Basophil activation test with food additives in patients with chronic urticaria

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Abstract

Background: Chronic urticaria (CU) is defined as the urticaria persisting for more than 6 weeks. However the etiology is frequently deemed as unclear, as most classical investigations failed to find the causes. Some patients with CU complain about aggravation of their symptoms after meal ingestion. However, it seems that clinically relevant allergies to food itself are thought to be rare (less than 10%) in patients with CU. Food additives are the substances artificially added in the food and a few of these are known to be implicated in allergic or allergy-like reactions. However, the role of food additives in CU is also still under investigation. Basophil activation test (BAT) is an *in vitro* diagnostic tool to identify the activation of basophil, and is now increasingly applied in various fields of allergic researches. We aimed to explore the association between food additives and CU using BAT.

Methods: The BAT was performed with 15 common food additives in 15 patients with CU who had histories of recurrent aggravation after various food intakes without definite food-specific IgE.

Results: Among the 15 patients studied, only two patients (13.3%) presented positive BAT to the food additives. One patient responded to monosodium glutamate, showing 18.7% of CD203c basophil expression. Another patient showed a positive BAT to sodium benzoate. Both patients had clinical correlations with the agents, which were partly proven by elimination diets.

Conclusion: The present study suggested a potential role of the BAT with food additives in the evaluation of possible causes in CU.

Keywords: Chronic urticaria, food additives, hypersensitivity, basophil activation test, monosodium glutamate, sodium benzoate

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LIST OF TABLES AND FIGURES

Introduction

Chronic urticaria (CU) is defined as the urticaria persisting for more than 6 weeks. However, the etiology is frequently deemed as unclear, as most classical investigations failed to find the causes (1). Although approximately one-half of CU cases have been shown to have an autoimmune etiology, the remaining cases have no known etiology or culprit agent despite thorough evaluation (1). The literature shows that 30% of patients believe that food might be a cause of their CU because variations in the diet, especially those that contain high levels of spices, seasonings, or natural histamine-like substances aggravated their symptoms (2, 3). However, clinically relevant allergies to foods are thought to be rare (occurring in less than 10% of cases) in patients with CU (4).

Food additives are substances used as sweeteners, flavorings, coloring agents, antioxidants or preservatives (5). There are thousands of substances (6), while relatively few have been identified in the significant adverse reactions (7). Of these, tartrazine, benzoate, monosodium glutamate (MSG), sulfite, aspartame, nitrites, salicylate and some colorants have been tipped as the potential culprits for adverse food reactions (5). Their potential roles in CU pathogenesis have been suggested by a few studies (8-13), but they are still under investigation. The reasons for the lack of evidence could be attributed to the practical difficulty of performing the gold standard test, the double-blind placebo-controlled food challenge (DBPCFC) (14, 15), but they are still under investigation. In addition, the methodological issues

such as inconsistent inclusion criteria and the absence of a standardized challenge protocol exist, and the outcome of the DBPCFC is not always satisfactory (11, 16, 17). The skin prick test, which is widely used to screen sensitized allergens, is not useful for discriminating food additives hypersensitivity (13). Assuming the culprit additive depends solely on the patient's subjective symptoms, which are sometimes vague and provoked by unrelated foods. Therefore, the selection of potential culprits among various kinds of food additives is also problematic.

The flow cytometric basophil activation test (BAT) is an in vitro tool to assess the expression of basophil activation markers after antigen stimulation. The test has strengths in that it can detect not only IgE-mediated responses (18-20) but also non-IgE-mediated immediate hypersensitivity (21-26), which means that it can be applied to allergic diseases of which the mechanism is not clearly known. Moreover, the BAT is not time consuming and able to examine multiple antigens at the same time.

In this regard, we supposed that the BAT may have potential applications for the diagnosis of food additives hypersensitivity in CU patients. The present study aimed to explore the association between food additives and CU assisted by the BAT.

Methods

Study Population

From October 2011 through March 2013, a total of 15 CU patients (duration >6 weeks) from the division of allergy and clinical immunology of Seoul National University Bundang Hospital (SNUBH) were prospectively and consecutively enrolled in this study. They were included if they reported recurrent aggravations of urticaria by various kinds of food from history including food diary but did not have clear evidence of food-specific IgE by skin prick test or food-specific IgE measurements. Skin prick tests were performed for 55 kinds of common food allergens in Korea (Allergopharma, Reinbeck, Germany)(27), and food-specific IgE were measured by the RIDA Allergy Screen (R-biopharm, Darmastadt, Germany) or UniCAP system (Thermo Fischer, Uppsala, Sweden). In case of positive skin tests or specific IgE tests, clinical correlation was determined by allergy specialists. All patients voluntarily participated in this study, and provided written informed consent. This study was approved by the institutional review board of SNUBH.

Sample Analysis

The BAT was carried out using a commercially available Flow-CAST® kit

(Buhlmann, Schonenbuch, Switzerland) according to the manufacturer's instruction. A total of 15 food additives, which had been previously reported to cause hypersensitivity reactions, were tested using commercial allergens of the CAST®-Allergens (Buhlmann, Schonenbuch, Switzerland), which included MSG, sodium nitrite, tartrazine, sodium salicylate, potassium metabisulfite, sodium benzoate, and food colorant mix Ⅰ(quinolone yellow, sunset yellow FCF, chromotrope FB, amaranth and new coccine), and food colorant mix Ⅱ(erythrosine, patent blue V, indigo carmine, and brilliant black BN)(28).

Briefly, the BAT was performed using the following steps. The patients' blood was processed within 2 hours after sampling in EDTA tubes. After removing the erythrocytes, the sample was treated with stimulation buffer solution. The cell suspensions were divided into 11 tubes containing two positive controls, one negative control, and the 15 kinds of food additives described above, respectively. For the positive controls, monoclonal antibodies to high affinity IgE receptors and nonspecific N-formyl-methionyl-leucyl-phenylalanine (fMLP) were used. The stimulation buffer was added to the tubes for background and negative control. The results of the BAT were expressed as percentages of basophils expressing CD203c which was known to be increasingly expressed on the basophil surface after allergenic stimulation in sensitized individuals and was regarded as a basophil activation marker. The expression of CD203c was detected with anti-CD203c-phycoerythrin. The stimulation index (SI) was calculated as follows:

Stimulation index (SI) = percentage of basophils activated by the food additive / percentage of activated basophils in the negative control.

The BAT was determined to be positive if the basophil activation was ≥ 5% and the SI was ≥ 2, in accordance with the manufacturer's instructions.

Results

Characteristics of Study Subjects

A total of 15 patients with CU were enrolled in this study (Table 1). They had a mean age of 38.7 ± 13.2 years, and 80.0% of them were women. The mean duration of symptoms was 33.0 ± 17.7 months. Only one patients (6.3%) showed elevated peripheral eosinophil counts and 7 patients (46.6%) had elevated total IgE levels ≥ 250 IU/mL. None had anti-thyroid hormone antibodies. Five patients (33.3%) were positive in skin testing or UniCAP to the food allergens, but all of them did not show any clinical correlations with urticaria.

Table 1. Baseline characteristics of 15 chronic urticaria patients

	Total (n=15)
Age, year	38.7 ± 13.2
Sex, M:F	3:12
Asthma	3 (20.0%)
Allergic rhinitis	5 (33.3%)
Drug hypersensitivity	5 (33.3%)
Whole blood cell counts	7817.9 ± 2751.5
Peripheral eosinophil counts	119.2 ± 143.6
Total IgE (IU/mL)	319.8 ± 316.4
Positive skin prick test to food allergens[†]	4/11 (36.4%)
Positive specific IgE to food allergens[‡]	1/8 (12.5%)

Values are presented as means ± standard deviation

†The skin prick test was performed with a standardized technique using 55 kinds of commercially available extracts of a food allergen panel (Allergopharma, Reinbeck, Germany) as well as histamine and saline as a positive and negative control, respectively.

‡Food-specific IgE was measured by using the RIDA Allergy Screen (R-biopharm, Darmastadt, Germany) or UniCAP (Thermo Fischer, Uppsala, Sweden).

The results of the BAT, skin test and specific IgE test in each patient are summarized in Table 2. Two patients (13.3%) showed positive BAT to any of food additives. The detailed histories of these cases are described as follows.

Case 1.

A 37-year-old woman visited the outpatient allergy clinic because of urticaria. She suffered from recurrent generalized urticaria, rash, and facial angioedema. She complained that the urticarial aggravation occurred particularly when eating high-seasoned, spicy, or Chinese foods. Previously, she had been treated for allergic rhinitis and also had experienced severe urticaria with a generalized rash after taking non-steroidal anti-inflammatory drugs (NSAIDs). Peripheral eosinophil counts and serum total IgE in her blood were 97.2/μL and 97 kU/L, respectively. T4 and thyroid stimulating hormone were also within normal range. A skin prick test showed weakly positive reactions (wheal size ≥ 3mm but allergen/histamine ratio < 1) to silk worm pupa, herring, cabbage, celery and walnut; however, none of them

provoked urticaria in the history. In the BAT, a total of 18.7% of basophils were activated after stimulation with MSG (Figure 1); however, the other 14 additives did not induce significant basophil activation, compared to the negative control (1.3%).

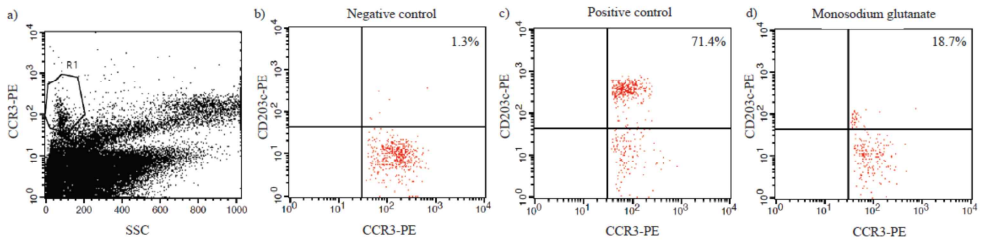

Figure 1. Basophil activation test results for patient 1 showing monosodium glutamate (MSG) hypersensitivity. (a) The basophils were identified as SSC^{low} $CCR3^{high}$ from the gated lymphocytes, (b) As a negative control, background basophil activation with stimulation buffer only (1.3%), (c) As a positive control, basophil activation with anti-IgE antibody (71.4%), (d) The percentage of activated basophils stimulated with MSG was 18.7%. The stimulation index (SI) with MSG was 15.0.

Case 2.

A 19 year-old female high school student with recurrent urticaria visited the allergy clinic. She complained of recurrent episodes of localized urticaria and erythematous rashes on face. She had been experiencing urticarial symptoms that had developed after eating a school meal. Her past medical history or family history was unremarkable for allergic diseases. Laboratory findings including peripheral

eosinophil count and serum total IgE were within the normal range. The result of an autologous serum skin test was also negative. There were no food allergens which positively reacted to the skin prick test. In BAT, only sodium benzoate activated basophils significantly (37.5% activation; Figure 2). After she abstained from the food additives by avoiding the relevant processed foods, her urticarial symptoms were resolved dramatically without further anti-histamine medications.

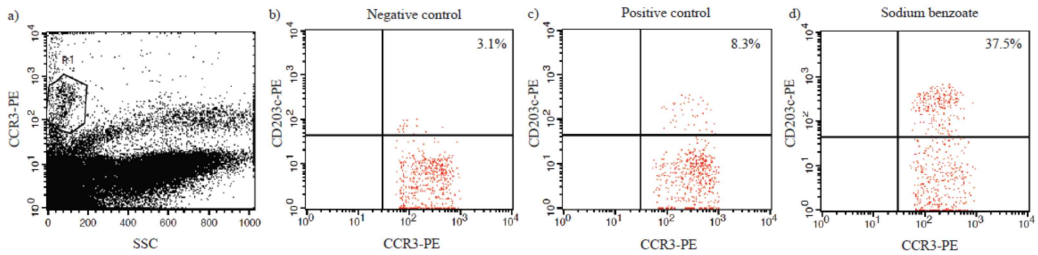

Figure 2. Basophil activation test results for patient 2 showing sodium benzoate hypersensitivity. (a) The basophils were identified as SSC^{low} $CCR3^{high}$ from the gated lymphocytes, (b) As a negative control, background basophil activation with stimulation buffer only (3.1%), (c) As a positive control, basophil activation with anti-IgE antibody (8.3%), (d) The percentage of activated basophils treated with sodium benzoate was 37.5%. The stimulation index (SI) with sodium benzoate was 12.1.

Table 2. Summary of the basophil activation tests and other profiles of the study participants

Patient	Sex	Age	Peripheral eosinophil	Total IgE	SPT	MAST	UniCAP	PB	PC	FC I	FC II	MSG	Nitrite	Tartra-zine	Sali-cylate	Sulfite	Sodium benzoate
1	F	37	97.2	97	+			1.3	71.7	2.0	2.0	18.7	1.7	2.4	1.5	0.6	1.9
2	F	19	89.0	28	-			3.1	8.3	1.6	1.6	0.5	0.0	0.4	2.5	0.0	37.5
3	F	45	98.3	59	-			2.3	60.1	3.1	2.3	2.7	3.3	2.2	4.2	2.3	1.1
4	M	43	43.5	31	-			4.2	76.6	3.9	4.3	4.2	3.1	2.9	2.9	2.5	2.9
5	M	22	150.0	773			-	1.5	76.8	1.8	2.3	4.0	1.8	1.6	1.8	1.1	4.4
6	F	32	98.3	685				1.2	61.1	1.1	0.9	0.5	0.0	0.9	0.2	0.4	0.2
7	F	66	96.6	400	+		-	0.6	59.6	2.1	0.4	0.0	0.0	0.4	0.2	0.0	0.0
8	F	32	166.5	324	-			1.4	69.7	0.5	4.5	2.1	0.5	0.7	0.2	0.2	0.4
9	F	29	25.9	612			Peach (class 3)	0.2	73.1	0.5	0.2	0.2	0.2	0.7	0.2	0.4	0.2
10	F	30	13.3	54	-	-		2.2	26.3	2.9	4.8	2.7	0.0	0.5	2.7	0.5	2.6
11	F	35	90.0	7	-			0.0	75.9	0.3	0.0	0.1	0.0	0.1	0.3	0.5	0.0
12	F	34	0	940	+	-	-	0.1	86.4	0.3	0.1	0.0	0.1	0.0	0.0	0.1	0.1
13	F	56	610.7	540	+		-	0.0	86.0	0.3	0.8	0.1	0.3	0.0	0.1	0.3	0.3
14	M	43	97.4	177		-		0.6	45.1	0.7	0.4	0.0	0.9	0.3	0.3	0.4	0.4
15	F	57	111.8	69.3	-		-	0.4	79.4	0.6	3.6	0.6	0.6	0.3	0.1	0.2	0.1

F: female, M: male, SPT: skin prick test, MAST, multiple allergen stimulation test, PB: patient's background, PC: patient's control, FC I: food colorant mix 1, FC II: food colorant mix II. Peripheral blood counts and total IgE are presented as cells/μ and IU/mL respectively.

Discussion

The present study explored the proportion of patient with food additives hypersensitivity in 15 CU patients via the utilization of the BAT. All of them had recurrent urticaria aggravated by various food intakes, which did not have evident food-specific IgE against common food allergens. A total of 13.3% of participants showed a positive BAT to any of the tested 15 common food additives, which may suggest a potential clinical utility of the BAT in such cases.

CU can be provoked by specific physical factors, such as pressure, heat, cold, sunlight and even non-specific stimuli (1). However, for most of the patients with CU, it did not have a well-described cause. Recent advances in the pathogenesis of CU revealed that 45% of patients presented autoimmune markers such as anti-FcεRI-α, anti-IgE or thyroid autoantibodies, and an autologous serum skin test was positive in 4.1-76.5% of cases (29). However, the etiology of the remaining 55% of patients is still unknown and remained ‘idiopathic’.

Many patients regard that urticaria are attributed to food allergies because their symptoms seem to fluctuate according to their diet. There are several reports that 22.5-30.0% of patients regard food as the cause of CU (2, 3). However, contrary to these patients’ beliefs, it seems that only less than 10% of CU might be associated with IgE mediated food allergies (30). Kobra and his colleagues examined the results of DBPCFC and only 10% of patients who complained about food-provoked aggravation of urticaria, reproduced their symptoms (4, 31).

The clinical features of CU that can be associated with diet could be somewhat different from those of food allergies. Food allergy is mediated by an IgE-dependent allergic mechanism and is more likely to result in acute urticaria as a generalized allergic reaction. Food allergy also develops in response to only certain foods and/or some foods or vegetables that share antigenic similarity (32). However, in CU, similar cutaneous reactions could be developed by several apparently unrelated foods and is especially more common as a reaction to commercially prepared forms of foods that are tolerated when prepared at home (33). These distinct clinical features in CU could arise from food additives, and not from the food itself.

About 3,968 substances are registered in Everything Added to Food in the United States which is regularly updated by the U.S Food and Drug Administration (6). Despite the great number of food additives, only a few have been implicated in adverse reactions and are mainly mediated through non-IgE mediated immunologic or non-immunologic mechanisms (5). There have been several case reports on urticaria, angioedema, asthmatic reactions and anaphylaxis caused by food additives (8-13, 16, 17). In some of these studies, they performed the oral provocation test for food additives and revealed that hypersensitivity to food additives is the cause of CU. However, some of these studies have limitations (5, 8, 9, 33, 34). The challenge procedure was executed under poorly controlled circumstances, which were not double-blinded or placebo-controlled. There have been large-scale studies on the prevalence of food additives hypersensitivity in the general population and it has been reported to be quite low (less than 1%, usually 0.18~0.2%) (35-37). However, children with atopy seemed to show a higher prevalence of hypersensitivity to food

additives (36). However, these studies also had limitations due to the fact that the criteria for patient selection and study design were not consistent.

The DBPCFC is the gold standard to diagnose hypersensitivity to foods or food additives (32). However, it is difficult to execute this on patients with CU in actual clinical practice. Patients with CU are usually taking an antihistamine, which should be discontinued for a certain period. However this may cause an increase in the activity of CU, and thus cause a false positive reaction to the provocation. On the contrary, if the antihistamine is not sufficiently stopped, a false negative result can occur even though food additives are the cause of CU. It is also difficult to conduct the test in a placebo-controlled manner due to the fact that food additives have their own taste and smell. Additionally, in many cases, it is difficult to select candidate additives solely based on the patients' symptoms and food diaries.

A pseudoallergen-free diet can be an alternative option to determine the possibility of hypersensitivity to food additives as a cause of CU. Previous studies reported that a pseudoallergen-free diet could be effective to reduce the severity of CU (10, 16, 38-40). Margel et al. tested a pseudoallergen-free diet on 140 CU patients, and 34% showed significant improvement on urticarial severity and/or quality of life (40). However, a pseudoallergen-free diet restricts all preserved and processed food, and even all spices and herbs, eggs, cakes, biscuits, tomatoes, fresh and dried fruits, except for salt and chives. Therefore, it is hard to carry out in real life and might cause nutritional imbalances (38).

There are many attempts to develop *in vitro* methods to diagnose the cause of CU. Recently, basophils are gaining much attention due to their important roles in CU (41-44). Basophils play an important role in traditional IgE-mediated food allergies (45). Due to the biological response in which activated basophils plays an important role in causing an allergic reaction, it would be more suitable to measure basophil responsiveness after antigenic stimulation rather than to measure the level of specific IgE in order to evaluate the clinical reactivity of CU. The BAT measures the level of expression of CD63 or CD203c on basophil surfaces with flow cytometry after stimulation of blood cells with allergen (19, 20, 46). CD63 or CD203c, which is used as a basophil activation marker, exists within the secretory vesicles inside the basophil at resting stage, and when the basophil is activated causing secretion of the vesicles, the CD63 or CD203c moves to the surface of cell membrane. The level of degranulation of mediators as a result of basophil activation is known to be directly proportional with the expression CD63 and/or CD203c (47-49).

There are numerous reports on the usefulness of the BAT in various allergic diseases. It proved to be especially useful in diagnosing bee or wasp venom anaphylaxis (50). The BAT could be used to test the induction of tolerance in children with cow's milk allergy (51). The BAT is also effective in diagnosing food allergies such as IgE-mediated reactions against pollen-derived food (19, 20) or wheat (46). It is also useful to test non-allergic or pseudoallergic reactions including drugs (26) such as muscle relaxants (22), antibiotics (23), NSAIDs(24), and even radiocontrast media (21).

However, the BAT for food additives has not been sufficiently studied yet. Garcia-Ortega and his colleagues confirmed using the BAT that hypersensitivity to sodium metabisulfite induced CU (52). Ebo et al. reported the case of a patient who had a history of recurrent anaphylaxis after eating cheese, and found that hypersensitivity to the natural dye annatto (Ceska Annato WS E160b), which was positive on the BAT, was the cause of anaphylaxis (53). In our study, the BAT was performed on CU patients whose symptoms were suspected to be related to hypersensitivity to food additives. The culprit was not clearly identified through their history, food diaries, skin prick tests for common food allergens or other additional laboratory tests. Of these patients, one patient showed a positive BAT to MSG and another to sodium benzoate. In these two patients, no symptom developed when they ate at home, but the conditions deteriorated when they ate outside the home, especially for Chinese foods or soups with plenty of seasonings and spices.

The present study has several limitations. First, we did not perform the DBPCFC for the two positive cases as the patients did not agree to perform the oral provocation tests with each additive. Instead, we instructed them on how to restrict the specific additives in their daily diet. Eventually, their symptoms improved after starting a specific food additive-free diet. Second, our low percentage of participants with the positive food additives BAT (13.3%) could raise questions on its diagnostic utility. Several factors are presumed to be responsible. We tested only 15 kinds of common food additives, which could be insufficient for screening purposes. Another possibility could be due to false negative results of the BAT, as shown in our previous study for taurine(54). In addition, less stringent inclusion criteria could

have influenced the positivity of BAT, and have limited our interpretation on the diagnostic utility. We suppose that the positivity might be increased if the subjects were more specifically selected for food additives reactions with oral provocation tests. Nevertheless, our two positive cases were clinically meaningful, as they could have remained unresolved without the diagnostic investigations. Third, we only performed the BAT for 15 patients with CU. This small sample size limits the interpretation of our results. Due to the quite low prevalence of food additives hypersensitivity and it's as-yet undetermined role, a large-scale study is needed to further evaluate the potential utility of BAT with food additives.

Despite the issues surrounding the use of the BAT for food additives, it could be a good alternative to the oral provocation test as a means to evaluate hypersensitivity to food additives in patients with CU. First, it can be applied not only in IgE-mediated, but also in non-IgE mediated reactions, which means that it can be applied to allergic diseases for which the underlying mechanism is not clearly. In addition, BAT results are not affected by anti-histamine or steroid use. Therefore it can prevent the exacerbation of urticaria stemming from the discontinuance of such drugs as well as false negative results for oral provocation tests due to insufficient discontinuation of drugs. The BAT can be used to evaluate multiple candidate allergens or materials simultaneously and independently. It is not easy to identify the culprits in cases of food additives hypersensitivity. Therefore using the BAT as

a screening tool for hypersensitivity to various additives could prevent the need to conduct consecutive oral provocation tests for the numerous potential candidate additives.

Conclusion

The pathogenesis of CU has not been clearly determined, and various environmental factors are suspected to be involved. In our explorative study, two of the 15 (13.3%) participants showed positive results in a BAT using food additives. Although these positive results may appear to be low, they were clinically meaningful as the conditions of these two patients could have remained as ‘idiopathic’ or ‘unexplained’ without the identification of the possible causes by the BAT. It warrants further studies evaluating the diagnostic utility of the BAT for food additives in patients with CU.

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국문 초록

서론: 만성두드러기는 6 주 이상 반복적으로 두드러기가 발생하는 질환으로, 발생기전과 유발요인을 규명하기 위한 연구가 활발하게 진행되었으나 아직까지 명확하게 밝혀지지 않았다. 만성두드러기 환자의 일부에서 음식물과의 연관성이 의심되나, 실제로 식품알레르기가 진단되는 경우는 드문 것으로 알려져 있다. 식품 첨가물은 다양한 목적으로 음식물에 첨가되는 물질로, 일부에서 알레르기 또는 알레르기양 반응을 유발할 수 있는 것으로 알려져 있다. 하지만 아직까지 식품 첨가물에 대한 과민반응이 실제로 만성두드러기를 유발하는 원인인지에 대해서는 충분히 연구되지 않았다. 호염기구 활성시험은 항원 자극 후 호염기구의 활성 정도를 측정하는 생체외 실험기법으로, 최근 다양한 알레르기 질환의 진단 및 평가에 사용되고 있다. 본 논문에서는 음식물 연관성을 보이는 만성두드러기 환자에게 식품 첨가물에 대한 호염기구 활성시험을 통해 만성두드러기와 식품첨가물의 관련성을 알아보고자 하였다.

방법: 식품알레르기는 배제되었지만 음식물 연관성이 의심되는 만성두드러기 환자 15 명을 대상으로, 15 개의 흔한 식품첨가물에 대한 호염기구 활성시험(basophil activation test)을 시행하였다.

결과: 15 명의 만성두드러기 환자들 중 2 명이 식품첨가물에 대한 호염기구 활성시험에서 양성소견을 보였다. 한 명은 글루탐산모노나트륨(monosodium glutamate) 처리 후 호염기구 활성지표인 CD203c 를 발현한 호염기구가 18.7%로 증가했고, 자극지수(stimulation index)는 15.0 이었다. 다른 환자는 벤조산나트륨(sodium benzoate)에 양성 반응을 보였고, 37.5%의 호염기구가 CD203c 를 발현했고, 자극지수는 12.1 이었다. 두 환자 모두에서 호염기구

활성시험에서 양성으로 나왔던 첨가물 제한 식이 (elimination diet) 후 만성두드러기가 유의하게 호전되었다.

결론: 식품첨가물에 대한 호염기구 활성시험은 음식 연관성을 보이는 만성두드러기 환자에서 식품 첨가물에 대한 과민반응을 진단하는데 도움이 될 수 있을 것이다.

주요어: 만성두드러기, 식품 첨가물, 과민반응, 알레르기, 호염기구 활성시험, 글루탐산모노나트륨, 벤조산나트륨

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