# The Relationship of Irritable Bowel Disease with Zonulin Protein A and Pyroptosis

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*Article*

# The Relationship of Irritable Bowel Disease with Zonulin Protein A and Pyroptosis

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**Abstract**

Background/Objectives: Evidence exists suggesting that the pathophysiology of IBS is multifaceted, involving mucosal inflammation, visceral hypersensitivity, microbial dysbiosis, and alterations in intestinal permeability. In our study, we found that the zonulin protein plays a role in IBS, and that pyroptosis can be triggered by inflammatory responses that lead to increased intestinal permeability and impaired intestinal permeability.Methods: Forty-four patients over 18 years of age who did not exhibit alarm symptoms, did not have systemic diseases affecting bowel movements, were not using medications that could affect bowel movements, and were not pregnant, and forty-four healthy individuals who signed the voluntary consent form were included in the study on a voluntary basis after undergoing colonoscopy and receiving a diagnosis of IBS according to the ROME-IV criteria. Levels of IL-1β, Nirp3, Gasdermin-D, and Zonulin Protein-A were examined in the patients, and serum levels were measured according to the normal reference range determined by our laboratory.Results: When the patient and control groups were compared, IL-1β levels were found to be statistically significantly higher in the patient group (p=0.000). NLRP3 levels were found to be statistically significantly higher in the patient group (p=0.000). The mean Gasdermin-d level was found to be statistically significantly higher in the patient group (p=0.009). The mean Zonulin Protein-A level was found to be statistically significantly higher in the patient group (p=0.001).Conclusion: In the etiopathogenesis of IBS, zonulin protein a and pyroptosis-related marker levels (Nlrp3, Il-1b, Gasdermin-D); The fact that it was detected significantly higher in patients with ibs compared to healthy controls supports our predictions that pyroptosis can be induced by inflammatory responses that may cause increased intestinal permeability and intestinal permeability, in which zonulin protein plays a role in ibs.

**Keywords:** Irritable Bowel Syndrome (IBS); cytokines; IL-1β; Nlrp3; Zonulin Protein-A; Gasdermin-d

## 1. Introduction

Irritable Bowel Syndrome (IBS) is a common gastrointestinal disorder that negatively impacts quality of life and whose etiology is not fully understood. Clinically, it is characterized by recurrent abdominal pain or discomfort, changes in bowel habits, and relief of symptoms after defecation in the absence of detectable organic pathology in standard diagnostic tests [1–3]. IBS is classified as a functional gastrointestinal disorder and is often associated with psychological stress and emotional disturbances [4,5]. According to the current Rome IV criteria, IBS is divided into four subtypes according to the dominant bowel habit: constipation-dominant (IBS-C), diarrhea-dominant (IBS-D), mixed type (IBS-M), and unclassified (IBS-U). Among these, IBS-D is the most common, accounting for approximately 40% of cases [6]. Subtype classification is based on the stool consistency reported by the patient using the Bristol Stool Form Scale [7]. Furthermore, IBS can be subdivided into sporadic, post-infectious (PI-IBS), or associated with Inflammatory Bowel Disease (IBD-IBS) [8,9].

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Zonulin is considered a serum marker of intestinal permeability and is the human analog of Vibrio cholerae zonula occludens toxin. It regulates the function of tight junctions between cells and plays a crucial role in maintaining intestinal barrier integrity [10,11]. Disruption of this barrier—often referred to as "leaky gut"—plays a role in the pathogenesis of IBS. Pyroptosis is a newly recognized form of programmed cell death, distinct from apoptosis and necrosis. It has emerged as an important innate immune mechanism in vertebrates [12]. Pyroptosis occurs through the activation of Pattern Recognition Receptors (PRRs) in response to microbial or host-derived danger signals, leading to the production of inflammatory cytokines and membrane pore formation [13,14].

## 2. Materials and Methods

This prospective study was conducted between April 1, 2018, and July 1, 2019, in the Internal Medicine and Gastroenterology outpatient clinics of the Research and Application Hospital. A total of 88 participants were included: 44 patients diagnosed with IBS according to ROME-IV criteria and 44 healthy control subjects. All participants were over 18 years of age, had no alarm symptoms, no systemic diseases affecting bowel habits, were not pregnant, and were not using medications that could alter gastrointestinal motility. Participants were selected voluntarily and randomly. The IBS patient group consisted of individuals under investigation for chronic diarrhea, with negative microbiological stool tests and normal colonoscopy findings, and with infectious and non-infectious organic causes ruled out. Venous blood samples were collected from all participants. Serum was separated by centrifugation and stored at -80°C until analysis. On the day of testing, samples were thawed at room temperature and analyzed using commercial ELISA kits. The following measurements were performed: NLRP3: Measurement range 2–600 pg/mL, accuracy 1.06 pg/mL; Zonulin Protein A: Measurement range 0.3–90 pg/mL, accuracy 0.08 ng/mL; IL-1β: Measurement range 20–6000 pg/mL, accuracy 10.07 pg/mL; Gasdermin-D: Measurement range 0.05–15 ng/mL, accuracy 0.017 ng/mL. All tests were performed according to the manufacturers' protocols. Statistical evaluations were performed using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA). Demographic characteristics and laboratory findings were summarized through descriptive statistical methods. Comparisons between groups were made using independent t-tests. Pearson correlation coefficients were calculated to assess the relationships between biochemical markers. A p-value of $<0.05$.

## 3. Results

A total of 88 participants were included; 44 were IBS patients (Group 1) and 44 were healthy control subjects (Group 2). The demographic characteristics of the groups were comparable. In the study, complete blood count, albumin, TSH, CRP, and sedimentation rate were evaluated in both groups. The mean age and gender distribution were homogeneous between the groups. Group 1 consisted of 28 men and 16 women, while Group 2 consisted of 21 men and 23 women. There was no significant difference in gender ratio between the groups (p=0.190). The mean age was 37.75±13.69 in Group 1 and 34.02±10.47 in Group 2 (p=0.261). The demographic data of the groups are shown in Table 1.

**Table 1. Age and Gender Findings in Patient and Control Groups.**

| | Group 1 Patient (n=44) | Group 2 Control (n=44) |
|---|---|---|
| Age(years) | 37,75±13,69 | 34,02±10,47 |

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| | | |
|---|---|---|
| Gender | | |
| Male | 28(%63,6) | 21(%47,7) |
| Female | 16(%36,4) | 23(%52,3) |

The mean WBC count was 8.31±2.22 $e^3$ u/L in Group 1 and 7.27±1.93 in Group 2 (p=0.020). The mean hemoglobin count was 14.56±1.89 g/dL in Group 1 and 13.46±1.89 g/dL in Group 2 (p=0.007). The mean neutrophil count was 4.77±1.66 $e^3$ u/L in Group 1 and 4.49±1.18 g/dL in Group 2 (p=0.246). The mean lymphocyte count was 2.72±0.79 $e^3$ u/L in Group 1 and 2.26±1.18 g/dL in Group 2 (p=0.001). Mean platelet count was 307.7±80.5 $e^3$ u/L in Group 1 and 269.20±70.2 in Group 2 (p=0.019). Mean eosinophil count was 0.16±0.11 $e^3$ u/L in Group 1 and 0.13±0.11 in Group 2 (p=0.075). Mean TSH was 2.0±1.12 $U^3$ u/mL in Group 1 and 1.98±0.93 in Group 2 (p=0.815). Mean CRP was 0.61±2.07 mg/dL in Group 1 and 0.67±0.79 in Group 2 (p=0.160). Sedimentation rate was found to be 10.25±11.11 in Group 1 and 6.5±7.92 in Group 2 (p=0.169). Mean albumin was 4.42±0.31 g/dL in Group 1 and 4.73±0.28 g/dL in Group 2 (p=0.044). Mean IL-1β was 154.81 ± 53.35 pg/mL in Group 1 and 111.51 ± 29.96 pg/mL in Group 2 (p=0.000). Mean Gasdermin-D was 1.225 ± 0.609 ng/mL in Group 1 and 0.861 ± 0.48 ng/mL in Group 2 (p=0.009). The mean Zonulin Protein A was found to be 4.30 ± 1.90 pg/mL in Group 1 and 3.015 ± 1.67 pg/mL in Group 2 (p=0.001). The mean NLRP3 was found to be 38.85 ± 24 pg/mL in Group 1 and 22.5 ± 10.78 pg/mL in Group 2 (p=0.000). Anti-gliadin antibody, tissue transglutaminase, stool parasite, and stool virus were within normal limits in all patients. Laboratory data for the groups are shown in Table 2.

**Table 2. Comparison of Laboratory Data Between Two Groups.**

| | Group 1 (Patient) | Group 2 (Control) | P |
|---|---|---|---|
| Wbc | 8,31±2,22 | 7,27±1,93 | **p=0.020** |
| Hgb | 14.56±1,89 | 13,46±1,89 | **p=0,007** |
| Nt | 4,77±1,66 | 4,49±1,18 | p=0,246 |
| Lym | 2,72±0,79 | 2,26±1,18 | **p=0,001** |
| Plt | 307,7±80,5 | 269,20±70,2 | **p=0,019** |
| Eos | 0,16±0,11 | 0.13±0,11 | p=0,075 |
| Tsh | 2,0±1,12 | 1,98±0,93 | p=0,815 |
| Crp | 0,61 ±2,07 | 0,67±0,79 | p=0,160 |
| Sedim | 10,25 ±11,11 | 10,25 ±11,11 | p=0,169 |

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| | | | |
|---|---|---|---|
| Alb | 4,42±0,31 | 4,73±0,28 | **p=0,044** |
| IL-1β | 154,81±53,35 | 111,51±29,96 | **p=0,000** |
| Gasdermin-d | 1,225±0,609 | 0,8611±0,48 | **p=0,009** |
| Zonulin protein a | 4,30±1,90 | 3,015±1,67 | **p=0,001** |
| NLRP-3 | 38,85±24,51 | 22,5±10,78 | **p=0,001** |

A significant positive correlation was found between WBC and neutrophil levels (r: 0.903, p: 0.00). A significant positive correlation was found between Zonulin Protein A and lymphocyte levels (r: 0.394, p: 0.008). A significant positive correlation was found between WBC and lymphocyte levels (r: 0.618, p: 0.00). A significant positive correlation was found between lymphocyte and neutrophil levels (r: 0.319, p: 0.035). A significant positive correlation was found between WBC and platelet levels (r: 0.323, p: 0.033). A significant positive correlation was found between IL-1β and NLRP3 (r: 0.855, p: 0.00). A significant positive correlation was found between IL-1β and Zonulin Protein A (r: 0.652, p: 0.00). A significant positive correlation was found between Zonulin Protein A and NLRP3 (r: 0.590, p: 0.00). A significant negative correlation was found between gasdermin-d and WBC levels (r: -0.305, p: 0.044). The available data are summarized in Table 3.

**Table 3. Correlation Findings Between Laboratory and Clinical Data.**

| | Il-1β | Gasdermin d | Z. protein a | Nlrp3 | Wbc | Nötrofil | Lenfosit |
|---|---|---|---|---|---|---|---|
| Il-1β | | r:-0.014 p:0.929 | r:0.652 p:0.001 | r:0.855 p:0.001 | r:-0.045 p:0.770 | r:-0.059 p:0.702 | r:0.210 p:0.171 |
| Gasdermin d | r:-0.014 p:0.929 | | r:-0.040 p:0.796 | r:0.018 p:0.906 | r:-0.305 p:0.044 | r:-0.226 p:0.140 | r:-0.253 p:0.098 |
| Z. protein a | r:0.652 p:0.001 | r:-0.040 p:0.796 | | r:0.590 p:0.001 | r:0.059 p:0.703 | r:-0.078 p:0.613 | r:0.394 p:0.008 |
| Nlrp3 | r:0.855 p:0.000 | r:0.018 p:0.906 | r:0,590 p:0.000 | | r:-0.041 p:0.792 | r:-0.035 p:0.821 | r:0.111 p:0.472 |
| Wbc | r:-0.045 p:0.770 | r:-0.305 p:0.044 | r:0.059 p:0.703 | r:-0.041 p:0.792 | | r:0.903 p:0.00 | r:0.618 p:0.00 |
| Nötrofil | r:-0.059 p:0.702 | r:-0.226 p:0.140 | r:-0.078 p:0.613 | r:-0.035 p:0.821 | r:0.903 p:0.00 | | r:319 p:0.035 |
| Lenfosit | r:0.210 p:0.171 | r:-0.253 p:0.098 | r:0.394 p:0.008 | r:0.111 p:0.472 | r:0.618 p:0.00 | r:319 p:0.035 | |

## 4. Discussion

The pathophysiology of IBS involves mucosal inflammation, altered intestinal permeability, microbial dysbiosis, and immune dysregulation. This study is the first to simultaneously assess serum levels of Zonulin Protein A and pyroptosis-related markers NLRP3, IL-1β, and Gasdermin-D in IBS patients. Our findings show that levels of all four markers are significantly higher in IBS patients compared to healthy controls. The strong correlations observed, particularly between IL-1β, Zonulin, and NLRP3, suggest that inflammation-induced pyroptosis may contribute to increased intestinal permeability, a key feature in IBS pathogenesis. While the role of pyroptosis in other inflammatory and autoimmune diseases such as metabolic syndrome, atherosclerosis, and inflammatory bowel disease (IBD) has been previously established [15–26], its association with IBS

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has not been investigated in previous clinical trials. Pyroptosis mediated by caspase-1 activation and gasdermin-D leads to membrane pore formation, cellular lysis, and the release of pro-inflammatory cytokines; these mechanisms may explain the persistent low-grade inflammation in IBS.

Previous research has shown altered cytokine profiles in IBS, including increased levels of IL-6, IL-8, IL-1β, and TNF-α, and decreased levels of the anti-inflammatory IL-10 [19–21]. Our study complements these data by suggesting pyroptosis as a mechanistic pathway through which these cytokines may be elevated. Furthermore, zonulin, known to regulate tight junction permeability, has been found to be elevated in diseases such as celiac disease, type 1 diabetes, and colorectal cancer, supporting its role as a non-invasive biomarker for intestinal barrier dysfunction [27–29]. Our results suggest that zonulin may also serve as an important marker in IBS and could potentially identify a subgroup of patients with tight junction dysregulation. Overall, our data support a novel IBS pathogenesis model involving zonulin-mediated disruption of intestinal barrier integrity, which can trigger immune responses and pyroptosis via NLRP3 inflammasome activation.

## 5. Conclusions

These findings pave the way for targeted therapies aimed at modulating tight junction proteins and inflammatory cell death pathways in IBS. In our study, the involvement of inflammation in the etiology of IBS, the association of inflammation-induced pyroptosis with IBS, and the significantly higher levels of biomarkers involved in pyroptosis in IBS patients compared to healthy controls support our predictions of a possible relationship between IBS and pyroptosis, and are promising for supporting therapeutic targets for IBS. However, the signaling mechanism of pyroptosis has not yet been fully elucidated. Further studies on pyroptosis signaling pathways are needed to support therapeutic targets for IBS.

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**Informed Consent Statement:** Due to retrospective design, patient informed consent was not required. In this prospective arm of study, all patients had signed informed consent. All patients' information is strictly confidential.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author due to confidentiality agreements.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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