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Factors associated with circulating zonulin in inflammatory bowel disease

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ABSTRACT – Background – Inflammatory bowel disease (IBD) comprises the spectrum between Crohn's disease (CD) and ulcerative colitis (UC), a condition whose prevalence in countries such as Brazil has increased significantly in recent years. Changes in the intestinal epithelial barrier function and, consequently, an increase in intestinal permeability, have been suggested as important factors in the pathogenesis of different autoimmune conditions, including IBD. Therefore, there is a need for a practical tool to assess gut barrier integrity in these patients. **Objective –** To study factors associated with serum zonulin levels, a marker of intestinal permeability, in patients with IBD. **Methods –** This was a cross-sectional observational study that included 117 patients with IBD and 32 healthy controls. Disease activity was assessed by the Simple Clinical Colitis Activity Index (SCCAI) in UC and by the Harvey-Bradshaw Index (HBI) in CD subjects. Zonulin levels were measured by ELISA and inflammatory cytokines by Cytometric Bead Array, using commercially available kits. **Results –** The mean age of IBD patients was 44.0±15.9 years, 66.7% were female, 57 subjects were diagnosed with CD and 60 with UC. At evaluation, clinical remission was observed in 56.7% of CD patients and in 59.2% of UC subjects. No differences were observed in zonulin levels when comparing IBD patients with the control group (95.28 ng/mL vs 96.61 ng/mL, *P*=0.573) and when comparing patients with CD to those with UC (79.68 ng/mL vs 106.10 ng/mL, *P*=0.887). Among IBD group, zonulin concentrations were higher among females, correlated positively with body mass index (BMI) and age; and negatively with hemoglobin and hematocrit. In patients with UC, zonulin correlated negatively with hemoglobin, hematocrit, and albumin; and positively with BMI and SCCAI. Among CD patients, zonulin was positively correlated with age and BMI, but not with HBI. No correlations were observed between zonulin and circulating cytokines in IBD patients. **Conclusion –** In this cohort mostly comprised of patients in clinical remission, serum zonulin levels were not higher in patients with IBD than healthy controls, and correlated with variables not linked to baseline disease, such as sex, age and BMI. However, zonulin correlated with clinical and laboratory parameters of disease severity and activity among subjects with UC, but not among patients with CD. These findings indicate a potential role for zonulin as a biomarker in IBD, particularly in UC.

Keywords – Inflammatory bowel disease; intestinal permeability; zonulin; inflammatory cytokines; disease activity.

INTRODUCTION

Inflammatory bowel disease (IBD) comprises the spectrum between ulcerative colitis (UC) and Crohn's disease (CD). Both are immune-mediated conditions characterized by chronic intestinal inflammation, being associated with greater morbidity and mortality and high costs to the health system[1]. The pathophysiology of IBD is not yet fully understood, but genetic, environmental and intrinsic factors related to the individual with this condition contribute to the development of intestinal inflammation. Changes in the intestinal epithelial barrier function and, consequently, an increase in intestinal permeability, have been suggested as important factors in the pathogenesis of different autoimmune conditions, including IBD[2,3].

The intestinal epithelial barrier separates the external microenvironment from the deeper layers of the host's mucosa. Thus, the intestinal lumen, which is located close to the apical region of epithelial cells and contains a variety of macromolecules, bacterial compounds and antibodies, remains isolated from more internal regions of the organism, such as the lamina propria. For this to occur, it is necessary the integrity of the epithelial barrier, a property acquired through cell adhesion that is present through the junctional complex, composed of the structures tight junctions, adherens 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, being responsible, in part, for the intestinal permeability[4,5].

Zonulin is a 47-kDa molecular weight protein described by Wang et al. which has the ability to increase intestinal permeability. Its identification was described by studies that investigated the pathophysiology of *Vibrio cholerae*, and identified a secondary enterotoxin, called zonula occludens toxin (Zot) and its endogenous homologous, then called zonulin[6]. The mechanism by which zonulin would modulate intestinal permeability, increasing it, was proposed

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by Fasano et al. This group proposed that zonulin would bind to the epidermal growth factor receptor (EGFR), activating it; also, zonulin would have the ability to activate this receptor through binding to the protease-activated receptor type 2 (PAR2)[2]. The activation of these receptors would culminate in an intracellular signaling process that results in a decrease in the interaction between proteins in tight junctions and in an increase in intestinal permeability.

A better understanding of the role of intestinal permeability in IBD is needed. The investigation of a potential biomarker related to increased intestinal permeability and its relationship with clinical activity and with well-established circulating inflammatory markers, such as C-reactive protein and cytokines, may bring new evidence about the pathophysiology of IBD. To date, there are few studies that have consistently evaluated zonulin concentrations in patients with IBD. The role and relevance of the assessment of zonulin concentrations in the management and follow-up of these patients is also not well established.

The aims of this study were to study serum zonulin concentrations in patients with IBD and its correlation with clinical indices of disease activity and circulating inflammatory markers, to compare the serum concentration of zonulin between patients with IBD and a control group and to compare the serum zonulin concentration according to the IBD phenotype and also regarding the demographic and clinical characteristics of IBD.

METHODS

Ethical considerations

This study is in accordance with the resolution of the National Health Council No. 196 of 10/10/1996. The protocols for this study were approved by the Ethics and Research with Human Beings Committee of the Federal University of Santa Catarina (CEPSH-UFSC) and are registered under the number 2.308.479. The written informed consent form was read and signed by all patients.

Patients

This was a cross-sectional observational study that included 117 patients (≥18 years of age) with IBD who were under regular follow-up at the inflammatory bowel disease Outpatient Clinic of the University Hospital of the Federal University of Santa Catarina, Brazil. All patients were under regular follow-up and had the diagnosis of IBD established through the combination of clinical, laboratory, radiological, endoscopic and histological findings. Patients in the following situations were excluded: hospitalization not related to IBD in the last 30 days, infections in the last 7 days, previous or current diagnosis of malignant neoplasms, and refusal or inability of the patient to understand the informed consent.

Thirty-two sex- and age-matched healthy subjects served as a control group. Exclusion criteria for the control group were: hospitalization in the last 30 days, infections in the last 7 days, diagnosis of any major chronic disease, previous diagnosis of any chronic inflammatory disease, previous or current diagnosis of malignant neoplasms, and refusal or inability of the patient to understand the informed consent.

The sample size was calculated separately for comparison of zonulin levels between cases and control group, and for the comparison of zonulin levels among IBD patients in clinical remission versus clinical activity. The minimum sample size for each group was estimated in 27 individuals for comparison between IBD and controls, and in 47 patients for comparison between IBD groups.

Methods

Clinical and demographic variables

The variables age, sex, comorbidities, subtype of inflammatory bowel disease (CD or UC), time since diagnosis, current therapy, current or previous smoking, previous hospital admissions and surgical history related to IBD, location, and behavior of the IBD were collected. Each patient also underwent physical examination to obtain variables such as weight, height, body mass index (BMI), blood pressure, heart rate, axillary temperature, and abdominal physical examination. Clinical scores to assess disease activity were then applied to all patients. The Simple Clinical Colitis Activity Index (SCCAI) was applied for those diagnosed with UC and the Harvey-Bradshaw Index (HBI) for those diagnosed with CD[7,8]. Patients were classified as being in clinical remission when SCCAI was ≤2 for UC or when IHB was ≤4 for CD[9,10].

Routine laboratory tests

All subjects underwent peripheral blood collection to perform the routine laboratory tests (blood count, aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, alkaline phosphatase and albumin). C-reactive protein (CRP) levels were measured by the automatic analyzer Dimension RxL Max® (Siemens), with an analytical sensitivity of 0.5 mg/dL.

Zonulin levels

The tests were performed in serum samples collected after clinical evaluation and stored at -80°C. Serum zonulin levels were measured by sandwich ELISA using a commercially available assay (Human Zonulin ELISA Kit - Elabscience®) on samples collected in conjunction with the routine laboratory tests.

Circulating cytokine measurements

The quantitative determination of circulating cytokines in the serum was performed using a Cytometric Bead Array (CBA) kit (BD Biosciences®; San Diego, CA). The fluorescence produced by the CBA beads was measured on a FASCSVerse Flow Cytometer (BD FACSVerseTM, San Jose, CA, USA) and analyzed using Software FCAP Array 3.0 (BD Biosciences). The minimum detection values were 18.9 pg/mL for IL-17A, 4.5 pg/mL for IL-10, 2.4 pg/mL for IL-6, 4.9 pg/mL for IL-4, 2.6 pg/mL for IL-2, 3.8 pg/mL for TNF, and 3.7 pg/mL for IFN-γ.

Statistical Analyses

Normality of the variable distribution was determined using the Kolmogorov-Smirnov test. The correlation between the numerical variables was evaluated using Spearman's correlation coefficient. Continuous variables were compared using Student's *t*-test in the case of a normal distribution or if not, using the Mann-Whitney test. Categorical variables were evaluated by the chi-square test or Fisher's exact test, as appropriate. All tests were performed using SPSS software, version 17.0 (SPSS, Chicago, IL, USA). A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Characteristics of included patients and zonulin levels in study groups

The main characteristics of the patients included in the study are depicted in TABLE 1. None of individuals in IBD or healthy control groups had history of current or recent non-steroidal anti-

TABLE 1. Characteristics of included patients.

Variable	Patients (n=117)
Age (years), mean ± SD	44.0±15.9
Female, n (%)	78 (66.7)
Diagnosis, n (%)	
Ulcerative colitis	57 (48.7)
Crohn's disease	60 (51.3)
Time since diagnosis (years), median	7.0
Comorbidities	
Systemic arterial hypertension, n (%)	27 (23.1)
Dyslipidemia, n (%)	18 (15.4)
Depressiom, n (%)	14 (12.0)
Bone metabolism disease, n (%)	12 (10.3)
Diabetes, n (%)	10 (8.5)
Primary sclerosing cholangitis, n (%)	3 (2.6)
Active alcohol consuption, n (%)	36 (30.8)
Current smoking, n (%)	5 (4.3)
Previous smoking, n (%)	46 (39.3)
Previous surgery, n (%)	19 (16.2)
BMI, mean ± SD	26.7±5.6
Laboratory evaluation	
Hemoglobin (mg/dL), median	13.1
Hematócrito (%), median	39.6
CRP (mg/dL), median	3.2
Albumin (g/dL), median	3.8
Zonulin (ng/mL), median	95.28
TNF (pg/mL), median	6.17
IFN-γ (pg/mL), median	14.53
IL-17A (pg/mL), median	26.38
IL-10 (pg/mL), median	7.44
IL-6 (pg/mL), median	7.70
IL-4 (pg/mL), median	15.74
IL-2 (pg/mL), median	10.32

SD: standard deviation, BMI: body mass index; CRP: C-reactive protein; TNF: tumor necrosis factor; IFN-γ: gamma-interferon; IL-17A: interleukin 17A; IL-10: interleukin 10; IL-6: interleukin 6; IL-4: interleukin 4; IL-2: interleukin 2.

inflammatory drugs use. In patients with CD, the most common location of involvement was the terminal ileum (33%). The most frequent pattern or phenotype of the disease was inflammatory, observed in 45% of patients. When evaluating the presence or not of clinical activity through the HBI, more than half of the patients (56.7%) with CD were in clinical remission, defined as HBI ≤4.

Regarding UC, the most common pattern of involvement was pancolitis or extensive colitis (57.1%). As for the assessment of disease activity in patients with UC, 77.2% of them were in clinical remission (≤3 daily evacuations and absence of blood in the stool); when the SCCAI activity score was applied, 59.2% of the patients were in clinical remission.

Serum zonulin Concentration in individuals non-IBD

In the control group, the median serum concentration of zonulin was 96.61 ng/mL. There were no significant differences in median zonulin levels when comparing IBD patients with the control group (95.28 ng/mL vs 96.61 ng/mL, *P*=0.573) (FIGURE 1).

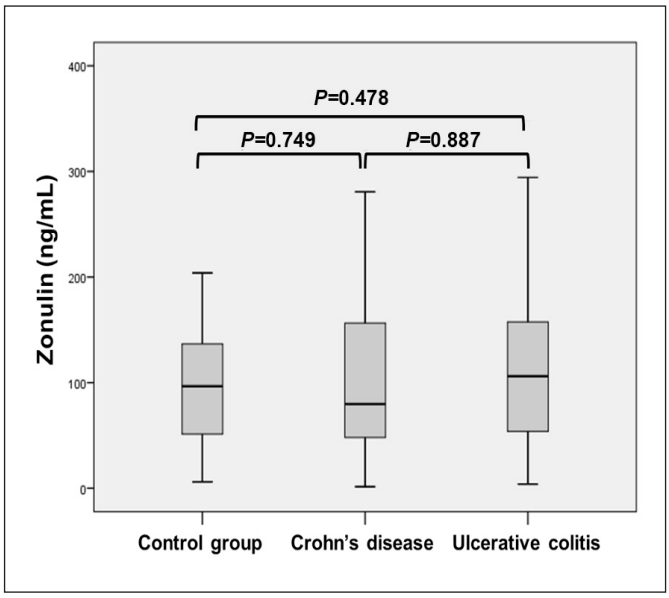

FIGURE 1. Comparison of serum zonulin levels according to study groups. No differences were observed when comparing control group with Crohn's disease patients (*P*=0.749) and with ulcerative colitis group (*P*=0.478). Likewise, zonulin levels were similar in both groups with IBD (*P*=0.887).

Serum zonulin concentration in all patients with IBD

In patients with IBD, zonulin levels were significantly higher among females when compared to males (126.05 ng/mL vs 46.65 ng/mL, *P*<0.001). No differences were observed when comparing patients with CD to those with UC (79.68 ng/mL vs 106.10 ng/mL, *P*=0.887). Likewise, no differences were noted for zonulin levels according to history of IBD-related previous surgical procedures (66.62 ng/mL vs 106.95 ng/mL, *P*=0.118) or hospitalizations (90.03 ng/mL vs 97.63 ng/mL, *P*=0.814).

Spearman correlation analysis was performed between serum zonulin concentrations and demographic, clinical, and laboratory variables (TABLE 2). In the IBD group as a whole, zonulin was positively correlated with age (r_s=0.184; *P*=0.047) and BMI (r_s=0.573; *P*<0.001). On the other hand, a weak negative correlation was observed between zonulin and hemoglobin (r_s=-0.254; *P*=0.006) and hematocrit (0.252; *P*=0.006). No correlations were observed between zonulin and circulating cytokines.

Serum zonulin concentration in patients with UC

Regarding the 57 patients with UC, serum zonulin presented a weak negative correlation with hemoglobin (r_s=-0.289; *P*=0.029), hematocrit (r_s=-0.299; *P*=0.024), and albumin values (r_s=-0.344; *P*=0.009) (TABLE 2). Conversely, zonulin levels positively correlated with BMI (r_s=0.412; *P*=0.002), CRP (r_s=0.299; *P*=0.024), and with the SCCAI clinical activity index (r_s=0.271; *P*=0.041). No correlations were observed between zonulin and circulating cytokines in UC patients.

Serum zonulin concentration in patients with CD

Among the 60 patients diagnosed with CD, significant positive correlations were observed between zonulin and age (r_s=0.349; *P*=0.006) and BMI (r_s=0.679; *P*<0.001) (TABLE 2). No significant correlations were observed between zonulin and other studied variables, including HBI and circulating cytokines.

TABLE 2. Correlation between serum zonulin concentration and the variables age, body mass index, and laboratory parameters in patients with inflammatory bowel disease.

Variable	IBD zonulin		UC zonulin		CD zonulin	
	r_s	*P*	r_s	*P*	r_s	*P*
Age	0.184	0.047	-0.003	0.981	0.349	0.006
BMI	0.573	<0.001	0.412	0.002	0.679	<0.001
Hemoglobin	-0.254	0.006	-0.289	0.029	-0.223	0.087
Hematocrit	-0.252	0.006	-0.299	0.024	-0.227	0.081
CRP	0.142	0.126	0.299	0.024	0.016	0.903
Albumin	-0.179	0.054	-0.344	0.009	-0.042	0.750
TNF	-0.084	0.374	-0.001	0.994	-0.164	0.216
IFN-γ	-0.042	0.657	0.016	0.905	-0.075	0.573
IL-17A	0.155	0.100	0.195	0.153	-0.114	0.391
IL-10	-0.066	0.486	-0.032	0.816	-0.102	0.443
IL-6	0.047	0.617	0.160	0.245	-0.045	0.738
IL-4	-0.116	0.219	-0.046	0.738	-0.121	0.360
IL-2	-0.116	0.220	-0.061	0.658	-0.169	0.200
SCCAI	-	-	0.271	0.041	-	-
HBI	-	-	-	-	0.108	0.412

IBD: inflammatory bowel disease; UC: ulcerative colitis; CD: Crohn's disease; BMI: body mass index; SCCAI: Simple Clinical Colitis Activity Index; HBI: Harvey-Bradshaw index; CRP: C-reactive protein; TNF: Tumor necrosis factor alpha; IFN: interferon; IL: interleukin; SCCAI: Simple Clinical Colitis Activity Index; HBI: Harvey-Bradshaw index.

DISCUSSION

The burden of inflammatory bowel disease is increasing globally and there is a great need for new tools to help manage these patients. Increased intestinal permeability is an important event in several chronic inflammatory conditions, and is thought to play a major role in IBD. Therefore, finding a simple noninvasive test to evaluate gut permeability might contribute for a better understanding of the disease and lead to an improvement in clinical management.

In the present study we evaluated serum zonulin levels, a marker of intestinal permeability, in 117 patients with IBD followed at our outpatient clinic. Among patients with CD, we observed that the most frequently affected location was the terminal ileum (L1), with 33% of cases. In turn, when the behavior of the disease was evaluated, the most prevalent was inflammatory (B1), which was observed in 45% of cases. More than half of the patients with CD (56.6%) were in clinical remission of the disease, demonstrating an IHB score ≤4 points. Through these characteristics, we concluded that our sample of patients with CD consisted mainly of patients with a less severe disease and with a lesser extent of the disease, resulting in a lower score in the disease activity score. On the other hand, when considering the diagnosis of UC, the pattern of involvement most commonly found in this series was pancolitis (E3), a finding compatible with the profile of patients who are expected to be followed up in a tertiary medical center[11].

In the present study, no differences in zonulin levels were observed when IBD patients were compared to controls, and when UC patients were compared to CD. In a recent study, Caviglia et al. evaluated 118 IBD patients and observed similar zonulin levels when comparing UC and CD[12]. However, contrary to what we observe here, in the Italian study significantly higher zonulin levels were observed in IBD subjects when compared to healthy controls[12]. This difference can be explained by the distinct profiles of the cohorts evaluated in both studies. Here, more than half of patients were in remission in contrast with only 20% in the Italian cohort.

When evaluating the IBD group as a whole, a moderate positive correlation was observed between serum zonulin and BMI. A previous study evaluating one hundred and twenty-three Caucasian men without IBD demonstrated that zonulin levels increase in parallel with BMI, waist/hip ratio (an important marker of visceral adiposity and insulin resistance), fasting insulin concentrations, triglycerides, uric acid, and IL-6 concentrations. On the other hand, zonulin concentrations were negatively correlated with HDL-cholesterol concentrations and with insulin sensitivity[13]. Obesity, per se, seems to influence intestinal permeability – some studies in animal models have already demonstrated that a high saturated fat diet results in changes in the intestinal microbiota and in an increase in intestinal permeability[14]. This increase in intestinal permeability, in turn, contributes to the installation of a low-grade inflammatory state and insulin resistance[15].

Zonulin levels were also positively correlated with age in IBD patients. This finding is similar to a study including 37 healthy subjects where zonulin levels were 22% higher among older adults as compared to young ones[16]. Although the data is limited, these findings may indicate intestinal permeability may contribute, at least partially, to age-related increases in chronic inflammation and associated disorders.

When UC patients were evaluated separately, zonulin concentrations correlated with variables classically associated with disease severity and activity, such as SCCAI score, CRP, albumin, hemoglobin, and hematocrit concentrations. Such associations were not observed among patients with CD, as no significant correlations were observed between zonulin and variables related to IBD severity, including IHB. It is possible to hypothesize that this result might be attributed to the characteristics of our patients with UC; the most common pattern of involvement was pancolitis (E3), representing, a priori, a more extensive and severe disease and, conceivably, more symptomatic. On the other hand, CD is characterized by greater heterogeneity and, in general, smaller segments of the gastrointestinal tract are affected, possibly with less impact on intestinal permeability. There are few studies evaluating the relationship between circulating zonulin levels and clinical activity in IBD. Caviglia et al. found no correlation between zonulin levels and clinical activity index[12]. However, results were not reported separately for UC and CD. On the other hand, a more recent study from the same group found that serum zonulin levels were higher among IBD patients in active phase, although, again, the results were not evaluated according to disease phenotypes[17].

It is important to emphasize that the evaluation of intestinal permeability can be performed using other methods, the main one being based on the ingestion of solutes with different absorptive properties and their subsequent measurement in the urine. The main test used in clinical studies is the lactulose/mannitol excretion ratio, which is considered a non-invasive test, but it is difficult to perform due to the need for prolonged fasting and the need for periodic collection of urine[18]. A previous study evaluated lactulose/mannitol excretion ratio among subjects with CD and celiac disease found that changes in barrier function seem to persist, even among CD patients in clinical remission[19]. Also, a Finnish study observed that the lactulose-mannitol ratio correlated positively with endoscopic disease activity, but correlations with clinical index or CRP were

poor[20]. The assessment of serum zonulin would have the advantage that, in addition to being non-invasive, it is easier to perform.

We acknowledge some limitations to our analysis. First, given its cross-sectional nature, the relationship between the studied biomarkers and relevant clinical outcomes cannot be addressed. Secondly, regular alcohol consumption was not considered exclusion criteria from the study, even though it was previously demonstrated to affect gut permeability. However, the effect of alcoholism on zonulin levels appears to be limited, as previously demonstrated by a study including subjects with alcohol use disorder[21]. Finally, the studied sample consisted mostly of patients in clinical remission of IBD, which may have made it difficult to analyze the impact of disease activity on serum zonulin concentrations. Studies that will investigate zonulin concentrations in different profiles of patients with IBD, especially including endoscopic and histological analysis, will be important to better define the role of this biomarker in IBD.

In conclusion, in this cohort, zonulin levels were associated with variables not related to the baseline disease such as sex and BMI. Zonulin also correlated with clinical and laboratory parameters of disease severity and activity among subjects with UC, but not in CD. Nonetheless, serum zonulin was not correlated with circulating cytokines. These findings indicate a potential role for zonulin as a biomarker in IBD, particularly in UC.

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Authors' contribution

Lacombe LAC: study design, clinical data collection, survey execution, writing of the text, statistical analysis, approval of the final version of manuscript. Matiollo C: Blood sample handling, execution of laboratory tests. Rosa JS, Felisberto M, and Dalmarco EM: execution of laboratory tests. Schiavon LL: study design, survey execution, writing and critical review of text, statistical analysis, approval of the final version of manuscript.

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RESUMO – Contexto – A doença inflamatória intestinal (DII) compreende o espectro entre a doença de Crohn (DC) e a colite ulcerativa, condição esta cuja prevalência em países como o Brasil vem aumentando significativamente nos últimos anos. Alterações na função da barreira epitelial intestinal e, consequentemente, um aumento da permeabilidade intestinal, têm sido sugeridos como fatores importantes envolvidos na patogênese de diferentes condições autoimunes, dentre elas, a DII. Desta forma, existe a necessidade de uma ferramenta prática para avaliar a integridade da barreira epitelial intestinal nestes pacientes. **Objetivo –** Estudar os fatores associados com os níveis séricos de zonulina, um marcador da permeabilidade intestinal, em pacientes com DII. **Métodos –** Estudo observacional transversal que incluiu 117 pacientes com DII e 32 indivíduos que compuseram o grupo controle. A atividade da doença foi avaliada pelo *Simple Cliniical Colitis Activity Index* (SCCAI) na colite ulcerativa e pelo índice de Harvey-Bradshaw (IHB) em pacientes com DC. Os níveis de zonulina foram quantificados por ELISA e os níveis das citocinas inflamatórias pelo *Cytometric Bead Array*, utilizando kits comercialmente disponíveis. **Resultados –** A média de idade dos pacientes com DII foi de 44,0±15,9 anos, 66,7% eram do sexo feminino, 57 pacientes eram portadores de DC e 60 pacientes eram portadores de colite ulcerativa. No momento da avaliação clínico-laboratorial, 56,7% dos pacientes com DC encontravam-se em remissão clínica e, dentre os pacientes com colite ulcerativa, 59,2% deles assim se encontravam. Não foram observadas diferenças nos níveis séricos de zonulina entre pacientes com DII e grupo controle (95,28 ng/mL vs 96,61 ng/mL; *P*=0,573), assim como entre pacientes com DC e pacientes com colite ulcerativa (79,68 ng/mL vs 106,10 ng/mL, *P*=0,887). Dentre os pacientes com DII, as concentrações de zonulina foram mais elevadas no sexo feminino e correlacionaram-se positivamente com o índice de massa corporal (IMC) e com a idade, correlacionando-se negativamente com os níveis de hemoglobina e hematócrito. Nos pacientes com colite ulcerativa, as concentrações de zonulina correlacionaram-se negativamente com os parâmetros hemoglobina, hematócrito e albumina e, positivamente, com o IMC e com o SCCAI. Dentre os pacientes com DC, a zonulina sérica correlacionou-se positivamente com a idade e com o IMC, mas não com o IHB. Não foram observadas correlações entre os níveis de zonulina e as citocinas circulantes nos pacientes com DII. **Conclusão –** Nesta coorte constituída majoritariamente por pacientes em remissão clínica, os níveis séricos de zonulina não se mostraram aumentados em pacientes com DII em relação a indivíduos controles e correlacionaram-se com variáveis não relacionadas à doença de base, como com o sexo, com a idade e com o IMC. No entanto, os níveis séricos de zonulina correlacionaram-se com parâmetros clínicos e laboratoriais de gravidade e atividade da doença dentre os pacientes com colite ulcerativa, mas não dentre os pacientes com DC. Estes achados indicam um potencial papel da zonulina sérica como um biomarcador na DII, principalmente na colite ulcerativa.

Palavras-chave – Doença inflamatória intestinal; permeabilidade intestinal; zonulina; citocinas inflamatórias; atividade da doença.

REFERENCES

1. Windsor JW, Kaplan GG. Evolving Epidemiology of IBD. Curr Gastroenterol Rep. 2019;21:40.
2. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev. 2011;91:151-75.
3. 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.
4. Farquhar MG, Palade GE. Junctional complexes in various epithelia. J Cell Biol. 1963;17:375-412.
5. Odenwald MA, Turner JR. The intestinal epithelial barrier: a therapeutic target? Nat Rev Gastroenterol Hepatol. 2017;14:9-21.
6. 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.
7. Harvey RF, Bradshaw JM. Methods and devices – A simple index of Crohn's-disease activity. Lancet. 1980;315:514.
8. Walmsley RS, Ayres RC, Pounder RE, Allan RN. A simple clinical colitis activity index. Gut. 1998;43:29-32.
9. Turner D, Seow CH, Greenberg GR, Griffiths AM, Silverberg MS, Steinhart AH. A systematic prospective comparison of noninvasive disease activity indices in ulcerative colitis. Clin Gastroenterol Hepatol. 2009;10:1081-8.
10. Best WR. Predicting the Crohn's disease activity index from de Harvey-Bradshaw Index. Inflamm Bowel Dis. 2006;12:304-10.
11. Gomes TNF, de Azevedo FS, Argollo M, Miszputen SJ, Ambrogini O Jr. Clinical and Demographic Profile of Inflammatory Bowel Disease Patients in a Reference Center of São Paulo, Brazil. Clin Exp Gastroenterol. 2021;17:91-102.
12. Caviglia GP, Dughera F, Ribaldone DG, Rosso C, Abate ML, Pellicano R, et al. Serum zonulina in patients with inflammatory bowel disease: a pilot study. Minerva Med. 2019;110:95-100.
13. Moreno-Navarrete JM, Sabater M, Ortega F, Ricart W, Fernández-Real JM. Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance. PLoS One. 2012;7:e37160.
14. Cani PD, Bibiloni R, Knauf C, Waget A, Neytrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes. 2008;57:1470-81.
15. Genser L, Poitou C, Brot-Laroche É, Rousset M, Vaillant JC, Clément K, et al. L'altération de la perméabilité intestinale : chaînon manquant entre dysbiose et inflammation au cours de l'obésité ? [Alteration of intestinal permeability: the missing link between gut microbiota modifications and inflammation in obesity?]. Med Sci. 2016;32:461-9.
16. Qi Y, Goel R, Kim S, Richards EM, Carter CS, Pepine CJ, et al. Intestinal permeability biomarker zonulin is elevated in healthy aging. J Am Med Dir Assoc. 2017;18:810.e1-810.e4.
17. Caviglia GP, Rosso C, Stalla F, Rizzo M, Massano A, Abate ML, et al. On-treatment decrease of serum interleukin-6 as a predictor of clinical response to biologic therapy in patients with inflammatory bowel diseases. J Clin Med. 2020;9:800.
18. Schoultz I, KEITA ÅV. The Intestinal Barrier and Current Techniques for the Assessment of Gut Permeability. Cells. 2020;9:1909.
19. Vilela EG, Torres HOG, Ferrari MLA, Lima AS, Cunha AS. Gut permeability to lactulose and mannitol differs in treated Crohn's disease and celiac disease patients and healthy subjects. Braz J Med Biol Res. 2008;41:1105-9.
20. Halme L, Turunen U, Tuominen J, Forsstrom T, Turpeinen U. Comparison of iohexol and lactulose-mannitol test as markers of disease activity in patients with inflammatory bowel disease. Scand J Clin Lab Invest. 2000;60:695-701.
21. Donnadieu-Rigole H, Pansu N, Mura T, Pelletier S, Alarcon R, Gamon L, et al. Beneficial effect of alcohol withdrawal on gut permeability and microbial translocation in patients with alcohol use disorder. Alcohol Clin Exp Res. 2018;42:32-40.