Ig E Reactivity to Carbohydrate Moieties of Glycoproteins in Wheat Allergy

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Directed by Professor Kyu-Earn Kim

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ABSTRACT

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RATIONALE: In current available diagnostic tests, wheat proteins do not include all the allergens derived from wheat protein fractions with different solubility. The role of whole gliadin to wheat allergy has not evaluated, yet. The

carbohydrate moieties of different glycoprotein such as cross-reactive carbohydrate determinants and galactose alpha-1,3-galactose can induce IgE responses with diversity of clinical significance. In this study, we purposed to evaluate the allergenicity of gliadin to wheat allergy. Possible participation of the glycan from gliadin of wheat in their IgE-binding capacity was also investigated in children with wheat allergy.

METHODS: Total IgE, wheat specific IgE, careful history taking and oral food challenge were done for 52 children. They were characterized as wheat allergy patients, wheat IgE high group, wheat positive control and negative control. SDS-PAGE and glycan detection of gliadin were evaluated. IgE binding to gliadin and deglycosylated gliadin were measured by immunoblotting and ELISA.

RESULTS: Gliadin specific IgE was detected in all patients with wheat allergy and not correlated with wheat specific IgE. The range of glycan was almost

overlapping with whole gliadin bands. Removal of glycan from gliadin reduced allergenicity of gliadin. In gliadin, allergenicity of glycan portion was larger in wheat allergy patients than wheat IgE high group.

CONCLUSIONS: We suggest that the examination of IgE reactivity to whole gliadins are useful to diagnosis of wheat allergy. We concluded that almost all gliadins might be glycosylated and N-glycan of gliadin might have allergenicity as possible carbohydrate epitope in wheat allergy children.

Key words: carbohydrate epitope, cross-reactive carbohydrate determinants, food allergy, galactose alpha-1,3-galactose, gliadin, glycan, oral food challenge, wheat allergy

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I. INTRODUCTION

Wheat is one of the major crops consumed by humans. In Korea, we've also ingested wheat with various types like noodle, bread or cookie, etc. Wheat is

associated with three categories of allergic disease such as food allergy, baker's asthma and WDEIA (wheat-dependent exercise-induced anaphylaxis). Wheat is one of most common foods causing food allergy in children.[1] According to multicenter study in 2010, wheat is the fourth most common food allergen in Korean children.[2] Clinical manifestations of food allergy to wheat are similar to those of other food allergies, with symptoms on the skin, gut and respiratory tract.

Wheat protein represents about 10%-15% (dry weight) of wheat grain.[3] Wheat proteins are classified based on extraction in different solvents, which are albumin, globulin, gliadin and glutenin.[4] Water and dilute salt-soluble fractions of wheat are albumins and globulins, whereas ethanol and dilute acid-soluble wheat fractions are gliadins and glutenins.[4] Albumins and globulins include only 15%-20% of total protein, but most protein components are prolamins (gliadins plus glutenins).[3]

Prolamines are a group of plant storage proteins having a high proline content and found in the seeds of cereal grains such as gliadin in wheat, hordein in barley, secalin in rye, zein in corn, kafirin in sorghum and as a minor protein, avenin in oats. They are characterised by a high glutamine and proline content and are generally soluble only in strong alcohol solutions.[5]

Gliadins are members of the prolamin of superfamily.[6] The term 'gluten' contains approximately equal amounts of gliadin and glutenin and is the major determinant of the properties of wheat flour conferring cohesiveness and viscoelasticity that allows its dough to be processed into many kinds of food.[7] Gliadins are monomeric proteins that are grouped into 3 types– α /ß-, γ -and ω -gliadins–according to their biochemical characteristics and electrophoretic mobility at low pH.[8] The α /ß-, γ -and ω -gliadin types are separated and distinguished based on their amino acid sequences. α-/β-gliadins are soluble in low-percentage alcohols. γ-gliadins are ancestral form of cysteine-rich gliadin

with only intrachain disulfide bridges. ω-gliadins are soluble in higher percentages, 30–50% acidic acetonitrile. Glutenins form polymers maintained by interchain disulphide bridges and are classified into high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits after reduction and separation using sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[8]

The diagnosis of food allergy to wheat is hampered by the very low positive predictive value of serum specific IgE to wheat mainly due to the cross-reactivity with grass pollen and to the incomplete representation of wheat proteins in the currently available diagnostic tests, which do not include all the allergens derived from wheat protein fractions with different solubility.[9] Current in vitro test reagents for the diagnosis of wheat allergy mainly contain water-soluble wheat protein and a small amount of gluten, so there are some limitations to diagnose.[3] Recent studies have shown that specific IgE to gliadin

can be an indicator for risk of severe immediate reaction-like anaphylaxis and wheat dependent, exercise-induced anaphylaxis (WDEIA).[6] A recombinant ω-5-gliadin, an important allergen for wheat-dependent exercise-induced anaphylaxis and other allergies to ingested wheat, is available on ImmunoCAP test and has been found to be a useful marker for clinical reactivity in wheat-sensitized individuals.[4] Its role as an allergen has not been elucidated yet. The role of whole gliadin to wheat allergy has not evaluated, either.

The term glycan refers to a polysaccharide or oligosaccharide.[10] Glycans can be homo- or heteropolymers of monosaccharide residues, and can be linear or branched.[10] Glycan may also be used to refer to the carbohydrate portion of a glycoconjugate, such as a glycoprotein, glycolipid, or a proteoglycan.[10] Glycans can be found attached to proteins as in glycoproteins and proteoglycans.[10] N-Linked glycans are attached in the endoplasmic reticulum to the nitrogen (N) in the side chain of asparagine in the sequon.[10] The sequon is a Asn-X-Ser or Asn-

X-Thr sequence, where X is any amino acid except proline and may be composed of N-acetyl galactosamine, galactose, neuraminic acid, N-acetylglucosamine, fructose, mannose, fucose, and other monosaccharides.[10] However, O-linked glycan is the addition of sugar chains on the hydroxyl oxygen (O) on the side-chain of hydroxylysine, hydroxyproline, serine or threonine.

Many or most of the allergens we inhale or ingest are glycosylated with oligosaccharides.[11] Despite this, the current evidence show that the IgE antibodies associated with allergic disease are specific for protein epitopes and most research has focused on protein epitopes.[11]

The presence of IgE antibodies to carbohydrate antigens was first identified from in vitro experiments looking at cross-reactivity between different plant-derived antigens.[12] In part because of this approach, the carbohydrate epitopes identified were generally, or exclusively, cross-reactive, which led to the

designation *cross-reactive carbohydrate determinants* (CCDs).[11] It is well known that the carbohydrate moieties present on many plant foods can induce anti-glycan IgE responses. But, the clinical significance of these cross-reactive carbohydrate determinants is unclear.[12, 13] By contrast, recent work has shown that IgE antibodies specific for the carbohydrate galactose-α-1,3-galactose (α -gal) are capable of eliciting serious, even fatal, reactions.[14, 15]

In this study, we purposed to evaluate the allergenicity of gliadin to wheat allergy. Possible participation of the glycan from gliadin of wheat in their IgE-binding capacity was also investigated with sera of children with wheat allergy.

II. MATERIALS AND METHODS

1. Patients and sera

The Sera from 52 patients who visit to the allergy clinic for food allergy or other allergic disease at the Severance hospital of Yonsei university and Ilsan paik hospital of Inje university were obtained. After venous blood was drawn, serum was separated and stored at –20℃ until analysis.

In this study, we divided the patients into 4 groups such as wheat positive control, negative control, wheat IgE high group, and wheat allergy patients. Eight patients who had clinical history of immediate hypersensitivity reactions following wheat ingestion and four patients who showed positive results to OFC (oral food challenge) to wheat were grouped as wheat allergy patients. Nine patients with high wheat specific IgE who had never eaten wheat due to severe atopic dermatitis and five patients who had no symptoms after wheat ingestion

in spite of high wheat specific IgE were grouped as wheat IgE high group. Ten patients with high wheat specific IgE who had unclear history for wheat allergy were grouped as wheat positive control and their sera were mixed as wheat positive pooled serum. Sixteen children who didn't showed any symptoms after wheat ingestion were grouped as negative control.

In order to confirm or exclude wheat allergy, the diagnosis was based on case history, wheat specific IgE concentration and oral food challenge. In wheat allergy patients, eight patients with a strong convincing history of immediate objective hypersensitivity reaction following wheat ingestion such as anaphylaxis, angioedema, or urticatia and with high concentration of wheat specific IgE above laboratory cut point were considered as wheat allergy and didn't carry out OFC. In wheat allergy patients, OFC to wheat was done for confirmed diagnosis to four patients with history of multi food allergy, positive wheat specific IgE under laboratory cut point and no experience of wheat

ingestion.

2. ImmunoCAP test for total and specific IgE to wheat

Total and specific IgE level to wheat were measured using ImmunoCAP test (Pharmacia, Diagnostics, Uppsala, Sweden). For negative control, specific IgE to alternaria, Dermatophagoides pteronyssinus, Dermatophagoides farinae, pollens or other foods according to their clinical history were measured instead of wheat specific IgE. According to the manufacturer's instructions, specific IgE levels of over 0.35 kU_A/L were considered positive.

3. Open oral food challenges to wheat

Oral food challenges to wheat will be performed as open challenge in a hospital setting and supervised by a physician in accordance with the guidelines of the Korean Academy of Pediatric Allergy and Respiratory Diseases. Patients were challenged with boiled wheat noodle. Total dose of wheat noodle for OFC was adjusted according to age (90-200g). The test was started by consuming

one strand of boiled wheat noodle, and this amount was doubled every 15 min until symptoms arose or until the entire test meal was eaten (Table 1).

Table 1. Protocol of oral food challenge

Time(min)	doses
0	1 strand (1.353 g)
15	1/48 of total dose*
30	1/24 of total dose*
45	1/8 of total dose*
60	1/4 of total dose*
75	rest
135	(observation)

* Total dose of wheat noodle for OFC was adjusted according to age (90-200g)

4. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of gliadin

Gliadin (Sigma-Aldrich, St. Louis, MO, USA) was purchased commercially. Constitutive proteins of gliadin (Sigma-Aldrich, St. Louis, MO, USA) were separated by SDS-PAGE. According to Laemmli's method, gliadin was

dissolved in loading buffer (60 mM of Tris-HCl, 25% glycerol, 2% sodium dodecyl sulfate [SDS], 14.4 mM of 2-mercaptoethanol, and 0.1% bromophenol blue) and reacted in boiling water for 8 minutes. Samples then are separated on 4-20% gradient gels by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and protein bands are stained with coomassie blue.

5. Glycan detection in gliadin

Glycans which were included in gliadin were identified by using a commercially available kit (DIG Glycan Differentiation Kit, Roche, Mannheim, Germany). Detection of glycan was performed by following the manufacturer's instructions. Gliadins were transferred to polyvinylidene difluoride (PVDF) membrane (Immobilon P, Millipore Co., Billerica, MA, USA) after SDS-PAGE. The specific binding of lectins to carbohydrate moieties was used to identify glycan and its structure. For differentiating between carbohydrate structures,

lectins which selectively recognize the terminal sugars were used.

6. IgE Immunoblotting of gliadin and deglycosylated gliadin

Gliadin was dissolved in a sample buffer for SDS-PAGE and boiled for 8 min. To each lane of a 4-20% gradient gel, 10μg of gliadin was applied. After electrophoresis under constant-current conditions (150 V), separated proteins were transferred onto polyvinylidene difluoride membranes (Immobilon P, Millipore Co., Billerica, MA, USA) by applying direct current (250 mA) for 90 min. To examine the allergenicity of gliadin, IgE bindings to gliadin by immunoblotting were evaluated with pooled serum from wheat positive control, pooled serum from negative control, and buffer control.

To estimate the significance of glycan as epitope in gliadin-IgE binding, glycan was removed from gliadin. For glycan removal from gliadin, gliadin were oxidized by soaking a membrane in 20 mM $NaIO_4$/50 mM acetate buffer (pH 4.5) for 2 h at room temperature. Gliadin-IgE bindings were evaluated by

immunoblotting with both gliadin and $NaIO_4$ treated gliadin. Each membrane was successively washed with TBS containing 0.5% Tween-20 (TBS-T). After that, 4 mL of serum diluted in the blocking buffer (1:9) was placed on the respective membranes and was reacted overnight at 4℃. The membranes were incubated for 1 h with alkaline phosphatase-labeled anti-human IgE antibodies (Sigma-Aldrich, St. Louis, MO, USA) diluted (1:1,000) in the blocking buffer. The membranes were developed in NBT/BCIP solution (Roche Diagnostics, Mannheim, Germany). To confirm glycan removal, glycan detection were tried by using a DIG Glycan Differentiation Kit (Roche Diagnostics, Mannheim, Germany) following the supplier's instructions. With individual serums from wheat IgE high group and wheat allergy patients, IgE bindings to gliadin and deglycosylated gliadin were compared by Immunoblotting. We couldn't perform IgE immunoblotting with sera of patient No. 7, 9-12, 14 and 26 due to the lack of serum.

7. ELISA of gliadin and deglycosylated gliadin

We observed IgE bindings to gliadin and deglycosylated gliadin with individual serum from wheat IgE high group and wheat allergy patients by Immunoblotting with our above experimentss. By ELISA, we compared IgE bindings to gliadin and deglycosylated gliadin with individual serums from wheat IgE high group and wheat allergy patients once again.

Gliadin were dissolved in 100 mM carbonate buffer (pH 10.0) at a concentration of 3μg/mL. Gliadin were distributed into 96-well microtiter plates (Costar Co, NY, USA) and incubated overnight at 4℃. The wells were then washed 5 times with TBS-T (Tris-buffered saline containing 0.5% Tween-20), and the unoccupied areas were blocked by reacting with 200 μL of 5% BSA/TBS-T for 2 h at room temperature. After successive washings with TBS-T, a serum diluted in the blocking buffer (1:10) was distributed into the wells and reacted for 1h at room temperature. Following successive washings with TBS-T,

the wells were blocked again with the blocking buffer for 30 min. After that, 100 μL of biotynylated anti-human IgE antibodies (Sigma-Aldrich, St. Louis, MO, USA) diluted in the blocking buffer (1:500) was distributed into every well and incubated at room temperature for 1 h. The wells were washed again with TBS-T, and 100 μL of streptavidin-HRP (R&D system, Minneapolis, MN, USA) was distributed into them. After 30min of development at room temperature, the absorbance at 450 nm was measured with a VersaMax microplate reader (Molecular devices, CA, USA).

To remove glycan from gliadin, glycan of gliadin adsorbed onto microtiter plates was oxidized by adding 10 μL of 40 mM $NaIO_4$ in 50 mM acetate buffer (pH 4.5). After 24 h of incubation at room temperature, the wells were thoroughly washed with TBS-T.

8. Statistical analysis

Data were expressed as mean ± SD. Statistical analysis comparing gliadin ,

gliadin ratio and allergenicity of glycan among different groups were assessed by Student's t-test. Statistical analysis comparing IgE binding to gliadin and IgE binding to deglycosylated gliadin was assessed by paired t-test. Correlation coeffecients were determined by using the Spearman rank correlation test. P values of less than .05 were considered to be stastically significant.

III. RESULTS

1. Characterization of patients

The mean age of the patients was 2.06 years (0.53 to 7.5 years). Ten patients with high wheat specific IgE who had unclear history for wheat allergy were grouped as wheat positive control (Table 2).

Table 2. Characteristics of wheat positive control (n = 10)

Age (years)	Sex	Diagnosis	Total IgE (IU/mL)	Wheat IgE (kU_A/L)	Clinical symtoms after ingestion of wheat
1	M	AD, BA, FA	>5000	>100	unclear
6	M	AD, AR, BA, FA	>5000	>100	unclear
0.5	M	AD, AR, BA	>5000	>100	unclear
1	M	AD	2741	>100	unclear
2.8	M	AD, BA, FA	3470	>100	unclear
1	M	AD	>5000	56.6	unclear
1	F	AD, BA, FA	1446	52.7	unclear
1	M	AD	1488	73.5	unclear
0.6	M	AD	1140	60.3	unclear
1.8	M	AD	4540	99.6	unclear

AD, atopic dermatitis; AR, allergic rhinitis; BA, bronchial asthma; FA, food allergy

Sixteen children who didn't showed any symptoms after wheat ingestion were grouped as negative control (Table 3).

Table 3. Characteristics of negative control (n = 16)

Age (years)	Sex	Diagnosis	Total IgE (IU/mL)	Specific IgEs to others* (kU_A/L)	Clinical symtoms after ingestion of wheat
0.42	M	AD, BA	2	all negative	none
4	F	AR	2.9	all negative	none
2	M	BA	30.6	all negative	none
2	F	CC	70.3	all negative	none
2	F	BA	48.3	all negative	none
2	M	CC	94.3	all negative	none
2	F	BA, AR	74.2	all negative	none
3	M	AR	7.1	all negative	none
1	M	AD	135	all negative	none
4	F	AR	4.69	all negative	none
1	M	r/o BA	28.9	all negative	none
1	M	BA, AR	6.22	all negative	none
2	M	BA	20.8	all negative	none
1	F	BA	19	all negative	none
4	F	AR	719	all negative	none
1	F	r/o BA	5.9	all negative	none

AD, atopic dermatitis; AR, allergic rhinitis; BA, bronchial asthma; CC, chronic cough; FA, food allergy

* specific IgE to alternaria, Dermatophagoides farinae, Dermatophagoides pteronyssinus, pollens or other foods

Nine patients with high wheat specific IgE who had never eaten wheat due to severe atopic dermatitis and five patients who had no symptoms after wheat ingestion in spite of high wheat specific IgE were grouped as wheat IgE high

group (Table 4).

Table 4. Characteristics of wheat IgE high group (n = 14)

No.	Age(years)	Sex	Diagnosis	Total IgE (IU/mL)	Wheat IgE (kU_A/L)	Clinical symtoms after ingestion of wheat
1	0.6	M	AD, FA	>5000	>100	never eaten
2	1	M	AD, FA	>5000	>100	never eaten
3	0.92	M	AD	>5000	82.1	never eaten
4	1.75	M	AD	>5000	> 100	never eaten
5	0.75	M	AD, FA	773	48.8	never eaten
6	0.65	M	AD	5000	30.4	never eaten
7	0.53	M	AD, AR	1488	73.5	never eaten
8	2.1	M	AD, AR,	3190	38.8	never eaten
9	2	F	AD,FA	>5000	>100	never eaten
10	2.1	F	AD, FA	515	73.5	No symptoms
11	0.66	F	AD	798	22.4	No symptoms
12	5	M	AD,FA	501	22.6	No symptoms
13	2.1	M	AD, FA	3757	43	No symptoms
14	2.1	M	AD, FA	3606	46.7	No symptoms

AD, atopic dermatitis; AE, angioedema; Ana, anaphylaxis; AR, allergic rhinitis; BA, bronchial asthma; FA, food allergy

Eight patients who had clinical history of immediate hypersensitivity reactions following wheat ingestion and four patients who showed positive results to OFC to wheat were grouped as wheat allergy patients (Table 5).

Wheat allergy patients showed variable clinical symptoms after ingestion of wheat. Patients with No. 17, 20, 22, 24 and 25 showed even anaphylaxis due to wheat ingestion (Table 5).

Table 5. Characteristics of wheat allergy patients (n = 12)

No.	Age (years)	Sex	Diagnosis	Total IgE (IU/mL)	Wheat IgE (kU_A/L)	Clinical symtoms after ingestion of wheat (OFC reaction)
15	1.3	F	AD, FA	151	10.9	AE
16	7.5	M	AD,AR,FA	1124	9.26	U
17	6.1	M	AD,AR,BA,FA	1422	32.9	Ana
18	1	M	AD, BA, FA	42.7	0.67	(U)
19	5	M	AD,AR,BA,FA	203	1.49	(U)
20	1	F	AD, FA	94	15.9	Ana
21	5	M	AD,AR,BA,FA	199	2.17	(U)
22	1	F	FA	116	36.6	Ana
23	3	M	AD, FA	323	11.1	U, AE
24	2	F	AD, CU, FA	219	82.6	Ana
25	2	M	AD, FA	296	97.5	Ana
26	1	M	AD, FA	289	4.07	(U, Sn)

AD, atopic dermatitis; AE, angioedema; Ana, anaphylaxis; AR, allergic rhinitis; BA, bronchial asthma; CU, chronic urticaria; FA, food allergy; (Sn), sneezing during OFC; U, urticaria; (U), urticaria during OFC; OFC, oral food challenge

2. The results of oral food challenges

For patients with No 18, 19, 21 and 26, OFC to wheat performed and all patients were wheat OFC positive. Patient No. 18 showed very tiny wheal on Lt axilla which was supposed as urticaria or insect bite on the dose of 1/24 of total dose. His symptoms was not clear to be positive reaction. After 15min, same dose were repeated and there were no additional symptoms. But, one more wheal on right side cheek appeared after complete ingestion of whole dose (90g). After a few minutes, urticaria appeared on trunk, too. We gave oral antihistamine (hydroxyzine syrup 0.5mL/kg) and observed till his urticaria disappeared.

Patient No. 19 showed very mild perioral rash on the dose of 1/24 of total dose. His symptoms was not clear to be positive reaction. After 15min, same dose were repeated and urticaria appeared on his face. Patient No. 21 showed urticaria on the dose of 1/24 of total dose. Patient No. 26 showed urticaria and

sneezing on the dose of 1/24 of total dose.

3. Proteins, glycans and IgE bindings of gliadin

Constitutive proteins of gliadin were separated by SDS-PAGE and protein bands ranging from 25 to 60 kDa were observed (Fig. 1). The allergenicity of gliadin were evaluated by immunoblotting with pooled sera from wheat positive control. We observed IgE bindings between gliadin and pooled serum from wheat positive control through whole range of gliadin. However, gliadin didn't show IgE binding to both pooled serum from negative control and buffer control (Fig. 1).

Glycan which was included in gliadin was detected by DIG Glycan Differentiation Kit. The range of glycan was almost overlapped with gliadin as from 25 to 60 kDa (Fig. 1).

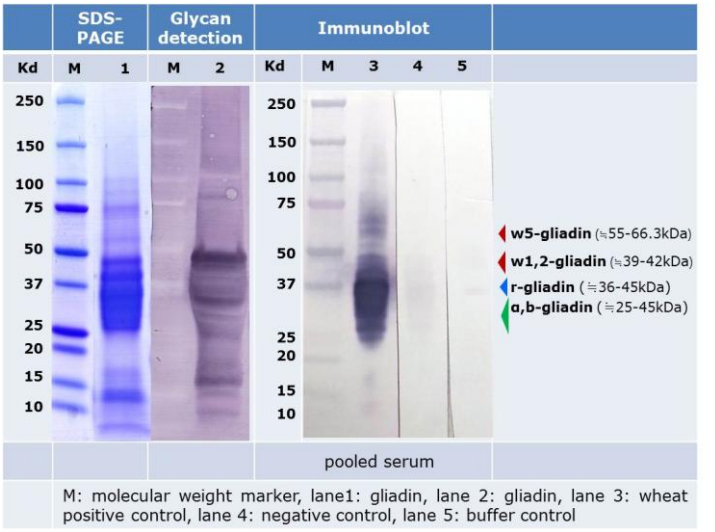

Fig. 1. Protein bands of gliadin ranging from 25 to 60 kDa by SDS-PAGE. Glycan which was included in gliadin was detected by DIG Glycan Differentiaion Kit and the range of glycan was almost overlapped with gliadin as from 25 to 60 kDa. IgE bindings between gliadin and pooled serum from wheat positive control through whole range of gliadin were observed by immunoblotting.

4. The correlation of wheat specific IgE and gliadin specific IgE and gliadin ratio according to wheat allergy

In wheat IgE high group, wheat specific IgE by ImmunoCAP test was significantly correlated with gliadin specific IgE by ELISA (r=0.77, p=0.001) (Fig. 2).

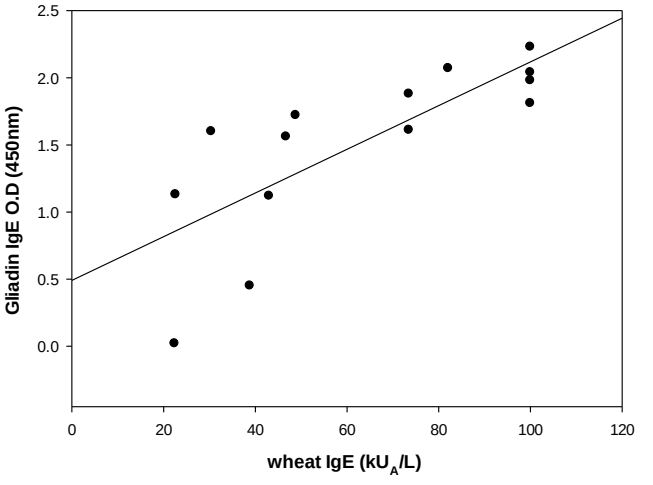

Fig. 2. Correlation between wheat specific IgE and gliadin specific IgE in wheat IgE high group. Specific IgE level to wheat were measured using ImmunoCAP test and specific IgE level to gliadin were measured by ELISA. In wheat IgE high group, wheat specific IgE was significantly correlated with gliadin specific IgE (r=0.77, p=0.001).

In wheat allergy patients, wheat specific IgE by ImmunoCAP test was not correlated with gliadin specific IgE by ELISA (r=0.43, p=0.16) (Fig. 3).

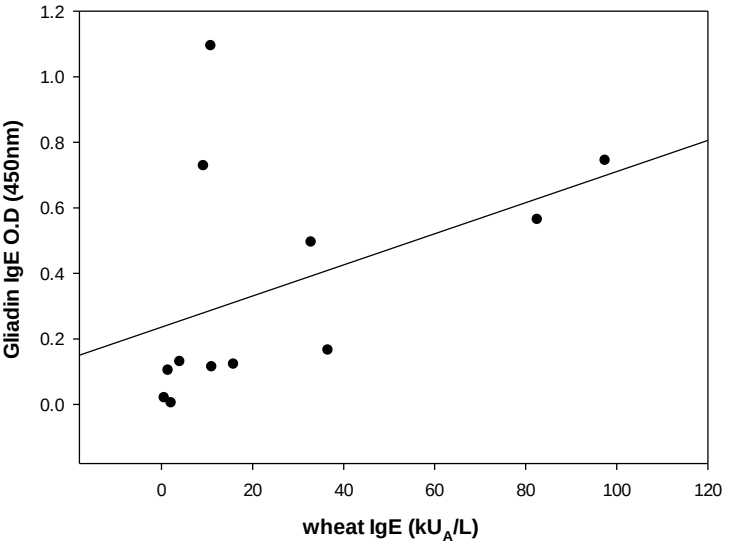

Fig. 3. Correlation between wheat specific IgE and gliadin specific IgE in wheat allergy patients. Specific IgE level to wheat were measured using ImmunoCAP test and specific IgE level to gliadin were measured by ELISA. In wheat allergy patiens, wheat specific IgE was not significantly correlated with gliadin specific IgE (r=0.43, p=0.16).

We compared gliadin ratio (gliadin specific IgE/wheat specific IgE) between wheat allergy patients and wheat IgE high group. Wheat allergy patients showed the tendency of higher gliadin ratio than wheat IgE high group (Fig. 4).

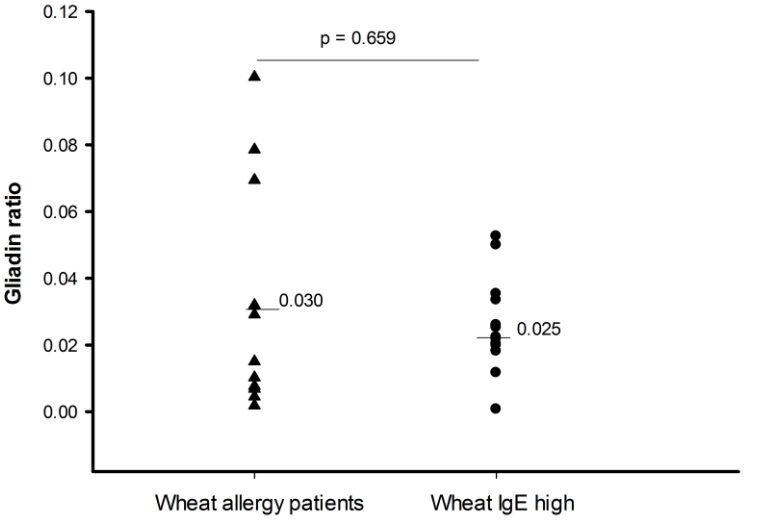

Fig. 4. Comparison of gliadin ratio between wheat allergy patients and wheat IgE high group. Gliadin ratio = gliadin specific IgE/wheat specific IgE. Specific IgE level to wheat were measured using ImmunoCAP test and specific IgE level to gliadin were measured by ELISA. Wheat allergy patients showed the tendency of higher gliadin ratio than wheat IgE high group.

5. Allergenicity of gliadin

We observed IgE bindings between gliadin and pooled sera from wheat positive control by immunoblotting with our above results. By ELISA, we evaluated IgE bindings to gliadin once again. IgE bindings between gliadin and individual serums from wheat allergy patients and wheat IgE high group were evaluated by ELISA. For comparison, IgE bindings between gliadin and individual serums from negative control were evaluated by ELISA. In wheat

allergy patients and wheat IgE high group, IgE reactivity to gliadin were detected in all sera and significantly higher than wheat negative controls(0.98 ± 0.68 vs 0, p=0.001). IgE reactivity for gliadin was not observed in negative control (Fig. 5).

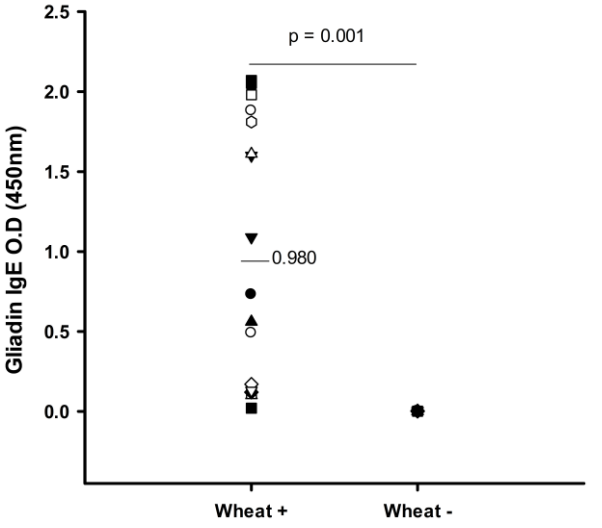

Fig. 5. IgE bindings between gliadin and individual serums from wheat allergy patients and wheat IgE high group by ELISA (n = 26). IgE bindings between gliadin and individual serums from negative control were evaluated by ELISA (n=16). In wheat allergy patients/wheat IgE high group, IgE reactivity to gliadin were detected and significantly higher than negative control. IgE reactivity to gliadin was not observed in negative controls.

6. The effect of deglycosylation to allergenicity of gliadin

For the evaluation of allergenicity of glycan, we compared IgE bindings to

gliadin with IgE bindings to deglycosylated gliadin. For removal of glycan from gliadin, gliadin was oxidized by $NaIO_4$. With individual sera from wheat IgE high group, IgE bindings was evaluated by immunoblotting and ELISA. Glycan removal was confirmed by glycan detection. Before evaluation with individual sera, we evaluated IgE binding with pooled serum from wheat positive control. With pooled serum from wheat positive control, IgE binding with gliadin in immunoblotting was showed with ranging from 25 to 60kD. After glycan removal by $NaIO_4$ treatment, intensity of band was decreased. With individual sera from wheat IgE high group, IgE bindings to gliadin were evaluated in immunoblotting. IgE bindings were showed in all sera with ranging from 10 to 100 kD. After glycan removal by $NaIO_4$ treatment, intensity of bands were decreased in all sera(Fig. 6A). IgE binding was also evaluated by ELISA. Deglycosylated gliadin were significantly less recognized by the IgE antibodies from all sera of wheat IgE high group than gliadin (1.51 ± 0.18 vs 1.30 ± 0.23,

p<0.001)(Fig. 6B).

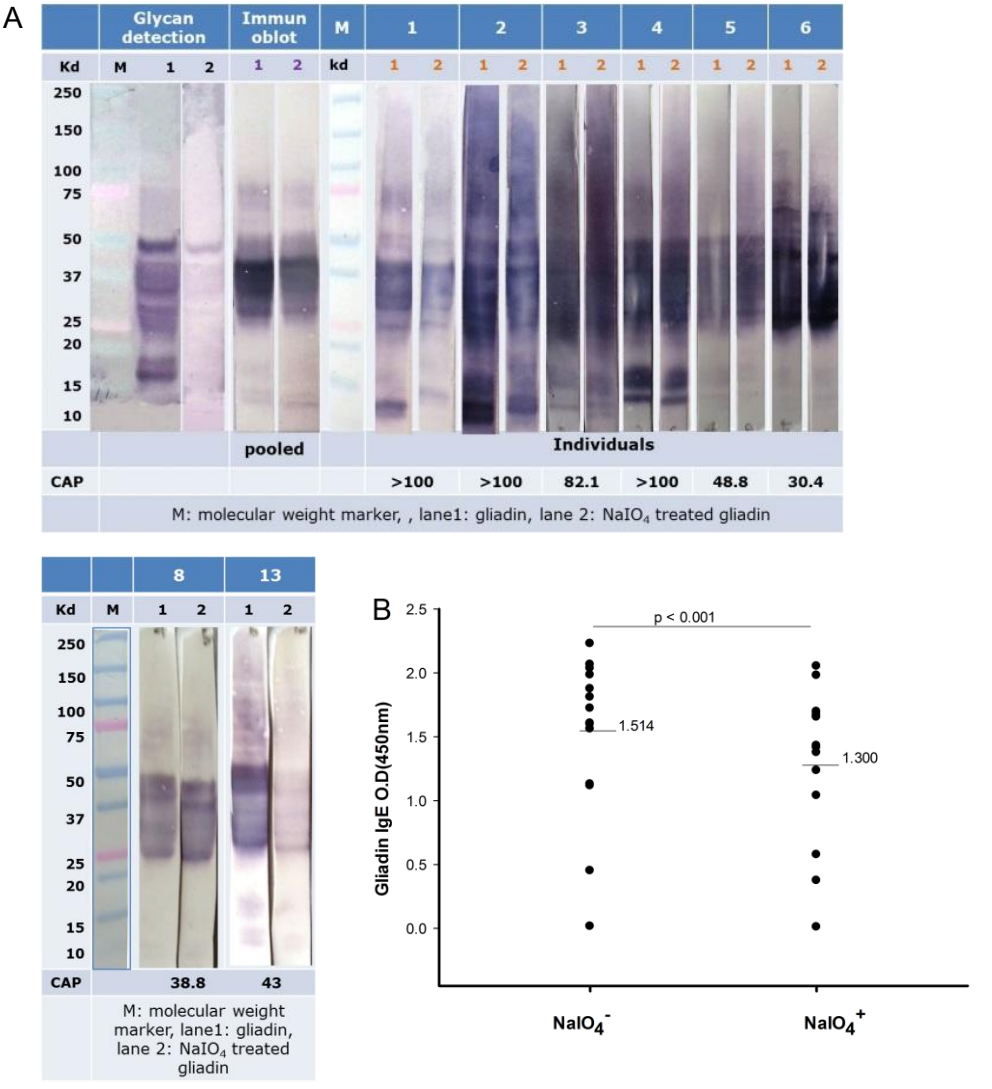

Fig. 6. Comparison of IgE binding between gliadin and deglycosylated gliadin with sera from wheat IgE high group by immunoblotting and ELISA. (A) IgE bindings in immunoblotting were showed with ranging from 10 to 100 kD. After glycan removal with $NaIO_4$ treatment, intensity of bands were decreased. (B) IgE bindings to gliadin in ELISA were significantly decreased after glycan

removal.

For the evaluation of allergenicity of glycan, we compared IgE bindings to gliadin with IgE bindings to deglycosylated gliadin. For removal of glycan from gliadin, gliadin was oxidized by $NaIO_4$. With sera from wheat allergy patients, IgE bindings were evulated by immunoblotting and ELISA. Glycan removal was confirmed by glycan detection (Fig. 7A). With individual sera from wheat allergy patients, IgE bindings to gliadin were evaluated by immunoblotting. IgE bindings were showed in all sera with ranging from 25 to 60 kD (Fig. 7A). After glycan removal by $NaIO_4$ treatment, intensity of bands were decreased in all sera (Fig. 7A). IgE binding was also evaluated by ELISA. Deglycosylated gliadin were significantly less recognized by the IgE antibodies from all sera of wheat allergy patients than gliadin (0.36 ± 0.35 vs 0.24 ± 0.31, $p=0.021$)(Fig. 7B).

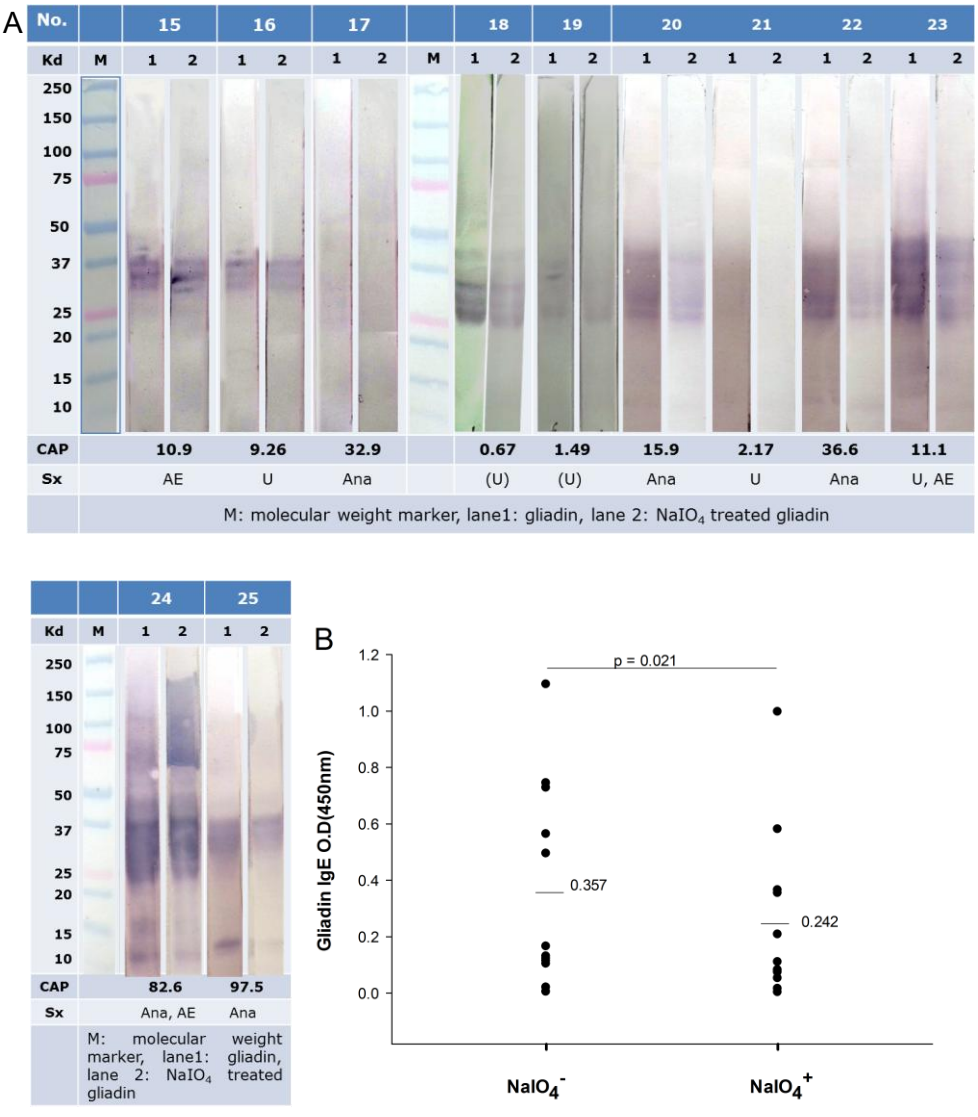

Fig. 7. Comparison of IgE binding between gliadin and deglycosylated gliadin with sera from wheat allergy patients by immunoblotting and ELISA. (A) IgE bindings in immunoblotting were showed with ranging from 25 to 60 kD. After glycan removal with $NaIO_4$ treatment, intensity of bands were decreased. (B) IgE bindings to gliadin in ELISA were significantly decreased after glycan removal. AE, angioedema; Ana, anaphylaxis; U, urticaria; (U), urticaria during oral food challenge.

7. The allergenicity of glycan portion in gliadin

We compared allergenicity of glycan portion in gliadin between wheat allergy patients and wheat IgE high group. In gliadin, IgE bindings of glycan portion were larger in wheat allergy patients than wheat IgE high group (0.34 ± 0.19 vs 0.17 ± 0.12, p=0.011)(Fig. 8).

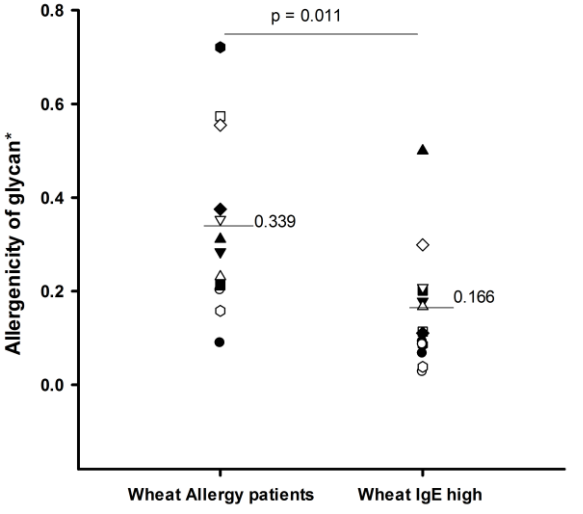

Fig. 8. Comparison of the allergenicity of glycan portion in gliadin between wheat allergy patients and wheat IgE high group. In gliadin, IgE bindings of glycan portion were larger in wheat allergy patients than wheat IgE high group. *Allergenicity of glycan = (IgE binding to gliadin – IgE binding to deglycosylated gliadin)/ IgE binding to gliadin

We also compared allergenicity of glycan portion in gliadin among wheat allergy patients, wheat IgE high- NE (never eaten) group and wheat IgE high-

NoSx (no symptoms) group. In gliadin, IgE bindings of glycan portion were larger in wheat allergy patients than wheat IgE high-NE (never eaten) group (0.34 ± 0.19 vs 0.12 ± 0.06, p=0.004)(Fig. 9). In gliadin, IgE bindings of glycan portion seems to be larger in wheat allergy patients than wheat IgE high-NoSx (no symptoms) group (0.34 ± 0.19 vs 0.24 ± 0.17, p=0.341)(Fig. 9).

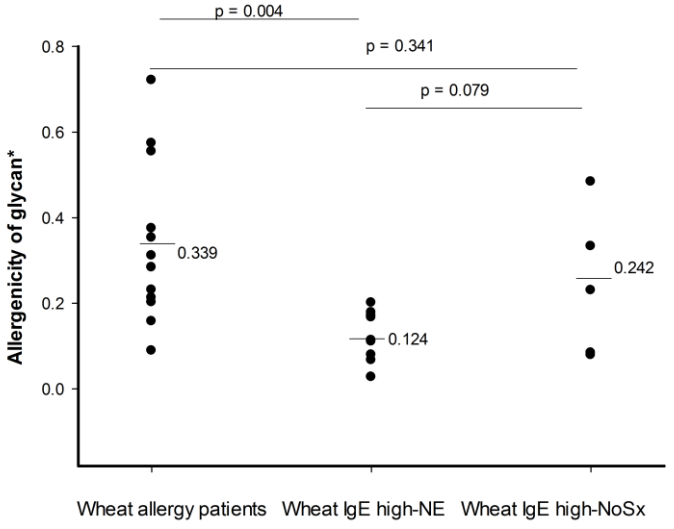

Fig. 9. Comparison of the allergenicity of glycan portion in gliadin among wheat allergy patients, wheat IgE high- NE (never eaten) group and wheat IgE high- NoSx (no symptoms) group. In gliadin, IgE bindings of glycan portion were larger in wheat allergy patients than wheat IgE high group. *Allergenicity of glycan = (IgE binding to gliadin – IgE binding to deglycosylated gliadin)/ IgE binding to gliadin

IV. DISCUSSION

Wheat allergy is primarily an IgE-mediated response. Several wheat flour proteins have been identified as allergens on the molecular level with IgE from patients with baker's asthma or with wheat-dependent food allergy.[16] In albumin/globulin fractions, the most important allergens are the α -amylase/trypsin inhibitor subunits.[9] They are responsible for baker's asthma and also for wheat food allergy, as demonstrated by James et al.[17] in children with atopic dermatitis, and by Simonato et al.[18] and Armentia et al.[19] in adults. Sander et al. also revealed that the α -amylase inhibitors are the major group of wheat proteins responsible for baker's asthma.[4] Sander's study showed 75% bakers had IgE to at least one of the 19 single allergens.[4] HRP (horseradish peroxidase) and MUXF (25% each), followed by WTAI-CM1 (20%), thiol reductase (16%), WTAI-CM3 (15%), WTAI-CM2 and thioredoxin (12.5%), WMAI-28, triosephosphate-isomerase, ab-gliadin (10%), 1-cys-peroxiredoxin

(7.5%), dehydrin, serpin, glyceraldehyde-3-phosphate-dehydrogenase (5%), ω-5-gliadin, nsLTP and profilin (2.5%).[4] 38% of bakers had IgE to any α-amylase inhibitor.[4]

Until now, Studies for searching more significant wheat allergen among several wheat proteins has usually done in adult patients with baker's asthma. But, we evaluated wheat allergen in children with food allergy due to wheat, in this study.

Among several wheat proteins, we focused on gliadin, in this study. Several studies demonstrated that gliadins involved in IgE-mediated reactions to ingested wheat in children with atopic dermatitis[20], recurrent urticaria[21] and in wheat-dependent, exercise-induced anaphylaxis (WDEIA).[22] Recently, Bittner et al. cloned and expressed a 20 kDa fragment of an αβ-gliadin that reacted in an ELISA with 18 (12%) of 153 bakers with occupational asthma.[23] Using recombinant DNA technology, specific IgE to αß-gliadin detected in 10% of the

sera and specific IgE to ω-gliadin detected in 2.5% of the tested sera from baker's asthma.[4] In Sandiford's study of baker's asthma, specific IgE to α-gliadin and to total glutenins are detected in all sera and IgE to β-, γ-, fast ω-, and slow ω-gliadin were present in lower numbers of sera.[24] For food allergy to wheat, detection of IgE to ω -5 gliadin seemed to be associated with responsiveness to the challenge test and was particularly useful in infants with a suspicion of wheat allergy.[25]

Salt insoluble wheat protein fractions contains gliadin and glutenin. There was only a few studies for evaluation of significance of glutenin. A 42-kDa LMW glutenin subunit was described as a major allergen in patients with gastrointestinal symptoms after wheat ingestion. More recent studies suggested a role for HMW glutenin subunits in WDEIA.[9]

Testing of IgE reactivity in patients with different clinical profiles of wheat allergy (food, WDEIA, baker's asthma) to salt-soluble and salt-insoluble protein

fractions from wheat flour revealed a high degree of heterogeneity among recognized allergens in groups with different clinical profiles, as well as within each group.[3] However, mainly salt-soluble proteins seem to be associated with baker's asthma, and prolamins with WDEIA, whereas both protein fractions reacted to IgE from food-allergic patients.[3] As preliminary study, the IgE reactivity of both salt soluble fraction and salt insoluble fraction of wheat were evaluated by immunoblotting with pooled sera of children with wheat allergy. We could find widely ranged distribution of specific IgE- protein bands in both salt soluble fraction and salt insoluble fraction of wheat. These results correspond well with those of the earlier study which reported that both protein fractions reacted to IgE from food-allergic patients.

Current in vitro test reagents for the diagnosis of wheat allergy mainly contain water-soluble wheat protein and a small amount of gluten, so there are some limitations to diagnose.[3] To reveal the significance of IgE reactivity to salt

insoluble fraction of wheat, I tried to gather so-called ‘false-negative patients’ who showed immediate reaction after ingestion of wheat, but had negative results in wheat ImmunoCAP test. Previous study showed the existence of ‘false-negative patients’,[25] but I failed to gather these patients in this study. However, the results of this report revealed that all patients with wheat allergy showed IgE reactivity to gliadin, and there was some patients who showed very low wheat specific IgE levels in ImmunoCAP test and positive results in oral food challenge. These results support the hypothesis that gliadin is significantly important allergen among wheat protein fractions and could be the cause of ‘false negative patients’ when wheat allergy patients were tested with wheat ImmunoCAP test. However, the water/salt insoluble proteins share cross-reacting epitopes with water/salt soluble proteins.[24] Individuals who are hypersensitive to water/salt soluble wheat proteins produce specific IgE to water/salt insoluble wheat proteins.[24] So, all patients of wheat allergy in this

study showed positive results in both wheat specific IgE with ImmunoCAP test and gliadin-IgE reactivity with immunoblotting and ELISA.

Recently, ImmunoCAP test to ω-5 gliadins and gluten were developed and can be used commercially, but there are only a few institute that can evaluate ImmunoCAP test to ω-5 gliadins and gluten. The significance of ω-5 gliadins in wheat allergy was begun to be evaluated with developed new commercial test. But, in previous studies of baker's asthma, specific IgE to αβ -gliadin and γ-gliadin were also detected in patient's sera.[4, 23, 24] There are no commercial test which measure IgE to whole gliadin. Prolamin allergens are identified from 11 to 90 kDa by immunoblotting[18] and αβ –gliadin, γ-gliadin, ω1,2- gliadin. ω5-gliadin are usually detected in 25 - 45kDa, 36-45kDa, 39-42kDa, and 55-66.3kDa, respectively. When constitutive proteins of gliadin were separated by SDS-PAGE, in this report, protein bands ranging from 25 to 60 kDa were observed. IgE bindings between gliadin and pooled serum from wheat allergy

patients were observed through whole range of gliadins as well as range of ω-5 gliadin. These results support the hypothesis that whole gliadins are significantly important allergen as well as ω-5 gliadin. This is the first time that IgE reactivity to whole gliadines was shown in children with wheat allergy. All patients with wheat allergy showed IgE reactivity to gliadin and gliadin didn't show IgE binding to both pooled serum from negative control. So, IgE reactivity to gliadin seems to be specific with wheat allergy patients. Correlations between wheat specific IgE levels and gliadin specific IgE levels were also examined. In wheat IgE high group, wheat specific IgE levels in ImmunoCAP test were significantly correlated with gliadin specific IgE levels in ELISA. But, in wheat allergy patients, wheat specific IgE levels in ImmunoCAP test were not correlated with gliadin specific IgE levels in ELISA. So, wheat specific IgE levels in ImmunoCAP test didn't reflect IgE reactivity to gliadin. To compare the importance of IgE reactivity to gliadin between wheat

allergy patients and wheat IgE high group, we evaluated gliadin ratio (gliadin specific IgE/wheat specific IgE). Wheat allergy patients showed the tendency of higher gliadin ratio than wheat IgE high group. These results also support the hypothesis that gliadin is significantly important allergen among wheat protein fractions in wheat allergy. In front pages of this report, we already revealed that all patients with wheat allergy showed IgE reactivity to gliadin, and there was some patients who showed very low specific wheat IgE levels in ImmunoCAP and positive results in oral food challenge. For these several reasons, we suggest that the examination of IgE reactivity to whole gliadins were useful to diagnosis of wheat allergy.

Most of the allergens we inhale or ingest are glycosylated with oligosaccharides that are at least potentially immunogenic.[11] Despite this, the normal teaching and the bulk of the current evidence show that the IgE antibodies associated with allergic disease are specific for protein epitopes

whose structure is defined by the amino acid sequence and/or tertiary structure of a section of the protein.[11] Because identified IgE antibodies against carbohydrate epitopes seem to have limited clinical relevance[12, 26] and most research which focused on protein epitopes used recombinant molecules in which the oligosaccharides may not be the same as those on the natural molecules.[27, 28] But, it is not difficult to argue that oligosaccharides are immunogenic. The A and B antigens of red blood cells are excellent examples, but there are many others .[29, 30]

In general, prolamins from wheat, barley or rye are considered not to be glycosylated. However, several immunochemical studies did give some indication for glycosylation among gliadin, glutenin or hordein families.[31, 32] In α- gliadins, carbohydrates have been detected, but without covalent binding.[33] Some wheat proteins belonging to both the gliadin and glutenin families showed a reaction with anti-(xylose containing N-glycans) antibodies and so these

proteins are thought to be glycosylated.[34] In this study, we could find widely ranged distribution of gliadins and glycan were mostly overlapping with gliadins. So, we concluded that whole range of gliadins contains glycan.

Plant protein N-glycosylation has long been recognized as a common post-translational modification of proteins destined to the vacuole and the extra-cellular environment.[35] N-linked glycans are extremely important in proper protein folding in eukaryotic cells.[10] N-linked glycans also play an important role in cell-cell interactions.[10] Carbohydrate moieties of plant and invertebrate glycoproteins are very abundant environmental immune determinants. IgE in some human sera reacted with an antigen present in a large number of unrelated foods : porato, spinach, wheat, buckwheat, peanut, honey, and others.[12] The best recognized of the CCDs is MUXF3, which is present on many different plant proteins but was first defined on a protein (bromelain) derived from pineapple stem.[36] The antigen, which was periodate-sensitive and heat-stable, was also

found in pollen.[12] The IgE response to pollen allergens often includes IgE antibodies specific for glycosylation motifs on the pollen proteins.[11] Even more surprisingly, these antibodies often reacted in vitro with bee and vespid venom and were sometimes apparently induced by Hymenoptera stings. N-linked glycans in plant and Hymenoptera (e.g. wasps, bees and other stinging insects) allergens are commonly known as cross-reactive carbohydrate determinants (CCDs).[37] These oligosaccharides known as CCDs are present on many different species[11] and may induce extensive IgE cross-reactivity.[37] Specific IgE to CCDs was found to be present in up to 63% of patients attending an allergy clinic due to hymenoptera venom allergy and in up to 73% of subjects with allergy to 5 or more pollens.[37] Extensive evidence indicates that the stings of bees and other venomous insects can induce IgE antibody responses to CCDs that cross-react with plant glycoproteins.[38] It has also become clear that carbohydrates are a factor in parasitic diseases; nematode N-glycans and

glycolipids sometimes carry phosphorylcholine, a component also present on the surfaces of some pathogenic bacteria, and appear to be immunomodulatory.[39] In previous study, using the ELISA inhibition test, rice ELISA was inhibited by other cereals, such as barley, wheat and buckwheat, up to 75.7%, 33.3% and 18.4%, respectively and significant inhibitions were noted barley and wheat flour (66.2% and 59.1%).[40] But, the IgE from patients sensitized to wheat cross-react with γ3-hordein of barley due to sequence homology with wheat allergens rather than through shared carbohydrate determinants.[34]

IgE to CCDs were also found in patients with wheat specific IgE. In CCD sensitized patients, in vitro IgE cross-reactivity may cause false-positive specific IgE determinations and therefore, food challenge with CCD-containing allergens may not induce clinical symptoms in patients IgE sensitized to CCDs.[37] In this study, there were five children who showed no symptoms after

wheat ingestion even with high wheat specific IgE levels. In outpatient's clinic, we often met children who showed no symptoms after wheat ingestion with low wheat specific IgE levels. Among CCDs, most studies about CCDs used well-recognized CCDs, such as HRP and MUXF. Sander's study with baker's asthma showed 25% of bakers had IgE to CCDs such as HRP and 25% of bakers had IgE to another CCDs such as MUXF.[4] Thirty percent patients of baker's asthma had IgE to at least one CCD (HRP and MUXF).[4] Sander's study also showed eight of ten patient's who had IgE to wheat and mild asthma reacted exclusively to CCDs.[4] Another study also revealed that patients allergic to a water-soluble fraction from wheat flour were sensitive to pineapple enzyme, bromelain.[41] In this study, we didn't examine IgE to either HRP or MUXF. However, IgE to CCDs such as HRP and MUXF could be reasons of false-positive patients who showed no symptoms after wheat ingestion even with positive wheat specific IgE levels.

Seven N-linked glycosylation sites were found in wheat protein. Three of these sites were dominated by variant forms of the XylMan3FucGlcNAc2, i.e. the HRP-type of glycan. Complex-type glycans with one or two additional GlcNAc were observed, however in trace amounts only.[35] At four sites the glycan consisted of a single GlcNAc residue.[35] Glycan type appears to be site-specific indicating that N-glycan processing depends on the surrounding peptide sequence or protein structure.[35] In this study, we focused glycan in gliadin and it was revealed high mannose N-glycan chains. We didn't know whether glycan in gliadin was same with one of well-recognized CCDs or not. However, immunologic significance of glycan in gliadin seemed to be different with that of well-recognized CCDs.

IgE antibodies to plant-derived cross-reactive carbohydrate determinants usually seem to have only minor clinical significance. The IgE binding seems to lack specificity.[12] Digestion and absorption of carbohydrates is generally rapid,

and most of the glycosylation on proteins or lipids is thought to be cleaved off the parent molecules.[42] However, it is important to remember that the relationship to the parent molecule may alter the antigenicity of the sugars, just as the presence of an oligosaccharide may alter the antigenicity of the associated peptide.[43, 44] In this study, IgE bindings between gliadin and sera from wheat allergy patients were observed through whole range of gliadins and those range were mostly overlapping with the ranges of glycan. So, we thought glycan could be the possible epitope of gliadin.

Recently, researchers have become aware of an oligosaccharide that is common to all mammals, except the higher apes, and that can be the target for IgE antibodies.[14, 45] Unlike IgE antibodies to plant-derived cross-reactive carbohydrate determinants which have not been related to anaphylaxis, they are related with anaphylaxis and are the cause of two novel forms of anaphylaxis in the southeastern United States. One was reactions during the first infusion of the

monoclonal antibody cetuximab and the other was adult-onset delayed anaphylaxis to red meat.[11] The IgE antibodies against cetuximab were shown to be specific for an oligosaccharide, α-gal, which is present on the Fab portion of the cetuximab heavy chain. [11, 14] Adult-onset delayed anaphylaxis to red meat is also severe reaction related to IgE antibodies to the carbohydrate epitope α-gal.[15] It has recently been reported that some patients with cat allergy have IgE antibodies that bind to a carbohydrate epitope on cat IgA, a major component of cat epithelium–derived allergy extracts.[46]

The cross reactivity between α-gal and CCDs were evaluated in previous study.[15] Screening of sera from the 24 patients with IgE antibodies to α-gal revealed that only 3 of the 24 had cross-reactivity to bromelain, which contains both xylose and core-3-linked fucose.[15] Moreover, sera with high titer-specific IgE antibodies to bromelain did not contain IgE antibodies to α-gal.[15] We didn't evaluate α-gal in this study. So, we couldn't demonstrate the cross-reactivity of

glycan in gliadin and α-gal.

To evaluate IgE reactivity to glycan of gliadin, we compared IgE binding between gliadin and deglycosylated gliadin with immunoblotting and ELISA. After glycan removal by $NaIO_4$ treatment, intensity of bands in immunoblotting were decreased in both wheat allergy patients and wheat IgE high group. With ELISA, deglycosylated gliadin were significantly less recognized by the IgE antibodies from both wheat allergy patients and wheat IgE high group than gliadin. Our results supported the view that glycan of gliadin might involve the allergenicity of gliadin whether patients with positive wheat specific IgE had symptoms of wheat allergy or not. Then, we intended to evaluate the difference of the significance of glycan in gliadin depending on the symptoms after wheat ingestion in patients. So, we compared allergenicity of glycan portion in gliadin between wheat allergy patients and wheat IgE high group. In gliadin, IgE bindings of glycan portion were larger in wheat allergy patients than wheat IgE

high group. So, we could conclude that glycan of gliadin had more significant allergenicity in wheat allergy patients.

Further research will reveal the exact structure of glycan in gliadin and compare with the structure of well-recognized CCDs. We will try to reveal the reason of difference of allergenicity between glycan in gliadin and well-recognized CCDs.

V. CONCLUSIONS

The purpose of this study is to evaluate the allergenicity of gliadin to wheat allergy and to investigate the allergenicity of glycan which included in gliadin as carbohydrate epitopes in wheat allergy patients. In this study, we revealed that gliadin IgE was detected in all patients with wheat allergy and not correlated with wheat specific IgE. And there were some patients who showed very low specific wheat IgE levels in ImmunoCAP test and positive results in oral food challenge. For these several reasons, we suggest that the examination of IgE reactivity to whole gliadins were useful to diagnosis of wheat allergy. The range of glycan was almost overlapping with whole gliadin bands. So, we revealed that almost all gliadins might be glycosylated. Deglycosylation of gliadin reduced allergenicity of gliadin. In gliadin, allergenicity of glycan portion was larger in wheat allergy patients than wheat IgE high group. So, we could conclude that glycan of gliadin had more significant allergenicity in

wheat allergy patients.

We concluded that N-glycan of gliadin might have allergenicity as possible carbohydrate epitope in wheat allergy children.

Further research will reveal the exact structure of glycan in gliadin and compare with the structure of well-recognized CCDs. We will try to reveal the reason of difference of allergenicity between glycan in gliadin and well-recognized CCDs.

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ABSTRACT (IN KOREAN)

밀 알레르기에서 당단백 항원의 탄수화물 IgE 반응성

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목 적: 밀단백에는 용해도에 따른 다양한 단백분획이 포함되는데, 현재 상용되는 검사의 밀단백은 이들을 골고루 포함하지 못한다. 소아의 밀 알레르기에서 이들 밀 단백 중 전체 gliadin의 역할은 아직 규명된 바 없다. Cross-reactive carbohydrate determinants와

galactose alpha-1,3-galactose 같은 당단백의 탄수화물들은 IgE 반응성을 가지나 임상적인 중요도는 다양하다. 밀 알레르기 소아에서 gliadin의 glycan의 IgE 반응성에 대해서는 아직 연구된 바 없다. 본 연구에서는 밀 알레르기에서 gliadin의 항원성을 규명하고 gliadin의 glycan의 IgE 반응성을 알아보고 탄수화물 항원결정기(epitope)로써의 가능성에 대해 밝히고자 한다.

방 법: 52명의 소아에게 총 IgE 농도, 밀 특이 IgE, 자세한 병력청취, 식품경구유발시험을 시행하고 밀알레르기 환아군, 밀 IgE 증가군, 밀 IgE 양성대조군, 음성대조군으로 나눈다. Gliadin의 단백분획을 SDS-PAGE로 분석하고, gliadin내의 glycan을 분석한다. Immunoblotting과 ELISA로 gliadin과 glycan을 제거한 gliadin의 IgE 반응성을 측정한다.

결 과: 모든 밀 알레르기 환아에서 gliadin specific IgE 가 측정되었고, wheat specific IgE의 농도와 유의한 상관관계가 없었다. Gliadin

단백띠의 전영역과 glycan이 검출되는 영역이 거의 일치하였다. Gliadin에서 glycan을 제거하면 항원성이 감소하였다. Gliadin에서 glycan 부분의 항원성은 밀 IgE 증가군 보다 밀알레르기 환아에서 더 높았다.

결 론: 전체 gliadin에 대한 IgE 측정은 밀 알레르기 진단에 유용하다. 거의 모든 gliadin은 당화되어 있으며, gliadin의 N-glycan은 밀 알레르기 환아에서 탄수화물 항원결정기로서의 항원성을 가지는 것으로 추측된다.

핵심되는 말: 탄수화물 항원결정기(epitope), cross-reactive carbohydrate determinants, 식품 알레르기, galactose alpha-1,3-galactose, gliadin, glycan, 식품 경구유발시험, 밀 알레르기