

# The Role of antiFcεRIα Autoantibodies Detection and Autologous Serum Skin Test in Comparison To Histamine Release Assay in Diagnosis of Chronic Autoimmune Urticaria

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Chronic autoimmune urticaria is manifested by wheals and itching for 6 weeks, which is mediated mainly by autoantibodies against IgE receptors (FcεRIα). We aimed to assess the role of IgG autoantibody against FcεRIα in combination with autologous plasma skin test (APST), and autologous serum skin test (ASST) for autoimmune urticaria (AIU) diagnosis. This study was a case control study of 47 chronic spontaneous urticaria (CSU) patients and 47 healthy controls. Patients and control were subjected to ASST, APST, and ELISA assay of serum autoantibodies to FcεRIα. Histamine release assay (HRA) as the gold standard method for autoimmune urticaria diagnosis was performed after basophil percoll isolation and subjected to sera of both patients and control. The validity of ASST and autoantibodies to FcεRIα were determined in comparison to HRA. Autologous serum skin test was positive in 30 (63.8%) of CSU patients, and 12.7% of the healthy control ( $P=0.000$ ). IgG autoantibodies to FcεRIα were demonstrated in 55.5% of the patients and were more common in patients with positive (80%) than negative ASST (11.7%) ( $P=0.000$ ). There was significant positive correlation between "ASST" and "APST" positivity, and clinical severity as Spearman coefficient for this correlation was 0.477 ( $P=0.001$ ). There was a significant association between IgG positivity to FcεRIα and disease severity as Spearman coefficient was 0.360 ( $P=0.02$ ). Combined "ASST", "APST" and FcεRIα autoantibody tests revealed 100% sensitivity and 100% specificity for autoimmune urticaria diagnosis. In conclusion, combined anti-FcεRIα assay, with ASST, and APST improved the diagnosis of chronic autoimmune urticaria among patients with chronic spontaneous urticaria.

Chronic urticaria has been classified into two main categories: "chronic spontaneous urticaria" (CSU) and "chronic inducible urticaria" [1]. Inducible urticaria are cases in which urticaria wheals are initiated by a certain inducing factor (e.g. compression, scratch, hot, cold, and vibration). Prevalence of chronic urticaria is estimated to be from 0.5 to 5% of the general population [2].

Spontaneous urticaria is relatively a new term, and defined as chronic skin condition associated with eruptive and itchy wheals for more than 6 weeks with no well-defined extrinsic causes for mast cell and basophil degranulation [3]. It is manifested by characteristic wheals which last no more

than 24 h with or without angioedema with an estimated prevalence of 0.5-1.8% [4].

An important category of chronic spontaneous urticaria is autoimmune urticaria (AIU) which constitutes about 50% of the cases [5]. Previous studies had focused on the mechanisms leading to mast cell and basophil activation in AIU; either due to autoimmunity type I in which IgE auto-antibodies directed at self-antigens, or type II autoimmunity due to IgG directed against the alpha subunit of the IgE receptor I (anti- FcεRIα) which is mostly functional and characterizes spontaneous urticaria, or to IgE itself [6, 7]. IgE-bound FcRI cross-linking on the basophil surface elicits degranulation by calcium-dependent

mechanism, and release of potent inflammatory mediators, histamine, lysosomal enzymes, and proteoglycans [8].

The diagnostic features of autoimmune urticaria were positive functional autoantibodies (basophil histamine release test), positive autologous serum skin test (ASST) to determine the *in vivo* mast cell activation, and positive immunological detection of autoantibodies against FcεRIα receptors as determined by “European Academy of Allergy and Clinical Immunology” (EAACI) [9, 10]. Positive ASST in acute spontaneous urticaria cases increases the chance of developing chronic condition [11]. Functional autoantibodies against FcRI or IgE were commonly screened by ASST testing of patients with CSU [12, 13]. However, it was noticed that 20–30% of allergic patients without CSU and 40–55% of healthy control may have positive ASST, leading to conflicting opinions regarding the ASST specificity of in diagnosing AIU [14].

Different immunological techniques are used for autoimmune urticaria diagnosis with different sensitivities and specificities including: immunoblotting assay and ELISA [15]. Functional antibodies testing by basophil activation or histamine release which is the gold standard method is suggested to be more consistent in diagnosing autoimmune CU patients but these test are practically difficult, which restricts the routine daily practice [16].

The present study aimed to assess the role of IgG autoantibody against FcεRIα in combination with autologous plasma skin test (APST), and autologous serum skin test (ASST) for chronic autoimmune urticaria (AIU) diagnosis.

## Patients and Methods

### Sample Size Calculation

Sample calculated to be (94) cases, divided into 2 groups (47 in each one) calculated using Open Epi I program at confidence interval 95% and power of test 80%, as percentage of autoantibodies in CSU is 25% versus 3 % in healthy controls (6).

### Patients and Control Subjects

This study is a case control, conducted over two years. During the study period, patients with CSU from the allergy and immunology unit and Dermatology outpatient clinic were recruited. Healthy volunteers also participated in the study as control group after obtaining informed consent, and approval of the IRB of the Faculty of Medicine, Zagazig University # 6312.

This study included healthy control group (47 individuals) and 47 CSU patients with symptoms for 6 weeks or more without evident external factors. A detailed history was taken and physical examination was done for all the patients. Patients inclusion criteria were; patients aged 18 to 60 years, avoidance of antihistamines for 4 days, and corticosteroid for 10 days, or other immunosuppressive drugs stoppage for 2 months before the study. Exclusion criteria were; inducible urticaria, lacking information of laboratory tests, other diseases as lymphoma, leukemia, atopic dermatitis, or other itchy skin disease.

All patients were subjected to the following investigations: differential leucocyte count, erythrocyte sedimentation rate (ESR), C reactive protein (CRP), antithyroid antibodies (ATA), and anti-nuclear antibody (ANA).

### Clinical Scoring of CSU Patients

Patient’ symptoms; itching and wheals were documented each morning and evening daily for seven days to assess UAS 7. Urticaria activity score 7 (UAS 7) was calculated as the mean of the morning and evening scores daily over 7 days [17]. The recorded score was determined according to the EAACI and Global Allergy and Asthma European Network guidelines (EAACI/GA2LEN) as presented in table (1) [18]. Patients defined as refractory to antihistamines, if had persistent symptoms of urticaria despite treatment with 4 fold doses of antihistamines [17].

### Autologous Serum Skin Test (ASST)

All participants were subjected to autologous serum skin test (ASST). Blood specimens were allowed to clot for 30 min. Then, centrifuged at 2500 rpm for 10 min (2 ml were stored at -20°C for ELISA and HRA). Autologous serum volume of 50 µl was injected intradermal, in volar aspect of forearm in uninvolved skin about 2 cm below the cubital fossa, using a 1 ml insulin syringe. Similarly, 50 µl of 0.9% sterile normal saline (negative control) was injected intradermal proximally, and at a distance of at least 5 cm, 50 µl of histamine (10 µg/ml) (Omega diagnostics, UK) was injected distally as a positive control. Result was determined by measuring the diameter of wheal at 30 min, and the positive test was characterized by wheal-and-flare reaction >1.5 mm more than negative control. Patients were divided into the CU-ASST (+) and CU-ASST (-) groups [19].

### Autologous Plasma Skin Test (APST)

From all subjects, additional 5 milliliters of blood were drawn into heparinized collection tube to prepare autologous plasma. Autologous plasma of 50 µl volume was intradermal injected into the forearm volar aspect. Also, negative control and positive control (Omega diagnostics, UK) were used as ASST. APST was reported as positive with wheal diameter exceeding 1.5 mm more than negative control at 30 min [7].

### Anti- FcεRIα Autoantibody assay

All subjects were subjected to detection of anti-FcεRIα antibodies using ELISA assay (Human high affinity immunoglobulin epsilon receptor subunit alpha autoantibody ELISA Kit; Bioassay technology laboratory, Changhai, China) and the patients were divided into the CU Anti- FcεRIα Autoantibody (+) and CU Anti- FcεRIα Autoantibody (-) groups.

Serum samples were diluted 5-folds by adding 10 µL sample to 40 µL of sample diluent and added to the antigen on the wells pre-coated with FcεRIα antigens. Fifty µl of negative control (assay buffer, conjugate and substrate), and positive controls (Anti-FcεRIα Autoantibody containing serum) were pipetted to the wells and protected in dark for 2 h at RT. Unbound antibody was washed by 350 µl wash buffer. A Horseradish Peroxidase (HRP) coupled antibody (50µl) was then added and incubated for 30 minutes. Substrate was then added (for 10 min) and color developed. The reaction was stopped by addition of acidic stop solution and color changed into yellow that was measured at 450 nm within 15

minutes after adding the stop solution. The optical density (OD) of samples, positive and negative controls were determined to detect anti- Fcε RIα antibodies in the serum samples.

Cutoff Value equals to average Negative Control OD plus 0.15 according to manufacture instructions. The sample was considered positive if OD of sample  $\geq$  Cutoff Value.

### Passive Histamine Release Assay (HRA)

Histamine release assay measures the amount of released histamine from basophils obtained from healthy donor after incubation with serum from chronic urticaria patient and healthy control to test the presence of functional anti-FcεRIα antibodies.

The first step was basophil isolation. Sixteen ml of blood samples were collected from 4 healthy individuals and was overlaid on Percoll gradient (15 mL of 53% Percoll prepared overlaid on 15 mL 62% Percoll). The basophil layer, was located 1 cm above the interface after centrifugation [20]. The purified basophils were suspended in Phosphate buffered saline buffer (PBS) [21].

To test the presence of functional anti-FcεRIα antibodies, 40 µl of diluted serum (1:4) was added to 40 µl of basophils suspension and incubated at 37°C for an hour. Chilling on ice was performed to stop activation. Each well content was centrifuged at 3000 g for 5 min at 4 °C. Then, supernatant was collected. The cell sediment was lysed using three freeze-thaw cycles. Then, cells were suspended in 200 µl Phosphate buffered saline buffer (PBS), centrifuged for 10 min at 2000 g, and the histamine in the supernatants (induced) and filtrate was quantified [22].

Portions of the basophil cells were incubated without any addition at 37°C with (spontaneous degranulation) and with 1 g/ml of the calcium ionophore (internal positive control causing degranulation of 40% of histamine releaser or non-releaser basophil). After incubation process, all samples were added to 200 µl of Phosphate buffered saline buffer (PBS), centrifuged for 5 minutes, and histamine in the supernatants and filtrate was quantified (Total) was quantified [23].

Released histamine was measured by ELISA (Histamine (HIS) ELISA kit, Biovision, Milpitas, USA) according to manufacture instruction. Briefly, 50 µl of each histamine standard (1.56-100 ng/mL), controls (Blank; substrate only, negative control;

contains assay buffer, conjugate and substrate), and samples were pipetted into the wells of the Histamine Microtiter strips coated with anti-rabbit IgG antibody for one hour. Fifty µL of Biotinylated Detection Antibody working solution were added to the wells and the plate was incubated for one hour. Then, the plate was washed, and Horseradish Peroxidase conjugated Streptavidin was added to all wells. After that, addition of TMB substrate was followed by stop solution, the resulting yellow color was read at 450 nm and read on the standard curve.

Results were expressed as percentage of anti-FcεRIα mediated HR that equals (induced HR – spontaneous HR) divided by total histamine content and multiplied by 100 [23]. A cut-off value of 10% release was used based on receiver operator characteristics (ROC) that were calculated for patients and control [14].

#### Statistical Analysis

The collected data were analyzed by computer using Statistical Package of Social Services version 25

Table 1. Urticaria activity score 7 (UAS7) of disease severity according to the EAACI/GA2LEN guidelines [18]

| Score   | Wheals   | Pruritis  |
|---------|--|---|
| Score 0 | None   | None  |
| Score 1 | Mild (<20 wheals/24 h)                                       | Mild (present but not annoying or troublesome)                            |
| Score 2 | Moderate (20-50 wheals/24 h)                                 | Moderate (troublesome, not interfere with normal daily activity or sleep) |
| Score 3 | Intense (>50 wheals/24 h or large confluent areas of wheals) | Intense (severe pruritus, interfere with normal activity or sleep)        |

## Results

The present study included two groups: Chronic spontaneous urticaria (CSU) adult patients who were referred to the outpatient immunology and allergy unit (47 patients) and healthy control group (47 healthy adult). Patients and control groups demographics characteristics and results of laboratory tests were analyzed and it was found that the age mean of patients with CSU was 39.25±7.3 years old and that of healthy control was 38.4±6.9 years old. There was no significant difference between both groups regarding Age ( $P = 0.850$ ). In both groups, there were more females as female's frequency in

[SPSS]. Continuous quantitative variables were stated as range, median and mean±SD and categorical qualitative variables were determined as absolute number and percentage. Suitable significance statistical tests were used as Chi-square test ( $\chi^2$ ) was used for comparing categorical variables or Fisher's exact test was used when expected cell in less than 5 in 2X2 tables. Mann-Whitney U (MW test) was used for numerical variables comparison as it is non-parametric equivalent of t test if the data cannot be assumed to have a normal distribution. Correlation was determined by Spearman rank correlation coefficients. Performance characteristics of anti-FcεRIα antibodies ELISA, ASST, APST were determined by Receiver operating characteristic (ROC) analysis to determine the area under curve (AUC), sensitivity, specificity, and accuracy and Interactive do diagram was done on Medcalc program. The results were statistically significant if probability < 0.05.

patients with CSU and healthy control were 63.8% and 55.3%, respectively. This finding was statistically insignificant ( $P=0.529$ ).

Regarding tests for diagnosis of chronic autoimmune urticaria as ASST, APST, and FcεRIα autoantibodies detection, there was statistically significant differences observed between chronic spontaneous urticaria group and healthy control. Odds ratio of ASST, APST, and anti- FcεRIα autoantibodies positivity among CSU cases were significantly higher than healthy control (OR=12.05, 12.05, 3.6; respectively) as presented in table 2.

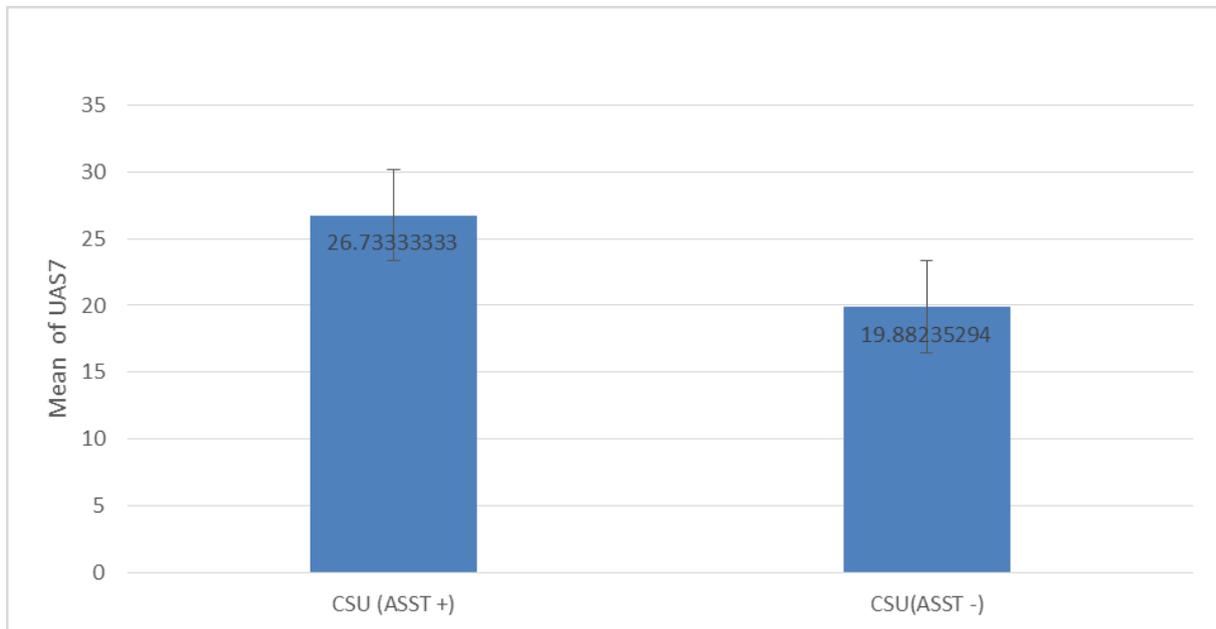
Table 2. Laboratory data among patients with chronic spontaneous urticaria and healthy controls

| Variable                    | CSU group<br>[N=47] |      | Healthy control group<br>[N=47] |      | P- value | OR<br>[C.I]     |
|-----------------------------|---------------------|------|---------------------------------|------|----------|-----------------|
|                             | NO.                 | %    | NO.                             | %    |          |                 |
| ASST                        | 30                  | 63.8 | 6                               | 12.7 | 0.000    | [4.2-34.2]      |
| APST                        | 30                  | 63.8 | 6                               | 12.7 | 0.000    | [4.2-34.2]      |
| Anti- FcεR1α autoantibodies | 26                  | 55.3 | 12                              | 25.5 | 0.003    | 3.6[1.51-8.6]   |
| CRP                         | 4                   | 8.5  | 10                              | 21.2 | NS       | 0.33[0.09-1.15] |
| ESR                         | 11                  | 32.4 | 10                              | 21.2 | NS       | 1.13[0.42-2.98] |
| Histamine release assay     | 26                  | 55.3 | 0                               | 0.0  | 0.000    | 3.23[2.26-4.6]  |

CI: Confidence Interval, ASST: Autologous Serum Skin Test, APST: Autologous Plasma Skin Test, CSU: Chronic Spontaneous Urticaria, CRP: C Reactive Protein, ESR: Erythrocyte Sedimentation Rate.  $P > 0.05$  is not significant (NS).

Patients who were ASST positive constituted 63.8% of CSU patients. The ASST positive CSU patients group were clinically and laboratory different from ASST negative CSU group as regarding symptoms that last more than 5 days/week, and UAS7 (fig.1), ATH, ANA, anti- FcεR1α autoantibodies and antihistamines refractory

response. These differences were highly statistically significant as showed in table 3. APST was positive in 63.8% of patients and 12.7% of control. There was significant positive correlation between ASST and APST positivity on one hand and UAS 7 on the other hand as Spearman coefficient for this correlation was 0.477 ( $P=0.001$ ).



**Figure 1.** Mean of urticaria activity score of CSU patients in relation to ASST. CSU: Chronic Spontaneous Urticaria, UAS7: Urticaria Activity Score 7, ASST: Autologous Serum Skin Test. This figure shows that Mean of UAS 7 was  $25.7 \pm 7.8$  among CSU patients with positive ASST, while it was  $18 \pm 7.2$  among CSU patients with ASST negative.

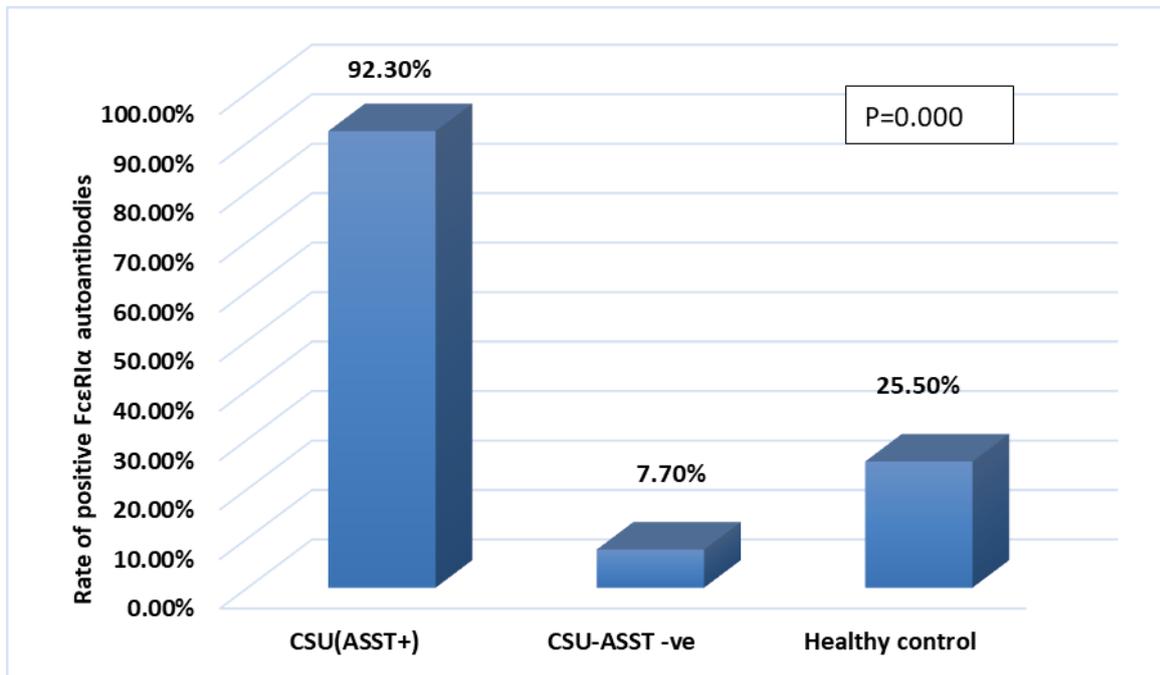
Table 3. Differentiation between ASST positive and ASST negative patients with chronic spontaneous urticaria according to their clinical and laboratory findings

| Parameters                         | ASST positive<br>[N=30] |       | ASST negative<br>[N=17] |      | P value | OR [C.I.]       |
|------------------------------------|-------------------------|-------|-------------------------|------|---------|-----------------|
|                                    | NO.                     | %     | NO.                     | %    |         |                 |
| Age                                | 39.6 ± 8.11             |       | 37.7 ± 5.6              |      | NS      | -----           |
| Sex                                |                         |       |                         |      |         |                 |
| Male                               | 9                       | 30    | 6                       | 35.2 | NS      | 0.78[0.22-2.7]  |
| Female                             | 21                      | 70    | 11                      | 64.3 |         |                 |
| Night symptoms                     | 17                      | 56.6  | 10                      | 58.8 | NS      | 0.91[0.27-3.0]  |
| Symptoms 5 d/week                  | 25                      | 83.3  | 4                       | 23.5 | 0.000   | 16.2[3.7-71.0]  |
| UAS 7                              | 25.7 ± 7.8              |       | 18 ± 7.2                |      | 0.001   | -----           |
| Angioedema                         | 12                      | 40    | 9                       | 52.9 | NS      | 0.59[0.17-1.9]  |
| ATH                                | 13                      | 43.3  | 0                       | 0.0  | 0.001   | 2[1.42-2.79]    |
| ANA                                | 10                      | 33.3  | 0                       | 0.0  | 0.008   | 1.85[1.37-2.49] |
| Anti- FcεRIα autoantibodies        | 24                      | 80    | 2                       | 11.7 | 0.000   | 30[5.34-168.4]  |
| ESR                                | 9                       | 30    | 2                       | 11.7 | NS      | 3.21[0.6-17.06] |
| CRP                                | 4                       | 13.3  | 0                       | 0.0  | NS      | 1.65[1.29-2.0]  |
| Antihistamines refractory response | 24                      | 80    | 4                       | 23.5 | 0.000   | 13[3.09-54.5]   |
| APST                               | 30                      | 100.0 | 0                       | 0.0  | 0.000   | -----           |
| Histamine release assay            | 26                      | 86.6  | 0                       | 0.0  | 0.000   | 5.25[2.17-12.6] |

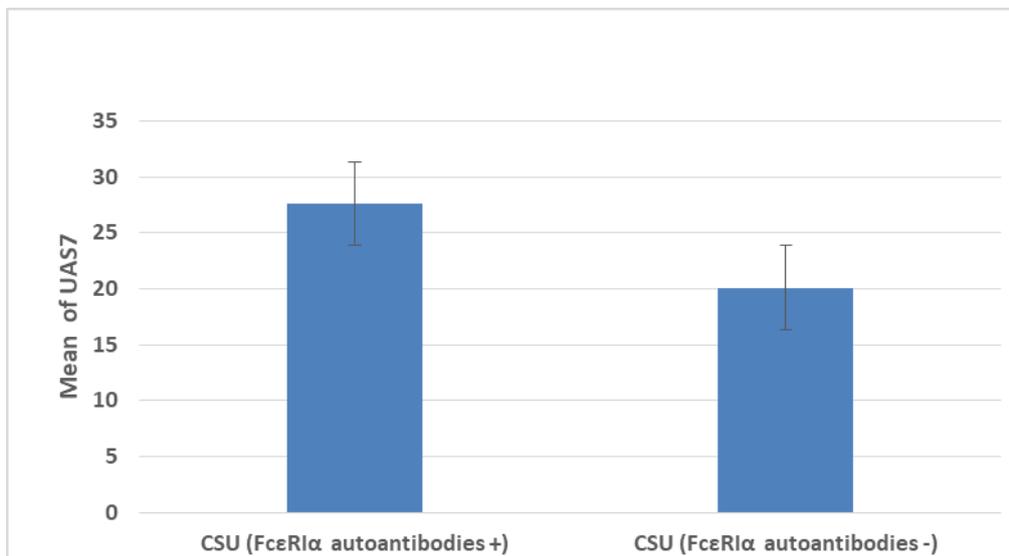
UAS7: Urticarial Activity Score 7, ATH: Antithyroid Antibodies, ANA: Antinuclear Antibodies, ESR: Erythrocyte Sedimentation Rate, CRP: C Reactive Protein, APST: Autologous Plasma Skin Test.  $P > 0.05$  is not significant (NS).

Autoantibodies against FcεRIα detection by ELISA assay revealed positivity in 55.3% of CSU patients and in 25.5 % of healthy control. Specifically, 92.3% of anti- FcεRIα autoantibodies positive patient were ASST positive (Fig.2). It was found that there was statistically significant differences between FcεRIα autoantibody positive and FcεRIα Autoantibody negative patients with chronic spontaneous urticaria as regarding symptoms

more than 5 days/week, and UAS 7 (fig.3). Also, differences in ATH, ANA, ASST, APST and antihistamines refractory response were highly statistically significant as  $P < 0.05$  as presented in table 4. There was a significant positive correlation between anti- FcεRIα autoantibodies positivity on one hand and UAS 7 on the other hand as Spearman coefficient for this correlation was 0.360 ( $P = 0.02$ ).



**Figure 2.** Proportion of positive FcεR1α autoantibodies in relation to ASST positivity among CSU and control groups. CSU: Chronic Spontaneous Urticaria, ASST: Autologous Serum Skin Test. This figure shows proportion of positive FcεR1α autoantibodies in relation to ASST where 92% of positive FcεR1α autoantibodies were ASST positive vs 7.7% of positive FcεR1α autoantibodies were ASST negative, among healthy control 12 out of 47 (25.5%) cases were positive FcεR1α autoantibodies.



**Figure 3.** Mean of urticaria activity score (UAS 7) in relation to FcεR1α autoantibodies among the CSU group. CSU: Chronic spontaneous Urticaria, UAS7: Urticaria Activity Score 7. This figure shows that Mean of UAS 7 was 27.6 ± 5 among CSU with positive FcεR1α autoantibodies, while it was 20.1 ± 7.3 among CSU patients with FcεR1α autoantibodies negative CSU patient.

Table 4. Differentiation between anti- FcεRIα autoantibodies positive and anti- FcεRIα autoantibodies negative patients with chronic spontaneous urticaria according to their clinical and laboratory findings

| Parameters                    | Anti- FcεRIα autoantibodies |      |                 |      | P value | OR [C.I.]       |
|-------------------------------|-----------------------------|------|-----------------|------|---------|-----------------|
|                               | Positive N=26]              |      | Negative [N=21] |      |         |                 |
|                               | NO.                         | %    | NO.             | %    |         |                 |
| Age                           | 37.9 ± 8.11                 |      | 40.14 ± 6.1     |      | NS      | -----           |
| Sex                           |                             |      |                 |      |         |                 |
| Male                          | 5                           | 19.2 | 10              | 47.6 | NS      | 0.26[0.07-0.95] |
| Female                        | 21                          | 80.7 | 11              | 52.3 |         |                 |
| Night symptoms                | 15                          | 57.6 | 12              | 57.1 | NS      | 1.02[0.32-3.2]  |
| Symptoms 5 d/week             | 21                          | 80.7 | 8               | 38   | 0.006   | 6.8[1.8-25.3]   |
| UAS7                          | 27.62 ± 5.0                 |      | 20.1 ± 7.3      |      | 0.000   | -----           |
| Angioedema                    | 10                          | 38.4 | 11              | 52.3 | NS      | 0.56[0.17-1.8]  |
| ATH                           | 13                          | 50   | 0               | 0.0  | 0.000   | 2.6[1.7-4.0]    |
| ANA                           | 10                          | 38.4 | 0               | 0.0  | 0.001   | 2.31[1.5-3.3]   |
| ASST                          | 24                          | 92.3 | 6               | 28.5 | 0.000   | 30[5.34-168.4]  |
| APST                          | 24                          | 92.3 | 6               | 28.5 | 0.000   | 30[5.34-168.4]  |
| ESR                           | 7                           | 26.9 | 4               | 19   | NS      | 1.5[0.38-6.2]   |
| CRP                           | 4                           | 15.3 | 0               | 0.0  | NS      | 1.9[1.4-2.6]    |
| Antihistaminics Refractivness | 22                          | 84.6 | 6               | 28.5 | 0.000   | 13.7[3.3-57.1]  |
| Histamine Release Assay       | 24                          | 92.3 | 2               | 9.5  | 0.000   | 114[14.6-885.8] |

CI: Confidence Interval, UAS7: urticarial activity score 7, ATH: antithyroid antibodies, ANA: Antinuclear antibodies, ASST: Autologous Serum Skin Test, APST: Autologous Plasma Skin Test, ESR: Erythrocyte Sedimentation Rate, CRP: C Reactive Protein.  $P > 0.05$  is not significant (NS)

Autoimmune urticaria constituted 55.3% of chronic spontaneous urticarial as determined by histamine release assay. 86.6% of ASST positive patients were positive for HRA, and 92.3% of anti- FcεRIα autoantibodies positive patient were HRA positive. Histamine release percentage in patients ranged from 15% to 70% with a mean of  $32.76 \pm 22.27$  which was significantly higher than that of control group that was  $6.96 \pm 1.82$  (t test= 7.91,  $P=0.000$ ).

Also, there was strong positive correlation between HRA, and UAS 7 as Spearman coefficient for this correlation was

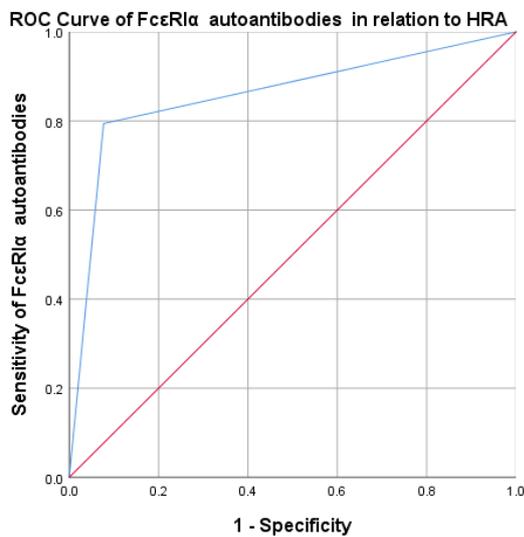
0.47 ( $P=0.03$ ). There was significant positive correlation between anti-FcεRIα Autoantibodies & HRA as Spearman coefficient was 0.82 ( $P=0.000$ ), and that between ASST and HRA was 0.84 (0.000).

The accuracy of ASST, APST, and anti-FcεRIα autoantibodies detection in diagnosis of AIU was analyzed and it was found that the combination of anti- FcεRIα autoantibodies, ASST and APST attained the sensitivity and specificity of 100% and 100% respectively. These results were presented in table 5, figure 4, 5, 6

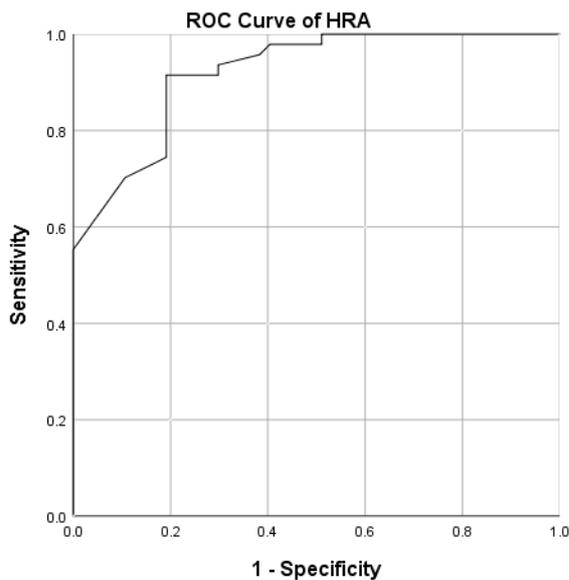
Table 5. ROC test analysis of anti- FcεR1α autoantibodies alone and in combination with one or more specific parameters in relation to Histamine release assay

| Diagnostic tests                         | Sensitivity | specificity | PPV    | NPV    |
|--|-------------|-------------|--------|--------|
| Anti- FcεR1α autoantibodies              | 92.3%       | 90.5%       | 92.3%  | 90.5%  |
| Anti- FcεR1α autoantibodies + ASST       | 92.3%       | 100.0%      | 100.0% | 91.3%  |
| Anti- FcεR1α autoantibodies + ASST +APST | 100.0%      | 100.0%      | 100.0% | 100.0% |

PPV: Positive Predictive Value, NPV: Negative Predictive Value, ASST: Autologous Serum Skin Test, APST: Autologous Plasma Skin Test.

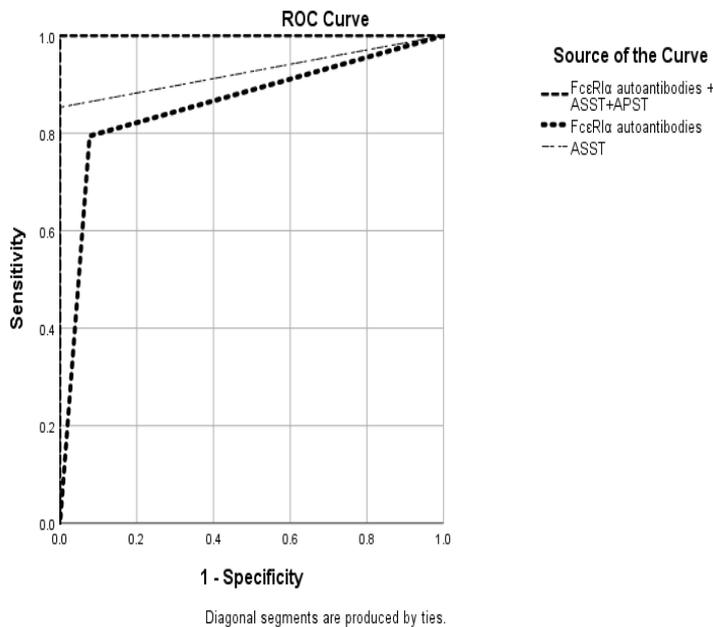


**Figure 4.** Roc Curve of FcεR1α autoantibodies Assay in relation to Histamine release assay. HRA: Histamine Release Assay, AUC; Area under curve. It shows that the sensitivity of FcεR1α autoantibodies in predicting truly positive case in relation to HRA was 92.3%, Area under Roc Curve= 0.859.



**Figure 5.** Roc Curve of Histamine release assay in Autoimmune Urticaria diagnosis. HRA: Histamine release assay. It shows that Cutoff of HRA > 9.75% was predicting AIU cases with sensitivity of 70.2%, and specificity of 89.4%, Area under Roc Curve= 0.92, with statistical significance.

Diagonal segments are produced by ties.



**Figure 6.** Roc Curve of FcεR1α autoantibodies ELISA, ASST and FcεR1α autoantibodies +ASST + APST in relation to Histamine release assay(HRA). ASST: Autologous Serum Skin Test, APST: Autologous Plasma Skin Test. It shows that the sensitivity of FcεR1α autoantibodies in predicting truly positive AIU cases in relation to HRA was 92.3%, Area under Roc Curve= 0.85, AUC=0.926 in ASST, in combining FcεR1α autoantibodies +ASST +APST, sensitivity increased to be 100% and AUC =1.

## Discussion

Chronic autoimmune urticaria is associated with autoantibodies presence, including antibodies to the high-affinity IgE receptor in about 35% of patients and/or anti-IgE antibodies in about 10% of patients and characterized by recurrent itchy wheals and/or angioedema [24,25]. These autoantibodies can activate cutaneous mast cells and blood basophils. Recent understandings of the pathogenesis of CU based on autoimmune process provide the possibility of developing novel treatment strategies. For this, there is urgency to understand the immune-pathogenesis mechanisms of autoimmune urticaria in patients with CSU [26].

Diagnosis of patients with autoimmune urticaria is very critical as they may need higher antihistamines regimen, systemic corticosteroids and immunomodifying drugs during active episodes. The ASST is used as

a selection tool of AIU patients to detect the pathogenic autoantibodies in CSU patients [27, 28].

In agreement with the result obtained by Magen & his colleagues, this study result demonstrated ASST positivity in 63.8% of patients with CSU [12], which was higher than that demonstrated by other previous studies, which stated that ASST was positive in 40% of patients [29, 30]. The current study and in association with another research, demonstrated nearly similar result for ASST positivity among healthy control (12.7%) [19]. Also, study by Sajedi & his colleagues demonstrated a higher occurrence of AIU (80%) in CSU patients and a higher rate of APST positivity (86%) [31]. An explanation for these discrepancies could be from variant patients determination criteria and test result interpretative criteria [19]. Also, unidentified serum factors may be responsible for ASST positivity [32].

In consistence with a study conducted earlier to assess autoreactivity by APST and ASST in clinical practice, the current result concluded that, there was no statistically significant difference between APST and ASST positivity (60.06 %,  $P = 0.593$ ) [33]. Also, another study revealed, that APST positivity rate was 43%, and ASST was 37% ( $P > 0.5$ ) of patients with CSU [30].

However, a research conducted by Kumaran & others revealed that, 90% of patients were APST (+), 68% were ASST (+) [11]. The reason for high positivity of APST may due to activation of the coagulation flow. These autoantibodies, activate eosinophils, which stimulates the coagulation cascade which will induce more mast cells activation [10].

As regarding mean age, this study result showed insignificant difference between ASST positive and negative patients as revealed by Pokhrel & his colleagues [34]. However, a previous study demonstrated that ASST positive group was younger than ASST negative group ( $31.85 \pm 12.32$  years vs  $34.40 \pm 11.38$  years) [29].

In association with previous studies, the current result demonstrated a female majority in autoimmune urticaria cases [64%] [34,30]. The suggested mechanisms for female prevalence may aggressive inflammation mediated by leptin, TNF- $\alpha$ , IL-6, and Toll-like receptors. As female has higher leptin levels, break of auto tolerance occurs more frequently in female [30]

As regarding clinical manifestation, disease severity, and occurrence of symptoms more than 5 days/week, this study demonstrated significant differences between ASST positive and negative patients. Also, other researches result showed that urticaria lesions in ASST positive patients were more severe than those in ASST negative patients regarding

duration and frequency [29, 35]. Also, another research noticed that ASST response was associated with a prolonged duration of disease ( $P=0.002$ , 95% CI, 56.64–26.22) [36]. However, other studies revealed that there was no difference between ASST positive and negative patients in the severity of urticaria lesions [37, 34]. This difference may due to sample size variation [38].

This research and in consistence with study of Chanprapaph & other researchers, concluded that, there was no significant differences between ASST positive and negative patients in ESR and CRP results [30]. This could be due to the high referral rate and persistent patients attending allergy unit. Also, due to continuous T-cell stimulation and T cell polyclonal activation [34].

The current study result are consistent with previous reports, that concluded a significant correlation between CSU with a positive ASST result and autoimmune diseases with serum markers as antinuclear and antithyroid antibodies [39, 40, 41]. However, other studies, reported that autoimmunity was found to be associated with both ASST positive and ASST negative groups and found no statistical difference in the frequencies of antithyroid antibodies, and antinuclear antibodies between the two groups due to production of polyclonal autoantibodies by immune cells [42,29,30]. This difference may have resulted from variant ethnicity of studies population in different researches [30].

In spite of screening of autoimmune chronic urticaria by ASST and APST as useful tools for diagnosis, autoantibodies to IgE and Fc $\epsilon$  receptor are supportive for diagnosis [25]. These tests are practically easy, revealed mast cell degranulation, and predict the remission rate in CSU [43]. The negative result of ASST and APST indicates

a good prognosis, and better response to therapy [44]

In agreement with previous researches conducted earlier, this study result revealed that the patients carrying anti-FcεRIα autoantibodies were more than healthy control subjects and demonstrated these antibodies in 68.4% of chronic urticaria patients and ranged from 0% to 43% in healthy control [45, 46,47]. However, others reported that, the patients carrying anti-FcεRIα antibodies did not differ from healthy control [20]. IgG autoantibodies detection in CSU means that autoimmunity is important mechanism of pathogenesis [18]. Another study demonstrated that 32.8% of patients with CSU showed IgG autoantibody against FcεRIα and in only 3.1% of healthy controls. Also, they demonstrated, that these autoantibodies were more frequently occurring in ASST (+ve) patients [15].

The current result, and in association with previous studies, revealed that functional IgG anti- FcεRIα autoantibodies presence was correlated to disease activity as those patients were described to have more severe symptoms [14,38]. Also, there was a significant correlation between UAS 7 and ASST and APST. However, another research revealed that there was no correlation was found between FcεRIα autoantibody, urticaria scoring, antihistamines response, or the presence of other autoantibody [42].

Functional anti-FcεRIα autoantibodies presence were determined by blood basophils cells using the HRA [9]. Current study data, and result obtained previously indicated that up to 55.3%, 40%, and 50%; respectively of adult CSU cases may be due to basophil activating antibodies [27, 48].

However, previous studies reported that the basophil activation by HRA was 55.7%

in patients with CSU, and 54.4% of the control group, with no statistical difference. The difference may due to the fact that these antibodies are complement-fixing of IgG1 and IgG3 subtypes [49, 9].

In agreement with Viswanathan & his colleagues, our result confirmed that basophil histamine release assay had significant association with resistance to antihistamines and disease severity [50]. However, another groups has shown that there was no correlation between basophil histamine-releasing and the presence of FcεRIα autoantibodies among patients with chronic urticaria and disease severity [51]. However, both the BHRA and the basophil CD63 assay require long time, high experience, and laboratory skills [9]

In this study and in agreement with another study, we observed that ASST(+ve) patients showed increased basophil activation, with significant statistical difference [52]. The research conducted previously demonstrated that Autologous serum skin test (ASST) that was used to detect auto-antibodies had a sensitivity of 80% [19]. Also, Konstantinou & his colleagues concluded that, autologous serum skin test [ASST], for diagnosis of AIU cases, had sensitivity and specificity of 70%, with positive predictive value of 85% [39]. Also, another study revealed that the negative predictive value of the ASST was 82.5% [36].

This study demonstrated that sensitivity of anti- FcεRIα autoantibodies in predicting truly positive case in relation to HRA was 92.3%. Also, other researchers demonstrated that, the sensitivity and specificity of anti-FcεRIα autoantibodies were 70%, and 80%; respectively [9]. However, it was relatively higher than what has been demonstrated earlier (32.8% and 55% respectively) [14]. Thus their detection is important indicator of

autoimmune etiology that is dependent on histamine release by basophil cell response [9].

This is supported by a previous result, which revealed that serum autoantibodies to FcεRIα were presented in 43% of CSU patients and none of healthy controls. Sensitivity and specificity of autoantibodies alone was 47%, 100%, respectively [48]. It was found that combined autoantibody and T-cell responses to FcεRIα increased the sensitivity [48]. This study, revealed that autoantibodies detection combination with ASST+ APST enhances the sensitivity and specificity of autoantibodies to be 100% & 100%, respectively. Also, it was found that measurement of cell mediated mechanism in combination with the FcεRIα autoantibody enhanced the diagnostic validity of this test for CSU diagnosis [48].

In conclusion, anti FcεRIα autoantibodies detection has a diagnostic role in chronic autoimmune urticaria disease and their diagnostic efficacy increased by combining their detection with ASST, and APST. Also, these combined tests could be used as alternative to histamine release assay for autoimmune urticaria confirmation.

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