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ORIGINAL ARTICLE

Interleukin-23/ Interleukin -17 Axis in Chronic Spontaneous Urticaria: A Case- Control Study

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ABSTRACT

Background: *Chronic Spontaneous Urticaria (CSU) is a common health problem and its clear etiology is not established yet. Several theories have been tried to illustrate its etiology and pathogenesis. Autoantibodies and inflammatory cytokines like IL-23 and IL-17 are hypothesized to take part in CSU pathogenesis and outcome.* ***Objectives:*** *To detect serum levels of IL-23 and IL-17A among CSU patients and to determine its correlation with disease severity and its relation to autoreactivity.* ***Methodology****: Serum levels of IL-23 and IL-17A were measured in 23 patients with CSU (CSU group) and 23 healthy controls (control group). In CSU group, Weekly Urticaria Activity Score (UAS7) was recorded to assess disease severity. Autologous Serum Skin Test (ASST) was performed to assess autoreactivity. CSU patients´ group was subdivided, based on ASST, into positive ASST ($ASST^+$) and negative ASST ($ASST^-$) subgroups. Correlation of serum IL-23 & IL-17A levels, with UAS7 and ASST response were analyzed.* ***Results****: CSU group had higher serum IL-17A and IL-23 levels than control group (P=0.000). $ASST^+$ CSU had higher serum IL-17A and IL-23 levels than $ASST^-$ ones (P=0.000). Additionally, UAS7 was higher in ASST+ subgroup than ASST- subgroup (32+11.7 versus 16.27+ 9.92; P =0.005). There was significant positive correlation between disease severity and serum levels of both IL-17A and IL-23 among CSU patients (r= 0.626 & P= 0.001 and r=0.515 & P= .012, respectively).* ***Conclusion:*** *Increased serum IL-17A and IL-23 levels may constitute two major determinants of CSU pathogenesis and severity.*

INTRODUCTION

Chronic Spontaneous Urticaria (CSU), is characterized by recurrent wheals with or without angioedema for six weeks or more without an obvious stimulus. Its definite ateiopathology is complex and it may be attributed to participation of different immunologic, inflammatory and coagulation reactions which finally lead to basophil and mast cell degranulation with wheal formation[1].

At least 50 % of CSU pathogenesis is attributed to autoimmunity. The mechanism of this autoimmunity is unclear but can be explained by the presence of IgE against auto-allergens (type I hypersensitivity) and/or IgG autoantibodies against IgE or its receptor on mast cells and basophils (type II hypersensitivity) [2].

Autologous serum skin test (ASST) is a screening, in vivo, test for serum auto-reactivity in patients with CSU that is able to detect autoantibodies[3].

However, autoantibodies are not detectable in many patients of CSU suggesting that the pathogenesis of the disease might be attributed to other mechanisms. Therefore, the detection of other biomarkers beyond autoantibodies should be cosidered[4].

Interleukin (IL)-23 is mainly released by macrophages and dendritic cells. It stimulates the development and expansion of T helper (Th) 17 cells which control the production of pro-inflammatory cytokine IL-17. IL-17 is involved in immunopathology of many chronic inflammatory and autoimmune diseases by acting on fibroblasts, epithelial cells, and synoviocytes, which in turn, lead to the release of some pro-inflammatory cytokines such as; IL-6, IL-1 ,tumour necrosis factor (TNF)α, and different T cell- and neutrophil-attracting chemokines including chemokine ligand (CCL)2 and CCL7. Moreover, IL-17 stimulates antibody production by acting on IL-17 receptor on follicular dendritic cells, B cells and T cells[5, 6].

This relationship between IL-23 and Th17 suggested that the IL-23/IL-17 axis is a fundamental pathway driving various autoimmune processes [5]. There are some theories about the pathogenic role of the IL-23/IL-17 axis in CSU. Moreover, high levels of IL-17and IL-23 were found among CSU patients with positive correlation to the disease severity denoting their possible value as biomarkers for disease severity[7].

This study aimed to evaluate the role of IL-23/IL-17 axis as an inflammatory biomarker for aetiopathogenesis and clinical outcome of CSU patients.

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METHODOLOGY

Study design and setting

This case- control study was conducted over three months, from September 2020 to December 2020. It was carried out at Allergy and Immunology Unit, Medical Microbiology & Immunology Department, and Dermatology & Venereology Department, Faculty of Medicine, Zagazig University Hospitals.

Ethical consideration

The study was approved by the institutional review board (IRB) no 6540/2020, Faculty of medicine, Zagazig University. This study was carried out in accordance with the revised Declaration of Helsinki. All patients and controls provided an informed written consent.

Subjects and Inclusion criteria

Twenty-three CSU patients were enrolled in CSU group and 23 apparently healthy participants of matched age and sex were enrolled in the control group. Patients were recruited by systemic random sampling from those attending Allergy and Immunology Unit. CSU was defined by the recurrence of spontaneous wheals, in absence of any obvious cause, lasting <24 hour for six weeks or more [8].

CSU group was further subdivided based on the ASST response into positive ASST ($ASST^+$) and negative ASST ($ASST^-$) subgroups.

Exclusion criteria

We excluded patients suffering from any chronic inflammatory, autoimmune diseases, urticarial vasculitis, drug-induced urticaria, physically induced urticaria, associated allergic dermatitis, or any pruritic skin diseases. Also, patients received corticosteroids or immunosuppressant medication the last six weeks were excluded from the study. Pregnant and lactating women were excluded.

In order to exclude known causes of urticaria, all CSU patients were subjected to the following: full detailed history, complete blood count, urine analysis, stool analysis, hepatic functions, hepatitis serology, serum creatinine, complement 3, rheumatoid factor, antinuclear antibodies, C-reactive protein, antistreptolysin-O, erythrocyte sedimentation rate, and *Helicobacter pylori* antigen stool test. Any patient with abnormal blood chemistry tests, blood hematology tests, stool analysis or urine analysis was also excluded. Moreover, skin prick test was done to rule out the allergic causes[9].

UAS7 and ASST were assessed in all CSU patients. Blood samples were drawn from all participants to assess the serum IL-23 and IL-17A levels.

Sample collection

Five ml blood were collected by venipuncture under complete aseptic conditions. Samples were allowed to clot to be centrifuged at 1000 xg for 10 minutes. Then, sera were collected and 50 ul of fresh serum was used immediately in ASST. The remaining sera was preserved at -20 °C to be tested by Enzyme-linked Immunosorbent assay (ELISA) for IL-23 and IL-17A.

Weekly Urticaria Activity Score (UAS7)

It was estimated according to EAACI/GA2LEN/EDF/WAO Guidelines by asking the patients to determine the number of wheals and pruritus intensity that they suffered from, during 24 h for seven days preceding blood sampling (Table i). Then, weekly UAS was estimated by sum of scores of the seven consecutive days (over a week) with a score ranging from 0 to 42. It was graded as: 30–42 (severe), 15–29 (moderate) and 0–14 (mild) [8].

Table i: Urticaria activity score during 24 h

Wheals	Score	Pruritus intensity	Score
No wheals/24 h	0	No	0
<20 wheals/24 h	1	Mild (present but not annoying)	1
20-50 wheals/ 24 h	2	Moderate (annoying but not interfering with sleep or	2
> 50 wheals/24 h	3	normal daily activity)	
		Severe(interfering with sleep or normal daily activity)	3
Total daily score*	0-6		

*Sum of score: 0–6 for each day is summarized over consecutive seven days.

Autologeous Serum Skin Test (ASST)

ASST was done according to EAACI/GA2LEN/EDF/WAO Guidelines. The patients were asked to stop antihistamines for one week before blood collection. Fifty ul of autologous fresh serum were injected intradermally. The wheal and flare reactions were read at 30 min. Saline solution (0.9% weight/volume NaCl) was applied as negative control while histamine (10 mg/ ml) was applied as a positive control. A positive response was considered when the mean wheal perpendicular diameter was ≥1.5mm compared to the saline response [8, 10].

Serum level of IL-23

Human IL-23 ELISA KIT, INOVA No. 18, Keyuan Road, Daxing Industry Zone, Beijing, China, was used to detect serum level of IL-23 according to manufacturer's instructions. This quantitative ELISA kit was based on biotin double antibody sandwich technology and the results were expressed in pg/ml.

Serum level of IL-17A

IL17-A was measured by commercially available quantitative ELISA Kit supplied by Thermo Fisher Scientific (Bender MedSystems gmbH/Campus Vienna Biocenter 2/1030 Vienna, Austria) according to the manufacturer's instructions. ELISA kit was based on biotin double antibody sandwich technology and the results were expressed in pg/ml.

Statistical analysis

Continuous variables were reported as mean value and standard deviation, while categorical variables were reported as numbers and percentages. Mann Whitney test and t-test were used to detect difference for quantitative variables between two groups as appropriate. Chi-square test ($\chi 2$) was used to compare proportions. The strength of the correlation between two continuous sets of data was detected by Pearson's correlation coefficient (r). Statistical packages (EPI-info Version 6.04 and SPSS Version 20 inc. Chicago, USA) were used to analyze collected data. A p-value<0.05 was considered to be statistically significant at 95% confidence interval.

RESULTS

Characteristics of the study participants:

The mean age of CSU patients and controls were 35 and 31.7 years respectively. Thirteen (56.5%) patients with CSU were males while 15 (65.2%) healthy participants were males in the control group. Both CSU group and control group were matched in age and sex (*P*= 0.197; 0.546 respectively). ASST was positive in 52.2% of CSU patients. Regarding urticaria severity among CSU patients, 47.8% were mild, 26.1% were moderate and 26.1% were severe (table 1). CSU group was further subdivided, based on the ASST response, into ASST+ (n=12) and ASST-(n=11) subgroups. The characteristics of both subgroups were illustrated in table 2.

Table 1: Baseline characteristics of the study population

Variables	CSU group (n=23)	Control group (n=23)
Age (years) mean ± SD	35 ±8.1	31.7±8.7
Sex, male, no (%)	13 (56.5)	15 (65.2)
ASST $^{+}$, no (%)	12 (52.2)	0
UAS7, mean ± SD	22.13+12.8	0
Severity, no (%)		
Mild	11 (47.8)	0
Moderate	6 (26.1)	0
Severe	6 (26.1)	0

SD, standard deviation; CSU, chronic spontaneous urticaria; ASST, autologous serum skin test; UAS7, weekly urticaria activity score.

Table2: Characteristics of the ASST^{+} CSU and ASST^{-} CSU subgroups

	ASST^{+} CSU subgroup (n = 12)	ASST^{-} CSU subgroup (n = 11)
Age (years) mean± SD	32.5±8.97	37.7±6.6
Sex, male, no (%)	7 (53.3)	6 (54.5)
UAS7, mean ± SD	32+11.7	16.27+ 9.92
Severity, no (%)		
Mild	4 (33.3)	7 (63.6)
Moderate	3 (25)	3 (27.3)
Severe	5 (41.7)	1 (9.1)

SD, standard deviation; CSU, chronic spontaneous urticaria; ASST, autologous serum skin test; UAS7, weekly urticaria activity score.

Serum levels of IL-17A and IL-23

The mean serum levels of IL-17A and IL-23 were significantly higher in CSU group than control group (*P* =0.000). Additionally, the mean serum levels of IL-17A and IL-23 were significantly higher in ASST $^{+}$ CSU subgroup than ASST $^{-}$ CSU subgroup (*P* =0.000) (table 3, 4).

Urticaria severity

Concerning disease severity and ASST, UAS7 was significantly higher in ASST^{+} CSU subgroup than in ASST^{-} CSU subgroup (32+11.7 versus16.27+ 9.92; *P*=0.005) (Table 2). There was a significant positive correlation between UAS7 and serum level of IL-17 in CSU group (r= 0.626 & *P*= 0.001). Also, there was a statistically significant positive correlation between UAS7 and serum level of IL-23 in CSU group (r= 0.515& *P*= 0.012) (Figure 1, 2).

Table3: Serum levels of IL-17A, and IL-23 among the studied groups

Cytokine (pg/ml)	CSU group (n = 23)	control group (n = 23)	*P*-value
IL-17A	35.043±7.4	24.935±7.5	0.000**
IL-23	35.174±7.4	15.783±6.98	0.000**

Data was expressed as mean ± SD, CSU, chronic spontaneous urticaria

Table 4: Serum levels of IL-17A, and IL-23 among ASST+ CSU patients, ASST- CSU patients

Cytokine (pg/ml)	ASST^{+} CSU subgroup (n = 12)	ASST^{-} CSU subgoup (n =11)	*P*-value
IL-17A	39.92± 6.2	29.73±4.2	0.000**
IL-23	40.79± 6.04	29.045 ±0.96	0.000**

Data was expressed as mean ± SD, ASST, autologous serum skin test; CSU, chronic spontaneous urticarial

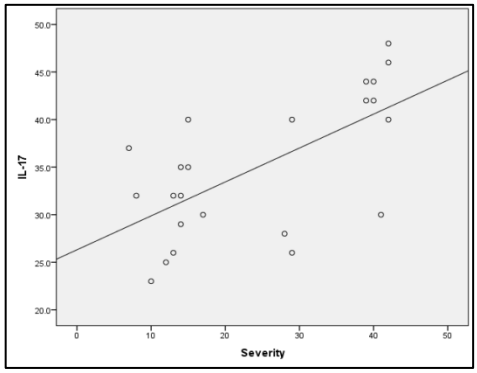

Fig. 1: Correlation between serum level of IL-17A (pg/ml) and weekly urticaria activity score (UAS7) among CSU patients (r= 0.626 & *P*= 0.001)

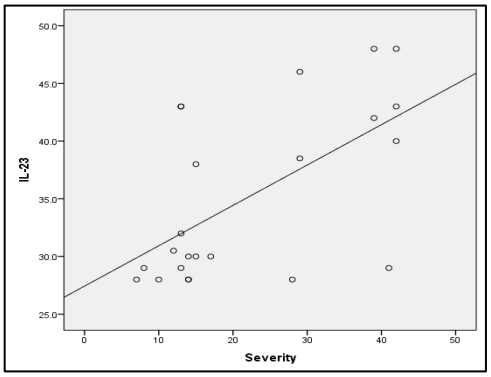

Fig. 2: Correlation between serum level of IL-23 (pg/ml) and weekly urticaria activity score (UAS7) among CSU patients (r=0.515& *P*= 0.012)

DISCUSSION

As a common disease, there is a great need to understand the pathogenesis of CSU and to detect possible biomarkers indicating disease severity. Symptoms of CSU develop due to basophil and mast cells degranulation which might be attributed to the collaboration of several mechanisms of the immune system, autoantibodies, cytokines, complement and coagulation systems. Many mediators are related to CSU severity suggesting their possible value as biomarkers for the disease severity[7].

In this case-control study, the role of IL- 17/ IL-23 axis in CSU disease activity was determined. In the present study, CSU severity was assessed by UAS7 where 47.8%, 26.1% and 26.1% were mild, moderate and severe, respectively. Autoreactivity was determined by ASST where 52.2% of CSU patients were ASST^{+} and it was found that UAS7 was significantly higher in ASST $^{+}$ CSU than ASST $^{-}$ CSU patients. Our finding goes hand in hand with the results of previous studies [9, 11, 12]. This could point to the role of Th17 cells and make their suppression one of the potential therapeutic trials that should be considered particularly in severe CSU, especially in ASST^{+} CSU patients.

In the current study, the mean serum levels of IL-17A and IL-23 were significantly higher in CSU group than control group. This agrees with other studies[9, 13, 14, 15]. Dos Santos and his colleagues [16] demonstrated no obvious changes in the number of Th17 cells in CSU patients. However, previous two studies reported that

Th17 response is not effective in CSU pathogenesis and they found that serum levels of IL-17 and IL-23 were significantly lower among CSU group when compared with the control group and they attributed that to consumption of IL-23 and IL-17 in the inflammatory process [3, 17].

The altered IL-17 and IL-23 levels among CSU patients could be attributed to dysfunction of innate immune response among CSU with subsequent functional impairment of dendritic cells that alters the cytokine release by T cells, mainly of IL-17A [16]. A point that needs to be further investigated among CSU patients in order to explain the changes in cytokine profile of Th17.

In this study, the mean serum IL-17A and IL-23 levels were higher in $ASST^+$ CSU subgroup than $ASST^-$ CSU subgroup. This agrees with previous studies [9, **18**]. This comes in accordance with the known role of Th17 cells and their cytokines in promoting inflammation and autoimmunity [18].

In our study, serum IL- 17A and IL- 23 levels were positively correlated with UAS7 that denotes the key role of IL-23/IL-17 axis in the pathophysiology and severity of CSU. This comes in accordance with Sharma and his colleagues[**13**] who reported a significant positive correlation between serum IL-17, IL-18, IL-23 and TNF-α levels and severity of CSU. Atwa and her colleagues[**9**] have found similar results when they investigated the relation of Th17 cells with CSU development and severity. Additionally, the results of our study come in accordance with another study stated that Th1, Th2 and Th17 responses were responsible for the inflammatory process in CSU where that serum IL-17 and IL-4 levels showed a positive correlation with the disease severity [**15**].

CONCLUSION

IL- 17/ IL- 23 axis can contribute to the pathogenesis and disease activity of CSU where it might be utilized as an indicator for disease severity.

Recommendation

The role of other biomarkers in CSU pathogenesis and outcome should be assessed. Further studies, using biological agents targeting IL-23 and IL-17 should be investigated, as new treatment strategies in CSU.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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