Original Article

Comparative Study of Positive Versus Negative Autologous Serum Skin Test in Chronic Spontaneous Urticaria and its Treatment Outcome

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Abstract

Background: Chronic urticaria (CU) is defined as urticaria persisting daily or almost daily for more than 6 weeks and affecting 0.1% of the population. Mast cell degranulation and histamine release are of central importance in the pathogenesis of CU. About 40-50% of the patients with chronic idiopathic urticaria (CIU) or chronic spontaneous urticaria (CSU) demonstrates an immediate wheal and flare response to intradermal injected autologous serum. This led to the concept of autoimmune urticaria (AIU). **Aims:** To determine the occurrence, clinical features, associated clinical conditions, comorbidities of AIU, and to compare this with CSU. This study aimed to find the frequency of autologous serum skin test (ASST)-positive patients among patients with CSU and to identify the clinical and laboratory parameters associated with positive ASST and to compare the treatment outcome. **Materials and Methods:** A prospective correlation study in 110 patients with CSU was conducted, after screening 200 CU patients attending the outpatient Department of Dermatology during from January 2012 to May 2013. Patients were subjected to ASST, complete blood counts, urine routine examination, liver function tests, renal function tests, thyroid function tests (T3, T4, and TSH), and urine analysis. **Results:** Out of 200 CU patients screened, 90 patients had excludable causes based on detailed history and skin prick test, and the remaining 110 patients were considered to have CSU. These 110 patients were further subjected to ASST, serum immunoglobulin E (IgE), and peripheral blood eosinophilia. ASST was positive in 48 patients and negative in 62 patients. Frequency of urticarial attacks and associated diseases such as abnormal thyroid function tests in both ASST-positive and ASST-negative patients did not show any statistical significance. Only attacks of angioedema in ASST-positive individuals were higher and were statistically significant. In the ASST-positive group, 31 (81.25%) patients showed improvement with first-line antihistamines, along with oral prednisolone and injection Histaglobulin and 10 (10.41%) patients did not show any improvement. Thirty-three (43.54%) patients in the ASST-negative group showed improvement while 13 (30.62%) patients did not show improvement. **Conclusion:** ASST is considered a screening test for AIU, which decreases the rate of diagnosis of "idiopathic" form of CU. ASST-positive patients in addition to antihistamines, were treated with short course of oral steroids and weekly Histaglobulin injections for 5 weeks followed by the 3rd and 6th months.

Keywords: Autoimmune, autologous serum skin test, chronic spontaneous urticaria (CSU)

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Introduction

Urticaria is characterized by short-lived swellings of the skin due to transient leakage of plasma into the surrounding superficial dermis. Most types of urticaria are due to activation of dermal mast cells. 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.

Chronic idiopathic urticaria (CIU) or chronic spontaneous urticaria (CSU) is manifested as widespread, short-lived (lasting less than 24 h) wheals occurring daily or almost daily for at least 6 weeks and where a predominant physical cause has been excluded.[1]

Chronic urticaria (CU) affects 0.1% of the population.[2-4] Recent reports have indicated the presence of autoantibodies in about one-third of the patients with CIU. These histamine-releasing autoantibodies are directed against the alpha subunit of the high affinity immunoglobulin E (IgE) receptor FceR1 α. Patients with autoantibodies in their sera have no distinctive diagnostic clinical features and tend to have more severe and unremitting urticaria and usually pose a therapeutic challenge to the treating physician.[5-7] Although basophil histamine release assay is currently the "gold standard" for detecting functional autoantibodies in the serum of patients, it is a time-consuming procedure and difficult to standardize. Western blotting, enzyme-linked immunosorbent assay (ELISA), and flow cytometry using chimeric cell lines expressing the human Fc epsilon receptor I alpha (FceRI α) may be useful in the future.

The autologous serum skin test (ASST), which indicates the presence of functional circulating autoantibodies to FceRI α and/or to IgE, is the best test *in vivo* clinical test for the detection of basophil histamine-releasing activity *in vitro*. ASST has a sensitivity of 70% and specificity of 80%. Disease activity of urticaria correlates directly with the serum levels of FceRI α autoantibody.[8]

As the conventional approach of management of CSU may be unsuccessful, ASST is especially important from the therapeutic point of view as it can help dermatologists to commit themselves to initiate immunosuppressive therapy in such patients.

Materials and Methods

This was a prospective correlation study conducted in 110 CSU patients from January 2012 to May 2013. Two hundred patients of CU presenting with widespread, short-lived (lasting less than 24 h) wheals occurring daily or almost daily for at least 6 weeks were screened, attending the Outpatient Department of Dermatology, RRMCH were included in this study. Approval of the Institutional Ethics Committee of RRMCH was obtained for conducting this study and written informed consent was taken from each patient. Antihistamines were withdrawn 48 h before ASST was performed. None of the patients were taking corticosteroid or immunosuppressive drugs 4 weeks before the study.

All patients were exposed to

- Thorough history taking including duration of the disease, frequency of attacks associated with angioedema, dermographism, or systemic symptoms (fever, joint pain, and abdominal pain).
- General and dermatological clinical examinations.
- Routine investigations included urine and stool analysis, complete blood count (CBC), liver function test (LFT), renal function test (RFT), and thyroid function test (TFT) (T3, T4, and TSH). Investigations for thyroid antibodies, C3 and C4 complement levels, and antinuclear antibodies (ANAs) were not done due to logistical problems.
- Tests for hepatitis C virus and hepatitis B virus.

Patients with physical urticaria, food and drug allergies, and urticarial vasculitis were ruled out after taking detailed history and relevant investigations. Also, all patients underwent skin prick test and patients found positive for common allergens were excluded.

The remaining patients with CSU were admitted in the hospital and exposed to:

- Peripheral blood absolute eosinophil count.
- Serum IgE level.
- ASST technique by using insulin syringe.

Autologous serum skin test technique

Samples of 5 mL of venous blood was drawn into sterile vacutainers without a clotting accelerator and allowed to clot at room temperature for 30 min. The serum was sent to a clinical laboratory for centrifugation at 2,000 rpm for 15 min. Samples of 0.05 mL of autologous serum and 0.9% sterile normal saline were injected seperately intradermally over the uninvolved volar aspect of the forearm leaving a gap of at least 3 cm between the two injection sites. After 30 min, the wheal formed at each injection site was measured. A positive ASST was defined as a serum induced wheal of 1.5 mm or more than the saline induced response at 30 min. During the initial screening of CU patients, skin prick test for allergens was done in all patients to identify any cause and to classify CSU. Clinically all these patients had long-standing classical history of urticarial wheals and flare with few episodes of angioedema; hence, positive

control using injection histamine was not done while performing ASST.

All the patients were grouped into ASST-positive and ASST-negative after the initial tests and the results, along with all other parameters, were compared. Both the ASST-positive and ASST-negative groups were treated with similar drugs in addition to antihistamines; they received short course oral steroids, tablet prednisolone 20 mg once daily for 1 week, along with weekly Histaglobulin injections subcutaneously for 5 weeks and at the 3rd month and 6th month and the outcome was noted. The treatment response was assessed weekly for the first 5 weeks and later during the 3rd month and 6th month.

Statistical studies

Data entry and analyses were performed using statistical package for social sciences (SPSS) version 10 (IBM Corporation, USA). Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on mean ± standard deviation (SD) (minimum-maximum), and results on categorical measurements are presented in number (%). Significance was assessed at 5% (*P* value). $P \leq 0.05$ indicated a significant result. Chi-square/Fischer exact test has been used to find the significance of study parameters on categorical scale between two or more groups. Student's *t*-test (2-tailed, independent) has been used to find the significance of study parameters on a continuous scale between the two groups, intergroup analysis.

Results

One hundred and ten patients with CSU were evaluated, out of 200 patients with CU initially screened for this study. The remaining 90 patients had an excludable cause, after through history and skin prick test. These 110 patients were subjected to ASST, absolute eosinophil count (AEC), serum IgE, and peripheral blood eosinophilia. Positive ASST was seen in 48 (43.62%) patients and negative ASST was found in 62 (56.36%) patients. Clinical data of patients with positive ASST versus negative ASST are shown in [Table 1].

Out of 110 patients, 37 (33.63%) patients were males and 73 (66.36%) patients were females. The male:female ratio was 1:1.9. Negative ASST was found in 40 females (67.74%) and 22 males (32.25%). Positive ASST was found in 33 (64.58%) females and 15 (35.41%) males. However, there was no significant difference between both the genders ($P = 0.641$).

Out of 110 patients, the minimum age was 5 years and maximum age was 60 years. The average age observed was 30.23 years. The mean age of patients with ASST-positive patients was 28.54 ± 13.50 years and the mean age of patients with ASST-negative patients was 31.55 ± 14.43 years but this difference was not significant ($P = 0.262$) [Table 2].

Table 1: Comparison of study variables between autologous serum skin test-positive and autologous serum skin test-negative patients

Study variables	ASST-positive *n* = 48 (%)	ASST-negative *n* = 62 (%)	*P* value
Age in years, Mean+SD†	28.54±13.50	31.55+14.43	Chi-square: 2.3 *P*=0.262
Sex			Chi square: 0.217 *P*=0.641
Male	15 (35.41)	22 (32.25)	
Female	33 (64.58)	40 (67.74)	
Frequency of attacks			Chi-square: 2.79 *P*=0.599
Everyday	20 (41.66)	19 (30.6)	
Alternate days	14 (29.16)	23 (37.09)	
Once in 3 days	11 (22.91)	15 (24.1)	
Once in 1 week	3 (6.25)	5 (8.06)	
Distribution of wheals			
Generalized	46 (97.91)	49 (95.16)	
Localized	2 (2.08)	13 (4.83)	
Associated disease (Abnormal TFT)	8 (16.66)	5 (8.06)	*P*=0.7
H/o‡ drugs	7 (14.58)	4 (6.45)	
Family H/o	13 (27.08)	7 (11.29)	
Angioedema	13 (33.33)	8 (14.51)	*P*=0.031*
Presence of diurnal variation	12 (25)	19 (25.80)	

*$P \leq 0.05$ = Statistically significant, ASST = Autologous serum skin test, TFT = Thyroid function test, †SD = Standard deviation, ‡H/o = History of

The minimum duration of an illness was 2 months while the maximum was 120 months. An average duration of illness was 16.34 months. However, there was no significant increase in duration of the disease in the ASST-positive group in comparison to that of the ASST-negative group (P = 0.498). The frequency of urticarial attacks in both ASST-positive and ASST-negative groups did not show any statistical significance. Thirteen (33.33%) patients in the ASST-positive group and eight (14.51%) patients in the ASST-negative group had angioedema; this was statistically significant (P = 0.031).

Associated diseases of thyroid abnormality were noted in eight (16.66%) of the ASST-positive patients and five (8.06%) of the patients in the ASST negative group, which was statistically not significant (P = 0.166). Seven patients were hypothyroid with elevated TSH levels and one was hyperthyroid in the ASST-positive group. All five patients in the ASST-negative group were hypothyroid. There was nonsignificant difference between ASST-positive and ASST-negative groups regarding routine investigations. (P = 0.7) [Table 3].

Patients with an autoimmune urticaria (AIU) have more severe unremitting urticaria. ASST-positive patients in addition to antihistamines were treated with short course oral steroids, along with weekly Histaglobulin injections subcutaneously for 5 weeks and at the 3rd month and 6th month. ASST-negativ e patients who did not show improvement with first-line antihistamines were also administered with oral steroids and injection histaglubulin, and showed significant improvement in the disease activity.

In the ASST-positive group, 31 (81.25%) patients showed improvement after treatment with injection Histaglobulin in addition to antihistamines, 10 (10.41%) patients did not show any improvement while seven (8.33%) patients were lost to follow-up. Thirty-three (43.54%) patients in the ASST-negative group showed improvement while 13 (30.62%) patients did not show improvement and the remaining 16 (25.80%) were lost to follow-up. Out of 48 ASST-positive patients, 31 patients showed significant improvement in disease activity upon addition of oral steroids and injection Histaglobulin, along with first-line antihistamines. Thirty-three ASST-negative patients out of 62, who did not show improvement with first-line antihistamines or who failed to achieve remission were also given oral steroids tablet prednisolone 20 mg once daily for 1 week and injection Histaglobulin and showed a marked reduction in symptoms such as the appearance of wheals, duration and frequency of occurrence of the disease; they were assessed during the regular weekly follow-up for the first 5 weeks and later at the 3rd month and 6th month. However, this was not statistically significant between positive and negative ASST groups regarding the treatment outcome (P = 0.328) [Table 4].

Table 2: Age distribution of patients

Age in years	Positive ASST n = 48 (%)	Negative ASST n = 62 (%)
1-10	5 (10.41)	4 (6.45)
11-20	7 (14.58)	9 (14.51)
21-30	17 (33.33)	22 (35.48)
31-40	11 (22.91)	11 (17.74)
41-50	5 (10.41)	10 (16.12)
51-60	3 (8.33)	6 (9.67)

Chi-square = 2.3, P value = 0.8, ASST = Autologous serum skin test

Discussion

The basophil histamine release assay is currently the gold standard for detecting functional autoantibodies in the serum of patients with CU. But the disadvantages of this procedure are that it is difficult to standardize as it requires fresh basophils from healthy donors, is time-consuming, and it remains confined to research centers. Western blot, ELISA, and flow cytometry may be useful for screening in the future but these need to be validated.

Table 3: Investigations in autologous serum skin test-positive and autologous serum skin test-negative patients

Investigations	Total no. of patients n = 200 (%)	ASST-positive n = 48 (%)	ASST-negative n = 62 (%)	*P value
Raised AEC	33 (16.25)	13 (27.08)	7 (11.29)	0.806
Peripheral blood eosinophilia	37 (18.5)	11 (22.91)	13 (20.96)	0.730
Raised IgE†	83 (41.5)	29 (60.41)	27 (43.54)	0.883
Thyroid abnormality	19 (9.5)	8 (16.66)	5 (8.06)	0.166
Other investigations				
Low hemoglobin	16 (8)	4 (8.33)	3 (4.83)	
Raised WBC	19 (9.5)	2 (4.16)	5 (8.06)	
Raised ESR	29 (14.5)	5 (10.41)	7 (11.29)	
Raised ASLO	23 (11.5)	6 (12.5)	4 (6.45)	

*$P \leq 0.05$ = Statistically significant, ASST = Autologous serum skin test, AEC = Absolute eosinophil count, WBC = White blood cell, ESR = Erythrocyte sedimentation rate, ASLO = Antistreptolysin O, †IgE = Immunoglobulin E

Table 4: Response to treatment and follow-up in autologous serum skin test-positive and autologous serum skin test-negative patients

Treatment outcome follow-up Tablet prednisolone 20 mg OD† × 7 days and injection Histaglobulin subcutaneous 1,2,3,4,5 weeks and 3,6 months	ASST-positive *n* = 48 (%)	ASST-negative *n* = 62 (%)	Chi-square: 2.2, **P* = 0.328
⁺Improved	31 (81.25)	33 (43.54)	
No improvement	10 (10.41)	13 (30.62)	
Lost to follow-up	7 (8.33)	16 (25.80)	

**P* ≤ 0.05 = Statistically significant, ASST = Autologous serum skin test, ⁺Criteria for assessment during follow-up: Reduced wheals/flare, frequency and duration of disease, angioedema; †OD = Once daily

ASST is the simplest and the best *in vivo* clinical test for the detection of basophil histamine-releasing activity. ASST is simple, semi-invasive, inexpensive, and easy to perform. Results can be obtained within 30 min. The test could be useful in India as it is economical and cost-effective in comparison to basophil histamine release assay.[9,10] The finding of ASST positivity in 43.62% of the patients in our study was comparable with 27-60% positivity reported previously.[1,2,11]

Sabroe *et al*. found evidence of functional autoantibodies in 31% of the 107 patients with CU.[12] Zweiman *et al*. reported basophil histamine-releasing activity in 30% of 70 CU sera[13] while Tong *et al*. found that 52% of 50 CU sera released histamine from basophils.[14]

A study conducted by Sabroe *et al*.[8] and Azim *et al*.[15] concluded that patients with autoantibodies in their sera have more severe attacks according to several parameters including frequency, duration of individual episodes, and more episodes of angioedema in ASST-positive patients but this was statistically not significant. This was in concordance with our study regarding frequency and duration of individual episodes but there was more occurrence of angioedema in ASST-positive patients in our study, which was statistically significant.

Regarding the sex and age distribution between ASST-positive group and ASST-negative group, there was no significant difference in our study.

The association of CU with thyroid autoimmunity has been studied by Leznoff *et al*.[16] and it was postulated that thyroid autoimmunity may play a role in the pathogenesis of CU and angioedema. A study conducted by Noemi Bakos *et al*.[17] observed a relationship between AIU and autoimmune thyroiditis. In a study conducted by Krupashankar *et al*.[18] a significantly higher incidence of *H. pylori* antibodies and thyroid antibodies were detected in ASST-positive patients in comparison to ASST-negative patients. Autoimmune diseases such as thyroid disease, vitiligo, diabetes mellitus, pernicious anemia, and rheumatoid arthritis were reported more commonly in patients with AIU.[18] However, in our study there was no significant difference in thyroid levels in both the groups.

In our study group, patients who were both ASST-positive and ASST-negative having severe unremitting urticarial attacks not subsiding to antihistamines received immunotherapy in the form of short course of oral corticosteroids for 1 week, along with subcutaneous injection Histaglobulin weekly for 5 weeks followed by the 3rd month and 6th month, and showed improvement in the form of decreased severity of symptoms, appearance of wheals, and duration and frequency of occurrence of the disease assessed during the follow-up for receiving the treatment.

Histaglobulin, a product of the serum institute of India contains histamine with human immunoglobulin. When administered subcutaneously, it forms antibodies against histamine, which increases the histamine-binding capacity of the serum. It is indicated for the treatment of atopic dermatitis, allergic rhinitis, and chronic allergic states.[19,20] This form of immunotherapy could prove to be useful in India as it is economical, cost-effective, and can be an alternative to the use of expensive drugs such as cyclosporine and omalizumab in the treatment of CU. Further large scale studies can substantiate the use of immunotherapy in AIU, which are resistant to conventional anti-histamine therapy.

Conclusion

ASST is considered a screening test for AIU, which decreases the rate of diagnosis of "idiopathic" form of CU. Our study showed that there is no difference statistically between ASST-positive and ASST-negative patients in terms of epidemiologic details except for the frequency of attacks of angioedema. In our study, the follow-up period of patients was only 6 months but there is a need for more studies and a longer follow-up period after treatment to conclude the significance of ASST and its treatment outcome with Histaglobulin immunotherapy.

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Conflicts of interest

There are no conflicts of interest.

References

1. Mamatha G, Balachandran C, Prabhu S. Chronic idiopathic urticaria: Comparison of clinical features with positive autologous serum skin test. Indian J Dermatol Venereol Leprol 2008;74:105-8.
2. Krupashankar DS, Shashikala K, Madala R. Clinical and investigative assessment of patients with positive versus negative autologous serum skin test: A study of 80 south Indian patients. Indian J Dermatol 2012;57:434-8.
3. Goh CL, Tan KT. Chronic autoimmune urticaria: Where we stand? Indian J Dermatol 2009;54:269-74.
4. Greaves MW. Chronic urticaria. N Engl J Med 1995;332: 1767-72.
5. Niimi N, Francis DM, Kermani F, O'Donnell BF, Hide M, Kobza-Black A, *et al*. Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in chronic urticaria. J Invest Dermatol 1996;106:1001-6.
6. Grattan CE, Wallington TB, Warin RP, Kennedy CT, Bradfield JW. A Serological mediator in chronic idiopathic urticaria — A clinical, immunological and histological evaluation. Br J Dermatol 1986;114:583-90.
7. Fiebiger E, Maurer D, Holub H, Reininger B, Hartmann G, Woisetschläger M, *et al*. Serum IgG autoantibodies directed against the alpha chain of Fc epsilon RI: A selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? J Clin Invest 1995;96:2606-12.
8. Sabroe KA, Grattan CE, Francis DM, Barr RM, Kobza Black A, Greaves MW. The autologous serum skin test: A screening test for autoantibodies in chronic idiopathic urticaria. Br J Dermatol 1999;140:446-52.
9. Patil S, Sharma N, Godse K. Autologous serum therapy in chronic urticaria. Indian J Dermatol 2013;58:225-6.
10. Kocatürk E, Aktaş S, Türkoğlu Z, Kavala M, Zindanci I, Koc M, *et al*. Autologous whole blood and autologous serum injections are equally effective as placebo injections in reducing disease activity in patients with chronic spontaneous urticaria: A placebo controlled, randomized, single-blind study. J Dermatolog Treat 2012;23:465-71.
11. Vohra S, Sharma NL, Mahajan VK, Shanker V. Clinicoepidemiologic features of chronic urticaria in patients having positive versus negative autologous serum skin test: A study of 100 Indian patients. Indian J Dermatol Venereol Leprol 2011;77:156-9.
12. Sabroe RA, Seed PT, Francis DM, Barr RM, Kobza Black A, Greaves MW. Chronic idiopathic urticaria: Comparison of the clinical features of patients with and without anti-FcepsilonRI or anti-IgE autoantibodies. J Am Acad Dermatol 1999;40:443-50.
13. Zweiman B, Valenzano M, Atkins PC, Tanus T, Getsy JA. Characteristics of histamine-releasing activity in the sera of patients with chronic idiopathic urticaria. J Allergy Clin Immunol 1996;98:89-98.
14. Tong LJ, Balakrishnan G, Kochan JP, Kinét JP, Kaplan AP. Assessment of autoimmunity in patients with chronic urticaria. J Allergy Clin Immunol 1997;99:461-5.
15. Azim ZA, Mongy SE, Salem H. Autologous serum skin test in chronic idiopathic urticaria: Comparative study in patients with positive versus negative test. J Egypt Women Dermatol Soc 2010;7:129-33.
16. Leznoff A, Josse RG, Denburg J, Dolovich J. Association of chronic urticaria and angioedema with thyroid autoimmunity. Arch Dermatol 1983;119:636-40.
17. Bakos N, Hillander M. Comparison of chronic autoimmune urticaria with chronic idiopathic urticaria. Int J Dermatol 2003;42:613-5.
18. Krupashankar DS, Ramnane M, Rajouria EA. Etiological approach to chronic urticaria. Indian J Dermatol 2010;55:33-8.
19. Gushchin IS, Luss LV, Il'ina NI, Pakhomova LA. Therapeutic effectiveness of histaglobin preparations in patients with allergic rhinitis and chronic urticaria. Ter Arkh 1999;71:57-62.
20. Kaur M, Sharma G, Goel AK, Dewan SP. Clinical efficacy and safety of histaglobulin and fexofenadine in patients with chronic idiopathic urticaria. Indian J Pharmacol 2003;35:195-6.