

Altered Systemic Adipokines in Patients with Chronic Urticaria

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Keywords

Chronic urticaria · Adipokines · Lipocalin-2 · Severity · Quality of life

Abstract

Background: Increasing evidence suggests that adipokines affect immune responses and chronic urticaria (CU) is associated with an altered immune response related to chronic systemic inflammation. Our objectives were to investigate whether adipokines are involved in CU pathogenesis and to outline relationships between adipokines and urticaria severity and quality of life. **Methods:** Serum adiponectin, leptin, lipocalin-2 (LCN2), interleukin (IL)-10, IL-6, and tumor necrosis factor (TNF)- α concentrations were measured by enzyme-linked immunosorbent assays in 191 CU patients and 89 healthy controls. The effect of LCN2 on N-formyl-methionine-leucine-phenylalanine (fMLP)-induced neutrophil chemotaxis was assessed using migration assays. CU severity was assessed based on the urticaria activity score (UAS). To explore relationships between adipokines and UAS and the chronic urticaria-specific quality of life (CU-QoL) questionnaire, a structural equation model was used. **Results:** Mean levels of serum LCN2, TNF- α , IL-6, and IL-10 were significantly higher in CU patients than in controls. Adiponectin levels were significantly lower in patients with CU than in controls. While serum IL-6 levels were significantly higher in

refractory CU patients, compared to responsive CU individuals, LCN2 levels were significantly lower. LCN2 inhibited fMLP-induced neutrophil migration. LCN2 showed a direct relationship with UAS ($\beta = -0.274, p < 0.001$), and UAS was found to contribute to CU-QoL ($\beta = 0.417, p < 0.001$). **Conclusions:** Our results highlighted an imbalance in pro- and anti-inflammatory adipokines in CU patients. We suggest that LCN2 could be a differential marker for disease activity and the clinical responses to antihistamine treatment in CU patients. Modulation of systemic inflammation may be a therapeutic strategy for treating severe, refractory CU.

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Introduction

Chronic urticaria (CU) is a common skin disorder defined by recurrent wheals and pruritus of at least a 6-week duration [1]. Considerable evidence has shown that mast cells play a key role in CU, although the causes of mast cell activation are still under investigation. Recent studies suggest that 35–55% of CU patients have circulating autoantibodies to IgE and/or the high-affinity IgE receptor Fc ϵ RI, and these autoantibodies exhibit histamine-releasing activity [2]. While the pathogenesis of CU is not completely understood in most patients, histological examination of CU patients has revealed perivascular infiltra-

tion of CD4⁺ and CD8⁺ T lymphocytes, eosinophils, basophils, mast cells, and neutrophils [3]. Accordingly, studies have deemed CU to be associated with an altered immune response related to chronic systemic inflammation [4, 5]. Low-grade or subclinical inflammation can stimulate mast cell activation upon the release of adipokines, including interleukin (IL)-6, IL-9, and IL-33 [6]. In CU patients, increased IL-6 and C-reactive protein levels have been found to be significantly associated with urticaria severity [7].

We previously demonstrated that 29.8% of CU patients have metabolic syndrome and that patients with metabolic syndrome present with poor clinical outcomes for CU, such as a higher mean urticaria activity score (UAS) and uncontrolled CU [8]. Most proinflammatory adipokines are overproduced in metabolic syndrome, while anti-inflammatory adipokines, such as adiponectin and IL-10, are downregulated [9, 10]. This suggests a possible relationship between adipokines and CU pathogenesis.

Lipocalin-2 (LCN2), also known as neutrophil gelatinase-associated lipocalin and siderocalin, is stored in secondary granules that contain lactoferrin, calprotectin (S100A8/A9), or Mac-1 (CD11b/CD18) [11]. LCN2 is released from neutrophils upon stimulation [12]. Meanwhile, several studies have found serum LCN2 levels to be correlated with metabolic syndrome, insulin resistance, obesity, and certain inflammatory diseases [13–15]. LCN2 has also been shown to induce endothelial dysregulation and cardiomyocyte apoptosis [16]. Additional research demonstrated that LCN2 ameliorates the outcomes of an experimental renal ischemia mouse model by rescuing mouse proximal tubules from acute tubular necrosis [17].

Accumulating evidence has demonstrated that adipokines are associated with allergic inflammation and mast cells, particularly in asthma [6]. However, studies have yet to determine whether adipokine dysregulation is associated with CU. We sought to investigate the potential involvement of adipokines in the pathogenesis of CU. In addition, we explored the relationships between adipokines and urticaria severity and quality of life in patients with CU.

Materials and Methods

Subjects

We performed a hospital-based cross-sectional study of 191 patients with CU, defined as CU with no external triggers (120 females and 71 males, median age 40 years, range 19–60 years) and

92 healthy controls (63 females and 29 males, median age 38 years, range 26–54 years). Subjects aged ≥ 19 years and CU patients with wheals and itching for at least 6 weeks were recruited. Patients with other chronic skin diseases and those with clinical evidence of urticarial vasculitis or physically induced urticaria were excluded. All subjects provided written informed consent at the time of enrollment. The normal controls had no previous history of inflammatory or allergic diseases or urticaria.

Assessment of Urticaria Activity Score and Therapeutic Response

CU disease activity was assessed in relation to UAS within 1 week prior to outpatient clinic visits, with total scores of 0–15 [18]. UAS is used to score wheals according to quantity (0, no wheals; 1, <20 wheals; 2, 20–50 wheals; and 3, >50 wheals), distribution range [0, none; 1, <25% of the body surface area (BSA); 2, 25–50% of the BSA; and 3, >50% of the BSA], mean diameter (0, no wheals; 1, <1 cm; 2, 1–3 cm; and 3, >3 cm), and duration (0, no wheals; 1, <4 h; 2, 4–12 h; and 3, >12 h) and to score pruritus according to intensity (0, no pruritus; 1, mild; 2, moderate; and 3, severe) within 1 week prior to outpatient clinic visits. Disease-specific quality of life in patients with CU was measured at enrollment using the computerized chronic urticaria-specific quality of life (CU-QoL) questionnaire [19], which consists of 4 domains: emotional distress, stigma, urticaria symptoms, and food or environmental distress. Refractory CU was defined as patients whose urticaria symptoms were not controlled by increasing the antihistamine dose to 4-fold within 3 months. In contrast, patients who responded well to antihistamines were classified as having responsive CU [20]. The required antihistamine doses used to control urticaria symptoms for a subsequent 3 months after enrollment were calculated as the equivalent dose (mg/day) of loratadine. Similarly, the dose requirement of systemic steroids was calculated as the prednisolone equivalent dose (mg/month) [21].

Measurement of Serum Adipokines

Serum adiponectin, leptin, LCN2, IL-10, IL-6, and tumor necrosis factor (TNF)- α levels were measured by ELISA using commercially available reagents (R&D Systems Inc., Minneapolis, MN, USA). For detection of adiponectin (range 3.9–250 ng/mL) and leptin (range 15.6–1,000 pg/mL), serum samples were diluted 100 times. For detection of LCN2 (range 0.2–10 ng/mL), sera were diluted 20 times. Undiluted serum samples were measured for IL-10 (range 7.8–500 pg/mL), IL-6 (range 9.38–600 pg/mL), and TNF- α (range 15.60–1,000 pg/mL). Serum total IgE levels were measured using the ImmunoCAP system (Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer's instructions (measuring range 2–5,000 kU/L).

Neutrophil Migration Assay

Polymorphonuclear neutrophils (PMN) were isolated from the peripheral blood of healthy donors by density gradient centrifugation, followed by dextran sedimentation and hypotonic lysis of red blood cells, as described previously [12]. Cells comprising >95% neutrophils were suspended in RPMI 1640 with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were preincubated with anti-LCN2 (ab41105; Abcam, Cambridge, MA, USA) for 18 h at 37°C in a humidified incubator containing 5% CO₂. Viable cells were visualized by incubation with 2 μ M calcein-acetoxy-

Table 1. Clinical characteristics of the study subjects

	Refractory CU (n = 72)	Responsive CU (n = 119)	p value (refractory vs. responsive)	CU (n = 191)	Controls (n = 92)	p value (CU vs. controls)
Male:female ratio	32:40	39:80	0.123	71:120	29:63	0.426
Age, years	32 (23–52)	41 (19–60)	0.654	40 (19–59)	38 (26–54)	0.148
Total IgE, kU/L	19.9 (0–282)	108.0 (3–2,586)	0.160	133.0 (3–2,586)	20.0 (0–282)	<0.001
Duration, months	11.0 (0–480)	6.0 (1–240)	0.175	7.0 (1–480)		
Angioedema, n (%)	50 (69.4)	26 (21.8)	<0.001	76 (39.8)		
UAS (0–15)	13 (5–15)	10 (0–15)	<0.001	11 (1–15)		
CU-QoL (0–100)	52.9 (0–97)	69.1 (15–97)	0.001	64.7 (0–97)		
Dose requirement						
Antihistamines ^a , mg/day	40.0 (8.2–66.8)	21.8 (3.1–56.7)	<0.001	24.4 (3.1–66.8)		
Systemic steroids ^b , mg/week	7.7 (0–100)	0	<0.001	0.2 (0–100)		
Patients on systemic steroids, %	74.0	0				
Patients on cyclosporine, %	18.0	0				
Patients on LTRA, %	18.0	1.7				

Values are presented as medians (ranges), determined using the Mann-Whitney U test or Fisher's exact test, unless otherwise stated. CU, chronic urticaria; UAS, urticaria activity score; CU-QoL, chronic urticaria-specific quality of life; LTRA, leukotriene receptor antagonist. ^a Loratadine equivalent dose. ^b Prednisolone equivalent dose.

methyl (Sigma Aldrich, St. Louis, MO, USA) for 30 min. Migration assays were performed using the ChemoTx[®] system (Neuroprobe, Bethesda, MD, USA). For chemotaxis, 50 µL of the cell suspension (6×10^6 cells/mL) were added to a microplate. N-formyl-methionine-leucine-phenylalanine (fMLP) at 10^{-7} M was then added to the lower compartment as a chemoattractant. Cells were allowed to migrate for 3 h. Then, the filter was removed and 50 µL of the cell suspension in the lower compartment were added to Nunclon Delta Surface 96-well plates (Thermo Scientific, Waltham, MA, USA). Fluorescence intensity in the migrated cells was determined using a microplate reader. Experiments were performed in triplicate using cells from 6 subjects.

Statistical Analysis

Data for continuous variables are presented as medians (range). Prevalence rates are listed as percentages. The Mann-Whitney U test or one-way ANOVA with a post hoc LSD test was used for between-group comparisons of continuous variables. Categorical variables were compared using Fisher's exact test. Spearman's ρ test was used for correlation analysis. $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS software (version 19 for Windows; SPSS, Chicago, IL, USA).

To explore the associations between adipokines in relation to UAS and CU-QoL, path analysis using Amos 21.0 software (Small Waters Corp., 2009) was performed. Structural equation modeling was used to evaluate the fit of the final model by examining a number of statistics. To examine the magnitude of the discrepancy between the sample and fitted covariance matrices, the χ^2 test was used when a nonsignificant test indicated that the model and data were consistent [22]. The comparative fit index examines the dif-

ference in overall fit among the models, and values >0.95 indicate a good model fit. The root mean square error of approximation evaluates the approximate fit of the model, with values <0.005 suggesting a reasonable goodness of fit [22].

Results

Clinical Characteristics of the Study Subjects

Table 1 presents the clinical characteristics and serum adipokine levels of the patients with CU classified into the responsive and refractory groups, as well as the healthy controls (controls). The mean duration of urticaria in CU patients at enrollment was 28.9 months. The median UAS and CU-QoL were 11 (range 1–15) and 64.7 (range 0–97), respectively. Of 191 patients, 72 (37.7%) were classified into the refractory CU group. The serum total IgE levels of the CU patients were significantly higher than those of the controls (236.0 ± 344.1 vs. 44.3 ± 61.5 kU/L, $p < 0.001$). There was no significant difference in mean age, gender, or total IgE levels between patients with refractory and responsive CU (Table 1). Patients with refractory CU showed a higher prevalence of angioedema ($p < 0.001$). As expected, UAS and CU-QoL were significantly different between the 2 treatment response groups. Patients with refractory CU had higher UAS and lower CU-QoL

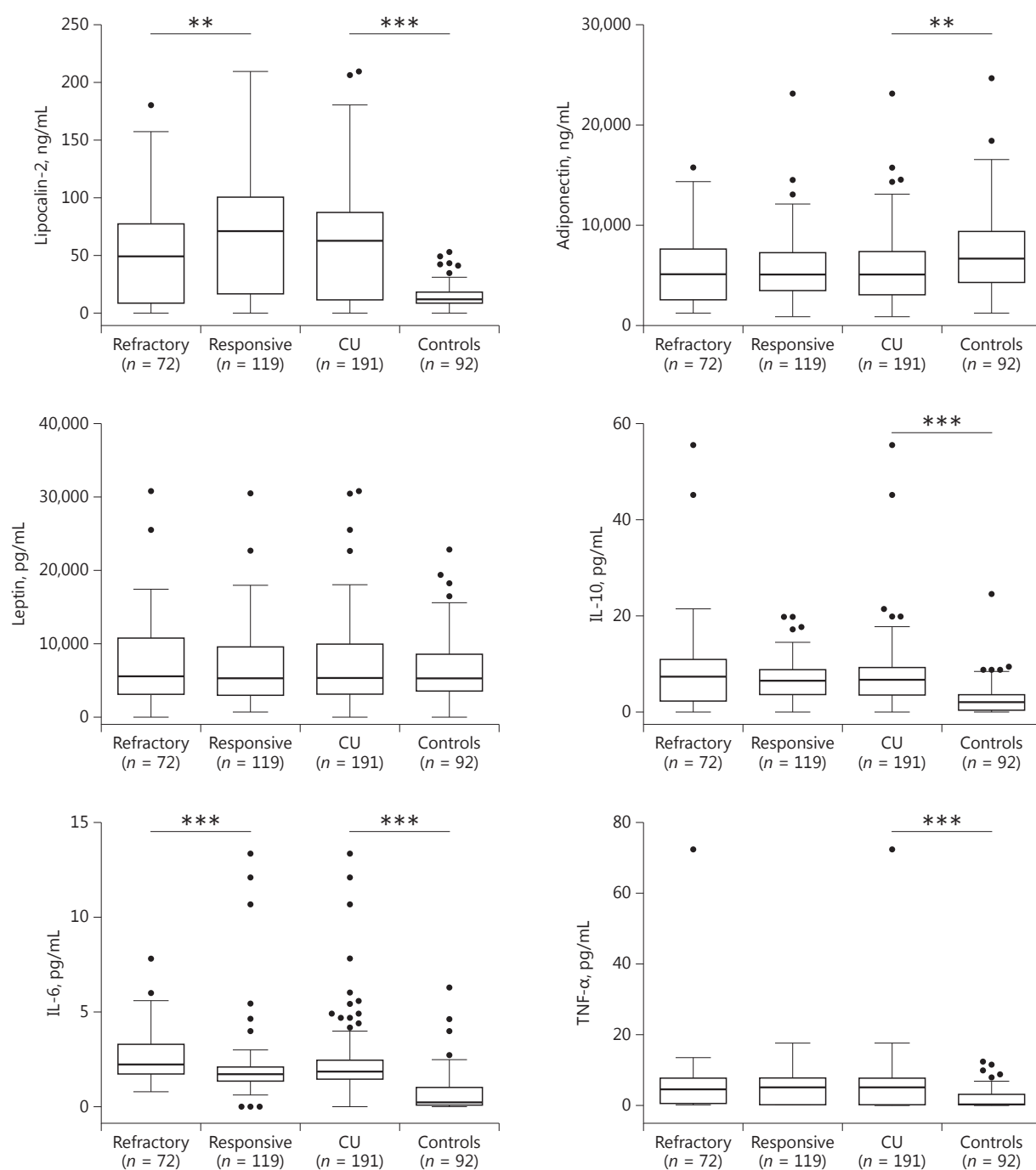


Fig. 1. Serum levels of adipokines in patients with refractory and responsive chronic urticaria. CU, chronic urticaria; Refractory, refractory chronic urticaria; Responsive, responsive chronic urticaria; IL, interleukin; TNF- α , tumor necrosis factor- α . *p* values were calculated using the Mann-Whitney U test. * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001.

Fig. 2. Correlations between serum adipokines and clinical outcomes of chronic urticaria patients. Correlation analysis using Spearman's ρ test revealed a negative correlation between chronic urticaria-specific quality of life (CU-QoL) and urticarial activity score (UAS). Interleukin (IL)-6 showed a positive correlation with UAS, while lipocalin-2 (LCN2) showed a negative correlation. LCN2 was related to other adipokines, including leptin, IL-10, and IL-6. Data are presented as Spearman's ρ . * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

CU-QoL							
-0.352***	UAS						
0.164	-0.226**	Lipocalin-2					
-0.029	0.026	0.238**	Leptin				
0.048	-0.078	-0.112	0.050	Adiponectin			
0.207*	0.006	0.477***	0.240**	-0.057	IL-10		
-0.090	0.248**	0.327***	0.152*	-0.202**	0.301***	IL-6	
-0.119	0.115	0.124	-0.004	-0.154*	0.192**	0.341***	TNF- α

scores compared to those with responsive CU (13 vs. 10, $p < 0.001$, and 52.9 vs. 69.1, $p = 0.001$). With regard to treatment, patients with refractory CU had greater needs for antihistamine (mg/day) and systemic corticosteroids (mg/week) than patients with responsive CU ($p < 0.001$ for each). In the refractory CU group, the percentages of patients who received systemic corticosteroids, cyclosporine, and leukotriene receptor antagonist were 74, 18, and 18%.

Comparison of Serum Adipokines between Refractory and Responsive CSU

LCN-2, IL-10, IL-6, and TNF- α levels were significantly increased in sera from patients with CU versus controls (Fig. 1). In contrast, adiponectin levels were significantly reduced in CU patients versus controls. There was no difference in serum leptin levels between CU and controls. Among adipokines, LCN2 levels decreased significantly in refractory CU patients compared to patients who responded to antihistamines ($p = 0.003$). In contrast, IL-6 was significantly higher in the refractory group than in the responsive CSU group ($p = 0.001$). Meanwhile, there were no significant differences in IL-10, adiponectin, leptin, and TNF- α levels between the 2 treatment response groups.

Correlations between Serum Adipokines and Clinical Outcomes of CSU

With regard to CU-QoL, a significant and negative correlation was found with UAS (Spearman's $\rho -0.352$, $p < 0.001$), whereas a positive correlation was observed with IL-10 levels (coefficient 0.248, $p < 0.001$; Fig. 2). With regard to UAS, a significant positive correlation was noted with IL-6 (coefficient 0.248, $p < 0.01$), while a sig-

nificant negative correlation was observed with LCN2 levels (coefficient -0.226 , $p < 0.01$). Among the adipokines evaluated, LCN2 was significantly related to leptin (coefficient 0.238, $p < 0.01$), IL-10 (coefficient 0.477, $p < 0.001$), and IL-6 (coefficient 0.327, $p < 0.001$).

LCN2 Inhibits fMLP-Induced Migration of Human PMN

To investigate the chemotactic effect of LCN2, we pre-incubated PMN with LCN2 at different concentrations (0.1, 1, and 10 $\mu\text{g/mL}$) and then investigated neutrophil chemotaxis using fMLP as a chemoattractant. We found that LCN2 inhibited fMLP-induced neutrophil migration in a dose-dependent manner ($p < 0.05$ for each; Fig. 3).

Structural Equation Modeling

A structural equation model was developed to evaluate the relationship between adipokines and UAS and CU-QoL (Fig. 4). To investigate the determinants of CU-QoL, the final outcome of CU, we considered that each adipokine by itself or through direct or indirect interaction with other adipokines could influence disease activity. The goodness of fit of the model was demonstrated using several fit statistics. A χ^2 test of absolute fit was not significant ($p = 0.839$), indicating that this model was a good fit for the data. This was further supported by a root mean square error of approximation value < 0.001 (90% CI 0.000–0.065) and a comparative fit index value > 0.95 . The model had a relatively small Akaike information criterion value (i.e., 66.501), suggesting that this simple model may provide a good explanation of the relationships among CU-QoL, UAS, and adipokine levels. The regression coefficient β represents the index of path direction and the

Fig. 3. Effects of lipocalin-2 (LCN2) on N-formylmethionine-leucyl-phenylalanine (fMLP)-induced neutrophil migration. Cells were either untreated or primed with LCN2 at different concentrations (0.1, 1, and 10 $\mu\text{g/mL}$) for 18 h prior to the migration assay. After incubation with calcein-acetoxymethyl, cells were placed into the upper chamber of chemotaxis plate. fMLP at 10^{-7} M was used as a chemoattractant. Cells were allowed to migrate within 3 h. Data are presented as fold changes of migrated cells compared to the positive control (fMLP only) (means $\pm$ SEM). Six independent experiments in triplicate were conducted and the means of individual experiments were analyzed. *p* values were calculated by one-way ANOVA with a least significant difference post hoc test. ** *p* < 0.01 compared to the negative control (medium group); # *p* < 0.05 compared to the positive control (fMLP only). NS, not significant compared to the negative control (medium).

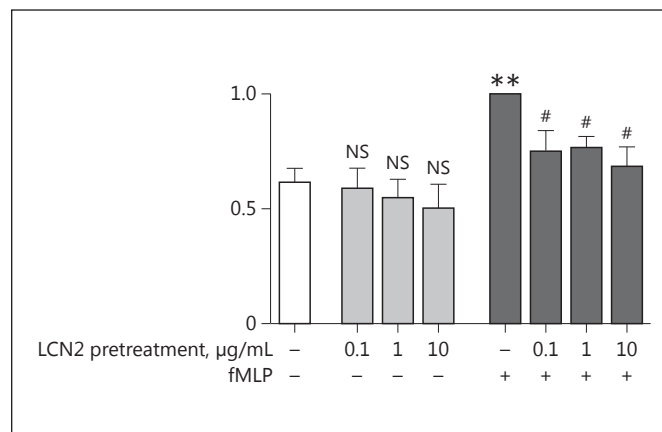
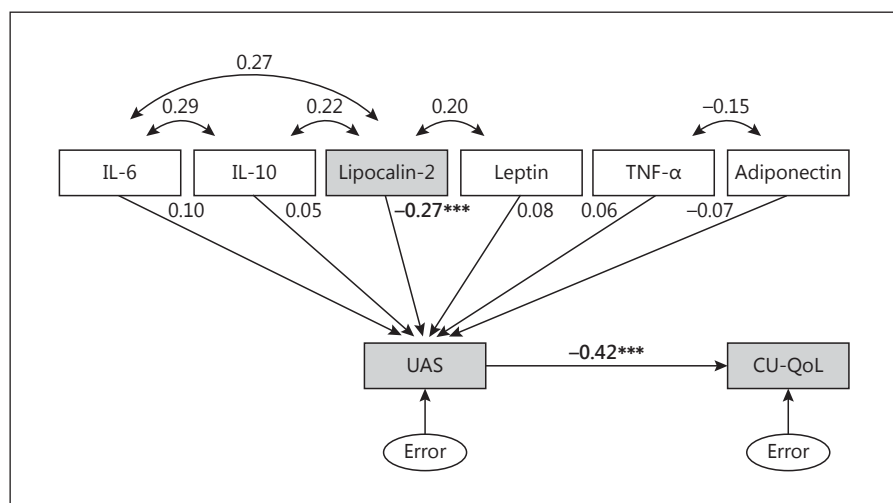


Fig. 4. Structural equation modeling to estimate relationships among urticarial activity score (UAS), chronic urticaria-specific quality of life (CU-QoL), and adipokines. Lipocalin-2 was found to be a significant predictor of UAS ($\beta = -0.274$, *p* < 0.001). UAS contributed significantly to CU-QoL ($\beta = -0.417$, *p* < 0.001). The numbers on the arrows indicate the regression coefficient (β) of the structural equation model. Positive numbers mean a positive correlation between 2 factors, whereas negative numbers mean negative correlations. The direction of the arrows represents cause and effect. *** *p* < 0.001.



intensity of the relation between 2 factors. LCN2 was found to be a significant predictor of UAS ($\beta = -0.274$, *p* < 0.001), while other adipokines, such as leptin, adiponectin, IL-6, IL-10, and TNF- α , did not contribute directly or indirectly to UAS. A significant contribution of UAS to CU-QoL was also identified in the model ($\beta = -0.417$, *p* < 0.001).

Discussion

The inflammatory response in CU is characterized by an imbalance between proinflammatory cytokines (IL-6 and TNF- α) and anti-inflammatory adipokines (adiponectin and IL-10). We found increased levels of IL-6, TNF- α , and LCN2, as well as reduced levels of adiponec-

tin, in sera from CU patients compared to controls. In CU, augmented levels of proinflammatory cytokines might be involved in skin lesions and interactions with immune cells [8, 23]. In the present study, elevated levels of LCN2 in patients with CU were inversely correlated with disease activity scores for urticaria (UAS), while a significant positive correlation was observed between IL-6 levels and UAS. Furthermore, LCN2 was significantly increased in patients with responsive CU compared to those with CU refractory to antihistamines.

LCN-2, a component of the innate immune system, plays an important role in the acute-phase response to induction of apoptosis and infection [16]. Similarly to acute-phase proteins, LCN2 functions to enhance host defenses and limit the potential harmful effects of the inflammatory response in healthy tissues [24]. LCN2 is ex-

pressed at a higher level in both tissues and body fluids in several acute and chronic inflammatory, ischemic, and metabolic disorders [25, 26]. Notably, LCN2 was shown to be upregulated in response to cellular stress and to play an important role in promoting protective effects in a liver injury model. Others have suggested that LCN2 might act as an intrinsic “help me” sensor that recruits inflammatory cells to damaged tissue [27].

Increased levels of LCN2 were also previously observed in patients with psoriasis, another skin disorder showing abnormal keratinocyte differentiation and inflammation [15]. Psoriasis itself might act as an inflammatory stimulus to induce LCN2 production in neutrophils [28]. Also, histamine was found to upregulate the expression of LCN2 in a human breast cancer cell line [29]. Histamine might cause bidirectional effects on neutrophil respiratory burst activation via cell surface H₁ and H₂ receptors [30]. Therefore, we assume that, to some extent, histamine may be involved in LCN2 production in neutrophils.

Meanwhile, in the present study, serum levels of adiponectin were reduced in CU patients, suggesting an anti-inflammatory role of adiponectin in CU. Adiponectin can exert both proinflammatory and anti-inflammatory effects [31, 32]. In metabolic diseases, TNF- α and IL-6, which are secreted from mast cells in the skin and from macrophages in adipose tissue, are implicated in inhibition of the local production of adiponectin [33, 34]. In sera from the CU patients included in this study, serum levels of IL-10 increased and were positively correlated with CU-QoL. Although IL-10 is upregulated in metabolic syndrome and obese asthma patients, a subgroup of CU patients with a positive autologous serum skin test showed attenuated serum levels of IL-10 that were associated with quick relief of the inflammatory milieu [9, 10, 23].

To identify potential serological markers for the determination of severe or refractory CU, we established a structural equation model to estimate the relationship among disease activity, CU-QoL, and serum adipokine levels. Considering that some significant correlations were noted not only among adipokines themselves but also among clinically important outcomes, including UAS and CU-QoL, we set the order of parameters in the univariate analysis model. Essentially, we assumed that adipokines can affect UAS itself or via interactions with other adipokines. Previously, UAS was identified as an important predictor of CU-QoL and a prognostic factor to monitor the response to treatment [19, 35]. Our model revealed that LCN2 was the only adipokine affecting

UAS, even though various complex interactions among adipokines were considered. Increased LCN2 in sera from patients with CU was associated with a decrease in UAS and subsequently improvement in CU-QoL.

Thus, we suspect that serum levels of LCN2 may be useful as a biomarker to determine disease activity and identify individuals who would show a favorable clinical response to antihistamine treatment among patients with CU. CU is a complex disease that involves various, integrated inflammatory pathways [36]. Recent guidelines recommend nonsedating H₁-antihistamines as the first-line and major treatment to control urticarial symptoms; however, a substantial proportion of patients show little benefit from antihistamine therapy [37]. Furthermore, as there is no reliable biomarker for the prediction of responses to antihistamines, clinicians have no choice but to apply the same stepwise treatment to every CU patient. Consequently, there is a great need for new therapeutic strategies and the development of a biomarker that can predict or monitor the therapeutic responses of patients with refractory CU.

The role of LCN2 in the pathogenesis of CU remains to be determined. LCN2 is able to bind to fMLP, platelet-activating factor, leukotriene B₄, and lipopolysaccharides, thereby inhibiting inflammatory responses [38]. Additionally, LCN2 might promote the migration of human and murine neutrophils via Erk 1/2-mediated signaling [12]. In CU, the urticarial wheal is characterized by perivascular infiltration, including neutrophils as a prominent population [2]. Thus, we investigated the effects of LCN2 in fMLP-induced neutrophil migration. We found that pretreatment with LCN2 significantly suppressed neutrophil chemotaxis induced by fMLP in a dose-dependent manner. fMLP-induced neutrophil chemotaxis required involvement of the phosphoinositide-3 kinase pathway [39]. In murine breast cancer cells, LCN2 has been found to inhibit the PI3K/Akt pathway [40]. Besides, LCN2 has been reported to have both anti- and proinflammatory effects [41]. In our study, we found a decreased level of serum LCN2 in patients with refractory CU and a significantly negative association between LCN2 and urticaria activity, advocating the anti-inflammatory effects of LCN2 in CU. Thus, we speculate that priming of neutrophils with LCN2 may inhibit fMLP-induced neutrophil migration. The regulatory effect of LCN2 on neutrophil chemotaxis might link LCN2 to refractory CU. However, further studies are necessary to reveal the underlying mechanism.

This study has several strengths and limitations. We noted distinct adipokine profiles between responsive and

refractory CU subjects. Moreover, this is the first report on LCN2 as a potential biomarker for the effectiveness of antihistamine therapy in CU, as well as the association of LCN2 with CU outcomes. However, this was a hospital-based, cross-sectional study, and therefore we could not demonstrate a causal link between LCN2 and CU.

In conclusion, our findings highlight an imbalance between pro- and anti-inflammatory adipokines in patients with CU, and further differences were observed according to severity and treatment responses to antihistamines. Although its biological activity is not specific to CU, serum levels of LCN2 may be a useful biomarker for the prediction of responses to antihistamine treatments in patients with CU.

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Author Contributions

H.K.T.T. wrote the first draft of this paper and performed experiments. D.L.P. designed and performed experiments. G.Y.B. collected samples and clinical data. H.Y.L. performed the statistical analyses. H.-S.P. collected samples and interpreted data. Y.M.Y. designed experiments, interpreted data, and revised this paper.

Disclosure Statement

The authors declare no conflicts of interests in relation to the content of this report.

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