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Original Research Article

Autologous serum skin test in chronic spontaneous urticaria and its correlation with biochemical markers- A descriptive study from a tertiary hospital of North India

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ABSTRACT

Background: Autoimmune urticaria (AIU) is an important subset of chronic spontaneous urticaria (CSU) and can be relatively difficult to manage. Autologous serum skin test (ASST) has served as a useful bedside test to detect AIU and its comparison with various blood biomarkers may unlock avenues in diagnosing autoimmune urticaria.

Objective: Aim of our study was to assess role of ASST in CSU and its correlation with relevant biomarkers.

Materials and Methods: It was a hospital-based study in which 58 patients were enrolled. ASST was done in all with routine investigations including Absolute Eosinophil Count (AEC), Thyroid stimulating hormone (TSH) and Immunoglobulin E(IgE).

Results: ASST was positive in 44.8% out of 58 patients. ASST positive patients showed longer disease duration, higher severity and more generalised disease than the negative group. AEC was relatively lower, although statistically insignificant in ASST positive patients (284.21±57.43 cells/ul) as compared to AEC in ASST negative patients (310.64±71.05 cells/ul) (p=0.131). TSH on the other hand showed comparable values in both the groups (p=0.744). Mean serum IgE showed statistically significant difference between those who were ASST positive (297.96 ±42.34 IU/ml) vs ASST negative. (323.57 ±44.16 IU/ml) (p=0.029*).

Conclusions: ASST can serve as a useful test to diagnose patients with autoimmune urticaria which can often be more severe and prolonged as well as being refractory to conventional treatment. Lower values of AEC and IgE in ASST positive patients may complement the diagnosis of autoimmune urticaria.

1. Introduction

Chronic spontaneous urticaria (CU) is a potentially distressing dermatosis presenting with wheals and itching with or without angioedema.[1] Autoimmunity contributes to a large number of cases (30-50%) characterised by the presence of antibodies directed against FcεR1α receptor located on mast cells and basophils or less commonly against immunoglobulin E (IgE) itself.[2]The detection of these antibodies is important since it is difficult to identify subset of patients with autoimmune urticaria clinically. Autoimmune Urticaria consists of two sub types; auto antibody type (type 2b aiCU) and auto allergic CU (type 1 aiCSU).[3] Also, such patients are poor responders to conventional doses of antihistaminic drugs and may require corticosteroids or immunomodulatory drugs in some cases which otherwise are not recommended in routine management of chronic urticaria.[4]

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The in vitro tests like basophil histamine assay lack standardisation and are technically demanding besides being not easily available.[5] Autologous serum skin test (ASST) is a simple and easy to perform in vivo intradermal test using patient's own serum to demonstrate circulating Immunoglobulin G (IgG) antibodies against FcεR1α receptor and IgE.[6] ASST has a sensitivity of approximately 70% and a specificity of 80% thus exhibiting reasonable accuracy as a diagnostictest.[7] Test positivity of chronic urticaria patients using ASST varies in various studies from as low as 4.1% to 76.5%.[8,9] In a study by Godse et al. from Mumbai incidence was 26.6% which was in concordance with some studies from western countries.[2] There are a few studies from southern India which show ASST positivity ranging from 34% to 50%.[7,10] The variation in the prevalence can be possibly attributed to ethnic and genetic factors. India being a large country is known for its genetic heterogeneity and south Indian population differs from north Indian population in genetics, dietary preferences and environmental exposures. Besides this there is paucity of such studies conducted in north India. With this view this study intended to assess the prevalence of autoimmune urticaria and utility of ASST as a simple, low-cost alternative from the northernmost state of India. Besides this, we compared various blood parameters with ASST to test their utility as potential markers in diagnosing chronic autoimmune urticaria.

2. Materials and Methods

It was a prospective study undertaken in dermatology department of a tertiary care hospital of North India. The study was completed within the period six months from September 2019 to February 2020. Sample size was calculated using Epi Info Program (Version 7.4.2) using Confidence interval of 99 percent, power of study at 80 percent and taking point prevalence value of exposure as 1 percent. A total of 58 cases were selected for the study after fulfilling the selection criteria and ASST was done in all. Patients with daily or almost daily occurrence of urticarial wheals for six weeks or more were included in the study. Patients less than18 years, pregnant and lactating women, patients having physical urticaria, urticarial vasculitis; patients with known drug allergies or food allergies; patient taking antihistamine in the last three days, patient taking any corticosteroids, immunosuppressant drug in the last two weeks, severely ill patients, and patients with Human Immune Deficiency Virus Infection were excluded from the study. The data was obtained from the subjects after obtaining an informed consent and patient confidentiality was maintained. There were no ethical issues. A data was collected in the form of an anonymous proforma filled by the researcher which included relevant demographic data, history and clinical examination and results of laboratory investigations obtained from the subjects. A detailedl history regarding possible eliciting factors of urticaria, time of onset, duration of individual wheal in hours, duration of disease in months, size and distribution of wheals; associated angioedema etc were noted. General and dermatological examination was done. Symptoms and signs were graded on the basis of Urticarial activity score (UAS) ranging from 0 to 3 each. Urticarial score was calculated in aggregate for a week with the score ranging from 0 to 42.[11] To determine the extent of cutaneous involvement, we divided the skin surface in 5 body regions (head-neck region, chest with upper back, abdomen with lower back, upper limbs and lower limbs). The disease was classified as generalised when urticarial wheals involved more than 2 out of 5 regions. Laboratory investigations which were advised for all included: complete blood count, Thyroid stimulation hormone (TSH), Absolute Eosinophil Count (AEC), Serum Immunoglobulin E (IgE), urine examination, stool examination. Finally, ASST was performed.[7] 5 ml of venous blood was collected in a sterile vacutainer and allowed to clot at room temperature for 30 min. Serum was centrifuged at 2000 rpm for 15 min and 0.05 ml of autologous serum was injected intradermally, in uninvolved skin, using a 1 ml insulin syringe (30-gauge needle) into the left forearm 2 cm below the cubital fossa. Similarly, 0.05 ml of 0.9% sterile normal saline (negative control) was injected intradermally proximally into the right forearm. Test arm and control arm were kept separate to ensure uniformity in study protocol for the sake of convention, for ease of administration of injections and reading the result and to avoid confusion in interpretation as has been followed by majority studies on the subject in India.[2,7] A serum induced erythematous weal with a diameter of 1.5 mm more than the saline induced response within 30 min was taken as positive. [Figure 1]. Positive control (histamine) was not used due to unavailability and since result interpretation requires comparison with the negative control only (saline); thus, use of a positive control could be omitted.

2.1. Statistical analysis

Data was entered and analysed using Microsoft Excel and Statistical Product and Service Solutions (SPSS 22.0). Numerical data was calculated in the form of mean, standard deviation and categorical data as frequencies and proportions. Chi square test was used to test association between categorical variables. Mann Whitney U test was used for continuous variables. Findings with p value of <0.05 was taken as significant. Variables including epidemiological (age, gender), clinical (age of onset, site, severity and angioedema) and biochemical (TSH, AEC, IgE) were compared between ASST positive and negative group.

3. Results

The study included 58 subjects out of which 39(67.24%) were females and 19(32.76%) were males. Male to Female ratio was 1:2. [Figure 2] The age of patients ranged from 18 to 68 years with mean age of 33.9 years (standard deviation 13.4 years). More than half (57%) of patients belonged to the age group between 20-40 years. [Figure 3]

ASST was positive in 26 (44.8%) out of 58 patients and negative in 32 (55.2%). On comparing various clinical and epidemiological factors with ASST test, it was found that age and gender distribution was comparable in both ASST positive and ASST negative patients and there was no statistical difference. Similarly, age of onset of the disease did not vary significantly between the two groups. Patients who were ASST positive showed a higher mean duration of the disease (1.5 ±0.9 years) than the test negative group (1.0 ± 0.6 years), however the difference did not reach the level of statistical significance.

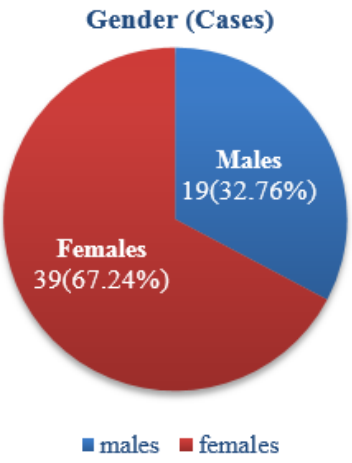

Fig. 1: Pie chart showing gender distribution of patients.

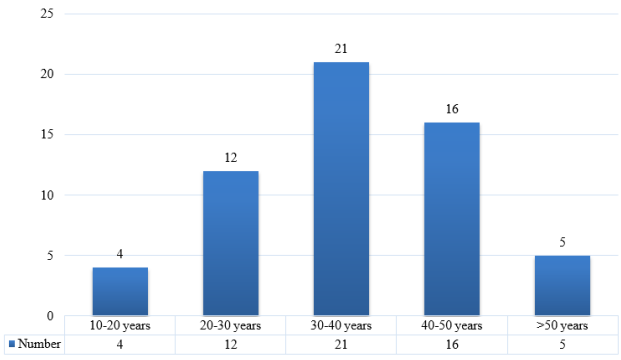

Fig. 2: Bar chart showing age distribution of patients.

On comparing the severity of chronic urticaria using UAS with respect to ASST test result, it was observed that

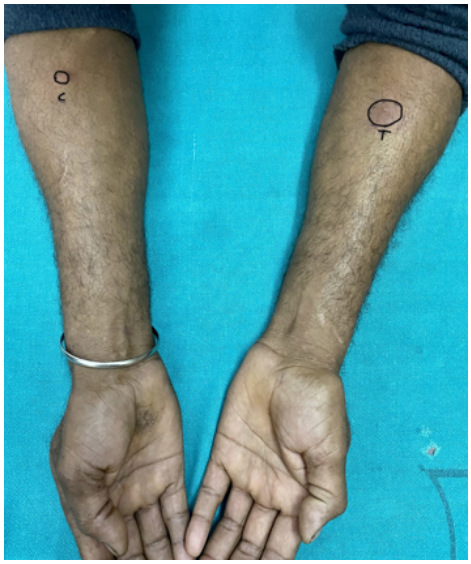

Fig. 3: Image of a patient with a positive ASST.

patients who tested positive for ASST had a higher severity (UAS[7] score >16 in 45.6%) as compared to test negative patients (UAS[7] score >16 in 18.4%). The difference was statistically significant (p=0.026). Moreover, the 30.8% patients of ASST positive had generalised involvement while 9.4% patients among ASST negative group had generalised involvement. The difference was statistically significant (p=0.033). Two patients from ASST positive group while 3 from ASST negative group had angioedema and there was no statistical difference. [Table 1]

On comparing various blood parameters with ASST test result, it was observed that mean AEC was relatively lower, although in the normal range in ASST positive patients (284.21±57.43 cells/ul) as compared to AEC in ASST negative patients (310.64±71.05 cells/ul). However, the difference did not reach the level of statistical significance (p=0.131). TSH on the other hand showed comparable values in both the groups (p=0.744). Mean serum IgE however showed statistically significant difference between those who were ASST positive (297.96 ±42.34 IU/ml) vs ASST negative. (323.57 ±44.16 IU/ml) (p=0.029*). ASST positive patients had lower mean IgE levels as compared to ASST negative patients.

4. Discussion

Chronic spontaneous urticaria is one of the common and distressing chronic dermatosis both for the patient and treating dermatologist. With advancement in understanding of chronic urticaria, a distinct subgroup known as Autoimmune Urticaria (AIU) is now considered a major contributor and can be relatively resistant to conventional management with normal doses of antihistaminic drugs. It may require use of corticosteroids and immunomodulatory

Table 1: Comparison of various clinical and epidemiological factors based on ASST.

Feature	ASST positive	ASST negative	p value
Age (yrs.),			
mean±SD	34.6±13.5	33.2±13.8	0.980
Gender			
Female	19(73.1%)	20(62.5%)	0.397
Male	7(26.9%)	12(37.5%)	
Mean			
Age of onset (yrs.)	31.2±11.7	33.8±12.20	392
Duration of urticaria (yrs)			
Mean and SD	1.5±0.9	1.0±0.6	0.768
UAS[7]			
0-16	15(54.4%)	27(81.6%)	0.026*
16-42	11(45.6%)	5(18.4%)	
Site of lesion			
Localized	18(69.2%)	29(90.6%)	0.033*
Generalized (>2/5 regions)	8(30.8%)	3 (9.4%)	
Angioedema			
Present	2(7.7%)	3(9.4%)	0.816
Absent	24(92.3%)	29(90.6%)	
Total	26(100%)	32(100%)	

ASST: Autologous serum skin test, Min: Minimum, Max : Maximum.
*P value of <0.05 is taken as significant.

Table 2: Relationship of various blood parameters with ASST.

Biochemical variable	ASST positive	ASST negative	P value
AEC [cells/uI]	284.21±57.43	310.64±71.05	p=0.131
TSH [uIU/ml]	3.23 ±1.52	3.1±1.49	p=0.744
Total IgE [IU/ml]	297.96 ±42.34	323.57 ±44.16	p=0.029*

Mann Whitney U Test
AEC: Absolute eosinophil count, TSH: Thyroid Stimulating Hormone, IgE: Immunoglobulin E.
*P value of <0.05 is taken as significant.

drugs especially during exacerbations.[12,13] ASST which detects the circulating antibodies in such patients can serve as a useful bedside test to detect patients of AIU.[10] In our study, ASST was found to be positive in 26 (44.8%) out of 58 patients suffering from chronic urticaria. Various studies from abroad and some from south India have shown similar positivity rate.[7,13] Interestingly, AIU also contributes to a similar percentage of cases of CU (30-50%) which further supports the utility of ASST test in detecting autoimmune urticaria.[2] In our study, we found that in patients who tested ASST positive, chronic urticaria lasted for a longer time (1.5 ±0.9 years) as compared to the test negative group (1.0 ± 0.6 years), however the difference did not reach the level of statistical significance perhaps due to a small sample size. Association of duration of diseases with ASST positivity was also demonstrated by some previous studies by Sabroe et al. and Fusari et al.[14,15] However, Kulthanan et al.and Vikrakumar et al. showed no such association.[16,17]

Severity of chronic urticaria in our study was calculated using UAS,[7] it was observed that patients who tested positive for ASST had a higher urticaria severity (UAS[7] score >16 in 45.6%) as compared to test negative patients (UAS[7] score >16 in 18.4%). The difference was statistically significant (p=0.026). Moreover, patients having ASST positive had more generalised involvement when compared to ASST negative group. The difference was statistically significant (p=0.033). Various studies in the past have also shown that cases of AIU diagnosed by ASST show a higher disease severity and extent of involvement.[2,10,17]

We also tried to elicit relationship between various blood parameters with ASST. In our study, we found that both AEC and IgE showed lower mean values in ASST positive patients versus test negative patients. In case of AEC the difference did not reach the level of statistical significance (p=0.131) however the serum total IgE mean value showed statistically significant difference between those who were ASST positive versus ASST negative(p=0.029*). TSH on the other hand showed comparable values in both the groups (p=0.744). Some studies have however demonstrated high IgE and thyroid abnormalities in ASST positive patients which is contrary to our results.[18] We did not observe any association of ASST with thyroid dysfunction perhaps because of a small sample size and the absence of antithyroid antibodies in our workup. We believe that

ASST positive subgroup of chronic urticaria more or less represents the patients of Autoimmune Urticaria (type 2b aiCU) which is characterised by autoantibody mediated eosinopenia or low normal eosinophil count as compared to auto allergic CU (type 1 aiCSU). Similar observations were made by Kolkhir et al. who concluded in that eosinopenia patients of chronic urticaria is associated with type IIb autoimmunity, high disease burden and poor response to treatment and recommended that eosinophils should be explored as potential biomarkers in CU.[19] This also explains the reason for relatively low IgE in ASST positive patients. The increased IgG autoantibodies may saturate the FcεR1α receptor sites causing receptor mediated down regulation or bind directly to IgE antibody thus decreasing their availability in the serum. These observations can pave way to explore the role of AEC, serum IgE and other biomarkers to screen patients with autoimmune Urticaria which along with ASST may complement in establishing the diagnosis of AIU.

Limitations of our study included a small sample size; in vitro tests were not done; other potential markers like anti-thyroid antibody levels (anti TPO), D Dimer and IL 6 were not done.

We thus conclude that ASST is a useful test in detecting autoimmune chronic urticaria which differs from other forms of chronic urticaria in being more prolonged, severe and generalised besides being refractory to antihistamines. Thus, ASST may help in timely recognition and management of this subgroup. Also, blood parameters like AEC and IgE can potentially serve as valuable diagnostic ensemble along with ASST.

5. Acknowledgements

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6. Source of Funding

None.

7. Conflict of Interest

None.

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