International Journal of Research in Dermatology
Attada S et al. Int J Res Dermatol. 2022 May;8(3):301-307
http://www.ijord.com

DOI: https://dx.doi.org/10.18203/issn.2455-4529.IntJResDermatol20221001

Original Research Article

Comparison of chronic idiopathic urticaria patients with positive and negative autologous serum skin test

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Received: 13 February 2022
Revised: 17 March 2022
Accepted: 19 March 2022

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ABSTRACT

Background: Chronic urticaria, with or without angioedema, has been defined as daily or almost daily symptoms and signs recurring for more than six weeks. Chronic urticaria has an idiopathic etiology in majority. This is called as chronic idiopathic urticaria (CIU) or chronic spontaneous urticaria (CSU). Aim and objectives were to compare the clinical features in CIU patients with a positive autologous serum skin test (ASST) and negative ASST.
Methods: Fifty clinically diagnosed patients of CIU attending the department of DVL OPD at tertiary care teaching hospital, were enrolled based on the inclusion and exclusion criteria.
Results: ASST was positive in 48% (24/50) of patients with CSU. In study of 50 patients, 66% were females and 34% were males. The mean age of patients was 36.8 years. The disease's median duration was 10 months and 11 months in ASST positive and ASST negative groups respectively. Of the 50 patients, 20 patients showed a positive response to the test for simple dermographism, of which 13 were ASST positive. Of the 50 patients, 8 patients gave a history of atopy, of which 5 were in the ASST positive group, 3 were in ASST negative group.
Conclusions: In the present study ASST was positive in 48% of patients with CIU. Majority of ASST positive patients had almost daily attacks. ASST positive patients had a longer duration of persistence of wheals. ASST is a fairly good indicator of autoimmune etiology for chronic urticaria.

Keywords: CIU, ASST, Autoimmune

INTRODUCTION

Urticaria is a common, heterogeneous group of disorder with a large variety of underlying causes. It is characterized by the sudden appearance of transient wheals, each lasting 1-24 hours (h) and/or angioedema lasting up to 72 hours.[1] Chronic urticaria, with or without angioedema, has been defined as daily or almost daily symptoms and signs recurring for more than six weeks.[2] Chronic urticaria has an idiopathic etiology in majority. This is called as CIU or more recently, CSU.[3]

CSU affects 0.5-1% of individuals and significantly reduces the quality of life (QOL).[2] The 30-50% of patients with CSU have an autoimmune basis. They have autoantibodies directed at the FcεR1 receptor located on mast cells and basophils or less commonly against IgE.[4] This subgroup is referred as autoimmune urticaria.

CSU is also associated with antithyroid antibodies in approximately 27% of cases and other autoimmune conditions such as vitiligo, rheumatoid arthritis, pernicious anemia, and insulin-dependent diabetes mellitus.[5,6] The basophil histamine release assay is the gold standard for detecting those functional autoantibodies. This bioassay is challenging to perform and is confined to research centres.[7] Western blot, ELISA and flow cytometry may be useful for screening in the future, needs to be validated.

ASST is a simple *in-vivo* clinical test for the detection of basophil histamine-releasing activity.[8] In 1986, Grattan et al were the first to use ASST to differentiate autoimmune urticaria from CSU.[9] ASST has a sensitivity of approximately 70% and a specificity of 80%.[10] It may be used as an accurate predictive clinical test to indicate functional circulating autoantibodies.[11]

A positive ASST is defined as a red serum-induced wheal with a diameter of 1.5 mm or more than the saline induced response at 30 mins.[12] Diagnosing CAU patients becomes essential as they need high doses of antihistamines and systemic corticosteroids during acute exacerbations. Immunomodulatory drugs have therapeutic benefit in autoimmune urticaria patients recalcitrant to therapy and significantly impaired quality of life.[13]

In this study, we present the proportion of patients with CIU having autoantibodies in their sera using ASST and the comparison of clinical features between test positive and negative groups.

METHODS

Study design

It is a cross sectional study. This study was conducted in the department of DVL of a teaching hospital in Chinakakani, A. P. from September 2020 to December 2021. Ethical clearance was obtained from institutional ethics committee.

Inclusion criteria

Men and women between 12-60 years of age, subjects with a history of wheals for >6 weeks and subjects who gave their consent for the test were included in the study.

Exclusion criteria

Subjects with a history of physical urticaria other than simple dermographism-due to heat, cold, sun exposure, pressure, urticaria due to medications, insect bites, food allergy, or other known etiology, cholinergic urticaria, hereditary angioedema and subjects who are pregnant and lactating were excluded from the study.

Sample size

All newly diagnosed, as well as old cases of CIU attending DVL OPD during the study period. All patients were detailed about the study and informed consent was taken.

Data collection

A predesigned Proforma was used for all cases including detailed history, clinical examination findings and necessary laboratory investigations.

Analysis

Data analysis was done in Microsoft excel. Chi-square test of association and z test of proportion was used for statistical analysis.

ASST methodology

About 5 ml of venous blood was collected in a sterile vacutainer and allowed to clot at room temperature for 30 min. Serum was centrifuged at 2000 rpm for 15 min and 0.05 ml of autologous serum was injected intradermally, in uninvolved skin, using a 1 ml insulin syringe (30-gauge needle) into the right forearm 5 cm below the cubital fossa. Similarly, 0.05 ml of 0.9% sterile normal saline (control) was injected intradermally 2 cm proximally. A serum induced erythematous wheal with a diameter of 1.5 mm more than the saline induced response within 30 min was taken as positive.

RESULTS

Fifty patients diagnosed with chronic urticaria who met the inclusion criteria were studied, and the following results were observed.

Out of 50, 17 were males, 33 females. The age of the youngest patient was 14 years, and that of the oldest was 60 years. Out of 50 patients, 24 showed a positive ASST response (48%) in wheal and flare response, which was 1.5 mm more than the saline control read at 30 minutes.

Figure 1: Positive ASST.

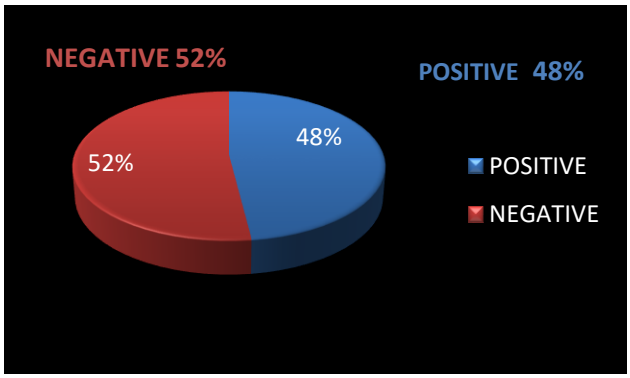

Figure 2: ASST results.

Patients were divided into two groups, i.e., ASST positive and ASST negative, based on the results, to evaluate various epidemiological and clinical parameters.

Age distribution

The mean age of the patients was 36.8 years. The age of the youngest patient was 14 years and that of the oldest was 60 years. Mean and standard deviation of ASST positive group-4.8 and 2.38. Mean and standard deviation of ASST negative group-5.2 and 1.30. A t test for two independent means was used. P=0.4, statistically not significant. There was no significant difference found between age groups and ASST results.

Table 1: Age distribution among ASST positive and ASST negative groups.

Age distribution (Years)	ASST positive	ASST negative
11-20	2	3
21-30	5	6
31-40	8	6
41-50	6	5
>50	3	6

Gender distribution

Of 50 patients studied, 17 were males and 33 were females. Out of 17 males, 8 (47.05%) were ASST positive. Out of 33 females, 16 (48.48%) were ASST positive.

Table 2: Gender distribution among ASST positive and negative groups.

Gender distribution	ASST positive (%)	ASST negative (%)
Male	8 (47.05)	9 (52.94)
Female	16 (48.48)	17 (51.51)

Z test of proportion is used. P=0.92. Among males and females, statistically, no significant difference was found among ASST positive and negative groups.

Duration of disease

According to the duration of disease, patients were divided into four groups. The median duration was ten months and 11 months in ASST positive and ASST negative groups, respectively. Chi-square test is used. P=0.508. Not statistically significant. There is no significant difference between ASST positive and negative groups in duration of disease.

Simple dermographism

Of the total 50 patients, 20 patients showed a positive response to the test for simple dermographism, of which 13 were in the ASST positive group and seven from the negative group. A statistically significant difference in the presence of simple dermographism was not found among the groups (p=0.93, chi-square test).

Table 3: Duration of disease in years in ASST positive and ASST negative groups.

Duration of disease (Years)	ASST positive (%)	ASST negative (%)
0-1	13 (54.1)	14 (53.88)
1-2	8 (33.33)	5 (19.2)
2-3	1 (4.1)	3 (11.5)
>3	2 (8.33)	4 (15.38)

Table 4: Percentage of individuals with simple dermographism in ASST positive and negative group.

Simple dermographism	ASST positive (%)	ASST negative (%)
Positive	13 (54.1)	7 (26.92)
Negative	11 (45.8)	19 (73.07)

Frequency of attacks

The frequency of attacks per week was divided into three groups; significant relation was noted between frequency of attacks and ASST positivity (p=0.023, chi-square test).

Table 5: Frequency of attacks in ASST positive and negative groups.

Variables	ASST positive (%)	ASST negative (%)
Daily	17 (70.8)	13 (50)
1-3/week	6 (25)	11 (42.3)
1-3/month	1 (4.1)	2 (7.6)

Median duration of persistence of wheals

The median duration of persistence of wheals in the ASST positive group was 2 hours and the ASST negative was ½ hr. ASST positive patients had a longer duration of persistence of wheals when compared to ASST negative group. A highly significant association was noted between ASST positivity and duration of persistence of wheals (p=0.04, chi-square test)

Table 6: Median duration of persistence of wheals in ASST positive and negative groups.

The median duration of persistence of wheals	ASST positive	ASST negative
	2 hours	30 mins

Distribution of lesions

Most of the patients had wheals distributed all over the body. Of the total 50 patients, 37 had wheals distributed

all over the body, of which 18 were in the ASST positive group, 19 in the negative group. There was no statistically significant difference noted between ASST positive and negative groups (p=0.876, chi-square test).

History of atopy

Of the total 50 patients, eight patients gave a history of atopy, of which five were in the ASST positive group; three were in ASST negative group. A statistically significant correlation was not observed between the history of atopy and the ASST result (p=0.370, chi-square test).

Table 7: Statistics of ASST positive and negative individuals with a history of atopy.

H/O atopy	ASST positive (%)	ASST negative (%)
Present	5 (20.8)	3 (11.5)
Absent	19 (79.1)	23 (88.4)

Angioedema

Of the 50 patients, 9 patients (18%) had a history of angioedema. The 41 (82%) had no history of angioedema. Of 24 ASST positive patients, 4 (16.6%) had H/O angioedema and 20 (83.3%) had no H/O angioedema. No statistically significant correlation was noted between the two groups and a history of angioedema (p=0.813, chi-square test).

Table 8: Statistics of ASST positive and negative individuals with a history of angioedema.

H/O angioedema	ASST positive (%)	ASST negative (%)
Present	4 (16.6)	5 (19.2)
Absent	20 (83.3)	21 (80.7)

Thyroid function tests

TFTs were done on all patients. Abnormal TFT was obtained in 2 of 24 ASST positive patients and 2 of 26 ASST negative patients p=0.933, statistically not significant.

DISCUSSION

Urticaria is a prevalent condition that affects up to 20% of the population in their lifetime.[14] In about 80% of cases, no external allergen or contributory process is identified and thus termed CIU or CSU.[14] About 30-50% of CSU patients have circulating histamine, releasing autoantibodies to high-affinity IgE receptors on basophils and mast cells called autoimmune urticaria.[7]

ASST is the most straightforward test for detecting that auto antibodies.[7] It is an in-vivo intradermal test for measurement of histamine activity, released by basophilic degranulation. Besides detecting autoimmune basis, ASST helps identify the amount of severity and prognosis of CIU and approach specific therapeutic options. The present study was conducted based on ASST to assess its association with various clinical parameters of CIU.

ASST positive

In the present study, 48% of patients with CIU had shown positive ASST. Earlier studies reported about 25-50% of CIU patients to be ASST positive. A positive test is suggestive of an autoimmune basis but not diagnostic for a patient's urticaria. Confirmation is needed by in vitro testing of the patient's serum for anti-FcεR_1 or anti-IgE autoantibodies.

Vohra et al in their study, had 46% cases with ASST positivity demonstrating an autoimmune process initiating urticaria.[15] Kumar et al study found 41.6% were ASST positive.[16] The reported prevalence of ASST positivity in chronic urticaria patients varies from 35 to 58% in various studies.[17-19] Bakos et al had 54% and Colgecen et al had 63% positivity of ASST in autoimmune urticaria.[20,21] The percentages of patients with CAU among CSU patients vary widely in different studies conducted in India and abroad.

Present study is consistent with Vohra et al and Kumar et al in ASST positivity.[15,16]

Age distribution

In the present study, the age distribution was divided into five groups ranging from 12 years to more than five years. The age of the youngest patient was 14 years, and that of the oldest was 60 years. The mean age of the patients studied was 36.8 years. Of 24 ASST positive, the majority belongs to the age group of 31-40 years. T test for two independent means was used p=0.44, which is statistically not significant. There was no statistically significant difference in age distribution between ASST positive and negative groups.

In Mamata et al study, there was no significant difference in age distribution among patients with and without auto antibodies.[22] Of the total 100 patients studied, more number belongs to 21-30 years. and 41-50 years. The mean age of presentation was 34 years.

In Kumar et al study, the age range was 18-75 years. The mean age of patients was 36.7 years.[16] The majority belonged to 21-30 years.

Gender distribution

In the present study, out of the 50 patients studied, 17 (34%) were males, 33 (66%) were females. Out of 17 males, 8 (47.05%) were ASST positive and 9 (52.94%) were ASST negative. Out of 33 females, 16 (48.48%) were ASST positive, and 17 (51.51%) were ASST

negative. Of 24 ASST positive patients, 8 were males, which constitutes about 33.33% and 16 were females, which constitutes approximately 66.66%. The female preponderance of ASST positivity is observed in the present study.

Kumar et al studied a total of 48 patients. There were 20 (41.6%) males and 28 (58.3%) females. The male: female ratio was 1:1.4.[16] Sabroe et al studied female preponderance.[23] Beevi et al study showed 34% males, 66% females.[7]

Duration of disease

In the present study, the median duration of disease was 10 months and 11 months in ASST positive and ASST negative groups, respectively. Based on the duration of the disease mainly divided into four groups.

Of 24 ASST positive patients' majority (54.1%) were presented with less than or equal to 1-year duration of disease, 33.33% were presented with 1-2 years duration of disease.

Of 26 ASST negative patients majority (53.8%) were presented with less than or equal to the 1-year duration of disease. The 19.2% were presented with 1-2 years duration of disease.

Chi-square test is used. P=0.508, which is not statistically significant. Statistically, a significant difference was not found in disease duration between ASST positive and negative groups.

Mamatha et al study reported median duration was 12 months and 15 months in ASST positive and ASST-negative groups, respectively.[22] There was no significant difference in the duration of disease between the two groups. Bajaj et al study showed the duration of urticaria ranged from 6 months to 32 years (median 30 months) in ASST positive patients and six months to 10 years (median: 36 months) in ASST negative patients.[24]

Simple dermographism

In the present study, 40% (20/50) showed a positive response to simple dermographism. Of which 13 were in the ASST positive group and seven from the negative group. A statistically significant difference in the presence of simple dermographism was not found among the groups. Among 24 ASST positive patients, 13 (54.1%) with positive, simple dermographism and 11 (45.8%) with negative dermographism. Out of 26 ASST negative patients, 7 (26.92%) with positive dermographism and 19 (73.07%) with negative dermographism.

Mamatha et al studied 100 patients.[22] The 32 patients showed a positive challenge test for simple dermographism, which occurred more frequently in ASST-negative patients than in those who were ASST-positive. Rathoriya et al study of total 53 patients 30 (56.6%) showed positive dermographism response.[25] Of 23 ASST positive patients, 14 (60%) with positive, simple dermographism, 09 (39%) with negative dermographism. Of 30 ASST negative patients, 16 (53%) with positive dermographism, 14 (46%) with negative dermographism.

The present study is consistent with Rathoriya et al study.[25]

Frequency of attacks

In the present study, the frequency of attacks per week was divided into three groups as daily, 1-3 attacks per week, 1-3 attacks per month. Of 24 ASST positive group 17 (70.8%) had daily attacks. Six (25%) had 1-3 attacks/week, 1 (4.1%) had 1-3 attacks/month. Of 26 ASST negative group 13 (50%) had daily attacks. The 11 (42.3%) had 1-3 attacks/week, 2 (7.6%) had 1-3 attacks/month. A statistically significant relation was observed in the present study between the frequency of attacks and ASST positivity (p=0.023, chi-square test).

Mamatha et al showed patients with positive ASST had more frequent attacks, which was statistically significant compared to the ASST-negative group.[22] Sabroe et al showed that the frequency of disease was significantly more in ASST positive patients.[23]

Median duration of persistence of wheals

In the present study, ASST positive patients had a longer duration of persistence of wheals when compared to ASST negative group. The median duration of persistence of wheals in the ASST positive group was 2 hours and ASST negative was ½ hour. A highly significant association was noted between ASST positivity and duration of persistence of wheals.

Mamatha et al study found wheals lasted for a significantly longer duration in patients with positive ASST, the median duration being four hours for ASST-positive compared to two hours in ASST-negative individuals.[22] Kumar et al study did not show any difference between ASST positive and negative patients regarding the duration of wheals.[16] The duration of wheals ranged from 10 min to 6 hour with a median of 1 hour.

Distribution of lesions

In the present study, of the total 50 patients, 37 had wheals distributed all over the body, of which 18 were in the ASST positive group, 19 in the negative group. Of 24 ASST positive patients, 18 (75%) had lesions distributed all over the body and 6 (25%) had lesions only on the face and extremities. Of 26 ASST negative patients, 19 (73.07%) had lesions distributed all over the body and 7

(26.9%) had lesions only on the face and extremities. Statistically, a significant difference was not observed between ASST positive and negative groups regarding the distribution of lesions (p=0.876, chi-square test).

In Mamatha et al study, sixty-eight of 100 patients gave details regarding the affected sites.[22] There was no significant difference between ASST positive and negative patients regarding the affected areas. The majority of patients (90%) had generalized lesions involving the trunk and extremities. Rathoriya et al study showed that out of 23 ASST positive patients, 19 (82%) patients had lesions over the whole body, whereas only 4 (17%) had lesions over the face, limbs. The distribution of lesions in ASST positive patients was comparable to that of ASST negative patients.[25]

Present study results are comparable with Mamatha et al study, Rathoriya et al study.[22,25]

H/O atopy

Of the total 50 patients, 8 (16%) patients gave a history of atopy, of which five were in the ASST positive group, 3 were in ASST negative group. Of 24 ASST positive patients, 5 (20.8%) gave a history of atopy and 19 (79.1%) had no atopy history. Of 26 ASST negative patients, 3 (11.5%) had a history of atopy and 23 (88.4%) had no history of Atopy. No statistical correlation between the history of atopy and ASST was noted (p=0.370, chi-square test).

Kumar et al study found regarding atopy and there was no significant difference between serum positive and serum negative groups. It is 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 a high level of IgE can prevent the binding of anti-FcεR1α antibodies to the receptor, already saturated by immunoglobulin.[16] Kulthanan et al and Caproni et al studies also found no significant difference between serum positive and serum negative groups.[24,26]

Rathoriya et al study of 23 ASST positive patients, family history of atopy was present in 8 (35%) patients and absent in 15 (65%) patients.[25] No relation found between results of ASST and the presence of the family history of atopy. The present study was consistent with these studies.

Angioedema

In the present study, 9 (18%) had a history of angioedema, of which 4 (44.4%) were in the ASST positive group, 5 (55.5%) in the ASST negative group. Of 24 ASST positive patients, 4 (16.6%) had H/O angioedema, 20 (83.3%) had no H/O angioedema. Of 26 ASST negative patients, 5 (19.2%) had H/O angioedema, 21 (80.7%) had no H/O angioedema. Statistically there was no significant difference between ASST-positive or negative patients regarding the history of angioedema (p=0.813, chi-square test).

In Mamatha et al study, angioedema occurred in 15 of our 100 patients.[22] Significant difference was not found between ASST-positive or negative patients in the incidence, frequency of angioedema. In study done by Sabroe et al angioedema occurred in 93 out of 107 cases.[23] No significant difference was found between ASST-positive or negative patients regarding incidence, frequency of angioedema in this study.

Thyroid function tests

In the present study of 50 patients, 4 had abnormal thyroid function tests, of which two were in the positive group; two were in the negative group. Of 24 ASST positive patients, 22 (91.6%) had normal TFT, 2 (8.3%) had abnormal TFT. Of 26 ASST negative patients, 24 (92.3%) had normal TFT, 2 (7.69%) had abnormal TFT. Statistically significant difference was not found between the two groups (p=0.813, chi-square test).

Leznoff and Sussman have studied the association of chronic urticaria with thyroid autoimmunity.[27] They postulated that thyroid autoimmunity might play a role in the pathogenesis of chronic urticaria and angioedema. Kumar et al studied a total of 48 patients.[16] Found abnormal thyroid function test (TFT) value and thyroid autoantibodies in two patients. In this study, TFT and thyroid autoantibodies were measured only if indicated; hence, the finding's significance cannot be commented upon.

Limitation of the present study: Though ASST is an effective test to predict the presence of autoantibodies in CIU, positive ASST does not always mean autoimmune urticaria due to altered prevalence of ASST positivity in CIU as well as varied sensitivity and specificity from different studies. Thyroid autoantibodies which are confirmatory to diagnose thyroid disease were not done routinely in all patients.

CONCLUSION

In the present study ASST was positive in 48% of patients with CIU. There was overall female preponderance in CIU. Majority of ASST positive patients had almost daily attacks. ASST positive patients had a longer duration of persistence of wheals. ASST is a fairly good indicator of autoimmune etiology for chronic urticaria.

The natural course of CIU is usually episodic and self-limited in most patients, but the disorder can cause marked physical and psychological distress. ASST is simple, cheap and effective diagnostic method to detect the presence of autoimmunity in CIU patients. ASST helps in identifying the severity of disease and choosing appropriate therapeutic options accordingly.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

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Cite this article as: Attada S, Rao GV, Kilaru KR, Nukala K. Comparison of chronic idiopathic urticaria patients with positive and negative autologous serum skin test. Int J Res Dermatol 2022;8:301-7.