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A MODERN APPROACH TO THE DIAGNOSIS AND PREVENTION OF RECURRENCE OF ULCERATIVE COLITIS: ZONULIN AS A PROGNOSTIC MARKER OF INTESTINAL PERMEABILITY

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Abstract. The aim of the study was to determine the diagnostic role of zonulin in the feces of ulcerative colitis patients. The results of the study showed a significantly higher level of zonulin in the feces of ulcerative colitis patients in the acute stage (133.2±72.3 ng/ml) than among patients in remission (92.8±39.6 ng/ml) (P≤0.05). Correlation analysis of zonulin and fecal calprotectin showed a positive correlation in both acute and remission stages. Fecal zonulin levels can be used to determine intestinal permeability and as an additional diagnostic marker of the level of inflammation in the intestine of ulcerative colitis patients.

***Key words**: zonulin, fecal calprotectin, ulcerative colitis, diagnostic marker.*

Ulcerative colitis (UC) is an immune-mediated disease characterized by chronic intestinal inflammation associated with increased morbidity and mortality and significant healthcare costs [1]. The pathophysiology of UC is not fully understood, but genetic, environmental, and host factors contribute to the development of intestinal inflammation. Alterations in intestinal epithelial barrier function and, consequently, increased intestinal permeability have been proposed as important factors in the pathogenesis of various autoimmune diseases, including UC [2, 3].

The intestinal epithelial barrier ensures separation of the external microenvironment from the deeper layers of the host mucosa. Thus, the intestinal lumen, located near the apical portion of epithelial cells and containing a variety of macromolecules, bacterial compounds, and antibodies, remains isolated from more internal tissues of the body, such as the intestinal lamellar membrane. For normal functioning, the integrity of the epithelial barrier is essential, which is ensured by cellular adhesion and a functional complex consisting of tight junctions, adhesive junctions, and desmosomes. These protein structures have dynamic properties and influence the ability of the intestinal epithelium to control the passage of substrates through the paracellular pathway, partially responsible for intestinal permeability [4, 5].

Zonulin (pre-haptoglobin 2) is a protein found in an uncleaved form in human serum [6] and is highly correlated with bacterial DNAemia [7]. It is a physiological mediator that reversibly regulates intestinal permeability by regulating intercellular tight junctions [8] and is considered a biomarker of intestinal barrier dysfunction [6].

There are several pilot studies to determine the role of zonulin in a limited number of patients with inflammatory bowel diseases, the results of which are controversial and require further study [9–11].

The aim of the study was to determine the diagnostic role of zonulin in feces in patients with UC.

Materials and methods. The study was conducted at the Republican Clinical Hospital No. 1 and the Tashkent Medical Academy in 2021–2022. Patients with UC in the acute (n=20) and remission (n=15) stages were examined. The diagnosis of UC was confirmed using standard clinical, endoscopic, radiographic, and pathological criteria according to the Montreal Classification of Ulcer Disease (UC) severity. UC categories include proctitis, left-sided colitis, and extensive or pancolitis. Disease activity was measured using the Mayo Clinic scoring system, which consists of 4 items: stool frequency, rectal bleeding, flexible proctosigmoidoscopy results, and patient functional assessment. Disease duration was measured in years from the onset of symptoms.

Zonulin concentration (ng/mL) was determined using an ELISA kit using the Human Zonulin ELISA Kit (Germany) according to the manufacturer's instructions. The assay utilized a competitive binding method. Biotinylated zonulin was added to samples, standards, positive and negative controls as a competitor for the sample's own zonulin. Color intensity was inversely proportional to the zonulin concentration in the sample. Samples were read at 450 nm, and a 4-parameter algorithm was used to generate a standard curve and calculate data. All assays were performed in duplicate within 24 hours of sample collection using the same lot numbers. According to the manufacturer's recommendations, fecal zonulin levels of 83.15, 83.15-110.0, and greater than 110.0 ng/mL were considered normal, elevated, and very high, respectively.

Fecal calprotectin analysis was performed using a fluorescent immunoassay kit for calprotectin at the Gunchamed Clinic (Tashkent, Uzbekistan). Fecal calprotectin levels were quantified in μg/g. Diagnostic fecal calprotectin levels are: 5–50 μg/g is normal, 50-100 μg/g is weakly positive, and >100 μg/g is positive.

Processing of primary data, correlation analysis and comparison of mean values were carried out using the IBM SPSS Statistics 23 program.

Results and discussions. The results of the study showed a significantly higher level of zonulin in the feces of patients with UC in the acute stage (133.2±72.3 ng/ml) than among patients in remission (92.8±39.6 ng/ml) (P≤0.05). The same trend was found for fecal calprotectin among patients with UC in the acute stage (1349.2±592.0 μg/g) and remission (218.6±108.0 μg/g) (≤0.001). Correlation analysis of zonulin and fecal calprotectin showed a positive correlation both in the acute stage and in the remission stage (Table 1).

Table 1. Fecal zonulin and fecal calprotectin levels in patients with UC

Groups	Fecal zonulin level, ng/ml (M±m, max-min)	Fecal calprotectin level, μg/g (M±m, max-min)	Pearson correlation (zonulin/ calprotectin)
patients with UC in the acute stage (n=20)	133,2±72,3 (55,9-300,0)	1349,2±592,0 (554,1-2019,0)	0,645*
patients with UC in remission (n=15)	92,8±39,6 (34,7–161,1)	218,6±108,0 (97,5–419,2)	0,519**
T-test (P)	≤0,05	≤0,001	–

*Correlation is significant at the 0.01 level; **Correlation is significant at the 0.05 level.

Our findings indicate an association between the level of inflammation and intestinal permeability in patients with UC. Similar results were obtained by Lacombe et al. (2022) [9], who found that zonulin correlated with clinical and laboratory parameters of disease severity and activity among patients with UC, but not among patients with Crohn's disease. When comparing zonulin levels among patients with inflammatory bowel disease and individuals without any health complaints, the authors found no significant differences.

It is important to emphasize that intestinal permeability can be assessed using other methods based on the ingestion of solvents with different absorbent properties and their subsequent measurement in urine. The main test used in clinical studies is the lactulose/mannitol excretion ratio, which is considered a non-invasive test but is difficult to perform due to the need for prolonged fasting and periodic urine collection [12]. A previous study assessing the lactulose/mannitol excretion ratio among patients with Crohn's disease and celiac disease showed that changes in barrier function appear to persist even among patients with Crohn's disease in clinical remission [13]. Furthermore, a Finnish study noted that the lactulose/mannitol ratio was positively correlated with endoscopic disease activity, but the correlation with the clinical index or C-reactive protein was weak [14]. The advantage of zonulin assessment is not only that it is non-invasive, but also that it is easier to perform.

Conclusion. Fecal zonulin levels can be used to determine intestinal permeability and as an additional diagnostic marker for intestinal inflammation in patients with UC. Zonulin determination is a noninvasive diagnostic method that plays an important role in assessing the severity of inflammatory bowel disease during pathogenetic therapy.

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