ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2006) 24: 201-206

Autologous Serum Skin Test in Chronic Idiopathic Urticaria: Prevalence, Correlation and Clinical Implications

Kanokvalai Kulthanan, Sukhum Jiamton, Taniya Gorvanich and Sumruay Pinkaew

SUMMARY Some cases of chronic idiopathic urticaria (CIU) have histamine-releasing IgG autoantibodies in their blood. This disease subgroup is called "autoimmune urticaria". To date, the autologous serum skin test (ASST) is the best *in vivo* clinical test for the detection of basophil histamine-releasing activity *in vitro*. This study aimed to find the prevalence of ASST positive cases in Thai patients with CIU, to identify factors related to the positivity of ASST and to find the clinical implications of ASST in CIU. A retrospective study was performed among 85 CIU patients who attended the Urticaria Clinic at the Department of Dermatology, Siriraj Hospital and were willing to perform ASST, from January 2002 to December 2003. Twenty-one (24.7%) patients had a positive ASST. There was no significant difference between patients with positive ASST and negative ASST as to the severity of the disease (wheal numbers, wheal size, itching scores and the extent of body involvement) as well as the duration of the disease.

Urticaria is characterized by short-lived swellings of the skin due to transient leakage of plasma into the surrounding superficial dermis. The mast cell is the major effector cell in this disease. Numerous chemotactic factors, enzymes, cytokines and proteoglycans exist preformed in cutaneous mast cell granules or are newly generated from the membrane of the mast cells and can be readily released. Episodes of urticaria occurring at least twice a week for six weeks are considered to be chronic. After taking a thorough history and undergoing physical examination, appropriate screening investigations and provocative test results, about 70% of the patients with chronic urticaria (CU) were diagnosed to have chronic idiopathic urticaria (CIU) as no cause could be identified. Some cases of CIU were reported to have histamine-releasing IgG autoantibodies in their blood.[1] This disease subgroup is called "autoimmune urticaria". These autoantibodies were estimated to be present in at least 30 percent of patients with CIU.[2-6] Although, the basophil histamine release assay is currently the "gold standard" for detecting functional autoantibodies in the serum of patients[7], it is a time consuming procedure and difficult to standardize. Western blotting, enzyme-linked immunoabsorbent assay and flow cytometry using chimeric cell lines expressing the human FcεRIα may be useful in the future, however, they still need to be validated.[7] The autologous serum skin test (ASST), which indicates the presence of functional circulating autoantibodies to FcεRIα and/or to IgE, is the best *in vivo* clinical test for the detection of basophil hista-

From the Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.
Correspondence: Sukhum Jiamton
E-mail: srsjt@mahidol.ac.th

mine-releasing activity *in vitro*.[8] ASST has a sensitivity of approximately 70% and a specificity of 80% when the positive result is defined as a pink, serum-induced wheal with a diameter of 1.5 mm or greater than an adjacent normal saline control injection at 30 minutes.[9] Disease activity of urticaria correlates directly with the serum levels of the FcεRIα autoantibody. Sabroe *et al*.[8,9] reported that patients with autoantibodies had more severe urticaria according to several parameters than patients without autoantibodies. However, some reports showed no significant difference between patients with and without autoantibodies, but those with autoantibodies tend to have more severe disease.[9] The ASST is useful for patients with severe CIU and recalcitrant course under consideration for immunomodulatory treatment.[10, 11]

To date there are still no data on ASST in CIU in the Thai population. This study aimed to find the prevalence of the ASST positive cases among Thai patients with CIU and to identify factors related to the positivity of ASST as well as clinically important findings of ASST in CIU.

MATERIALS AND METHODS

This study was approved by the Siriraj Hospital Institutional Review Board. Records of patients with CIU who attended the Urticaria Clinic and performed ASST at the Department of Dermatology, Siriraj Hospital from January 2002 to December 2003 were retrospectively reviewed. CIU was defined as urticaria with episodes occurring at least twice a week for 6 weeks without a specific cause identifiable after full evaluation.[1,7]

Inclusion criteria were patients with CIU, aged at least 18 years, and willing to perform the ASST. We excluded patients with urticarial vasculitis, patients with predominant co-existing physical urticaria and patients who were pregnant or in the lactation period. All participants were negative for hepatitis B antigen and antinuclear antibody. Written informed consent was obtained from all patients before performing ASST. All patients discontinued short acting antihistamines for at least three days, and long acting antihistamine for at least sevens days before skin tests were performed.

Autologous serum skin test technique

Venous blood was placed in sterile plastic tubes and allowed to clot at room temperature for 30 minutes. Then the serum was separated by centrifugation at 500 x *g* for 15 minutes and kept in aliquots for use in the ASST. Sample (50 µl) of autologous serum and 0.9% sterile saline (for negative control) were separately injected intradermally into the volar aspect of the patient's forearm skin with 27G needles, leaving gaps of at least three centimeters. A skin prick test with histamine (10 µg/µl) was used as a positive control. Areas known to have been involved in spontaneous wheals in the last 24 hours were avoided. Skin prick tests with histamine were interpreted after 15 minutes. Wheals and flare responses were measured after 30 minutes. A positive ASST was defined as a serum-induced wheal which was both red and had a diameter of 1.5 mm or more than the saline-induced response at 30 minutes.[8]

The clinical details noted were a personal or family history of atopy and the duration in months since the onset of urticaria. The number and size of the wheals, severity of itching and extent of body involvement were scored as follows: number of wheals, 0 = no wheals, 1 = 1-5, 2 = 6-15, 3 = 16-25, 4 = > 25; wheal size, 0 = none, 1 = < 5 mm, 2 = 5-20 mm, 3 = > 2 cm, 4 = > 4 cm; itching, 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe; extent of body involvement, 0 = none, 1 = 1-10%, 2 = 11-30%, 3 = 31-50%, 4 = > 50%. Associated systemic symptoms and angioedema were also recorded.

Statistical methods

All statistical analyses were two-sided at a 95% confidence level using SPSS version 10 for windows. ASST data for positive and negative test results were analyzed for demographic data such as sex, mean age (SD), days in a week with wheals, course of disease, outcome of disease, personal history of allergic rhinitis, asthma, atopic dermatitis and allergic conjunctivitis, family history of atopy, angioedema and urticaria with systemic symptoms.

In addition, positive ASST and negative ASST were analyzed for their scores of urticarial

symptoms, *i.e*. wheal number, wheal size, extent of body involvement and itching.

RESULTS

Eighty five patients were enrolled in the study. Sixty-nine (81%) were female. The mean age (SD) of the patients was 34.9 (0.9) years with a range of 18 to 70 years. The mean (SD) total duration of the disease was 15.6 (2.0) months ranging from 3 months to 9 years. Twenty-one (24.7%) of the 85 subjects had a positive reaction to the ASST. The serum-positive group was composed of 16 women and 5 men (mean age 38.8 years), while the 64 ASST-negative subjects were 53 women and 11 men (mean age 33.6 years). There were 24 patients (28.2%) with a positive family history of atopy and 19 patients (22.4%) with a positive personal history of atopy. The ASST was positive in 8 (38.1%) of the former and in 9 (19.1%) of the latter, which showed no significant difference.

Table 1 shows characteristics of those with positive ASST and negative ASST. There was no significant difference between patients with positive ASST and negative ASST according to sex, age at the time of assessment, course of disease, clinical outcome, personal and family histories of atopy and associated systemic symptoms including angioedema. There was no significant difference between the two groups in the total duration of the disease at the time of assessment. The mean total duration of the group with positive ASST was 14.3 months and of the group with negative ASST was 16.1 months. There was no significant difference between the two groups in the mean number of days with wheals (frequency) per week.

Table 2 compares the scores of the urticarial symptoms of the patients with positive ASST to those with negative ASST. There was no significant difference between patients with positive ASST and negative ASST as to the wheal number, wheal size, itching scores and the extent of body involvement.

There was no relationship between wheal diameter of positive ASST and severity (body surface area of urticaria) (p = 0.8), or wheal diameter of positive ASST and duration of disease (p = 0.9). Also, there was no significant difference of wheal size of positive ASST among chronic urticaria patients whose disease was cleared (mean wheal size 6.1) or not improved (mean wheal size 3.5) (p = 0.1). The prevalence of thyroid autoantibodies in patients with positive ASST was 9.5% and in those with negative ASST 3.1%. However, there was no statistically significant difference (p = 0.23).

DISCUSSION

The prevalence of ASST positive cases among Thai patients with CIU in our study was 24.7%. Sabroe *et al*.[8] reported that one-third of their patients with CIU had circulating functional antibody against the high affinity IgE receptor FcεRIα or IgE. Asero *et al*.[12] reported that 205 out of 306 (67%) Italian patients with CIU showed a positive ASST. Nettis *et al*.[13] reported that serum from 42 of 102 Italian patients with CIU (41.2%) induced a wheal-and-flare response. Bakos *et al*.[14] reported 26 of 48 (54.2%) positive ASST in Hungarian patients with chronic urticaria. Caproni *et al*.[15] showed that 23 of 68 Italian patients (34%) with CIU had a positive reaction to ASST. Vedanthan showed that 70% (50/74) of adult American patients with CIU had positive ASST. Zuberbier *et al*.[16] reported the presence of anti- FcεRIα autoantibodies in 17 of 48 German patients (35%) with chronic urticaria using a sandwich ELISA technique. Sabroe *et al*.[9] reported that 33 of 107 patients with CIU (31%) had functional autoantibodies on the basis of the serum evoked histamine release in vitro from the basophils of 2 healthy donors.

The findings of our study agreed with previous studies of Caproni *et al*.[15] in terms of the female predominance of the disease (CIU) and the percentages of ASST positive cases. Our study also showed no significant difference between subjects with and without antibodies when taking mean age, sex distribution and clinical morphology of individual wheals into account. The majority of parameters examined to define the severity of urticaria showed no significant difference between patients with a wheal-and flare response to ASST and patients with a negative response, *e.g.* wheal amount, wheal size, area of body involvement and itching. From our study, there were no significant differences between the serum-positive and serum-negative groups with regard to

Table 1 Characteristics of ASST positive and ASST negative patients

Characteristics	All patients (n = 85)	Positive ASST (n = 21)	Negative ASST (n = 64)	*p* value
Sex				0.5
Male, n (%)	16	5 (31.3)	11 (68.7)	
Female, n (%)	69	16 (23.2)	53 (76.8)	
Age, mean (SD), **years**	34.9 (10.8)	38.8 (8.7)	33.7 (11.2)	0.058
Days with wheals/week, mean (SD) days	4.5 (2.9)	4.1 (3.1)	4.6 (2.9)	0.6
Personal history				
Atopy	21 (24.7)	4 (19.1)	17 (26.6)	0.5
Allergic rhinitis, n (%)	15 (17.9)	3 (14.3)	12 (19.1)	0.6
Asthma n (%)	3 (4.8)	0	3 (4.8)	0.3
Atopic dermatitis n (%)	1 (1.6)	0	1 (1.6)	0.5
Allergic conjunctivitis, n (%)	0	0	0	NA
Angioedema, n (%)	6 (7.4)	2 (9.5%)	4 (6.4%)	0.6
Urticaria with systemic symptoms, n (%)	21 (24.7)	0	21 (32.8)	0.2
Course of disease*, months	n = 83	n = 20	n = 63	
mean (SD)	26.8 (41.3)	27.4 (31.0)	26.7 (44.3)	0.9
median	14	14	13	
minimum, maximum	1, 300	3, 121	1,300	
Outcome of disease				0.8
Improved/ heal	63 (74.1)	16 (76.2)	47 (73.4)	
Not improved	7 (8.2)	1 (4.8)	6 (9.4)	
Lost to follow up	15 (17.7)	4 (19.1)	11 (17.2)	
Family history				
Atopy, n (%)	24 (28.2)	8 (38.1)	16 (25.0)	0.3
Allergic rhinitis, n (%)	10 (11.8)	2 (9.5)	8 (12.5)	0.7
Asthma, n (%)	10 (11.8)	4 (19.1)	6 (9.4)	0.2
Atopic dermatitis, n (%)	0	0	0	NA
Urticaria/angioedema, n (%)	7 (8.2)	2 (9.5)	5 (7.8)	0.8

*Course of disease: time from the patient visited urticaria clinic to clearance of lesions
NA, not available

associated systemic symptoms including angioedema and atopy frequency.

Sabroe *et al*.[8,9] reported in 1999 that patients with autoantibodies had more severe urticaria than patients without autoantibodies according to several parameters: they had higher urticaria scores; the urticaria involved more sites; it was more itchy at its worst; and there was a higher incidence of associated systemic symptoms particularly flushing and gastrointestinal symptoms. Differences between patients with and without autoantibodies in the number of sites involved and in the number of systemic symptoms were more pronounced for patients still within the first 12 months of their disease. This suggests that patients with autoantibodies may have their most severe attacks close to the onset of the disease. However, even within the first 12 months of the disease's course, our patients with autoantibodies did not have more severe urticaria than patients without autoantibodies. Caproni *et al*.[15] did not demonstrate any significant difference between the serum-positive and serum-negative cases with regard to mean age, sex distribution, angioedema and atopy. However, all patients with positive ASST presented more severe clinical features than those with serum-negative.

However, Nettis *et al*.[13] noted that the majority of parameters used to define the severity of urticaria showed no significant difference between patients with positive and negative ASST. The only

Table 2 Scores of urticaria symptoms for ASST positive and negative patients by wheal number, wheal size, extent of body involvement and itching

Characteristics	Score					*p* value
	0	1	2	3	4	
Number of wheals*, n (%)						0.8
Positive ASST	7 (33.3)	5 (23.8)	2 (9.5)	1 (4.8)	6 (28.6)	
Negative ASST	20 (31.8)	13 (20.6)	13 (20.6)	4 (6.4)	13 (20.6)	
Wheal size,** n (%)						1.0
Positive ASST	7 (33.3)	3 (14.3)	4 (19.1)	2 (9.5)	5 (23.8)	
Negative ASST	20 (31.8)	7 (11.1)	17 (26.9)	5 (7.9)	14 (22.2)	
Extent of body involvement*,** n (%)						0.1
Positive ASST	7 (33.3)	7 (33.3)	4 (19.1)	1 (4.8)	2 (9.5)	
Negative ASST	18 (28.6)	32 (50.8)	10 (15.9)	3 (4.8)	0	
Itching**,** n (%)						0.4
Positive ASST	2 (14.3)	10 (71.4)	0	2 (14.3)	0	
Negative ASST	5 (13.5)	18 (48.7)	8 (21.6)	5 (13.5)	1 (2.7)	

*number of wheals, 0 = no wheals, 1 = 1-5, 2 = 6-15, 3 = 16-25, 4 = > 25;
**wheal size, 0 = none, 1 = < 5 mm, 2 = 5-20 mm, 3 = >2 cm, 4 = >4 cm;
***extent of body involvement, 0 = none, 1 = 1-10%, 2 = 11-30%, 3 = 31-50%, 4 = > 50%
****itching, 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe.

significant difference they noted between the two groups was the incidence of angioedema. Our study results were similar to Nettis *et al.*[13] except that there was no significant difference in the incidence of angioedema between the two groups.

It was expected that the atopic subjects would belong mostly to a less severe disease subgroup because it has been demonstrated that the atopic subject's trend toward high levels of IgE can prevent the binding of anti- FcεRIα antibodies to the receptor, already saturated by immunoglobulins. Our study like Nettis *et al.*[13] and Caproni *et al.*[15] showed no significant difference between the serum-positive and serum-negative groups with regard to atopy frequency.

Bakos *et al.*[14] reported no laboratory differences between the autoimmune and non-autoimmune groups except for the basophil count. He observed a relationship between autoimmune urticaria and autoimmune thyroiditis. Numerous studies have found a prevalence of 14-20% of thyroid autoimmunity in chronic urticaria patients. Positive autologous serum skin testing was previously described in patients with autoimmune urticaria associated with thyroid autoimmunity. In our study, the prevalence of thyroid autoantibodies in patients with positive ASST was 9.5% and in those with negative ASST 3.1%. However, there was no statistically significant difference.

Routine treatment for autoimmune and non-autoimmune chronic urticaria is the same. However, immunotherapies using plasmapheresis, intravenous gamma globulin, cyclosporine and methotrexate have been successfully reported in the treatment of severe antihistamine-resistant CIU. Bagenstose *et al.*[17] reported that only patients with autoimmune chronic urticaria resistant to H_1-antagonist monotherapy might benefit from the addition of the leukotriene D4-receptor antagonist zafirlukast to their treatment regimen. These results also suggest that a routine screening of patients with chronic urticaria with ASST might be useful in formulating therapeutic algorithms in the management of chronic urticaria.

In summary, may be due to the limited number of patients, we could not demonstrate significant differences between patients with positive and negative ASST.

ACKNOWLEDGEMENTS

The authors would like to express sincere thanks to Dr. Puan Sutthipinitharm, Head of the Department of Dermatology and to the administrative staff of the department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University.

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