

## Autologous serum skin test versus histamine release test in the diagnosis of Chronic autoimmune urticaria

Al-Sadat Mosbeh,<sup>1</sup> MD, Ibrahim G. Ramadan,<sup>2</sup> MD, Abdelaleem Elgendy,<sup>3</sup> MD

<sup>1</sup>Department of Dermatology and Venereology, <sup>2</sup>Department of Internal Medicine, <sup>3</sup>Department of Clinical Pathology Al-Azhar University Hospitals, Faculty of Medicine Al-Azhar University, Cairo, Egypt

### ABSTRACT

**Background:** Diagnosis of chronic autoimmune urticaria (CAU) is difficult; autologous serum skin test (ASST) seems to be a useful screening test which should be completed with specific and confirmatory test. Histamine release test (HR-test) is a gold standard, specific and confirmatory test to detect autoimmune urticaria.

**Aim:** This study was designed to compare an *in vivo* ASST with an *in vitro* HR-test for the diagnosis of CAU and to evaluate antithyroglobulin antibody (Anti-Tg) in patients and controls.

**Patients and Methods:** A total of 27 patients with chronic idiopathic urticaria (CIU) and 10 healthy controls were submitted for ASST, HR-test and antithyroglobulin antibody (Anti-Tg).

**Results:** A Positive ASST was detected in 14 patients (51.85%) and 4 healthy controls (40%), the difference was not significant ( $P$  value = 0.253). A HR-test showed positive result in 10 patients (40.74%), but no positive results in the controls, the difference was statistically significant ( $P$  value < 0.005). A Positive Anti-Tg was seen in 4 patients (14.11%), but no positive results was seen in control group, the difference was statistically significant ( $P$  value < 0.005). All patients with positive Anti-Tg were also positive for ASST (28.57%) and HR-test (40%).

**Conclusion:** The ASST is a screening test but HR-test is a specific, dependable and confirmatory test for the diagnosis of CAU. Also, the HR-test is guaranteed in the diagnosis of CAU for patients on continuous medications such as antihistamines and corticosteroids. Antithyroid antibodies should be screened in all patients with chronic urticaria (CU) even in euthyroid state.

**KEY WORDS:** Chronic urticaria (CU), chronic autoimmune urticaria (CAU), chronic idiopathic urticaria (CIU), autologous serum skin test (ASST), histamine release test (HR-test), antithyroglobulin antibody (Anti-Tg)

### INTRODUCTION

Chronic urticaria (CU) refers to continuous or intermittent wheals existing for > 6 weeks that may be caused by physical stimuli, allergy, systemic illness, drugs, or infection. However, in the majority of cases (80% - 90%) no eliciting cause is identified.<sup>1</sup> CU may be divided into chronic autoimmune urticaria (CAU) and chronic idiopathic urticaria (CIU).<sup>2,3</sup>

The earliest direct evidence for CAU comes from studies that showed that the injection of patient

serum into the dermis of the skin caused a wheal and flare response. This *in vivo* test, the autologous serum skin test (ASST), was first used in the 1940s to diagnose CAU. The ASST seem to be useful screening test, which should be completed with confirmatory assay.<sup>4</sup> The gold standard for determining an autoimmune cause of CU is the histamine release test (HR-test).<sup>5</sup>

Moreover, an association between CU and autoimmune thyroid disease has been recognized since the early 1980s. Specifically, it has been

---

**Correspondence:** Dr. Al-Sadat Mosbeh, Department of Dermatology and Venereology, Al-Azhar University Hospitals, Faculty of medicine Al-Azhar University, Cairo, Egypt. Email: sadatmosbeh@gmail.com

shown that a higher percentage of patients with CU show autoantibodies against either thyroid peroxidase (TPO) or thyroglobulin (Tg) than a normal healthy population.<sup>6</sup> There is also evidence that HLA DRB4 class II alleles may predispose individuals to CAU.<sup>7</sup>

In current study, we compared an *in vivo* ASST and an *in vitro* HR-test for the diagnosis of CAU. We also evaluated antithyroglobulin antibody (Anti-Tg) levels in the same patients and controls.

### PATIENTS AND METHODS

A total of 27 patients with CIU and 10 healthy volunteers (control group) were enrolled in this study. The patients were collected from Al-Azhar University hospitals during the period from year 2012 to 2014. All Patients who were included in the study met the clinical criteria for CIU (characteristic wheals lasting < 24-48 hours occurring most days for > 6 weeks) with exclusion of any known cause of chronic urticaria such as food allergy, drugs, infectious agent, and parasitic infestation. Routine investigations including complete blood count, random blood sugar, urine analysis, liver functions, kidney functions, thyroid profile (T3, T4 and TSH) and hepatitis markers were done to exclude non-idiopathic chronic urticaria. Patients with physical urticaria, urticarial vasculitis, children and pregnant women were excluded from the study. An informed consent was obtained from all patients and controls. Patients were advised to stop antihistamines for 2 days and corticosteroids for one week before the blood sample was collected. All the patients and controls underwent ASST, HR-test and Anti-Tg.

Five ml of venous blood was drawn into sterile tubes without clotting accelerator, and allowed to

clot at room temperature for 30 minutes. Serum was separated by centrifugation at 500g for 15 minutes and divided into aliquots for use in the HR-test, ASST and Anti-Tg.

**Histamine release test (HR-test):** 50%, 25% and 12.5% serum dilutions were incubated with dextran-sediment basophils from healthy donor and histamine release over 16.5% was considered positive. The donor basophils were selected on the basis of histamine release response to anti IgE. Histamine was determined by fluorometry by glass fiber method (HR-test; RefLab, Copenhagen, Denmark). Test results were reported as positive or negative. In the laboratory, serum and donor basophils were incubated and histamine release of more than 16.5% was considered positive. All positive HR-test reactions were detected at the 12.5% serum dilution.<sup>8</sup>

**Autologous serum skin test (ASST):** 0.05mL of serum and 0.9% sterile saline as a control were injected intradermally into volar forearm skin with at least 3 cm between injection sites. A reading of the wheal was taken after 30 minutes. A wheal and flare of more than 1.5 mm diameter than that of the control was considered positive.<sup>9</sup>

**Antithyroglobulin antibody (Anti-Tg):** Level of serum Anti-Tg was measured on the Immulite 200 (Siemen, Los Angles, CA, USA). The value  $\geq$  40 IU/ml was considered positive.<sup>6</sup>

### RESULTS

The study included 27 patients, of which there were 15 females (55.5%) and 12 males (44.5%). The control group was formed by 10 age and sex

**Table 1** Comparison of ASST, HR test and Anti-Tg in both patients and controls

	ASST		HR test		Anti-Tg	
	Positive	Negative	Positive	Negative	Positive	Negative
<b>Patients</b>	N = 14 51.85 %	N = 13 48.14	N = 10 40.74%	N=17 62.96%	N=4 14.11%	N=23 85.18
<b>Controls</b>	N=4 40%	N=6 60%	N=0 0%	N=10 100%	N=0 0%	N=10 100%
<b>P value</b>	= 0.253		< 0.005		< 0.005	

matched healthy volunteers. The age of patients ranged from 20 - 44 years with an average age of 36.2 years. Controls group included 6 females (60%) and 4 males (40%) with an average age 35.3 years.

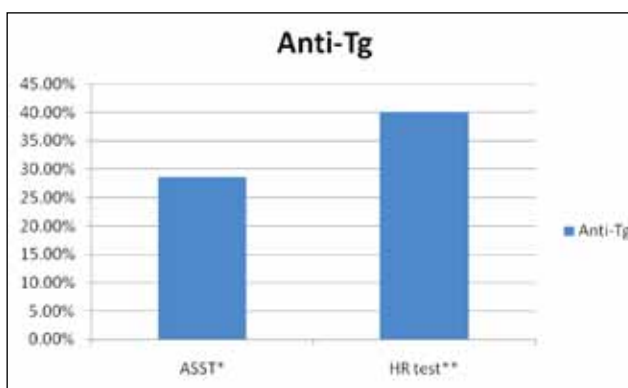
Out of 27, 14 patients (51.85%) and 4 controls (40%) showed positive ASST, the difference was not statistically significant ( $P$  value= 0.253). While, 10 patients (40.74%) showed positive HR-test, but none of controls was positive for HR-test, the difference was significant statistically ( $P$  value < 0.005). The data has been summarized in table.

One patient was taking anti-histamine (Cetirizine 10mg/day) and another patient was on systemic steroid (prednisolone 20mg/day), and the medications could not be stopped due to intolerable itching. These patients showed negative results for ASST and positive results for HR-test.

Anti-Tg test was positive in 4 patients (14.11%), in spite of the normal thyroid profile, while all controls were negative for Anti-Tg, the difference was statistically significant ( $P$  value < 0.005) (Table 1). Patients who showed positive Anti-Tg were also positive for ASST (28.57%) and HR-test (40%). (Fig. 1).

## DISCUSSION

It is difficult to establish the etiology in patients of chronic urticaria (CU). There is a strong evidence for an autoimmune basis for a group of patients

**Fig. 1** Percentage of Anti-Tg in positive ASST and HR tests

with CU, Involvement of functional autoantibodies against the high-affinity IgE receptor ( $Fc\epsilon R1\alpha$ ) of dermal mast cells and basophils has been documented and the term CAU identifies a subset of patients in which these autoantibodies are the cause of the lesions, as suggested by a positive response to intradermal injection of autologous serum *in vivo*.<sup>2,3</sup> Although numerous attempts have been made to devise specific *in vitro* tests for CAU, including enzyme-linked immunosorbent assay and other immunobinding assays, the current gold standard laboratory test is the demonstration and measurement of histamine release from target basophils or dermal mast cells by flow cytometry.<sup>10,11</sup> A positive ASST is suggestive but not diagnostic of an autoimmune urticaria and confirmation is needed by HR-test. Autoimmune and non autoimmune cases are indistinguishable both clinically and histologically, however later tends to have more severe urticaria.<sup>12</sup>

In agreement with previous studies,<sup>12-14</sup> HR-test is considered a specific and confirmatory standard test for the diagnosis of autoimmune chronic urticaria. We found that there was discordance between the results of HR-test and ASST. This may be contributed to that ASST being an *in vivo* test, which measures the response of mast cells, in addition bradykinin, thrombine and C5a may

be generated during clotting process leading to vascular permeability, edema and mast cell degranulation plus the irritant effect of serum which has a role in the genesis of the positive reaction. Whereas, HR-test is *in vitro* test which use donor basophils that were selected on the basis of histamine release response to anti IgE.<sup>13,15,16</sup>

It is interesting to note that patients with chronic urticaria have involvement of skin mast cells but have less involvement of other tissue mast cells, such as those in the lung and gastrointestinal tract.<sup>13</sup>

Two patients who could not stop antihistamines and corticosteroids in this study showed a positive HR-test but a negative ASST, these results were in agreement with the study of Hyry et al. An explanation of these results may be that the donor basophils in HR-test are not inhibited.<sup>8</sup> Histamine release test can be used in the diagnosis of autoimmune urticaria in patients on continuous medication such as antihistamines and corticosteroids.

The frequency of thyroid-specific autoantibodies in chronic urticaria patients, as shown in some of the previous studies<sup>17-19</sup> varies from 12.1% to 26.7%. In the current study the percentage of anti-thyroid antibodies in patients with chronic urticaria was found to be 14.11%. This does not suggest only a cause and effect relationship between autoimmune chronic urticaria and autoimmune thyroid disease, but it also suggests that individuals that are predisposed to one autoimmune disease are at risk of other types of autoimmune disorders. Although most patients with thyroid autoantibody were euthyroid, the detection of thyroid autoantibodies may precede the development of hypothyroidism, and this test can serve as a marker to be followed serially over time.<sup>13</sup> Patients with chronic urticaria should be screened for antithy-

roid antibodies even in euthyroid state.

Diagnosis of autoimmune chronic urticaria is important in clinical practice, it reduces the need for extensive testing in search of an exogenous cause and, ultimately may reduce unnecessary laboratory testing. In addition, immunomodulatory drugs may be initiated sooner in chronic urticaria patients who are known to have an autoimmune process.<sup>20,21</sup> So, histamine release test is beneficial for patients with chronic urticaria to diagnose autoimmune urticaria.

We believe that the ASST is an invasive technique as it an *in vivo* clinical test. Also, it is a screening and non specific test for the diagnosis of autoimmune urticaria, it cannot be used for patient on continuous medication, it requires well trained performers, there is also a risk of injecting wrong serum into a patient. While, the HR-test is a non invasive as it an *in vitro* laboratory assay, it is measured by flow cytometry. It is specific, dependable, confirmatory and standard test for the diagnosis of autoimmune urticaria. In addition, HR-test is helpful in the diagnosis of autoimmune urticaria for patients with continuous medication such as antihistamines and corticosteroids.

## CONCLUSION

The ASST is a screening test, while HR-test is a specific, dependable, confirmatory and standard test for the diagnosis of chronic autoimmune urticaria. Also, the HR-test is guaranteed in the diagnosis of autoimmune urticaria for patients with continuous medication such as antihistamines and corticosteroids. Antithyroid antibodies should be screened in all patients with chronic urticaria even in euthyroid state.

**REFERENCES**

1. Kikuchi Y, Kaplan AP. Mechanisms of autoimmune activation of basophils in chronic urticaria. *J Allergy Clin Immunol* 2001; 107:1056-62.
2. Kaplan AP, Greaves M. Pathogenesis of chronic urticaria. *Clin Exp Allergy* 2009; 39:777-87.
3. Kaplan AP. Chronic urticaria: pathogenesis and treatment. *J Allergy Clin Immunol* 2004; 114:465-74.
4. Soundararajan S, Kikuchi Y, Joseph K, et al. Functional assessment of pathogenic IgG subclasses in chronic autoimmune urticaria. *J Allergy Clin Immunol* 2005; 115:815-21.
5. Kaplan AP, Joseph K. Basophil secretion in chronic urticaria: autoantibody-dependent or not? *J Allergy Clin Immunol* 2007; 120:729-30.
6. Zauli D, Grassi A, Ballardini G, et al. Thyroid autoimmunity in chronic idiopathic urticaria: implications for therapy. *Am J Clin Dermatol* 2002; 3:525-28.
7. O'Donnell BF, O'Neill CM, Francis DM, et al. Human leucocyte antigen class II associations in chronic idiopathic urticaria. *Br J Dermatol* 1999; 140:853-58.
8. Hyry H, elg p, Ranki. A commercial histamine release test and autologous serum skin test in the diagnosis of autoimmune urticaria. *Allergy* 2006; 61:1146-47.
9. Sabroe RA, Grattan CE, Francis DM, Barr RM, Kobza BA, Greaves MW. The autologous serum skin test: a screening test for autoantibodies in chronic idiopathic urticaria. *Br J Dermatol* 1999; 140:446-52.
10. Sabroe RA, Fiebiger E, Francis DM, Maurer D, Seed PT, Grattan CE, et al. Classification of anti-FcεRI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol* 2002; 110:492-99.
11. Puccetti A, Bason C, Simeoni S, et al. In chronic idiopathic urticaria autoantibodies against FcεRII/CD23 induce histamine release via eosinophil activation. *Clin Exp Allergy* 2005; 35:1599-1607.
12. Platzer MH, Grattan CEH, Poulsen LK, et al. Validation of basophil histamine release against the autologous serum skin test and outcome of serum-induced basophil histamine release studies in a large population of chronic urticaria patients. *Allergy* 2005; 60:1152-56.
13. Michelle L, Jhon F., Leonard C. Comparison of the in vivo autologous skin test with in vitro diagnostic test for diagnosis of chronic autoimmune urticaria. *Allergy Asthma Proc* 2009; 30:28-34.
14. Godse KV, Nadkarni, Jani G. comparison of commercial histamine release test and autologous serum skin test in the diagnosis of autoimmune urticaria 2010; 76:413-15.
15. Asero R, Roboli p, Tedeschi, et al. chronic urticaria: A disease at a crossroad between autoimmunity and coagulation. *Autoimmune Rev* 2007; 7:71-76.
16. Sabroe RA, Greaves MW. chronic idiopathic urticaria with functional autoantibodies. *Br J Dermatol* 2006; 154:813-19.
17. Leznoff A, Josse RG, enburg J, et al. Association of chronic urticaria and angioedema with thyroid autoimmunity. *Arch Dermatol* 1983; 119:636-40.
18. Verneuil L, Leconte C, Ballet JJ, et al. Association between chronic urticaria and thyroid autoimmunity: A prospective study involving 99 patients. *Dermatology* 2004; 208:98-103.
19. Rumbly JS, Katz JL, Schocket AL. Resolution of chronic urticaria in patients with thyroid autoimmunity. *J Allergy Clin Immunol* 1995; 96:901-05.
20. Sands MF, Blume JW, Schwartz SA. Successful treatment of 3 patients with recurrent idiopathic angioedema with omalizumab. *J Allergy Clin Immunol* 2007; 120:979-81.
21. Spector SL, Tan RA. Effect of omalizumab on patients with chronic urticaria. *Ann Allergy Asthma Immunol* 2007; 99:190-93.