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A potential role of gliadin extract skin prick test in IgE-mediated wheat allergy

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Abstract

Background: Wheat extracts containing both water/salt and alcohol soluble proteins may increase extract's accuracy for diagnosing IgE-mediated wheat allergy.

Objective: This study aimed to determine the performance of new invented in-house prepared wheat extracts for skin prick test (SPT).

Methods: Children aged 1-18 years with history of immediate wheat allergy were recruited. Four in-house prepared wheat extracts (wheat-Coca-10%EtOH, and 3 new invented extracts, wheat-salt, gliadin, and glutenin) and a commercial wheat extract were used for SPT. Serum specific IgE (sIgE) to wheat and omega-5 (ω-5) gliadin were also determined. Oral food challenge (OFC) with wheat flours was performed in all patients except those with history of wheat-induced anaphylaxis or with recent symptoms within the past 6 months.

Results: Thirty-one children were recruited. Of those, 14 were excluded from OFC (12 with history of anaphylaxis and 2 with recent symptom). OFC was positive in 8 of 17 children. Of the 5 extracts and sIgE to wheat and ω-5 gliadin, gliadin extract provided the best SPT performance with 84.2% sensitivity, 88.9% specificity, 94.1% positive predictive value (PPV), 72.7% negative predictive value (NPV), 7.59 positive likelihood ratio (LR), 0.18 negative LR, and 85.7% accuracy.

Conclusion: Compared to other in-house and commercial wheat extracts and sIgE to wheat and ω-5 gliadin, SPT with an in-house gliadin extract yielded the highest performance for the diagnosis IgE-mediated wheat allergy.

Key words: Gliadin, Glutenin, Oral Food Challenge, Skin Prick Test, Specific IgE to ω-5 gliadin

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Introduction

Wheat (Triticum aestivum) is one of the most common causes of food allergy in childhood. Wheat allergy affects about 0.1-4% of children, and about 11-20% of children with food allergy.[1-4] Wheat allergy is presented with various manifestations in one or more systems, including cutaneous, gastrointestinal, respiratory, and cardiovascular symptoms of immediate or delayed onset. The symptoms vary from mild to life-threatening. Immediate reactions to wheat are caused by IgE, and allergic patients often develop symptoms within the first two years of life.[1,5] These patients usually develop clinical tolerance

by 16 years of age, with a resolution rate of 29%, 56%, and 65% by age 4, 8, and 12 years, respectively.[6,7] Thai patients have a similar rate of resolution, which is 27%, 45.7%, and 69% by age 4, 5, and 9 years, respectively.[8]

The current strategy for managing wheat allergy focuses primarily on strict avoidance of wheat allergens.[5,9] However, since wheat is a commonly used ingredient in various types of foods, wheat allergic patients often have difficulty avoiding wheat.[1] It was reported that wheat allergic patients have approximately 1 unintentional exposure episode every 4 years,

of which 45% resulted in anaphylaxis.[7]

Diagnosis of food allergy is an essential step for determining the cause of allergic reactions to food. Upon visiting an allergist, an interview to gather information about the patient's history of food allergy will be conducted, and several tests will be performed. Since there is significant discordance between reported food reactions and the actual incidence of food allergy, interview for history of food allergy cannot be solely depended upon.[9,10] Skin prick test (SPT) and serum specific IgE (sIgE) are required to determine food sensitization. The oral food challenge (OFC) test is essential for a definite diagnosis of food allergy.[9,11] However, despite yielding the highest accuracy, OFC is associated with risk of anaphylaxis and it is time-consuming.[10] Thus, both SPT and sIgE with high accuracy are important diagnostic tools with low to no risk of anaphylaxis.

Unlike diagnosis of food allergy caused by other major sources, such as egg or peanut, the roles of SPT and sIgE in determining wheat allergy are more complicated. Wheat grain consists of carbohydrate and proteins, which are categorized as salt/water soluble and salt/water insoluble or alcohol soluble proteins.[1] Most commercial wheat extracts contain mostly salt/water soluble proteins. However, the wheat allergens reported to cause wheat allergy, as well as anaphylaxis, are mostly salt/water insoluble proteins.[5,9,12-14] Therefore, the use of commercial extracts to determine wheat sensitization may yield less accurate results.[1,12-14] Wheat extract that solubilizes both water/salt and alcohol soluble proteins would improve the accuracy of diagnosis of wheat allergy. Previous study reported in-house prepared wheat extract in Coca's solution and 10% alcohol (WC10Et) containing both water/salt and alcohol soluble proteins.[15] SPT results using WC10Et yielded better sensitivity and accuracy than those observed when using commercial wheat extract.[15] However, WC10Et had poor specificity (66.7%), poor negative predictive value (NPV; 66.7%), and a small positive likelihood ratio (LR; 2.75).[16] To improve the overall performance of in-house prepared wheat extract, a new formula of solutions was developed to extract a higher amount of alcohol soluble proteins, gliadins, and glutenins. Thus, this study aimed to determine the overall performance of these newly developed in-house wheat extracts.

Methods

Subjects

The protocol for this study was approved by the Siriraj Institutional Review Board (SiRB) of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (COA no. Si303/2560). Written informed consent from guardians, and assent from children older than 7 years of age were obtained prior to inclusion in the study.

Children age 1-18 years with history of immediate wheat allergy who received treatment at the Pediatric Allergy Clinic of the Division of Allergy and Immunology, Department of Pediatrics, Faculty of Medicine Siriraj Hospital during 2017-2018 were recruited. Children with underlying diseases, such as chronic renal disease, chronic liver disease, chronic cardiovascular diseases, or uncontrolled asthma, were excluded.

Data collection

Demographic and clinical data were obtained from all study patients. Immediate wheat allergy was defined as any allergic symptoms, such as urticaria, angioedema, tongue swelling, rhinoconjunctivitis, stridor, coughing, wheezing, collapse, tachycardia, hypotension, or anaphylaxis, which developed within 2 hours of wheat ingestion. Anaphylaxis was defined according to National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium criteria.[17]

Skin prick testing

Three newly developed in-house wheat extracts [wheat-salt (WS), gliadin (Glia), and glutenin (Glu)], an in-house prepared WC10Et, and a commercial wheat extract (ALK-Abelló A/S, Hørsholm, Denmark) were used for SPT. Histamine (10 mg/ml) and saline were used as positive and negative control, respectively. Participants were asked to stop antihistamine for ≥ 7 days before SPT.

All in-house prepared extracts used durum wheat semolina flour as a starting material at 0.1 g/mL of extract solution. WC10Et was prepared as described by Pacharn, et al.[15] The newly developed extracts were prepared in 3 steps starting from extracting wheat water/salt soluble proteins, followed by extracting wheat gliadins before extracting wheat glutenins. Extraction of wheat water/salt soluble proteins was performed by dissolving wheat flour in 30 mM Tris pH 8.0, 500 mM NaCl with stirring for 2 hours before centrifugation. The pellet from wheat-salt (WS) extract was further dissolved in Tris based solution (pending Thai petty-patent) containing SDS and glycerol with stirring for 1 hour before centrifugation. The pellet from Glia extract was further dissolved in alkali solution pH > 9 (pending Thai petty-patent) containing NaCl and 10% EtOH with stirring for 1 hour before centrifugation. No reducing agents were used as a component of Glia or Glu extract solution. Extracts for SPT were prepared by diluting total extracted proteins to a final concentration of 1 mg/mL, after which they were filtered through a sterile 0.2 micrometer filter and stored at -20°C. All chemical components of all solutions are certified safe for use in humans. The identity of extracted proteins in WS, Glia, and Glu extracts was confirmed by liquid chromatography–mass spectrometry (LC-MS).

Specific IgE

Serum specific IgE against total wheat allergens and ω5-gliadin were measured by ImmunoCAP assays (Phadia, Uppsala, Sweden; lower detection limit < 0.35 kAU/L) according to the manufacturer's recommendations.

Oral food challenge test

Oral food challenge (OFC) test with 31 grams of wheat (2 slices of bread) was performed with recruited children, except those with history of wheat-induced anaphylaxis or recent symptom within the past 6 months. During OFC, wheat flour was gradually increased from 100 mg to 500 mg, and then to 1, 2, 4, 8, and 15.4 g at 15-20 minutes intervals. Heparin lock was inserted and maintained for all participants before starting the first dose of OFC. Vital signs, symptoms, and signs

were recorded every 15-20 minutes. Emergency resuscitation equipment and medicines were available in case of anaphylaxis.

SDS-PAGE gel analysis of protein in extracts and Identification of proteins in extracts by Liquid Chromatography-Tandem Mass Spectrophotometry (LC-MS/MS)

Twenty μg of total proteins from WS, Glia and Glu extracts, were resolved per well of 12% SDS-PAGE gel at constant current. Resolved proteins in SDS gel were stained in solution containing Coomassie Brilliant Blue G250 for 1 hour before was washed in distilled water. For each extract, several protein bands were excised and treated as followed. An excised gel piece was submerged in 50 mM ammonium bicarbonate solution containing 50% Acetonitrile (ACN) until colorless and incubated in 10 mM dithiothreitol (DTT) for 15 minutes at 60°C. The gel piece was added in 50 mM ammonium bicarbonate solution containing 55 mM iodoacetamide and incubated for 30 minutes at room temperature in the dark. The gel piece was dried using 100% ACN before resuspended in 50 mM ammonium bicarbonate containing 0.1 mg/mL trypsin (Sigma-Aldrich, USA) and incubated at 37°C overnight. The reaction was mixed with ACN at 1:1 (v/v) ratio and incubated for 20 minutes. The solution was dried in centrifugal concentrator at 45°C before resolved peptides in 0.1% formic acid was injected into an Ultimate 3000 nano-LC system (Dionex; Surrey, U.K.) coupled with MicroToF Q II mass spectrometer (Bruker; Bremen, Germany). The mass spectra data were acquired using Hystar software (Bruker Daltonics, Germany) and were converted by Compass DataAnalysis software (Bruker Daltonics, Germany). The converted files were analyzed with Mascot server (version 2.6.2.1, Matrix Science, USA) to search matched sequences in NCBI database with 95% confidence.

Statistical analysis

Data analysis was performed using a computer base and SPSS statistical software (version 16.0). Categorical data are described as frequency and percentage. Continuous data are given as mean and standard deviation (SD) for data with normal distribution, and as median and range for non-normally distributed data. Comparisons of mean wheal diameter (MWD) of SPT and level of sIgE between groups of wheat allergy and control (negative OFC) were made using Mann-Whitney U test. Differences between groups were considered significant at a p-value of ≤ 0.05.

Results
Subjects

Thirty-one children with history of wheat allergy were recruited. Baseline demographic and clinical characteristics of all participants are shown in **Table 1**. The median age of children was 64 months, and 15 (48.4%) were male. The median onset of wheat allergy was 6.5 months, and the median age at wheat introduction was 6 months. Asthma, allergic rhinitis, atopic dermatitis, and food allergy was found in 16%, 64.5%, 42%, and 74% of participants, respectively. Fifteen children (48.4%) had family history of atopy. The most common symptom associated with wheat ingestion was cutaneous (100%), followed by respiratory (33%) and gastrointestinal (23%).

Table 1. Baseline demographic and clinical characteristics of study patients

Patient characteristics	Total (n = 31)	Anaphylaxis (n = 13)	Mild reaction (n = 9)	Control (n = 9)	p-value
Male gender, n (%)	15 (48.4%)	3 (23.1%)	6 (66.7%)	6 (66.7%)	0.05
Age (months), median (range)	64 (14-156)	94 (16-154)	47 (14-74)	64 (18-156)	0.03
Age at onset (months), median (range)	6.5 (3-12)	7 (5-12)	6 (6-12)	7 (5-12)	0.97
Age at wheat introduction (months), median (range)	6 (2-12)	7 (5-12)	6 (6-12)	6 (2-12)	0.89
Personal history of atopy, n (%)					
Asthma	5 (16.1%)	2 (15.4%)	0 (0.0%)	3 (33.3%)	0.23
Allergic rhinitis	20 (64.5%)	9 (69.2%)	5 (55.6%)	6 (66.7%)	0.89
Atopic dermatitis	13 (41.9%)	3 (23.1%)	5 (55.6%)	5 (55.6%)	0.22
Other food allergy	23 (74.2%)	11 (84.6%)	5 (55.6%)	7 (77.8%)	0.35
Family history of atopy, n (%)	15 (48.4%)	5 (38.5%)	3 (33.3%)	7 (77.8%)	0.14
History of symptom(s), n (%)					
Cutaneous	30 (100%)	13 (100%)	9 (100%)	9 (100%)	-
Respiratory	10 (33.3%)	9 (69.2%)	0 (0.0%)	1 (12.5%)	0.01
Gastrointestinal	7 (23.3%)	3 (23.1%)	0 (0.0%)	0 (0.0%)	0.02
Cardiovascular	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-

A p-value < 0.05 indicates statistical significance

Oral food challenge (OFC) and group classification

A flow chart of patient recruitment and wheat challenge outcome is shown in **Figure 1**. Excluding the 12 children who had history of anaphylaxis and the 2 children who had strong history of recent wheat allergy, the remaining 17 children underwent OFC with wheat. Eight of those (47%) had positive results, and one developed anaphylaxis. The anaphylactic patient developed generalized urticarial rash and vomiting after ingesting the 6th dose (8 g) of wheat (cumulative dose: 15,600 mg). Her symptoms resolved after a dose of intramuscular epinephrine, intravenous chlorpheniramine, and ranitidine. The other patients who had positive OFC developed only urticarial rash without angioedema or organ involvement. The mean wheat dose that elicited symptom was 4.4 g (cumulative dose: 8.4 g).

Twelve children with history of anaphylaxis, and one child who developed anaphylaxis upon OFC were classified into the wheat anaphylaxis group (n = 13). Two children with strong history of recent symptom within 6 months, and 7 children with positive OFC without anaphylaxis were classified into the mild reaction group (n = 9). Children who had negative OFC were classified into the control group (n = 9). When comparing patient characteristics among the 3 groups, female gender, age at inclusion, and history of respiratory and gastrointestinal systems were all found to be significantly higher in the anaphylaxis group than in the other 2 groups (**Table 1**; $p < 0.05$).

Predictive diagnostic capacity of SPT and sIgE

The protein profile of 30 μg of extract was determined by SDS-PAGE (**Figure 2**). The results of LC-MS from excised SDS-PAGE gel pieces containing proteins confirmed that WS extract contained albumins, globulins, and alpha-amylase/trypsin inhibitor. Glia extract contained α/β, γ, and ω-gliadin, whereas Glu extract contained low-molecular-weight (LMW) and high-molecular-weight (HMW) glutenin subunit.
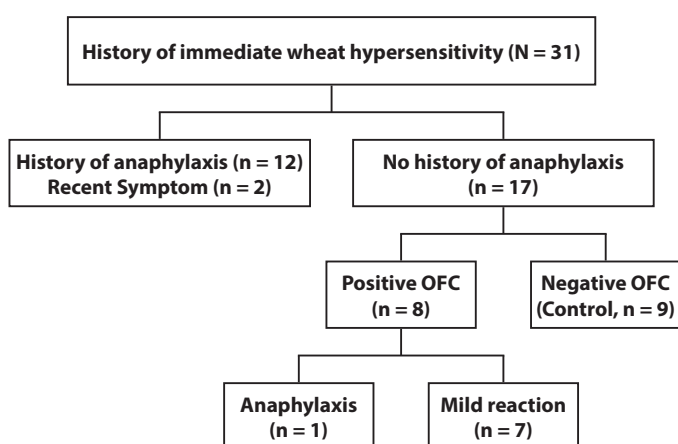

Figure 1. Flow diagram of patient recruitment and group allocation. Twelve children with history of anaphylaxis and one child who developed anaphylaxis upon oral food challenge (OFC) were classified into the wheat anaphylaxis group (n = 13). Two children with strong history of recent symptom within 6 months, and 7 children with positive OFC without anaphylaxis were classified into the mild reaction group (n = 9). Children who had negative OFC were classified into the control group (n = 9).
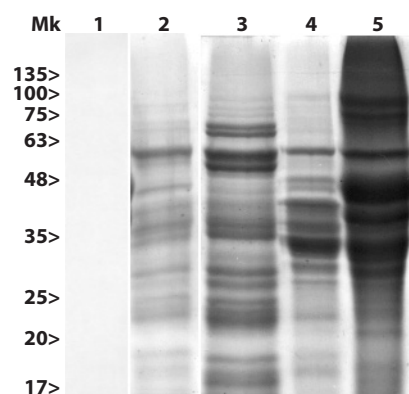

Figure 2. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of all wheat extracts used in this study. Each lane contained 30 μg/well of protein. Note: 1 = Commercial extract; 2 = Wheat-Coca's solution/10% EtOH extract; 3 = Wheat-Salt extract; 4 = Gliadin extract; 5 = Glutenin extract.

Table 2. Predictive ability of skin prick test (SPT) using 4 in-house prepared wheat extracts and a commercial wheat extract, and sIgE to wheat proteins and ω-5-gliadin

Test (cutoff)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR	-LR	Accuracy (%)
Skin prick test							
Wheat-Coca's solution/10% EtOH (MWD 4 mm)	78.9%	77.8%	88.2%	63.6%	3.55	0.27	78.6%
Wheat-salt extract (MWD 2.5 mm)	84.2%	77.8%	88.9%	70.0%	3.79	0.20	82.1%
Gliadin extract (MWD 2.5 mm)	84.2%	88.9%	94.1%	72.7%	7.59	0.18	85.7%
Glutenin extract (MWD 2.5 mm)	78.9%	88.9%	93.8%	66.7%	7.11	0.24	82.1%
Wheat commercial solution (MWD 2 mm)	55.0%	88.9%	91.7%	47.1%	4.96	0.51	65.5%

Table 2. (Continued)

Test (cutoff)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR	-LR	Accuracy (%)
Specific IgE							
Wheat proteins (0.6 kUA/l)	89.5%	66.7%	85.0%	75.0%	2.69	0.16	82.1%
ω-5 gliadin (0.20 kUA/l)	73.7%	88.9%	93.3%	61.5%	6.64	0.30	78.6%

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; MWD, mean wheal diameter

The performance of the different SPT extracts and sIgE to wheat and ω-5 gliadin to diagnose immediate wheat allergy is shown in **Table 2**. Receiver operating characteristic (ROC) curve analysis was used to identify the optimum MWD cutoff to diagnose immediate wheat allergy from different extracts. The optimum MWD for WC10Et, WS, Glia, Glu and commercial extracts was 4, 2.5, 2.5, 2.5, and 2 mm, respectively. The optimal cutoff for sIgE to wheat and ω-5 gliadin was 0.6 and 0.2 kAU/L, respectively. Among all of the tested extracts, SPT with Glia extract provided the best performance, with 84.2% sensitivity, 88.9% specificity, 94.1% positive predictive value (PPV), 72.7% negative predictive value (NPV), 7.59 positive

likelihood ratio (LR), 0.18 negative LR, and 85.7% accuracy.

Comparisons of SPT and sIgE in patients with different severity and controls

The MWD from different wheat extracts in anaphylaxis, mild reaction, and control patients are shown in **Figure 3**. The MWDs from all in-house extracts, but not commercial extract, were significantly larger in the anaphylaxis group than in the control group ($p < 0.05$). In addition, the MWDs from Glia and Glu extract were significantly larger in the mild reaction group than in the control group ($p < 0.05$).
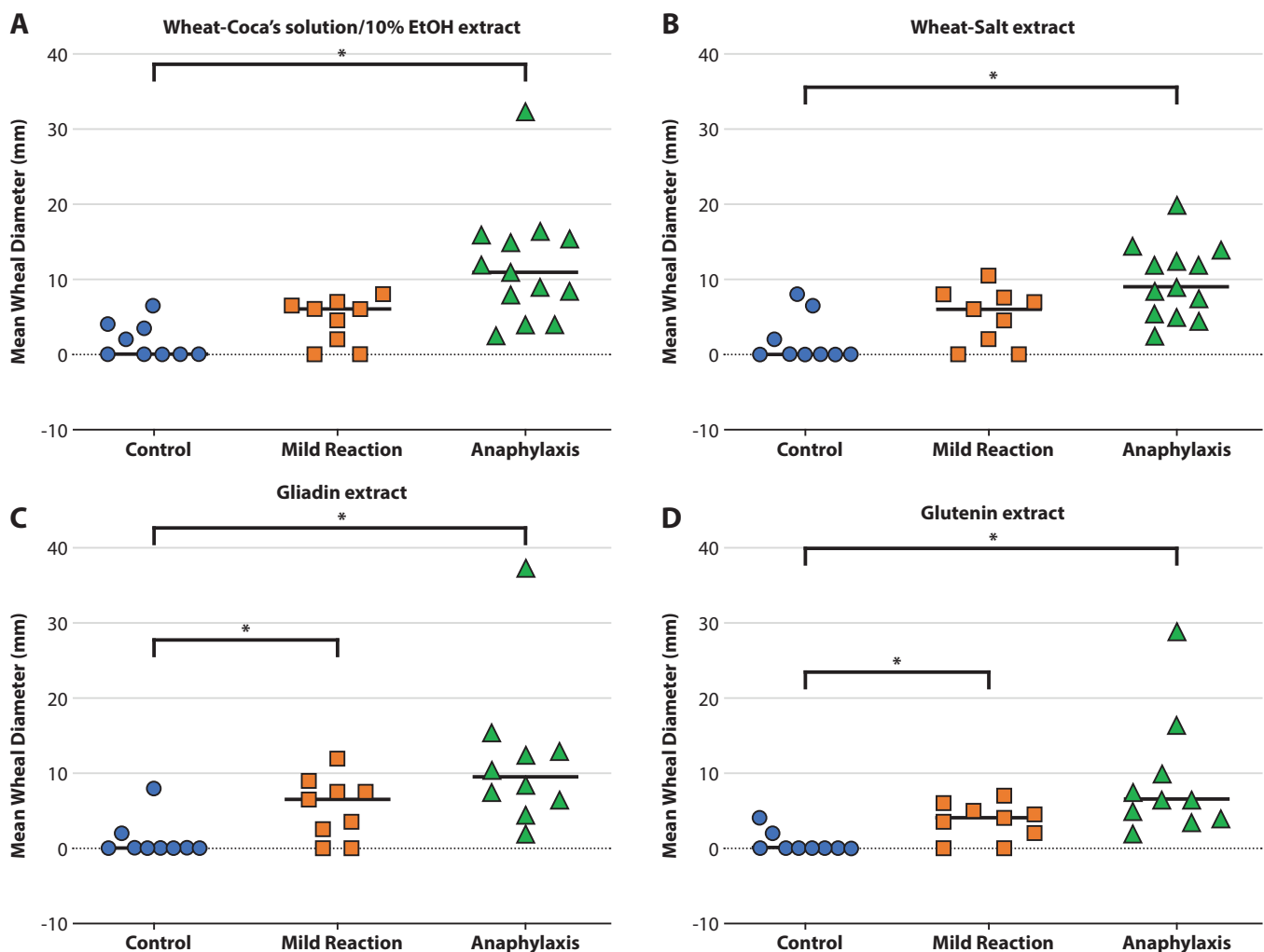

Figure 3. Comparison of mean wheal diameter from skin prick test using 5 extracts (A-E) among the control, mild reaction, and anaphylaxis groups. Note: Note: A) Wheat-Coca's solution/10% EtOH extract; B) Wheat-Salt extract; C) Gliadin extract; D) Glutenin extract; E) Commercial extract.
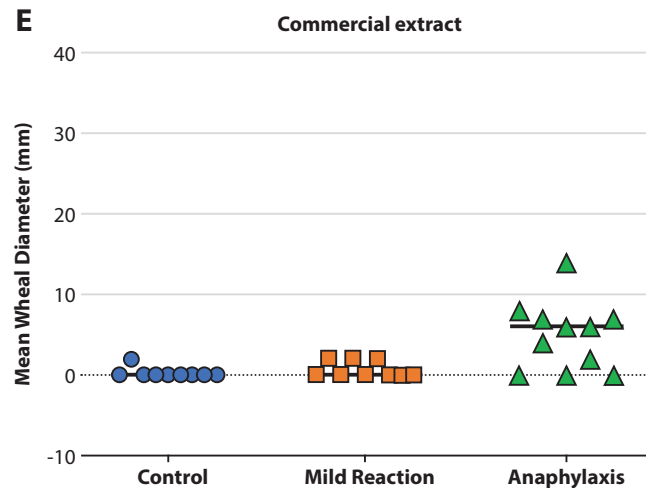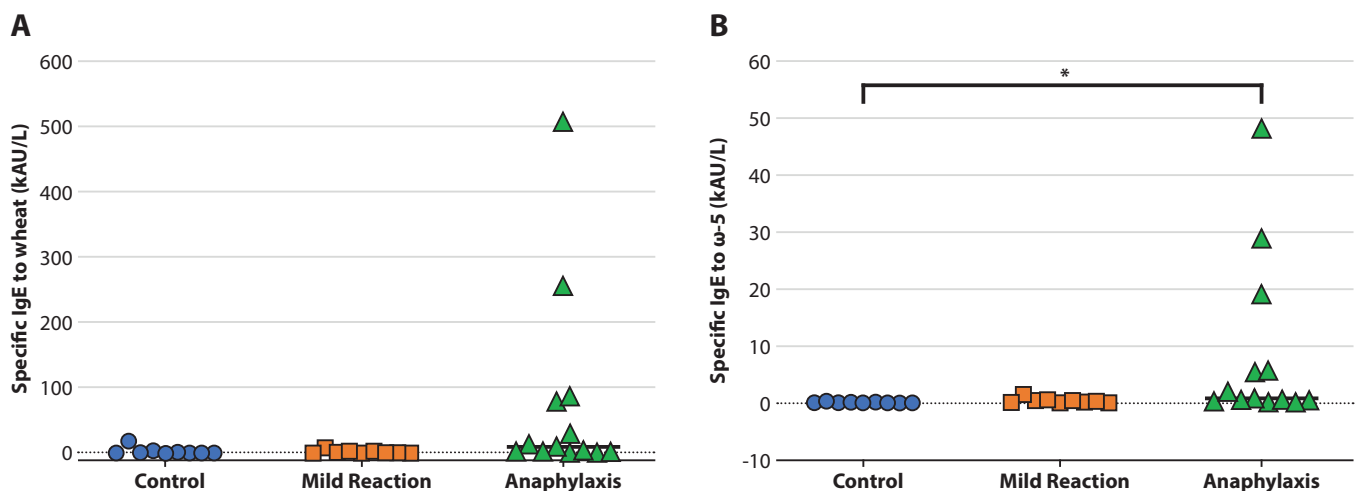
Figure 3. (Continued)

Figure 4. Comparison of serum specific IgE to wheat and to ω-5 gliadin among the control, mild reaction, and anaphylaxis groups. Note: A) specific IgE to wheat; and, B) specific IgE to ω-5 gliadin.

The levels of sIgE to wheat and to ω-5 gliadin in anaphylaxis, mild reaction, and control patients are shown in **Figure 4**. The level of sIgE to ω-5 gliadin, but not to wheat, was significantly higher in the anaphylaxis group than in the control group ($p < 0.05$).

The limitation of this study is a small sample size. A large scale multicenter study to identify the best diagnostic test for wheat allergy and wheat induced anaphylaxis is needed.

In conclusion, compared to other in-house and commercial wheat extracts and sIgE to wheat and ω-5 gliadin, SPT with an in-house gliadin extract yielded the highest performance for the diagnosis IgE-mediated wheat allergy.

Discussion

In this study, we found that almost half of children with history of wheat allergy developed allergic reaction upon OFC. This rate of challenge-proven allergy is similar to the rate reported by Perry. et al. who found 43% of food challenges to be positive.[10] One patient in our study developed anaphylaxis despite a previous history of only urticaria after wheat ingestion. This finding supports previous studies which found that children with previous mild reactions to food may suffer more

severe reactions, and that anaphylaxis is unpredictable.[18,19]

The role of SPT and the level of sIgE for the diagnosis of wheat allergy are problematic. First, cross-reactivity among several grass pollens, such as Poaceae family and wheat, can lead to false-positive test in patients with grass sensitization.[20] Second, sIgE to wheat levels may remain high even though children have already outgrown their wheat allergy.[12] Third, the level of sIgE to wheat that predicts 95% of wheat allergy can be as high as 100 kUA/L.[13,21,22] Furthermore, wheat grain proteins have four main fractions, which are salt/water soluble albumins (15%), salt/water soluble globulins (7%), salt/water insoluble gliadins (33%), and salt/water insoluble glutenins (45%).[1]

Gliadins and glutenins, both of which are members of the cereal prolamin family, are important major wheat allergens. Gliadins that solubilize in alcohol-containing solution are identified as α/β, γ, and ω-gliadin, with molecular weights of 30-45, 35-38, and 43-67 kDa, respectively.[23,24] Glutenins, which are polymeric proteins, can be divided into high- and low-molecular-weight (HMW and LMW) groups, with molecular weights of 75-110 and 30-40 kDa, respectively.[1,23,24] SDS-PAGE analysis (**Figure 2**) showed that all in-house extracts contained more

proteins than the commercial extract (especially proteins at 30-50 kDa), which resulted in better performance of in-house wheat extracts compared to that of commercial extract during SPT in patients with IgE-mediated wheat allergy. Moreover, gliadin and glutenin extracts could discriminate patients with mild allergic reaction or anaphylaxis from control group. Since most commercial wheat extracts for SPT only have salt/water soluble wheat allergen, some authorities have suggested that commercial wheat extracts for SPT should not be used due to very low specificity for diagnosis of wheat allergy.[1,23]

It was reported that the use of a level of specific IgE to wheat proteins in clinical diagnosis could be challenging due to the low specificity. Our results showed a level of sIgE to wheat proteins had high sensitivity (89.5%), but poor specificity (66.7%), which is similar to the findings of a previous study.[5] Specific IgE to wheat proteins is commonly detected among atopic children of all ages without true food allergy, and it is estimated that 65% of patients with grass pollen allergy have false-positive IgE to wheat.[13,23] Even after children outgrow their wheat allergy, sIgE may remain detectable or at a high level.[12]

Omega-5 gliadin is a potent sensitizer in adults with exercise-induced anaphylaxis, and in children with IgE-mediated immediate wheat allergy.[14] The results of our study showed that sIgE to omega-5 gliadin had higher specificity, PPV and positive LR compared to sIgE to wheat. This results were consistent with reports that suggested that ω-5 gliadin is a more specific marker for wheat allergy diagnosis than whole wheat proteins.[1,23]

The limitation of this study is a small sample size. A large scale multicenter study to identify the best diagnostic test for wheat allergy and wheat induced anaphylaxis is needed.

In conclusion, compared to other in-house and commercial wheat extracts and sIgE to wheat and ω-5 gliadin, SPT with an in-house gliadin extract yielded the highest performance for the diagnosis IgE-mediated wheat allergy.

Acknowledgements

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