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Cytokine Profiles of Chronic Urticaria Patients and The Effect of Omalizumab Treatment

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ABSTRACT

Introduction: Cytokines are key mediators in immunological and inflammatory conditions, including chronic spontaneous urticaria (CSU).

Objectives: To investigate Th1, Th2, and Th17 cytokine profiles in CSU and to evaluate the possible effect of omalizumab treatment.

Methods: Patients who were followed up for CSU, as well as healthy volunteers, were included in the study. To assess urticaria activity, the 7-day-Urticaria Activity Score (UAS-7), the Urticaria Control Test (UCT), and the Chronic Urticaria Quality of Life Questionnaire (CU-QoL) were filled. Serum levels of IL-6, IL-17, IL-31, eotaxin, RANTES, TNF-α, and TSLP were analyzed by ELISA and compared in CSU and control groups. The patients were analyzed in two groups as the omalizumab group and the non-omalizumab group based on their treatment status.

Results: Total IgE, ESR, CRP, RANTES, and TNF-a were significantly different in the overall comparison of the three groups: CSU-receiving omalizumab, CSU-not receiving omalizumab, and control groups ($P < 0.01$, 0.015, <0.01, <0.01 and <0.01 respectively). Total IgE, CRP, RANTES, and TNF-α values were similar in those who received and did not receive omalizumab, yet these biomarkers were significantly higher in both groups than in the control group ($P < 0.05$). Statistical significance in ESR was observed only between the CSU-receiving omalizumab group and the control group ($P = 0.01$). Within the CSU patients, there was a slight but significant correlation between UCT and TNF-α ($P = 0.008$, $r = 0.32$) and IL-17 ($P = 0.06$, $r = 0.33$) levels.

Conclusions: The investigated cytokine profile in CSU patients may differ from healthy controls, particularly with the higher levels of RANTES and TNF-α, and omalizumab treatment does not seem to affect that profile in CSU patients.

Introduction

Chronic spontaneous urticaria (CSU) is a common clinical condition characterized by itchy wheals and/or swelling that resolves within 24 hours and lasts for at least six weeks [1]. Symptoms are associated with impaired quality of life, and as a result, there is a high probability of experiencing depression, anxiety, and sleep disorders. Treatment response and monitoring of disease activity in CSU are mostly based on the use of patient-reported outcomes. [2]. However, the main limitation of these extensive questionnaires for the assessment of CSU is their subjective nature.

Recently, studies have highlighted that certain cytokines can be considered as potential biomarkers related to the disease [3]. Moreover, the emergence of such biomarkers contributes to studies on new biological agents in the management of resistant CSU and supports personalized treatment and follow-up [4, 5].

Activation and degranulation of mast cells and migration of basophils to the tissue are frequently observed in the inflammation of urticaria. In addition, uncontrolled T-cell activation has been reported in skin biopsies from the lesional/non-lesional areas and peripheral circulation of CSU patients [6]. In this regard, a high percentage of inflammatory cells are composed of T lymphocytes and monocytes, suggesting that T cells play an important role in the pathogenesis of CSU [7].

As a Th1 mediator, tumor necrosis factor-α (TNF-α) is a candidate biomarker for urticaria released by other inflammatory cells that can be detected in the area of urticaria lesions together with skin mast cells [8]. The fact that inflammatory mediators such as IL-6 and IL-31, which are involved in Th2 inflammation, have higher levels in urticaria patients and regress with treatment or remission, promises that these and similar biomarkers may be important in the etiopathogenesis and treatment modalities of CSU [4, 9-12].

Many chemokines and enzymes involved in the pathogenesis of urticaria are also being investigated. Among the chemokines, RANTES is a protein that especially attracts T cells to the site of inflammation and has been proposed to be of potential importance in the etiopathogenesis of CSU without correlation with disease activity and treatment response [13]. Eotaxin is a chemokine that specifically regulates eosinophil selection and recruitment. Circulating eotaxin levels have been shown to increase during exacerbations of atopic dermatitis and acute urticaria, implying that it probably serves to recruit and activate eosinophils and represents a biomarker of lesional activity [14].

Many markers have been explored for conceivable relations with CSU activity, but the number of studies that predict response to treatment and assess the levels of Th1, Th2, and Th17 cytokines together is limited, and the results showed a variation in cytokine profiles between CSU patients and healthy control groups, with no common and definite increase or decrease for these markers [15-19]. The objective of this study was to evaluate the Th1, Th2, and Th17 serum cytokine levels in CSU and to document the possible effect of omalizumab on these biomarkers.

Methods

Study Subjects

The present case-control study included CSU-diagnosed patients, aged ≥18 years, and followed up at the Adult Allergy and Immunology Clinic between December 2019 - December 2021. The diagnosis of CSU was made based on the recurrence of spontaneous wheals lasting 24 hours for at least 6 weeks, according to the guidelines [1]. Patients who had inducible or known caused- urticaria, accompanied collagen tissue disease or urticarial vasculitis, patients receiving corticosteroid therapy for any reason, and pregnant women were excluded from the study. Hemogram, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), D-dimer, thyroid stimulating hormone (TSH), anti-TPO, liver, and kidney function tests were performed on each patient.

Omalizumab was administered subcutaneously at 300 mg once a month for the indication of chronic urticaria in patients whose symptoms have not been controlled with antihistamines as stated in the guidelines [1]. Patients were analyzed according to their omalizumab treatment status by dividing them into two groups, as those who received omalizumab and those who did not. In order to evaluate the effectiveness of the treatment for the omalizumab group, patients who had been receiving omalizumab therapy for at least 4 months were included.

Age- and gender-matched healthy volunteer participants were recruited as the control group after being informed about the procedures and purpose of the study. Venous blood samples (10 mL) from patients and controls were centrifuged at 1500 g for 10 minutes, and samples were aliquoted and stored at -80°C.

Assessment of Urticaria Activity

To evaluate urticaria activity, the 7-day-Urticaria Activity Score (UAS-7), Urticaria Control Test (UCT) scores, and Chronic Urticaria Quality of Life Questionnaire (CU-QoL) were filled out [20-23].

Protein Assessment by Enzyme-Linked Immunosorbent Assay (ELISA)

Serum levels of IL-6, IL-8, IL-17, IL-25, IL-31, IL-33, Eotaxin, RANTES, GM-CSF, TNF-α, TSLP were analyzed in triplicate by ELISA using a commercial kit (IL-6: Abcam; IL-8, IL-17, IL-25, IL-31, IL-33, Eotaxin, RANTES, GM-CSF, TSLP: R&D Systems; TNF-α: Mabtech) using the En-Sight Multimode Plate Reader (PerkinElmer) according to the manufacturer guidelines.

Statistical Analysis

IBM SPSS Statistics, version 25.0 software (IBM Corp.), was utilized for the statistical analyses. The normal distribution of continuous variables was checked via the Kolmogorov-Smirnov test and analyzed as mean ± standard deviation (SD) in a normal distribution, otherwise as median [lower-upper quartile] values. Patient demographics were analyzed using the Chi-square test to compare distributions of gender and comorbidities, and the Mann-Whitney U test to compare the UCT, UAS-7, CU-QoL scores, and CSU duration in both groups. Spearman's rank correlation test was applied for correlation analysis. The Kruskal-Wallis with Dunn test was used to compare the biomarkers of CSU patients who received omalizumab and those who did not receive omalizumab with those of the control group. Values of $P < 0.05$ were interpreted as statistically significant.

The study was approved by the Ethics Committee of Hacettepe University Hospital (Approval No: GO-19/1032) and all participants gave written consent before the study procedures.

Results

The study included 78 patients with a diagnosis of CSU followed in the Hacettepe University Adult Allergy and Immunology Clinic and 18 healthy participants.

The mean age of CSU patients was 40.2 ± 12.5 years and 57 (73.1%) of them were female (Table 1). The median CSU duration of the patients was 48 (IQR: 15-120) months. Comorbidities were determined as, 12 (15.4%) asthma, 11 (14.1%) allergic rhinitis, 8 (10.3%) hypertension, 5 (6.4%) diabetes mellitus, 10 (12.8%) hypothyroidism, 5 (6.4%) rheumatological, 4 (5.1%) cardiac, 3 (2.6%) renal, and 1 (1.3%) psychiatric disorder. There was no difference in comorbidities between the two groups receiving and not receiving omalizumab. Of the patients, 42 (53.8%) were receiving omalizumab, with a median duration of 18 months (9-30) and a CSU duration of 66 (31.2-138) months.

The median UCT score of the patients was 10 (5-13), and the median UAS-7 score was 15 (4-27). UCT scores were

Table 1. Comparisons of the patient characteristics according to the status of omalizumab therapy.

Variables	Total (N = 78)	Omalizumab group (N = 42)	Non-omalizumab group (N = 36)	P value
Age, mean ± SD, years	40.2 ± 12.5	42.4 ± 13.7	38.5 ± 11.9	0.19
Female gender, N (%)	57 (73.1)	33 (78.6)	24 (66.7)	0.30
CSU duration, median (IQR), months	48 (15-120)	66 (31.2-138)	24 (2-96)	0.001
Comorbidities, N (%)				
Asthma	12 (15.4)	9 (21.4)	3 (8.6)	0.20
Rhinitis	11 (14.1)	5 (11.9)	6 (17.1)	0.53
Hypertension	8 (10.3)	6 (14.3)	2 (5.6)	0.18
Diabetes mellitus	5 (6.4)	4 (9.5)	1 (2.8)	0.36
Hypothyroidism	10 (12.8)	6 (14.3)	4 (11.1)	0.74
Rheumatologic disorders	5 (6.4)	3 (7.1)	2 (5.6)	0.57
Cardiac disorders	4 (5.1)	2 (4.8)	2 (5.6)	0.63
Renal disorders	2 (2.6)	1 (2.4)	1 (2.8)	0.71
Psychiatric disorders	1 (1.3)	1 (2.4)	0 (0)	n/a
UCT score, median (IQR)	10 (5-13)	12 (8-14)	8 (2-11)	<0.001
UAS-7 score, median (IQR)	15 (4-27)	8.5 (1-27)	22 (13-27)	0.04
CU-QoL score %, median (IQR)	27.1 (15.2-45.7)	22.8 (14.4-45.3)	33.6 (17.3-56.5)	0.14

CSU = Chronic Spontaneous Urticaria; CU-QoL = Chronic Urticaria Quality of Life; IQR = Interquartile range; n/a = non applicable; SD = standard deviation; UAS-7 = 7-day Urticaria Activity Score; UCT = Urticaria Control Test.

higher in the group receiving omalizumab (median [IQR]: 12 [8-14] versus 8 (2-11), P < 0.001). UAS-7 scores were lower in the omalizumab group (8.5 (1-27) versus 22 [13-27], P = 0.04). There was no significant difference between the quality-of-life scores in both groups (Table 1).

In the comparison of the CSU and control groups; CRP, ESR, D-dimer, and Total IgE levels were higher in CSU patients (n=78) compared to the control group (N = 18) (P values, respectively, 0.001, 0.06, 0.02 and <0.001). Among the biomarkers, RANTES and TNF-α levels were more elevated in CSU patients (both P values <0,001). The eosinophil counts and percentages, eotaxin, IL-6, TSLP, IL-17, and IL-31 levels were similar between the CSU and control groups (Figure 1). There was no significant difference in eosinophil count/percentage, Total IgE, erythrocyte sedimentation rate, CRP, eotaxin, IL-6, TSLP, RANTES, TNF-α, IL-17, and

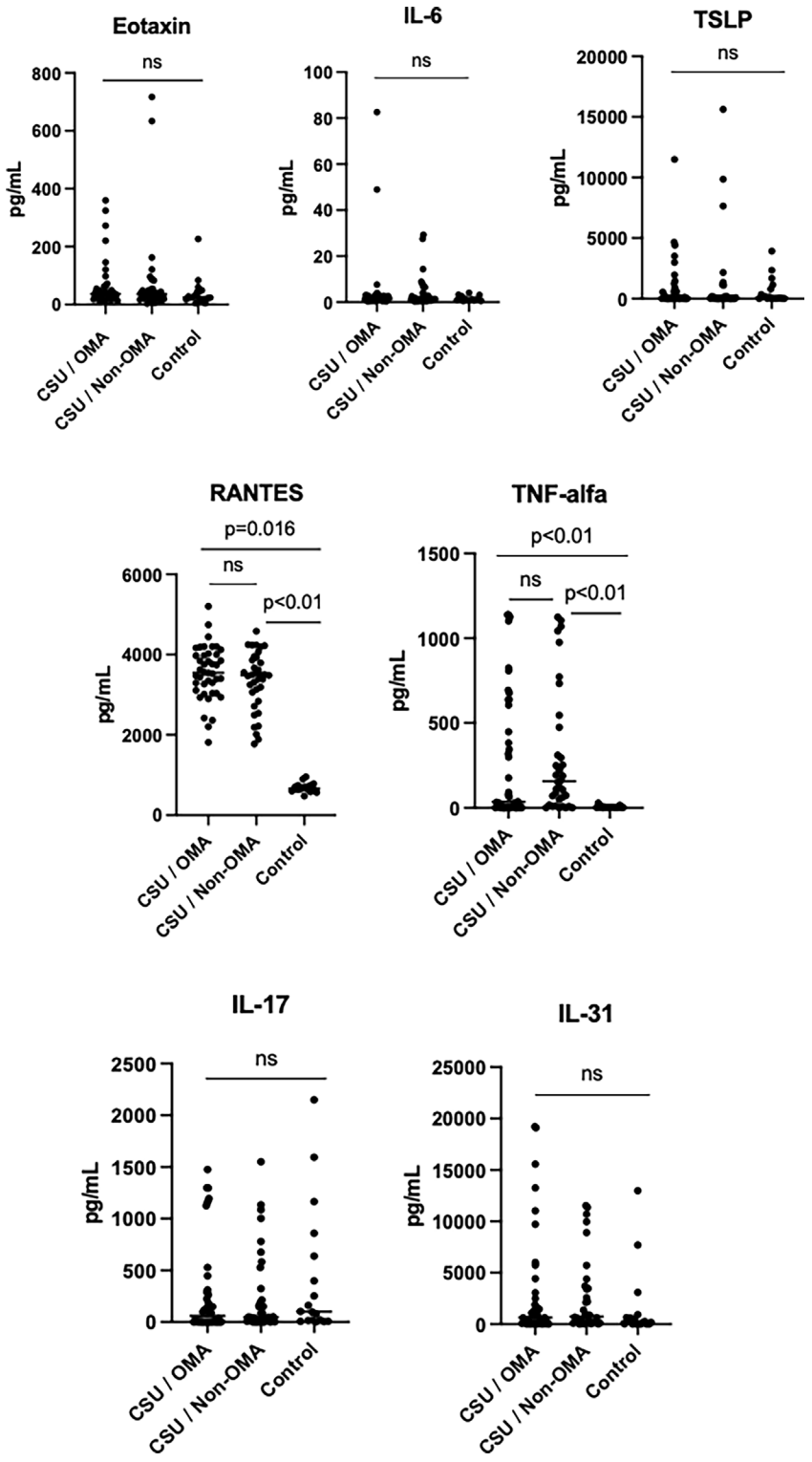

Figure 1. Comparison of biomarker levels in chronic spontaneous urticaria and control groups
CSU = chronic spontaneous urticaria; OMA = omalizumab; ns = non-significant.

IL-31 levels between the groups that were receiving and not receiving omalizumab (Figure 1 and Table 2).

In the overall comparison of the three groups: CSU-receiving omalizumab, CSU-not receiving omalizumab, and control groups, Total IgE, ESR, CRP, RANTES, and TNF-α were significantly different (P <0.01, 0.015, <0.01, <0.01 and <0.01 respectively). Total IgE, CRP, RANTES, and TNF-α values were similar between those who received and did not receive omalizumab, but both groups were significantly different from the control group in terms of these biomarkers. Total IgE, CRP, RANTES, and TNF-a were higher in patients receiving and not receiving omalizumab compared to the control group (Table 2). Statistical significance in ESR was observed only between the CSU-receiving omalizumab group and the control group (CSU/OMA versus control, P = 0.01). D-dimer levels between patients and healthy controls were higher in the total CSU group (P = 0.02); however, this did not constitute as evidence for a correlated association and lost significance in the overall comparison of the three groups (P = 0.05).

Within the CSU patients, there was a slight but significant correlation between UCT scores and TNF-α (P = 0.008, r = 0.32) and IL-17 (P = 0.06, r = 0.33) levels (Figure 2). Serum levels of IL-8, IL-25, IL-33, and GM-CSF were also analyzed by ELISA; however, their levels were below the detection ranges of the kits.

Table 2. Comparisons of biomarker profile by omalizumab status and control.

Variables*	CSU / OMA (n = 42)	CSU / Non-OMA (n = 36)	Control (n = 18)	P[a]			P[b]
				OMA vs Control	Non-OMA vs Control	OMA vs Non-OMA	
Age, years	42.4 ± 13.7	38.5 ± 11.9	38 (30-47)	ns	ns	ns	0.37
Female gender, n (%)	33 (78.6)	24 (66.7)	38 (30-47)	ns	ns	ns	0.43
Eosinophils, cells/μL	100 (100-215)	100 (100-200)	115 (100-200)	ns	ns	ns	0.88
Eosinophils, %	1.60 (0.82-2.62)	1.55 (1.02-2.67)	1.5 (1.2-2.6)	ns	ns	ns	0.96
D-dimer, μg/mL	0.35 (0.24-0.51)	0.41 (0.23-0.69)	0.24 (0.19-0.38)	ns	ns	ns	0.05
Total IgE, IU/mL	232 (87.1-358)	105 (37.4-248)	23.6 (11.9-50.6)	<0.01	<0.01	ns	<0.01
ESR, mm/h	10.5 (6-20)	10 (4-15)	3 (2-6)	0.01	ns	ns	0.015
CRP, mg/L	0.43 (0,29-0.7)	0.54 (0.27-1.03)	0.20 (0.11-0.37)	0.01	<0.01	ns	<0.01
Eotaxin, pg/mL	36.2 (25.1-54.5)	35.7 (19.4-50.5)	24.8 (17-46.5)	ns	ns	ns	0.37
IL-6, pg/mL	1.44 (0.82-2.76)	1.37 (0.90-3.93)	1.0 (0.7-1.6)	ns	ns	ns	0.41
TSLP, pg/mL	27.8 (0.72-582.9)	12.2 (0.32- 149.3)	93.6 (6.5-793.0)	ns	ns	ns	0.41
RANTES, pg/mL	3548 (3108-4003)	3483 (2950-3894)	664.4 (640-743)	<0.01	<0.01	ns	<0.01
TNF-α, pg/mL	35.1 (0.29-605.3)	156.7 (33.08-392)	1.8 (1.5-2.91)	0.016	<0.01	ns	<0.01
IL-17, pg/mL	58.6 (1.75-263.8)	47.7 (8.2-268.8)	100 (15.8-639)	ns	ns	ns	0.31
IL-31, pg/mL	632.2 (100.8-2498)	743.2 (121.3-3447)	213.1 (9-633.6)	ns	ns	ns	0.21

*Categorical variables were presented with frequencies and percentages (%) and continuous variables as mean ± standard deviation or median (IQR).

CSU; Chronic spontaneous urticaria, OMA: Omalizumab

Pa; Pairwise comparisons between the groups

Pb; Kruskal-Wallis test for nonparametric variables for the comparison of the three groups

C; Chi-Square Test

ns: non-significant

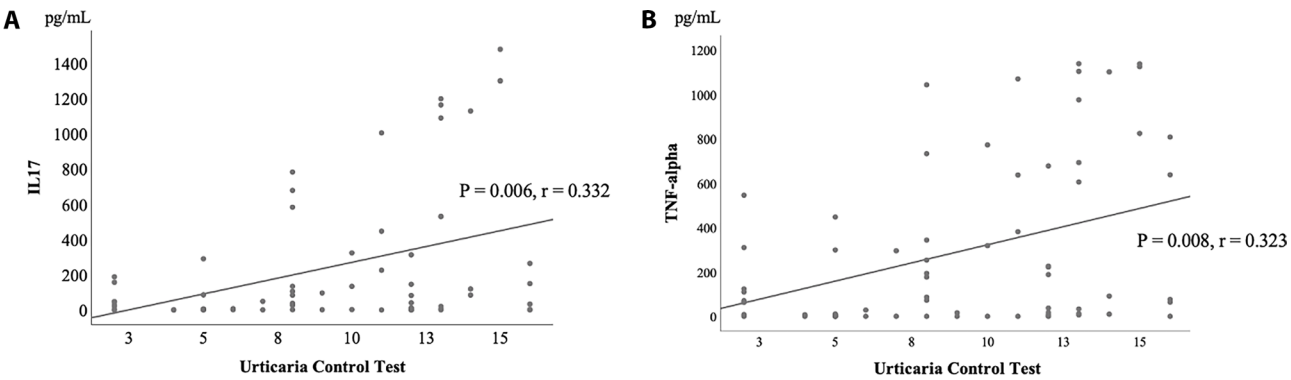

Figure 2. Correlation graphs between urticaria control test and IL-17 /TNF-α levels.

Conclusions

In the current study, the biomarker profile in CSU was investigated by comparing the Th1, Th2, and Th17 cytokines, which are considered to be valuable in the pathogenesis, in CSU patients and healthy controls. Of the biomarkers studied (TNF-α, IL-6, IL-31, TSLP, IL-17, eotaxin, RANTES), TNF-α and RANTES were considered as potential biomarkers of CSU, while it was observed that the effect of omalizumab treatment was not through these biomarkers. Consistent with the literature, in our study, TNF-α levels were significantly higher in the CSU patient group [24]. This can be explained by the theory previously presented by Walsh et al. that dermal mast cells include a high amount of immunoreactive TNF-α in their granules and that they can be rapidly released into the extracellular area by degranulation [25]. In the comparison of patients receiving and not receiving omalizumab treatment, although TNF-α levels were lower in those receiving omalizumab treatment, no statistically significant change was observed. Similarly, in the study by Noga et al, in which 19 participants were compared, no significant difference was observed in TNF-α levels between the omalizumab and placebo groups [26]. However, in a case series of patients who did not respond to omalizumab, TNF-α antagonists were noticed to be efficacious in 60% of 20 CSU patients [27]. This suggests that TNF-α may be a potential biomarker for determining disease activity, but it is insufficient to assess omalizumab treatment response, but may be an alternative pathway in patients resistant to omalizumab.

It has been reported that IL-6 which is known to play a crucial role in inflammatory responses, is also detected in high concentrations in CSU patients, and it has been stated that IL-6 signaling may be considered in the pathogenesis and inflammatory activation of this disease [28]. High IL-6 level has been related to disease severity and activity in many studies, and it has been proposed that IL-6 may be a good biomarker for CSU disease [15,29, 0]. On the other hand, there are also studies showing that IL-6 is not related to disease activity in CSU [31,32]. In a study by Noga et al, investigating the omalizumab effect on inflammatory mediators in moderate and severe allergic asthma, no statistically significant change was observed in IL-6 levels after omalizumab treatment [33]. In the present study, IL-6 levels were also comparable between the study groups. Additionally, similar results were observed in those receiving and not receiving omalizumab. Therefore, IL-6, which has many important physiological and anti-inflammatory functions such as inflammation, fever, and tumorigenesis, has not been considered as a specific and reliable marker for CSU.

TSLP is a cytokine with a critical role in regulating inflammatory responses. TSLP was observed higher in the lesions than in non-lesional skin areas of CSU patients [34]. Therefore, pharmacological inhibition of the interaction between TSLP and its receptor or antibody-mediated TSLP neutralization was discussed to be a possible option in the treatment of CSU patients [35]. However, the number of studies examining serum TSLP levels in CSU is limited. Metz et al. reported no significant increase in TSLP levels in CSU patients [32]. In our study, TSLP levels showed variation in healthy and CSU samples, and no significant difference was detected between the groups. This suggests that since TSLP is a more target-oriented molecule, its levels in CSU patients may be more significant in assessing lesioned skin rather than as a serum biomarker.

It has been shown that IL-31, mainly secreted by Th2 cells, causes pruritus by activating the IL-31 receptor on sensorial neurons, hence, it is thought to be an important interleukin in skin-related inflammation such as atopic dermatitis [36-38]. Additionally, studies have shown that anti-IL-31 treatment significantly reduces/prevents itching [36,39-42]. Moreover, in addition to the lesioned skin samples of CSU patients, high levels of IL-31 were also detected in serum samples [38,43]. Besides, in a study conducted with 15 CSU patients who were highly responsive to omalizumab treatment, a decrease in IL-31 levels was observed after treatment [40]. In our current study, there was a tendency for higher IL-31 levels in CSU patients. If the sample size in the control group was larger, it is believed that the IL-31 levels between the CSU and control groups would have reached a significant difference.

Studies investigating the function of cytokines in CSU indicated that plasma levels of IL-17 were significantly increased in relation to disease severity compared to the control group [6,38,44]. Besides, there are studies showing that plasma IL-17 levels in CSU patients are similar, and even lower than, healthy controls [45]. Previous studies demonstrated that the levels of IL-17 were higher in autologous serum skin testing-positive CSU patients [8]. In our study, there was no difference in IL-17 levels between CSU and control groups, but a significant correlation was observed between IL-17 level and urticaria control test among CSU patients, which supports previous studies [8]. This may be due to the fact that autologous serum skin testing was not performed in our study and IL-17 levels were not measured by classifying accordingly.

Eotaxin is a potent agonist for the Cysteine-Cysteine (CC) chemokine receptor-3, which attracts eosinophils to the skin, thus suggesting its involvement in the pathophysiology of CSU. In a study by Tedeschi et al, it was indicated that serum eotaxin levels in 100 CSU patients were slightly higher in chronic urticaria patients and were correlated with the disease severity. In our analysis, eotaxin levels were comparable in healthy controls and CSU patients.

RANTES promotes the mast cell progenitors for differentiation and activation, thereby inducing the histamine release, and

is therefore considered to be involved in CSU pathogenesis [46]. In a study by Puxeddu et al, levels of RANTES and IL-8 were quantified in CSU patients, and RANTES was observed to be remarkably higher in CSU compared to healthy controls. However, no association was found between other markers and RANTES [13]. Consistent with these findings, in the current study, RANTES levels were detected to be higher in CSU patients regardless of disease activity.

This study had certain limitations. First, due to the cross-sectional study design, the study outcomes were not fully sufficient to evaluate the efficacy of omalizumab treatment on biomarkers. It would be more reliable to analyze the present outcomes with longitudinal studies. However, despite its cross-sectional design, the fact that patients have been on omalizumab treatment for a long time and the cytokine profiles of those receiving and not receiving omalizumab were similar despite better clinical scores, suggests that omalizumab might be effective through different pathways than these cytokines. The second limitation was the limited number of participants in the control group. Some p-values could be more significant with a larger sample size in the control group.

In conclusion, our results suggest that the investigated cytokine profile in CSU patients may differ from healthy controls and omalizumab does not seem to affect that profile in CSU patients.

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