Journal of Pakistan Association of Dermatologists. 2023;**33(4)**:1529-1534.

Original Article

The autologous plasma skin test and autologous serum skin test positivity rate in chronic spontaneous urticaria

Sarah Diba, Nopriyati, Inda Astri Aryani, Muhammad Athuf Thaha, Stella Sunur, Deddy

Department of Dermatology and Venereology, Faculty of Medicine Universitas Sriwijaya/ Dr. Mohammad Hoesin General Hospital, Palembang, South Sumatera, Indonesia.

Abstract

Background The autologous plasma skin test (APST) and autologous serum skin test (ASST) are simple, semi-invasive, inexpensive, and easy to perform diagnostic test for chronic spontaneous urticaria (CSU). However, the positivity rate of both tests was varied. The study aims to compare APST and ASST positivity rate in CSU patients, and correlate with urticaria activity.

Methods This study included 36 CSU patients underwent testing with APST and ASST. Urticaria activity was assessed by weekly urticaria activity score (UAS7). We observed positivity APST and ASST among moderate-severe and mild urticaria activity.

Results Twenty-two of 36 CSU patients was female, with mean age was 28± 9.969 years old. The positivity rate of APST was higher than ASST (83.33% vs. 66.67%). Positive APST with negative ASST were 25%, meanwhile negative APST with positive ASST were 8.33%. Not only were no statistically significant differences in APST and ASST, but also age, sex, and ASST diameter with urticaria activity (p = 0,378, p = 0.968, p = 1.000, p = 0.826, respectively). The positiveness rates of APST were statistically significant higher in moderate-severe activity than mild activity CSU patients (p = 0.008).

Conclusion In conclusion, positivity rate of APST was higher than ASST among patients with CSU. Furthermore, positivity APST was associated with moderate-severe urticaria activity, and may considered as a screening test to predict urticaria activity in CSU patients.

Key words
Chronic spontaneous urticaria; Screening test; Autologous plasma skin test, Autologous serum skin test, Urticaria activity score.

Introduction

Chronic urticaria (CU) is a mast cell driven skin disease characterized by the development of wheals, angioedema, or both for more than 6 weeks, and classified as spontaneous (CSU) and inducible (CIndU). CSU comes with known cause (type I autoimmunity and type IIb autoimmunity, or mast cell–activating autoantibodies) and unknown causes, while CIndU is provoked by, for example, cold, heat, pressure, friction, or contact e.g. with proteins, among other factors.[1,2] Both types can be present concomitantly.[3]

The prevalence of CSU in the general population ranges between 0.5%-1%,[3] and shows an increasing trend.[4,5] Two mechanism have been postulated to be relevant in patient with CSU. A type I autoimmunity can activate mast cells and

Address for correspondence
Dr. Sarah Diba
Department of Dermatology and Venereology
Dr. Mohammad Hoesin General Hospital
Jl. Jendral Sudirman Km. 3,5, Palembang
South Sumatera, Indonesia
Email: sarah_diba_dr@yahoo.com

basophils by crosslinking IgE autoantibodies (AAbs). A type II autoimmunity, IgG-AAbs bind to IgE or to FcεRI, which might involve complement C5a and C5aR receptor. IgG-AAbs against FcεRII might activate eosinophils and induce subsequent mast cell degranulation.[2] The presence of these autoantibodies is demonstrated by papule and erythematous reaction by intradermal injection of autologous serum or plasma.[6]

The gold standard to established the diagnosis of are basophil histamine release assay and flow cytometric basophil activation test.[7] Autologous serum skin test (ASST) is an *in vivo* test and serve as an effective clinical screening tool for autoreactivity of mast cell activating autoantibodies in patients with CSU.[8,9] Not only eosinophils activated by autoantibodies, but also expressed tissue factor, resulting in thrombin generation. The generation of thrombin has been shown increase vascular permeability and mast cell degranulation. This activation of blood coagulation has been added to be the others mechanism involves in CSU.[10] Therefore, another test for CSU is the autologous plasma skin test (APST) (86-90%), which resulted in more positive responses than ASST (56-68%) as plasma contains more complement and coagulation factors.[7,8,11] The study aims to compare the positivity rate of APST and ASST in patients with CSU, and correlate with urticaria activity.

Methods

A cross-sectional study was conducted at the dermatology outpatient clinic in Dr. Mohammad Hoesin Palembang General Hospital Palembang from October 2020 to September 2021. All patients were subjected to complete history taking, thorough general and dermatologic examination, and physical challenge tests. CSU was diagnosed based on a history of continuous or recurrent urticaria for more than 6 weeks duration, with negative physical challenge tests. Urticaria activity was measured by urticaria activity score over 7 days (UAS7). CSU patients aged more than 18 years old were included. Patients with physical urticaria or urticarial vasculitis, pregnant and lactating women, patients with a history of type I hypersensitivity, and those on antihistamine, systemic corticosteroid, and immunosuppressive therapy were excluded. The study was approved by the Health Research Ethics Committee Dr. Mohammad Hoesin General Hospital Palembang (No.102/kepkrsmh/2020) and a written informed consent was obtained from all patients.

APST and ASST were performed as per standard guidelines. The plasma was prepared by collecting venous blood (4 ml) in a sterile tubes contained sodium citrate 3.2%. These samples were centrifuged for 15 min at 1.250 RPM to obtain plasma. To obtain serum, venous blood (3 ml) was taken in a sterile glass tube without clotting accelerator and allowed to clot at room temperature for 30 minutes. Serum was separated by centrifugation at 1.250 RPM for 15 minutes. Histamine diphosphate (10 μg/ml) and sterile physiological saline (0.9%) were used as positive and negative controls, respectively.

Sterile saline, autologous plasma, autologous serum, and histamine diphosphate were taken with separate insulin syringes (0,1 cc form each solution), and were injected intradermally at least 3-5 cm apart onto the flexor surface of the forearm of patient. The result was accessed at 30 minutes after injection. Test was considered positive if the wheal of autologous plasma and autologous serum was at least 1.5 mm more than those of negative control with sterile physiological saline and also if histamine wheal was at least 3 mm more than the negative control.

Statistical analyses were performed using Statistical Package for Social Sciences Statistics Version 22.0 (IBM Corp., Released 2015, Armonk, NY, USA). Descriptive statistics for studied variables were presented as mean, standard deviation, minimum and maximum values The association between demographic characteristics, and wheal diameter with APST and ASST was assessed using the Fisher exact, Mann-Whitney test, and unpaired *t*-test. A *P*-value of <0.05 was considered significant. Pearson correlation was used to analyze correlation between APST and ASST with urticaria activity.

Table 1 Clinical variables of the study population.

Characteristic	*n = 36*
Age(yrs),mean±SD (min-max)	28± 9.969 (18–59)
17 – 25	20 (55.6)
26 – 35	9 (25)
36 – 45	5 (13.9)
46 – 55	1 (2.8)
>55	1 (2.8)
Sex (%)	
Male	14 (38.9)
Female	22 (61.1)
Urticaria Activity (%)	
Severe	14 (38.9)
Moderate	15 (41.7)
Mild	7 (19.4)
Wheal diameter, mean (min-max)	
APST	5.2 (0.7 – 11.1)
ASST	7.9 (4.2 – 15.2)

Results

A total of 36 patients were included in the study, 22 (61.1%) were females, and 14 (38.8%) were males with mean age was 28±9.969 years old. The most common age range was found at 17-25 years with 20 patients (55.6%). In this study, 14 (38.9%) of the patients were classified as severe, 15 patients (41.7%) as moderate, and 7 patients (19.4%) as mild CSU activity. The range of wheal diameter in APST were 0.7–11.1 mm, while in ASST were 4.2–15.2 mm, as shown in **Table 1**.

APST was positive in 30 patients (83.33%) and ASST was positive in 24 patients (66.67%) out of 36 patients. Twenty-one patients (58.33%) were positive to both ASST and APST, 3 patients (8.33%) were negative to both ASST and APST, 9 patients (25%) were APST positive and ASST negative, and 3 patients (8.33) were negative for APST and positive ASST. There was no significant difference between APST and ASST (p = 0.378) (**Table 2**).

The mean diameter of wheal of APST was significantly larger in moderate-severe activity than mild activity (5.3 mm and 4.4 mm; p= 0.001). However, there was no significance difference of age, sex, and ASST wheal diameter in the two groups (p=0.968, p=1.000, p=0.826, respectively). The positiveness rates of APST were significantly higher in the patient with moderate-severe activity than mild activity (p= 0.008), with moderate correlation (r= 0.534). There was a very weak correlation between ASST and urticaria activity (r = 0.248) (**Table 3**).

Discussion

The Diagnosis of CSU is often delayed, and associated with severely impaired quality of life.[3,12] The diagnostic workup of CSU includes a thorough history, physical examination (including review of pictures of wheals and/ or angioedema), basic tests, and the assessment of disease activity, impact, and control. The basic tests include a differential blood count and CRP and/or ESR, in all patients, and total IgE and IgG-anti-TPO.[1] APST and ASST are simple, semi-invasive, inexpensive, and easy to perform diagnostic test for CSU, however APST claimed to be a more sensitive and reliable *in vivo* skin test than ASST.[12]

In this study, a total of 36 CSU patients are dominated by female (61.1%) with mean age

Table 2 Relationship between APST and ASST in CSU patients

	ASST (%)		*Total (%)*	*p-value*
	Positive	*Negative*		
APST (%)				
Positive	21 (58.33)	9 (25)	30 (83.33)	0.378[a]
Negative	3 (8.33)	3 (8.33)	6 (16.67)	
Total	24 (66.67)	12 (33.3)	36 (100)	

[a] Fisher's exact test.

Table 3 Comparison of clinical variables between moderate-severe and mild urticaria activity.

Characteristic	*Urticaria activity*		*p-value*	*Correlation (r)*
	Moderate-severe	*Mild*		
Age, year, mean (min-max)	25 (18-59)	24 (21-54)	0.968[a]	
Sex				
Male	11	3	1.000[b]	
Female	18	4		
Wheal diameter, mean, (min-max)				
APST	5.3 (0.7 – 11.1)	4.4 (1.2 – 5.9)	0.001*[c]	
ASST	7.8 (4.2 – 15.2)	9.2 (4.2 – 10.1)	0.826[a]	
APST				
Positive	27	3	0.008*[b]	0.534[d]
Negative	2	4		
ASST				
Positive	21	3	0.190[b]	0.248[d]
Negative	8	4		

[a]Mann-Whitney test; [b]Fisher's exact test; [c]Unpaired *t*-test; [d]Pearson correlation.

28±9.969 years old, as reported in other study.[6,13,14] There was 38.9% were classified as severe, 41.7% as moderate, and 19.4% as mild urticaria activity. The mean of wheal diameter in ASST slightly larger than APST (APST=5.2 mm; ASST=7.9 mm). The result of this study was diverse to Asero *et al.* and Aktar *et al.*, which report diameter APST is larger than ASST.[15,16] APST was positive in 83.33% patients and negative in 16.67% patients, whereas positive and negative ASST found in 66.67% and 33.33% patients, respectively. Positive APST with negative ASST found on 25% patients, and negative APST with positive ASST found on 8.33% patients. There was not significant difference between APST and ASST (p=0.378). The results of this study seem to be similar to the previous studies, which indicate that APST is a sensitive method for detection of functional auto antibodies in patients with CSU.[12,15,17] However, this finding is not always consistent.[13,18]

The APST diameter was significantly correlated with moderate-severe urticaria activity (p=0.001), meanwhile there was no correlation between age, sex, and ASST diameter with urticaria activity (p=0.968; p=1.000; p=0.826, respectively) In this study found association of positivity APST with moderate-severe urticaria activity (p=0.008), with moderate correlation (r=0.534). In contrast with this study, Thadanipon *et al.* reported positivity ASST was associated with disease activity.[18] This finding was in accordance with Cugno *et al.* which reported mechanism of CSU is not only type 1 and 2 autoimmunity, but also blood clotting activation.[2,10]

Limitation of this study not included laboratory tests for autoimmune disease related CSU and gold standard examination. Further studies regarding the prognostic and management significance of the autoreactivity demonstrated by positive APST should be performed with a

standardization of the APST examination, larger sample size, and long-term follow-up.

Conclusion

This study found positivity rate of APST was higher than ASST among patients with CSU. Furthermore, positivity APST was associated with moderate-severe urticaria activity. APST may considered as a screening test to predict urticaria activity in CSU patients.

References

1. Zuberbier T, Abdul Latiff AH, Abuzakouk M, Aquilina S, Asero R, Baker D, *et al*. The international EAACI/GA2LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria. Allergy. 2022;77(3):734–66.
2. Kolkhir P, Church MK, Weller K, Metz M, Schmetzer O, Maurer M. Autoimmune chronic spontaneous urticaria: What we know and what we do not know. J Allergy Clin Immunol. 2017;139(6):1772–81.
3. Curto-Barredo L, Riba Archilla L, Roura Vives G, Pujol RM, Giménez-Arnau AM. Clinical features of chronic spontaneous urticaria that predict disease prognosis and refractoriness to standard treatment. Acta Derm Venereol. 2018;98(7):641–7.
4. Lapi F, Cassano N, Pegoraro V, Cataldo N, Heiman F, Cricelli I, *et al*. Epidemiology of chronic spontaneous urticaria: Results from a nationwide, population-based study in Italy. Br J Dermatol. 2016;174:996–1004.
5. Chu CY, al Hammadi A, Agmon-Levin N, Atakan N, Farag A, Arnaout RK, *et al*. Clinical characteristics and management of chronic spontaneous urticaria in patients refractory to H1-Antihistamines in Asia, Middle-East and Africa: Results from the AWARE-AMAC study. World Allergy Organ J. 2020;13(4).
6. Alpay A, Solak Tekin N, Tekin IÖ, Altinyazar HC, Koca R, Çnar S. Autologous serum skin test versus autologous plasma skin test in patients with chronic spontaneous urticaria. Dermatol Res Pract. 2013;2013.
7. Baig SA, Balachandran C, Nayak US. Comparative evaluation of autologous serum skin test and autologous plasma skin test in chronic urticaria. J Pakistan Assoc Dermatologists. 2013;23(4):378–83.
8. Ismail R, Jamil A, Nor N, Bakhtiar M. A comparison of autologous serum, plasma, and whole blood for intradermal autoreactivity testing in patients with chronic spontaneous urticarial: A cross-sectional study. J Dermatol Dermatol Surg. 2022;26(1):6.
9. Niu X, Zhu L, Shi M, Zhang Y, Gao X, Qi R. Association of positive and negative autologous serum skin test responses with clinical features of chronic spontaneous urticaria in Asian patients: A systematic review and meta analysis. Exp Ther Med. 2019;17:2603–13.
10. Cugno M, Marzano A v, Asero R, Tedeschi A. Activation of blood coagulation in chronic urticaria: Pathophysiological and clinical implications. Intern Emerg Med. 2010;5:97–101.
11. Kumaran M, Mangal S, Narang T, Parsad D. Autologous serum and plasma skin tests in chronic spontaneous urticaria: A reappraisal. Indian Dermatol Online J. 2017;8:94–9.
12. Dogheim N, Gheida S, Ghaly N, Ibrahim A, El-Enin A. Chronic idiopathic urticaria: autologous skin tests and treatment. Egyptian J Dermatol Venereol. 2014;34(1):46–52.
13. Boonpiyathad T, Sangasapaviliya A. Autologous serum and plasma skin test to predict 2-year outcome in chronic spontaneous urticaria. Asia Pac Allergy. 2016;6(4):226–35.
14. Nopriyati N, Thaha A, Tjekyan S. Hubungan autologous serum skin test/ASST dengan keparahan klinis urtikaria kronik idiopatik di RSUP Dr. Moh Hoesin Palembang. MAKARA J Heal Res. 2008;12(1):27–35.
15. Asero R, Tedeschi A, Riboldi P, Cugno M. Plasma of patients with chronic urticaria shows signs of thrombin generation, and its intradermal injection causes wheal-and-flare reactions much more frequently than autologous serum. J Allergy Clin Immunol. 2006;117(5):1113–7.
16. Aktar S, Akdeniz N, Ozkol HU, Calka O, Karadag AS. The relation of autologous serum and plasma skin test results with urticarial activity score, sex and age in

patients with chronic urticaria. Postep Derm Alergol. 2015;32(3):173–8.

17. Sajedi V, Movahedi M, Aghamohamadi A, Ghareguzlou M, Shafiei A, Soheili H, *et al*. Comparison between sensitivity of autologous skin serum test and autologous plasma skin test in patients with chronic idiopathic urticaria for detection of antibody against IgE or IgE receptor (FcεRIα). Iran J Allergy Asthma Immunol. 2011;10(2):111–7.
18. Thadanipon K, Wattanakrai P. Comparison between autologous serum skin test and autologous plasma skin test in Thai chronic urticaria patients. J Med Assoc Thai. 2017;100(9):1014–20.