

Meeting Report of the Special Committee for the Safety of Protein Hydrolysates in Cosmetics

6th Meeting

Date and Time: 11:00-15:30, October 8, 2012

Venue: Meeting Room of the Japanese Society of Allergology Office

Attendee: 16 committee members including President, 2 observers

Kayoko Matsunaga (Chair), Michiko Aihara, Yoshiro Ikezawa, Yasuharu Itagaki, Reiko Kishikawa, Mitsuru Sawa, Shinichi Sugiura, Hiroyuki Tanaka, Yuko Chinuki, Reiko Teshima, Michihiro Hide, Atsuki Fukushima, Yuma Fukutomi, Eishin Morita, Akiko Yagami, Kazuo Akiyama (President of the Japanese Society of Allergology)

Absentee: Atsuo Urisu (presence by proxy), Zenichiro Kato

Observer: Masashi Nakamura (Fujita Health University)

Kazumi Sasaki (National Institute of Technology and Evaluation)

Minutes:

Session 1- Progress of antigen analysis and animal experiment was reported by the following members.

1. Dr. Teshima

Study on immunological reaction to quasi drug/cosmetics components using animal model: As to commercially available hydrolyzed wheat products and acid hydrolyzed gluten contained in cosmetics, percutaneous sensitizing potency was tested using mice. Comparing the result with that of Glupearl 19S revealed high sensitizing potency of HWP-1 that has high molecular weight component as is the case with Glupearl 19S. As to percutaneous sensitizing potency of heat- and various time-treated acid- hydrolyzed gluten(acid-HGs), it was highest when hydrolyzed for 0.5 hour and it went weaker as hydrolysis duration got longer.

Analysis of serum IgE response of patients sensitized to soap containing acid-hydrolyzed wheat: In the EXiLE (IgE Cross linking-induced Luciferase Expression) test, IgE of patients sensitized to old "Cha no Shizuku" soap products showed strong reaction to Glupearl 19S and acid-HGs hydrolyzed for 0.5 to 12 hours under the conditions of 0.1N of HCL at 100°C. It is estimated that some kind of new epitope had been generated through hydrolysis. EXiLE test response in HW ("Cha no Shizuku"-HWP sensitized patients) group was distinctively differentiated from CW (children with food allergy) or AW (adults with WDEIA) group. Detection sensitivity was also higher than Western blotting or dot blotting and EXiLE test is considered to be a useful method.

Dr. Sawa (ophthalmologist) suggested that antigen specific secretory IgA of mucous membranes of eyes (mucosa-associated lymphoid tissue) be studied as periorbital edema is expressed due to wheat absorbed through intestine. He referred to the assay to determine eosinophil found at mouse conjunctiva.

2. Dr. Itagaki

In the ongoing antigen analysis, research is focused on deamidation conducted to improve gluten's solubility, foaming and other properties.

The result is going to be reported at the next meeting.

3. Dr. Tanaka

To inspect if food allergy symptoms develop when percutaneously sensitized animal model is provided with antigen, study is going on concerning the following: (1) setting of optimum percutaneous sensitization protocol; (2) effect of oral antigen challenge on mice having systemically sensitized to hydrolyzed wheat; (3) effect of oral antigen challenge on mice having percutaneously sensitized to hydrolyzed wheat.

Marked influence was not observed in both body temperature change and fatality rate, which presumably resulted from (1) percutaneous sensitization and (2) wheat component elimination from feed. As the total IgE increase was equal to or higher than that observed in intra-abdominal sensitization, the influence of (2) is suspected.

Feed containing wheat is going to be used next time to study sensitization by intermediates.

4. Dr. Kato (document presented)

- 1) Gene analysis: Analyzing HLA gene by means of PCR Luminex
- 2) Treatment method development: Establishing treatment method for allergy induced by specific hydrolysate based on HLA steric structure.

Session 2- Data collected up to September 30 via doctor registration site - reported by Dr. Matsunaga

Breakdown of symptoms in 705 positive cases (out of 1451 positive cases composed of 1389 females and 62 males, aged 45.2 in average)

Allergic symptom expression: During/after face washing and after wheat intake: 523(67%)

After wheat intake (no symptom during/after face washing): 177(30%)

During face washing (no symptom after wheat intake): 5(1%)

Symptoms after face washing: No symptom: 190(27%)

Swelling of eyelids and periorbital area: 257(36%)

Urticaria/itching/redness: 231(33%)

Uncertain: 27(4%)

Symptoms after wheat intake: Anaphylactic shock: 178(25%)

Dyspnea/vomiting/diarrhoea: 190(27%)

Others (urticarial, periorbital swelling, nasal congestion, running nose, itching, redness, etc.): 318(45%)

No symptom: 5(1%)

Uncertain: 14(2%)

Previous history of allergy: No history: 290(41%)

Previous history: 380(54%) (Pollinosis/allergic rhinitis: 279(40%); Atopic dermatitis: 39 (6%); Urticaria: 24 (3%); Others: 38 (5%))

Breakdown of symptoms in 214 negative cases :

Symptoms after face washing: No symptoms: 3 (1%); Periorbital swelling: 85 (40%);

Urticaria/itching/redness/others: 60 (28%); Uncertain: 66 (31%)。

Symptoms after wheat intake : Uncertain: 153 (71%); No symptom: 15 (7%);

Urticaria/periorbital swelling/nasal congestion/running nose/itching/redness/others: 30 (14%);

Dyspnea/vomiting/ diarrhea: 14 (7%); Anaphylactic shock: 2(1%)

Previous history of allergy : No history: 75 (35%); Previous history: 126 (59%);

Pollinosis/allergic rhinitis: 88 (41%); Atopic dermatitis: 5 (3%);

Urticaria: 9 (4%); Others: 24 (11%)

Session 3- Case review reported by clinical doctor members

1. Dr. Kishikawa from Fukuoka National Hospital

As a result of skin test conducted on 232 patients who came to the hospital from 2008 to September, 2012, 137 tested positive and 95 tested negative. (Out of these 95 negative cases, 73 tested negative despite the presence of actual symptoms, 3 were just worried although they had no symptoms, and 19 were diagnosed with other diseases.)

The report included 4 skin test negative cases where challenge test was conducted, out of which 2 patients tested positive and 2 negative (dust mite allergy and bronchiectasis cases), 3 atypical cases and ELISA test performance.

48-year-old female: SPT test result was positive and ELISA concerning Glupearl 19S was negative → Food challenge test result was negative and challenge test with 500 mg aspirin was negative, and the patient's clinical course is now being followed up.

As to cases where more than one year have passed after examined at our hospital, controlled intake is more prevalent than totally refraining from eating wheat-containing food, which is employed by patients with serious symptoms in fear of intake by mistake. There are cases where disorders have remitted through intake avoidance and regular medication.

2. Dr. Chinuki and Dr. Morita (Shimane University)

Diagnosis of 43 users of "Cha no Shizuku" examined at the university

Positive cases (34): Patients without wheat allergy treatment history: 17

 Patient recovered from wheat allergy: 8

 Patients who are not followed up due to geographical or other reasons: 9

Negative cases (5): Despite clinical symptoms present, both the SPT and serum diagnosis results were negative (3 cases were also judged negative in the basophil activation test).

Cases without clinical symptoms (patients were just worried over nothing): 2

Cases diagnosed with other diseases based on the clinical symptoms: 2

It is under consideration if basophil activation test can be utilized as remission indication.

3. Dr. Hide (Hiroshima University)

3 individuals completely recovered from clinical symptoms, out of 98 patients diagnosed as typical cases.

→ In 2 cases, no Glupearl antibody was found in HRT and ELISA. In one case, the patient was judged to have recovered although HRT/ELISA results were positive.

Many cases showed negative results in HRT/CAP-RAST.

Out of 60 cases for which ELISA to detect Glupearl antibody was performed, 14 showed negative results afterward.

Out of 28 cases for which HRT to detect Glupearl antibody was performed at least 2 times (excluding cases of low responder), 4 cases were confirmed negative.

4. Mr. Nakamura (Fujita Health University)

ELISA results as to 878 test bodies submitted from 179 institutions nationwide

Though SPT and ELISA results were highly correlated with each other, there were a few exceptional cases. Even typical cases had variations.

- Typical case : Positive in Both SPT and ELISA (330 cases)

In many cases, "gluten sIgE > wheat sIgE". In some cases, "wheat sIgE > gluten sIgE". When patients tested positive for $\omega 5$, the sIgE levels against wheat, gluten and Glupearl 19S were high.

- Atypical case 1 : Positive in SPT; negative in ELISA (99 cases)

Most patients tested negative for sIgE antibody to wheat and gluten. When sIgE was detected, "gluten sIgE < wheat sIgE" in relatively many cases. Change into negative conditions may have been obtained over time.

- Atypical case 2 : Negative in SPT; positive in ELISA (12 cases, out of which 3 cases overlap)

Many cases were difficult to diagnose by means of SPT due to internal use of anti-allergic medicine, mechanical urticaria, small wheal, being positive for "Cha no Shizuku" soap but negative for Glupearl 19S, and other reasons.

- Others

Cases with high ELISA values despite small amount of soap cakes used - report by Dr. Yagami at Fujita Health University.

It was proposed to discuss how to handle patients who report to other hospitals, the Japanese Society of Allergology and MHLW, insisting that their disorders result from "Cha no Shizuku" usage although negative results of SPT/ELISA reveals that their symptoms are irrelevant to "Cha no Shizuku" soap.

Because challenge test was refused, there are not enough data to judge if they are "Cha no Shizuku"-induced cases or not.

5. Dr. Aihara (Yokohama City University)

1) Clinical courses and prognoses of typical cases

The following are the clinical courses of 21 patients who were diagnosed with immediate wheat allergy induced by hydrolyzed wheat (Glupearl 19S) as of September, 2012

Change in clinical symptoms

- 13 patients (62%) continued to eat wheat-containing foods and 9 persons developed allergic symptoms. Individuals without allergic symptoms had wheat-containing foods avoiding exercise or NSAIDs (nonsteroidal anti-inflammatory drugs).
- 3 persons developed allergic symptoms by eating foods containing wheat or hydrolyzed wheat without knowing.

Change in test results

- Out of 16 cases where wheat, gluten and ω -5 gliadin-specific IgE's could be determined over time, every level declined over 6 months to 1 year in 15 cases, but such declining trend could not be observed in one case.
- Glupearl 19S-specific IgE level decreased in 11 cases out of 14.
- In 4 cases, patients developed disorder by taking wheat-containing foods even after they tested negative for serum IgE antibody specific to wheat and gluten.
- In SPT performed on 4 cases, reaction grew weaker over time.

2) Atypical cases

Cases without eyelid edema when elicited

- Challenge test : After taking 20 g of wheat flour, wheal developed on the forearm.
- Though there is a report on contact urticaria expression after washing face with "Cha no Shizuku" soap, oral intake of wheat products mainly elicits disorders in digestive organs and wheals on trunk/four limbs, without facial symptoms.
- Wheat- and gluten-specific IgE levels became negative over time, but Glupearl 19S-specific IgE antibody level did not decline.

3) Cases which were not determined positive

- A. Cases where patients suspected wheat allergy caused by "Cha no Shizuku" soap or other products: 32
- B. Cases that had typical "Cha no Shizuku" related symptoms: 21
- C. Denied cases: 10 (Although these patients had suspected clinical symptoms, 5 were thought negative based on SPT and serodiagnosis results, and another 5 were diagnosed with other diseases.
- D. Case for which diagnosis could not be provided (test were not performed): 1

6. Dr. Fukutomi (National Hospital Organization Sagamihara National Hospital)

A. Parameter: 143

B. Certain cases: 90

C. Denied cases

- 1) Suspected clinical symptoms were observed, but SPT result was negative: 0
- 2) Upset over nothing: 5
- 3) Diagnosed with other diseases: 33
- 4) Delayed type reaction: 3
- 5) Not determinable: 2

Not a few patients suffer from allergic symptoms elicited by wheat intake (with no exercise) even after serum wheat- and gluten-specific IgE antibody level becomes too low to detect.

Session 4-General

In typical cases, stopping the applicable soap usage lowers the level of antibodies against wheat, gluten and Glupearl 19S, which allows most patients to eat wheat-containing foods without problems. But care must be taken as disorder may be repeated depending on exercise strength or physical conditions. Antibody level remains the same in some cases, and it takes time for the level to decline when the initial

value is high in other cases. If remission from wheat allergy is defined as conditions where no reaction is elicited after wheat intake followed by exercise, remission rate so far is about 3%. More study is required as to a few cases where eyelid edema develops despite no IgE antibody observed. It is necessary to evaluate the validity of challenge test in such cases. In atypical cases, other proteins may be responsible for the disorders, which must be studied further. Diagnostic criteria established by the Committee are applicable in examining patients, but not applicable to past anaphylaxis. How to note this should be considered.

The above mentioned matters are to be discussed in the next meeting scheduled for December 1 (12:10~13:10) in Osaka.