

Original Research Article

## **Diagnostic Role of Autologous Serum Skin Test in Chronic Urticaria and Comparison of Clinical Features with Positive Autologous Serum Skin Test**

Kavitha Dasari<sup>1\*</sup>, Narendar Gajula<sup>2\*</sup>, Razia begum<sup>3\*\*</sup>, Bandhavi Karri<sup>4\*</sup>

<sup>1</sup>Assoc. Professor, <sup>2</sup>Asst. Professor, <sup>3</sup>Senior Resident, <sup>4</sup>Post Graduate,  
\*Department of DVL, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar, Telangana, India.  
\*\*Govt General Hospital, Godavarikhani.

Corresponding Author: Kavitha Dasari

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### **ABSTRACT**

**Background:** Chronic idiopathic urticaria can pose a therapeutic challenge for the physician and can also be distressing for the patient. ASST helps in identification of auto immune urticaria and may stimulate the use of immunotherapy in severe disease that is unresponsive to conventional antihistamines.

**Aim:** To determine the incidence of positive Autologous Serum Skin Test (ASST) in Chronic idiopathic urticaria and comparison of clinical and laboratory parameters of patients with positive and negative autologous serum skin test.

**Methods:** In the present prospective observational study, 60 clinically diagnosed cases of chronic idiopathic urticaria attending the Department of Dermatology, Venereology, Leprology at Chalmeda Ananda Rao hospital were enrolled and ASST was done & results were compared with clinical & other lab parameters.

**Results:** out of 60 enrolled patients ASST was positive in 21(35%) patients and ASST was negative in 39 (65%) patients.

**Conclusion:** ASST was a simple and cost effective test which helps us in the classification of chronic urticaria and its treatment.

**Key Words:** chronic idiopathic urticaria, autologous serum skin test (ASST).

### **INTRODUCTION**

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. [1,2] Urticaria was considered idiopathic in most of the patients before autoimmunity was recognized as a cause. It is still not possible to ascribe a specific aetiology to around 50% of patients presenting with the ordinary presentation of chronic urticaria even after looking for autoreactivity with autologous serum skin test (ASST) or

testing for functional autoantibodies with the basophil histamine release assay. [3] CD203c is a basophil activation marker known to be upregulated by cross-linking of the FcεRIα receptor and may serve as a useful marker to identify chronic idiopathic urticaria. ASST is a simple in-vivo intradermal clinical test for the detection of basophil histamine releasing activity. [4] A positive result in the form of an immediate hypersensitivity reaction (read against a control) signifies the presence of circulating histamine releasing factors (autoantibodies) [5] hence; ASST is a useful diagnostic aid in identifying

chronic autoimmune urticaria. ASST as an investigative technique has been found to have a sensitivity of approximately 70 % and a specificity of 80%.<sup>[6]</sup> Though, basophil histamine release assay is the gold standard for detecting functional autoantibodies, the procedure is lengthy, requires fresh basophils from healthy donors and skilled expertise is desired. For this reason, the test is generally limited to research laboratory centers.<sup>[4,5]</sup> Identification of Autoimmune Urticaria may stimulate the use of immunotherapy in severe disease unresponsive to antihistamine therapy. The present study is undertaken to study the clinical features of Chronic Idiopathic urticaria and correlate them with the results of Autologous Serum Skin test.

## **MATERIALS AND METHODS**

In the present study 60 clinically diagnosed cases of chronic idiopathic urticaria attending the Department of Dermatology, Venereology, Leprology at Chalmeda Ananda Rao hospital were enrolled. The study period was two years (2012 October-14 September). This is a prospective observational study.

**Inclusion criteria:** All cases of chronic idiopathic urticaria i.e. recurrent urticarial wheals of > 6 weeks duration, both sex Age 18 and above.

**Exclusion criteria:** Physical cause of urticaria other than simple dermatographism. Food or drug allergy, Urticarial vasculitis, pregnant women, Age less than 18 years, Failure to discontinue drugs prior to the test. Following parameters are noted on entry into the study - Detailed history, Examination - systemic and cutaneous, Investigations, ASST Procedure. Physical urticaria, food & drug allergies, urticarial vasculitis were ruled out after taking detailed history & relevant lab investigations. Laboratory investigations to exclude systemic causes which included: Blood Picture (CBP), Complete Urine Examination (CUE), Liver Function Tests (LFT), Renal Function Tests (RFT),

Thyroid Function Tests (TFT), Erythrocyte Sedimentation Rate, and Antinuclear Antibody.

## **MATERIALS USED FOR AUTOLOGOUS SERUM SKIN TEST:**

1. Centrifuged serum of patient. 2. Sterile normal saline (0.9%).

**Procedure of Asst:** 2ml of patient venous blood is collected in a sterile glass tube without anticoagulant and allowed to clot for 30 minutes at room temperature. Serum is then separated by centrifugation (2000 rpm) for 15 minutes. Then take 0.05 ml of serum (equal to 2 Units of insulin syringe) and 0.9% of normal is injected separately intradermally over volar aspect of left forearm. A separate syringe should be used for each site of injection. Serum is injected proximally and saline is injected distally. The gap between two injection sites should be at least 5cm. After 30 min. observe for wheal formation at the site of serum injection. If Wheal formation is >1.5mm then Serum induced wheal is considered positive.

**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 nonparametric test. Man Whitney was used for other variables as they were not distributed normally.

## **OBSERVATIONS AND RESULTS**

**Asst Positivity:** ASST was positive in 21(35%) patients and ASST was negative in 39 (65%) patients.

**Age Distribution of the Patients:** Maximum cases were in the age group of 21-30 years (30%), followed by age group 31- 40 years (28.33%). The age of the youngest patient was 18 years and that of the oldest was 80 years. The mean age at onset of urticaria in ASST positive patients was 35.31+ 13.85 years, while it was 33.61 + 13.56 years in the ASST negative Patients.

**Gender Distribution:** Out of 60 patients 26 were females and 34 were males. ASST was positive in 9 (42.85%) female patients and 12 (57.14%) male patients. ASST was negative in 17 (43.58%) female patients and 22 (56.41%) male patients.

**Duration Of The Disease:** The mean duration of the disease in ASST positive patients was  $4.2 \pm 2.58$  and  $7.8 \pm 11.94$  in ASST negative patients. p value= 0.019 which was found to be statistically

significant. In the present study the duration of disease in ASST positive patients were long lasting as compared to ASST negative patients.

### Duration of the Wheals

Table-1: Duration of the Wheals

S.NO	Wheal duration	ASST positive	ASST negative
1	30sec-2Hours	4	23
2	2-4Hours	0	8
3	4-6Hours	5	4
4	6-8Hours	4	1
5	8-10Hours	3	0
6	>10Hours	5	3

Table-2: Frequency of Attacks

FREQUENCY OF ATTACKS				
S.NO.	Frequency of attacks	ASST Positive	ASST negative	P value
1	Daily	16(76.19%)	11(28.20%)	0.00037*
2	1-3/week	3(14.28%)	19(48.71%)	0.0082*
3	1-3/month	2(9.52%)	9(23.07%)	0.195

### Clinical Features:

Table-3: Clinical Features

S.NO.	Clinical features	ASST positive	ASST negative	P value
1	Abdominal pain	1 (4.76%)	3(7.69%)	0.31
2	Breathlessness	3 (14.28%)	5(12.82%)	0.47
3	Headache	6 (28.57%)	2 (5.12%)	0.15
4	Joint pains	2 (9.52%)	2 (5.12%)	1
5	Absent	9 (42.85%)	27 (69.23%)	0.0027*

### Urticarial Activity Score:

Table-4: Urticarial Activity Score

S.NO.	UAS	ASST positive	ASST negative	P value
1	3	0	6(15.38%)	0.014*
2	4	0	9(23.07%)	0.0027*
3	5	7(33.33%)	18(46.15%)	0.0027*
4	6	14(66.66%)	6(15.38%)	0.736

Urticarial Activity Score is >4 in all the ASST positive patients as compared to ASST negative patients, which is statistically significant.

**DERMOGRAPHISM:** It is positive in few patients, but these results were statistically insignificant. Angioedema-Angioedema occurred in 9.52% of ASST positive patients and 23.07% in ASST negative patients. So it is significant in ASST negative patients.

**MEDICAL ILLNESS:** Diabetes-I was present in 4.76% of ASST positive patients, while Diabetes-II is seen in 4.76% of ASST positive patients and 25.64% of ASST negative patients. Hypothyroidism is seen in 28.57% of ASST positive patients and 10.25% of ASST negative patients. Anemia is seen in 19.04% of ASST positive patients and 10.25% of ASST negative patients. Leucocytosis is seen in 4.76% of ASST

positive patients and 7.69% of ASST negative patients. Both the values were insignificant. AEC was raised in 9.52% of ASST positive patients and 17.94% of ASST negative Patients, which was found to be insignificant. ESR was normal in all ASST positive patients, whereas it was raised in 10.25% of ASST negative patients, but these results were insignificant. ESR was normal in all ASST positive patients, whereas it was raised in 10.25% of ASST negative patients, but these results were insignificant. ANA profile abnormality was noticed in 2.56% of ASST negative patients. None of the ASST positive patients showed abnormal ANA profile.

## ILLUSTRATIONS



Fig-1: Positive ASST showing serum induced wheal 1.5 mm greater than normal saline wheal



Fig-2: Urticarial wheal

## DISCUSSION

Chronic urticaria in all of its manifestations is a common affliction. Although rarely life threatening wide spread urticaria and its associated angioedema can be both debilitating and frightening. Approximately 15-20% of the population may experience at least one episode of urticaria in their lifetime and about one quarter of these patients are likely to develop chronic urticaria. [6]

The rational therapy for urticaria is the identification and avoidance of causative agents that directly or indirectly precipitate the eruption. [7] In chronic urticaria the search for a cause is much more difficult. [8] Patients often seek medical attention with the hope that a reversible cause can be identified.

Being able to efficiently apply a cost-effective work up for urticaria is challenging. Therefore, the challenge for the clinician is to try to identify a cause that could lead to a specific treatment or avoidance strategy. [9] The basophil histamine release assay is currently the gold standard for detecting these functional auto-antibodies in the serum of patients with chronic urticaria.

However, this bioassay is difficult to standardize because it requires fresh basophils from healthy donors, is time-consuming and it remains confined to research centers. Western blot, enzyme linked immunosorbent assays (ELISA) and flow cytometry may be useful for screening in the future, but they need to be validated.

ASST is the simplest and the best in vivo clinical test for detection of basophil histamine releasing activity. ASST is simple, semi-invasive, inexpensive, and easy to perform. Results can be obtained within 30 minutes.

It draws attention towards underlying systemic, autoimmune and infective conditions. Provides evidence for rational use of immunomodulators to modify the course of chronic urticaria. 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. In the Present study ASST was positive in 21(35%) patients and ASST was negative in 39 (65%) patients. In the present study, CIU was found to be more common among the age groups of 21-30 years (30%), followed by 31-40years (28.33%) and 41-50years. In the present study, Out of 60 patients 26 were females and 34 were males. ASST was positive in 9 (42.85%) female patients and 12 (57.14%) male patients. ASST was negative in 17 (43.58%) female patients and 22 (56.41%) male patients. In the present study duration of the disease in ASST positive patient's mean  $\pm$  S.D is 4.2  $\pm$

2.58 and  $7.8 \pm 11.94$  in ASST negative. ( $p=0.019$ ), which is significant. The mean duration of the wheal in ASST positive patients is  $3.5 \pm 1.87$  and  $6.5 \pm 8.54$  in ASST negative patients. P value is 0.000, which is highly significant. Patients with ASST positivity were seen to have daily attacks of urticaria when compared to ASST negative patients. The percentages of patients with ASST positive were 76.19% whereas the percentage with ASST negative were 28.20% showing a significant P value. Of the Total patients presenting with 1-3 attacks per week 14.28% were ASST positive and 48.71% were ASST negative. The percentage of patients showing in 1-3 urticarial attacks per month were 9.52% ASST positive and 23.07% ASST negative. According to our study ASST positive patients presented with headache more frequently than ASST negative patients, whereas breathlessness was frequently seen in ASST negative patients, however both clinical features were statistically insignificant.

In the present study Urticarial Activity Score is  $>4$  in all ASST positive patients as compared to ASST negative patients, which is statistically significant.

In the present study dermatographism was present in the 4.76% of ASST positive patients and 7.69% of negative patients, which was statistically not significant. Dermatographic subjects comprise a special group. They do not have autoantibodies according to in vitro tests but manipulation of skin while injecting the sample may cause a wheal and flare response regardless of the substance injected and may be taken as false positive responses. [6]

According to the current study angioedema is present in 9.52% ASST positive patients and 23.07% in ASST negative patients. So it is statistically significant in the ASST negative patients.

In the present study anaemia was present in 19.04% of ASST positive patients and 10.25% of ASST negative patients. Leucocytosis is present in 4.76%

of the ASST positive patients and 7.69% of ASST negative patients. Statistical difference was not found regarding abnormal CBP. Pernicious anaemia is an important factor to rule out autoimmunity as a causative factor for autoimmune urticaria. In the present study absolute eosinophil count was raised in 9.52% of ASST positive patients and 17.94% of ASST negative patients. This was not statistically significant. Although blood eosinophil count was significantly higher in ASST positive group than in ASST negative group, the level in both groups was below the upper normal value. The reduction in blood eosinophil count in CIU is consistent with their presence in lesional skin biopsy. [8] The role of tissue eosinophilia is unclear, but it is possible that release of toxic eosinophil major basic protein and eosinophil cationic protein further augments histamine release from mast cells in the late phase of urticaria.

Thyroid abnormality was present in 33.33% of ASST positive patients and 15.38% of ASST negative patients. These results were not significantly important.

The present study did not find any difference in the incidence of thyroid disease. This is likely because an insufficient number of patients were included for the study of a disease of low incidence (thyroid autoimmunity) or because TFT and thyroid autoantibodies were not routinely measured for all patients. Thus, TFT alone is not enough to rule out thyroid disease and the thyroid antibody test should be carried out in all chronic idiopathic urticarial patients.

Statistical analysis of these patients did show a significant difference between patients with positive and negative ASST for frequency of attacks, urticaria severity score, duration of each wheal and duration of disease. In agreement with present study observations, other studies found that patients with autoantibodies in their sera (CIU, who have positive ASST results) have distinctive diagnostic clinical features that differentiate them from patients who

do not have these antibodies (CIU, who have negative ASST results).

### Comparison with Various Studies

Table-5 Comparison with Various Studies

Authors	Sabroe et al.	George et al. [10]	Vohra et al.	Krupashankar et al. [11]	Present study
Mean age of Presentation	45	34	14-63	14-75	18-80
Male/female Ratio	88/19	44/56	31/69	36/44	34/26
% of patients With Autoimmunity	31%	34%	46%	58.8%	35%
Correlation of frequency and positive ASST	Present	Present	Present	Present	Present
Correlation of duration and positive ASST	Present	Present	Absent	Absent	Present
Angioedema	93/107	15/100	55/100	33/80	11/60
Correlation of Systemic symptoms and positive ASST	Present	No	No	No	No
Thyroid Disease	No	No	No	No	No

### CONCLUSION

ASST is simple, cost-effective test for detecting CAU patients who have no distinctive clinical features differentiating them from CIU patients.

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