mo)	Clinical features					Diagnosis	Skin test wheal size (mm)		Specific IgE levels (kU_A/L)			
		Itching	Urticaria	Angioedema	Eczema	Nasal congestion		SPT	PTP	CM	ALA	BLG	Casein
1	M/76	+	+				Acute urticaria	12	ND	51.4	6.87	10.8	56.1
2	M/19				+		AD	ND	ND	79.5	58.9	13.2	4.65
3	M/28	+	+	+			Acute urticaria	6	6	7.2	0.9	0.84	9.8
4	F/11				+		AD	4	ND	5.36	4.9	8.35	0.52
5	M/34	+			+		AD	ND	ND	9.04	10.9	6.17	5.28
6	M/28				+		AD	ND	ND	50.2	2.71	67.1	9.81
7	M/29				+		AD	0	0	15.3	1.12	0.5	0.36
8	M/24		+	+			Acute urticaria	ND	ND	2.97	1.08	0.13	1.37
9	F/20	+			+		AD	ND	0	1.27	0.02	2.04	0.19
10	F/99	+			+		AD	ND	9	1.37	0.03	0.04	1.94
11	M/36	+			+		AD	5	0	2.48	0.27	0.47	0.54
12	F/8	+			+	+	AD	ND	ND	4.5	0.08	1.19	1.22
13	M/6	+			+		AD	ND	ND	16.9	0.19	0.45	0.25

ND: not done

Table 3. Characteristics and allergologic testing in patients with egg allergy

Patient	Sex/age (mo)	Clinical features							Diagnosis	Wheal size (mm)			Specific IgE levels (kU_A/L)		
		Itching	Urticaria	Angioedema	Eczema	N/V	Diarrhea	Edema		SPT	PTP	Egg	Ovomucoid	Ovalbumin	Conalbumin
1	F/29					+	+		Acute GI reaction	8	25	7.59	8.16	2.75	0.47
2	F/22		+						Acute urticaria	8	ND	2.61	2.19	1.54	0.07
3	M/11				+				AD	ND	ND	7.91	9.81	3.09	0.46
4	M/11	+			+				AD	ND	ND	6.18	0.61	7.2	0.11
5	M/76	+	+						Acute urticaria	9	ND	22.4	18.2	3.61	4.45
6	M/19				+				AD	ND	ND	100	100	92.7	3.36
7	M/22							+	EGID	0	5	2.95	0.12	2.11	0.19
8	M/28				+				AD	0	ND	2.02	0.99	1.91	0.03
9	M/7				+				AD	ND	ND	7.75	2.52	4.12	0.23

Table 3. Characteristics and allergologic testing in patients with egg allergy (Continues)

Patient	Sex/age (mo)	Clinical features							Diagnosis	Wheal size (mm)			Specific IgE levels (kU_A/L)		
		Itching	Urticaria	Angioedema	Eczema	N/V	Diarrhea	Edema		SPT	PTP	Egg	Ovomucoid	Ovalbumin	Conalbumin
10	F/34	+			+				AD	0	7	100	9.5	98.1	40.9
11	F/22		+			+			Anaphylaxis	0	13	0.69	0.85	0.39	0.02
12	F/22				+				AD	0	6	0.65	0.03	0.02	0
13	M/34	+			+				AD	ND	ND	6.24	1.54	6.46	0.84
14	M/34				+				AD	ND	ND	100	100	100	1.39
15	M/76		+	+					Acute urticaria	13	38	6.24	1.25	7.24	1.9
16	M/29				+				AD	0	13	1.72	2.31	1.24	0.07
17	M/24		+	+					Acute urticaria	ND	ND	10.3	3.32	10.5	0.33
18	F/18	+			+				AD	5	ND	1.3	1.15	0.23	0.01
19	F/18	+			+				AD	6	ND	1.94	2.47	1.04	0.03
20	F/72		+	+		+	+		Acute urticaria	ND	ND	1.19	3.36	0.6	0.05
21	F/32	+	+						Anaphylaxis	8	20	16.7	9.94	9.73	5.01
22	F/36				+				AD	0	13	9.48	3.85	11.1	4.34
23	F/9				+				AD	ND	ND	2.49	0.15	3.2	0.02
24	F/20	+	+						Acute urticaria	ND	ND	13.5	19.9	3.32	0.19
25	F/99	+	+	+					Acute urticaria	ND	17	2.88	2.74	2.33	0.89
26	M/36	+	+			+	+		Anaphylaxis	8	19	15.2	9.23	15.3	0.75
27	M/28				+				AD	0	10	0.58	0.33	0.81	0.17
28	F/17		+	+					Acute urticaria	5	10	0.75	6.23	1.2	0.11
29	M/8				+				AD	ND	ND	7.47	0.01	11.9	0.14
30	F/10	+			+				AD	ND	ND	1.25	0	1.69	0.09
31	F/8	+	+	+					Acute urticaria	ND	ND	28	3.75	4.48	0.14
32	F/14	+			+				AD	0	10	3.27	1.02	3.23	0.1

SPT to cow's milk and egg white

The median wheal size of the SPT for cow's milk and egg white was 5 mm and 2.5 mm, respectively (**Table 1**). Interestingly, the SPT wheal size for raw egg white was greater than commercial extract in all patients (**Table 3**).

Serum sIgE to cow's milk and allergen components

The sensitization rates against cow's milk and egg components are shown in **Figure 2a**. The sensitization rate to BLG and casein was 91.7%, followed by ALA (66.7%). The median sIgE levels were 7.2, 1.08, 1.19 and 1.37 kU_A/L for cow's milk, ALA, BLG and

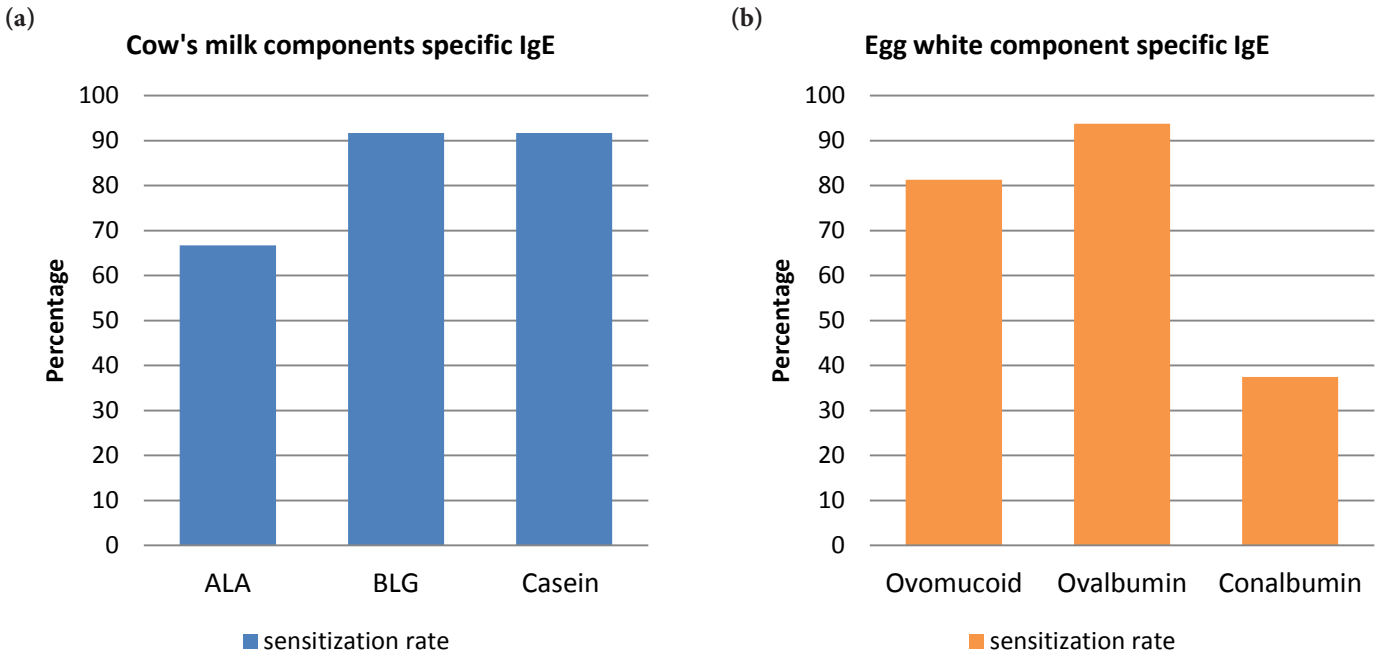


Figure 2. Percentages of positive sIgE against cow's milk components (a) and egg white components (b)

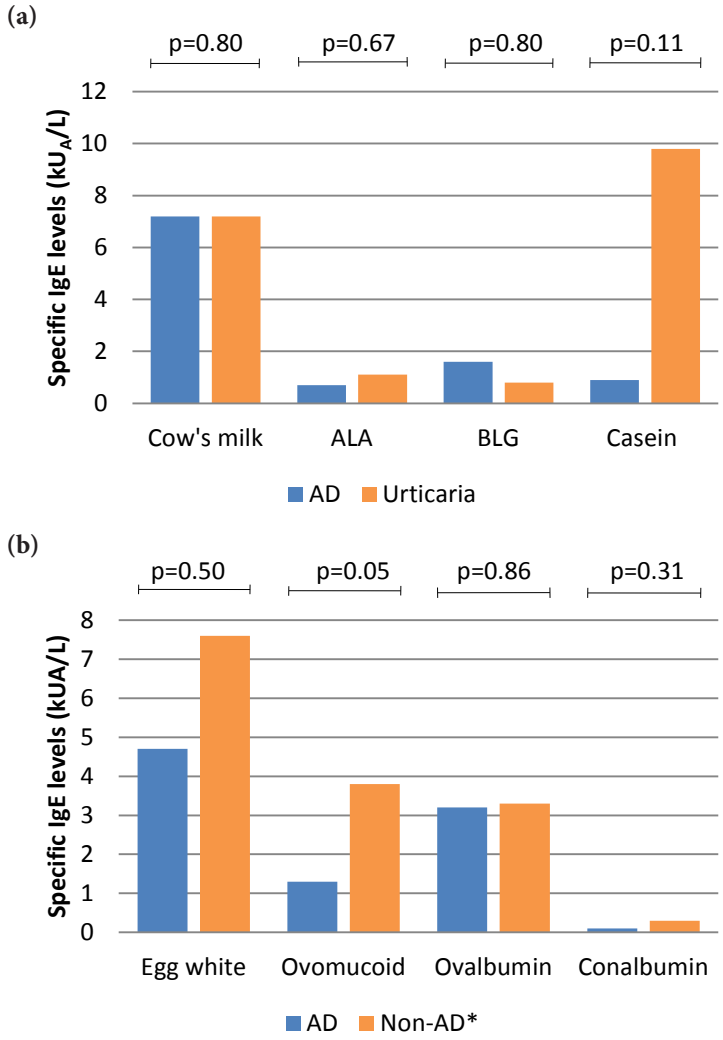


Figure 3. Levels of cow's milk sIgE and CRDs in the AD group (n=10) and acute urticaria groups (n=3) (a) and levels of egg-sIgE and CRDs in the AD group (n=18) and non-AD groups (n=13) (b)
*Non-AD = acute GI symptom (n=1), acute urticaria (n=9) and anaphylaxis (n=3)

casein, respectively. Thirteen cow's milk allergic subjects were divided into two groups: the AD group (n = 10) and the urticaria group (n = 3). Patients in the urticaria group had higher levels of casein sIgE than patients in the AD group, but this difference was not significant (median 9.8 kU_A/L vs. 0.9 kU_A/L, p = 0.11). There were no differences in cow's milk, ALA and BLG sIgE levels between the two groups (**Figure 3a**).

Serum sIgE to egg white and allergen components

The median sIgE levels were 6.21, 2.49, 3.21 and 0.18 kU_A/L for egg white, ovomucoid, ovalbumin and conalbumin, respectively. The highest percentage of sensitization was sIgE against ovalbumin (93.8%), followed by ovomucoid and conalbumin (81.3% and 37.5%, respectively) (**Figure 2b**). The egg allergic subjects were divided into two groups: the AD group, consisting of patients who were diagnosed with isolated AD (n = 18) and the non-AD group, patients diagnosed with acute gastrointestinal symptoms, acute urticaria or anaphylaxis (n = 13). The level of ovomucoid sIgE was significantly higher in the non-AD group than in the AD group (median 3.8 kU_A/L vs. 1.3 kU_A/L, p = 0.048). There were no significant differences in the median levels of sIgE against egg white, ovalbumin and conalbumin between the two groups (**Figure 3b**).

Discussion

Studies in Italy, Japan and Spain have shown that the most common cow's milk component provoking an allergic reaction is casein.[7-9] In our study, BLG and casein were the common allergens that caused sensitization among the three cow's milk components, and the level of casein sIgE in the patients with urticaria (isolated IgE-mediated cow's milk allergy) was higher than the levels of ALA and BLG sIgE and higher than the level of casein sIgE in the patients with AD, but there was no significant difference due to the small sample size. This indicates that there is a different pattern of cow's milk protein sensitization in different diagnoses of cow's milk allergy. Four (30%) of the 13 cow's milk allergic patients in our study had no predominant sIgE to the three cow's milk components, which may indicate that they had predominant sIgE to other cow's milk components, such as lactoferrin, immunoglobulin or bovine serum albumin.

Our study confirmed that ovomucoid and ovalbumin are the important egg allergens. We found that the higher the level of ovomucoid sIgE, the more likely the association with isolated IgE-mediated egg allergy (urticaria and anaphylaxis). Additionally, no patients with an egg allergy had predominant sIgE against conalbumin; thus, conalbumin sIgE may be useless in the diagnosis of an egg allergy in our patients.

Several studies have reported the importance of ovomucoid sIgE in predicting heated egg tolerance.[10,11] However, the sensitization pattern against allergen components varies between countries. The positive decision point of an ovomucoid sIgE level greater than 10.8 kU_A/L was a predictor of reactions to heated egg white.[10] This may not be applied to our population. For example, a patient in this study (patient 5) who had 18.2 kU_A/L of ovomucoid sIgE tolerated the heated egg (brownie). In contrast, a patient (patient 1) with 8.16 kU_A/L of ovomucoid sIgE developed a reaction after the heated egg challenge (cookie).

There are some limitations to this study. Due to the small sample size, we could not make a significant comparison between the groups.

In conclusion, most of the patients in our study were sensitized to two or more allergen components. BLG and casein for cow's milk and ovomucoid and ovalbumin for egg were the common allergen components causing sensitization in cow's milk and egg allergic patients. Among the patients with cow's milk allergy, the level of sIgE against casein in the urticaria group tended to be higher than in the AD group and in patients with an egg allergy; the non-AD group had a significantly higher ovomucoid sIgE level compared with the AD group.

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