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## A study on validity of autologous serum skin test in chronic idiopathic urticaria

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### Abstract

**Background and Aim:** Chronic urticaria is a common distressing dermatosis characterized by spontaneous occurrence of wheals lasting for less than 24 hours, with or without angioedema occurring daily or almost daily for more than 6 weeks. The main aim of the present study is to investigate the positivity of ASST and clinical and severity in chronic idiopathic urticaria.

**Methodology:** A total number of 100 cases of chronic idiopathic urticaria reporting to the Dermatology Venereology Leprology Department, Kamineni institute of medical sciences were studied.

**Results:** 46 of them were females and 54 were males. Maximum cases were in the age group of 21-30 years (33%), followed by age group 31- 40 years (26%). The age of the youngest patient was 14 years and that of the oldest was 65 years. Autoantibodies, detected by ASST, were seen in the sera of 43% patients with CIU, comparable to available reports in literature. Presence of autoantibodies was unrelated to gender and the mean age of onset of the disease was earlier in ASST positive patients. Presence of these autoantibodies was significantly associated with more duration of disease, more duration of wheal, frequent attacks of disease and UAS > 5. Angioedema and Abnormal Thyroid profile were significant in ASST positive patients. Dermographism occurred more frequently in ASST Negative patients. There was difference in presentation of clinical symptoms between ASST positive and negative patients but these were not statistically significant. There was difference in CBP, AEC, ESR, RBS Sr IgE Levels and ANA between positive and negative ASST patients, but these results were statistically insignificant.

**Conclusion:** Thus, ASST identifies a sub group of patients with CIU with more severe disease, who are likely to be more symptomatic and would require more aggressive treatment.

**Keywords:** Urticaria, ASST, Angiodema, Thyroid, Autoantibodies

### Introduction

Urticaria is a common condition – with lifetime incidence of approximately 15%, with females being affected more than males <sup>[1]</sup>. Urticaria is a heterogenous group of diseases defined in common as presence of short lived, blanchable erythematous, edematous, cutaneous swellings or wheals secondary to transient dermal edema and vasodilatation <sup>[2]</sup>. The name “Urticaria” is derived from the Latin word URTICA - meaning to burn or hives <sup>[3]</sup>. Mast cells play a central role in the pathophysiology Pruritus, the primary symptom is associated with significant morbidity <sup>[4]</sup>. Approximately 40% of patients with urticaria also experience angioedema (swelling that occurs beneath the skin) <sup>[5]</sup>.

Urticaria is generally classified as acute or chronic, depending on the duration of symptoms and the presence or absence of inducing stimuli <sup>[6]</sup>. Chronic urticaria is a common distressing dermatosis characterized by spontaneous occurrence of wheals lasting for less than 24 hours, with or without angioedema occurring daily or almost daily for more than 6 weeks <sup>[7, 8]</sup>. Chronic idiopathic urticaria, which is synonymous with chronic spontaneous urticaria, is a sub-type of chronic urticarial <sup>[9]</sup>.

In Chronic autoimmune urticaria, circulating immunoglobulin G (IgG) autoantibodies react to the alpha subunit of the high-affinity IgE receptor on dermal mast cells and basophils, leading to chronic stimulation of these cells and the release of histamine and other inflammatory mediators which cause urticaria and angioedema <sup>[10]</sup>.

The Autologous Serum Skin Test (ASST) has been widely adopted internationally as a clinical test to demonstrate circulating endogenous proinflammatory or wheal-inducing

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factors in urticaria patients since it was first described in 1986 [11]. It is regarded as a reliable *in vivo* test for chronic idiopathic urticaria with diagnostic, therapeutic and prognostic implications. A positive autologous serum skin test (ASST) is considered to reflect the presence of anti-FcεRI and/or anti-IgE autoantibodies that are capable of activating mast and basophil cell degranulation [12]. Therefore, ASST can be used as a predictive clinical test to diagnose autoimmune urticaria [13, 14].

Chronic urticaria is often associated with significant morbidity and a diminished quality of life [15]. It is important to determine whether chronic idiopathic urticaria is autoimmune in origin or not. This is especially important since immunosuppressive therapies may be tried if conventional approaches of management are unsuccessful [16]. The present study is undertaken to study positivity of autologous serum skin test in patients suffering with chronic idiopathic urticarial.

Hence the present study emphasize the positivity of ASST and clinical and severity in chronic idiopathic urticaria.

### Materials and Methods

In the present study 100 clinically diagnosed cases of Chronic Idiopathic Urticaria attending the Department of Dermatology, Venereology & Leprosy at kamineni institute of medical sciences, Narketpally were taken.

The study period was from October 2016 – September 2018 This is an cross sectional study.

### Inclusion criteria

All cases of chronic idiopathic urticaria that is recurrent urticarial wheals of > 6 weeks duration were taken.

Both Male and Female patients were included.

Patients between the age of 10 – 70 yrs were included.

Patients who are not on treatment were included.

### Exclusion

Patients who are on treatment for urticaria were excluded.

Patients on steroids, immunosuppressive treatment and chemotherapy are excluded.

Patients who fail to discontinue medication prior to the test were excluded.

### Following details and parameters are noted prior to the test

Detailed History

Cutaneous and Systemic examination

Investigations

### Details included

Informed consent was taken from all patients

1. Duration of disease
2. Duration of wheals
3. Frequency of attacks
4. No of wheals per attack
5. Intensity of Pruritus
6. Associated Angioedema Or Dermographism
7. Associated Systemic Symptoms (fever, joint pain, abdominal pain etc.)
8. Provoking physical factors
9. Food and Drug Intolerance
10. Associated Medical Illness
11. Urticarial Activity Score

Laboratory investigations which are done to exclude

### Systemic causes were

Complete Blood Picture (CBP)

Complete Urine Examination (CUE)

Erythrocyte Sedimentation Rate (ESR)

Random Blood Sugar (RBS)

Liver Function Tests (LFT)

Renal Function Tests (RFT)

Absolute Eosinophil Count (AEC)

Stool for Ova and Cysts.

Serum IgE Levels

Thyroid Function Tests (TFT)

### Urticarial Activity Score [7]

The Urticarial Activity Score (UAS) consisted of the sum of the wheal number score and the itch severity score

**Table 1:** Urticarial activity score interpretation

Score	Wheals	Pruritis
	None	None
1	Mild (<20 Wheals/24hr)	Mild
2	Moderate (20-50 Wheals/24hr)	Troublesome but does not interfere with sleep
3	Intense (>50 Wheals/24 Hrs Or Large Confluent Area Of Wheals)	Severe pruritus, which is sufficient troublesome to interfere with normal daily activity or sleep

One has to add both these scores viz., for both the number of wheals and severity of itch, on a given day.

### Autologous serum skin test interpretation [17, 18]

It is based on wheal size and color

a) Wheal Size

0 NEGATIVE CONTROL

1 WHEAL 1.5mm > NEGATIVE CONTROL

2 WHEAL 3-5mm > NEGATIVE CONTROL

3 WHEAL 6-10mm > NEGATIVE CONTROL SCORE RANGE 0 TO 3

b) Redness Score

0 SKIN COLORED

1 PINK

2 RED (ERYTHEMA)

### Statistical analysis

Descriptive statistics was used to summarize data for comparison between ASST positive and ASST negative group. Chi Square test was used for categorical variables and non parametric test. Man Whitney was used for other variables as they were not

### 1. Asst positivity

ASST was positive in 43 (43%) patients and ASST was negative in 57 (57%) patients.



**Positive asst**



Negative asst

2. Age wise distribution of patients

Table 2: Age Wise Distribution

Sl.no	Age	Asst Positive	Asst Negative	Total Patients	Percentage
1	10-20	4	11	15	15%
2	21-30	18	15	33	33%
3	31-40	12	14	26	26%
4	41-50	6	8	14	14%
5	51-60	2	5	7	7%
6	61-70	1	4	5	5%

Maximum cases were in the age group of 21-30 years (33%), followed by age group 31- 40 years (26%). The age of the youngest patient was 14 years and that of the oldest was 65 years. The mean age at onset of urticaria in ASST positive patients was 33.06 ± 11.5 years while it was 35.24 ± 14.8 years in the ASST negative patients. In the Present Study, age of onset of disease was earlier in positive patients.

Table 3: Gender distribution

Sl.no	Gender	Total	Asst Positivity	Asst Negativity
1	FEMALE	46	20	26
2	MALE	54	23	31

Out of 100 patients 46 were females and 54 were males. ASST was positive in 20 (46.52%) female patients and 23 (53.48%) male patients. ASST was negative in 26 (45.62%) female patients and 31 (54.38%) male patients.

Table 4: Duration of the disease

Sl no	Duration of disease	Asst Positive	Asst Negative	Total
1	3months - 1yr	11	26	37
2	1yr - 3yrs	13	13	26
3	3yr - 5yrs	12	10	22
4	> 5yrs	7	8	15

The mean duration of the disease in ASST positive patients was 3.35 ± 2.71 yrs and 2.54 ± 2.58 yrs in ASST negative patients with P value= 0.0214 which was found to be statistically significant. In the present study the duration of disease in ASST positive patients was more as compared to that of ASST negative patients.

Table 5: Duration of the wheals

Sl no	Duration of wheal	Asst positive	Asst negative	Total
1	30secs - 2hrs	14(36.6%)	30(52.7%)	44
2	2-5hrs	11(25.6%)	9(15.8%)	20
3	5-8hrs	6(14%)	6(10.6%)	12
4	8-10hrs	10(24%)	10(17.6%)	20
5	> 10hrs	2(4.7%)	2(3.6%)	4

The mean duration of the wheal in ASST positive patients was 4.5 ± 3.38 hrs and in ASST negative patients was 3.42 ± 3.22 hrs with P value= 0.024 which was found to be

statistically significant. In the present study the duration of wheal in ASST positive patients was long lasting as compared to ASST negative patients.

Table 6: Frequency of Attacks

Sl no	Frequency of attack	Asst positive	Asst negative	Total
1	Daily	26(60%)	20(35%)	46
2	1-4 episodes/week	11(26%)	23(40%)	24
3	1-4episodes/month	6(14%)	14(25%)	30

In the present study the daily attacks of urticaria was more common in ASST positive patients when compared to ASST negative patients. The percentage of patients with ASST positive were 60% where as the percentage of ASST negative were 35% showing a significant P value = 0.0411. The percentage of patients having 1-3 attacks per week were 26% in ASST positive and 40% in ASST negative patients. The percentage of patients showing in 1-3 urticarial attacks per month were 14% in ASST positive and 25% in ASST negative patients

Table 7: Angioedema

Sl no	Angioedema	Asst positive	Asst negative	Total
1	Present	8(18.6%)	3(5.27%)	11
2	Absent	35(81%)	54(94.7%)	89

Angioedema occurred in 18.6% of ASST positive patients and 5.26% in ASST negative patients with statistically significant P value of 0.022.

Table 8: Dermographism

Sl no	Dermographism	Asst positive	Asst negative	Total
1	Present	5(11.6%)	8(14%)	13
2	Absent	38(88.4%)	49(86%)	87

Dermographism was positive in 11.6% of ASST Positive patients and 14% of ASST Negative patients. But these results were statistically insignificant (P Value = 0.3709)

Table 9: Clinical Features

Sl no	Clinical features	Asst positive	Asst negative
1	Fever	5 (11.6%)	1 (1.8%)
2	Headache	1 (2.3%)	12 (21%)
3	Shortness of breath	4 (9.3%)	10 (17.6%)
4	Atopy	7 (16.3%)	2 (3.5%)
5	Joint pains	7 (16.3%)	4 (7%)
6	Abdominal pain	5 (11.6%)	1 (1.8%)
7	Absent	14 (32.6%)	27 (47.3%)

ASST positive patients presented with fever, atopy and joint pains more frequently than ASST negative patients, whereas breathlessness and headache was frequently seen in ASST negative patients, however both clinical features were statistically insignificant.

Table 10: Medical illness

Sl No	Medical illness	Asst positive	Asst negative
1	Hypertension	2 (4.6%)	3 (5.3%)
2	Diabetes Mellitus	5 (11.6%)	10 (17.54%)
3	Hypothyroidism	8 (18.6%)	3 (5.3%)
4	Absent	28 (65.2%)	41 (71.86%)

Hypertension was present in 4.6% of ASST Positive patients

and 5.3% of ASST Negative patients. Diabetes Mellitus was present in 11.6% of ASST Positive patients and 17.54% of ASST Negative patients. Hypothyroidism was present in 18.6% of ASST Positive patients and 5.3% of ASST Negative patients.

**Table 11:** Uas Score

Sl no	Uas score	Asst positive	Asst negative
1	3	1 (2%)	8 (14%)
2	4	8 (19%)	14 (25%)
3	5	21 (49%)	29 (50%)
4	6	13 (30%)	6 (11%)

Urticarial Activity Score > 5 was more common in the ASST positive patients as compared to ASST negative patients, which is statistically significant with P Value of 0.0272

**Table 12:** Complete blood picture

Sl no	Cbp	Asst positive (%)	Asst negative (%)
1	Anemia	12(28%)	12(21%)
2	Leucocytosis	8(18.60%)	9(15.40%)
3	Normal	23(53.40%)	36(63.20%)

Complete Blood Picture showed Anemia in 28% of ASST Positive patients and 21% of ASST Negative patients. Leucocytosis was positive in 18.6% of ASST Positive patients and 15.8% of ASST Negative patients. It was normal in 53.4% of ASST Positive patients and 63.2% of ASST Negative patients.

**Table 13:** Erythrocyte sedimentation rate

0	ESR	Asst positive (%)	Asst negative (%)
1	RAISED	3(7%)	6(10.50%)
2	NORMAL	40(93%)	51(89.50%)

Erythrocyte Sedimentation Rate was positive in 7% of ASST Positive patients and 10.5% of ASST Negative patients. It was negative in 93% of ASST Positive patients and 89.5% of ASST Negative patients. The values are not statistically significant (P Value = 0.285)

**Table 14:** Random blood sugar

Sl no	Rbs	Asst positive (%)	Asst negative (%)
1	Abnormal	5(11.60%)	10(17.50%)
2	Normal	38(89.40%)	47(82.50%)

Random Blood Sugar was positive in 11.6% of ASST Positive patients and 17.5% of ASST Negative patients. It was negative in 89.4% of ASST Positive patients and 82.5% of ASST Negative patients. Values are statistically insignificant. (P Value = 0.2164)

**Table 15:** Absolute eosinophil count

Sl No	Aec	Asst Positive (%)	Asst Negative (%)
1	Raised	4(9%)	7(12.30%)
2	Normal	39(91%)	50(87.70%)

Absolute Eosinophil Count was positive in 9% of ASST Positive patients and 12.3% of ASST Negative patients. It was negative in 91% of ASST Positive patients and 87.7% of ASST Negative patients with statistically insignificant P

Value = 0.3309.

**Table 16:** Stool for ova and cysts

Sl no	Stool for ova and cysts	Asst positive	Asst negative
1	Present	0	0
2	Absent	43	57

**Table 17:** Serum IgE levels

Sl no	Serum ige levels	Asst positive	Asst negative
1	Raised	2(4%)	0(0%)
2	Normal	41(96%)	57(100%)

Sr. IgE Level was positive in 4% of ASST Positive patients and 0% of ASST Negative patients. It was negative in 96% of ASST Positive patients and 100% of ASST Negative patients. The values are statistically insignificant. (P Value = 0.0912)

**Table 18:** Thyroid profile

Sl no	Thyroid profile	Asst positive (%)	Asst negative (%)
1	Abnormal	0	0
2	Normal	43	57

**Discussion**

Chronic urticaria (CU) is a common skin disorder affecting at least 0.1% of the population [57]. Mast cell degranulation and histamine release is of central importance in the pathogenesis of CU. About 70% of the patients with CU were diagnosed to have chronic idiopathic urticaria (CIU) as no cause could be identified [19].

About 40 - 50% of the patients with CIU, demonstrate an immediate wheal and flare response to intra-dermal injected autologous serum. This led to the concept of autoimmune urticaria (AIU). These patients demonstrate circulating histamine-releasing IgG autoantibody against the high affinity IgE receptor FcεR1α (35 - 40%) on dermal mast cells and basophils or less commonly to IgE itself (5 - 10%) [20].

Basophils and mast cells degranulation leads to release of histamine which is demonstrable by autologous serum skin test (ASST). The ASST; which indicate the presence of functional circulating autoantibodies to FcεR1α and/or to IgE is the best *in vivo* clinical test for detection of basophil histamine-releasing activity *in vitro*. Although basophil histamine-release assay is the gold standard for detecting functional autoantibodies in patient with CIU, this bioassay is difficult to standardize because it requires fresh basophils from healthy donors and is time consuming.<sup>14</sup> Western blot, enzyme linked immunosorbent assays (ELISA) and flow cytometry may be useful for screening in the future, but they need to be validated.

The present study has evaluated patients with chronic idiopathic urticaria by autologous serum skin testing and compared the clinical features and laboratory parameters of patients with positive and negative ASST results.

**1. Percentage**

In the Present study ASST was positive in 43 (43%) patients and ASST was negative in 57 (57%) patients. In study by Vohra *et al.* [13] ASST was positive in 46 (46%) and negative in the other 54 (54%) patients.

## 2. Age distribution of the patients

In the Present study, CIU was found to be more common among the age groups of 21-30 years (33%), followed by 31-40 years (26%). Mean age of onset was  $33.06 \pm 11.5$  yrs in ASST Positive patients and  $35.24 \pm 14.8$  yrs in ASST Negative patients indicating earlier age of onset in Positive patients.

Zeinab Abdel Azim *et al.* [21] found that the mean age in ASST positive group was  $34.3 \pm 10.2$  years, while that of ASST negative group was  $29.6 \pm 11.1$  years

## 3. Gender distribution

In the Present study, Out of 100 patients 46 were females and 54 were males. ASST was positive in 20 (46.52%) female patients and 23 (53.48%) male patients. ASST was negative in 26 (45.62%) female patients and 31 (53.38%) male patients.

Mamatha *et al.* [22] study observed that of a total of 100 patients, 56 were females and 44 males. 18 out of the 34 ASST-positive patients (52.94%) and 38 out of the 66 ASST-negative patients (57.58%) were female, but there was no statistical difference between males and females.

According to A. Ganapathi *et al.* [23], females had a higher percentage (46.4%) of ASST positivity than males (35%), but this was not statistically significant ( $P = 0.43$ ).

## 4. Duration of disease

In the Present study mean duration of the disease in ASST positive patients mean+ S.D is  $3.35 \pm 2.71$  yrs and  $2.54 \pm 2.58$  yrs in ASST negative ( $P$  value= $0.0214$ ), which is significant. Duration of Disease was more in positive patients than in negative patients.

A significant increase ( $P$  value=  $0.002$ ) in the duration of the disease in ASST positive group (median 4 years) in comparison to that of ASST negative group (median 1 year) was observed by Zeinab Abdel Azim *et al.* [21].

All the above studies are in correlation with the present study.

## 5. Duration of Wheal

The mean duration of the wheal in ASST positive patients is  $4.5 \pm 3.38$  hrs and in ASST Negative patients it is  $3.42 \pm 3.27$  hrs with  $P$  value of  $0.024$ , which is statistically significant, indicating duration of wheal is longer in Positive patients.

Mamatha *et al.* [22] observed that Wheals lasted for significantly longer duration in patients with positive ASST. The median duration being 4 hrs for ASST-positive patients compared to 2 hrs in ASST-negative individuals ( $P = 0.001$ ), which was statistically significant. These results were in correlation with the present study

## Frequency of attacks

The percentages of patients with ASST positive were 60% whereas the percentage with ASST negative were 35% showing a significant  $P$  value ( $0.0411$ ). 1-3 attacks per week 26% were ASST positive and 40% were ASST negative. The percentage of patients showing in 1-3 urticarial attacks per month were 14% ASST positive and 25% ASST negative. Patients with ASST positivity were seen to have daily attacks of urticaria when compared to ASST negative patients, indicating that frequent urticarial attacks are more in ASST Positive patients than that of ASST Negative patients

Zeinab Abdel Azim *et al.* [21] study shows that the percentage of patients with more frequent attacks ( $> 5$ /week) were significantly higher in positive ASST group (93.3%) compared to ASST negative (65%) group ( $p = 0.048$ ). This was statistically significant.

## Angioedema

According to the current study angioedema is present in 18.6% ASST positive patients and 5.26% in ASST negative patients with a statistically significant  $P$  value =  $0.02$

In Nettis *et al.* [25] study, the prevalence (69%) of angioedema is significantly higher in the ASST-positive cases as compared with that (43%) in the ASST-negative patients.

## Dermographism

In the present study dermographism was present in the 11.6% of ASST positive patients and 14% of negative patients, which was statistically not significant ( $P$  Value =  $0.3709$ )

Zeinab Abdel Azim *et al.* [21] study observed that 80% of ASST positive patients show dermographism, while 60% in the ASST negative group, This does not show any statistical significance.

## Clinical features

According to Present Study, ASST Positive patients presented with fever, atopy and joint pains more frequently than ASST negative patients, whereas shortness of breath and headache were frequently seen in ASST negative patients, however both clinical features were statistically insignificant.

Vohra *et al.* [26] study shows that Multiple symptoms were associated in 24(52%) ASST-positive and 20 (37%) ASST negative patients respectively. The gastrointestinal symptoms like abdominal pain, diarrhoea, indigestion (13 patients), general malaise, headache, loss of concentration, lassitude, feverish feel and feeling of hot or cold (45 patients), breathlessness/wheezing, palpitations (12 patients) and joint pains (two patients) were more frequent in the ASST-positive cases while symptoms like nausea/vomiting (three patients), flushing (seven patients), joint swelling (one patient) and syncope (three patients) were seen more often in those with negative ASST. However, the difference was not statistically significant ( $P = 0.18$ ).

Urticarial activity score

In the Present study Urticarial Activity Score is  $>5$  was more common in ASST positive patients as compared to ASST negative patients, which is statistically significant.

( $P$  Value =  $0.0272$ )

In Zeinab Abdel Azim *et al.* [21] there was significant increase ( $P$  Value=  $0.005$ ) in UAS in ASST positive group (UAS mean  $\pm$  SD  $4.5 \pm 0.5$ ) in comparison to ASST negative group (UAS mean  $\pm$  SD  $3.9 \pm 0.7$ ).

## Complete blood picture

In the Present Study, Anemia was present in 28% of ASST positive patients and 21% of ASST negative patients. Leucocytosis is present in 18.6% of the ASST positive patients and 15.8% of ASST negative patients. But these values were not statistically significant where  $P$  value was  $0.611$

Krupa shanker *et al.* [27] study found that leucocytosis was present in the 12.1% of the ASST negative patients and

none of them with ASST positive. They also found that the level of haemoglobin was low in 3.1% of ASST negative patients and 6.4% in ASST positive patients. But this was not statistically significant. This is in correlation with the present study.

### Erythrocyte sedimentation rate

In the Present Study, Erythrocyte Sedimentation Rate was 7% in ASST Positive patients and 10.5% in ASST Negative patients which was higher in ASST Negative patients.

### Absolute eosinophilic count

In the Present Study AEC was raised in 9% of ASST positive patients and 12.3% of ASST negative patients. This was not statistically significant (P Value = 0.3309) Krupa shanker *et al.* [27] study observed that raised AEC was seen in 12.1% of ASST negative patients and 8.5% of ASST positive patients, which is not statistically significant.

### Thyroid profile

In the Present Study, Thyroid abnormality was present in 18.26% of ASST positive patients and 5.26% of ASST negative patients. This was statistically significant with P Value = 0.0221.

Mamatha *et al.* [22] observed abnormal TFT values in 3 ASST-positive patients. This study did not find any difference in the incidence of thyroid disease. The above results were not in correlation with the present study. This might be due to low incidence of thyroid abnormalities in ASST positive patients in that study.

### Conclusion

ASST is a simple, out-patient procedure, inexpensive, semi invasive and easy-to-perform test It can be used as a simple screening test to diagnose chronic idiopathic urticaria patients. It is a cost-effective test for detecting Chronic Autoimmune Urticaria patients who have no distinctive clinical features differentiating them from Chronic Idiopathic Urticaria patients. It is especially important from the therapeutic point of view as it may help the dermatologist to initiate immunosuppressive therapy in such patients.

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### Conflict of Interest

None

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