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Chronic Spontaneous Urticaria: A Closer Look at Antinuclear Antibodies and Their Autoimmune Implications

Haniyeh Shahabi[1], Maryam Mahlooji Rad[2], Habib Soheili[3], and Tooba Ghazanfari[1]

[1] Department of Immunology, Immunoregulation Research Center, Shahed University, Tehran, Iran
[2] Asthma and Allergy Center, Tehran Medical Sciences Branch of Academic Center for Education, Culture and Research (ACECR), Tehran, Iran
[3] Department of Allergy and Immunology, Children's Medical Center, Pediatrics Center of Excellence, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Autoimmune activities in chronic spontaneous urticaria (CSU) are claimed to be one of the most common causes of disease pathogenesis. This study aims to evaluate the prevalence and patterns of antinuclear antibodies (ANA) in patients with CSU, investigate the relationship between ANA positivity and autologous serum skin test (ASST) results, and explore the implications of these findings for understanding the potential autoimmune nature of CSU, particularly in relation to immunoglobulin E (IgE) levels.

We analyzed data from 60 patients with CSU at Jahad Daneshgahi Clinic, Tehran, Iran. Patients were categorized based on ASST results (30 positive and 30 negative). Laboratory evaluations included ANAs via indirect immunofluorescence using the HEp-20-10 biochip kit. Data analysis was performed using chi-square and Mann-Whitney U tests.

Among the 60 CSU patients, 37 were ANA-positive, with common patterns being nuclear fine-speckled and nucleolar. A decrease in IgE levels among ANA-positive patients compared to ANA-negative ones was also observed.

Our study uncovered a notable 61.6% prevalence of ANA positivity among CSU patients, exceeding previously reported rates. The identification of nuclear fine-speckled and punctate nucleolar patterns may indicate associations with specific autoimmune conditions that warrant further investigation. Additionally, the observed lower IgE levels in ANA-positive patients suggest a distinct immunological profile, potentially reflecting type IIb autoimmunity.

Keywords: Autoimmune urticaria; Anti-nuclear antibodies; ANA patterns; Chronic spontaneous urticaria

INTRODUCTION

Chronic spontaneous urticaria (CSU) is a debilitating skin disorder that can affect individuals of all age groups, particularly women. Approximately 1% of the global population is affected by this disorder. Physicians

Corresponding Author: Tooba Ghazanfari, PhD;
Department of Immunology, Immunoregulation Research Center, Shahed University, Tehran, Iran. Tel: (+98 912) 2185 915, Fax: (+98 21) 5121 3564, Email: tghazanfari@yahoo.com

diagnose this disorder based on the presence of wheals, angioedema, or both on patients' skin surfaces for more than 6 weeks without any triggering factor.[1] Although the pathogenesis of the disease is not yet fully understood, CSU is mainly considered a mast cell-driven disease[2] and 2 primary mechanisms have been proposed to explain it. First, there is the dysregulation of intracellular signaling pathways, particularly in mast cells and basophils, which can impair the functionality or trafficking of these cells. The second mechanism that has gained significant attention involves autoimmune theories related to the formation of autoantibodies targeting various types of autoantigens like fragment crystallizable epsilon receptor I alpha subunit (FcεRIα) or immunoglobulin (Ig) E on mast cells or basophils.[1]. The initial evidence highlighting the significance of autoimmune events in CSU was observed through the elevation of thyroid autoantibody levels, thyroid dysfunction,[2] and the positivity of autologous serum skin test (ASST),[3] an in-vivo test.[4]

Within the autoimmune framework, there are 2 main types of CSU, namely auto-allergic, that relates to IgE productions against autoantigens like IL-24,[5] and type IIb in which autoantibodies mainly IgG against FcεRI or IgE are observable.[6] Better response to omalizumab (anti-IgE) treatment has been observed in auto-allergic patients and greater severity of symptoms and longer duration of the disease has been seen in type IIb.[6,7] Recent studies suggest these types might overlap, meaning some patients could have the characteristics of both;[8-10] therefore, further examination of autoimmune response mechanisms remains imperative for accurately identifying and treating the disease in these patients.

There are a few studies considering the positivity of antinuclear antibodies (ANA) among CSU patients.[11-13] ANAs are related to various autoimmune diseases, including systemic lupus erythematosus (SLE),[14,15] Sjögren's syndrome, systemic sclerosis, and so on.[16] While ANA is present in some CSU patients, there is no full grasp of how it affects the disease. Based on laboratory findings, the positivity of ANA could even be observed among healthy individuals. However, whether the positivity of ANA can influence intricate immunological pathways involved in CSU is still unclear and holds paramount importance that might shed light on the disease pathogenesis and pinpoint potential therapeutic targets. Understanding the distribution and implications of ANA patterns can aid in identifying patients at risk for more severe or systemic autoimmune manifestations, ultimately guiding more effective and personalized treatment strategies. This study examines patterns of ANA fluorescence to see if there are any clues about how ANA might impact CSU.

MATERIALS AND METHODS

Patients

We analyzed the data of 60 patients with CSU who attended Jahad Daneshgahi Clinic in Tehran City, Iran. The study was approved by a Specialist in Allergy and Clinical Immunology.

The inclusion criteria were the patient's consent to participate in the study, diagnosis of chronic hives, and no recent intake of systemic corticosteroids and immunosuppressive drugs in the past month. Meanwhile, the performance of ASST was noticed. The exclusion criteria were patients' dissatisfaction with continuing cooperation in the study, undergoing treatment with anti-IgE antibodies and immunosuppressive drugs and if necessary, use of oral corticosteroids, and antihistamines in the 4 days preceding the start of the tests with the approval of a physician were noticed.

The presence of CSU was confirmed as the recurrence of hives, with or without angioedema, remaining for at least 6 weeks.

Study Design

The main objective was to investigate the ANA fluorescent patterns and distribution in CSU patients with positive and negative ASST results. Accordingly, patients were divided into 2 main groups based on their ASST results (30 with positive and 30 with negative test results).

Autologous Serum Skin Test

ASST was performed based on the international guideline.[17] In brief, 0.05 mL of autologous patients' serum and plasma were injected intradermally. Subjects who produced wheals ≤1.5 over the negative control, which was normal saline, were considered positive for the test. Histamine was used as a positive control.

Antinuclear Antibodies Indirect Immunofluorescence Testing and Evaluation

To evaluate ANA pattern expression, we chose the indirect immunofluorescence (IIF) method. We purchased the HEp-20-10 liver biochip (Monkey) (Euroimmune AG, Luebeck, Germany) kit and the test

was performed according to the manufacturer's instructions. In brief, we collected the patient's serum sample in 1:100 dilution with phosphate buffer solution. The test result was evaluated by fluorescence microscope (Eurostar III Plus). The results obtained from examining the BIOCHIP slides under fluorescent light were classified into positive or negative patterns, revealing a wealth of information beyond the mere presence or absence of autoantibodies. This comprehensive test identifies the presence of autoantibodies and provides details about their levels and the HEp-2 IIFA pattern. The fluorescence intensity of the positive control was set at 1:640, and the semiquantitative scoring of fluorescence intensity at 400× ranged from 1:100 to 1:640. Adherence to the standards of the International Consensus on ANA patterns[18] was ensured throughout the assessment. Hep-2 cell autoantibody screening test, the IIFA pattern was assigned an alphanumeric code, ranging from AC-1 to AC-29. These 29 patterns were further categorized into four groups as follows: nuclear patterns, cytoplasmic patterns, mitotic patterns, and multiple patterns.[18]

Statistical Analysis

The Statistical Package for Social Science (IBM SPSS version 26; IBM Corp, New York, NY) was used to analyze the data. To investigate the correlation between ASST and ANA test results, we used the chi-square test. To compare the serum factors parameters, we did the nonparametric tests by performing the Mann-Whitney-U test. Meanwhile, the statistical significance was indicated by $p \leq 0.05$.

RESULTS

A total of 110 patients with CSU were evaluated. Fifty patients were excluded from the study because of the undergoing treatment or their dissatisfaction with continuing cooperation in the study. Subsequently, 60 patients were included, 30 with positive ASST, and 30 with negative test results. Forty-nine patients were female (81.7%) and 11 patients were male (18.3%). The mean age among ASST-positive patients was 38.60 and among ASST-negative ones was 39.03 years (Figure 1).

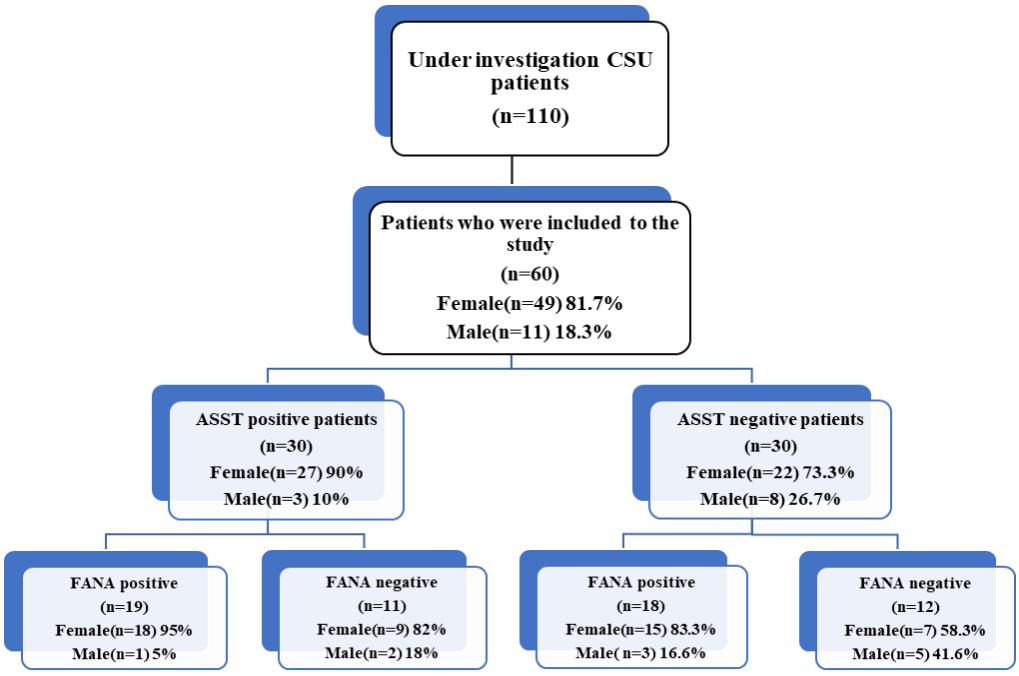

Figure 1. Diagram displaying the criteria used to determine patient eligibility for the study. According to our statistical calculations, a total of 60 patients were required for inclusion in the study. However, based on our exclusion criteria, we eliminated patients who were currently undergoing treatment or had received any treatment within 4 days prior to the laboratory tests. Additionally, due to the discomfort associated with the ASST procedure, some patients opted to withdraw from the study. Consequently, we initially screened 110 patients in order to identify our target of 60 participants.

Among the 60 patients, 23 (38.3%) cases were ANA negative, and 37 (61.6%) cases were positive for the test. The most commonly observed pattern among ANA-positive patients was nuclear fine-speckled, and punctate nucleolar, and homogenous nucleolar in the same proportion (38.89% and 13.89%), respectively (Figure 2). Microscopic fluorescent picture of these 3 patterns have been taken and are shown in (Figure 3).

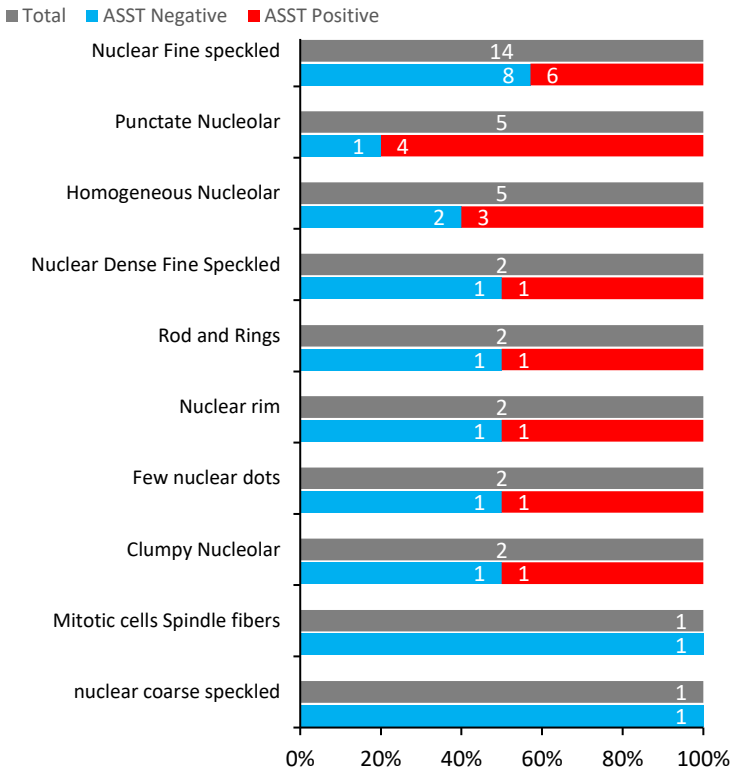

Figure 2. Antinuclear antibody pattern distributions among chronic spontaneous urticaria patients in total and based on their autologous serum skin test (ASST) results. Data are shown in numbers and percentages.

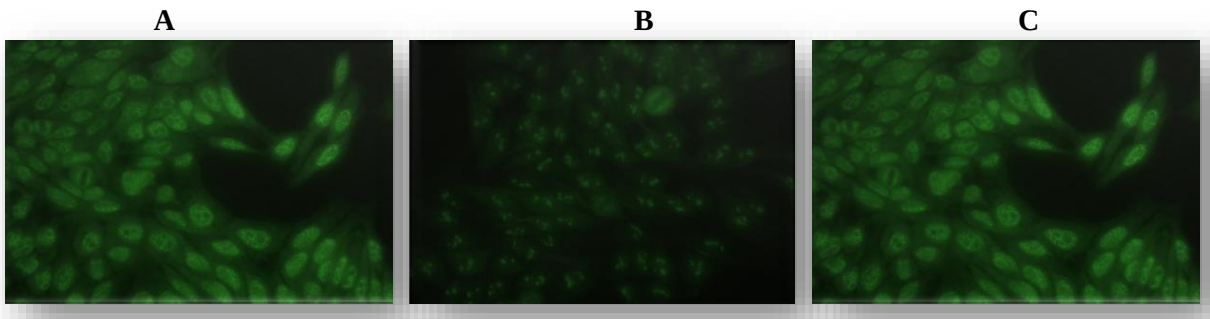

Figure 3. The antinuclear antibodies (ANA) patterns observed in 3 patients with chronic spontaneous urticaria. These 3 photos display 3 distinct patterns based on the binding behavior of ANA. A. Nuclear fine speckled pattern: fine tiny speckles are dispersed throughout the entire nucleoplasm. B. Punctate nucleolar: densely packed but distinct grains are visible in the nucleoli of interphase cells. In metaphase cells, up to 5 bright pairs of nucleolar organizer regions (NOR) can be observed within the chromatin. The cytoplasm of mitotic cells may show slight positivity. C. Homogenous nucleolar: the entire nucleolus exhibits diffuse fluorescence, whereas no staining is observed in the metaphase plate.

A total of 31.6% of cases were positive for both tests. Between those who were positive for both the ASST and FANA, the most commonly observed pattern was nuclear fine-speckled and punctate nucleolar (31.6% and 21%) (Figure 2). Although the correlation between the positivity of ASST and FANA was not significant (Figure 4).

A titer of 1:100 was detected in 26.3% of ASST-positive patients (n=5/19) and 16.7% of ASST negative ones (n=3/18). 57.9% (n=11/19) and 44.4% (n=8/18) of ASST positive and negative patients had a titer of 1:160 respectively. A titer of 1:320 was seen in 27.8% of ASST negative patients (n=5/18) and 10.5% of ASST positive ones (n=2/19). Also, 5.3% of ASST positive patients (n=1/19) and 5.5% of ASST negative patients (n=1/18) had a titer of 1:640. There were just ASST negative patients who had 1:1000 (n=1/18) titer of ANA (Figure 5).

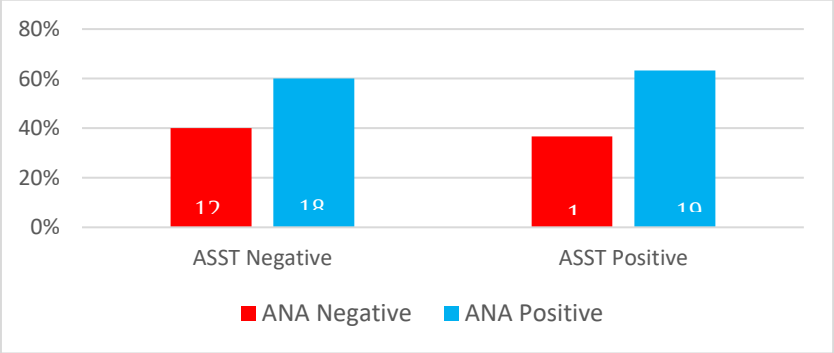

Figure 4. Prevalence of autologous serum skin test (ASST) and antinuclear antibodies (ANA) among 60 chronic spontaneous urticaria patients. Data are shown in number and percentage. Statistical analysis was performed using the chi-square test, with a *p* value of 0.711 indicating no significant association between ASST results and ANA status.

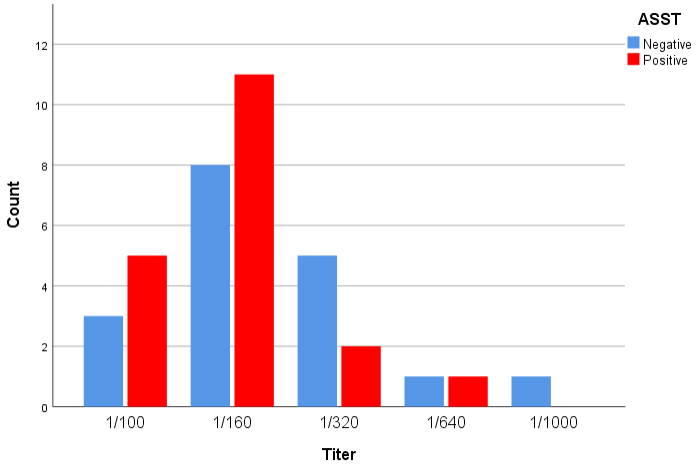

Figure 5. Anti-nuclear antibody titers in autologous serum skin test (ASST) positive and negative patients. Both ASST positive and ASST negative patients were studied for antinuclear antibodies and found to have an intensity of 1:100 to 1:160, 1:160 to 1:320, 1:320 to 1:640, 1:640 to 1:1000. Two groups were compared using chi-square test and no significant difference was found between the two groups (*p*=0.653).

The IgE mean level among our patients was 152.20 IU/mL and 22 (36.6%) patients had high IgE level (>160 IU/mL). We observed a decline in IgE levels among ANA-positive patients versus ANA-negative ones (135 IU/mL vs 178.39 IU/mL); however, the difference was not significant (Figure 6).

The ASST-positive group had significantly lower IgE levels compared with the ASST-negative group (p=0.001). The levels of IgE are presented in (Figure 6).

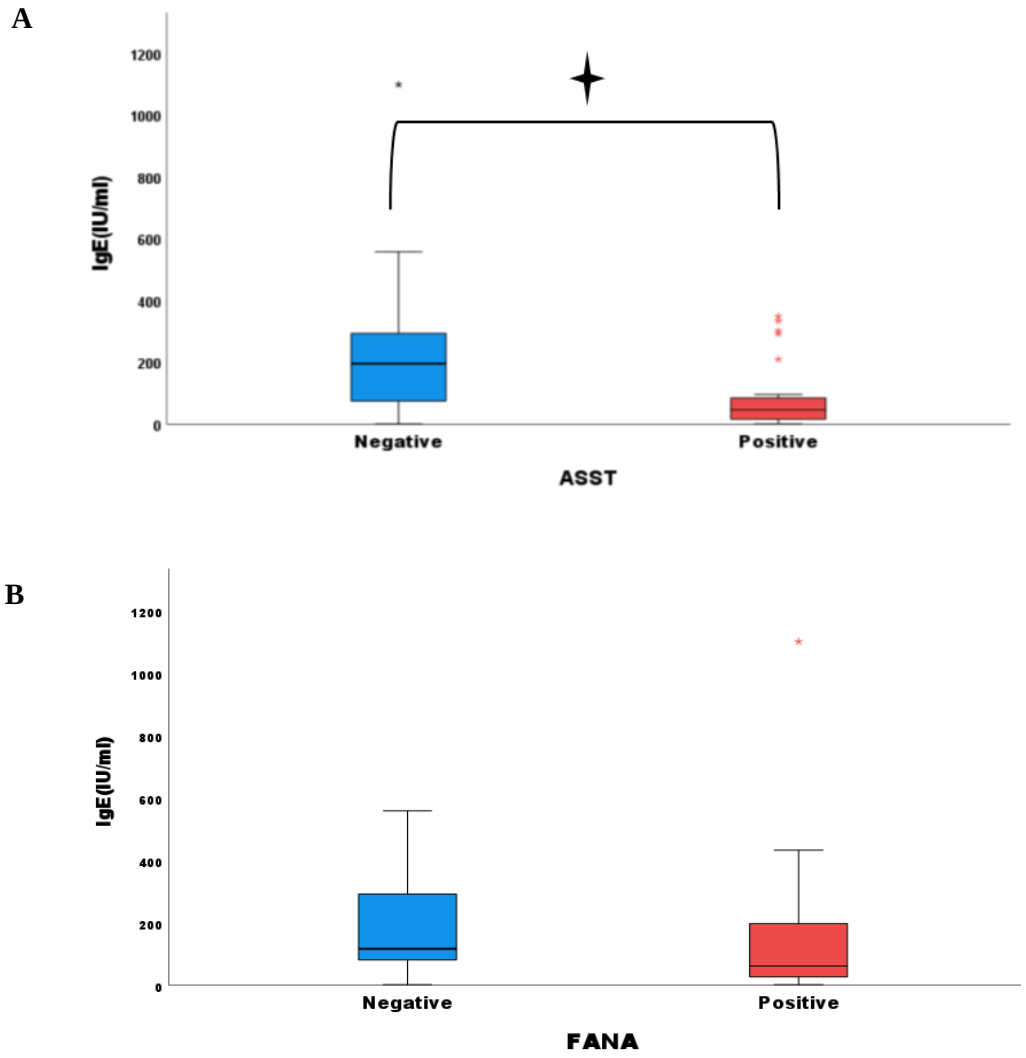

Figure 6. Correlations between autologous serum skin test (ASST) and immunoglobulin E (IgE) as well as fluorescent antinuclear antibodies (FANA) and IgE. A. Significant difference in IgE levels between ASST positive and negative groups is noted (p=0.001). B. No significant correlation between IgE levels in FANA positive and negative groups was observed (p=0.061).

DISCUSSION

Among our CSU patients, we observed a 61.6% prevalence of ANA test positivity. In various studies, the prevalence of ANA-positive CSU patients has shown fluctuations, ranging from 10.5% to 64%.[12,13,19-22] Among those who were positive for ANA and ASST, nuclear fine-speckled (31.6%) and punctate nucleolar (21%) were more common. We could not find any research regarding the specific investigation of ANA by

IIF test patterns in CSU patients. However, Magen et al evaluated 13 ANAs in CSU patients and they observed Sjögren's syndrome antibodies SS-A 60 and SS-B more than the rest.[11] According to the International Consensus on ANA pattern standards,[18] the nuclear fine-speckled pattern is associated with SS-A/Ro, SS-B/La, Mi-2, transcription intermediary factor (TIF1γ), TIF1β, Ku, and the punctate nucleolar pattern is associated with RNA polymerase I, human upstream binding factor (hUBF)/nucleolar organizer region 90 kDa antigen (NOR-90) antigens. Regarding the clinical relevance of these two patterns, the first one is related to various diseases particularly, sudden acquired retinal degeneration syndrome like Stevens-Johnson syndrome and SLE.[23] The second one is related to various situations like systemic sclerosis.[24,25] Different studies have highlighted the importance of ANA evaluation in CSU patients who are suspected of autoimmune diseases, specifically the Sjögren syndrome, rheumatoid arthritis, and thyroid autoimmune diseases.[11-13] However, based on American College of Rheumatology reports, positivity of ANA can be observed in up to 15% of healthy individuals. Besides, our experimental laboratory findings also show this factor positivity among healthy populations, further studies must be done to clarify whether these correlations are notable.

A total of 31.6 % of all patients were positive for both tests and among ASST-positive patients, 63.3% of them were also positive for the ANA test but the observed correlation was not significant. There are only a few studies regarding the correlation between the ANA and ASST results, 1 study reported higher ASST negative results among negative ANA patients (35.9%) versus ANA positive patients (22.2%), although the difference was not significant.[12] This high co-positivity rate between ASST and ANA recommends a potential link between autoreactivity (as indicated by ASST) and systemic autoimmunity (as indicated by ANA). Furthermore, the clinical implications of ANA positivity in ASST-positive CSU patients remain unclear. ANA positivity, commonly associated with systemic autoimmune diseases, might not have the same prognostic or therapeutic implications in the context of CSU. As ASST is an in vivo test, the skill of the one who performs the test can influence the test result. Future studies should elucidate the clinical relevance of these findings and explore targeted therapeutic strategies for patients with autoimmune features.

We observed a decline in IgE levels among ANA-positive patients compared to ANA-negative ones (135.93 IU/mL vs 178.39 IU/mL). The observed *p* value of 0.061 suggests that the sample size may not have been sufficient to detect a statistically significant result. It is possible that a larger sample size could yield different results, particularly in light of the observed decrease in antibody levels. This trend could suggest a different immunological profile in ANA-positive patients, potentially aligning with the concept of type IIb autoimmunity. According to our findings, a high level of IgE was seen in 36.6% of patients. Several studies have mentioned the higher prevalence of auto-allergic phenomena in CSU patients compared to type IIb. As there are difficulties regarding IgE autoantibody measurements in the laboratory, an increase in IgE level has been considered a diagnostic tool for auto-allergic CSU. According to our findings, most of our patients had normal levels of IgE (mean=152.20 IU/mL). Besides, recent studies have highlighted the possible overlap between these two subgroups as well as the presence of non-autoimmune groups.[8]

In the other part of our study, lower IgE levels in ASST-positive patients were observed, which was following most of the earlier studies. The reason for this decline is not yet fully understood; however, Hae-Sim Park et al. emphasized the difference between serum total IgE versus serum free IgE and claimed patients with low serum IgE levels might have higher serum total IgE than serum free IgE levels that should be taken into consideration.[26]

Our study had some limitations due to the patient population. Therefore, access to CSU patients was challenging as they typically sought specialists other than immunologists and allergists, specifically dermatologists. Furthermore, specialized centers for treating these patients are also scarce. Because of limited access, we could not extend our investigation to evaluate the specific autoantibodies. However further analyses of these could greatly enhance the depth of our research and its applicability to patient care.

Our study underscores the prevalence of ANA positivity. ANA-positive patients showed distinctive patterns (nuclear fine-speckled, nucleolar) and lower IgE levels. These findings suggest an important role of ANA positivity in the development of CSU, highlighting the importance of targeted therapies tailored to these mechanisms for improving treatment outcomes. For future research, we suggest focusing on deeper

mechanistic insights of ANA positivity and personalized treatment approaches based on autoimmune profiles in CSU.

STATEMENT OF ETHICS

All the patients provided informed consent. The study was approved by the Ethics Committee of Shahed University (IR.SHAHED.REC.1401.101).

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This study received no funding from any sources.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

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