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Original research article

AUTOLOGOUS SERUM SKIN TEST IN CHRONIC IDIOPATHIC URTICARIA

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Abstract

Aim: The present study is undertaken to study positivity of autologous serum skin test in patients suffering with chronic idiopathic urticaria

Methodology: This is an experimental study. In the present study 100 clinically diagnosed cases of Chronic Idiopathic Urticaria attending the Department of Dermatology, Venereology & Leprosy at KIMS, Narketpally were taken. The study period was from 2019 December - 2020 August.

Results: A total number of 100 cases of chronic idiopathic urticaria reporting to the Dermatology Venereology Leprology department, Osmania General Hospital were studied. 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. 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: Positive autologous serum skin test was seen in 60% of patients. It is a simple, practicable *in-vivo* intradermal clinical test to differentiate chronic idiopathic urticaria from autoimmune urticaria.

Keywords: ASST, CIU, UAS, IgE, urticarial

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Introduction

Urticaria is a common condition - with lifetime incidence of approximately 15%, with females being affected more than males [1]. 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 [2]. The name "Urticaria" is derived from the Latin word URTICA - meaning to burn or hives [3]**.** Mast cells play a central role in the pathophysiology Pruritus, the primary symptom is associated with significant morbidity [4]. Approximately 40% of patients with urticaria also experience angioedema (swelling that occurs beneath the skin) [5].

Urticaria is generally classified as acute or chronic, depending on the duration of symptoms and the presence or absence of inducing stimuli [6]. 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 [7, 8]. Chronic idiopathic urticaria, which is synonymous with chronic spontaneous urticaria, is a sub-type of chronic urticaria [9].

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 [10, 11].

It is also associated with antithyroid antibodies and autoimmune conditions such as vitiligo and rheumatoid arthritis [10, 11]. It has also been proposed that Helicobacter pylori, which has an immunogenic cell envelope, may play an indirect role in the etiology of chronic autoimmune urticaria by reducing immune tolerance and inducing autoantibody formation [12].

The Autologous Serum Skin Test (ASST) has been widely adopted internationally as a clinical test to demonstrate circulating endogenous proinflammatory or wheal-inducing factors in urticaria patients since it was first described in 1986 [13]. 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- FceRI and/or anti-IgE autoantibodies that are capable of activating mast and basophil cell degranulation [14]. It has a sensitivity of 70% and a specificity of 80%.**[15]** Though, basophil histamine release assay is the gold standard for detecting functional autoantibodies, the procedure is lengthy, requires fresh basophils from healthy donors, skilled expertise is desired and generally limited to research laboratory centers. Therefore, ASST can be used as a predictive clinical test to diagnose autoimmune urticaria [16, 17].

Chronic urticaria is often associated with significant morbidity and a diminished quality of life.**[18]** 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 [19].

The present study is undertaken to study positivity of autologous serum skin test in patients suffering with chronic idiopathic urticaria

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Aims and Objectives

1. To study the positivity of asst. in chronic idiopathic urticaria.
2. Correlate the result clinical and severity aspects of the Disease.

Materials and Methodology

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

The study period was from 2019 December - 2020 August.

This is an experimental study.

Inclusion criteria

All cases of chronic idiopathic urticaria i.e 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 criteria

- Physical causes of urticaria other than simple dermographism were excluded.
- Patient with Food or drug allergy were excluded.
- Patients of age less than 10yrs or more than 70 yrs were excluded.
- Pregnant and lactating women were excluded.
- 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

1. Detailed History
2. Cutaneous and Systemic examination
3. Investigations

Laboratory investigations which are done to exclude systemic causes were:

1. Complete Blood Picture (CBP).
2. Complete Urine Examination (CUE).
3. Erythrocyte Sedimentation Rate (ESR).
4. Random Blood Sugar (RBS).
5. Liver Function Tests (LFT).
6. Renal Function Tests (RFT).
7. Absolute Eosinophil Count (AEC).
8. Stool for Ova and Cysts.
9. Serum IgE Levels.
10. Thyroid Function Tests (TFT).
11. Antinuclear Antibody (ANA).
12. Urticarial Activity Score [7].
13. The Urticarial Activity Score (UAS) consisted of the sum of the wheal number

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score and the itch severity score.

Table 1: Urticarial activity score interpretation

Score	Wheals	Pruritis
0	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

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.

Observations and Results

ASST positivity

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

Fig 1: Positive ASST

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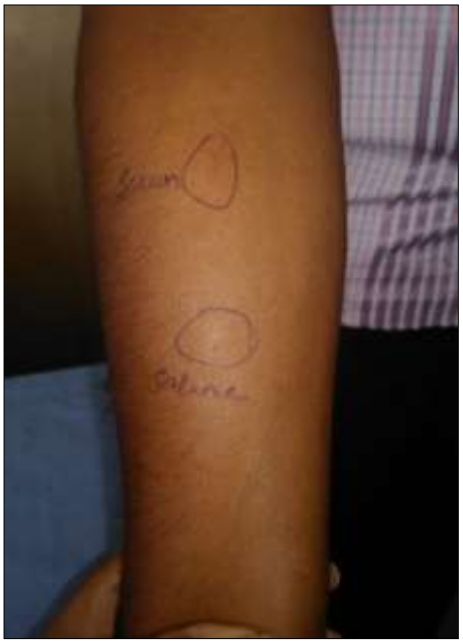

Fig 2: Negative ASST

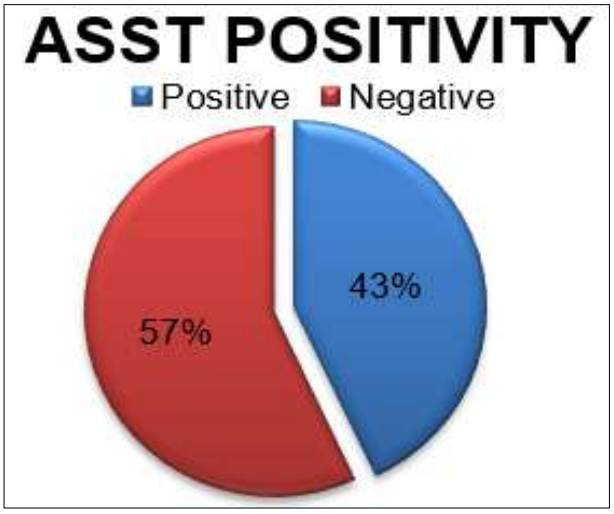

Chart 1: Percentage of ASST Positivity

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

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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: Duration of the disease

Sl. No.	Duration of disease	ASST Positive	ASST Negative
1.	3 months – 1 yr	11	26
2.	1 yr – 3 yrs	13	13
3.	3 yr – 5 yrs	12	10
4.	> 5yrs	7	8

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 4: Duration of the wheals

Sl. No.	Duration of wheal	ASST positive	ASST negative
1.	30 secs – 2 hrs	14	30
2.	2-5 hrs	11	9
3.	5-8 hrs	6	6
4.	8-10 hrs	10	10
5.	> 10 hrs	2	2

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 5: Frequency of attacks

Sl. No.	Frequency of attack	ASST positive	ASST negative
1.	Daily	26	20
2.	1-4 episodes/week	11	23
3.	1-4episodes/month	6	14

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

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percentage of patients showing in 1-3 urticarial attacks per month were 14% in ASST positive and 25% in ASST negative patients

Table 6: Angioedema

Sl. No.	Angioedema	ASST positive	ASST negative
1.	Present	8	3
2.	Absent	35	54

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 7: Dermographism

Sl. No.	Dermographism	ASST positive	ASST negative
1.	Present	5	8
2.	Absent	38	49

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).

Clinical features

Table 8: Clinical features

Clinical features	ASST positive	ASST negative
Fever	5 (11.6%)	1 (1.8%)
Headache	1 (2.3%)	12 (21%)
Shortness of Breath	4 (9.3%)	10 (17.6%)
Atopy	7 (16.3%)	2 (3.5%)
Joint Pains	7 (16.3%)	4 (7%)
Abdominal Pain	5 (11.6%)	1 (1.8%)
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.

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Table 8: 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 9: 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 10: Complete blood picture

Sl. No.	CBP	ASST positive	ASST negative
1.	Anemia	12	12
2.	Leucocytosis	8	9
3.	Normal	23	36

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.

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Table 11: Absolute eosinophil count

Sl. No.	AEC	ASST positive	ASST negative
1.	Raised	4	7
2.	Normal	39	50

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 12: Serum IgE levels

Sl. No.	Serum IgE levels	ASST positive	ASST negative
1.	RAISED	2	0
2.	NORMAL	41	57

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 13: Thyroid profile

Sl. No.	Thyroid profile	ASST positive	ASST negative
1.	Abnormal	8	3
2.	Normal	35	54

Thyroid Profile was positive in 18.6% of ASST Positive patients and 5% of ASST Negative patients, where as it was negative in 81.4% of ASST Positive patients and 95% of ASST Negative patients. And the values are statistically significant. (P Value = 0.0221).

Table 14: Ana profile

Sl. No.	Ana profile	ASST positive	ASST negative
1.	Abnormal	0	2
2.	Normal	43	55

ANA Profile was positive in 0% of ASST Positive patients and 3% of ASST Negative patients. It was negative in 100% of ASST Positive patients and in 97% of ASST Negative patients. And the values are statistically insignificant. (P Value = 0.0904)

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Discussion

Chronic urticaria (CU) is a common skin disorder affecting at least 0.1% of the population. 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 FceR1α (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 FceR1α 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.**[17]** Western blot, enzyme linked immunosorbent assays (ELISA) and flow cytometry may be useful for screening in the future, but they need to be validated.

Treatment for all forms of CU should include a sound, basic lifestyle. These include avoidance of stress, over-tiredness, alcohol, NSAIDS, and tight fitting garments. Second generation nonsedating H1 antihistamine are recommended as first line medications for initial treatment. New effective and safe therapeutic options have emerged for treatment of patients with CSU refractory to the standard dosage of an H1 antihistamine. Up dosing with a second generation non-sedating H1 antihistamine such as Desloratadine in 2, 3, or 4 times the licensed dosage is recommended as second line treatment. Omalizumab injections subcutaneously at monthly intervals are recommended as a novel effective and safe therapeutic option for CSU refractory to the above

As conventional approach of management of CIU may be unsuccessful, ASST is especially important from the therapeutic point of view as it can help the dermatologist to commit himself to initiate immunosuppressive therapy in such patients [21].

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

In study by Vohra *et al.* [16] ASST was positive in 46 (46%) and negative in the other 54 (54%) patients.

Study by Mamatha *et al.* [21] in prospective demonstrated that in a total of 100 patients with chronic idiopathic urticaria, 34 (34%) patients showed positive for ASST.

In the Present study mean duration of the disease in ASST positive patients mean+ S.D is 3.35 ± 2.71 yrs and 2.54±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)

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was observed by Zeinab Abdel Azim *et al*. [22].

In Staubach *et al*. [23], found that the duration of the disease was longer in ASST positive patients than that of ASST negative patients.

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*. [22] 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.

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

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*. [25] 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).

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*. [22] study there was significant increase (P Value = 0.005) in UAS in ASST positive group (UAS mean ± SD 4.5 ± 0.5) in comparison to ASST negative group (UAS mean ± SD 3.9 ± 0.7).

Vohra *et al*. [25] study showed urticaria activity score (UAS) of ≥5 in 44 (96%) ASST positive and in 19 (35%) ASST-negative patients, while it was <5 in two (4%) ASST-positive and 35 (65%) ASST negative patients respectively.

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).

Arun Verma *et al*. [26] study AEC was raised in 24 out of 200 patients. It was stastistically insignificant.

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

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significant.

In the Present Study, ANA profile was positive only in 2 patients of ASST Negative patients, which is statistically insignificant. (P Value = 0.094).

Mamatha, *et al*. [21] study showed, of the 10 patients tested, ANA was positive in 1 patient who also had positive ASST. This study did not find an increased incidence of other autoimmune diseases.

In E. Toubi et at study [28], ANA were found positive in 6/139 (4%) of patients compared to 1/60 (1.7%) of normal controls. Both duration and severity were not associated with ANA positivity.

Conclusion

To conclude, autologous serum skin test (ASST) was positive in significant number of patients (60%) in the present study. It can reasonably be used as a predictive clinical test to diagnose autoimmune urticaria, especially in situations where the basophil histaminereleasing test is not available. From management aspect of view, in ASST positive patients immunosuppressive therapy may be started if conventional line of management have failed.

Conflict of Interest: None.

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