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Fecal zonulin is elevated in Crohn's disease and in cigarette smokers

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ABSTRACT

Objectives: Human zonulin is a protein that increases permeability in the epithelial layer of the small intestine by reversibly modulating the intercellular tight junctions. There is not sufficient information available about zonulin's participation in inflammatory bowel diseases (IBD). The aim of this study was therefore to investigate fecal and serum zonulin in IBD patients and its relation to the disease localization, behavior and smoking status.
Design and methods: Forty IBD patients and forty healthy persons were examined for fecal and serum zonulin concentrations by competitive ELISA (DRG International Inc). Values were correlated to IBD type, localization and behavior, and smoking.
Results: Serum and fecal zonulin were significantly higher in patients with Crohn's disease compared to ulcerative colitis ($p = 0.038$ for fecal zonulin, and $p = 0.041$ for serum zonulin concentrations). No association of serum or fecal zonulin was found with respect to IBD localization and behavior. The only difference was found with respect to smoking. Both the IBD cohort and healthy smokers showed significantly higher fecal zonulin levels (median 203 ng/mL) compared to non-smokers (median 35.8 ng/mL), $p < 0.001$.
Conclusions: Fecal and serum zonulin levels are elevated in patients with active Crohn's disease but not with ulcerative colitis. High fecal zonulin levels in smokers irrespective of IBD point to the significant and undesirable up-regulation of gut permeability in cigarette smokers.

1. Introduction

Human zonulin is a 47-kDa human protein that increases permeability in the epithelial layer of the small intestine [1]. It is the only physiological mediator known that increases gut permeability by reversibly modulating the intercellular tight junctions, whereas proper functioning of the tight junctions is crucial for maintaining normal physiologic processes in the intestinal tract [2].

Increased serum/plasma zonulin levels have been described in celiac disease [3], type 1 and 2 diabetes [4,5] or in obesity-associated insulin resistance [6,7]; and circulating plasma zonulin has been suggested as a potential marker of intestinal permeability [8–10]. However, there is insufficient information about zonulin's participation in some important states of intestinal inflammation

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Table 1
Descriptive characteristics of examined IBD patients.

Diagnosis	Crohn's disease	32
	Ulcerative colitis	8
Gender	Male	19
	Female	21
Disease duration, years	Mean	6
	Range	2 –15
Behavior of CD	B1, non-stricturing non-penetrating Crohn's disease	16
	B2, stricturing Crohn's disease	12
	B3, penetrating Crohn's disease	4
Localization	L1, ileal Crohn's disease	8
	L2, colonic Crohn's disease	6
	L3, ileocolonic Crohn's disease	14
	L4, upper digestive Crohn's disease	4
	E1, ulcerative proctitis	1
	E2, left-sided ulcerative colitis	3
	E3, extensive ulcerative colitis	4
Concomitant medication	Systemic corticosteroids	14
	Local corticosteroids	6
	Azathioprine	20
Previous bowel surgery	No	31
	Yes	9
Smoking	No	31
	Yes	9

(e.g., inflammatory bowel diseases, IBD), and there is ambiguous information about serum and fecal zonulin in IBD [11].

The aim of this study was to investigate fecal and serum zonulin in IBD patients and its relation to the disease localization and behavior. Moreover, the relationship between fecal and serum zonulin and smoking status was examined.

2. Materials and methods

2.1. Specimen characteristics

Forty consecutive IBD patients – 32 with Crohn's disease (CD) and 8 with ulcerative colitis (UC) in whom fecal and blood samples were available – were enrolled from a tertiary IBD clinical center. All IBD subjects were patients on induction infliximab therapy, and were examined before the second infliximab dose. Therefore, it was a relatively homogenous cohort of patients with severe acute gut inflammation or chronic disease resistant to corticosteroids and/or immunosuppressants. Patients´ baseline demographic and clinical characteristics are presented in Table 1. Disease behavior and localization was classified according to the Montreal classification [12].

Forty healthy persons (laboratory technicians and their family members) without personal or family history of IBD were matched by age and gender and examined as a control (CTRL) group. There were 27 active smokers and 13 non-smokers in the CTRL group.

None of 80 examined persons (IBD patients or healthy controls) had been noted to have celiac disease or diabetes during previous clinical examinations.

The study was approved by the Institutional Ethical Committee. The purpose and procedures of the study were explained to participants, who signed informed consent forms.

2.2. Fecal samples

Raw stool samples from the IBD and CTRL groups were frozen and stored at −80 °C within 12 h after the sampling. Before the laboratory analysis, stool samples were thawed, and mechanical homogenization was performed using an inoculation loop. The Fecal Sample Preparation kit (Roche Diagnostics, Germany) for the preparation of fecal eluates was used. In this system, 100 mg of stool sample is suspended in 5 mL of appropriate extraction buffer using a vortex and subsequently centrifuged for 5 min at 2000*g* using a refrigerated centrifuge. For subsequent ELISA analysis, stool sample supernatants (eluates) were used immediately after their preparation.

2.3. Serum samples

Blood samples were collected into commercially available serum-separating tubes (Vacutainer, Becton Dickinson, USA). After collection, the blood was allowed to clot at room temperature for 30 min. The clot was removed by centrifugation for 10 min at 2000 g using a refrigerated centrifuge. Serum samples were stored at −80 °C immediately after their preparation.