Dermatology Practical & Conceptual

Evaluation of Serum Zonulin Level and Intestinal Permeability in Patients with Chronic Spontaneous Urticaria and the Relationship Between Serum Zonulin Level and Disease Severity

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ABSTRACT

Introduction: An emerging hypothesis suggests a potential link between enhanced intestinal permeability and the advancement of chronic spontaneous urticaria (CSU).

Objective: This study aimed to investigate the role of intestinal permeability in the etiopathogenesis of CSU by measuring serum zonulin levels, a marker of intestinal permeability, in both CSU patients and control subjects. Additionally, the study sought to explore the correlation between the severity of the illness and zonulin levels.

Methods: The study involved 61 patients with CSU and 59 healthy control individuals. For the CSU patients, comprehensive data were collected encompassing various aspects: age at onset of the condition, duration of the most recent attack, presence of any comorbid conditions, dosage of antihistamines being used, and urticaria activity score as well as detailed personal and family medical histories. Additionally, demographic information for these patients was also meticulously documented.

Result: The study revealed a statistically significant difference in zonulin levels between the CSU patient group and the control group, with a p-value of 0.000, indicating a highly significant disparity. Furthermore, among the CSU patients, those who presented with angioedema exhibited considerably higher zonulin levels compared to those without angioedema. This variation in zonulin levels based on the presence of angioedema was also statistically significant, with a p-value of 0.023.

Conclusion: The observed results suggest that increased intestinal permeability, as indicated by elevated zonulin levels, may play a crucial role in the pathophysiology of both CSU and angioedema. This association highlights the potential significance of intestinal permeability in the development and manifestation of these conditions.

Introduction

Urticaria is an inflammatory disease affecting the superficial dermis, marked by itchy red papules and plaques that last less than 24 hours. It can also be accompanied by angioedema. If the condition persists for less than six weeks, it is classified as acute urticaria; if it lasts six weeks or longer, it is considered chronic urticaria. Chronic urticaria is further divided into two main categories: chronic spontaneous urticaria and chronic inducible urticaria [1]. Although 80% of individuals with chronic urticaria experience remission within a year, studies show that 11% still have the condition after five years [2]. The exact cause of chronic urticaria is unknown, but autoimmunity is believed to play a role in about 45% of cases [3].

There are two primary theories regarding the pathogenesis of chronic urticaria. The first theory suggests that mast cells and basophils have functional or trafficking defects due to dysregulated intracellular signaling pathways. The second theory posits the development of autoantibodies against FcεRIα or IgE in these cells [4]. For individuals with a genetic predisposition, recent research indicates that compromised gut barrier function may cause and worsen autoimmune disorders [5]. The intestinal barrier's function is regulated by immunological, biochemical, and physical factors. This barrier's physical structure includes enterocytes, tight junctions (TJ), adherent junctions, desmosomes, mucus, and commensal bacteria [6,7]. When the intestinal barrier is disturbed, a condition often referred to as a leaky gut occurs. Increased intestinal permeability allows commensal microorganisms, bacterial compounds, and foreign antigens to enter the body uncontrollably. The submucosal innate immune system is activated in this scenario, and antigen traffic cannot be regulated. If this cycle continues, the acquired immune system is also activated, leading to the release of cytokines such as IFN-γ and TNF-α and further antigen passage. This results in a vicious cycle of uncontrolled antigen traffic [5].

In our study, we measured zonulin levels in serum to evaluate intestinal permeability. Tight junctions between enterocytes provide intestinal barrier control, and zonulin is a protein that regulates antigen traffic by controlling these TJ [8]. Zonula occludens toxin (Zot) was discovered during vaccine development studies against *Vibrio cholerae* bacteria [8]. This discovery illuminated the mechanisms involved in regulating paracellular transit in the gut. Zot is an endogenous enterotoxin that rapidly and reversibly regulates TJ. It was believed to mimic a protein that regulates TJ, which was subsequently named zonulin. Proteomics analysis of human serum revealed that zonulin is pre-HP2, the precursor of haptoglobin (HP) [10]. Pre-HP2, identified as zonulin, has been shown to increase permeability in the jejunum and ileum in ex vivo studies. Zonulin also prevents microorganism colonization of the small intestine by increasing permeability, playing a role in natural immunity defense mechanisms [11]. Elevated zonulin levels have been observed in autoimmune diseases such as type 1 diabetes mellitus, multiple sclerosis, inflammatory bowel diseases, and celiac disease, in metabolic diseases like insulin resistance, type 2 diabetes mellitus, polycystic ovary syndrome, and in systemic infectious diseases such as sepsis, HIV, and intestinal diseases like irritable bowel syndrome and non-celiac gluten sensitivity [8].

Objective

Our objective was to evaluate the contribution of intestinal permeability to the etiopathogenesis of chronic spontaneous urticaria (CSU) and to investigate its correlation with disease severity. This study aimed to fill a gap in the existing literature and provide new insights into the underlying mechanisms of CSU.

Methods

Study Design and Participants

This study was conducted at the Dermatology Department of the Hospital from April 2021 to December 2021. It was approved by the non-interventional Clinical Research Ethics Board of the Hospital in accordance with the Declaration of Helsinki Principles [08.02.2021, GO 104/01]. Written informed consent was obtained from all participants before enrollment.

We recruited 122 participants, including 63 patients with CSU (30 males and 33 females aged 18–62 years, with an average age of 32.96) and 59 age- and sex-matched healthy controls without CSU or other skin diseases. Based on a power analysis using the G*Power 3.1.9.2 program, a total of at least 120 samples was determined to be sufficient, with an effect size of 80% power, a 5% margin of error, and

d=0.496. All participants were Turkish and residents of Ankara, Turkey. The diagnosis of CSU was based on clinical criteria. Patients who presented to the hospital and volunteered to participate in the study were included. Exclusion criteria for CSU patients included being younger than 18 or older than 65, using systemic antibiotic therapy, being obese, or having concomitant systemic inflammatory diseases, autoimmune diseases, current infections, or previous gastrointestinal tract diseases. The control group consisted of age- and sex-matched healthy subjects who met the same inclusion and exclusion criteria as the CSU patients.

Measures

Patients had their age, weight, sex, marital status, past medical history, and self-report documented. Questions were asked about the onset of their illnesses, the duration of their attacks, and whether angioedema coexisted with them. Based on their urticaria activity score, patients were categorized as having mild, moderate, or severe urticaria. The Weekly Itch Severity Score and the Weekly Hives Severity Score, score range of 0 to 21, are two validated patient-reported outcomes measured by the Urticaria Activity Score (UAS7). UAS7 total scores range from 0 to 42, with lower scores representing fewer symptoms. UAS7 score ≤6 is well controlled, 7-15 is mild, 16-27 is moderate, and 28-42 can be considered as severe urticaria.

Sample Collection, Biochemical and Molecular Analysis

All participants had venous blood samples taken, which were sent to the biochemistry lab within no more than two hours. Samples were centrifuged for 10 minutes at 3000 rpm. Serum samples were then separated into individual Eppendorf tubes and kept at -80 °C until the analysis day. Serum samples were extracted on the day of analysis according to the manufacturer's instructions and stored for 30 minutes at room temperature (18 °C–25 °C). The sandwich enzyme-linked immunosorbent assay (Sandwich-ELISA, Elabscience®, Catalog Number: E-EL-H5560, Wuhan, China) kit was used in the analysis to determine the amounts of human zonulin in serum samples. In our study, the sensitivity for detecting chitin was established at 0.47 ng/mL. This level of sensitivity ensures that even minimal concentrations of chitin can be accurately identified in our assays. Regarding the precision of the assay, according to the manufacturer's specifications, the intra-assay coefficient of variation (CV) ranged from 4.85% to 5.69%. The intra-assay CV is a measure of the variability in the assay results when the same sample is tested multiple times within the same assay. This level of variation indicates a reasonably consistent performance of the assay for repeated measurements. Furthermore, the inter-assay coefficient of variation (inter-assay CV) was documented to be between 4.35% and 5.19%. The reported range of the inter-assay CV reflects the consistency of the assay across different test runs and conditions. These coefficients of variation are crucial to evaluating the reliability and reproducibility of the assay's results, indicating that the assay demonstrates both precision and consistency in measuring chitin levels across multiple tests and conditions.

Statistical Analysis

The statistical analyses in this study were conducted using the Statistical Package for Social Sciences (SPSS) version 15.0, based in Chicago, Illinois. Frequencies and percentages are used to represent descriptive categorical data, while continuous data are expressed as mean ± standard deviation. For normally distributed data, parametric methods were employed. The independent samples *t*-test (t-table value) was used to compare the measurement values of two independent groups under parametric techniques. The normality of the continuous data distribution was assessed using the Kolmogorov-Smirnov test. Nonparametric methods were applied for data that did not fit a normal distribution, using the Mann-Whitney U test (Z-table value) to compare the measured values between two independent groups.

Pearson-χ^2 test statistics were used to examine the relationship between two qualitative variables. Binary logistic regression (LR): The backward LR model was used to determine the factors affecting disease risk status. P-values below 0.05 were considered statistically significant.

Results

Characteristics of Participants

In this study, we enrolled 63 patients diagnosed with CSU and 59 healthy controls, matched for age and sex. All participants were of Turkish descent and from the same geographic region. The median age of the patients was 32 years, with an age range of 18 to 62 years. Females constituted 52.4% of the patient group. The median body mass index (BMI) was recorded at 25 kg/m², ranging from 17.9 to 29.8 kg/m². Comparative analysis revealed no significant differences in age, sex, or BMI between the CSU patients and the control group. The demographic and clinical characteristics of the study groups are detailed in Table 1.

A key focus of our study was on serum zonulin levels, a marker of intestinal permeability. The median zonulin level in the CSU patient group was 49.3 ng/mL (range: 2.2-58.3 ng/mL), which was notably higher than in the control group, which had a median of 41.9 ng/mL (range: 2.2-56.3 ng/mL). This difference was statistically significant (*P*=0.000), suggesting a potential link between increased intestinal permeability and CSU. Our study also categorized CSU patients

Table 1. Demographics and Clinical Characteristics of Participants.

Characteristic	CSU patients (N=63)	Healthy controls (N=59)	P-Value	w
Male / Female (N)	30 / 33	29/ 30	0.865	
Age (range)	34.95 ± 11.68 (18-62)	34.93 ± 9.07 (18-53)	0.743	
BMI (body mass index)	24.79 ± 3.03	24.28 ± 3.19	0.360	
Cigarette pack year (+/-)	19/49	17/41	0.919	
History of alcohol use (+/-)	5/58	3/56	0.525	
Disease duration (month)	47.95± 89.34 (2-396)	-		
Last attack (week)	8.91±12.67(0-72)			
Plaque residence duration	4.46±4.02(0-24)			
Serum zonulin level (range)	45.99±10.71(2.2-58.3)	37.63±15.09(2.2-56.3)	*0.000	

Abbreviation: CSU: chronic spontaneous urticaria.

Table 2. Relationship Between Zonulin Levels, Sex, Disease Severity, Presence of Angioedema*.

Characteristic			Serum Zonulin Level	P-Value**
Sex	Female(n)	33	51.1 (30.3-58.3)	*0.006
	Male (n)	30	44.2 (2.2-56.6)	
Angioedema	(+)	22	51.6 (30.8-58.3)	*0.023
	(-)	41	47.5 (2.2-56.6)	
UAS7	Well controlled	11	43.78 ±14.94	0.187
	Mild urticaria	22	43.64±12.12	
	Severe activity urticaria	30	48.62±6.81	

*Mann-Whitney U test used for the comparison between study groups.
** Significant p-values are marked in bold; $P<0.05$.

as mild, moderate, or severe based on the UAS. However, no statistically significant correlation was found between serum zonulin levels and the severity of CSU in this stratification. Gender-based analysis within the CSU group revealed that serum zonulin levels were significantly higher in female patients compared to their male counterparts. Additionally, patients with CSU who also had angioedema exhibited significantly higher zonulin levels compared to those without angioedema ($P=0.023$). Table 2 provides a comprehensive breakdown of the correlations between zonulin levels, sex, the severity of CSU, and the presence of angioedema, offering further insights into the complexities of this condition.

Discussion

In this study, we examined serum zonulin levels to determine whether intestinal permeability contributes to the pathophysiology of CSU. We found that CSU patients had significantly higher serum zonulin levels than the control group. Factors such as pseudoallergens, dysbiosis, metabolic syndrome, autoimmune reactions, stress, and certain medications are considered predisposing factors in the etiology of CSU. Autoimmune immunity is a significant predisposing factor given the co-occurrence of autoimmune illnesses, the presence of many autoantibodies, the higher frequency in females, and the response to the IgE monoclonal antibody omalizumab. It is believed that the intestine plays a role in the development of autoimmune illnesses and greatly influences the onset of these processes. It has been shown that disruption of the integrity of the connections between enterocytes in the small intestine causes an increase in intestinal permeability, and zonulin is a protein that physiologically modulates tight junctions (TJ) between enterocytes [8,10,12]. Gliadin and bacteria are primary factors that cause zonulin to be released [8]. Enterotoxin generation from numerous enteric infections that impact the tight junction in the host's gut also contributes to zonulin production. In both culture models and ex vivo experiments, gliadin administered to the apical surface of cells promotes zonulin release through CXCR3 receptors [8].

Recent years have seen an association between zonulin increase and autoimmune diseases such as celiac disease, type 1 diabetes mellitus, inflammatory bowel diseases, and multiple sclerosis [8]. It is thought that the increase in intestinal permeability may occur alongside or before the clinical

presentation of these diseases and may be related to disease severity. Drago et al. conducted an ex vivo study examining duodenal biopsies from controls and celiac patients, finding that gliadin exposure led to a brief increase in intestinal permeability in both groups, with the rise being more pronounced and resistant in celiac patients due to zonulin release. This has prompted therapeutic investigations, such as the study of larazotide acetate, which blocks the zonulin receptor and is in phase III trials for treating celiac disease [10,14]. Another study showed that gluten-loaded celiac patients treated with larazotide acetate experienced a decrease in intestinal permeability, symptoms like diarrhea and bloating, and levels of anti-tissue transglutaminase antibodies and IFN-γ [15]. Animal experiments have demonstrated that intestinal permeability increases in first-degree relatives of irritable bowel disease (IBD) patients even without IBD and before its onset [16,17]. Our research suggests that patients with CSU have higher zonulin levels than the control group, indicating a significant role for increased intestinal permeability in the pathophysiology of CSU. However, serum zonulin levels did not correlate with disease severity, possibly due to insufficient patient numbers in each group according to UAS7.

Our study found significantly higher zonulin levels in female patients compared to males, a connection not previously researched. This suggests that the higher incidence of autoimmune disorders in females might be linked to increased intestinal permeability [18]. Nonetheless, male CSU patients also showed higher zonulin levels than the control group, supporting zonulin's involvement in CSU pathophysiology.

In our investigation, CSU patients with angioedema had considerably higher serum zonulin levels than those without, suggesting increased intestinal permeability. This could imply that zonulin may also increase vascular permeability, given that angioedema is associated with increased blood vessel permeability. Although there is no literature on zonulin's effect on blood vessels, one study showed it regulates lymphatic endothelial cell layer structure and permeability [19]. The literature has demonstrated that zonulin levels may rise before clinical manifestation [17].

In light of our findings, the hypothesis that a patient's elevated zonulin level could signal the onset of angioedema becomes more compelling. On the other hand, the findings of this study have major therapeutic implications for the management and therapy of CSU. The correlation that has been discovered between the severity of CSU and elevated serum zonulin levels, especially in patients with angioedema, implies that intestinal permeability may play a crucial role in the worsening of the condition. This realization makes room for cutting-edge treatment strategies that focus on gut integrity and health. When doing a thorough diagnostic workup on CSU patients, clinicians may want to assess intestinal permeability. Additionally, as an adjunct therapy in the management of CSUs, interventions that target intestinal permeability such as the use of probiotics, certain dietary modifications, or pharmaceutical drugs that are known to increase the function of the gut barrier might be investigated. This method recognizes the connection between immunological function and gut health and not only provides a fresh approach to treating CSU but also fits with a more comprehensive understanding of patient health. However, it is important to note that, while our study provides a promising direction, clinical application of these findings should proceed with caution until further research corroborates the efficacy and safety of such interventions in CSU patients. Further research should explore the mechanisms linking serum zonulin levels and CSU severity and examine the potential of adjusting intestinal permeability as a treatment strategy for CSU. Long-term studies could provide deeper insights into the timing of intestinal permeability changes and CSU symptom onset or exacerbation. Our research lays the groundwork for a fresh understanding of CSU pathophysiology and suggests that intestinal permeability is a key factor in the disease's course. This discovery opens new avenues for future studies and potential treatments, aimed at improving the lives of those suffering from this chronic and often debilitating condition.

Limitations

It is crucial to acknowledge the limitations of our study. The results of our study may not be broadly applicable as they may be due to the small sample size and demographic homogeneity (all Turkish participants came from the same area). To confirm and build on our findings, larger sample sizes and a more diverse population would be needed in future research.

Conclusion

This study enhances our understanding of CSU by demonstrating a significant relationship between elevated serum zonulin levels and CSU severity, especially in patients with angioedema. These results highlight the potential role of intestinal permeability in the origin and progression of CSU, offering new insights into its etiopathogenesis. Our findings suggest that changes in intestinal permeability may influence immune responses in CSU patients, pointing to new therapeutic approaches that target this pathway.

Patent Consent: The authors acquired consent for the publication of identifying patient data or other materials, and included it with the manuscript when they submitted it to the journal. The consent stated that all patients provided their approval knowing that the information could be made public.

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