

# Autologous Serum Skin Test for Initial Identification of Auto-Antibodies in Chronic Urticaria

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## Abstract

**Background:** Autologous serum skin test (ASST) is easy to perform, cost effective measure for initial identification of auto-antibody in patients suffering from chronic urticaria which may occur due to autoimmune trigger by spontaneously developed auto-antibodies against Fc $\mu$ RI receptor of skin mast cells.

**Objective:** This review is designed to see the positivity of ASST in patients suffering from chronic urticaria.

**Materials & Methods:** This a retrospective data analysis conducted from the records of patients suffered from at least two episodes of urticaria or more in a week for 6 weeks and who did ASST aged more than 10 years between July 2015 to June 2017. These subjects were not pregnant or lactating mother, did not had urticarial vasculitis, predominant co-existing physical urticaria and had negative Hepatitis B antigen and anti-nuclear antibody reports.

**Results:** Total 53 were included in this study of them 33 were female. Mean age was 32 years with a standard deviation (SD) of 11 years. Among study patients 16 (30.2% of total) had a positive ASST result. No age or sex difference was observed in positive ASST cases. In positive cases significant (p-value: <0.001) mean induration difference of 2.75mm with a SD of 1.00 mm observed than induration produced by negative control.

**Conclusion:** ASST is easy and effective tool for initial identification of auto-reactive urticaria.

**Key Words:** autologous serum skin test, chronic urticaria, chronic spontaneous urticaria.

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## Introduction:

Urticaria is very common short-lived skin swellings due to transient leakage of plasma into the surrounding superficial dermis and its estimated lifetime prevalence is 20%.<sup>1</sup> Release of numerous preformed chemotactic factors, enzymes,

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cytokines and proteoglycans from cutaneous mast cell granules or are newly generated from mast cell membrane mediates urticaria. Episodes of urticaria occurring at least twice a week for six weeks are considered to be chronic. More than 50% of the patients suffering from chronic urticaria (CU) were diagnosed to have chronic spontaneous urticaria (CSU) as no cause could be identified.<sup>2</sup> About one third of all cases of CSU are the result of an autoimmune trigger. Around 30-40% of patients with CU spontaneously develop autoantibodies directed at the receptor Fc $\mu$ RI located on skin mast cells.<sup>3</sup> Chronic stimulation of this receptor leads to chronic hives. Simultaneously patients may have other autoimmune conditions like autoimmune thyroiditis, celiac disease, type 1 diabetes, rheumatoid arthritis, Sjogren's syndrome or systemic lupus erythematosus. A subset of patients with CU has complement activating (C5a), histamine releasing IgG autoantibodies that degranulate mast cells and basophils by binding to the  $\alpha$ -chain of the high-affinity IgE receptor.<sup>4</sup>

A clinical test to demonstrate circulating endogenous wheal-inducing factors in urticaria, the so-called autologous serum skin test (ASST), was developed in 1980.<sup>5</sup> Though Basophilic Histamine release assay is "Gold Standard" in detection of functional antibodies from serum of patients is time consuming and requires flowcytometry and standardization. Western blotting, enzyme-linked immunosorbent assay and

flow cytometry using chi-merci cell lines expressing the human Fc $\mu$ RI $\pm$  useful for further identification and need validation.<sup>6</sup> ASST has approximate sensitivity 70% with specificity 80% and indicates the presence of functional circulating autoantibodies against Fc $\mu$ RI $\pm$  and/or IgE, is the best *in vivo* clinical test in detection of histamine release activity *in vitro* when the positive result is defined as a pink, serum-induced wheal with a diameter  $\geq$ 1.5 mm than adjacent wheal of normal saline control injection after 30 minutes.<sup>7-8</sup> ASST is easy to perform and need no costly instrument. In low resource country like Bangladesh, it may be an alternative for initial detection of functional antibodies in CSU patients and useful for consideration of immunomodulatory treatment. Till there is no data on ASST in Bangladesh, this retrospective database study was designed to see the positivity of ASST of CU patient in Bangladesh.

#### Materials & Methods:

This is a retrospective data analysis of records of patents aged more than 10 years attended in Dermatology outpatient services of Farazy Hoapital limited, Block-E, Banosree, Dhaka, Bangladesh between July 2015 to June 2017 with history of more than 6 weeks urticaria or suspected urticaria occurring at least twice a week who did the ASST. Patients with urticarial vasculitis, predominant co-existing physical urticaria, patient who were pregnant or in lactation period and patients who were positive for Hepatitis B antigen and antinuclear antibody were excluded. Written informed consent was taken from every patient before performing ASST. Seven days before performing ASST all forms of anti-histamines were discontinued and all forms of immunosuppressive drugs were stopped at least two months before performing ASST.

#### Autologous serum skin test technique:

Two milliliter venous blood was collected from antecubital vein of patients and placed in a sterile plastic tubes and allowed for clotting for 30 minutes at room temperature. After clotting serum was separated by centrifugation at 2000 rpm for 15 minutes and kept in aliquots for use in the ASST. 0.05 milliliter autologous serum was injected intradermally into the volar aspect of forearm skin, avoiding the areas of wheeling within past 24 hours and equal volume 0.9% sterile saline (for negative control) were separately injected with 27G needles, leaving gaps of at least three centimeters. No positive control was used. Wheals and flare responses were measured after 30 minutes. A positive ASST was defined as a serum-induced wheal which was both red and had a diameter of 1.5 mm or more than the saline-induced response at 30 minutes<sup>7</sup>. No control patient was taken and no specificity or sensitivity was measured against any gold standard methods of identification of spontaneous antibody in patients of CSU.

#### Statistical analysis:

All statistical analyses were conducted using SPSS version 22 for windows with two-tailed 95% confidence interval. ASST data for positive and negative test results were analyzed for demographic data such as sex, mean age, related clinical indications. Continuous data is presented as mean, standard deviation (SD) and categorical data is presented percentages. *P*-value is extracted by using independent sample *t*-test for continuous data and chi-square test for categorical data.

#### Results:

Fifty-three ASST reports of chronic urticaria patients is analyzed who meet the inclusion criteria. Among them 20 (37.7%) were female and rest were male. Male, female ratio was 1.65 and mean age 32 years with a SD of 11 years. (Table-1).

**Table-1**  
*Characteristics of ASST positive and ASST negative patients.*

Characteristics	All patients, n=53 (100%)	Positive ASST, n=16 (30.2%)	Negative ASST, n=37(69.8)	<i>p</i> - <i>value</i>
<b>Sex</b>				
Male, n (%)	20 (37.7)	8 (15.1)	12 (22.6)	0.226 <sup>ns</sup>
Female, n (%)	33 (62.3)	8 (15.1)	25 (47.2)	
<b>Age in years, (mean<math>\pm</math>SD)</b>	32 $\pm$ 11	34 $\pm$ 10	31 $\pm$ 11	0.471 <sup>ns</sup>
<b>Related clinical indications, n (%)</b>				
Chronic urticaria	34 (64.2)	11 (20.8)	23 (43.4)	0.201 <sup>ns</sup>
Atopic dermatitis with chronic urticaria	12 (22.6)	2 (3.8)	10 (18.9)	
Dermato-graphism with chronic urticaria	1 (1.9)	0 (0.0)	1 (1.9)	
Chronic urticaria with allergic rhinitis	1 (1.9)	0 (0.0)	1 (1.9)	
Psoriasis with atopy with chronic urticaria	2 (3.8)	2 (3.8)	0 (0.0)	
Atopic dermatitis with chronic urticaria with dermato-graphism	1 (1.9)	1 (1.9)	0 (0.0)	
Infectious eczematoid dermatitis with suspected urticaria	1 (1.9)	0 (0.0)	1 (1.9)	
Seborrheic dermatitis with atopic dermatitis	1 (1.9)	0 (0.0)	1 (1.9)	

ASST= autologous serum skin test, n= number of cases, SD=standard deviation, ns= not significant.

**Table-II**  
*Test results of study patients (n=53)*

Interpretations in mm	Test result	n	Mean	±SD	Min-	Max	<i>p-value</i>
Negative Control	Positive	16	7.06	±1.73	3-	9	0.009 <sup>s</sup>
	Negative	37	5.84	±1.41	3-	8	
Total		53	6.21	±1.60	3-	9	
Test induration	Positive	16	9.81	±1.87	7-	14	<0.001 <sup>s</sup>
	Negative	37	6.62	±1.36	4-	9	
Total		53	7.58	±2.12	4-	14	
Difference induration	Positive	16	2.75	±1.00	2-	5	<0.001 <sup>s</sup>
	Negative	37	1.00	±0.00	1-	1	
Total		53	0.30	±0.46	1-	5	

n= number of cases, SD=standard deviation, Min= minimum, Max=maximum, s= significant

Sixteen patients of chronic urticaria were found positive ASST with significantly high (*p*-value: <0.001) mean induration 9.81±1.87mm and difference of induration 2.75±1.00 mm than negative control induration produced by 0.9% normal saline (table: II). All the patients experienced more than two episodes of urticaria in a week for six weeks among them 34 (64.2%) had only chronic urticaria, 12 (22.6%) patients had atopic dermatitis with urticaria and rest of the patients experienced dermato-graphism, allergic rhinitis, psoriasis, atopic dermatitis, infectious eczematoid dermatitis, seborrheic dermatitis along with chronic urticaria (Table 1).

Among 16 ASST positive patients 8 (15.1%) patients were male and same number of patients were female and mean age of positive ASST patients were 34 years with SD of 10 years. No significant difference observed between male and female positive ASST cases. No significant difference also observed regarding age in ASST positivity (Table 1).

Eleven patients (20.8%) out of 34 chronic urticaria patients, were found ASST positive. Two patients (3.8%) of psoriasis with chronic urticaria were positive for ASST and no significant difference found among patients of different presentations (table:1).

#### **Discussion:**

After introduction of ASST in 1986 several analyses done worldwide with a view to initial identification of auto-reactive antibody in CU patients and no previous study done in Bangladesh before.<sup>9</sup> We found ASST positivity in this retrospective study of Bangladeshi population is 30.2%. Sabroe *et al.* reported that one-third of their patients with CSU had circulating functional antibody against the high affinity IgE receptor FcμRI± or IgE.<sup>7</sup> Nettis *et al.* reported

that serum from 42 of 102 Italian patients with CSU (41.2%) induced a wheal-and-flare response.<sup>1</sup> Krause K *et al.* reported 24.7 % ASST positivity in Thai patients.<sup>9</sup> Caproni *et al.* showed that 23 of 68 Italian patients (34%) with CSU had a positive reaction to ASST.<sup>10</sup> A review of more than 50 worldwide different analysis of ASST positivity study by Krause K *et al.* showed mean positivity is 45.9% and positivity reported 35%-45% in most of the study among chronic spontaneous urticaria patients<sup>9</sup>. Godse K V showed 25%-45% patient of chronic urticaria had positive ASST.<sup>11</sup> So, our findings in Bangladeshi population is more or less similar with other study.

Findings in terms of the female predominance of chronic urticaria is similar with Caproni *et al.* and Kulthanan K *et al.*<sup>10,12</sup> But no significant sex difference observed (*p*-value:0.226) among subjects with positive or negative ASST and also no significant age difference (*p*-value:0.471) observed among positive or negative ASST subjects. These finding are similar to Krause K *et al.* findings.<sup>9</sup>

ASST is a recommended test of screening for identification of functional antibody in CU patients as ASST negativity excludes presence of functional antibodies in around 90% cases.<sup>13</sup> So, from similar ASST positivity of this study with other studies, we recommend the use of this simple screening tool for initial detection of functional antibodies in Bangladesh.

However, in this study no comparison done regarding specificity or sensitivity with any gold standard methods of antibody detection and finally a multicenter comparative study of ASST with Basophilic Histamine release assay involving larger number of subjects recommended in Bangladesh.

**Conclusion:**

Autologous serum skin test is cost effective, easy and safe test to detect auto-reactive antibody against mast cell membrane receptor with good positivity rate compared with other studies. We can safely use ASST for initial screening to identify auto-reactive antibody in chronic urticaria patients of Bangladesh. We hope that this retrospective analysis report will encourage further studies and understanding of the factors implicated in the CU.

**Conflict of interest:** None.

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