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A study on serum zonulin in chronic spontaneous urticaria patients

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Abstract

Chronic spontaneous urticaria (CSU) is a widespread disease with a complicated heterogenous pathophysiology. Increased intestinal permeability i.e., leaky gut has been linked to the pathology of many diseases. Zonulin was recently used as a marker for leaky guts. This study aimed to assess the relation between serum zonulin level and CSU and its possible relationship with disease activity. This was a comparative cross-sectional study, which included 97 CSU adult patients and 87 apparently healthy controls. CSU patients had significant lower zonulin level than controls (p<0.001). The median of serum zonulin level was equal to 2.93 ng/ml with interquartile range (IQR) (1.40-4.19) in the CSU group and of 3.92 ng/ml with IQR (2.97-4.69) in the control group. We found a positive correlation between serum zonulin and C-reactive protein with Pearson correlation coefficient of 0.2, (p=0.04). No significant correlation was found between serum zonulin level and urticaria activity score 7 or total immunoglobulin E level. In conclusion, this study found that serum zonulin level is lower in CSU patients than in controls which could be attributed to food restriction, severity of the CSU disease and/or drug intake in the CSU cases.

Keywords: Chronic spontaneous urticaria, Zonulin, urticaria activity score 7, Intestinal barrier, leaky gut, C reactive protein

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Introduction

Urticaria is a disease characterized by itchy areas of erythema and wheals with or without angioedema (swelling of the subcutaneous tissue and mucous membranes). Chronic urticaria can be labelled when the disease duration exceeds six weeks. If there is no obvious triggering agent it is called chronic spontaneous urticaria (CSU).[1]

CSU is a chronic disease that can cause disability of the patient and affect his quality of life. It can be debilitating, difficult to treat, and disappointing for both patients and physicians.[2] The humanistic and economic burden of CSU has been underestimated. It significantly influences performance at school and at work and is associated with a high consumption of medical resources, high treatment costs and other direct and indirect costs to society.[3]

Although CSU is a common disease, its pathogenesis is not yet fully understood. It has been previously confirmed that the disease is derived by mast cells,[4] stimulated by Type I and II hypersensitivity reactions in the form of immunoglobulin E (IgE) against external or auto-allergens and IgG autoantibodies against IgE or its receptors, respectively.[4,5] The latter is called autoimmune urticaria.[6] Other factors that can have a role in mast cell activation, may include infections, psychiatric problems and stress, auto-immune diseases, hyperlipidemia, obesity, malignancies and coagulation.[7,8] Also, further factors were studied last decade as pseudo allergy [9], HLA-DRB1,[10] Vitamin D deficiency[11] and gut dysbiosis.[12]

The relationship between allergy and intestinal permeability is an interesting subject of research. An increase in the intestinal permeability i.e., leaky gut, may result in facilitating the entry of allergenic proteins from the intestinal lumen into the systemic circulation, leading to activation of the adaptive immune system, allergen sensitization and/or extra-intestinal inflammation.[13]

Serum zonulin is a regulatory protein for gut permeability secreted from intestinal cells that control tight junctions and intestinal barrier, hence controlling gut permeability.[14] It has been used as a marker for damaged intestinal barrier in many diseases such as celiac disease,[15] type-1-diabetes,[16] inflammatory bowel disease/colitis,[17] multiple sclerosis,[18] obesity/insulin resistance, type-2-diabetes,[19,20] asthma,[13] coronary artery disease,[21] septicemia,[22,23] human immunodeficiency virus,[24] irritable bowel syndrome [25], non-celiac gluten sensitivity,[26] necrotizing enterocolitis,[27] and autoimmune (rheumatoid arthritis) arthritis.[14, 28]

The current research provided an important opportunity to advance the understanding of CSU pathogenesis through studying the possible relationship between serum zonulin and CSU activity. To the best of our knowledge this is the first study conducted in Egypt.

Subjects and Methods

This comparative cross-sectional study included 97 CSU adult patients attending the Allergy and Clinical Immunology Clinic of Ain Shams University, and 87 apparently healthy controls. The CSU patients were diagnosed according to the European Academy of Allergy and Clinical Immunology (EAACI) guidelines in 2018. [29] Patients had a history of itchy urticarial wheals fleeting in nature, which usually do not persist more than 24 hours. Attacks might be associated with angioedema, with no definite eliciting physical factor and for a duration exceeding 6 weeks.

The following patients were excluded from the study, including chronic inducible urticaria (as cholinergic urticaria, aquagenic urticaria and pressure urticaria), other allergic diseases, autoimmune diseases, malignancy and patients with symptoms suggesting gastrointestinal diseases.

All enrolled individuals were subjected to full clinical examination and history taking laying stress on criteria to fulfil the diagnosis of CSU. Provoking factors included food, inhalants, stress and drugs, and relation of symptoms to diurnal variation, travelling and menstruation. The severity of wheals and pruritus were assessed and documented using the urticaria activity score UAS 7.[30]

Skin tests

The following skin tests were performed for all patients:

Skin prick test (SPT)

The SPT was carried out for common food and aeroallergens including house dust mites, mixed molds, candida, Aspergillus, Hay dust, cockroach, pollens, rabbit hair, wool, pigeon feather, cat hair, penicillium, milk, banana, fish, nuts, chicken, wheat, strawberry and eggs. SPT was performed minimally after 4–6 weeks of a systemic allergic reaction and stopping the antihistaminic for 5 days.

The test was done on the volar surface of the forearm, 2 – 3 cm from the wrist and the cubital fossae. A drop of each of the allergen test solutions, along with saline and 10 mg/ml histamine solution were placed on the skin in identical order in all patients and immediately pricked by a single-head metal lancet

penetrating the epithelial layer of the skin without causing bleeding. Each test result was checked after 15 to 20 minutes. Positive results were when the wheal of the test was similar in size or bigger than that of histamine. [31]

Autologous serum skin test (ASST)

A blood sample (2 ml) was collected from each patient into sterile glass tubes without additives, allowed to clot at room temperature for 30 min then centrifuged at 450–500 g for 10 min, and serum collected. The patient's volar aspect of the forearm skin was cleaned with antiseptic and intradermally injected with 0.05 ml of normal saline as a negative control, 0.05 ml of 0.5–1 µg histamine as a positive control and 0.05 ml of undiluted serum (3–5 cm away from each other).

After 30 min, the test sites were observed and the mean of the maximum perpendicular diameters of any red wheal reactions to the ASST and the normal saline control skin test were calculated. ASST was considered positive if the difference between ASST mean wheal and normal saline mean wheal ≥ 1.5 mm.[32]

Laboratory investigations

The following laboratory tests were performed for all patients: Complete blood count (CBC) with differential was performed using automated hematology analyzer (Sysmex XN-1000, Sysmex corporation, Japan), according to the manufacturer's instructions. Erythrocyte sedimentation rate (ESR) by the Westergren method. Stool analysis for collected fresh stool samples, by macroscopic and microscopic examination of wet and iodine-stained films. *Helicobacter pylori* antigen in stool was performed using a rapid test (*H. pylori* Antigen Rapid Test Cassette, Hangzhou Clongene Biotech, China), according to the manufacturer's instructions. C-reactive protein (CRP) was measured using Chemo luminescence immunoassay and analyzer (Chemo luminescence immunoassay; Cobas e411, Roche diagnostics, Switzerland), according to the manufacturer's instructions.

In addition, total IgE and thyroid stimulating hormone (TSH) were measured in serum samples using chemiluminescence immunoassay analyzer (chemiluminescence analyzer, Cobas e411, Roche diagnostics, Switzerland), according to the manufacturer's instructions. Antithyroid peroxidase (anti-TPO) and antithyroglobulin (anti-TG) antibody were measured by commercially available ELISA kits (QUANTA Lite TPO ELISA, Inova Diagnostics, USA and QUANTA Lite Thyroid T ELISA kit, Inova Diagnostics, USA, respectively), according to the manufacturer's instructions.

Zonulin levels were measured in serum samples from all participants using a commercially available ELISA kits (Human Zonulin kit E3704, Bioassay Technology Laboratory, China), according to the manufacturer's instructions. The detection range of the kits was 0.3–90 ng/ml, with the lowest detection limit of 0.13 ng/ml. The final calculations were made after measuring optical density (OD) values using a microplate reader (Multiskan^{TM} FC microplate photometer, Thermo Fisher scientific, USA) set to 450 nm, and generating a standard curve against the kits standards OD values.

Statistical Analysis

Analysis of the data was done using the Statistical Package for the Social Sciences (SPSS) program version 27. Quantitative data are presented as the mean and standard deviation (SD) for parametric data, and median and interquartile range (IQR) for non-parametric data. Qualitative data are presented using count and percentage. Chi square test (or Fisher Exact test) was used to compare qualitative data between different groups. The Mann Whitney U test was used to compare non-parametric quantitative data between two independent groups. The Spearman's correlation test was used to measure linear correlation between different quantitative data. A p value ≤0.05 was considered statistically significant.

Results

Demographic and clinical data

This study comprised 97 CSU patients and 87 comparable age and sex, apparently healthy control individuals. There was female predominance in both cases and control groups

(79.4% and 75.3%, respectively). The mean age was 35.0 ± 11.6 years among the CSU cases compared to 37±12.5 years among the control group. Housewives constituted 51.5% of studied patients.

The median disease duration was 1.5 years (0.5-5 years), with progressive course in 78.4% of patients. The mean UAS7 score was 30.05 ± 13.37 where 69.1% of patients were classified as severe activity and only 9.3% well controlled.

Angioedema was a concern in 76.3% of our CSU patients. Common exacerbating factors as reported by patients were exercise, stress, food and drugs in 43.3%, 68%, 58.8% and 45.4% of patients, respectively. Positive family history of atopy was stated by 38.1 % of patients.

Laboratory characteristics

Regarding the main laboratory results in the patients' group, the *H. pylori* antigen in stool was detected in 35.1% of the study patients. Anti-TPO and anti-TG were high in 34% and 11.3% of the study patients, respectively. The CRP level was 7.82 ± 5.21 mg/dl, and ESR 28.34±13.51 mm/hr. The median total IgE was 88 IU/ml (ranged 43-191 IU/ml). The median eosinophils absolute value was 0.16 (0.06-0.3) $\times 10^3/\mu l$

After testing patients with common 24 food and aeroallergens by SPT, only 53.6% of patients had positive SPT. The most common positive results were for house dust mites, hay dust, cockroach, mixed molds, aspergillus, candida and pollens with a percentage of 30.9%, 15.5%, 13.0%, 12.4%, 11.3% and 11.3%, respectively. Also, ASST yielded positive results among 30.9% of the CSU patients.

Serum zonulin was significantly lower among the CSU cases than the control group [2.93 ng/ml (ranged 1.40-4.19 ng/ml) versus 3.92 ng/ml (2.97-4.69 ng/ml), respectively] (p=0.001, (Table 1). Upon correlating zonulin level with some parameters including age, disease duration, UAS 7 score, total IgE, eosinophils and CRP, only positive correlation was observed with CRP titer (r =0.2, p= 0.049), (Table 2). No significant relation was detected between zonulin level and other patients' parameters, as shown in Tables 3, 4 and Figure 1.

Table 1. Comparison of serum zonulin levels between chronic spontaneous urticaria cases and controls.

	Cases		Controls		*p* value
	Median	IQR	Median	IQR	
Serum Zonulin	2.93	1.40-4.19	3.92	2.97-4.69	0.001*

*Mann Whitney U test. $p \leq 0.05$ is significant.

Table 2. Correlation between serum zonulin level and other studied factors in the 97 study patients.

	Serum Zonulin	
	Spearman's correlation	*p* value
Age	-0.055	NS
Duration of CSU	-0.076	NS
UAS 7 score	-0.01	NS
Total IgE	0.041	NS
Basophils	-0.018	NS
Eosinophils	0.078	NS
CRP	0.200	0.049

UAS7: urticaria activity score 7, CRP: C reactive protein. $p > 0.05$ is not significant (NS).

Table 3. Relation between serum zonulin level and course of chronic spontaneous urticaria disease and the urticaria activity score 7 (UAS7).

		Serum zonulin		*p* value
		Median	IQR	
Course	Regressive	4.05	3.84-7.54	$NS^{\chi 2}$
	Stationary	2.13	0.90-2.97	
	Progressive	2.97	1.39-4.70	
UAS 7 score	Well controlled (1-6)	2.10	1.67-4.17	$NS^{\chi 2}$
	Mild activity (7-15)	2.29	0.83-3.64	
	Moderate activity (16-27)	3.48	0.77-8.00	
	Severe activity (28-42)	2.80	1.43-4.60	

χ2: Kruskal Wallis, test n= 97, IQR: inter quartile range. $p > 0.05$ is not significant (NS).

Table 4. Relation between serum zonulin level and other studied factors in the patients' group.

		Serum zonulin		*p* value
		Median	IQR	
Sex	Male	3.77	2.00-4.80	NS
	Female	3.36	1.86-4.52	
Severity	Mild	2.29	.83-3.64	NS
	Moderate to Severe	2.93	1.43-4.67	
Anti-thyroglobulin	Normal	2.87	1.37-4.17	NS
	Elevated	3.46	1.63-4.67	
ASST	Negative	2.77	1.30-4.17	NS
	Positive	3.36	1.90-5.15	
Angioedema	No	2.97	1.67-3.83	NS
	Yes	2.79	1.37-4.39	
H. pylori	No	2.93	1.43-4.67	NS
	Yes	2.19	1.37-3.64	
History of relation to food	No	3.17	1.52-4.84	NS
	Yes	2.33	1.37-3.85	
Skin test				
Mixed molds	Negative	2.80	1.30-4.19	NS
	Positive	3.00	2.17-5.08	
Pollens	Negative	2.85	1.37-3.93	NS
	Positive	2.97	1.57-14.24	
house dust mites	Negative	2.80	1.40-3.93	NS
	Positive	3.06	1.37-7.09	
hay dust	Negative	3.00	1.40-4.39	NS
	Positive	2.00	1.37-3.93	
Candida	Negative	2.79	1.40-3.85	NS
	Positive	4.92	0.83-13.65	
Cockroach	Negative	2.95	1.39-4.05	NS
	Positive	2.60	1.47-10.90	
Aspergillus	Negative	2.87	1.43-3.93	NS
	Positive	3.00	0.97-16.08	

IQR: interquartile range; ASST: autologous serum skin test. $p > 0.05$ is not significant (NS).

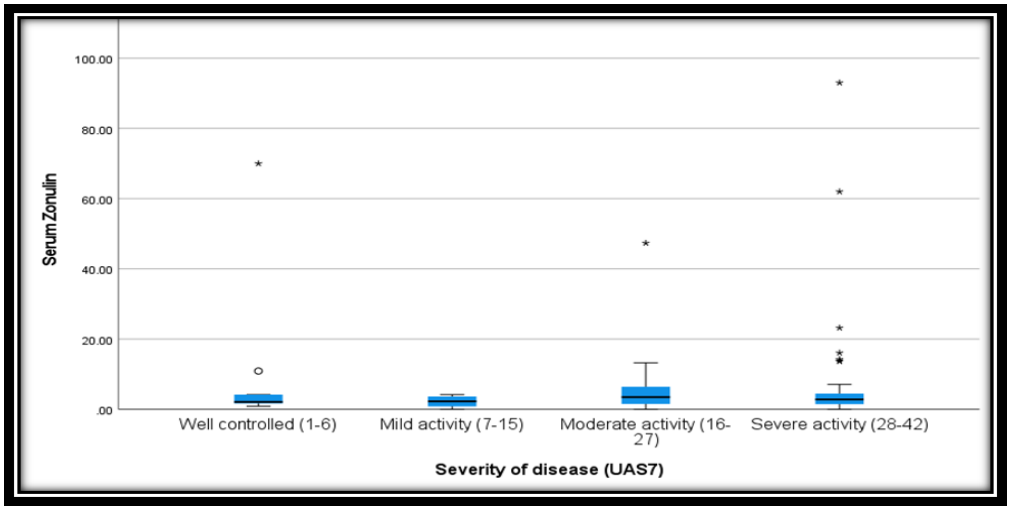

Figure 1. The relationship between zonulin level and Urticaria activity score 7 (UAS 7).

Discussion

Chronic urticaria is a very special disease due to its heterogenous etiology, increasing refractory cases and large effect on patients' quality of life. The current study aimed to assess the relation between serum zonulin level and CSU and its possible relationship with disease activity.

To our knowledge, serum zonulin a marker of leaky gut and increased intestinal permeability, was studied in many allergic and autoimmune diseases but not in urticaria.[33] These studies concluded that higher zonulin levels are present in patients with celiac disease,[15] type-1-diabetes,[16] inflammatory bowel disease/colitis[17], multiple sclerosis,[18] bronchial asthma,[13] atopic dermatitis,[34] systemic lupus erythematosus[35]and autoimmune (rheumatoid arthritis) arthritis[28,36] than in controls. These diseases are mostly autoimmune in nature and CSU is 20-50% autoimmune in nature and the other part is auto-allergic.[37] Hence, similar pathogenesis was suggested. This study, to the best of our knowledge, is the first to explore the relationship between serum zonulin and CSU.

The present research measured serum zonulin level in 97 CSU patients and 89 apparently healthy subjects comparable at age and sex. Contrary to expectations, this study demonstrated that CSU patients had lower zonulin levels in comparison to that of controls (p=0.001). These results could be attributed to more than one factor. Most of the included patients were receiving antihistamines and other drugs that might affect gut microbiota or zonulin affecting intestinal permeability. In 2016, a study was conducted to assess the gut microbiome variations in the average and healthy population. It also studied the effect of several drugs, including antibiotics, osmotic laxatives, inflammatory bowel disease medications, female hormones, benzodiazepines, antidepressants, and antihistamine. It was found that the intake of several of these substances was associated with variation in the microbiota composition.[38]

CSU patients were also used to follow a certain diet (avoiding allergenic foods). These regimens in turn can affect either gut microbiome or zonulin directly and thus affect the intestinal barrier. The effect of diet on serum zonulin was studied by Żak-Gołąb et al., 2013, which observed that plasma zonulin level was directly proportional to daily energy intake, and serum glucose concentration, but inversely proportional to the percentage of diet protein.[39] Another study done by *Del Bo'* et al., 2021, found that polyphenol rich diet can decrease zonulin levels enhancing the intestinal barrier.[40] In another study done for measuring serum zonulin during a weight loss program, serum zonulin level was found to be higher with the intake of non-nutritive sweeteners and markedly reduced with introduction of less sugar and fat.[41]

In the present study, another factor was that the most CSU patients (about 80%) had high UAS 7 denoting severe disease. There was no difference between patients with higher zonulin results when compared to those with mild disease activity.

Moreover, some studies suggested that serum zonulin level is not steady or stable and varies from time to time. In 2017, a study was done on celiac disease patients comparing serum zonulin levels to serum zonulin antibodies, and recorded measures at 0, 6, 14, 24 and 30 hours. The study found that zonulin level was higher in only 37% of celiac disease patients, while antibodies against zonulin were detected in up to 86% of patients with celiac disease. This could be attributed to the short half-life of serum zonulin in comparison to its antibodies. It was suggested that measurement of IgG and IgA antibodies against zonulin could be more efficient in evaluation of the loss of intestinal barrier integrity than a single measurement of zonulin level due to its fluctuation.[42]

We found no significant relation nor correlation between serum zonulin level and the duration or severity of the CSU disease, which could be attributed to that about 80% of our patients had moderate to severe disease according to UAS7, which makes comparing the results with mild cases partially inaccurate**.**

Moreover, no significant correlations were elicited between serum zonulin level and the course of the CSU disease or the presence of *H pylori* antigen in stool. This is in line with the finding of a study done in 2009 of zonulin level and *H pylori* which concluded that there were no difference in zonulin levels in *H. pylori*-negative and *H. pylori*-infected subjects by histopathology.[43]

Also, there was no relationship between serum zonulin level and common positive skin test results. This is in line with data reported by the study of Aktas et al., 2023, conducted to evaluate intestinal permeability in food allergy. They concluded that serum zonulin level was not associated with a number of food allergies nor with positive skin prick test for these foods.[44]

In the present study, there was no relationship between serum zonulin level and anti-TG, anti-TPO level or ASST results which may exclude any link between zonulin and autoimmune urticaria.

In our study, no significant correlation was found between serum zonulin level and the duration of the CSU disease, total IgE levels, and eosinophil or basophil levels. These are in line with findings of the study done to evaluate the relationship between asthma and serum zonulin.[13] In that study there was no statistically significant correlation between serum zonulin level and serum total IgE.[13] Also in 2018, a study was published aiming to assess the relation between atopic dermatitis and serum zonulin level in children. It concluded that serum zonulin was associated with the presence and severity of atopic dermatitis in children, and not related to total IgE level nor eosinophils.[34]

In our study, zonulin levels were not different between male and female CSU patients. In 2006, a study aimed to verify the association between serum zonulin levels and intestinal permeability in patients with type1 diabetes found that serum zonulin levels was not affected with sex of studied subjects.[45] In another study, exploring sex differences, and influences on zonulin and affective symptoms, serum zonulin was higher in females than in men independent from affective status.[46] Another study exploring factors associated with serum zonulin levels in patients with inflammatory bowel disease concluded that zonulin concentrations were higher among females.[47] These results could be attributed to that our patients were mostly females (79.4%), so the comparison between males and females could be inaccurate.

In our study, no significant correlation was found between serum zonulin level and age of patients. This finding is not in line with a study done to link leaky gut to the age-related inflammation, in which serum zonulin was higher in older subjects compared to younger adults.[48] However, that study included only 37 adult participants. Further studies with a larger study group are required.

In conclusion, our study found that serum zonulin level was lower in CSU patients than in controls. This could be due to multifactor e.g.

factors related to cases such as food restriction, severity of the disease and drug intake or short half-life of serum zonulin and its fluctuation. Serum zonulin level was not related to disease activity nor to the type of urticaria (autoimmune or not).

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Author Contributions

OMA; made substantial contributions to the design of the work. STK; made substantial contributions to the conception of the idea and substantial revision of the work. MAM; made substantial contributions to acquisition and analysis of data. MAE; to revising the work and interpretation of data. RHA; made substantial contributions to acquisition and analysis of data. MAZ; made substantial contributions to drafting and revising the work. The manuscript has been read and approved by all the authors, and the requirements for authorship as mentioned earlier in this document have been met, Each author declares that the manuscript represents honest work.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (FMASU MD 248/2021). Assurance No. FWA 00017585.

Informed consent

An informed written consent was obtained from each participant before being included in the study.

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