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Original Article

Assessment of Serum Zonulin Levels in Individuals Diagnosed With Chronic Spontaneous Urticaria

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ABSTRACT

Background: There have been reports indicating a correlation between heightened intestinal permeability and many autoimmune and chronic inflammatory disorders. The involvement of autoimmunity is now recognized as a significant factor in the development of chronic spontaneous urticaria (CSU). Zonulin is an important biomarker that regulates tight junction permeability within cells in the gastrointestinal tract, hence facilitating intestinal permeability.
Objective: To evaluate the correlation of CSU with intestinal permeability by measuring the serum levels of zonulin in patients diagnosed with CSU.
Methods: The study included 60 patients diagnosed with CSU and 64 age- and sex-matched healthy individuals as controls. Levels of serum zonulin were determined using the ELISA method.
Results: Although the serum zonulin value of the patients was higher compared to the controls, the difference did not reach a significant level (24.65±8.49 ng/ml vs. 21.03±7.36 ng/ml, p=0.077). The serum zonulin level had a significant correlation with the urticaria activity score in the CSU group (p=0.013). The results of the current study revealed that serum zonulin values significantly differed between patients with CSU and healthy controls.
Conclusion: This study is important in terms of being the first to investigate the serum zonulin levels in CSU. However, there is a need for further studies with larger patient groups.

Keywords: Chronic spontaneous urticaria; Intestinal permeability; Small intestine

INTRODUCTION

Chronic spontaneous urticaria (CSU) is a dermatological condition that presents with recurring itchy wheals and plaques, angioedema, or both, lasting more than six weeks. CSU is a multifaceted medical condition disease that is potentially related to imbalances in immunity, inflammation, and coagulation[1]. Although extensive studies have been undertaken on CSU, the pathogenesis of the disease is still largely unknown. However, well-developed hypotheses include those related to autoimmunity, histamine-releasing factors, and cellular defects. Recent research has demonstrated that the integrity of the intestinal barrier, which is subject to variations influenced by factors, such as dietary choices and contemporary way of life, is a significant contributor to the development of autoimmune diseases[2-11]. The intestinal epithelium plays a crucial role in the processes of nutritional digestion and absorption, as well as fluid-electrolyte hemostasis. It also acts as a protective barrier against microorganisms, antigens, and toxic substances in the gut lumen. The barrier function in the intestinal epithelium is mediated by tight junctions within cells, adherens junctions, and desmosomes. Zonulin has been described as the only physiological modulator in tight junctions within cells[12]. Elevated zonulin levels increase the intestinal permeability of antigens, stimulates mucosal and systemic inflammation, and triggers the development

of autoimmune diseases. Many scientific investigations have been conducted to evaluate the serum levels of zonulin in some dermatological diseases in which autoimmunity is involved in the etiopathogenesis, such as vitiligo, alopecia areata, psoriasis vulgaris, dermatitis herpetiformis, and rosacea[6-10]. Therefore, the primary objective of the present study was to assess the levels of serum zonulin in patients diagnosed with CSU. Additionally, we sought to investigate the potential effect of intestinal permeability on the development and severity of CSU, as well as to analyze its correlation with zonulin levels in this condition.

MATERIALS AND METHODS

This study prospectively included 60 patients who consecutively sought medical care at the dermatology outpatient clinic of Istanbul Medipol University between 2021 and 2023 with complaints of recurrent urticaria and/or angioedema persisting for a minimum duration of six weeks. CSU was diagnosed in accordance with the internationally accepted guidelines for the management of urticaria[1]. Patients with chronic inducible urticarial, systemic and/or autoimmune diseases, pregnant and lactating women, patients aged under 18, those with a history of drinking alcohol regularly and frequently, those using systemic immunosuppressives or immunomodulators, and those with a history of antibiotic, probiotic, or prebiotic use within the last three months were excluded from the study. The systemic treatments regularly used by the patients for urticaria were discontinued one week before serum samples were collected. Patients with moderate or severe urticaria and those who had received antihistamine or systemic steroid treatment for three or fewer days within the past week were included in the study. The control group was formed with 64 healthy individuals who had no previous urticaria attack, no known systemic and/or autoimmune disease, and no drug use and who had similar age and sex characteristics to the CSU group. The seven-day urticaria activity score (UAS7) was graded as well-controlled, mild, moderate, or severe, as previously described in the literature[13]. The research received ethical clearance approval from the related committee of Istanbul Medipol University, with decision number 719, and carried out in adherence to the ethical guidelines outlined in the Declaration of Helsinki. All patients and controls were informed about the study and provided consent for participation.

Age, sex, disease duration, treatments used, the presence of gastrointestinal system (GIS) symptoms (dyspepsia, diarrhea, constipation, and abdominal pain), additional allergic diseases (allergic rhinitis/conjunctivitis, asthma, or atopic dermatitis), thyroid function test results, and UAS7 were recorded.

In zonulin testing, blood samples of 10 ml were obtained from all patients and controls after a fasting period of 12 hours. The blood samples were then transferred into vacuum biochemistry tubes containing gel. After the samples were coagulated for 1 hour at room temperature, centrifugation at 1,000×g was applied for 20 minutes to separate the serum, which was then kept at −80°C until the time of analysis.

A human zonulin kit (Elabscience) based on the ELISA principle was used to measure zonulin levels. This kit is based on the sandwich-ELISA principle, with a range of detection spanning from 0.78 to 50 ng/ml and a sensitivity value of 0.47 ng/ml. Both intra- and inter-class coefficients of variation values <10%.

The data obtained from the study were analyzed by SPSS (IBM SPSS Statistics v. 26.0). In addition to descriptive statistics (frequency, mean, and standard deviation), Student's t-test was employed to compare parameters with a normal distribution, while the Mann–Whitney U test was undertaken to compare parameters without a normal distribution between two groups. The Pearson correlation test was conducted to examine the associations among parameters that exhibited a normal distribution, and Spearman's rho correlation analysis was utilized for the analysis of relationships among parameters that did not adhere to a normal distribution. The determination of the optimal cut-off values was undertaken using receiver operating characteristic (ROC) curves. The obtained findings were deemed statistically significant when the p value was <0.05.

RESULTS

The study included 60 patients with CSU (12 men, 48 women) and 64 healthy controls (18 men, 46 women). The mean ages of the patients and controls were 38.73±12.26 and 36.44±6.46 years, respectively. Age and sex did not significantly differ between the two groups. In the patient group, the mean duration of CSU was 38.13±36.51 months, and the mean UAS7 was 23.70±9.9. GIS symptoms were present in 43.3% of the patients in the CSU group.

Seventeen (28.3%) of the patients had other allergic diseases (allergic rhinitis, conjunctivitis, asthma, or atopic dermatitis) accompanying CSU. Upon evaluating the thyroid function test results of the patients, it was observed that 14 patients (23.3%) had hypothyroidism. There was no patient with hyperthyroidism. **Table 1** presents the demographic characteristics and clinical findings of the patients with CSU and controls. Although the mean zonulin value was higher in the CSU group compared to the controls, the difference was not statistically significant (24.65±8.49 ng/ml vs. 21.03±7.36 ng/ml, p=0.077) (**Table 2**). In the ROC curve analysis performed on zonulin levels to identify the CSU group, the ability of this value to differentiate between the two groups was statistically non-significant (p=0.094).

Table 1. Demographic and clinical data of the sample

Clinical features	CSU group	Control group
Age (yr)	38.73±12.26 (18–81/39)	36.44±6.46 (20–48/38)
Sex		
Male	12 (20.0)	18 (28.1)
Female	48 (80.0)	46 (71.9)
Disease duration	38.13±36.51	
GIS symptoms	26 (43.3)	
Other allergic disease	17 (28.3)	
Hypothyroidism	14 (23.3)	
Hyperthyroidism	0	
UAS7	23.70±9.9	
UAS7 ≤6	0	
UAS7 = 7–15	16	
UAS7 = 16–27	22	
UAS7 = 28–42	22	

Values are presented as mean ± standard deviation (min-max/median) or number (%).
CSU: chronic spontaneous urticaria, GIS: gastrointestinal system, UAS7: urticaria activity score average over seven days.

Table 2. Analysis of zonulin levels (mean ± SD)

	Control group	CSU group	Total	*p*-value*
Zonulin, ng/ml†	21.03±7.36	24.65±8.49	22.78±8.07	0.077

CSU: chronic spontaneous urticaria, SD: standard deviation.
*Statistical significance evaluated at p=0.05, †Student's t-test.

The UAS7 scores indicated that 16 patients with CSU had mild disease, 22 had moderate disease, and 22 had intense disease. There were statistically significant differences in serum zonulin levels according to the UAS7 (p=0.008), with the serum zonulin level being statistically significantly higher among the patients with severe disease compared to those with mild disease (p=0.002) (**Table 3**). Examination of the relationship between the zonulin level and age, disease duration, and the UAS7 in the CSU group revealed that the zonulin level had a positive correlation with the UAS7 (p=0.013) (**Table 4**). **Fig. 1** presents the correlation between the UAS7 and serum zonulin level. Upon sex-based analysis, zonulin levels did not statistically significantly differ between the female and male patients with CSU (p=0.304). No significant relationship was found between the serum zonulin level between the patients with and without accompanying allergic diseases (p=0.236). However, the serum zonulin value of the patient group with a diagnosis of hypothyroidism accompanied by CSU was found to be significantly higher than the group without hypothyroidism (p=0.017) (**Table 5**).

Lastly, concerning the presence of GIS symptoms in the CSU group, no significant difference was observed in zonulin values between the symptomatic and non-symptomatic patients (p=0.618).

Table 3. Correlation analysis between zonulin and age, disease duration, and the UAS7 among patients with chronic spontaneous urticaria

Variables	r	*p*-value
Disease duration*	0.14	0.475
Age†	0.16	0.385
UAS7†	0.45	0.013‡

*Pearson's correlation analysis; †Spearman's correlation analysis; ‡Statistical significance evaluated at p=0.05.
UAS7: urticaria activity score average over seven days.

Table 4. Serum zonulin levels of the patient groups according to the UAS7

UAS7 groups	Zonulin (ng/ml)		*p*-value
	Mean ± SD	Min–Max	
Well-controlled	None		
Mild	20.2±5.1	10.5–27.9	0.008*
Moderate	24.5±8.7	12.3–38.2	
Severe	28.6±8.3†	13.9–40.7	

UAS7: urticaria activity score average over seven days, SD: standard deviation.
*One-way analysis of variance; †Significant difference compared to the mild group (p=0.002).

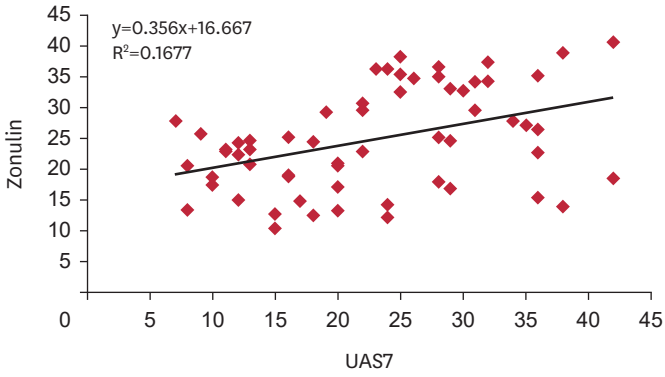

Fig. 1. Scatter plot of the correlation between the UAS7 and serum zonulin level of the patients diagnosed with chronic spontaneous urticaria.
UAS7: urticaria activity score average over seven days.

Table 5. Evaluation of serum zonulin levels according to TFT results and the presence of other accompanying allergic diseases

Co-diseases	Zonulin (ng/ml)		*p*-value*
	Mean ± SD	Min–Max	
TFT result			0.017
Normal	23.4±8.0	10.5–39.0	
Hypothyroidism	29.3±7.7	13.4–40.7	
Hyperthyroidism	None		
Allergic disease			0.236
Present	24.0±8.1	12.3–38.2	
Absent	26.8±8.4	10.5–40.7	

TFT: thyroid function tests.
*Student t-test.

DISCUSSION

Studies conducted in recent years have reported that increased intestinal permeability may be associated with many systemic, autoimmune, metabolic, neurological, psychiatric, and dermatological conditions. The diseases that have been subjected to investigation regarding the association between zonulin levels and intestinal permeability include type 1 diabetes mellitus,

rheumatoid arthritis, vitiligo, celiac disease, inflammatory bowel diseases, systemic lupus erythematosus, Alzheimer's disease, multiple sclerosis, schizophrenia, psoriasis, rosacea, dermatitis herpetiformis, atopic dermatitis, and alopecia areata[2,11]. The zonulin protein regulates tight junction permeability between intestinal absorptive cells. As a precursor of haptoglobin 2, it is responsible for the transactivation of epidermal growth factor receptor through PAR2. When both of these receptors are activated, intestinal permeability increases[12,14]. Prolonged zonulin upregulation of environmental triggers, including bacteria in the intestinal lumen and certain foods, such as gluten, results in elevated intestinal permeability and ongoing translocation of antigens from the intestinal lumen into the submucosa and circulation. This increased permeability causes stimulation of the immune system and leads to chronic systemic inflammation[15,16].

CSU is a prevalent medical condition disease that is marked by the presence of erythematous and pruritic wheals, angioedema, or a combination of the two and has a duration exceeding six weeks. Although the exact pathogenesis of CSU remains unknown, prior scientific works have shown a correlation between CSU and the Th2 cell immune response. It has been claimed that an autoimmune etiology may be seen at a rate reaching 40%, and this may be accompanied by subclinical infections and psychological variables. Several autoimmune conditions, including rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, and autoimmune thyroid disease, have also been linked to CSU[17,18]. Zonulin, a biomarker indicative of intestinal permeability, has been found related to a range of disorders, including autoimmune conditions[15]. In the literature, serum zonulin levels have been compared between control and patient groups in certain dermatological diseases for which autoimmunity is involved in the pathogenesis, and different results have been reported. Arslan et al.[10], who performed a comparison of the serum zonulin level between patients with vitiligo (n=41) and controls, reported a significantly higher level for this protein level in the former. In another study comparing 50 patients with psoriasis vulgaris to 32 healthy individuals, serum zonulin values were reported to be elevated higher in the psoriasis group compared to controls, and the difference was statistically significant[19]. In contrast, Hacınecipoğlu et al.[8] found that the mean serum zonulin level of 70 patients diagnosed with alopecia areata was lower than that of controls and that serum zonulin was not correlated with disease severity. In addition to autoimmunity, it is known that increased intestinal permeability causes chronic systemic inflammation. There exists a considerable body of work that examines the relationship between CSU and GIS. Many studies have shown a significant correlation between *Helicobacter pylori* infections and the formation of CSU[20]. Recently, research has also been undertaken to explore the association between gut microbiota and CSU, revealing notable differences in the gut microbiota of those diagnosed with CSU. In one of these studies, the authors noted significant decreases in *Subdoligranulum* and *Ruminococcus bromii* among individuals with a diagnosis of CSU[21].

Based on the coexistence of rosacea and GIS diseases, Yüksel and Ülfer[7] compared the serum zonulin values of patients diagnosed with rosacea to a control group and found a noteworthy elevation in the former. In the current study, we compared zonulin levels between 60 patients with CSU and 64 healthy controls. Although we found the mean serum zonulin value to be higher in the CSU group than in the control group, there was no difference of statistically significant nature in the comparison of the two groups (24.65±8.49 ng/ml vs. 21.03±7.36 ng/ml, p=0.077). This can be attributed to the small size of the CSU group in our sample. We also did not observe a significant relationship between the zonulin level and age, disease duration, or the presence of GIS symptoms among individuals diagnosed with CSU. However, there was a significant positive correlation between zonulin levels and the UAS7 in this group (p=0.013). Similarly, we found that, according to the UAS7, the serum zonulin level of the severe urticaria group was significantly higher than that of the mild urticaria group (p=0.002). Although this finding indicates a potential correlation between heightened intestinal permeability and the exacerbation of urticaria, it should be supported by further studies.

An important result of this study is that the serum zonulin level was found to be significantly higher in the group with thyroid disease accompanying CSU (p=0.017). It is known that thyroid disease is two to three times more common among patients with CSU than in the general population. It has also been emphasized that there is a relationship between autoimmune urticaria and autoimmune thyroiditis. Demir et al.[22], who examined the relationship between Hashimoto's thyroiditis and the serum zonulin level, reported that the zonulin level was significantly higher among the 77 patients with Hashimoto's thyroiditis compared to the 66 controls. These findings indicate the need to investigate changes in intestinal microbiota in autoimmune urticaria, as well as the zonulin level as a marker for this condition in larger samples.

We found only one study in the literature examining the relationship between zonulin and other allergic diseases, such as asthma, allergic rhinitis/conjunctivitis, and atopic dermatitis. In that study, the relationship between atopic dermatitis and serum zonulin was evaluated, and it was found that the serum zonulin level was associated with the presence and severity of atopic dermatitis. The authors suggested that increased intestinal permeability and elevated serum zonulin protein might be involved in the pathogenesis of atopic dermatitis by affecting tight junctions in the skin barrier[11]. In the current study, we did not detect a significant difference in the serum zonulin level between the patients with and without other allergic diseases.

One of the primary constraints of the present investigation concerns the limited sample size of patients with CSU.

In conclusion, this work has significance since it represents examination of the correlation between intestinal permeability and the serum zonulin level in CSU. The etiopathogenesis of CSU remains unknown. New data on intestinal barrier permeability in patients can open up new avenues for elucidating the pathogenesis and establishing treatment options. To this end, there is a need for comprehensive studies that will evaluate the permeability of the intestinal barrier and the presence of markers, e.g., zonulin, in patients with CSU.

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CONFLICTS OF INTEREST

The authors have nothing to disclose.

DATA SHARING STATEMENT

The data supporting the results of this study are available from the corresponding author upon reasonable request.

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