Original Article

Correlation Between Clinical Findings and Results of Autologous Serum Skin Test in Patients with Chronic Idiopathic Urticaria

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Objectives: To investigate the correlation between the clinical characteristics of chronic idiopathic urticaria (CIU) and the results of autologous serum skin test (ASST).

Methods: This prospective observational study was performed in the Motahari Allergy Clinic and included 69 patients referred to the clinic with a diagnosis of CIU. ASSTs were performed on all patients. We compared the disease characteristics, including duration severity, number of urticaria, and size of urticaria between those with positive and negative results of ASST.

Results: Of 69 patients with CIU, 39 (56.5%) were female and 30 (43.5%) were male with a mean age of 32.5 ± 12.3 (range 6–62) years. Patients with a positive ASST had a significantly higher number of wheals ($P = 0.043$) with larger sizes ($P = 0.031$). They also had higher frequencies of wheals (63.8% vs. 46.4% daily; $P = 0.039$) and higher disease activity scores (1.57 ± 0.9 vs. 2.01 ± 1.6; $P = 0.044$). The disease activity score was positively correlated with the size of the wheals, duration of the wheals, duration of the disease, and total number of wheals. Patients with positive ASST results had significantly higher frequencies of arthritis ($P = 0.027$).

Conclusion: Positive ASST results are associated with more severe disease determined by the larger wheals' size, longer duration of the disease, and higher frequencies of the disease. As ASST reveals the autoimmune basis of the CIU, it can be concluded that CIU patients with autoimmune basis will suffer from more severe disease.

Key Words: autologous serum skin test (ASST), chronic idiopathic urticaria (CIU), clinical findings

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Chronic idiopathic urticaria (CIU) is a skin disorder characterized by recurrent pruritic wheals, often accompanying angioedema lasting for at least six weeks.[1] CIU impairs the quality of life to a great extent and thus is one of the most frustrating skin diseases.[2] Studies in the field of its pathogenesis have revealed the autoimmune basis for the disease. It is shown that in about 25–35% of patients, the active IgG autoantibodies against IgE or the high affinity IgE receptor (FceRI) are responsible for the disease manifestations.[3–5] This autoimmune basis of the disease led to the development of a diagnostic test using autologous serum for inducing the wheal-and-flare skin reaction.[1] The autologous serum skin test (ASST) is used for diagnosis of CIU. Previous studies have shown that up to 50–80% of patients with CIU have positive ASST results.[1,6,7] The sensitivity and specificity of ASST are about 80% in the best conditions.[8]

The ASST has become a popular and readily available test for diagnosis of CIU in clinical practice. However, some authors have questioned the usefulness of the test because of the variability of its positivity in patients with CIU, ranging from 4% to 76% in the literature.[9] This may be explained due to patient selection and differences in methodology and re-

Key Points

- Chronic idiopathic urticaria (CIU) is a frustrating skin disorder characterized by recurrent pruritic wheals, often lasting for at least six weeks.
- Therapeutic autologous serum skin tests (ASST) can decrease the severity and duration of CIU by initiating immunosuppressive mechanisms.
- During the present study, some patients requested repeat ASSTs because they claimed that ASST had resulted in the decline of their symptoms.
- Positive ASST results are associated with more severe disease determined by larger wheal size, longer duration of the disease, and higher frequencies of the disease.

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sponse interpretation. Taskapan et al[10] reported that 52.5% of patients with CIU had positive ASST levels, while the positivity rate of ASST was found to be 55.5% in normal controls.

According to these conflicting studies, the value of ASST for CIU diagnosis is yet to be determined. Our knowledge regarding the clinical significance, as well as the correlation of ASST results with disease characteristics, is very limited. We conducted this study in order to investigate the correlation between the clinical characteristics of CIU and the results of ASST.

Materials and Methods

Study Subjects

This prospective observational study was performed from January 2009 through February 2010 in the Motahari Allergy Clinic, a tertiary health center affiliated with the University of Medical Sciences in Shiraz, Iran. We included a total of 69 patients (39 females and 30 males) referred to the clinic with a diagnosis of CIU. The diagnosis was based on the presence of recurrent, itchy hives lasting for at least six weeks and lacking angioedema. The study was approved by the Shiraz University of Medical Sciences Ethics Committee and all subjects gave their written consent. The protocol of the study was approved by the institutional review board at the university.

We excluded patients with physical urticaria and those with angioedema. We also excluded patients who were using corticosteroids, immunosuppressive drugs or antihistamines at the time of venipuncture. Patients with immunosuppressed conditions such as diabetes mellitus, kidney or bone marrow transplants, human immunodeficiency virus (HIV) and those with primary immunodeficiency diseases such as X-linked agammaglobulinemia, severe combined immunodeficiency (SCID), and common variable immune deficiency (CVID) were also excluded from the study.

Study participants received a detailed history regarding the nature of the CIU and its characteristics, and all patients underwent a precise physical examination in order to determine the clinical characteristics of the disease. We recorded the personal and family history of allergy and autoimmune diseases, duration of the disease, accompanying clinical signs and symptoms, duration of skin lesions, frequency of attacks, and skin manifestations. The disease activity was determined according to the EAACI /GA2LEN urticaria activity score[11] (Table 1) ranging from 0 to 3, which is indicative of mild, moderate, or intense disease. Patients were divided into two groups according to ASST results (positive ASST vs. negative ASST), and the clinical findings were compared between these two groups.

Methodology of ASST

Patients were asked to stop antihistamines and steroids at least 72 hours and one week, respectively, before performing the test.[12] To perform the ASST, 5 ml of venous blood was collected in a sterile vacutainer and allowed to clot at room temperature for 30 minutes. The sample was then centrifuged at 2000 rpm for 15 minutes in order to separate the serum. Autologous serum (0.05 mL) was injected intradermally using a 1 mL insulin syringe (30 gauge needle) to the left forearm 2 cm below the cubital fossa and similarly 0.05 mL of 0.9% sterile normal saline (control) was injected intradermally to the left forearm. In addition, 0.05 mL of histamine (10 μg/mL) was injected into the volar aspect of the right forearm as a positive control (Fig.). A serum-induced erythematous wheal with a diameter of 1.5 mm more than the saline-induced response within 30 minutes was taken as a positive test. Wheal and flare response was also measured at 30 minutes.

Table 1. Severity score of chronic urticaria according to EAACI/GA2LEN guidelines[11]

Scores	Wheals	Pruritus
0	None	None
1 Mild	<20 wheals/24 h	Present but not annoying or troublesome
2 Moderate	20–50 wheals/24 h	Troublesome but does not interfere with normal daily activity or sleep
3 Intense	>50 wheals/24 h or large confluent areas of wheals	Severe pruritus, interfering with normal daily activity or sleep

Statistical Analysis

All statistical analyses were performed with the Statistical Package for Social Sciences version 16.0 (SPSS® Inc., Chicago, IL). The results are expressed as mean value ± standard deviation (SD) for 95% confidence interval (CI)

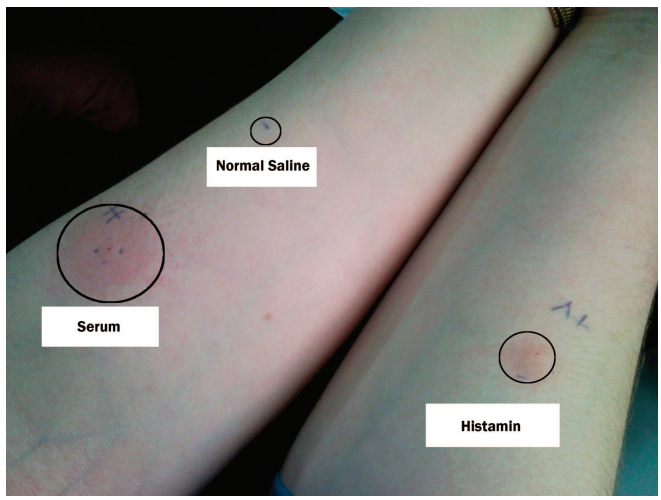


Fig. Positive response to intradermal autologous serum injection provoking a wheal reaction measuring 8 mm in diameter.

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with 5% degree of freedom or proportions. Chi-square test was used to compare the nonparametric data, including the prevalence of clinical signs and symptoms between those with positive and negative results for ASST. Independent t-test was used to compare the parametric data including age, duration of disease, duration of urticaria, and disease severity between two groups. A two-sided *P*-value of <0.05 was considered statistically significant.

Results

Overall, we included 69 patients with CIU; 39 (56.5%) were female and 30 (43.5%) were male, with a mean age of 32.5 ± 12.3 (range 6–62) years. Of the 69 patients with CIU, 41 (59.4%) tested positive for ASST. Table 2 summarizes demographic and disease characteristics of the patients and compares them between two study groups. Patients with positive ASST had significantly higher numbers of wheals ($P = 0.043$) with larger sizes ($P = 0.031$). They also had higher frequencies of wheals (63.8% vs. 46.4% daily; $P = 0.039$) and higher disease activity scores (1.57 ± 0.9 vs. 2.01 ± 1.6; $P = 0.044$). The disease activity score was positively correlated with size of the wheals ($r = 0.561$, $P = 0.002$), duration of the wheals ($r = 0.436$, $P = 0.013$), duration of the disease ($r = 0.312$, $P = 0.022$) and total number of wheals ($r = 0.351$, $P = 0.031$). Female patients (2.31 ± 1.5 vs. 1.24 ± 1.3; $P = 0.035$) and those with a positive family history of allergy (2.28 ± 1.6 vs. 1.34 ± 0.8; $P = 0.039$) also had higher scores of disease activity. There wasn't any correlation between age and disease activity score ($r = 0.021$, $P = 0.543$).

Table 3 compares the prevalence of clinical manifestations in patients with positive and negative ASST results. The most common clinical finding was pruritus, detected in 66 (95.7%) patients, followed by erythema in 61 (88.4%) patients, and fatigue in 38 (55.1%). Patients with positive ASST results had significantly higher frequencies of arthritis ($P = 0.027$). However, there wasn't any significant difference between both groups regarding the frequencies of other clinical findings.

Table 2. Demographic and disease characteristics of 69 patients with chronic idiopathic urticaria with positive and negative ASST results[a]

Characteristics	Total (n = 69)	ASST negative (n = 28)	ASST positive (n = 41)	P[c]
Mean age (yr)	32.5 ± 12.3	33.2 ± 14.2	32 ± 11.2	0.605
Sex				
Female	39 (56.5)	13 (46.4)	26 (63.4)	0.098
Male	30 (43.5)	15 (53.6)	15 (36.6)	
Duration of disease (mo)	40.1 ± 46.1	41.6 ± 36.9	38.8 ± 37.3	0.842
<6 mo	11 (15.9)	4 (14.3)	7 (17.1)	
0.5–1 yr	25 (36.2)	11 (39.3)	14 (34.1)	0.673
1–2 yr	12 (17.4)	5 (17.9)	7 (17.1)	
>2 yr	21 (30.4)	8 (28.6)	13 (31.7)	
Duration of wheals (hr)	18.8 ± 0.61	17.4 ± 6.2	19.6 ± 8.9	0.264
Total number of wheals	28.3 ± 2.3	24.8 ± 3.1	29.6 ± 4.7	0.043
Mean size of wheals (mm^2)	5.3 ± 1.6	3.11 ± 0.9	8.6 ± 4.6	0.031
Frequency of wheals				
Daily	41 (59.4)	13 (46.4)	28 (68.3)	
1–3 per wk	19 (27.5)	8 (28.5)	11 (26.8)	0.039
1–3 per mo	9 (13.1)	7 (25.1)	2 (4.9)	
Positive family history of allergy	29 (42)	9 (32.1)	20 (48.8)	
Disease activity score	1.89 ± 1.1	1.57 ± 0.9	2.01 ± 1.6	0.044
Mild	20 (28.9)	8 (28.5)	12 (29.2)	
Moderate	38 (55.1)	15 (53.7)	23 (56.1)	0.091
Intense	11 (16)	5 (17.8)	6 (14.7)	
Food or drug allergy	48 (69.6)	20 (71.4)	28 (68.3)	0.156
Additives food allergy[b]	22 (31.9)	7 (25)	15 (36.6)	0.211

Values are presented as n (%) and mean ± SD.

[a] *ASST, autologous serum skin test.*

[b] *In Iranian culture, spices such as pepper, turmeric, saffron, and sauce are categorized as food additives.*

[c] *P-value for comparison of the results between ASST positive and ASST negative patients.*

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Table 3. Comparing the frequency of clinical findings between those with positive and negative ASST results[a]

Clinical findings	Total (n = 69)	ASST negative (n = 28)	ASST positive (n = 41)	P^b
Pruritus	66 (95.7)	27 (96.4)	39 (95.1)	0.654
Erythema	61 (88.4)	24 (85.7)	37 (90.2)	0.395
Fatigue	38 (55.1)	15 (53.6)	23 (56.1)	0.462
Malaise	38 (55.1)	16 (57.1)	22 (53.7)	0.538
Hives	35 (50.7)	12 (42.9)	23 (56.1)	0.161
Reflux	29 (42)	14 (50)	15 (36.6)	0.243
Hair loss	25 (36.2)	8 (28.6)	17 (41.5)	0.233
Arthritis	24 (34.8)	6 (21.4)	18 (43.9)	0.027
Anxiety disorders	19 (27.5)	8 (28.6)	11 (26.8)	0.506
Fever	15 (21.7)	6 (21.4)	9 (22)	0.603
Nausea	13 (15.8)	7 (25)	6 (14.6)	0.199
Cough	8 (11.6)	4 (14.3)	4 (9.8)	0.581
Weight loss	7 (10.1)	4 (14.3)	3 (7.3)	0.275
Pigmentation	7 (10.1)	3 (10.7)	4 (9.7)	0.695
Vomiting	6 (8.7)	4 (14.3)	2 (4.9)	0.164

Values are presented as n (%).

[a]*ASST, autologous serum skin test.*

[b]P-*value for comparison of the results between ASST positive and ASST negative patients.*

Discussion

In the present study, we tried to investigate the correlation between the results of ASST and the clinical characteristics of the disease. We found that positive ASST results are associated with more severe disease determined by larger wheal size, longer duration of the disease, and higher frequencies of the disease. As ASST reveals the autoimmune basis of the CIU, it can be concluded that CIU patients with positive ASST will suffer from more severe disease. In other words, the autoimmune reaction will aggravate the clinical outcome and presentation.

ASST is a simple test which can be performed in the office with reliable results. It is based on the presence of autoantibodies against IgE or FceRI.[3–5] These antibodies have been recognized to play an important role in the pathogenesis of CIU.[4,5] However, its accuracy strictly depends on the methodology and case selection. In this study, the ASST was found to be positive in 59.4% of the patients, which is comparable to previous studies.[1,3,5–7] However, some other studies reported lower positivity for ASST in CIU patients.[12–14] In this regard, George et al[12] found that only 34% of the patients had a positive ASST. Sabroe et al[8] found functional autoantibodies in 31% of the CIU patients. Because of variability of the results of this test, some authors have questioned the usefulness of the test.[9] The range of ASST positivity in CIU patients is reported to be 4–76% in different studies. Taskapan et al[10] reported a high frequency of ASST positivity in healthy controls, compared to patients with nonallergic asthma and rhinitis, allergic rhinitis, or bronchial asthma and chronic idiopathic urticaria (55.5% vs. 52.5%). It can be concluded that ASST positivity might be a nonspecific phenomenon and thus its value should be re-evaluated.

The median duration of disease was found to be 41.6 ± 36.9 and 38.8 ± 37.3 months in those with ASST positive and negative results, respectively, which was consistent with Sabroe et al.[8] George et al[12] found that patients with a positive ASST had more frequent attacks compared to the ASST-negative group ($P = 0.038$). These results were further confirmed by Sabroe et al,[8] which showed that patients with autoantibodies in their sera have more severe attacks, according to several parameters including frequency, duration of individual episodes, and sites involved. These are in concordance with our findings, which noted frequent long-lasting episodes in ASST-positive patients.

The most common clinical finding in patients with CIU was found to be pruritus, followed by erythema and fatigue. Patients with ASST positive results had a significantly higher prevalence of arthritis compared to ASST negative patients. Juhlin[14] showed that ASST-positive patients had significantly more systemic symptoms; ie, gastrointestinal symptoms and flushing.[9] However, we did not find any association between clinical findings and the results of ASST, which is consistent with that being previously reported by George et al[12] Previous studies have shown that patients with CIU have increased risk for developing other autoimmune diseases such as thyroid disease, vitiligo, diabetes mellitus, pernicious anemia, and rheumatoid arthritis.[8,15] In this regard, it was shown by George et al[12] that ten patients tested positive for antinuclear antibodies (ANA), out of which one patient also had a positive ASST. Although a higher frequency of autoimmune disease has been reported in patients with autoimmune urticaria, they did not find an increased incidence of other autoimmune disease. None of our patients had simultaneous autoimmune disease or thyroid abnormalities, which are contrary to O'Donnell et al.[15]

As mentioned above, none of our patients had thyroid abnormalities determined by normal values for thyroid stimulating hormone (TSH). In this regard, Leznoff and Sussman[16] studied the association of chronic urticaria with thyroid autoimmunity with the hypothesis that thyroid autoimmunity plays an important role in the pathogenesis of CIU. They found that patients with thyroid autoimmunity had a higher prevalence of CIU and vice versa.[16] We failed to detect such an association, which might be explained due to our small study population. Thus, performing larger studies of thyroid dysfunction in CIU patients will shed some light on this issue.

ASST is a simple, inexpensive, and noninvasive test which can be performed easily in a clinic or office setting by the physician. It is the only available test known for diagnosing CIU in an outpatient setting. ASST also has therapeutic

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potential and is used as accessory management when conventional management is unsuccessful. Therapeutic ASST can decrease the severity and duration of CIU by initiating immunosuppressive mechanisms.[17] However, Staubach et al[18] did not find any evidence supporting the therapeutic potential of ASST. During the present study, some patients referred to our clinic requested repeat ASSTs because they claimed that ASST had resulted in the decline of their symptoms. The efficacy of repeated autologous serum injection in treatment of CIU needs to be evaluated in future studies.

Conclusion

Positive ASST results are associated with more severe disease determined by larger wheal size, longer duration of the disease, and higher frequencies of the disease. As ASST reveals the autoimmune basis of CIU, it can be concluded that the autoimmune reaction in CIU patients will aggravate the clinical outcome and presentation of the disease.

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