

Children with wheat anaphylaxis and with low wheat specific IgE have a different IgE immunoblot pattern than those with high wheat specific IgE

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Abstract

Background: Children with wheat anaphylaxis can present with a wide range of wheat-specific IgE (sIgE).

Objective: To identify differences in clinical features and predominant wheat allergens sensitized by these patients.

Methods: Children with history of wheat anaphylaxis were recruited. Skin prick test (SPT) to wheat, sIgE to wheat, omega-5 gliadin (ω 5G), lipid transfer protein (LTP) were investigated. Profiles of IgE-bound wheat allergens were studied to identify predominant wheat allergens.

Results: Twenty-nine children (17 males) aged 1-18 years were enrolled. Sixteen patients (55.2%) had wheat-sIgE > 100 kUA/L (WA_{hi}) and 13 patients (44.8%) had wheat-sIgE < 34 kUA/L (WA_{lo}). The median of peak wheat-sIgE in WA_{hi} and WA_{lo} were 340.5 kUA/L (IQR 184.3, 564.5) and 12.2 kUA/L (IQR 1.4, 41.3), respectively. Oral food challenge test (OFC) was carried out in 12 of 13 patients in the WA_{lo} group, all of which had positive results. Eight of these 12 patients developed anaphylaxis during OFC despite having wheat-sIgE less than 10 kUA/L. There were no differences in clinical characteristics and atopic history between WA_{hi} vs. WA_{lo} . Medium to low molecular weight gliadin (< 40 kDa) and glutenin (< 60 kDa) were commonly recognized by patients with WA_{hi} . IgE immunoblot pattern among the WA_{lo} group was more widely dispersed than those with WA_{hi} .

Conclusion: Wheat anaphylaxis can occur in patients with low wheat-sIgE. Predominant wheat allergens recognized by patients with WA_{lo} were different than those with WA_{hi} . Such difference could be responsible for anaphylaxis at even low levels of wheat-sIgE.

Key words: wheat hypersensitivity, food hypersensitivity, anaphylaxis, immunoblotting, gliadin

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Abbreviations:

SPT	Skin prick test
MWD	Mean wheal diameter
sIgE	Specific IgE
ω5G	Omega-5 gliadin
LMW	Low molecular weight
HMW	High molecular weight
WA _{hi}	Peak sIgE to wheat ≥ 100 kUA/L
WA _{lo}	Peak sIgE to wheat < 100 kUA/L
WDEIA	Wheat-dependent exercise-induced anaphylaxis

Introduction

Wheat is a common cause of food-induced anaphylaxis and food-dependent exercise-induced anaphylaxis.¹⁻⁴ A recent systematic review reported the incidence rate of food anaphylaxis to be 0.14 per 100 person-years. The highest rate was observed among children aged 0-4 years (7 per 100 person-years).⁵ In Thailand, self-reported prevalence of food allergy in children increased from 6.25% in 2005⁶ to 9.3% in 2012.⁷ Among this population, the prevalence of IgE-mediated food allergy confirmed by oral food challenge test was about 1.11%.⁷ The most common causes of food-induced anaphylaxis in Thai children were seafood, followed by wheat.^{8,9}

Since most children with wheat anaphylaxis developed their first symptoms during infancy,¹⁰ wheat-sIgE levels in these patients may not have fully reached their peak. Moreover, oral food challenge (OFC) is not desirable among these infants due to the risk of developing severe adverse reactions. The presence of omega-5 gliadin (ω5G), a marker for wheat-dependent, exercise-induced anaphylaxis (WDEIA),¹¹⁻¹⁴ has not been thoroughly studied in children with wheat anaphylaxis. Although Daengsuwan et al. reported that ω5G was a major allergen for wheat-induced anaphylaxis in Thai children in 2005,¹⁵ we, in 2016, demonstrated that ~35 kDa gliadin was another important allergen in children with wheat anaphylaxis.¹⁶ Moreover, alpha-amylase inhibitor,¹⁷ alpha, beta, and gamma-gliadins, and low-molecular-weight (LMW) glutenin subunits have been shown to be important wheat allergens recognized by children with wheat anaphylaxis.¹⁸

In our previous report of 100 children with IgE-mediated wheat allergy, skin prick test (SPT) size and wheat-sIgE were significant predictors for anaphylaxis.¹⁰ This finding is consistent with that by Rolinck-Werninghaus et al.,¹⁹ who demonstrated that levels of wheat-sIgE were related to the severity of the reactions. However, in clinical practice, we have observed some children developing wheat anaphylaxis despite having low wheat-sIgE. CRD may predict wheat anaphylaxis and determine severity. However, available data on this issue remains inconclusive.^{20,21} This study aims to identify differences in clinical features and predominant wheat allergens sensitized by children with wheat anaphylaxis who have low wheat-sIgE vs. high wheat-sIgE.

Methods

Subjects

Children diagnosed with wheat anaphylaxis were recruited from the Allergy Clinic of the Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University,

Bangkok, Thailand, between January 2011 to December 2017. The Siriraj Institutional Review Board (SIRB) reviewed and approved the study (COA no. si127/2017). Informed consent was obtained from parents or legal guardians, with assent obtained from all patients older than seven years of age. Anaphylaxis was diagnosed using the criteria proposed by the National Institutes of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network.²² We classified the symptoms of anaphylaxis into skin, respiratory, gastrointestinal, cardiovascular, and neurological systems. Syncope and unconsciousness were classified as neurological system involvement.¹ The severity of anaphylaxis was graded by using COFAR (Consortium for Food Allergy Research) grading system.²³ Peak wheat-sIgE was defined as the highest level of wheat-sIgE observed prior to the enrollment period. Patients were excluded if they had underlying diseases, such as celiac disease or autoimmune disease, or were lost to follow-up.

Skin prick test and specific IgE

Coca-10% EtOH wheat extract was prepared as previously described.¹⁶ SPT was performed on the volar aspect of the forearm using a monodentate lancet. Histamine dihydrochloride (10 mg/ml) was used as a positive control with normal saline as a negative control. A mean wheal diameter (MWD) of at least 3 mm larger than the negative control was considered a positive reaction.

Levels of sIgE to wheat, ω5G, and Tri a 14 (lipid transfer protein, LTP) were measured using ImmunoCAP® (Phadia, Uppsala, Sweden). sIgE levels greater than 0.35 kUA/L were considered positive. Current sIgE was the sIgE value within one year before the OFC or after the last reported anaphylaxis reaction. Peak sIgE was the highest documented value from medical records.

Preparation of Coca-10% EtOH wheat extracts for SPT

The Coca-10% EtOH wheat extract was prepared as follows:^{16,24} wheat flour (Kite® flour brand, Bangkok Thailand) was dissolved in Coca's solution containing 10% v/v absolute ethanol and was magnetically stirred for 1 hour at room temperature followed by centrifugation at 17,210 × g for 10 minutes. Pellet was discarded, and the supernatant was collected and stored at 4°C until the time of SPT.

Oral food challenge test (OFC)

OFC was performed in the hospital setting using the modified PRACTALL protocol.²⁵ All OFCs were open challenges. Wheat OFC schedule was as follows: 1 mg (approximately 1/15,000 of one slice of white bread), 3 mg, 10 mg, 30 mg, 60 mg, 100 mg, 300 mg, 1 gm, 3 gm (Kiteflour®, containing 13% wheat protein) introduced orally at 30-minute intervals. Patients may switch from wheat flour to white bread (Farm house®, Bangkok, Thailand containing 62% wheat) after reaching 1 gm or 3 gm of wheat flour. The starting dose for bread was 1.88 gm (1/8 slice), 3.75 gm, 7.5 gm, 15 gm, and 30 gm of wheat (approximately 3.9 gm of wheat protein or 2 slices of bread). The eliciting dose (ED) was the amount of wheat when patient developed an allergic reaction.

Wheat gliadin and glutenin extraction

Extracts of alcohol-soluble wheat gliadin and glutenin were prepared using a modified extraction protocol.^{16,26} Briefly, 100 mg wheat flour (Kite® flour brand) was mixed in 1 ml of 50 mM Tris-HCl pH 8, 0.5 M NaCl with continuous mixing for 1 hour at room temperature, followed by centrifugation at $17,210 \times g$ for 10 minutes at 4°C. This step was repeated prior to mixing the pellet with 1 ml of 50% (v/v) aqueous isopropanol, 50 mM Tris-HCl pH 8 with continuous mixing for 30 minutes at room temperature, followed by centrifugation at $17,210 \times g$ for 10 minutes at room temperature. The supernatant or 'gliadin extract' was collected. The pellet from the gliadin extraction was mixed with 50% (v/v) aqueous isopropanol, 50 mM Tris-HCl pH 8, 1% (w/v) DTT incubated at 60°C with vigorously mixing every 5-10 minutes for 1 hour, followed by centrifugation at $17,210 \times g$ for 10 minutes at room temperature. The supernatant or 'glutenin extract' was collected. Both gliadin and glutenin extracts were dried in a vacuum evaporator. Dried powder of gliadins and glutenins were dissolved in 50 mM Tris-HCl pH 7.5 containing 0.25% SDS and incubated at 65°C with vigorous shaking. The concentrations of dissolved proteins in both extracts were determined by bicinchoninic acid (BCA) protein assay.

Immunoblot with serum IgE against wheat proteins

Sera from patients were diluted at 1/10-1/200 based on ImmunoCAP® results using Phosphate Buffered Saline (PBS) (10 mM Na₂HPO₄, 2 mM KH₂PO₄, 2.7 mM KCl, 137 mM NaCl) containing 3% non-fat dry milk (buffer A) as solvent. Immunoblot was performed using 20 micrograms of total proteins from gliadin or glutenin extracts (see above) that were resolved from each well of 12% SDS-PAGE gel at constant current. Separated proteins in the gel were then electro-transferred onto a nitrocellulose membrane. The membrane was incubated in buffer A for 1 hour at 25°C (room temperature) followed by washing with PBS containing 0.2% v/v Tween-20 (buffer B). The membrane was incubated with diluted sera of patients and controlled in buffer A

overnight at 4°C. It was later washed with buffer B and was incubated with 1:10,000 diluted horseradish peroxidase (HRP) and conjugated mouse IgG anti-human IgE antibody (KPL; SeraCare Life Sciences, Inc., Milford, MA, USA) in buffer A for 1 hour. After washing, the membrane was incubated with HRP substrate (Millipore, MA, USA), and the emitted signal was captured by X-ray film.

Statistical Analysis

PASW Statistics version 18.0 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses. Demographic and clinical data were summarized using descriptive statistics. Categorical data are presented as numbers and percentages, and continuous data are presented as median and interquartile range (IQR). Chi-square test was used for comparisons of categorical data, and the Kruskal-Wallis test was employed for continuous data with non-normal distribution. All *p*-values less than 0.05 were considered statistically significant.

Results

Study Population

Twenty-nine children with ages ranging from 1 to 18 years (17 males 12 females) were recruited. From their current wheat-sIgE, we classified them into 2 groups, i.e., Group (1) those with current wheat-sIgE ≥ 100 kUA/L (WA_{hi}), Group (2) those with current wheat-sIgE ≤ 100 kUA/L (WA_{lo}). There were 16 (55.2%) children in the WA_{hi} group and 13 (44.8%) in the WA_{lo} group. The median age of patients was 8.7 years in the WA_{hi} group and 9.1 years in the WA_{lo} group. Most patients had concomitant allergic rhinitis, while asthma was observed more frequently among WA_{hi} (31.3%) than WA_{lo} (7.7%). Atopic dermatitis and other food allergy was more commonly observed among WA_{lo} (53.8%) than WA_{hi} (25%). However, these differences were not statistically different (*p* > 0.05). Most families introduced wheat into the diets of infants after 6 months of age (Table 1). Most patients developed their first episode of anaphylaxis during the first year of life (median age 7-8 months), and several children had more than one reaction per year.

Table 1. Demographic and clinical characteristics of the study population (N = 29).

Characteristics	Wheat anaphylaxis		P-value
	WA _{hi} (n = 16)	WA _{lo} (n = 13)	
Current age (years), median (IQR)	8.7 (6.5, 10.3)	9.1 (6.5, 10.3)	0.95
Male gender, n (%)	10 (62.5%)	7 (53.8%)	0.64
Personal atopy, n (%)			
Asthma	5 (31.3%)	1 (7.7%)	0.12
Allergic rhinitis	12 (75.0%)	9 (69.2%)	0.73
Allergic conjunctivitis	1 (6.3%)	1 (7.7%)	0.88
Atopic dermatitis	4 (25.0%)	7 (53.8%)	0.11
Other food allergy	4 (25.0%)	7 (53.8%)	0.11

Table 1. (Continued)

Characteristics	Wheat anaphylaxis		P-value
	WA _{hi} (n = 16)	WA _{lo} (n = 13)	
Family history of atopy, n (%)	8 (50.0%)	7 (53.8%)	0.84
Age of solid food introduction > 6 months, n (%)	4 (25.0%)	1 (7.7%)	0.22
Age of wheat introduction > 6 months, n (%)	9 (56.3%)	9 (69.2%)	0.48
Age of first anaphylaxis (months), median (IQR)	7 (6.0, 8.8)	8 (5.0, 11.5)	0.45
Time between wheat ingestion and reactions, n (%)			
< 15 minutes	5 (31.3%)	7 (53.8%)	
15-30 minutes	11 (68.8%)	5 (38.5%)	0.19
> 30 minutes	0 (0.0%)	1 (7.7%)	
Number of reactions per year > 1/year, n (%)	3 (18.8%)	3 (23.1%)	0.78

WA_{hi}, wheat anaphylaxis (peak sIgE to wheat ≥ 100 kUA/L);

WA_{lo}, wheat anaphylaxis (peak sIgE to wheat < 100 kUA/L);

IQR, interquartile range

Skin prick test and specific IgE to wheat

As can be seen from **Table 2**, median peak and current sIgE to wheat and ω5G among WA_{hi} were significantly higher than those with WA_{lo} (*p* < 0.001). Since current wheat-sIgE among WA_{hi} were over 100 kUA/L, OFC was deferred for all these patients. All WA_{hi} and 12/13 of WA_{lo} underwent SPT and had positive reaction to wheat. The MWD to wheat SPT in the WA_{hi} group was larger than the WA_{lo} groups, although this difference was not statistically significant (**Table 2**).

Levels of sIgE to Tri a 14 (lipid transfer protein, LTP) were measured in 28 patients. The result was positive in three patients (n = 2/15 from WA_{hi}, and n = 1/12 from WA_{lo} group). Among WA_{lo} patients, Six had positive ω5G-sIgE, and one had

positive LTP-sIgE. Patient #3 in the WA_{lo} group had a positive LTP result and had a negative result for ω5G-sIgE (**Table 3**).

OFC was performed in 12 of 13 WA_{lo} patients (**Table 3**). One patient in WA_{lo} was not challenged due to parental refusal. All 12 patients had positive OFCs, with nine developing anaphylaxis during OFC. Eight of these nine had current wheat-sIgE less than 10 kUA/L, the level of which was much lower than 26 kUA/L, the suggested cut-off point to predict positive wheat OFC.²⁷ Three of 12 patients developed urticaria during the OFC. Remarkably, patient #12 (**Table 3**), who had wheat-sIgE of only 1.51 kUA/L, developed protracted anaphylaxis. She required three doses of adrenaline injections to reverse anaphylaxis successfully.

Table 2. Results of skin prick test, specific IgE to wheat, and omega-5 gliadin (N = 29).

Test	Wheat anaphylaxis		P-value
	WA _{hi} (n = 16)	WA _{lo} (n = 13)	
MWD of SPT to wheat (mm)	11.3 (8.5, 15.0)	8 (6.8, 13)	0.17
Peak sIgE to wheat (kUA/L)	340.5 (184.3, 564.5)	12.2 (1.4, 41.3)	< 0.001
Peak sIgE to ω5G (kUA/L)	27.8 (15.6, 78.5)	4.2 (0.3, 7.9)	< 0.001
Current sIgE to wheat (kUA/L)	222 (127.8, 482.8)	3 (1.2, 5.4)	< 0.001
Current sIgE to ω5G (kUA/L)	20.8 (7, 67.8)	0.28 (0.1, 4.5)	< 0.001
SPT positive (MWD > 3 mm)	16 (100%)	12 (92.3%)	0.41
sIgE to wheat positive (≥ 0.35 kUA/L)	16 (100%)	13 (100%)	NA
sIgE to ω5G positive (≥ 0.35 kUA/L)	16 (100%)	6 (46.2%)	0.001

Data are presented as median and interquartile range (IQR) or number and percentage

WA_{hi}, wheat anaphylaxis (peak sIgE to wheat ≥ 100 kUA/L);

WA_{lo}, wheat anaphylaxis (peak sIgE to wheat < 100 kUA/L);

MWD, mean wheal diameter

Table 3. Patients with low level of wheat-sIgE who had positive oral food challenge test.

Pt. No.	Age of first anaphylaxis (mo)	Age of OFC (yr/mo)	Eliciting dose (mg)	Reaction during OFC (Grade of CoFAR)	MWD (mm)	sIgE (kUA/L)		
						wheat	Omega5	LTP
1	32	11/6	1880	ANA (gr 3)	9.0	1.24	6.42	0
2	12	5/0	100	U (gr 2)	7	0.55	0.03	0.01
3	6	11/1	3000	ANA (gr 3)	11.0	3.94	0.05	4.1
4	4	9/0	15000	ANA (gr 3)	17.5	6.48	2.6	0.21
5	8	8/10	60	ANA (gr 3)	7.5	34	10.9	NA
6	6	6/11	300	ANA (gr 3)	8.0	5.23	0.85	0.01
7	7	5/0	3000	U (gr 2)	15	2.96	0.07	0.02
8	3.5	7/5	15000	ANA (gr 3)	5.0	3.62	0.27	0
9	48	13/0	30000	U (gr 2)	8	0.75	8.21	0.02
10	11	7/7	3000	ANA (gr 3)	20.0	5.47	0.28	0.02
11	4	14/8	1880	ANA (gr 3)	6.5	1.24	0.19	0
12	11	1/10	200	ANA (gr 4)	10.5	1.51	0.43	0.04

No 7 – peak = 97.3 kUA/L at 7 months

No 5 – peak = 70.5 kUA/L at 4 years

OFC, Oral food challenge test; MWD, mean wheal diameter; ANA, anaphylaxis; U, urticaria; yr, year; mo, month; COFAR, Consortium for Food Allergy Research

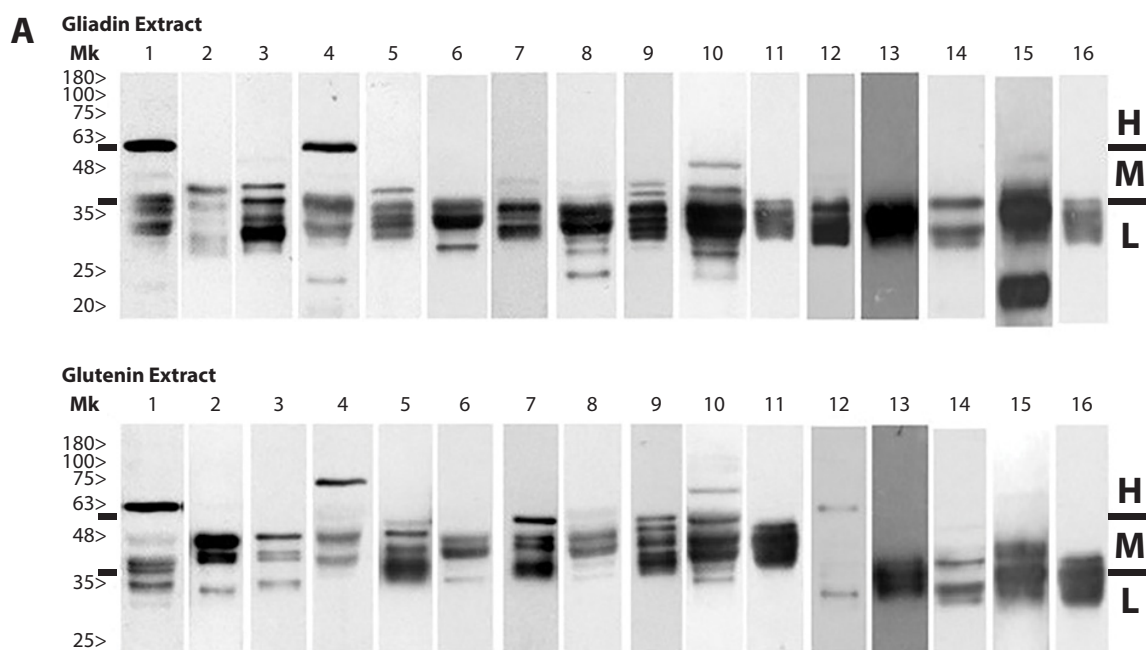


Figure 1. IgE immunoblotting to gliadin and glutenin extracts using sera from children with wheat anaphylaxis. Mk, marker; MW, molecular weight

Panel A: sera from WA_{hi} group (peak sIgE to wheat \geq 100 kUA/L)Panel B: sera from WA_{lo} group (peak sIgE to wheat < 100 kUA/L)L: proteins in MW range of < 40 kDa (α/β gliadin, LMW glutenin),M: proteins in MW range of 40-59 kDa (γ,ω gliadin, LMW glutenin),H: proteins in MW range of > 60 kDa (ω gliadin, HMW glutenin).

*Control sera was performed. No banding of IgE immunoblotting in both gliadin and glutenin extracts (data not shown).

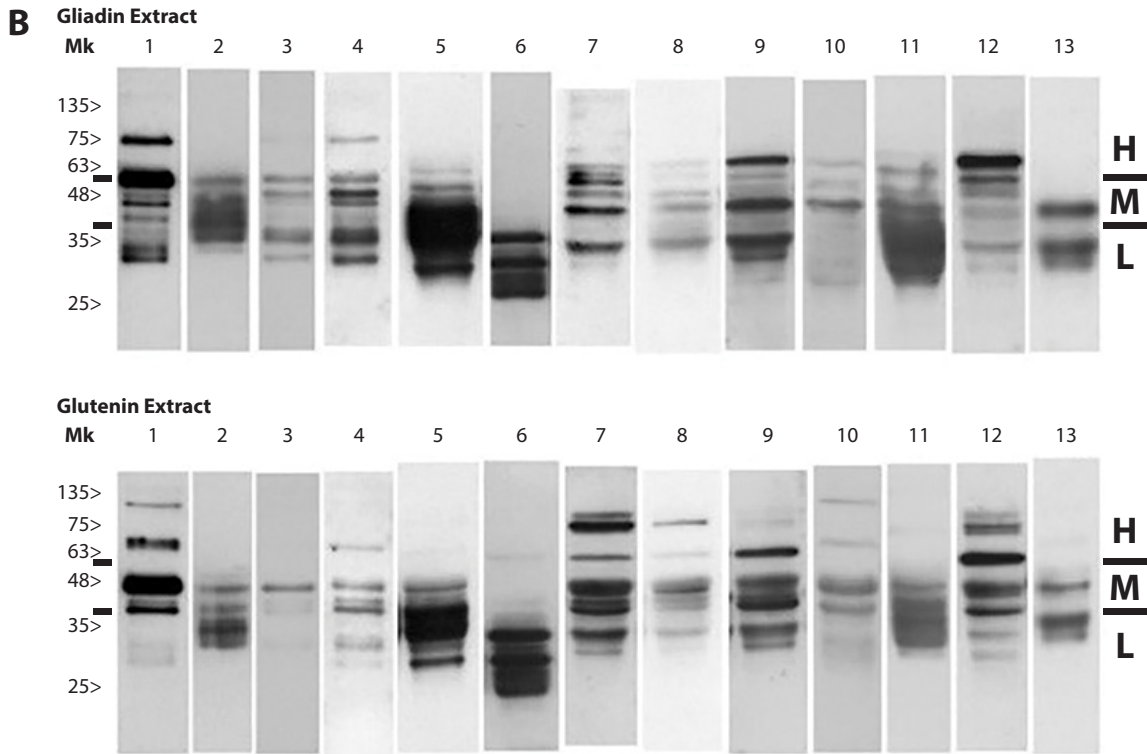


Figure 1. (Continued)

Table 4. Number of patients with positive profiles of different IgE bound allergens.

	IgE bound allergens by different				
	Gliadin			Glutenin	
MW(kDa)	> 60	40-59	< 40	> 60	< 60
Possible allergen	ω5G	Gamma	Alpha/beta	HMW	LMW
WA _{hi} (n = 16)	2 (12.5%)	16 (100%)	16 (100%)	1 (6.3%)	15 (93.8%)
WA _{lo} (n = 13)	11 (84.6%)	11 (84.6%)	11 (84.6%)	4 (30.8%)	12 (92.3%)

Subgroups of wheat anaphylaxis patients

Data are presented about the percentage of immunoblot in each group.

WA_{hi}, wheat anaphylaxis (peak sIgE to wheat ≥ 100 kUA/L);

WA_{lo}, wheat anaphylaxis (peak sIgE to wheat < 100 kUA/L);

MW, Molecular weight

Immunoblot with serum IgE against wheat proteins

Profiles of IgE-bound wheat proteins demonstrated different patterns of predominant allergens between 2 groups (Figure 1 and Table 4). Sera from patients in WA_{hi} group predominantly recognized protein of gliadin extracts in the range < 40 kDa (L) and 40-59 kDa (M) (Figure 1A and Table 4). Conversely, sera from WA_{lo} group recognized a more widely dispersed array of gliadin protein (from low to high molecular weights > 60 kDa). As for glutenins, both patient groups recognized mainly LMW glutenins (< 60 kDa), while those with HMW glutenins were recognized more common by WA_{lo} group.

Discussion

To our knowledge, this is the first study to demonstrate that wheat anaphylaxis can occur in patients with low wheat-sIgE levels. The clinical characteristics of WA_{lo} patients did not significantly differ from those WA_{hi}. Our patients were similar to those described in other studies, such as male predominance,^{1,20,28} family history of atopy,^{1,28} early onset of wheat anaphylaxis,¹ and the onset of symptoms after ingestion is less than 30 minutes. Allergic rhinitis was the most common atopic comorbidity in both WA_{hi} and WA_{lo} groups. In contrast, atopic dermatitis and other food allergy were found mainly in the WA_{lo} group. However, the difference was not statistically significant. Asthma was not commonly observed among our patients (7-31%) compared to those reported by Nilsson et al. (75%).²⁰ However, our patients were younger, and our sample size was smaller.

Eight of 13 children in our study developed anaphylaxis during OFC despite having current wheat-sIgE level below 10 kUA/L. Previous studies from Japan reported that the sIgE to wheat or ω 5G are related to the clinical reactivity to wheat allergy.^{29,30} In our previous study with WDEIA, wheat-sIgE was positive in 60.6%, while the positivity rate to ω 5G-sIgE was 87.9%. The question was raised whether ω 5G could be the culprit allergen for anaphylaxis in our WA_{10} patients. As in **Table 3**, only 2/12 WA_{10} patients (patients #5 and #9) had elevation of ω 5G-sIgE rendering the hypothesis unlikely. In addition, LTP-sIgE was positive in only one WA_{10} patient, indicating that LTP may not be the cause of anaphylaxis in these patients.

It can be clearly seen from **Figure 1**. (panel A) that all WA_{hi} patients recognized gliadin proteins of less than 48 kDa, most of which contain α , β , and γ gliadins.²⁶ Only 2 patients recognized 63 kDa proteins which include ω 5G. In contrast, a more diverse pattern is seen from panel B (gliadin extract – WA_{10}), suggesting a possible causal role of HMW gliadin in this group of patients. The discrepancy between the ImmunoCAP® vs. immunoblot results for sIgE to ω 5G in our study remains to be elucidated. In addition to LMW glutenins which were widely recognized by both WA_{hi} and WA_{10} patients, HMW glutenins (> 60 kDa) were also recognized by several WA_{10} patients. Although previously regarded as minor allergens,^{18,31} the role of HMW glutenins remains to be further examined. Further studies by using other novel techniques to identify specific IgE-binding epitopes could be beneficial to predict clinical severity, and treatment outcome.

This study has some limitations. First, our small sample size limited our ability to test for all potentially important differences and associations. Second, one patient in the WA_{10} group had recent anaphylaxis and was regarded as too young for OFC (less than 2 years old). Third, we did not evaluate the total IgE level in our subjects. So, we could not see compare the sIgE/total IgE level between 2 groups. Lastly, subjects with high wheat-sIgE levels were not challenge-proven, limiting the ability to directly compared between the WA_{10} and WA_{hi} groups.

In conclusion, We conclude that wheat anaphylaxis can occur in patients with low wheat-sIgE. Thus, wheat-sIgE levels alone should not be used to determine if a patient is no longer vulnerable to anaphylaxis. Clinical characteristics, SPT results, and wheat-sIgE levels cannot be clearly differentiated between patients with WA_{10} and WA_{hi} . Further studies focusing on pathomechanism for WA_{10} are warranted.

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Conflict of interest declaration

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References

1. Pourpak Z, Ghojzadeh L, Mansouri M, Mozaffari H, Farhoudi A. Wheat anaphylaxis in children. *Immunol invest.* 2007;36:175-82.
2. Srisuwatchari W, Vichyanond P. Oral food challenges: result of a 16-year experience at a major teaching hospital in Thailand. *Asia Pac Allergy.* 2018;8:e21.
3. Palosuo K. Update on wheat hypersensitivity. *Curr Opin Allergy Clin Immunol.* 2003;3:205-9.
4. Scherf KA, Brockow K, Biedermann T, Koehler P, Wieser H. Wheat-dependent exercise-induced anaphylaxis. *Clin Exp Allergy.* 2016; 46:10-20.
5. Umasunthar T, Leonardi-Bee J, Turner PJ, Hodes M, Gore C, Warner JO, et al. Incidence of food anaphylaxis in people with food allergy: a systematic review and meta-analysis. *Clin Exp Allergy.* 2015;45: 1621-36.
6. Santadusit S, Athapaisarsarudee S, Vichyanond P. Prevalence of adverse food reactions and food allergy among Thai children. *J Med Assoc Thai.* 2005;88 Suppl 8:S27-32.
7. Lao-araya M, Trakultivakorn M. Prevalence of food allergy among preschool children in northern Thailand. *Pediatr Int.* 2012;54:238-43.
8. Jirapongsananuruk O, Bunsawansong W, Piyaphanee N, Visitsunthorn N, Thongngarm T, Vichyanond P. Features of patients with anaphylaxis admitted to a university hospital. *Ann Allergy Asthma Immunol.* 2007; 98:157-62.
9. Nantanee R, Suratannon N, Chatchatee P. Characteristics and Laboratory Findings of Food-Induced Anaphylaxis in Children: Study in an Asian Developing Country. *Int Arch Allergy Immunol.* 2022;183:59-67.
10. Srisuwatchari W, Vichyanond P, Jirapongsananuruk O, Visitsunthorn N, Pacharn P. Characterization of children with IgE-mediated wheat allergy and risk factors that predict wheat anaphylaxis. *Asian Pac J Allergy Immunol.* 2022;40:263-268.
11. Matsuo H, Kohno K, Niihara H, Morita E. Specific IgE determination to epitope peptides of omega-5 gliadin and high molecular weight glutenin subunit is a useful tool for diagnosis of wheat-dependent exercise-induced anaphylaxis. *J Immunol.* 2005;175:8116-22.
12. Morita E, Matsuo H, Mihara S, Morimoto K, Savage AWJ, Tatham AS. Fast omega-gliadin is a major allergen in wheat-dependent exercise-induced anaphylaxis. *Journal of dermatological science.* 2003; 33(2):99-104.
13. Morita E, Chinuki Y, Takahashi H. Recent advances of in vitro tests for the diagnosis of food-dependent exercise-induced anaphylaxis. *J dermatol science.* 2013;71:155-9.
14. Thongngarm T, Wongsas C, Pacharn P, Piboonpocanun S, Sompornrattanaphan M. Clinical Characteristics and Proposed Wheat-Cofactor Challenge Protocol with a High Diagnostic Yield in Adult-Onset IgE-Mediated Wheat Allergy. *J Asthma Allergy.* 2020;13: 355-68.
15. Daengsuwan T, Palosuo K, Phankingthongkum S, Visitsunthorn N, Jirapongsananuruk O, Alenius H, et al. IgE antibodies to omega-5 gliadin in children with wheat-induced anaphylaxis. *Allergy.* 2005;60:506-9.
16. Pacharn P, Kumjim S, Tattiyapong P, Jirapongsananuruk O, Piboonpocanun S. Identification of wheat sensitization using an in-house wheat extract in Coca-10% alcohol solution in children with wheat anaphylaxis. *Asian Pac J Allergy Immunol.* 2016;34:153-8.

17. James JM, Sixbey JP, Helm RM, Bannon GA, Burks AW. Wheat alpha-amylase inhibitor: a second route of allergic sensitization. *J Allergy Clin Immunol.* 1997;99:239-44.
 18. Sievers S, Rawel HM, Ringel KP, Niggemann B, Beyer K. Wheat protein recognition pattern in tolerant and allergic children. *Pediatr Allergy Immunol.* 2016;27:147-55.
 19. Rolinck-Werninghaus C, Niggemann B, Grabenhenrich L, Wahn U, Beyer K. Outcome of oral food challenges in children in relation to symptom-eliciting allergen dose and allergen-specific IgE. *Allergy.* 2012;67:951-7.
 20. Nilsson N, Sjolander S, Baar A, Berthold M, Pahr S, Vrtala S, et al. Wheat allergy in children evaluated with challenge and IgE antibodies to wheat components. *Pediatr Allergy Immunol.* 2015;26:119-25.
 21. Phisitbuntoon T, Jirapongsananuruk O, Pacharn P, Wangthan U, Srisuwatchari W, Reamtong O, et al. A potential role of gliadin extract skin prick test in IgE-mediated wheat allergy. *Asian Pac J Allergy Immunol.* 2020.
 22. Lieberman P, Nicklas RA, Randolph C, Oppenheimer J, Bernstein D, Bernstein J, et al. Anaphylaxis--a practice parameter update 2015. *Ann Allergy Asthma Immunol.* 2015;115:341-84.
 23. Chinthrajah RS, Jones SM, Kim EH, Sicherer SH, Shreffler W, Lanser BJ, et al. Updating the CoFAR Grading Scale for Systemic Allergic Reactions in Food Allergy. *J Allergy Clin Immunol.* 2022;149:2166-70.
 24. Pacharn P, Siripipattanamongkol N, Pannakapitak N, Visitsunthorn N, Jirapongsananuruk O, Piboonpocanun S, et al. Accuracy of in-house alcohol-dissolved wheat extract for diagnosing IgE-mediated wheat allergy. *Asian Pac J Allergy Immunol.* 2020;38:102-7.
 25. Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, Sicherer S, Teuber SS, Burks AW, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report. *J Allergy Clin Immunol.* 2012;130:1260-74.
 26. van den Broeck HC, America AH, Smulders MJ, Bosch D, Hamer RJ, Gilissen LJ, et al. A modified extraction protocol enables detection and quantification of celiac disease-related gluten proteins from wheat. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2009;877:975-82.
 27. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol.* 2001;107:891-6.
 28. Keet CA, Matsui EC, Dhillon G, Lenehan P, Paterakis M, Wood RA. The natural history of wheat allergy. *Ann Allergy Asthma Immunol.* 2009;102:410-5.
 29. Yanagida N, Sato S, Takahashi K, Nagakura KI, Asaumi T, Ogura K, et al. Increasing specific immunoglobulin E levels correlate with the risk of anaphylaxis during an oral food challenge. *Pediatr Allergy Immunol.* 2018;29:417-24.
 30. Ito K, Futamura M, Borres MP, Takaoka Y, Dahlstrom J, Sakamoto T, et al. IgE antibodies to omega-5 gliadin associate with immediate symptoms on oral wheat challenge in Japanese children. *Allergy.* 2008; 63:1536-42.
 31. Makela MJ, Eriksson C, Kotaniemi-Syrjanen A, Palosuo K, Marsh J, Borres M, et al. Wheat allergy in children - new tools for diagnostics. *Clin Exp Allergy.* 2014;44:1420-30.
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