MSD

Medical Science and Discovery
ISSN: 2148-6832

Can Zonulin level be a new diagnosis and follow-up criterion in active ulcerative colitis?

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ABSTRACT

Objective: In this study, we compared the serum zonulin levels in patients diagnosed for the first time with active ulcerative colitis with those in healthy cases and attempted to determine whether serum zonulin levels were different in the active ulcerative colitis.

Material and Methods: A total of 53 naive patients admitted to our hospital between 2019 and 2020 and diagnosed with active ulcerative colitis by colonoscopy were included as a group of cases and 37 patients with no acute or chronic diseases whose colonoscopy was normal as the control group.

Results: The study was conducted on 90 cases, 65.5% male and 34.5% female. The patients with ulcerative colitis were compared with the control group in terms of serum zonulin levels. Average serum zonulin levels of the patients with ulcerative colitis (16.73 ± 5.49 ng/ml) were not significantly different than those in the control group 17.48 ± 8.31 ng/ml). Serum zonulin levels of the patients were also compared according to location and severity of disease and did not differ statistically significantly between the groups in terms of the Montreal Classification. When serum zonulin levels were grouped according to the Truelove and Witts criteria, there was no statistically significant difference between the patient groups themselves and the control group.

Conclusion: Serum zonulin levels were not greater in the patients with naive active ulcerative colitis compared to the healthy controls. Several previous studies have shown that serum zonulin levels are elevated in patients with ulcerative colitis, but more studies are needed on this subject.

Keywords: zonulin, inflammatory bowel diseases, ulcerative colitis

INTRODUCTION

The intestinal wall is composed of large, single-celled, thick epithelial cells surrounding the inner membrane of the intestine. Maintaining the integrity of this barrier and controlling its permeability is very important in terms of the regulation of the immune system and protection of it against pathogens. There are two ways in which elements pass through the intestinal lumen into the blood circulation. These two paths are the “transcellular pathway” with transporters along the brush border of enterocytes and the “paracellular pathway” through spaces between cells (**1, 2**). The paracellular pathway is controlled by gates with a protein structure called “tight junctions.” These dynamic structures are opened and closed in harmony with the nutritional status, physical activity, hormonal and neural signals, and inflammatory mediators (**3, 4**). Zonulin, a precursor to haptoglobins (HPs), which is called pre-haptoglobin-2 (pre-HP2), is a physiological and reversible modulator in a key position at these tight junctions. This protein is formed in the mucosa and directly controls the permeability of the intestine (**1, 5, 6**). In response to stimuli such as bacteria in the intestinal lumen or triggers in foods, zonulin is released into the lumen, binds to receptors on the apical surfaces of epithelial cells, and activates the pathways that cause deterioration of the integrity of tight junctions (**7, 8**). Genetic structure and environmental triggers may play a role in the pathogenesis of chronic inflammatory diseases (CIDs), which can be classified as allergic, autoimmune, and metabolic diseases. However, increased intestinal permeability is a common consequence of a number of complicated processes in such diseases (**3, 9, 10**).

Research Article

Received 17-01-2020

Accepted 07-02-2021

Available Online: 08-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.467

Long-term zonulin upregulation caused by environmental triggers leads to increased intestinal permeability and continuous passage of intestinal antigens to the submucosa. If intestinal permeability increases, a large amount of antigenic substances enter the systemic circulation. This can lead to chronic intestinal mucosal damage in the long term. Moreover, type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis, celiac disease and many other autoimmune diseases may develop due to the increased intestinal permeability (**9, 11**). Excessive permeability from the intestine has also been associated with chronic diseases such as irritable bowel syndrome, whose etiology is not well known. In a recent clinical study, serum zonulin levels were found to be higher in patients with inflammatory bowel disease (IBD) — in patients with ulcerative colitis (UC) and Crohn's disease (CD) both — than in normal healthy controls (**12**). This reinforces the hypothesis that certain zonulin-mediated pathways cause UC. In our study, we assessed whether serum zonulin levels of patients with active UC were different from those of normal healthy controls.

MATERIAL AND METHODS

Before the study began, ethics committee approval for the study was obtained from the ethics committee of our institution. The study was conducted on 37 healthy people in the control group and a total of 53 patients consisting of 21 women and 32 men diagnosed with naive active UC by colonoscopy, biopsy and laboratory findings from among patients admitted to our clinic between 2019 and 2020. The case and control group patients were included in the study voluntarily. Each patient was given an informed consent form before participation and was asked to sign it. Blood samples were taken and kept at -80 °C before starting treatment from the patients diagnosed with UC. As a control group, a total of 37 patients including 12 women and 25 men, who underwent a colonoscopy and whose colon mucosa was found to be normal, who did not have a CID, and whose laboratory values were normal, were included in the study. Similarly, blood samples taken from these patients were kept at -80 °C. Serum zonulin levels of these samples were determined by using the Sunred B10 Human Zonulin kit at the end of the study and were studied by using the ELISA method.

Statistical analyses: Statistical analyses were conducted in the Number Cruncher Statistical System (NCSS) 2007 software (Kaysville, Utah, USA). Descriptive statistical methods (means, standard deviations, medians, frequencies, ratios, minimum and maximum values) as well as comparative statistical methods were used when assessing the data of the study.

Whether the quantitative data were normally distributed was tested by using the Kolmogorov-Smirnov test, Shapiro-Wilk test and graphical evaluations. The quantitative data of the two groups with normal distribution were compared through student t-tests, and the data that were not normally distributed were compared through Mann Whitney U tests. Data of three or more groups that were not normally distributed were compared through Kruskal Wallis Tests. Qualitative data were compared through Pearson chi-square tests. Significance was assessed at least at $p < .05$ level.

RESULTS

The study was conducted on a total of 90 cases — 65.5% (n = 59) male and 34.5% (n = 31) female — at the Gastroenterology Clinic of Education and Research Hospital of a university. The ages of the cases ranged from 19.6 to 78.4 years, and the mean age was 37.61 ± 12.66 years.

Of the cases included in the study, 41.1% (n = 37) constituted the control group, and 58.9% (n = 53) constituted the group of patients with UC. The demographic characteristics of the case and control group patients included in the study are shown below in Table 1.

After the patients with UC were compared with the control group patients overall, they were also compared with the control group separately depending on disease location and severity. Disease locations were classified according to the Montreal Classification. Disease severity was assessed according to the Truelove and Witts criteria. Table 2 presents demographic data of the patient and control group cases according to disease location and severity.

When the cases were examined for disease locations according to the Montreal Classification, 28.3% (n = 15) of the cases with UC had ulcerative proctitis, 34.0% (n = 18) left-sided colitis, and 37.7% (n = 20) had pancolitis.

According to the Truelove and Witts (severity) criteria, 30.2% (n = 16) of the cases were mild, 37.8% (n = 20) moderate, and 32.0% (n = 17) were cases involving severe colitis.

The patients with UC were compared with the control group in terms of serum zonulin levels. Serum zonulin levels of the patients in the UC group were 16.73 ± 5.49 ng/ml on average, while serum zonulin levels of the patients in the control group were 17.48 ± 8.31 ng/ml on average. There was no statistically significant difference between the patients and the control group in terms of zonulin levels ($p > .05$).

Serum zonulin levels of the patients were also compared with those of the control group according to disease location and severity. Serum zonulin levels of the patients did not differ statistically significantly between the classes/groups that were classified in terms of the Montreal Classification ($p > .05$).

Serum zonulin levels showed no statistically significant difference between the patient groups themselves with UC that were grouped according to the Truelove and Witts criteria, and neither between them and the control group ($p > .05$).

The distribution of the control group and patient data according to the Montreal Classification and Truelove Witts criteria is shown in Table 3.

The graphical expression of serum zonulin levels of the patients with UC according to the location and severity of the disease is shown in Figure 1 (Montreal Classification: p= .375, Truelove and Witts: p= .796).

http://dx.doi.org/10.36472/msd.v8i2.467

Table 1. Distribution of demographic characteristics of cases (n = 75)

	Total (n = 90)	Control Group (n = 37, 41.1%)	Ulcerative Colitis (n = 53, 58.9%)
Age (Years)			
Min–Max (Median)	19.6–78.4 (35.7)	20.5–64.3 (40.4)	19.6–78.4 (30.5)
Mean±SD	37.61±12.66	41.23±11.83	34.05±12.99
Gender, n (%)			
Male	59 (65.5)	25 (67.6)	32 (65.5)
Female	31 (34.5)	12 (32.4)	21 (34.5)

Table 2. Distribution of patient and control groups according to disease location and severity

	Total (n = 90)		Control Group (n = 37, 41.1%)		Ulcerative Colitis Group (n = 53, 58.9%)	
	n	(%)	n	(%)	n	(%)
Montreal Classification (Localization)						
Control Group	37	(41.1%)	37	(100.0%)	0	(0.0%)
Ulcerative proctitis	15	(16.7%)	0	(0.0%)	15	(28.3%)
Left-sided colitis	18	(20.0%)	0	(0.0%)	18	(34.0%)
Pancolitis	20	(22.2%)	0	(0.0%)	20	(37.7%)
Truelove and Witts (Severity)						
Control Group	37	(41.1%)	37	(100.0%)	0	(0.0%)
Mild	16	(17.8%)	0	(0.0%)	16	(30.2%)
Moderate	20	(22.2%)	0	(0.0%)	20	(37.8%)
Severe	17	(18.9%)	0	(0.0%)	17	(32.0%)

Table 3. Distribution of data by Montreal Classification and Truelove and Witts Criteria

	Montreal Classification				
	Control Group (n = 37)	Ulcerative proctitis (n = 15)	Left-sided colitis (n = 18)	Pancolitis (n = 20)	p
Zonulin (ng/ml)					
Min–Max	11.4–49.4	10.7–30.2	10.8–24	10.1–33.3	.375
(Median)	(14.7)	(17.7)	(13.2)	(17.3)	
Mean±SD	17.48±8.31	18.11±6.36	14.63±3.74	17.70±6.02	
	Truelove and Witts Criteria				
	Control Group (n = 37)	Mild (n = 16)	Moderate (n = 20)	Severe (n = 17)	p
Zonulin (ng/ml)					
Min–Max	11.4–49.4	10.7–30.2	10.1–25.3	11.2–33.3	.796
(Median)	(14.7)	(13.9)	(14.8)	(16.3)	
Mean±SD	17.48±8.31	16.91±6.54	15.98±4.49	17.60±6.06	

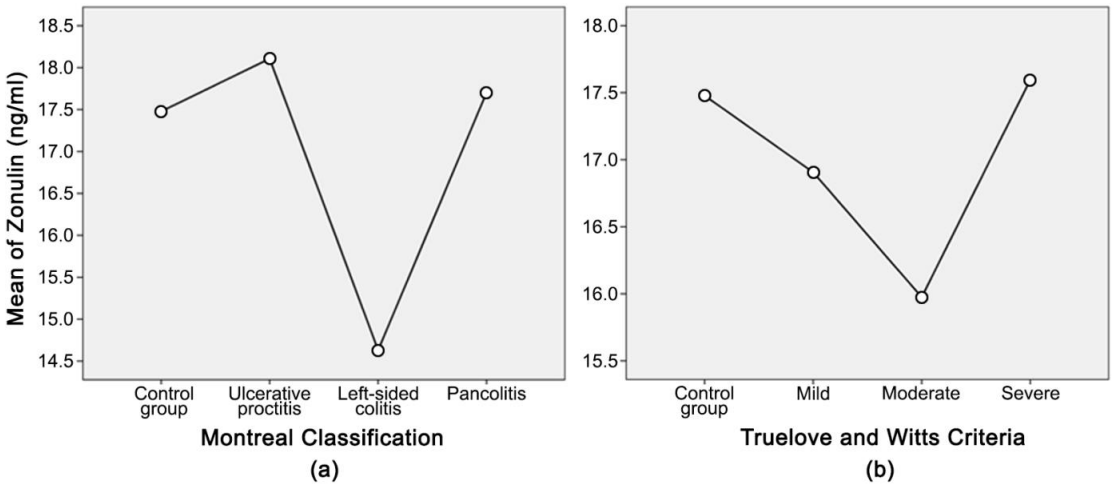

Figure 1. Distribution of Serum Zonulin levels according to **a.** Montreal Classification and **b.** Truelove and Witts Criteria

DISCUSSION

UC and CD are immune-mediated conditions characterized by chronic inflammation of the intestine, collectively called IBD. Their exact etiologies are unknown, but increased intestinal permeability has been shown to play a fundamental role in the pathogenesis of IBD. Zonulin is one of the little-known physiological mediators of paracellular intestinal permeability. Mature human HPs are heterodimeric plasma glycoproteins. Zonulin is a pre-HP2 isolated from human serum through proteomics studies (13). Most of the studies on the role of zonulin in IBDs are experimental animal model studies (14-17). There are not enough clinical trial studies conducted on humans (11, 13, 18). In the study of Caviglia et al. comparing UC and CD patients with healthy normal controls, serum zonulin levels were found to be significantly higher in UC and CD patients (12). In a study carried out by Vanuytsel et al., it has been shown that haptoglobin-2 (HP2) carries a higher frequency of risk allele in both UC and CD compared to healthy controls (13). In cirrhosis patients secondary to chronic hepatitis C, low serum zonulin levels were found, which were probably due to decreased production of this mediator in the liver (19). However, there is currently no study that reports serum zonulin levels of patients with UC to be normal or low compared to those of healthy controls.

In our study, the patients with naive active UC were compared with the healthy controls in terms of serum zonulin levels, but no significant difference was found between the patients and the control group. In addition, when the UC cases were grouped into different disease severities and locations, their serum zonulin levels were not different from those of the normal healthy controls. The results of our study suggest that serum zonulin levels cannot be a diagnostic and activity indicator of UC. As a matter of fact, it was shown in another clinical study that fecal zonulin in UC was a weaker marker than other parameters in assessing the response to infliximab (20). In a recent study by Wegh et al., serum zonulin was found to be a better indicator of intestinal permeability in patients with UC than fecal zonulin because of its compatibility with other intestinal permeability biomarkers (21). As can be seen, contradictory results have been obtained in a limited number of clinical studies on zonulin.

The sensitivity of the commercial ELISA kit, which was used to determine the level of serum zonulin, to the corresponding zonulin molecule may also be an important parameter determining the results. The development of methods that more precisely determine pre-HP2 levels may make zonulin a more sensitive parameter in IBD in the future (22).

However, the efficacy and diagnostic value of zonulin in UC is not a subject on which light has been fully shed. If HP2 is defined as a risk allele for IBD, the next question to answer is how HP affects the pathogenesis of the disease. This may be due to HP's possible immunomodulatory effect. Based on animal model studies, HP can be assumed to play a role in reducing the severity of inflammation and modulating the production of IL-17, which is a cytokine that is thought to be very important in the pathogenesis of IBD. However, clinical trials in humans are not yet available. In this respect, another hypothesis may be related to zonulin. Zonulin, described as pre-HP2, is defined as an important physiological instrument of paracellular intestinal permeability. Due to the permeability-enhancing effect of the carriers of the zonulin gene (genotype HP21 or HP22) on the intestinal barrier, it is likely that zonulin carries the risk of causing IBD. Further studies on the role of zonulin in IBD and its relationship to intestinal permeability will be important to find more answers for many interesting questions.

Author contributions: EA, GÜ; Both authors equally contributed to the conception and planning of the work that led to the paper and interpretation of the evidence it presents; and the writing of the paper/reviewing of successive versions.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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