

Short communication

Sensitivity and specificity of recombinant ω -5 gliadin-specific IgE measurement for the diagnosis of wheat-dependent exercise-induced anaphylaxis

Background: A recent study has shown that the measurement of specific IgE antibodies to B-cell epitope peptides of wheat ω -5 gliadin (Pep A) and high molecular weight glutenin subunit (Pep B) are useful to diagnose wheat-dependent exercise-induced anaphylaxis (WDEIA).

Aims of the study: We sought to compare the sensitivity and specificity of the *in vitro* tests for measuring the specific IgE antibodies to recombinant ω -5 gliadin (*r* ω -5 gliadin) with those for wheat, gluten, Pep A, and Pep B in identification of patients with WDEIA.

Methods: Fifty patients with WDEIA, 25 healthy subjects and 25 patients with atopic dermatitis with specific IgE antibodies to wheat but without experience of allergic reactions after ingestion of wheat products were enrolled in this study. The concentrations of specific IgE antibodies were measured using ImmunoCAPTM. The empirical receiver operating characteristics curves (ROC) for each test were prepared and the areas under the ROC curve (*AUC*) were compared.

Results: In patients with WDEIA, the sensitivities of the allergen-specific IgE tests for wheat, gluten, Pep A, Pep B and *r* ω -5 gliadin were 48%, 56%, 76%, 22%, and 80%, respectively. The seven of 10 WDEIA patients with no specific IgE antibodies to *r* ω -5 gliadin had specific IgE antibodies to Pep B. The highest *AUC* (0.850) was observed in the test for *r* ω -5 gliadin.

Conclusions: Measuring the concentration of specific IgE antibodies to *r* ω -5 gliadin is more useful than to wheat, gluten, or Pep A in the identification of patients with WDEIA.

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Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a severe form of allergy in which ingestion of wheat products before physical exercise induces anaphylactic symptoms, such as hypotension, dyspnea and generalized urticaria (1). Patients with WDEIA usually have IgE antibodies to proteins from wheat flour and the symptoms are induced by type I allergic reaction (2). The mechanism of eliciting anaphylactic symptoms by exercise in WDEIA is speculated that exercise facilitates allergen absorption into the blood from the gastrointestinal tract (3, 4). The most reliable treatment therapies for WDEIA are strict elimination of wheat products and/or a limitation of exercise after wheat ingestion. Therefore, it is important to

make a definitive diagnosis by a provocation test with ingestion of wheat followed by exercise. However, this provocation test possesses a risk because severe allergic reactions, such as anaphylactic shock, could be induced during the test. Sampson et al. (5) have shown the usefulness of measuring serum concentrations of food-specific IgE in predicting clinical reactivity for egg, cow's milk, peanut, and fish allergies. For serological diagnosis of wheat allergy including WDEIA, specific IgE to aqueous wheat proteins and wheat glutens, which are the water/salt insoluble wheat proteins, can be measured with commercially available tests. However, these tests are unsatisfactory for identification of patients with WDEIA because of their low sensitivity and specificity (6). Palosuo et al. (7) first reported that wheat ω -5 gliadin is a major allergen in WDEIA. We have clarified that approximately 80% of Japanese patients with WDEIA have specific IgE to wheat ω -5 gliadin and the rest of them have specific IgE to high molecular weight glutenin subunit (HMW-glutenin). Moreover, we have shown that the determination of

Abbreviations: *AUC*, area under the receiver operating characteristic curve; HMW-glutenin, high molecular weight glutenin subunit; Pep A, IgE-binding epitope peptide from ω -5 gliadin; Pep B, IgE-binding epitope peptide from high molecular weight glutenin subunit; *r* ω -5 gliadin, recombinant ω -5 gliadin; ROC, receiver operating characteristic curve; WDEIA, wheat-dependent exercise-induced anaphylaxis.

specific IgE to synthetic IgE-binding epitope peptides of ω -5 gliadin (Pep A) and HMW-glutenin (PepB) is highly useful to identify patients with WDEIA (8). Recently, we have produced a recombinant protein from the C-terminal half of ω -5 gliadin in *Escherichia coli* and demonstrated that the recombinant protein has sufficient ability of IgE-binding to detect the specific IgE to ω -5 gliadin in the sera of patients with WDEIA (9). In this study, we compared the sensitivity and specificity of the ω -5 gliadin-specific IgE test with those for wheat, gluten, Pep A, and Pep B in identification of patients with WDEIA.

Material and methods

Subjects and provocation test

The present study has been approved by the ethics committee of Shimane University Faculty of Medicine. Written informed consent was obtained from all the patients. Fifty patients with WDEIA who had recurrent episodes of allergic reactions, such as generalized urticaria, erythema, nausea, dyspnea and anaphylactic shock, induced by exercise after wheat ingestion were enrolled in this study. Thirty of them had been reported in a previous study (8). In 41 of the patients, a provocation test was used for the diagnosis but in nine patients, there were no provocation test performed because of the risk of eliciting severe anaphylactic reactions. The provocation tests, which include combinations of wheat ingestion, exercise and aspirin intake were performed as described previously (3). The type of wheat product, e.g. noodles, bread, and pasta, and the amount, 60–135 g as wheat flour, used in the provocation tests were decided based on clinical history. Treadmill exercise (Bruce protocol) was carried out 20–30 min after food intake. In those cases where the patients showed no symptoms after wheat ingestion followed by exercise, an administration of 500 mg of aspirin before wheat ingestion was added to the wheat and exercise-challenge protocol. A positive response to challenge was defined as the reproduction of allergic symptoms, such as urticaria, erythema, dyspnea, pruritus, nausea and anaphylactic shock. Twenty-five healthy subjects served as controls. Moreover, to evaluate the specificity of the ω -5 gliadin-specific IgE tests, we selected 25 patients with atopic dermatitis who had positive results with wheat-specific IgE test whereas they had

Table 1. Characteristics of the subjects

	WDEIA (n = 50)	AD* (n = 25)	Healthy controls (n = 25)
Sex			
Male	29	14	15
Female	21	11	10
Mean age (years, range)	30.7 (10–75)	24.8 (5–49)	33.4 (18–55)
Mean serum total IgE (IU/ml, range)	782 (22–14, 723)	7991 (278–21, 699)	154 (7.2–600)

*Patients with atopic dermatitis (AD) had wheat-specific IgE antibodies in their sera whereas they had no immediate allergic reactions to wheat.

experienced no episode of allergic reaction after ingestion of wheat products. The characteristics of the subjects are summarized in Table 1. Whole blood was obtained from the patients and serum was isolated and stored at -80°C until use.

Measurement of specific IgE

The ω -5 gliadin, Pep A (KPQQQSPQQQFPQQQIPQQQ) and Pep B (PTSPQQSGGQQPGQGQQ) were prepared as previously described (8, 9). Specific IgE antibodies for ω -5 gliadin, wheat, gluten, Pep A and Pep B in the sera were determined by ImmunoCAPTM according to the manufacturer's instructions (Phadia, Uppsala, Sweden).

Receiver operating characteristic curve (ROC) analysis

The empirical ROC curves were prepared and analyzed using data from patients with WDEIA and atopic dermatitis. In addition, an optimization of cut-off value was made by plotting sensitivity and specificity for antigen-specific IgE values, and choosing the cut off value as the point where both measures gave the best results.

Results

The concentration of wheat-, gluten-, Pep A-, Pep B- and ω -5 gliadin-specific IgE antibodies in the sera of subjects

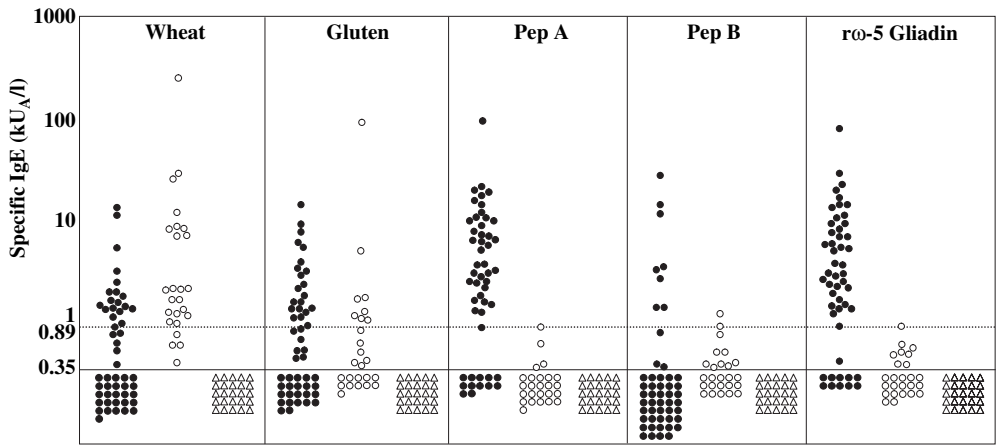


Figure 1. Concentration of specific IgE antibodies to wheat, gluten, Pep A, Pep B and ω -5 gliadin. Closed circles, open circles and open triangles represent patients with WDEIA, atopic dermatitis patients and healthy controls, respectively.

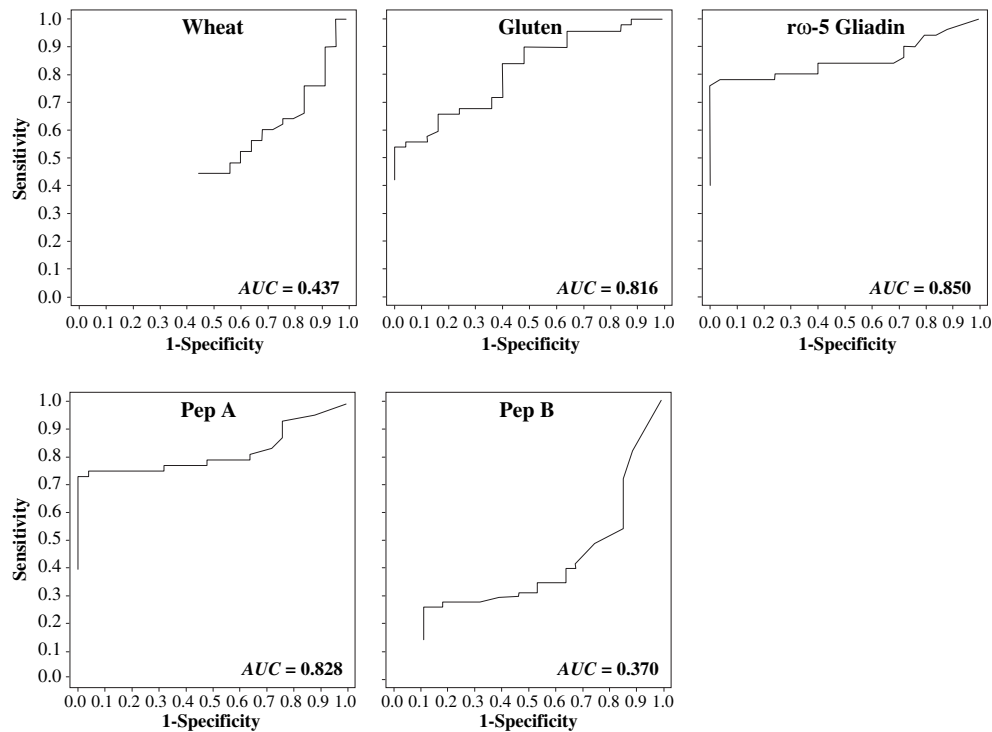


Figure 2. ROC curves for specific IgE test to wheat, gluten, Pep A, Pep B and ω -5 gliadin. AUCs were calculated to evaluate the performance of the tests.

are shown in Fig. 1. When a cut-off value in the test was set at 0.35 kU_A/l, no normal controls were positive in any of the tests. In the patients with WDEIA, the sensitivities of the test for wheat-, gluten-, Pep A-, Pep B- and ω -5 gliadin-specific IgE were 48%, 56%, 76%, 22%, and 80%, respectively. The specificity of each test was calculated using the data from the 25 patients with atopic dermatitis who had specific IgE to wheat and the calculated specificity were 44%, 84%, 60%, and 68% for gluten, Pep A, Pep B, and ω -5 gliadin, respectively. In addition, seven of 10 patients with WDEIA, who had no specific IgE antibodies (below 0.35 kU_A/l) to either ω -5 gliadin or Pep A, had specific IgE antibodies to Pep B (range from 0.38 to 11.4 kU_A/l). The remaining three patients with WDEIA did not have specific IgE to ω -5 gliadin, Pep A or Pep B.

To evaluate the diagnostic ability of the different *in vitro* tests in the diagnosis of WDEIA, ROC analysis for each *in vitro* test were performed. The areas under the curve (AUCs) received from the ROC analysis for each test showed the highest value for the ω -5 gliadin test (0.85) as against 0.828 for Pep A, 0.370 for Pep B, 0.816 for gluten, and 0.437 for wheat (Fig. 2). This procedure was used to obtain a cut-off value, 0.89 kU_A/l, giving the maximum efficiency of the ω -5 gliadin test in the diagnosis of WDEIA with a sensitivity of 78% and a specificity of 96%.

Discussion

The results of our study demonstrate that the sensitivity of the *in vitro* test measuring specific IgE antibodies to ω -5 gliadin is the highest in the identification of patients with WDEIA. Previously we reported that determination of specific IgE antibodies to Pep A, which is composed of the major IgE-binding epitope sequences from ω -5 gliadin, is useful in diagnosis of WDEIA. The specificity of the test for Pep A (84%) that was calculated, when comparing with the data from atopic dermatitis patients without immediate wheat allergy, was higher than that for the ω -5 gliadin test (68%). However, the AUC received from ROC analysis for ω -5 gliadin (0.850) was higher than that for Pep A (0.828), which strongly suggests that the diagnostic ability in measuring specific IgE antibodies to ω -5 gliadin is higher than that in Pep A when used in the diagnosis of WDEIA. The Pep A contains the major IgE-binding epitopes, QQIPQQQ, QQFPQQQ, and QQSPQQQ, in contrast to the ω -5 gliadin that, in addition, contain the minor IgE-binding epitopes, QQLPQQQ, QQYPQQQ, QQSPEQQ, PYPP, YQQYPQQ, QQPPQQ, and QQFHQQQ, previously identified (7, 8). Thus, such minor IgE-binding epitopes may also contribute to elicit allergic symptoms in some patients with WDEIA.

When using a cut-off value of 0.35 kU_A/l in the ω -5 gliadin specific IgE test, the sensitivity was 80% (40/50) and thus it seemed somewhat low for applying the test in the diagnosis of WDEIA. However, seven of the 10 patients who had no specific IgE antibodies to ω -5 gliadin had specific IgE antibodies to HMW-glutenin epitope peptide (Pep B). As HMW-glutenin is the second major allergen in WDEIA, following the ω -5 gliadin (8), the additional measurement of specific IgE antibodies to HMW-glutenin (Pep B) seemed to be essential in the diagnosis of WDEIA. The three WDEIA patients with no specific IgE antibodies to ω -5 gliadin, Pep A or Pep B suggest that there are other important wheat allergens involved in WDEIA. In fact, several gliadins in wheat, such as α -gliadin, β -gliadin, γ -gliadin, ω -1,2 gliadin, are considered to be allergens in WDEIA (7, 10–12).

Sampson et al. have shown a direct correlation between the food-specific IgE level and the probability of having clinical symptoms (13). The prediction of appearance of anaphylactic symptoms is very important to conduct safe provocation tests. In this study, we estimated that the cut-off value giving maximal efficiency in the diagnosis of WDEIA is 0.89 kU_A/l. Under this condition, 40 of 100 tested subjects had a positive result and 39 of these 40 subjects (97.5%) were diagnosed as

suffering from WDEIA. It is, therefore, considered that the determination of the concentration of specific IgE antibodies to ω -5 gliadin may be a good tool to predict a clinical reaction in the provocation test and thus unsafe provocation tests can be avoided in the diagnosis for WDEIA.

Specific IgE antibodies to ω -5 gliadin are detected not only in patients with WDEIA but also in children with immediate wheat allergy (14, 15) suggesting that production of ω -5 gliadin-specific IgE antibodies is not specific only for patients suffering from WDEIA. Furthermore, the results lead to the hypothesis that ω -5 gliadin is an allergen with the ability to induce relatively severe allergic symptoms, such as anaphylactic shock, but different pathogenic mechanisms may underlie the induction of the symptoms in WDEIA and common wheat allergy. However, additional studies are required to elucidate this hypothesis. In conclusion, the findings in this study suggest that measurement of the concentration of specific IgE antibodies to ω -5 gliadin is highly useful in the identification of patients with WDEIA and predicting a positive outcome in a provocation test. Moreover, the measurement of specific IgE antibodies to HMW-glutenin in addition to ω -5 gliadin improves the sensitivity of the *in vitro* test in the identification of WDEIA patients.

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