Comparison between Autologous Serum Skin Test and Autologous Plasma Skin Test in Thai Chronic Urticaria Patients

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Objective: *To determine the prevalence and compare the results of positive autologous serum skin test (ASST) and autologous plasma skin test (APST) in Thai chronic urticaria patients.*

Material and Method: *A retrospective review of all chronic urticaria patients attending the Urticaria Clinic who underwent ASST and APST between January 2010 and March 2014 was conducted. The data on their demography, disease severity, and results of ASST, APST, and other relevant tests were collected and analyzed using a statistical software.*

Results: *Seventy-nine patients, comprising 14 (17.7%) males and 65 (82.3%) females, were included in the present study. Their median (range) age was 37 years (18 to 73 years). The median (range) duration of the disease was two years (2 months to 30 years). ASST was positive in 48 patients (60.8%) whereas APST was positive in 50 patients (63.3%). The difference in prevalence between both tests was not statistically significant (p = 0.593). A statistically significant association with higher number of wheals during each attack of urticarial symptoms was found in ASST-positive group (p<0.001), but not in APST-positive group (p = 0.064).*

Conclusion: *ASST and APST can similarly detect autoreactivity in Thai chronic urticaria patients. Thus APST, the less established test between the two, is unnecessary.*

Keywords: *Autoimmune urticaria, Autologous plasma skin test, Autologous serum skin test, Chronic urticaria, Skin test*

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Chronic urticaria is a common skin disease estimated prevalence of 0.5 to 1%[1]. Chronic spontaneous urticaria is defined as recurrent urticaria occurring for at least six weeks independent of external stimuli[2]. Among Thai chronic urticaria patients, 75% have chronic spontaneous urticaria[3]. Approximate one third of chronic spontaneous urticaria patients have circulating autoantibodies against high affinity IgE receptor (FcεRI) and/or immunoglobulin E (IgE) which can release histamine from basophils and dermal mast cells[4]. This group of patients can be classified as having chronic autoimmune urticaria.

Tests for autoimmune urticaria includes in vivo testing with the autologous serum skin test (ASST) and autologous plasma skin test (APST), and in vitro testing focuses on basophil histamine release, isolation of autoantibodies, and markers of basophil activation[5]. In 2013, the European Academy of Allergology and Clinical Immunology (ECAAI) proposed the gold standard for diagnosis of autoimmune chronic urticaria[6] to include: (a) a positive bioassay (basophil histamine release assay or basophil activation marker expression) to demonstrate functionality in vitro, (b) positive autoreactivity (by means of a positive ASST) to demonstrate relevance in vivo to mast cell degranulation and vasopermeability, and (c) a positive immunoassay for specific immunoglobulin G (IgG) autoantibodies against FcεRIa and/or anti-IgE (western blot or ELISA) to demonstrate antibody specificity. Nevertheless, most in vitro tests are complex and not available in all practices. ASST is a relatively simple in vivo test capable of detecting the presence of histamine-releasing factors in chronic urticaria patients. It involves intradermal injection of autologous serum which can induce a wheal and flare response. Although ASST cannot be used solely as a means to diagnose chronic autoimmune urticaria, it could be used as a screening test[7,8]. Using the optimal cut-off point determined by Sabroe et al in 1999, the ASST has a sensitivity of 70% and specificity of 69%[8]. Subsequently, Asero et al found that APST gave a higher percentage of positive results than ASST due to the capability of thrombin, generated by the coagulation factors present in plasma but not in serum, to activate mast cells thus causing an increase in

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vascular permeability[9]. However, other studies produced varied results in this regard. Metz et al[10] and Sajedi et al[11] found that APST yielded more positive results than ASST. In contrast, Kocatürk et al[12] reported more positive results from ASST than APST. Also, the results of ASST and APST were similar in the studies by Yildiz et al[13] and Godse[14]. Therefore, there is still a controversy concerning the advantage of APST or ASST over the other.

The primary objective of the present study was to determine the prevalence and compare the results of positive ASST and APST in Thai chronic urticaria patients, in order to prove the advantage of APST over ASST in detecting autoreactivity. The authors also aimed to evaluate the associations between these skin test results and the patient's clinical features as well as other relevant laboratory tests.

Material and Method

The present study was a retrospective review of medical records. It conformed to the guidelines of the 1975 Declaration of Helsinki and was approved by the Ethical Review Committee on Research Involving Human Subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University. The medical records of all patients attended the Urticaria Clinic between January 2010 and March 2014 were reviewed retrospectively. The Urticaria Clinic is a specialized dermatology outpatient clinic in a government university medical school hospital, operating since January 2010. It is designated for the care of urticaria patients who do not respond to treatment with standard dose of antihistamines and/or need special test(s) for urticaria. The tests performed in the Urticaria Clinic include skin prick test, ice cube test, sandbag test, warm arm bath, epinephrine intradermal test, ASST, and APST. The tests were chosen at the discretion of the dermatologist attending the clinic, according to the patient's clinical findings. ASST and APST were prescribed to most patients with chronic spontaneous urticaria, and were always prescribed together. In other words, all patients who had ASST also had APST, and vice versa.

The Urticaria Clinic's patient registration form, which recorded the urticaria symptoms and signs at the initial visit to the Clinic along with the tests, were reviewed. The patients who fulfill the following criteria were included in the study. First, they must have suffered from urticaria, characterized by itchy wheals, at least two times per week for at least six weeks, either before or at the time of the initial visit. Second, they must have had ASST and APST. Patients with physical urticaria or urticarial vasculitis were excluded from the study.

The procedures for ASST and APST in our clinic were as follows. Treatment with antihistamines was discontinued at least seven days prior to the skin tests in all patients. Ten milliliters of venous blood from each patient was drawn. To prepare the autologous serum, 5 ml of blood was put into a plain sterile tube (BD Vacutainer® blood collection tubes, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) without any additive, accelerator, or anticoagulant. The other 5 ml used to prepare the autologous plasma was put into a sterile tube containing 10.8 mg of dipotassium ethylenediaminetetraacetic acid (K_2 EDTA) (BD Vacutainer® blood collection tubes, Becton, Dickinson and Company, Franklin Lakes, NJ, USA), then lightly agitated to be mixed with K_2 EDTA. Both tubes were allowed to stand at room temperature for 30 minutes, and then centrifuged at 500 x g for 15 minutes[8], after which autologous serum or plasma was collected. For skin testing, ASST and APST were administered by intradermal injection of 0.05 ml of autologous serum and autologous plasma, respectively, into the volar aspect of each forearm. As a negative control, sterile normal saline solution (NSS) of 0.05 ml volume was also injected intradermally using the same method into the volar aspect of one arm, distant from the injection site of autologous serum and plasma. Skin prick testing with histamine (10 mg/ml) was used as the positive control. The negative control for APST was performed by intradermal injection of 0.05 ml of diluted K_2 EDTA, which was prepared by mixing 5 ml of NSS with the K_2 EDTA in the same type of tube used to prepare autologous plasma. However, this negative control for APST was discontinued after being done in the first 20 patients, because none developed any wheal after 30 minutes at the injection site of diluted K_2 EDTA, but some patients experienced burning sensation during the injection. Skin test reading was made at 30 minutes after the injections. ASST was considered positive when the wheal induced by the serum was erythematous and at least 1.5 mm greater in diameter than the wheal induced by NSS[8]. APST was also deemed positive when the same criteria, regarding erythema, and plasma-induced wheal size relative to NSS-induced wheal, were met.

The clinical data on the patients' sex, age, duration of disease, history of angioedema, duration of daily attacks, frequency of attacks, dermographism, wheal size, number of wheals, degree of itching, impairment of sleep, and impairment of daily activities

were collected from the patients' Urticaria Clinic registration forms and medical records. The laboratory results of thyroid stimulating hormone (TSH) level, antithyroglobulin antibody, and antimicrosomal antibody tested prior to ASST and APST, were also retrieved from the hospital's electronic database of laboratory results, if available. These clinical and laboratory data were tested for their associations with the results of ASST and APST.

The statistical methods utilized in testing for associations in the present study included Pearson's Chi-squared test or Fisher's exact test for categorical variables, and Wilcoxon rank-sum test for continuous variables with non-normal distribution. McNemar's test was used to test for difference between two dependent proportions, i.e., proportions of patients with positive ASST vs. positive APST. All tests were two-sided at a 95% confidence level. The statistical analysis was performed with Stata Statistical Software Release 12 (College Station, TX: StataCorp LP). Based on the data from the study by Yildiz et al[13], a sample size of 70 patients was estimated to detect the difference of 12% between the prevalence of positive ASST and APST, at the power of 0.8 and confidence level of 95%.

Results

Seventy-nine patients were included in the present study. Among them, 14 were male (17.7%) and 65 were female (82.3%). The median (range) age was 37 years (18 to 73 years). The median (range) duration of the disease was two years (2 months to 30 years). ASST was positive in 48 patients (60.8%), whereas APST was positive in 50 patients (63.3%), as shown in Table 1. The difference between ASST and APST results was not statistically significant ($p = 0.593$).

The patients' demographic data, specific history with regard to urticaria, TSH level and result of antithyroid autoantibodies were shown in Table 2. Among the 24 patients with history of atopy, 23 had allergic rhinitis, and two had asthma. The history of atopy did not show statistical significance among patient groups with different skin test results. Only the number of wheals at the time of having ASST was significantly different between ASST (+) and ASST (-) groups ($p<0.001$), with higher number of wheals (more than 12) during each attack of urticarial symptoms experienced in the majority of patients (70.8%) with positive ASST. The difference in the number of wheals was not statistically significant between APST (+) and APST (-) groups ($p = 0.064$). Other characteristics, including sex, age, duration of disease, history of angioedema, duration of daily attacks, frequency of attacks, dermographism, wheal size, degree of itching, impairment of sleep, impairment of daily activities, abnormal TSH level, and antithyroid autoantibodies did not show statistically significant difference between ASST (+) and ASST (-) or between APST (+) and APST (-) groups.

Table 1. Results of ASST and APST in Thai chronic urticaria patients

	APST (+)	APST (-)	Total
ASST (+)	42	6	48 (60.8%)
ASST (-)	8	23	31 (39.2%)
Total	50 (63.3%)	29 (36.7%)	79 (100%)

ASST = autologous serum skin test; APST = autologous plasma skin test

Discussion

ASST is a simple in vivo screening test for assessing autoreactive urticaria that has been extensively reviewed by the Dermatology Section of the EAACI and the Global Allergy and Asthma European Network (GA²LEN) task force in 2009[15]. Asero et al[9] suggested that the coagulation cascade and generation of thrombin; a serine protease able to activate mast cells causing increase in permeability of endothelium might be involved in the pathogenesis of chronic urticaria. Thus, some studies demonstrated that intradermal testing with plasma (APST) resulted in more positive responses than ASST in chronic urticaria patients[9,11]. However, the superior results of APST over ASST was not consistent in other studies. The main objective of the current study is to compare the results of APST and ASST in Thai patients investigated for chronic spontaneous urticaria. The present study found that both tests gave similar results without statistically significant difference. The results are consistent with a number of previous studies, while differ from other previous studies. Comparison of APST and ASST results from the present study and previous studies are shown in Table 3. In summary, the results of the present study are comparable to those of Yildiz et al[13] in 2011, both in the similarity between the two tests and the percentage of positive tests. Godse[14] and Metz et al[10] also obtained similar results between APST and ASST, although at a lower percentage than the present study. The studies by Asero et al[9] and Sajedi et al[11] yielded higher percentages of positive APST than positive ASST although the difference was not significant in the latter. In contrast, Kocatürk et al[12] had considerably more

Table 2. Demographic data, severity of urticaria, and laboratory values of chronic urticaria patients according to results of skin tests

Characteristic	Total* n = 79	ASST (+) n = 48	ASST (-) n = 31	p-value	APST (+) n = 50	APST (-) n = 29	p-value
Sex, n (%)				0.130			0.599
Male	14 (17.7)	6 (12.5)	8 (25.8)		8 (16.0)	6 (20.7)	
Female	65 (82.3)	42 (87.5)	23 (74.2)		42 (84.0)	23 (79.3)	
Age (years), median (range)	37 (18 to 73)	37 (18 to 65)	36 (18 to 73)	0.655	35.5 (18 to 70)	44 (18 to 73)	0.360
Duration of disease, years, median (range)	2 (0.2 to 30)	2 (0.2 to 30)	3 (0.2 to 20)	0.976	2 (0.2 to 20)	2.5 (0.3 to 20)	0.890
History of angioedema, n (%)	21 (26.6)	15 (31.3)	6 (19.4)	0.243	14 (28.0)	7 (24.14)	0.708
History of atopy, n (%)	24 (30.4)	14 (29.2)	10 (32.3)	0.770	16 (32.0)	8 (27.6)	0.681
Duration of daily attacks, hours, median (range)	6 (0.2 to 24)	6 (0.5 to 24)	7 (0.2 to 24)	0.844	6 (0.5 to 24)	6 (0.2 to 24)	0.358
Frequency of attacks, days per week, median (range)	7 (1 to 7)	7 (1 to 7)	7 (1 to 7)	0.567	7 (1 to 7)	7 (1 to 7)	0.859
Dermographism, n (%)	28 (35.4)	15 (31.3)	13 (41.9)	0.334	16 (32.0)	12 (41.4)	0.401
Wheal size at time of having ASST and APST, n (%)				0.074			0.171
No wheals	6 (7.6)	1 (2.1)	5 (16.1)		4 (8.0)	2 (6.9)	
<1.25 cm	7 (8.9)	3 (6.3)	4 (12.9)		2 (4.0)	5 (17.2)	
1.25 to 2.5 cm	13 (16.5)	8 (16.7)	5 (16.1)		7 (14.0)	6 (20.7)	
>2.5 cm	53 (67.1)	36 (75.0)	17 (54.8)		37 (74.0)	16 (55.2)	
Number of wheals at time of having ASST and APST, n (%)				<0.001			0.064
None	5 (6.3)	1 (2.1)	4 (12.9)		3 (6.0)	2 (6.9)	
1 to 6	18 (22.8)	6 (12.5)	12 (38.7)		9 (18.0)	9 (31.0)	
7 to 12	14 (17.7)	7 (14.6)	7 (22.5)		6 (12.0)	8 (27.6)	
>12	42 (53.2)	34 (70.8)	8 (25.8)		32 (64.0)	10 (34.5)	
Degree of itching, n (%)				0.687			0.957
None	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	
Mild	14 (17.7)	9 (18.8)	5 (16.1)		9 (18.0)	5 (17.2)	
Moderate	31 (39.2)	17 (35.4)	14 (45.2)		19 (38.0)	12 (41.4)	
Severe	34 (43.0)	22 (45.8)	12 (38.7)		22 (44.0)	12 (41.4)	
Impairment of sleep, n (%)				0.431			0.202
None	21 (26.6)	13 (27.1)	8 (25.8)		14 (28.0)	7 (24.1)	
Mild	17 (21.5)	12 (25.0)	5 (16.1)		10 (20.0)	7 (24.1)	
Moderate	25 (31.7)	16 (33.3)	9 (29.0)		19 (38.0)	6 (20.7)	
Severe	16 (20.3)	7 (14.6)	9 (29.0)		7 (14.0)	9 (31.0)	
Impairment of daily activities, n (%)				0.737			0.579
None	18 (22.8)	9 (18.8)	9 (29.0)		10 (20.0)	8 (27.6)	
Mild	30 (38.0)	19 (39.6)	11 (35.5)		20 (40.0)	10 (34.5)	
Moderate	21 (26.6)	14 (29.2)	7 (22.6)		15 (30.0)	6 (20.7)	
Severe	10 (12.7)	6 (12.5)	4 (12.9)		5 (10.0)	5 (17.2)	
Abnormal thyroid stimulating hormone level [n = 69], n (%)	4 (5.8)	2 (4.7)	2 (7.7)	0.629	2 (4.4)	2 (8.7)	0.596
Positive antithyroglobulin antibody [n = 72], n (%)	11 (15.3)	5 (11.9)	6 (20.0)	0.508	4 (8.7)	7 (26.9)	0.084
Positive antimicrosomal antibody [n = 74], n (%)	9 (12.2)	5 (11.6)	4 (12.9)	1.000	5 (10.6)	4 (14.8)	0.716

* Number of patients who had thyroid stimulating hormone and antithyroid autoantibodies tested were less than 79, thus shown separately in each row

Table 3. Autologous skin test results from previous studies comparing percentages of positivity to APST and ASST in chronic urticaria patients

Author	Location	Year	Number of patients	% APST (+)	% ASST (+)	Wheal size criterion for positive test	Prior discontinuation of antihistamines (days)
Asero et al[9]	Italy	2006	71	86	53	3 mm in absence of reaction to NSS	5
Godse[14]	India	2008	30	46	46	1.5 mm larger than wheal by NSS	3
Metz et al[10]	Germany, Spain, United Kingdom	2009	200	43	37	1.5 mm larger than wheal by NSS	N/A
Yildiz et al[13]	Turkey	2011	42	62	62	1.5 mm larger than wheal by NSS	7
Kocatürk et al[12]	Turkey	2011	70	39	63	1.5 mm larger than wheal by NSS	3
Sajedi et al[11]	Iran	2011	58	78	66	3 mm larger than wheal by NSS	7
Present study	Thailand	2014	79	63	61	1.5 mm larger than wheal by NSS	7

NSS = normal saline solution; N/A = not applicable

positive ASST than positive APST in their patients with equal specificity of the two tests but a higher sensitivity of ASST compared to APST. Of note, the criterion for a positive ASST or APST and the period of discontinuation of antihistamine treatment before the skin tests varied among the studies, as indicated in Table 3. This could partly explain the difference in the results across the studies. The results of the present study support those of previous studies[10,13,14] and a recent consensus guideline[15] that did not recommend APST over ASST.

In the present study, ASST and APST were discordant in 14 patients (17.7%), including 6 (7.6%) with ASST (+)/APST (-) and 8 (10.1%) with ASST (-)/APST (+). This discord could result in part from the non-specific reactions possibly produced by K_2 EDTA[9] as well as from random error.

The prevalence of positive ASST in the present study was slightly higher than some previous studies, including the original study by Sabroe et al[8] which defined the optimal cut-off point for a positive ASST in 1999 and a previous study in Thai patients with chronic idiopathic urticaria by Kulthanan et al[16] in 2006. This may be due to the selection bias in the present study, of which the patients tended to have chronic urticaria unresponsive to conventional doses of antihistamines. There is controversy about the relationship between ASST positivity and disease severity. Nonetheless, the positivity of ASST in the present study was significantly associated with higher number of wheals during each attack of urticarial symptoms (p<0.001). This finding was in accordance with the study by Sabroe et al[17] which demonstrated that chronic idiopathic urticaria patients with anti-FcεRI or anti-IgE autoantibodies had significantly more wheals, wider distribution of wheals, more severe itching, and more systemic symptoms (gastrointestinal symptoms and flushing) than patients without autoantibodies.

Since the anticoagulant used in preparing plasma for APST was K_2 EDTA, not sodium citrate recommended in the literature, the readers should bear in mind that the results of APST in the present study may not be accurate. Another limitation exists in that some patients were not tested for TSH level and antithyroid autoantibodies due to the retrospective nature of the present study. These blood tests were not found to be associated with the results of either ASST or APST.

In 2013, the ECAAI proposed the gold standard for diagnosis of autoimmune chronic urticaria[6] to include: (a) a positive bioassay (basophil histamine release assay or basophil activation marker expression) to demonstrate functionality in vitro, (b) positive autoreactivity (by means of a positive ASST) to demonstrate relevance in vivo to mast cell degranulation and vasopermeability, and (c) a positive immunoassay for specific IgG autoantibodies against FcεRIa and/or anti-IgE (western blot or ELISA) to demonstrate antibody specificity. Therefore, using ASST alone without identifying the autoantibodies and demonstrating a positive functionality is not adequate in diagnosing chronic autoimmune urticaria. However, most in vitro tests are not available in general dermatology practices. The authors believe that ASST is an inexpensive, easily performed test that can simply demonstrate to the patient the endogenous cause of the urticaria which may eliminate the patients' frustration and unnecessary laboratory investigations requested to identify any exogenous causes of their chronic urticaria.

Conclusion

The present study found that ASST and APST produced similar results in Thai chronic urticaria patients, with prevalence of approximately 60% positivity. The results showed that the use of plasma instead of serum for intradermal testing is not necessary. In the present study, only the positive ASST was associated with higher number of wheals. Long-term follow-up and future studies regarding the prognostic and management significance of the autoreactivity demonstrated by positive ASST should be performed.

What is already known on this topic?

A large number of chronic urticaria cases are autoimmune in nature. The diagnosis can be supported by ASST and/or APST. Previous studies showed controversial results regarding the advantages of APST in comparison with ASST.

What this study adds?

ASST and APST produced similar results in Thai chronic spontaneous urticaria patients, with positivity of approximately 60%. Thus APST, the less established test between the two, is unnecessary.

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Potential conflicts of interest

None.

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การเปรียบเทียบระหว่างการทดสอบโดยการฉีดซีรัมและพลาสมาของตนเองเข้าในชั้นผิวหนังในผู้ป่วยโรคลมพิษเรื้อรังชาวไทย

กุลวัตร ธาดานิพนธ์, เพ็ญพรรณ วัฒนไกร

วัตถุประสงค์: *เพื่อหาความชุกของการมีผลทดสอบโดยการฉีดซีรัมของตนเองเข้าในชั้นผิวหนัง (autologous serum skin test, ASST) และการฉีดพลาสมาของตนเองเข้าในชั้นผิวหนัง (autologous plasma skin test, APST) เป็นบวกในผู้ป่วยโรคลมพิษเรื้อรังชาวไทย และเพื่อเปรียบเทียบผลของการทดสอบทั้งสองชนิดดังกล่าว*

วัสดุและวิธีการ: *ศึกษาย้อนหลังในผู้ป่วยโรคลมพิษเรื้อรังทุกรายที่เข้ารับการรักษาในคลินิกลมพิษและได้รับการทดสอบโดยทั้ง ASST และ APST ตั้งแต่ เดือนมกราคม พ.ศ. 2553 จนถึง มีนาคม พ.ศ. 2557 ข้อมูลเกี่ยวกับประชากรศาสตร์ ความรุนแรงของโรค ผลการทดสอบ ASST และ APST รวมทั้งการทดสอบอื่น ๆ ที่เกี่ยวข้องได้ถูกรวบรวมและวิเคราะห์โดยใช้โปรแกรมทางสถิติ*

ผลการศึกษา: *ข้อมูลจากผู้ป่วย 79 ราย ได้ถูกรวบรวมเข้าในการศึกษานี้ ซึ่งประกอบด้วยชาย 14 ราย (17.7%) และหญิง 65 ราย (82.3%) มีค่ามัธยฐาน (พิสัย) ของอายุ 37 ปี (18-73 ปี) และค่ามัธยฐาน (พิสัย) ของระยะเวลาที่เป็นโรคลมพิษเรื้อรัง 2 ปี (2 เดือน ถึง 30 ปี) ผู้ป่วย 48 ราย (60.8%) ได้ผลบวกต่อ ASST และ 50 ราย (63.3%) ได้ผลบวกต่อ APST ความแตกต่างระหว่างความชุกของการได้ผลบวกจากการทดสอบทั้งสองชนิดดังกล่าวไม่มีนัยสำคัญทางสถิติ ($p = 0.593$) พบความสัมพันธ์ที่มีนัยสำคัญทางสถิติระหว่างจำนวนรอยโรคระหว่างที่มีอาการลมพิษกับการได้ผล ASST เป็นบวก ($p<0.001$) แต่ไม่พบความสัมพันธ์ดังกล่าวกับการได้ผล APST เป็นบวก ($p = 0.064$)*

สรุป: *การทดสอบ ASST และ APST สามารถตรวจหาปฏิกิริยาต่อตนเองได้ใกล้เคียงกันในผู้ป่วยโรคลมพิษเรื้อรังชาวไทย จึงไม่มีความจำเป็นในการทำการทดสอบ APST ซึ่งเป็นที่ยอมรับน้อยกว่า ASST*