

Short communication

Three cases of childhood nocturnal asthma due to buckwheat allergy

Background: Buckwheat flour (BF) is known as a potent food allergen. Sensitization to it usually occurs by ingestion but also by inhalation in occupational or domestic exposure. Buckwheat chaff-stuffed pillows (BCP) can be contaminated with BF during the process of pilling, and many Korean children and adults use BCP for health reasons.

Methods and Results: We here present three cases of BF allergy in children using BCP, who had been treated as nonatopic asthmatics after undergoing the routine allergy skin tests and serologic tests. We took careful clinical histories, and performed skin prick tests (SPT), the radioimmunoassay (RIA) for specific IgE, the BCP-elimination test, the BF bronchial provocation test, and IgE Western blot. All three children showed positive skin reactions to BF, but none of them had positive reactions to house-dust mites. Nocturnal asthmatic symptoms were improved during 7 days of BCP elimination, and asthmatic responses were observed by bronchial provocation tests with homemade BF extract. Serum BF-specific IgE antibodies and several IgE-binding proteins were detected by RIA and Western blot analysis, respectively.

Conclusions: Thus, a small amount of BF attached to BCP can induce BF sensitization, and BCP should be considered a main cause of childhood nocturnal asthma in those asthmatics exposed to these pillows.

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Buckwheat flour (BF), a common foodstuff in Europe and Asia, is known as a highly potent food allergen. The sensitization to buckwheat usually occurs by ingestion, but also can occur by inhalation in occupational or domestic exposure to flour (1–8). In 1909, Smith described a young patient who showed severe manifestations of asthma, allergic rhinitis, urticaria, and angioedema after ingestion of a small amount of buckwheat, and the allergic symptoms were also induced by inhalation of BF (2). While there have been several related clinical reports on buckwheat allergy during the past 30 years, the lack of epidemiologic studies made it difficult to estimate the prevalence or incidence of allergic manifestations to buckwheat among the populations in different countries.

In South Korea, BF is an important food allergen in schoolchildren and adults, and the positive rate of skin test is about 5% (9). There are several case reports on buckwheat hypersensitivity in South Korea; two cases caused by the ingestion of noodles containing BF (*soba*) (10), one case caused by the inhalation of flour attached to buckwheat husk pillow (11), and one case of occupational asthma caused by the inhalation of BF in a noodle factory (12). In fact, it is unusual for young children to eat BF-containing foods in South Korea; however, many Korean children use buckwheat chaff-

stuffed pillows (BCP) as a traditional means to improve health and intelligence, particularly during infancy. For this reason, there is a possibility of sensitization to BF through the antigen attached to the buckwheat chaff. It may be a causative allergen as important as house-dust mites in infancy and young children. We here report a clinical and immunologic study on three cases of childhood nocturnal asthma, mainly due to BF sensitization by long-lasting BCP exposure.

Material and methods

Case reports

Patient E.J.H., a 5-year-old boy, had suffered every night from cough and wheeze after sleep for the last 3 years. He had twice undergone allergy skin tests and serologic tests for evaluation of allergy, but all test results were negative, because BF antigen was not included in the tests. Interestingly, he experienced symptom-free nights while staying at his grandparents' house, which was free of BCP. The nocturnal asthmatic symptoms appeared 6 months after BCP use. Patients Y.H.S. (a 7-year-old boy) and L.J.W. (an 8-year-old girl) also had been diagnosed and managed as having nonatopic asthma for 3 and 2 years, respectively, and their symptoms had been aggravated at night for 1 year. By careful history taking on the use of BCP, we confirmed that all three patients had been exposed to BCP for 2–6 years (E.J.H., 5 years; Y.H.S., 6 years; and L.J.W., 2 years). Accordingly, we performed SPT with 54 antigens, including BF (Bencard and Torii), and measured antigen-specific IgE antibodies to

Table 1. Results of skin prick test and serum antigen specific IgE and total IgE levels of subjects

Patient (sex/age)	Skin prick test* (mm)				Serum specific IgE (U/ml)			Serum total IgE (U/ml)
	Dp	Df	BF	Histamine	Dp	Df	BF	
E.J.H. (M/5 years)	4 × 2	3 × 3	8 × 5	6 × 3	<0.35	<0.35	95.7	299
Y.H.S. (M/7 years)	2 × 2	2 × 2	27 × 11	7 × 5	<0.35	<0.35	73.9	245
L.J.W. (F/8 years)	2 × 2	2 × 1	3 × 3	6 × 4	<0.35	<0.35	3.7	30

*Results show wheal size induced by skin prick test. Dp: *D. pteronyssinus*; Df: *D. farinae*; BF: buckwheat flour.

house-dust mites and BF by the AlaSTAT RIA method (DPC Co., Los Angeles, CA, USA). The results of the SPT and the RIA are shown in Table 1. All three patients showed positive skin reactions to BF, but were negative to the house-dust mites, *Dermatophagoides farinae* and *D. pteronyssinus*. We performed the BCP elimination test and the BF bronchial provocation test in three patients. IgE Western blots were done with the sera from patients and controls.

Preparation of BF extract

We obtained BF from the traditional market, and defatted it with ether. The defatted BF was soaked in 1:10 w/v in phosphate-buffered saline (PBS, pH 7.4), at 4°C for 24 h, and then centrifuged at 10 000 g at 4°C for 1 h. The supernatant was dialyzed against distilled water for 48 h (cutoff pore size with 3.5 kDa), and lyophilized at -70°C. The final protein content of crude extract was 220 µg/mg dry weight.

BCP elimination test and BF bronchial provocation test

To evaluate the effect of BCP elimination on the patients' nocturnal symptoms, BCP were removed from patients' bedding for 7 days. The presence of nocturnal symptoms (cough, wheeze, and awakening) were recorded for 14 days (7 days before and 7 days after BCP elimination). Finally, the numbers of symptom-free nights during each 7 tested days were counted. After 7 days of BCP elimination, BF bronchial provocation tests were carried out by a simple modification of the method of Chai et al. (13), with informed consent from the parents. Serial dilutions of homemade BF extract (1:10 000, 1:2000, and 1:1000 w/v in PBS) were used for provocation tests. With the DeVilbiss 646 nebulizer, each concentration of BF extract was inspired by the five-tidal-breaths method. FEV₁ or PEFR (for patient E.J.H.) was measured before and 10 min after the inhalation of each concentration. Inhalations were continued at 15-min intervals until there was a 20% fall or more from baseline FEV₁ or PEFR. The test which showed a 20% fall or more from baseline FEV₁ or PEFR was considered to show an immediate asthmatic response. Then FEV₁ or PEFR was measured every hour for 10 h for evaluation of the late asthmatic response.

IgE Western blot analysis

With the homemade BF extract, electrophoresis was performed by a modification of the method of Laemmli (14), with a Novex Mini-Cell system for 90 min at 18–30 mA. A wide-range standard of molecular weight (Mw) markers (4–250 kDa, Novex, San Diego, CA, USA) and BF solution were loaded onto a Novex precast Tris-Glycine gradient gel (4–20% acrylamide) for separation of antigen. Electrophoresis was performed by the method of Towbin et al. (15) for 90 min at 200 V with a Novex transfer apparatus. After transfer of separated proteins, the nitrocellulose membrane was treated with 0.5% Tris-3% bovine serum albumin solution for 4 h to block nonspecific protein binding. The diluted (1:20 v/v) sera from study patients, two positive controls (food-challenge-positive buckwheat-allergic patients), and two negative controls (buckwheat nonsensitive atopic children) were incubated with each cut of membrane for 16 h in a cold room. After washes with Tween 20

containing Tris-buffered saline (TBS-T), each membrane was incubated with 1:1000 v/v of alkaline phosphatase-conjugated antihuman IgE (Sigma, USA) for 2 h. For detection of IgE-binding bands, NBT/BCIP (Pierce, USA) was applied to each membrane for 4 min.

Results

BCP elimination test and BF bronchial provocation test

All three patients had dramatic improvement of nocturnal symptoms within 3 days after BCP elimination (Table 2). Symptoms, such as wheeze and awakening, disappeared from the first day of BCP elimination, and cough gradually disappeared during BCP elimination. With the BF bronchial provocation test, patients E.J.H. and Y.H.S. experienced dual asthmatic responses, and patient L.J.W. experienced an immediate asthmatic response (Table 2). Twenty percent or more decrements of FEV₁ or PEFR were observed at the concentration of 1:2000 in all three patients. Patient E.J.H. complained of persistent cough for 3 days after the provocation test.

IgE immunoblot analysis

More than 21 IgE-binding bands (ranging from 4 to 120 kDa) were identified by Western blot analysis (Fig. 1). On immunoblot with two sets of positive control sera obtained from patients with food-challenge-positive buckwheat allergy (lanes 1 and 2), the patterns of IgE immunoblot were very similar, and there were distinct bands with molecular masses of 120, 100, 98, 84, 79, 74, 70, 61, 59, 55, 53, 47, 45, 41, 36, 34, 31, 24, 22, 8–12, 6, and 4 kDa. On the two sets of immunoblot using the sera obtained from BCP-induced buckwheat-allergic patients (E.J.H., lane 3; Y.H.S., lane 4), there were several distinct IgE-binding bands with masses of 59, 53, 50, 47, 41, 36, 34, 6, and 4 kDa; the other bands were not clearly identified (Fig. 1). On the other two sets of immunoblot with negative control sera (lanes 5 and 6), there was a single band of 59 kDa, and this band was considered a nonspecific binding.

Discussion

In the present study, we confirmed the clinical relevance of BF attached to BCP as an important inhalant allergen in three asthmatic children. Interestingly, we

Table 2. Results of BCP elimination and provocation tests and BF bronchial provocation test

Case	Days nocturnal symptom-free*						BF bronchial provocation test
	Cough		Wheeze		Awakening		
	B	A	B	A	B	A	
E.J.H.	0	5	2	6	4	7	Dual asthmatic response
Y.H.S.	0	5	3	7	5	7	Dual asthmatic response
L.J.W.	0	5	5	7	6	7	Immediate asthmatic response

*B: for 7 days just before BCP elimination; A: for 7 days after BCP elimination.

found that BCP was the inducer of asthma in these three patients, who had been previously diagnosed as nonatopics, because of negative skin test results using test kits which excluded BF antigen.

There are several reports on occupational asthma caused by BF inhalation in the workplace, such as in cake or bread makers, noodle makers, health food makers, and pillow and cushion manufacturer (4–7, 12). In 1964, Matsumura et al. (8) reported six cases of

Japanese children with buckwheat asthma, possibly induced by BF attached to BCP, but they simply described clinical symptoms and the result of skin tests to BF. And there has been no further study focused on BCP-induced asthma until now. In 1994, we observed a young boy (E.J.H.) who suffered from chronic intractable asthma and had been treated for nonatopic asthma. By detailed history taking, we found that he had been using BCP for almost 5 years, and his first experience of asthmatic symptom had occurred 6 months after his exposure to BCP. Eventually, we confirmed that he had BF allergy caused by long-lasting exposure to BCP, and that he never ate food containing BF. Afterward, we found that one-third of the asthmatic children and/or their family members in Kyung-Gi province in South Korea used BCP to promote health (data not shown). In 1987, Hong et al. (16) reported that buckwheat chaff, rice chaff, cotton, sponge, and feather had been used as pillow-stuffing materials in South Korea, buckwheat chaff being the most common among them; up to 30.5% of Korean allergic asthmatic adults used BCP to improve health. However, Hong et al. (16) did not consider BF to be significant, because all patients showed stronger positive reactions to house-dust mites than to BF. But, interestingly, our three patients showed negative skin reactions and serum specific IgE responses to house-dust mites. Furthermore, within 3 days after elimination of BCP, without any other environmental control for house-dust mites from patients' bedding and bedrooms, all three patients were free of nocturnal symptoms. We also confirmed the BF-induced asthmatic responses by the bronchial provocation test. Eventually, patients had no complaints of significant asthmatic attacks without any medication, except intermittent β_2 -agonist inhalations for their acute asthma symptoms during 2 years of follow-up period. Unfortunately, however, we could not measure the amount of BF antigens in dust from their BCP.

In view of the prompt and profound effects of BCP elimination on nocturnal asthmatic symptoms, it should be noted that even a small amount of BF attached to BCP has strong allergenicity, and BF should be considered a potent sensitizing inhalant allergen in early life. In 1964, Matsumura et al. (8) observed that all six of their patients experienced

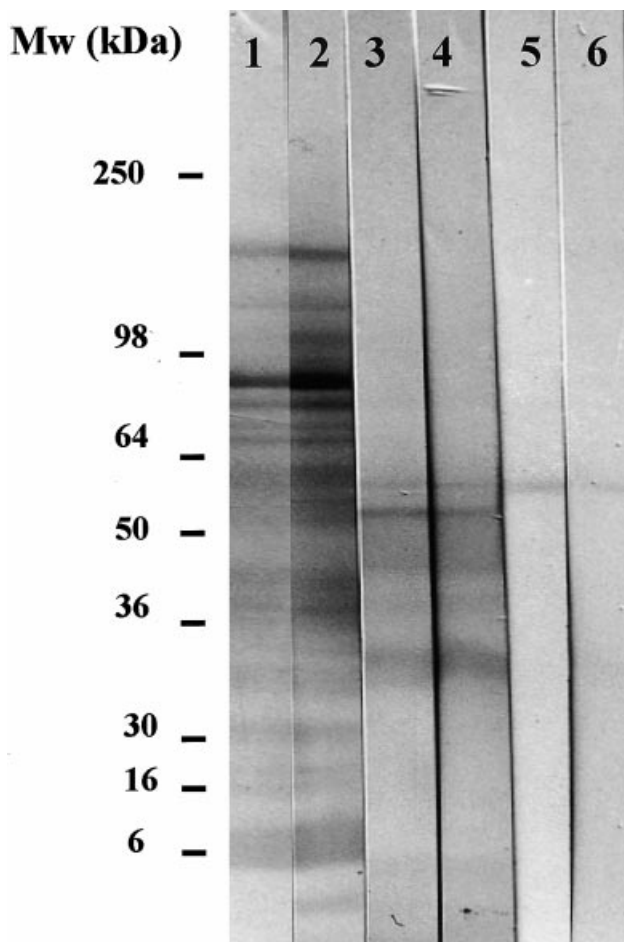


Figure 1. Immunoblot analysis of SDS-PAGE-separated components of homemade buckwheat flour extract. Positive control sera from buckwheat allergy caused by ingestion (lanes 1 and 2). Sera obtained from study patients (lane 3: E.J.H.; lane 4: Y.H.S.). Negative control sera from buckwheat-nonsensitive asthmatic children (lanes 5 and 6).

asthmatic symptoms just after getting into bed; their ages at first use of BCP were 1.5–2 years, the intervals of asthma onsets were 0.5–1 years, and their symptoms disappeared after the removal of BCP. In the present report, the clinical courses of our patients were very similar to the report of Matsumura et al. In addition to clinical symptoms and history of BCP exposure, we performed further evaluations, including IgE immunoblot analysis and the BF bronchial provocation test. However, we did not carry out oral challenge tests since those patients had no history of the ingestion of BF-containing foods.

In the present study, we confirmed IgE-mediated buckwheat allergy by detecting serum specific IgE antibodies by RIA, and immediate-type skin responses by skin prick tests and by IgE immunoblot analysis. The immunoblot showed at least eight IgE-binding bands such as 53, 50, 47, 41, 36, 34, 6, and 4 kDa; however, there was no significant binding with the 24-kDa component which had been identified previously as a major BF allergen (1). While using the sera from food-

allergy patients who served as the positive controls, we found at least 21 IgE-binding bands including the 24-kDa component. From the above result, we suggest that the ingestion of BF can induce more diverse IgE responses than antigen inhalation. However, with regard to the allergenicity of BCP, we need to evaluate the antigens from buckwheat husk itself, which is not related to BF attached to the BCP. In the present study, we could not identify the buckwheat husk-specific antigens and related IgE responses; thus, we could not evaluate the complete allergenic components of BCP in our study subjects. We need further research to identify the unique buckwheat husk allergens and their possible clinical relevance in BCP users.

In conclusion, a small amount of BF attached to BCP may induce strong sensitization to buckwheat and BCP may be a major cause of childhood nocturnal asthma. Therefore, a history of BCP exposure should be taken into consideration, and BCP should be included in the environmental education program for patients.

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