

Original Research Article

Autologous serum skin test in 250 patients of chronic spontaneous urticaria at tertiary care hospital in Gujarat

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ABSTRACT

Background: Chronic urticaria (CU) is defined as urticaria persisting daily as or almost daily for more than 6 weeks and affecting 0.1% of the population. In chronic autoimmune urticaria, immunoglobulin G (IgG) auto antibodies react to the alpha subunit of the high-affinity IgE receptor (FcεR1) on dermal mast cells and basophils, leading to chronic stimulation of these cells. This results in the release of histamine and other inflammatory mediators which cause urticaria and angioedema. More than half of the patients have autoimmune urticaria. ASST is the only in vivo test to detect autoimmune urticaria. The aim was to study the frequency of ASST positivity among patients with chronic spontaneous urticaria and to identify the clinical and laboratory parameters associated with positive ASST.

Methods: The present study is prospective observational study of chronic spontaneous urticaria. ASST was performed in 250 patients fulfilling exclusion and inclusion criteria. Baseline investigations (CBC, ESR, urine, stool, RBS and CRP) were done for all the patients whereas specific investigations (ANA, Thyroid Profile, IgE, Anti H.Pylori IgG and skin biopsy) were done in selected cases.

Results: Out of 250 patients examined, 60% had positive autologous serum skin test suggestive of autoimmune urticaria. H. pylori antibodies were significantly higher (29%) in ASST positive patients. While antinuclear antibody (ANA) was positive in 4% and thyroid antibodies were present in 7% of patients with positive ASST. Serum IgE was elevated in 32% ASST positive patients.

Conclusions: ASST is an easy, simple and cost effective screening test for chronic autoimmune urticaria.

Keywords: Chronic spontaneous urticaria, ASST

INTRODUCTION

Urticaria is a common, heterogeneous group of disorders with a large variety of underlying causes. It is characterized by the sudden appearance of fleeting wheals, each of which last 1-24 hours and/or angioedema lasting up to 72 hours.¹ Chronic urticaria, with or without angioedema, has traditionally been defined as daily or almost daily symptoms recurring for more than 6 weeks.² Chronic spontaneous urticaria (CSU) affects 0.5-1% of individuals (lifetime prevalence) and significantly

reduces quality of life (QOL).² Chronic spontaneous Urticaria is more common in adults, and affects women more frequently than men.³ Estimated order of frequency of different aetiologies of chronic urticaria include: autoimmune > idiopathic > pseudoallergic > chronic infection.⁴ Chronic spontaneous urticaria is classified further as chronic autoimmune urticaria and chronic idiopathic urticaria (CIU).³

Historically in 1986, Grattan et al were the first to use ASST to differentiate autoimmune urticaria from chronic

idiopathic urticaria.⁵ Autoimmune Urticaria/angioedema that accounts for about 30-50% of chronic spontaneous urticaria may be associated with other autoimmune conditions such as thyroiditis.²

The autologous serum skin test (ASST) as defined by Sabroe et al is currently the simplest and the best *in vivo* clinical test for detection of basophil histamine releasing activity.⁶ ASST has a sensitivity of approximately 70% and a specificity of 80% for *in vitro* basophil histamine release, when the serum response is at least 1.5 mm greater than the control saline skin test at 30 min.⁷ Autologous serum skin test (ASST) is used for detection of autoantibody against either IgE or high affinity IgE receptor. It is a useful screening test to evaluate the presence of serum histamine releasing factor, along with histamine releasing autoantibodies. The ASST is most widely used clinical test for urticaria and relatively safe and simple to perform.⁴

Chronic autoimmune urticaria is also associated with antithyroid antibodies in approximately 27% of cases, as well as other autoimmune conditions such as vitiligo and rheumatoid arthritis. It has also been proposed that *Helicobacter pylori* (*H.pylori*), which has an immunogenic cell envelope, may play an indirect role by reducing immune tolerance and inducing autoantibody formation. However, it is important to note that the limited number of studies conducted in this area have yielded conflicting results.³

METHODS

The prospective observational study carried out in department of Skin and Venereal Disease of BJ Medical College Ahmedabad at tertiary care hospital. Approval of the Institutional Human Ethics Committee was taken. Patients having CSU, fulfilling the inclusion criteria attending Dermatology OPD were enrolled. The study period was July 2011 to December 2013.

Inclusion criteria

Participant can be male or female, more than 18 years of age, daily or almost daily appearance of wheals for more than 6 weeks, willingness for injections.

Exclusion criteria

Patients with physical urticaria, urticarial vasculitis, children, pregnancy, willing to be pregnant, lactating women, urticaria with known etiology like food, drugs, any infections etc., systemic corticosteroid or immunosuppressive drug use in the past 6 weeks, other systemic illnesses requiring treatment.

All patients fulfilling the inclusion and inclusion criteria were enrolled. Informed and written consent were taken from patients in their language prior to enrolment in the study. Patient's confidentiality was maintained.

Participants were made to understand the process completely and all relevant questions were answered.

Demographic data of all individuals enrolled in the study were recorded. History regarding onset, frequency of disease, infection, gastrointestinal symptoms, aggravating and associated factors were taken. Assessment of the severity of the symptoms based on urticaria activity score (UAS) was done. As urticaria symptoms frequently change in intensity, overall disease activity is best measured by advising patients to document 24-hr self-evaluation scores for several days. Thus the UAS, is the sum score of 7 consecutive days.⁸

Table 1: Scoring system for urticaria patients.¹

Score	Wheal	Pruritus
0	None	None
1	Mild (<20 wheals/24h)	Mild(present but not annoying or troublesome)
2	Moderate(20-50 wheals/24h)	Moderate(troublesome but does not interfere with normal daily activity or sleep)
3	Intense(>50 wheals/ 24 h or large confluent areas of wheals)	Intense(severe pruritus, which is sufficiently troublesome to interfere with normal daily activity or sleep)
Sum of score : 0-6		

Baseline investigations (CBC, ESR, urine and stool routine micro), RBS, CRP was done for all the patients whereas specific investigations (ANA, thyroid profile, IgE, anti *H.Pylori* IgG, skin biopsy) were done in selected cases to exclude chronic urticaria cases that are not 'idiopathic'.

Pre – requisite for ASST

- Antihistamines were discontinued (at least two days for short acting antihistamines, 6 days for Desloratidine and 2 weeks for Doxepin).
- Systemic steroids were discontinued for at least 2 weeks.
- Forearm has to be free of wheals.

Preparation of serum

Carefully label all tubes and syringes with the patient's name. Collect 5 ml blood into sterile glass tubes without additives. Allow blood to clot at room temperature for 30 minutes. Centrifuge sample, at about 500 RPM for 10 minutes. Suck 0.2 ml serum into 1 ml tuberculin syringe in order to inject 0.05 ml intradermally into the volar aspect of forearm.

Skin testing technique

Forearm is chosen as the test site and cleaned with spirit swab. 0.05 ml of fresh undiluted serum is injected

intradermally. Negative control: intradermal injection of 0.05 ml of sterile saline. Positive control: intradermal injection of 0.05 ml of 10-20 ug/ml of histamine solution or a skin prick test with 10 mg/ml histamine solution (Practical tip: since histamine is not available easily this step may be skipped).

Skin test reading: at 30 minutes. Wheal and flare response was calculated by measuring two perpendicular diameters (d1 and d2) according to formula $\pi(d1+d2)/4$.⁹

Table 2: Interpretation of ASST.⁹

Positive test	Negative test	Uninterpretable
A serum Induced red wheal with diameter 1.5 mm or more than the saline induced response at 30 minutes with a positive histamine test.	Serum wheal response pale rather than uniformly red with a positive histamine test.	Red wheal develops at the saline injections sites or histamine skin prick test is negative.

Statistical methods

Chi- square test has been used to find association between ASST, ANA, Thyroid- antibodies, IgE and *Helicobacter pylori* IgG.

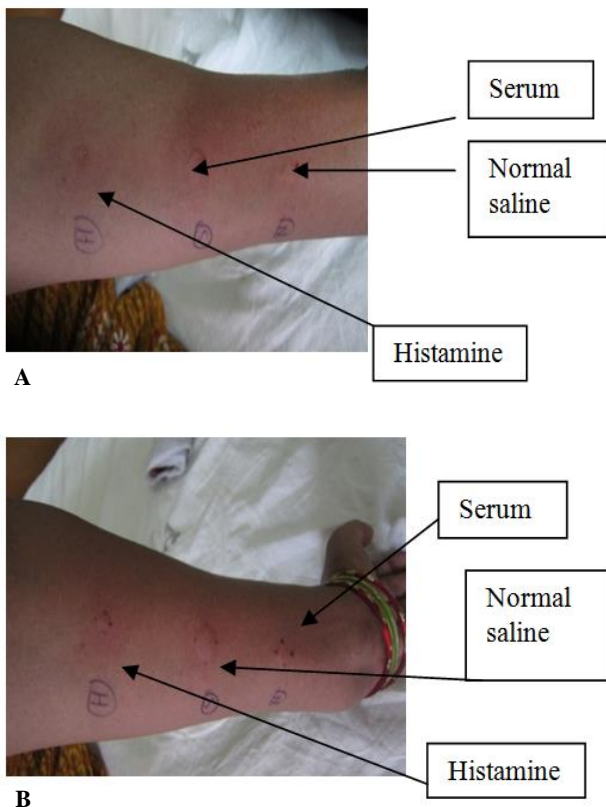


Figure 1: ASST: (A) immediate post injection; (B) 30 min post injection.

RESULTS

In present study, 250 patients who underwent ASST, 60% showed a positive test. Majority patients with chronic spontaneous urticaria belonged to the reproductive age group (20-40 years). Our study minimum age was 18 years and maximum was 75 years.

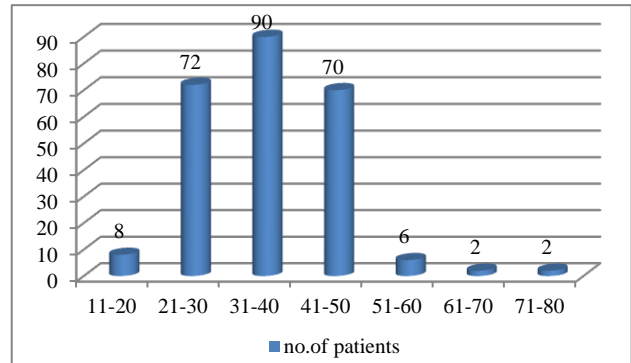


Figure 2: Age distribution.

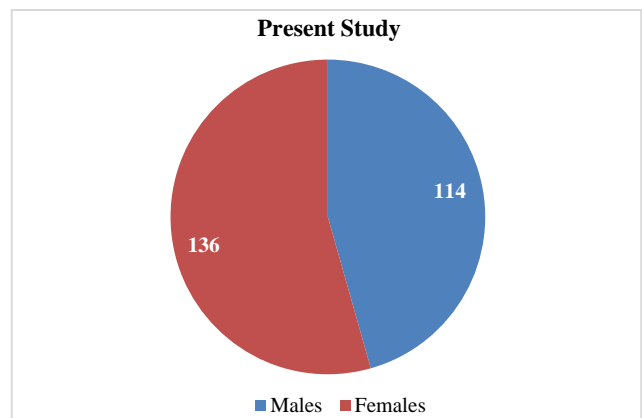


Figure 3: Comparison of sex distribution.

In present study the male: female ratio is 1:1.19, the minimum duration of the illness was 1&1/2 months while maximum was 144 month with an average duration of 23.05 months.

In our study, longer duration, angioedema and higher frequency correlated to a higher incidence of ASST positivity. Duration of each attack varied from minimum of half-an-hour to a maximum of more than 12 hours, but less than 24 hours. The average duration of each urticarial attack lasted for about 6.5 hours.

H. pylori antibodies were significantly higher (29%) for ASST positive patients when compared to ASST negative patients with a significant p value (<0.001). Similarly, antinuclear antibodies (ANA) were positive in 4% of patients, thyroid antibodies were present in 7% of patients and IgE was elevated in 32% of ASST positive patients with a significant p value.

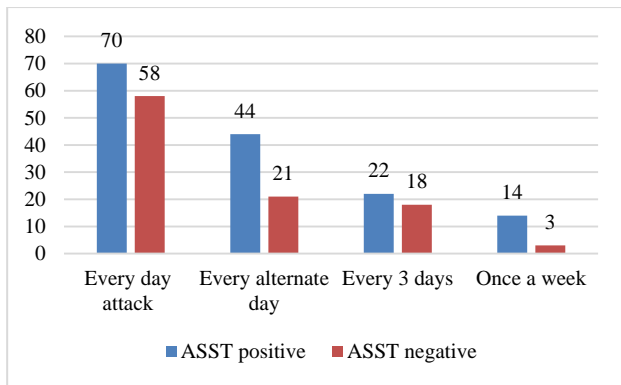


Figure 4: Frequency of attacks in ASST positive and ASST negative patients.

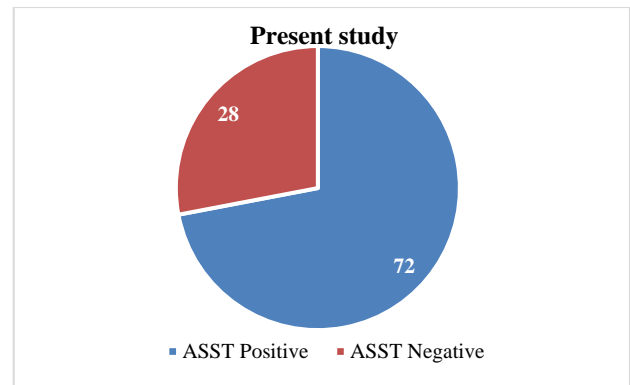


Figure 5: Angioedema in ASST positive and ASST negative patients.

Table 3: Special investigation.

Investigation	Asst +ve n=150 N (%)	Asst -ve n=100 N (%)	Chi square value at df=1	P value
ANA			24.04	<0.00001
Negative	144 (96)	100 (100)		
Positive	6 (4)	0 (0)		
Thyroid			339.56	<0.00001
Negative	124 (93)	98 (98)		
Positive	26 (7)	2 (2)		
IgE			5.76	<0.01
Negative	102 (68)	70 (70)		
Positive	48 (32)	30 (30)		
H. pylori			784	<0.00001
Negative	106 (71)	94 (94)		
Positive	44 (29)	6 (6)		

DISCUSSION

It has already been described that ASST with positive test being defined as one with serum induced wheals, which is both erythematous and has a diameter of 1.5 mm greater than saline response at 30 minutes which is a reasonably predictive clinical test to reveal functional circulating antibodies with a sensitivity of 65-71% and specificity 78-81%.¹⁰ In my study, 250 patients who underwent ASST, 60% showed a positive test comparable with study done by Shankar et al.¹¹

In this study, lesions lasting for a significantly longer duration and with higher frequency correlated to a higher incidence of ASST positivity as compared to ASST negative patients. *H. pylori* antibodies were significantly higher (29%) for ASST positive patients when compared to ASST negative patients showing a significant P value (<0.00001). It is postulated that *H. pylori* infection may induce development of pathogenic auto antibodies by molecular mimicry.^{12,13} Appelmek et al first demonstrated the molecular mimicry between *H. Pylori* and lipopolysaccharide (LPS) anti Lewis antibodies in autoimmune type-B gastritis.^{12,13}

In our study antinuclear antibodies (ANA) were found in 4% of patients, who were ASST positive with a significant p value (<0.00001).

Thyroid antibodies were present in 7% of patients with positive ASST, with a significant P value (<0.00001). IgE was elevated in only 32% ASST positive patients with a significant p value (<0.01). This suggest autoimmunity through cross reactivity or other mechanisms may play role in immunological hyper reactivity.¹¹

A study by Sabroe et al concludes that patients with auto antibodies showed frequent attacks.¹⁴ There was a statistically significant difference in TSH, thyroid antibodies and ANA between the ASST positive and negative groups, indicating a positive correlation between a positive ASST and auto immunity.

Patients with autoimmune urticaria have no distinctive, diagnostic, clinical or histopathological features which differentiate it from non autoimmune cases, although they tend to have more severe urticaria.¹⁵ Patients with positive ASST had more severe urticaria, more prolonged duration, more frequent attacks, angioedema and GI symptoms than negative ASST patients in our study.

CONCLUSION

The markers of autoimmunity may be found in many types of chronic urticaria, but ASST may be the only clinically demonstrable evidence of autoimmunity. ASST is considered as easy, simple to perform, cost effective screening test for an autoimmune urticaria. The diagnosis of autoimmune urticaria may allow use of an immunotherapy in severe disease unresponsive to other therapy. Patients with positive ASST may have more severe urticaria than negative.

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Conflict of interest: None declared

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

REFERENCES

1. Zuberbier T. A summary of the New International EAACI/GA2LEN/EDF/WAO Guidelines in Urticaria. WAO J. 2012;5:S1-S5.
2. Powell RJ. BASCI guidelines for management of chronic urticarial and angio-oedema. Clin Experimental Allergy. 2007;37:631-50.
3. Kanani A. Urticarial and angioedema. Allergy Asthma Clin Immunol. 2011;7(suppl);S9.
4. Zuberbier T, Grattan C, Maurer M (Eds.). Urticaria and angioedema, Springer Verlag Berlin Heidelberg 2010.
5. Grattan CE, Wallington TB, Warin RP, Kennedy CT, Bradfield JW. A serological mediator in chronic idiopathic urticaria: A clinical Immunological and histological evaluation. Br J Dermatol. 1986;114:583-90.
6. Ghosh S. What's new in urticarial? Indian J Dermatol. 2009;54(3):280-2.
7. Sabroe RA, Grattan CE, Francis DM, Barr RM, Kobza Black A, Greaves MW. The autologous serum skin test: Screening test for auto antibodies in chronic idiopathic urticaria. Br J Dermatol. 1999;140:446-52.
8. Zuberbier T. EAACI/GA2LEN/EDF/WAO guideline: definition, classification and diagnosis of urticaria. Allergy. 2009;64:1417-26.
9. Kaplan AP. Chronic urticaria and angioedema. N Engl J Med. 2002;246:175-9.
10. George M, Balachandran C, Prabhu S. Chronic idiopathic urticaria: Comparison of clinical feature with positive autologous serum skin test, KMC. Manipal. J Dermatol Venereol Leprol. 2008;74:105-8.
11. Krupa Shankar DS, Ramnane M, Rajouria EA. Etiological approach to chronic urticaria. Indian J Dermatol. 2010;55:33-8.
12. Ozkaya-Bayazit E, Demir K, Ozgüroğlu E, Kaymaklıoğlu S, Özarmağan G. H. pylori eradication in patients with chronic urticaria. Arch Dermatol 1998;134:1165-6.
13. Appelmelk BJ, Simoons-Smit I, Negrini R, Moran AP, Aspinall GO, Forte JG, et al. Potential role of molecular mimicry between H. pylori lipopolysaccharide and host blood group antigen in autoimmunity. Infect Immun. 1996;64:2031-40.
14. Sabroe RA, Seed PT, Francis DM, Barr RM, Black AK, Greaves MW. Chronic idiopathic urticaria: comparison of the clinical features of patients with and without anti FCεR1 or anti IgE auto antibodies. J Am Acad Dermatol. 1999;40:443-50.
15. Buss YA, Garrelfs UC, Sticherling M. Chronic urticaria which clinical parameters are pathogenetically relevant? A retrospective investigation of 339 patients. J Dtsch Dermatol Ges. 2007;5:22-9.

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