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Original Article

Impact of zonulin on the depressive and anxious states in patients with ulcerative colitis

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Abstract

Aim: The objective of this study is to investigate serum zonulin levels in patients diagnosed with ulcerative colitis and to examine the association between serum zonulin concentrations and the Beck Depression and Anxiety Inventory scores. Symptoms of depression and anxiety in patients with inflammatory bowel disease (IBD) are believed to be associated with increased intestinal permeability. Zonulin, a protein that modulates tight junctions between cells of the intestinal wall, has been studied as a marker of intestinal barrier integrity.
Materials and Methods: This study was designed as a prospective investigation, conducted among patients with ulcerative colitis (UC) who were in clinical remission, as determined by both clinical assessments and laboratory tests, between November and December 2017. Demographic and clinical data were collected. All consecutive patients were evaluated using the Beck Depression and Anxiety Inventories. Serum zonulin levels were measured in each patient.
Results: The mean age of the 50 patients was 47.3±12.2 years. The mean disease duration of these patients was 7.2±5.9 years. Mild depression was observed in 10 patients (20%), while moderate depression was present in 3 patients (6%). Similarly, mild anxiety affected 8 patients (16%), and moderate anxiety was noted in 6 patients (12%). Serum zonulin levels were significantly higher in obese patients compared to those with normal or overweight body mass indices ($p<0.001$). Serum zonulin levels showed a moderate positive correlation with body mass index ($r=0.586$, $p<0.001$), Beck Depression Inventory scores ($r=0.508$, $p<0.001$), and Anxiety Inventory scores ($r=0.437$, $p=0.002$).
Conclusion: Obesity was significantly associated with elevated serum zonulin levels. In addition, depression and anxiety scores were moderately correlated with serum zonulin levels in patients with ulcerative colitis.

Keywords: Inflammatory bowel disease, zonulin, ulcerative colitis, depression, anxiety

INTRODUCTION

Ulcerative colitis (UC) is an immune-mediated inflammatory condition (1-3). Its exact pathogenesis still not fully understood. However, numerous studies have explored the potential contributions of immunological, microbial, environmental, and genetic factors in the progression of UC (1,4,5).

The development of an abnormal immune response against the gastrointestinal tract microbiota has been considered one of the primary hypotheses in the development of inflammatory bowel diseases in genetically susceptible populations (2,5). Increased intestinal epithelial permeability may facilitate the translocation of microbial antigens and other luminal substances into the circulatory system (1,6,7). This dysfunction in the intestinal epithelial barrier can lead to abnormal interactions between luminal antigens and the mucosal immune system, further driving the pathophysiology of UC (3).

Depression and anxiety are commonly associated with chronic, long-standing diseases (8). As a chronic inflammatory condition, ulcerative colitis (UC) often leads to severe symptoms, contributing to psychological issues in affected patients (9-12).

The gut-brain axis is a term used to describe the relationship between alterations in the gut microbiome, intestinal permeability,

and their impact on mental health disorders such as depression and anxiety (8,12). Improvements in this axis may lead to enhanced cognitive function and alleviation of mental health symptoms.

Several biomarkers, including serum zonulin, intestinal fatty acid-binding protein, lipopolysaccharide-binding protein, fecal albumin, and calprotectin, have been studied in the context of assessing intestinal barrier integrity (5,6). Zonulin, a 47 kDa protein, is known to increase epithelial permeability in the small intestine by regulating intercellular junctions (1,6,13). Previous studies have demonstrated that zonulin increases intestinal permeability by reversibly opening intercellular junctions (14-16), which in turn elevates circulating inflammatory markers (5).The potential therapeutic use of zonulin inhibitors has been experimentally investigated in the context of various acute and chronic inflammatory diseases (17). All these data suggest that zonulin levels play a role in the diagnosis, clinical activity and treatment of UC (1,17).

This study investigates whether serum zonulin levels influence symptoms of anxiety and depression in UC patients.

MATERIALS AND METHODS

Study Design

This prospective, single-center, observational study included patients with ulcerative colitis (UC) aged 18 to 70 years, who were followed up at the gastroenterology outpatient clinic of the Department of Internal Medicine, Gazi University Faculty of Medicine, Ankara, between November and December 2017. All patients were confirmed to be in clinical remission based on the Truelove and Witts disease activity index. The study protocol was approved by the Gazi University Faculty of Medicine Clinical Research Ethics Committee (Approval Date: 23/10/2017, Approval No: 492). The study was conducted in accordance with the principles outlined in the Declaration of Helsinki, and written informed consent was obtained from all participants.

Ulcerative colitis (UC) diagnosis is based on laboratory, radiological, clinical, endoscopic, and histological findings. The clinical severity of UC is evaluated using the Truelove and Witts disease activity index, categorizing the condition as mild, moderate, or severe (18,19). Factors assessed in determining the severity of UC include the number of bloody stools per day, the frequency of positive fecal occult blood tests, heart rate, body temperature, hemoglobin levels, and erythrocyte sedimentation rate (ESR).

Indicators of remission include the following:

- Bloodless stools once or twice a day,
- Absence of fever and tachycardia,
- Normal hemoglobin levels and ESR values, or a return to normal levels,
- Weight gain (18).

The study's exclusion criteria included lack of consent, irregular follow-up visits, acute diseases, concurrent infections, malignancies, chronic psychiatric or neurological disorders, substance abuse, and the use of psychotropic medications within the past year.

Data Collection

Demographic characteristics, including gender, body mass index (BMI), and age, as well as clinical characteristics such as smoking status, disease duration, and medications used, were recorded. Patients were classified based on their BMI as follows:

- Underweight (BMI<18.5 kg/m²),
- Normal weight (BMI 18.5–24.99 kg/m²),
- Overweight (BMI 25.0–29.99 kg/m²),
- Obese (BMI≥30 kg/m²) (20).

All patients underwent a comprehensive physical examination. Erythrocyte sedimentation rate (ESR), hemoglobin levels, and C-reactive protein (CRP) levels were measured.

The Beck Depression-Anxiety Inventory was administered to all patients to assess their levels of depression and anxiety. The inventory consists of 21 items (21-23), each scored from zero to three, with a maximum possible score of 63. Based on the total score, patients' depression and anxiety levels were categorized as normal/minimal, mild, moderate, or severe. The Turkish validation studies for the Beck Depression-Anxiety Inventory have been previously conducted (24,25). Blood samples were collected from each participant after overnight fasting upon admission to measure serum zonulin levels. All serum samples were stored at -80°C until analysis. Serum zonulin levels were measured using the Zonulin enzyme-linked immunoassay (ELISA) Human Kit (Algen Diagnostik Medikal Ltd. Şti., Ankara, Türkiye). Following dilution of the samples according to the manufacturer's instructions, the serum zonulin levels were determined using the double-antibody sandwich technique (3). A four-parameter algorithm was employed for the standard curve, and optical density was measured at 450 nm. The manufacturer did not provide a specific reference range for zonulin (ng/mL). According to the manufacturer, the intra-assay and inter-assay coefficients of variation were 3.4% and 13.3%, respectively (3).

Statistical Analysis

Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as follows:

- Mean±standard deviation for continuous variables with normal distribution,
- Median (minimum–maximum values) for continuous variables without normal distribution,
- Number and percentage for categorical variables.

The normality of numerical variables was assessed using the Kolmogorov-Smirnov, Anderson-Darling, and Shapiro-Wilk tests. Independent samples with normally distributed numerical variables were compared using the t-test, while the Mann-Whitney U test was applied for groups where numerical variables were not normally distributed. For categorical variables in 2x2 tables, Fisher's exact test and Pearson's chi-square test were used for analysis.

When comparing two or more independent groups with numerical variables following a normal distribution, a one-way analysis of variance (ANOVA) was employed. The Games-Howell test was used in the parametric analysis to identify group differences when the data exhibited a heterogeneous distribution.

Pearson correlation coefficients were used to evaluate correlations between numerical variables. The correlations were categorized based on their coefficients as follows:

- No or very weak correlation (r<0.2),
- Weak correlation (r=0.2–0.29),
- Moderate correlation (r=0.4–0.59),
- Good correlation (r=0.6–0.79),
- Strong correlation (r=0.8–1.0).

A probability value (p) of ≤0.05 was considered statistically significant.

RESULTS

he average age of the 50 patients in the study was 47.3±12.2 years. Of these patients, 66% (n=33) were male, and 34% (n=17) were female. The mean BMI was 26.3±4.7 kg/m². Nearly 70% of the patients had a normal weight or were overweight. The mean disease duration was 7.2±5.9 years. Table 1 presents the distribution of demographic and clinical characteristics.

Table 1. Demographic and clinical characteristics of the patients.

Parameter		Value
	Age (year)	47.3±12.2
Age groups	≤40 years	14 (28)
	41-50 years	11 (22)
	51-60 years	18 (36)
	≥61years	7 (14)
Sex	Male	33 (66)
	Female	17 (34)
	Body mass index (kg/m²)	26.3±4.7
Body mass index groups	Underweight	1 (2)
	Normal weight	18 (36)
	Overweight	22 (44)
	Obese	9 (18)
Smoking	Smoker	7 (44)
	Ex-smoker	21 (42)
	Non-smoker	22 (14)
	Disease duration (months)	7.2±5.9
	Bloody stools	16 (32)
Medications	Topical aminosalicylates	15 (30)
	Oral aminosalicylates	14 (28)
	Corticosteroids	2 (4)
	Purine metabolites	9 (18)
	Tumor necrosis factor-α inhibitors	4 (8)

Table 2 presents the laboratory characteristics of the patients. The mean serum zonulin level was 23.4±7.6 ng/mL.

Table 2. Laboratory investigations of the patients

Parameter	Mean±standard deviation	Range
Hemoglobin (g/dL)	13.6 ± 1.5	10-16.6
Erythrocyte sedimentation rate (mm/hr)	11.92 ± 9.4	-2-47
C-reactive protein (mg/dL)	4.1 ± 2.9	1.5-19.0
Serum zonulin (ng/mL)	23.4 ± 7.6	3.3-39.7

Beck Depression-Anxiety Inventory scores revealed the following:

- **Depression:** 37 (74%) patients had normal/minimal depression, 10 (20%) patients had mild depression, and 3 (6%) patients had moderate depression.
- **Anxiety:** 35 (70%) patients had normal/minimal anxiety, 8 (16%) patients had mild anxiety, and 6 (12%) patients had moderate anxiety (Table 3).

Table 3. Distribution of the Beck Depression and Anxiety Inventory Scores

Categories		Mean±standard deviation (range)
Beck Depression Inventory score		6.6±5.5 (0-23)
Depression categories	None	37 (74)
	Mild	10 (20)
	Moderate	3 (6)
	Severe	0 (0)
Beck Anxiety Inventory score		6.9±6.6 (0-28)
Anxiety categories	None	35 (70)
	Mild	8 (16)
	Moderate	6 (12)
	Severe	1 (2)

The serum zonulin levels differed significantly among the groups based on BMI (p<0.001). Specifically, obese patients had higher serum zonulin levels compared to those who were normal weight or overweight (Table 4). In contrast, serum zonulin levels did not vary significantly between groups based on gender (p=0.816) or smoking status (p=0.850) (Table 4).

Table 4. Comparison of serum zonulin levels according to demographic and clinical characteristics of the patients

Variables		Serum zonulin levels (ng/mL) (Mean±standard deviation)	p
Sex	Male (n=33)	23.2±7.2	0.816
	Female (n=17)	23.7±8.6	
Obesity groups	Underweight (n=1)		<0.001
	Normal weight (n=18)	20.0±8.0	
	Overweight (n=22)	22.6±6.0	
	Obese (n=9)	32.0±3.8	
Smoking status	Non-smoker (n=22)	22.7±7.8	0.850
	Ex-smoker (n=21)	23.7±7.2	
	Smoker (n=7)	24.4±9.0	

Serum zonulin levels correlated with BMI (r=0.586, p<0.001), Beck Depression (r=0.508, p<0.001), and Anxiety Inventory (r=0.437, p=0.002) scores (Table 5). As the BMI values and the scores of the Beck Depression/Anxiety Inventory increased, serum zonulin levels also increased.

Table 5. Correlation of serum zonulin levels with numerical demographic, clinical and laboratory parameters

	Serum zonulin	
	r	p
Age	-0.142	0.327
BMI	0.586	<0.001
Disease duration	-0.003	0.986
Hemoglobin	-0.019	0.896
ESR	-0.124	0.389
CRP	-0.162	0.262
Beck Depression Inventory	0.508	<0.001
Beck Anxiety Inventory	0.437	0.002

DISCUSSION

The findings of this study revealed a moderate positive correlation between serum zonulin levels and both body mass index (BMI) and Beck Depression and Anxiety Inventory scores. However, no significant correlation was observed between serum zonulin levels and other patient or disease-related characteristics, including age, gender, smoking status, and disease duration.

Previous studies have hypothesized that chronic inflammation is a common etiology for depressive symptoms and poor outcomes in patients with inflammatory bowel disease (26). Several authors have also proposed reciprocal relationships between zonulin levels, intestinal barrier dysfunction, and inflammation (27). While zonulin may not be directly involved in the development of depressive and anxious symptoms, it could serve as a biomarker for intestinal barrier dysfunction, consistent with the findings of this study (1). Disturbances in intestinal permeability may lead to various extraintestinal manifestations of UC, accompanied by elevated serum zonulin levels.

Zonulin regulates endothelial and epithelial barrier functions. Zonulin is released into the circulation when the intestinal barrier is disrupted. On the other hand, microbial antigens cross the intestinal barrier, which activates T lymphocytes and triggers the release of proinflammatory cytokines into the circulation. These proinflammatory cytokines further increase intestinal permeability, creating a vicious cycle. The increased proinflammatory cytokines can easily cross the blood-brain barrier, affecting brain cells, neurotransmitter metabolism, pituitary-hypothalamus, and the adrenal system. These biological mechanisms may also explain the relationship between zonulin and depression and anxiety.

In a study involving patients with Crohn's disease (CD) and ulcerative colitis (UC), Iordache et al. (6) evaluated the relationship between gut permeability and depression in patients with inflammatory bowel disease using the Patient Health Questionnaire-9 and found that the Patient Health Questionnaire-9 scores were moderately correlated with serum calprotectin and lipopolysaccharide-binding protein levels in the positive direction, but not with serum zonulin levels. In contrast, the findings of this study demonstrated a moderate positive correlation between serum zonulin levels, body mass index (BMI), and Beck Depression and Anxiety Inventory scores. The discrepancies between the results of these studies may be attributable to differences in patient characteristics, patient-reported outcomes for depression, and the laboratory techniques used to measure zonulin levels (5).

Demographic and clinical factors influencing zonulin levels in inflammatory bowel disease (IBD) have been investigated in previous studies. However, the results have been inconsistent. While some found a relationship between zonulin levels, age, and gender, others did not (1,28). Lacombe et al. (1) demonstrated that zonulin levels did not vary based on the type of IBD. Studies examining zonulin levels in colorectal cancer and different types of IBD have also yielded conflicting results (3,7,16,29). In one such study, Caviglia et al. found a negative correlation between zonulin levels and disease duration in IBD (3).

Several studies determined that obesity correlated with zonulin levels in UC, as in this study (1,2). It has been speculated that obesity due to a high saturated fat diet might lead to an increased intestinal permeability resulting in changes in the intestinal microbiota (1). Nevertheless, large-scale studies are needed to elucidate the underlying pathophysiological mechanisms.

The first limitation was the relatively small sample size. Secondly, only patients with ulcerative colitis (UC) in remission were included. Thirdly, only serum zonulin levels were analyzed, as opposed to cecal measurements (3,7,13). The inclusion of additional biomarkers for intestinal permeability and the analysis of fecal samples could have provided more comprehensive results. Lastly, the effect of disease severity on zonulin levels could not be assessed due to the absence of UC patients with varying levels of disease severity.

CONCLUSION

In conclusion, this study demonstrated that serum zonulin levels were correlated with depression and anxiety scores in patients with ulcerative colitis (UC). Additionally, patients with higher body mass index (BMI) values had elevated serum zonulin levels . Therapeutic interventions aimed at preserving intestinal permeability may help alleviate depressive and anxious symptoms in UC patients. Future research is needed to clarify the pathophysiological mechanisms linking gut barrier dysfunction and psychiatric symptoms.

Learning Points

1. Dysfunction of the intestinal epithelial barrier may be associated with depression and anxiety in ulcerative colitis patients.

2. Biomarkers, including serum zonulin, have been investigated in assessing integrity of intestinal barrier.
3. Obese ulcerative colitis patients had significantly higher serum zonulin levels.
4. Serum zonulin levels correlated with body mass index, Beck Depression and Anxiety Inventory scores.
5. Therapeutic maneuvers protecting intestinal permeability may contribute to treating depressive and anxious symptoms in ulcerative colitis.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
The study protocol was approved by the Gazi University Faculty of Medicine Clinical Research Ethics Committee (Approval Date: 23/10/2017, Approval No: 492). The study was conducted in accordance with the principles outlined in the Declaration of Helsinki, and written informed consent was obtained from all participants.

REFERENCES

1. Lacombe LAC, Matiollo C, Rosa JSD, et al. Factors associated with circulating zonulin in inflammatory bowel disease. Arq Gastroenterol. 2022;59:238-43.
2. Wang X, Memon AA, Palmér K, et al. The association of zonulin-related proteins with prevalent and incident inflammatory bowel disease. BMC Gastroenterol. 2022;22:3.
3. Caviglia GP, Dughera F, Ribaldone DG, et al. Serum zonulin in patients with inflammatory bowel disease: a pilot study. Minerva Med. 2019;110:95-100.
4. Lacerda JF, Lagos AC, Carolino E, et al. Functional food components, intestinal permeability and inflammatory markers in patients with inflammatory bowel disease. Nutrients. 2021;13:642.
5. Power N, Turpin W, Espin-Garcia O, Smith MI; CCC GEM Project Research Consortium; Croitoru K. Serum zonulin measured by commercial kit fails to correlate with physiologic measures of altered gut permeability in first degree relatives of Crohn's Disease patients. Front Physiol. 2021;12:645303.
6. Iordache MM, Tocia C, Aschie M, et al. Intestinal permeability and depression in patients with inflammatory bowel disease. J Clin Med. 2022;11:5121.
7. Szymanska E, Wierzbicka A, Dadalski M, Kierkus J. Fecal zonulin as a noninvasive biomarker of intestinal permeability in pediatric patients with inflammatory bowel diseases-correlation with disease activity and fecal calprotectin. J Clin Med. 2021;10:3905.
8. Choi J, Kim JH, Park M, Lee HJ. Effects of flavonoid-rich orange juice intervention on major depressive disorder in young adults: a randomized controlled trial. Nutrients. 2023;15:145.
9. Abu Sneineh A, Haj Ali S, Al-Masri A, et al. Prevalence of anxiety and depressive symptoms in ulcerative colitis patients in Jordan and its relationship to patient-reported disease activity. Sci Rep. 2022;12:7682.
10. Mikocka-Walus A, Knowles SR, Keefer L, Graff L. Controversies revisited: a systematic review of the comorbidity of depression and anxiety with inflammatory bowel diseases. Inflamm Bowel Dis. 2016;22:752-62.
11. Barberio B, Zamani M, Black CJ, et al. Prevalence of symptoms of anxiety and depression in patients with inflammatory bowel disease: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2021;6:359-70.
12. Yuan X, Chen B, Duan Z, et al. Depression and anxiety in patients with active ulcerative colitis: crosstalk of gut microbiota, metabolomics and proteomics. Gut Microbes. 2021;13:1987779.
13. Cenni S, Casertano M, Trani M, et al. The use of calgranulin-C (S100A12) and fecal zonulin as possible non-invasive markers in children with inflammatory bowel disease: a clinical study. Eur J Pediatr. 2023;182:1299-308.
14. Wang W, Uzzau S, Goldblum SE, Fasano A. Human zonulin, a potential modulator of intestinal tight junctions. J Cell Sci. 2000;113 Pt 24:4435-40.
15. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev. 2011;91:151-75.
16. Kushlinskii NE, Gershtein ES, Zybina NN, et al. Blood serum zonulin in colorectal cancer, autoimmune bowel diseases, and irritable bowel syndrome. Bull Exp Biol Med. 2022;173:376-9.
17. Troisi J, Venutolo G, Terracciano C, et al. The therapeutic use of the zonulin inhibitor AT-1001 (larazotide) for a variety of acute and chronic inflammatory diseases. Curr Med Chem. 2021;28:5788-807.
18. Truelove SC, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. Br Med J. 1955;2:1041-8.
19. Basha OM, Hafez RA, Salem SM, et al. Impact of gut microbiome alteration in ulcerative colitis patients on disease severity and outcome. Clin Exp Med. 2023;23:63-1772.
20. Guardado J, Carchman E, Danicic AE, et al. Obesity does not impact perioperative or postoperative outcomes in patients with inflammatory bowel disease. J Gastrointest Surg. 2016;20:725-33.
21. Beck AT, Steer RA, Carbin MG. Psychometric properties of the Beck Depression Inventory: twenty-five years of evaluation. Clin Psychol Rev. 1998;8:77-100.
22. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. J Consult Clin Psychol. 1988;56:893-7.
23. Kohn PM, Kantor L, DeCicco TL, Beck AT. The Beck Anxiety Inventory-Trait (BAIT): a measure of dispositional anxiety not contaminated by dispositional depression. J Pers Assess. 2008;90:499-506.

24. Kapci EG, Uslu R, Turkcapar H, Karaoglan A. Beck Depression Inventory II: evaluation of the psychometric properties and cut-off points in a Turkish adult population. Depress Anxiety. 2008;25:E104-10.

25. Ulusoy M, Sahin NH, Erkmen H. Turkish Version of the Beck Anxiety Inventory. J Cogn Psychother .1998;12:163-72.

26. Moulton CD, Pavlidis P, Norton C, et al. Depressive symptoms in inflammatory bowel disease: an extraintestinal manifestation of inflammation?. Clin Exp Immunol. 2019;197:308-18.

27. Sturgeon C, Fasano A. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. Tissue Barriers. 2016;4:e1251384.

28. Qi Y, Goel R, Kim S, et al. Intestinal permeability biomarker zonulin is elevated in healthy aging. J Am Med Dir Assoc. 2017;18:810.e1-4.

29. Malíčková K, Francová I, Lukáš M, et al. Fecal zonulin is elevated in Crohn's disease and in cigarette smokers. Pract Lab Med. 2017;9:39-44.