

Original Research Article

A cross-sectional study on autologous: serum skin test in chronic urticaria in a tertiary care centre

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ABSTRACT

Background: Urticaria persisting daily for more than six weeks is termed as chronic urticaria. A significant number of them have an autoimmune basis, where ASST is used for detection of chronic autoimmune urticaria (CAU). The aim and objective of the study was to determine the frequency of autoimmunity with ASST in chronic urticaria patients.

Methods: This was a hospital based cross sectional study, 100 patients with chronic urticaria fulfilling the inclusion and exclusion criteria were enrolled in the study. Detailed history, physical examination and routine investigations were recorded and ASST was done for all patients.

Results: Thirty seven (37%) out of 100 CU patients were ASST positive. Statistical analysis of the clinical variables did not show significant difference between ASST positive and negative patients except for dermographism and mean duration of the disease.

Conclusions: ASST is a simple *in vivo* clinical test for detection of basophil histamine releasing activity. Diagnosing CAU is important as these patients may need high doses of anti-histamines, systemic corticosteroids and/or immunomodulatory drugs. Autologous serum therapy itself has a therapeutic role in treatment of CAU patients.

Keywords: Autoimmune urticaria, ASST, Chronic urticaria, Autoimmunity

INTRODUCTION

Urticaria is defined as a transient eruption of circumscribed oedematous and usually itchy swellings of the dermis. These lesions last for a few hours but not exceeding 24 hours.¹ AE accompanies 40-50% of urticaria and 10% experience AE alone.² About 1 in 5 people suffer from urticaria atleast once in their lifetime.³ About 30-50% of CSU have an autoimmune basis and are referred to as chronic autoimmune urticaria (CAU), it presents with continuous wheals associated with malaise, indigestion and sensation of hot and/or cold.⁴ A personal and/or family history of other autoimmune diseases. 27%

of CAU patients are associated with antithyroid antibodies or with conditions such as diabetes mellitus, pernicious anemia, vitiligo and rheumatoid arthritis.⁵ Immunologically, CAU is characterized by presence of functional autoantibodies that activate mast cells and basophils by crosslinking the high-affinity IgE receptor (FcεRIα). About 50% of CU have functional autoantibodies to FcεRI and 9% to IgE antibody itself.⁵ Currently, The Autologous serum skin test (ASST) has been widely adopted as a simple clinical test to demonstrate in-vivo basophil histamine releasing activity.⁶ ASST has approximately 70% sensitivity and 80% specificity.⁷ Diagnosing CAU is important as they

need high doses of antihistamines and systemic corticosteroids during acute exacerbations. Immunomodulatory drugs are of therapeutic benefit in recalcitrant autoimmune urticaria.⁵ Hence, this study was carried out to estimate the prevalence of autoimmunity in chronic urticaria patients in a tertiary care centre.

METHODS

This was a cross-sectional study conducted at Mahatma Gandhi Medical College and Research Institute, a tertiary care hospital. Study was initiated after obtaining Institutional Ethics committee clearance. Patients attending DVL OPD and diagnosed of chronic urticaria clinically were enrolled in the study based on the inclusion and exclusion criteria. The study period was between January 2015 to May 2016.

Inclusion criteria

Inclusion criteria were above 18 years of age; with no discernible cause of urticaria.

Exclusion criteria

Exclusion criteria were children's, pregnant and lactating women; patients with physical urticaria; urticarial vasculitis; patients with known drug allergies, food allergies; patients on corticosteroids and immunosuppressive drugs.

All patients fulfilling the inclusion and exclusion criteria were enrolled in the study. Informed consent was taken from the patients in their language prior to their inclusion in the study.

Demographic data of all individuals included in the study were recorded. Detailed history was taken including duration of the disease, frequency of attacks, associated angioedema, dermatographism, family history of urticaria, atopy and autoimmune diseases along with General and dermatological examination (skin, hair, nail, mucosa). Patients were subjected to a set of investigations which included complete blood count, absolute eosinophil count (AEC), Erythrocyte sedimentation rate (ESR), Blood sugar (Fasting and post prandial), Thyroid function test (TFT) and urine examination.

Prerequisites for ASST

1. Antihistamines should be withdrawn at least 2 to 3 days prior to the test.
2. Doxepin and Astemizole should be withdrawn 2 to 6 weeks prior to the test.
3. Test area should be free of lesion.

ASST was performed in our study population as per the guidelines quoted by Ghosh and Ghosh.⁸

Procedure

1. Two milliliters of venous blood was collected from the antecubital vein.
2. After clotting of blood at room temperature.
3. Serum was separated by centrifugation (2000 rpm for 10-15 min).
4. As much as 0.05 ml of serum was injected intradermally into the volar aspect of forearm, avoiding the areas of whealing within the past 24 h.
5. Equal amounts of normal saline [negative control] and histamine (10 µg/mL) [positive control] was injected intradermally 3 to 5 cm apart in the volar aspect of the same forearm.
6. Wheal and flare response was measured after 30 min.

Interpretation: A positive test is defined as a serum-induced wheal response with a diameter of more than 1.5 mm or more than that of the saline-induced response at 30 min. The maximum vertical (d1) and horizontal (d2) diameters of the wheals were measured.

Then the average diameter (D) was calculated $[D=(d1+d2)/2]$.⁸

All data were enrolled in a data collection proforma sheet and entered into Microsoft Excel sheet. Patient privacy and confidentiality were maintained.

Statistical analysis was carried out using SPSS version 20 (IBM SPSS, US) software. Continuous data were expressed as mean± standard deviation and median with range. Qualitative variables were expressed as percentage. Chi-square test, T-test, risk estimates were the other statistical methods used in the study.

RESULTS

One hundred consecutive patients of chronic urticaria fulfilling the inclusion and exclusion criteria were enrolled in the present study.

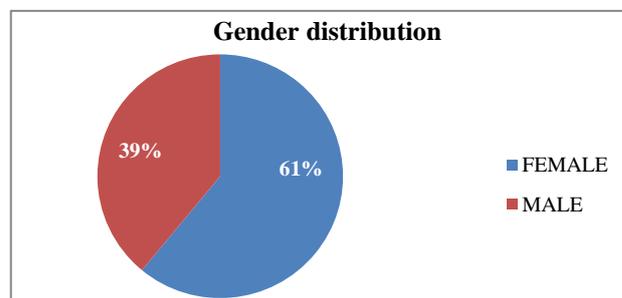


Figure 1: Gender distribution of patients with chronic urticaria.

Patient profile

Out of the 100 chronic urticaria patients 39 (39%) were males and 61 (61%) were females (1:1.56 male: female)

ratio) with mean age of the study population being 36.32 ± 12.85 years (ranging from 18 to 70 years with median of 34.50 years) (Figure 1).

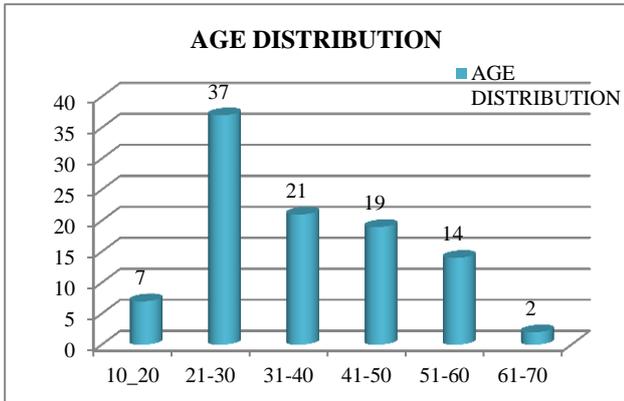


Figure 2: Age distribution of patients with chronic urticaria.

In our study, the maximum number of chronic urticaria patients belonged to the age group of 21-30 years (37%) followed by 31-40 years (21%) (Figure 2).

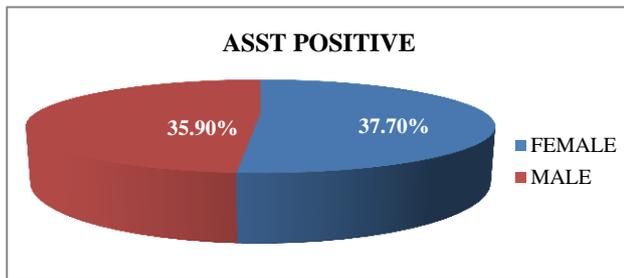


Figure 3: Gender distribution based on ASST positivity.

Gender distribution based on positive and negative ASST

The below mentioned figure shows that out of 37 ASST positive patients, 23 (37.7%) were females and 14 (35.9%) were males. In the present study there is no statistically significant gender difference ($p=0.855$) with respect to ASST positivity (Figure 3).

Table 1 shows the mean age distribution among chronic urticaria patients in our study was 36 ± 12.85 years and that of ASST positivity was 31.70 ± 12.85 years; mean duration of the disease in the study group was 52.01 ± 66.31 months and in ASST positive patients was 31.19 ± 37.5 months.

Age distribution based on positive and negative ASST

Figure 4 shows that in both ASST positive (20; 54.10%) and negative group (17; 45.90%) the maximum age distribution was between 21-30 years.

Table 1: Mean age and duration of the study population.

S.no	Study group	Mean age (years)	Mean duration (months)
1	All chronic urticaria	36.32 ± 12.85	52.01 ± 66.31
2	ASST positive	31.70 ± 9.83	31.19 ± 37.5
3	ASST negative	39.03 ± 13.62	64.24 ± 76.1

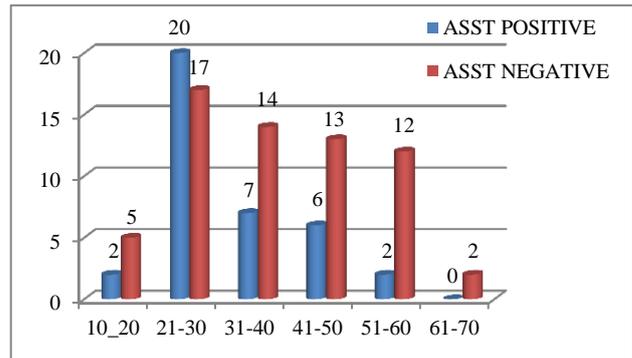


Figure 4: Age distribution based on positive and negative ASST.

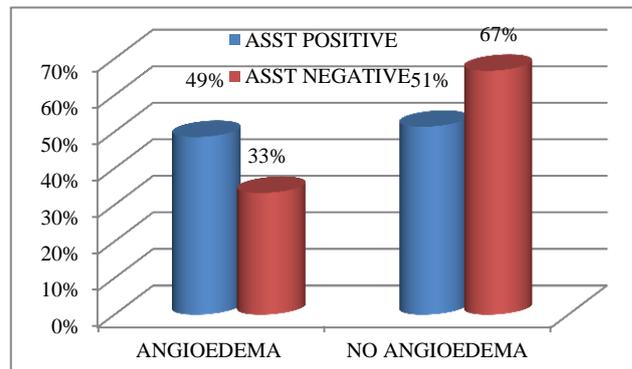


Figure 5: Frequency of ASST positivity in patients with angioedema.

Frequency of ASST positivity in patients with angioedema

Figure 5 shows that among the 100 chronic urticaria patients, 39 had history of angioedema, out of which 18 (48.6%) patients were positive for ASST and 21 (33.3%), were negative for ASST. There is no statistical significance with association of ASST positivity and angioedema ($p=0.13$).

Frequency of ASST positivity in patients with dermatographism

Among 100 chronic urticaria patients, 33 demonstrated dermatographism out of which 21 (63.6%) patients were ASST positive and 12 (36.4%) patients were ASST

negative. There is statistically significant association between dermographism and ASST positivity (p=0.00) (Figure 6).

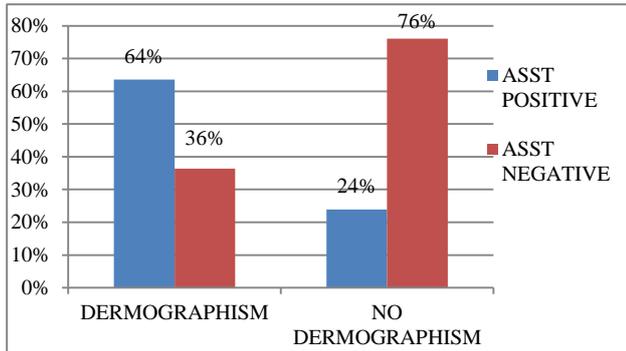


Figure 6: Frequency of ASST positivity in patients with dermographism.

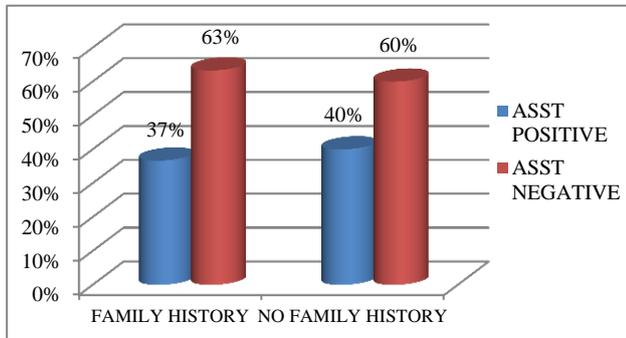


Figure 7: Frequency of ASST positivity in patients with family history of urticaria.

Frequency of ASST positivity in patients with family history of urticaria

Among the 100 chronic urticaria patients, 10 patients had family history of urticaria. Out of the 10, 4 (40.0%) patients were positive to ASST and 6 (60.0%) patients were negative to ASST. P value (0.83) not significant (Figure 7).

Frequency of ASST positivity in the presence of atopy

Out of 100 chronic urticaria patients 8 patients had history suggestive of atopy, among them 3 (37.5%) patients were positive for ASST and 5 (62.5%) were ASST negative. P value (0.97) not significant (Figure 8).

Frequency of ASST positivity in patients based on extent of involvement

Among 100 chronic urticaria patients 75 patients had whole body involvement with ASST positivity in 29 (38.7%) patients and 25 patients had involvement of face and extremities alone with ASST positivity in 8 (32.0%) patients. There is no statistical significance between

extent of involvement and ASST positivity (p=0.55) (Figure 9).

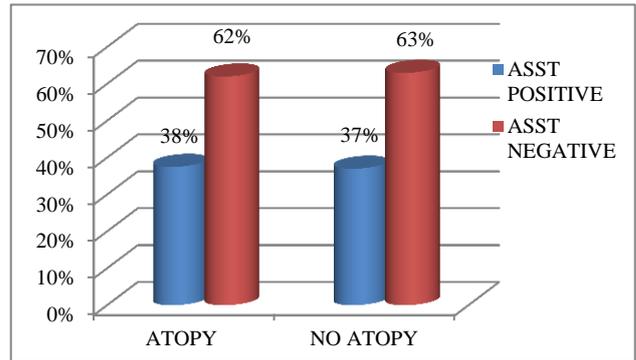


Figure 8: Frequency of ASST positivity in the presence of atopy.

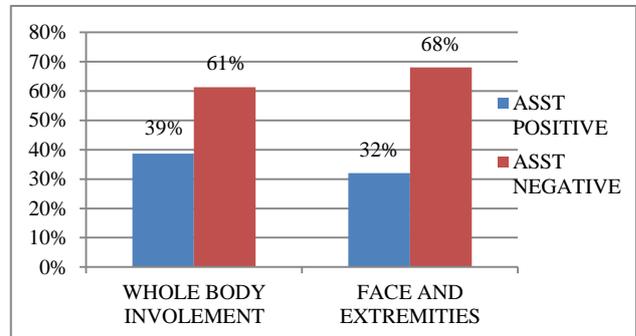


Figure 9: Frequency of ASST positivity based on extent of involvement.

Table 2: Urticaria severity score in ASST positive patients.

S.no	USS	ASST		Total (%)
		Positive (%)	Negative (%)	
1	≤5	2 (33.3)	4 (66.7)	6 (100)
2	6-10	30 (35.3)	55 (64.7)	85 (100)
3	>10	5 (55.6)	4 (44.4)	9 (100)
Total		37 (37.0)	63 (63.0)	100 (100)

Table 2 shows that among 37 ASST positive patients, 2 (33.3%) patients had urticaria severity score of ≤5; 30 (35.3%) had urticaria severity score of 6-10; 5 (55.6%) had urticaria severity score >10. In the present study there is no statistically significant association between USS and ASST positivity (p=0.47).

Frequency of ASST positivity in diabetes mellitus

Among 100 chronic urticaria patients 14 had diabetes mellitus, out of which 3 (21.4%) patients were positive to ASST and 11 (78.6%) patients were negative to ASST. There is no statistical significance between chronic urticaria associated with diabetes mellitus and ASST positivity (p=0.19) (Figure 10).

Table 3: Analysis of risk factor in chronic urticaria patients.

S.no	Risk factor	ASST				Odd's ratio	95% confident interval		P value
		Positive	%	Negative	%		Lower	Upper	
1	Gender								
	Female	23	37.7	38	62.3	0.92	0.4	2.13	0.85
	Male	14	35.9	25	64.1				
2	Atopy	3	37.5	5	62.5	1.02	0.23	4.55	0.97
3	Angioedema	18	48.6	21	33.3	1.89	0.82	4.34	0.13
4	Family history	4	40	6	60	1.15	0.3	4.37	0.83
5	Extent of involvement								
	Whole body	29	38.7	46	61.3	1.34	0.51	3.5	0.55
	Face and extremities	8	32	17	68	0.74	0.24	1.95	
6	Dermographism	21	63.6	12	36.4	5.57	2.25	13.78	0
7	Diabetes mellitus	3	21.4	11	78.6	0.41	0.1	1.6	0.19
8	Thyroid function test	3	75	1	25	5.47	0.54	54.64	0.1

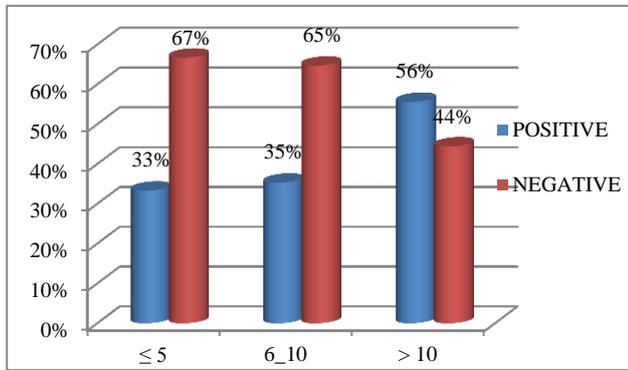


Figure 10: Urticaria severity score in ASST patients.

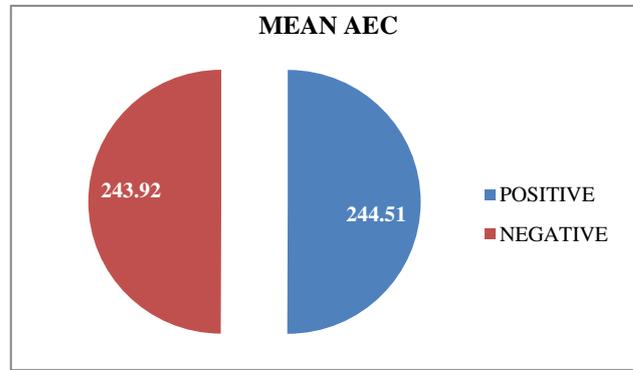


Figure 12: Frequency of ASST positivity with mean AEC.

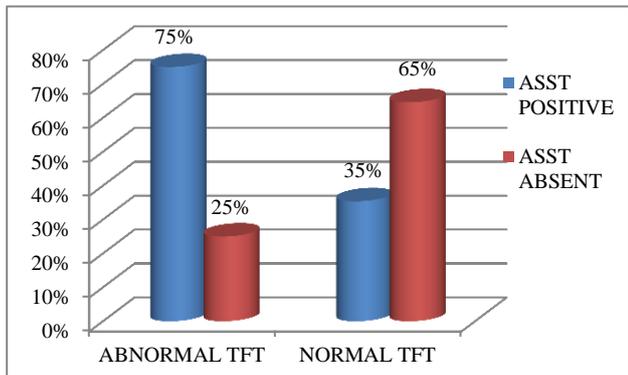


Figure 11: Frequency of ASST positivity in the presence of abnormal TFT.

Frequency of ASST positivity in the presence of abnormal TFT

Figure 11 shows out of 100 chronic urticaria patients 4 had abnormal thyroid function test out of which 3 (75.0%) patients were positive for ASST with no statistical significance (p=0.10).

Frequency of ASST positivity with mean AEC

The mean absolute eosinophil count was 244.51±61.83 in ASST positive patients and 243.92±74.66 in ASST negative patients. There is no statistical significance between mean AEC and ASST positivity (p=0.08) (Figure 12).

DISCUSSION

Urticaria is persisting daily or almost daily for more than six weeks is termed as chronic urticaria.⁹ Although, in many patients with CU the disease remains idiopathic, in the last few years an autoimmune basis has been proposed for the same. The autoimmune subgroup constitutes about 45% of CIU. Clinical examination of patients with CIU is generally unhelpful in distinguishing autoimmune from nonautoimmune etiology.⁹

Autologous serum skin test (ASST) is a simple in vivo clinical test for the detection of basophil histamine-releasing activity. Sabroe et al, reported that ASST has approximately 70% sensitivity and 80% specificity.⁷ Hence our study was carried out to determine the

prevalence of ASST positivity in CIU patients and its clinical significance.

Demographic characteristics

Gender distribution

In our study, out of 100 chronic urticaria patients, 61 (61%) were females and 39 (39%) were males [male:female ratio (1:1.5)]. The results in our study are comparable to the studies done by Ganguly et al in which out of 48 patients male:female ratio was 1:1.4.⁴ Kulthanan et al, reported out of 85 CU patients male:female ratio was 1:4.3.¹⁰ George et al, reported in their study out of 100 CU patients male:female ratio was 1:1.2 exhibiting female preponderance.¹¹ Whereas the study done by Sabroe et al showed a male predominance among 107 CIU patients with male:female ratio of 5:1.¹²

Among the 61 females with CU, 23 (37.7%) were positive to ASST and 38 (62.3%) were negative to ASST; out of 39 males, 14 (35.9%) were ASST positive and 25 (64.1%) were ASST negative. There was no statistical difference between gender distribution and ASST positivity ($p=0.855$). Female predominance in CU and ASST positivity may be related to the fact that autoimmune diseases are more common in females compared to males.

Age distribution

In our study, age distribution ranged between 18-70 years, with maximum number of chronic urticaria patients belonging to 21- 30 years (37%) followed by 31-40 years (21%). The mean age distribution in CU patients was 36.32 ± 12.85 years, similar to the study done by George et al, in which 81% belonged to similar age group.¹¹

Among the 37 ASST positive patients the mean age ranged between 31.70 ± 9.83 years. The present study is comparable to the studies done by Kulthanan et al, Sabroe et al, Vohra et al, Ganguly et al, in which the mean age distribution was 38.8 years, 45 years, 32.69 years and 36 years respectively.^{4,10,12,13}

Duration of chronic urticaria

In our study, the total duration of the disease ranged from 2 months to 30 years. The mean duration of the disease was 52.01 ± 66.31 months. The mean duration of the disease in ASST positive group was 31.19 ± 37.5 months while it was 64.24 ± 76.1 months in ASST negative group.

The present study is comparable to the studies done by Vohra et al and Kurt et al in which the mean duration of the disease in ASST positive patients was 40 months and 67.2 months respectively.^{13,14} But it was found to be higher on comparison with the study done by Hamamy et al in which the mean duration of the disease was 13

months.⁷ In our study it is seen that the mean duration of the disease is relatively low in ASST positive patients with p value of 0.005. This was similar to the study done by Sabroe et al.¹²

ASST positivity in CU

In our study, out of 100 CU patients 37 (37%) were ASST positive and 63 (63%) were ASST negative, which is comparable to the study done by Caproni et al who reported that out of 68 CU patients, 23 (34%) had a positive reaction to ASST.⁷ Asero et al reported out of 78 CU patients 35% were positive to ASST.⁷

Sabroe et al reported 24.7% prevalence of ASST positive patients in their study group.¹⁵ Godse reported out of 45 CU patients, 26.67% were positive to ASST.¹⁵

Nettis et al reported 42 (41.2%) ASST positivity out of 102 CU patients.¹⁶ Whereas, Bakos et al in his study reported a higher percentage of ASST positivity 54.2%.¹²

Angioedema

In our study, among the 100 CU patients, 39 had history of angioedema, out of which 18 (48.6%) patients were positive for ASST and 21 (33.3%) were negative for ASST. The present study is comparable to the studies done by Verma et al, Vohra et al, Ganguly et al in which patients with angioedema had ASST positivity of about 54.5%, 59%, and 60% respectively; but, comparatively lesser to the study done by Sabroe et al which showed 86.9% ASST positivity in patients with angioedema.^{4,14,17,18} However few studies done by Kulthanan et al and George et al reported a lower percentage of ASST positivity among patients with angioedema which was only 7.05% and 15% respectively.^{10,19} There is no statistically significant association between ASST positivity and angioedema ($p=0.13$) which is similar to previous studies.^{4,7,12,16,19}

Dermographism and ASST

In our study, out of 100 CU patients 33 demonstrated dermographism out of which 21 (63.6%) were ASST positive and 12 (36.4%) were ASST negative. There is statistically significant association between dermographism and ASST positivity ($p=0.00$). In contrast to our finding, studies done by Zeinab Abdel Azim et al and Kumar et al. did not show any statistically significant association between ASST positivity and angioedema.²⁰ Whereas George et al. reported that dermographism occurred more frequently in ASST negative group compared to ASST positive group.¹⁹

History of atopy

In our study, out of 100 patients, 8 presented with history of atopy, among them 3 (37.5%) patients were ASST positive and 5 (62.5%) were ASST negative.

The present study is comparable to the study done by Kulthanan et al who reported that among 24 patients with atopy, 8 (38.1%) were positive to ASST; and Krupashankar et al reported, among 15 patients with atopy, 8 (17.1%) were ASST positive.^{10,20} Similar to the earlier studies reported, we also observed in our study that there was no statistically significant ($p=0.97$) correlation between the frequency of atopy and ASST positivity.^{10,20-22}

Family history of urticaria

Among the 100 CU patients, 10 had family history of urticaria, among them 4 (40%) were ASST positive and 6 (60%) were ASST negative. The present study is comparable to the study done by Krupashankar et al in which among 27 patients with family history of CU, 18 (38.3%) were ASST positive.²³ But, less when compared to the study done by Verma et al in which among 35 patients with history of urticaria 60% had positive ASST.¹⁷ Kulthanan et al reported out 7 patients with family history of urticaria 2 (9.5%) were positive to ASST.¹⁰ Similar to previous studies, we also observed in our study that there was no statistically significant correlation between family history and ASST positivity ($p=0.83$).^{4,10,20,22,24}

Urticaria severity score (USS)

In our study, among 37 ASST positive patients 2 (33.3%) had urticaria severity score (USS) ≤ 5 ; 30 (35.3%) had USS between 6-10 and 5 (55.6%) patients had USS ≥ 10 , among 63 ASST negative patients 4 (66.7%) had USS ≤ 5 ; 55 (64.7%) had USS between 6-10; 4 (44.4%) patients had USS ≥ 10 .

The urticaria severity score in our study was increased compared to the study done Hamamy et al. in which among 22 ASST positive patients 2 (3.7%) showed USS ≤ 5 ; 10 (18.5%) with USS between 6-10 and 10 (18.5%) with USS ≥ 10 .¹⁴

Similar to studies done by Ganguly et al, Vohra et al, Al Hamamy et al, Krupashankar et al, Kurt et al, Kulthanan et al, our study also showed there was no statistical significant correlation between disease severity and ASST positivity ($p=0.47$).^{4,7,10,14,20,21}

Extent of involvement

In the present study out of 100 CU patients, 75 had urticaria involving all over the body, of which 29 (38.7%) were ASST positive and 46 (61.3%) were ASST negative and 25 patients had only involvement of face and extremities, of which 8 (32.0%) were ASST positive and 17 (68.0%) were ASST negative.

ASST positive patients with whole body involvement 14 (25.9%) was found to be higher when compared the study done by Al Hamamy et al while, face and extremity

involvement 8 (14.8%) was comparable to the present study.²² Similarly, study done by Vohra et al showed increased involvement of palms and soles in ASST positive patients.¹⁴ Similar to other studies our study also showed statistical insignificance between the extent of involvement and ASST positivity ($p=0.55$).

As reported by, Hamamey et al the probable reason for higher ASST positivity among patients with predominant involvement of face and extremities is that these patients may have higher risk of demonstrating functional antihistamine autoantibody activity than those with generalized urticarial distribution.⁷

Mean absolute eosinophil count

In the present study, the mean absolute eosinophil count was 244.51 ± 61.83 in ASST positive patients and 243.92 ± 74.66 in ASST negative patients. The mean absolute eosinophil count in our study is higher compared to the study done by Azim et al were mean AEC in ASST positive patients was 176.6 and in negative patients was 131.¹³ In the study done by Krupashankar et al reported that out of 8 patients with raised AEC, 4 (8.5%) were ASST positive.²³ Similar to previous studies there was no statistical significant correlation between mean AEC and ASST positivity ($p=0.08$) in our study.

Autoimmunity and ASST

In our study, out of 100 CU patients, 4 patients had abnormal thyroid function test of which 3 (75.0%) patients were ASST positive and 1 (25.0%) was ASST negative. 14 out of 100 CU patients had diabetes mellitus, among them 3 (21.4%) were ASST positive and 11 (78.6%) were ASST negative.

The present study is inconsistent with the study done by O'Donnell et al in which there was significant differences between ASST positive and ASST negative patients with regard to autoimmune thyroid disease.²⁵ But, was consistent with the study done by Ganguly et al in which there is no significant association between autoimmune diseases and ASST positive patients, and also with study done by Kurt et al suggested that chronic systemic diseases alone were not associated with positive ASST response.^{4,14} Similar to the above studies our study also had no statistical significance between ASST positivity and autoimmune systemic diseases like diabetes mellitus and abnormal thyroid test.

CONCLUSION

In our study, out of 100 chronic urticaria patients, 39 (39%) were males and 61 (61%) were females [1:1.56; male: female ratio]. The mean age group of 36.32 ± 12.85 years. The maximum number of CU patients belonged to the age group of 21-30 years (37%) followed by 31-40 years (21%). The mean duration of the disease was 52.01 ± 66.31 months (ranging from 2 months to 30

years). Out of 100 CU patients, 37% were ASST positive and 63% were ASST negative. In ASST positive patients, the mean age distribution was 31.70±12.85 years and the mean duration of disease was 31.19±37.5 months. There was statistical significance in mean duration of the disease and dermatographism in ASST positive patients. There was no significant association between ASST positivity and mean age distribution, disease severity, extent of involvement, angioedema, atopy, family history of urticaria, other autoimmune disease, and absolute eosinophil count.

Hence, ASST is a simple and cost-effective in-vivo clinical test used for assessing autoimmune etiology in patients with chronic spontaneous urticaria.

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Ethical approval: The study was approved by the institutional ethics committee

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