Positive association between leptin serum levels and disease activity on endoscopy in inflammatory bowel disease: A case-control study

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Abstract. Inflammatory bowel disease (IBD) includes ulcerative colitis (UC), Crohn's disease (CD) and indeterminate colitis. As these subtypes of IBD display important differences in the behavior of the natural course of the disease, the identification of non-invasive markers for IBD is important. The aim of the present study was to evaluate the serum levels of 10 adipokines and their association with endoscopic activity in IBD. The 10-protein profile (C-peptide, ghrelin, gastric inhibitory polypeptide, glucagon-like peptide-1, glucagon, insulin, leptin, plasminogen activator inhibitor-1, resistin and visfatin) was evaluated using serum from 53 participants (23 UC and 11 CD patients, as well as 19 controls) from Zacatecas (Mexico) by using the Bio-Plex Pro Human Diabetes 10-Plex Panel (Bio-Rad Laboratories, Inc.). Compared with those in the controls, leptin levels were significantly lower in patients with IBD ($P=4.9x10^{-4}$). In addition, serum leptin displayed differences between groups with and without disease activity on endoscopy ($P<0.001$). Among the study population, serum leptin levels of <5,494 pg/ml significantly increased the odds of IBD by 12.8-fold [odds ratio (OR)=12.8, 95% confidence interval (CI)=3.04-53.9, P=0.001]. In addition, patients with serum leptin levels of <2,498 pg/ml displayed 5.8-fold greater odds of disease activity on endoscopy among the study population (OR=5.8, 95% CI=1.52-22.4, P=0.013). No differences in the serum levels of the remaining proteins were identified between the groups. Among the study population, serum leptin was associated with an increased risk of IBD and with disease activity on endoscopy. Additional studies will be necessary to validate the use of leptin as a non-invasive biomarker of IBD severity.

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Introduction

Inflammatory bowel disease (IBD) involves ulcerative colitis (UC), Crohn's disease (CD) and indeterminate colitis. These subtypes of IBD display important differences in the behavior of the natural course of the disease, including frequent remissions and exacerbations, response to treatment and complications. Early diagnosis is critical for proper treatment (1). In Mexico, in the last 10 years there has been an increase with ~76 new UC cases per year, which is a dramatic increase compared with the previous decade that recorded an average of 28 cases per year, accumulating 150,000 cases in Mexico (2,3). Despite increased use of immunosuppressive therapy, the long-term risk of required intestinal resection and permanent ileostomy in CD is ~80 and 10%, respectively (4). In UC patients, the risk of required colectomy is ~1% per year according to population-based cohort studies in Northern Europe (5).

Remission in UC is defined as complete resolution of symptoms and endoscopic mucosal healing, whereas in CD, a clinical remission is considered as one with a clinical

disease activity index (CDAI) of <150 (6-8). In CD and UC, the early use of aggressive therapy, including the combination of thiopurines and anti-tumor necrosis factor-α (anti-TNF-α) is considered, with the aim of achieving deep and sustained remission (9). However, certain CD patients present with localized and uncomplicated (no perforation, no stricture) disease at diagnosis. Similarly, UC may manifest without disabling symptoms, biological abnormalities or severe endoscopic lesions at diagnosis. In those patients, the early and prolonged use of immunosuppressive therapy (anti-TNF-α), with its associated risk of serious infections and cancer, may not be appropriate, as the spontaneous evolution of the disease may have been benign (10,11).

The risk of over-treating patients may be reduced by accurately diagnosing them with a combination of clinical and endoscopic examination, as well as detection of serological markers present in different stages of the disease, which may predict the subsequent course of IBD; however, serological biomarkers for the severity of IBD have been far lees studied in UC than in CD, and it has been rarely applied in the clinical setting (12).

TNF-α is a cytokine involved in the regulation of a wide spectrum of biological processes, including cell proliferation, differentiation and apoptosis, as well as coagulation and lipid metabolism. TNF-α may decrease the appetite, body weight and body mass index (BMI), and induce the synthesis of catabolic hormones, including insulin-like growth hormone-1, and increase lipolysis in adipose tissue (13). As adipocytes have been recognized to actively participate in systemic immune responses via the secretion of peptides detectable in the systemic circulation, the so-called adipocytokines (adipokines) (14-16), increased lipolysis by increased TNF-α production results in impaired production of certain adipokines, such as leptin (13). In metabolism, the plasma levels of leptin serve as a signal of energy sufficiency to the hypothalamus, resulting in anorexia and increased energy expenditure when fat stores are exceeded (17). During inflammation, leptin may falsely signal an excess of fat mass to the hypothalamus and drive an inappropriate physiological response. In addition, if the TNF-α concentration diminishes when the immunologic response and inflammation decrease due to treatment with anti-TNF-α drugs, including infliximab (17-19), the deregulation of leptin by the use of biological therapy may be expected. Therefore, the regulation of leptin and other adipokines and its physiological consequences have been examined in human inflammatory diseases, including IBD (19-21). In one of these studies, Waluga *et al* (21), investigated serum adipokine levels (transforming growth factor-β1, adiponectin, leptin, chemerin, resistin and visfatin) in patients with IBD prior treatment and after achieving clinical remission. Their results suggested that IBD modulated serum adipokine levels by increasing resistin and visfatin release and suppressing leptin production. These authors proposed leptin concentrations in CD and UC subjects, may be the result of TNF-α hyperactivity leading to a decrease in leptin mediated chronic inflammation. Accordingly, the aim of the present study was to evaluate the serum levels of 10 adipokines and their association with disease activity on endoscopy in IBD. The modulation of the adipokines by IBD therapy was also evaluated.

Materials and methods

Participants and biological samples. A case-control study consisting of non-related subjects from the Zacatecas state in Mexico was performed. Subjects were recruited from the Gastroenterological service of the Zacatecas Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado (ISSSTE) General Hospital (Zacatecas, Mexico) between July and October 2016. The protocol was approved by the Committee on Education, Research, Training and Ethics of ISSSTE General Hospital (approval ID, OFC226/2016-2-001). All participants provided written informed consent for their participation in the study, in accordance with the Helsinki declaration. IBD was diagnosed according to clinical, endoscopic and pathological criteria as previously described (18). All patients with previous diagnoses of IBD were included in the case group (n=34; UC, n=23; CD, n=11). All the cases at the time of recruitment had already received pharmacological therapy according with the World Gastroenterology Organization Global guidelines (22). The control group (n=19) consisted of healthy subjects who were screened due to indications of colon cancer in accordance with the World Gastroenterology Organization guidelines (23) and absence of treatment with anti-inflammatory drugs. In the two groups, participants with comorbidities, including diabetes or autoimmune diseases, or with any associated inflammatory or infectious diseases, including tuberculosis, cytomegalovirus infection or urinary tract infection, were excluded.

Each of the participants donated a blood sample at the moment of their recruitment. Subsequently, they underwent a colonoscopy procedure according the guidelines of the American Society for Gastrointestinal Endoscopy (24). Tissue samples from colon segments were obtained for histopathologic evaluation using Multibite™ biopsy forceps (Boston Scientific, Boston, MA, USA) (24). Blood samples were centrifuged at 1,000 x g for 15 min at room temperature (RT). Serum was collected, aliquoted and stored at -80˚C until use. Epidemiological and clinical data, including clinical activity/disease activity on endoscopy by appropriate scales [UC Mayo endoscopic score (UC-MES), UC endoscopic index of severity (UCEIS), UC/CD Montreal classification, CD activity index (CDAI), simple endoscopic scale for CD (SES-CD) and Truelove-Witts score] (25,26), date of diagnosis, initial and current treatment, phenotype, extraintestinal manifestations of the disease and laboratory parameters, were obtained from clinical records. The presence of endoscopic activity for each IBD subtype was defined as the UC-MES and the UCEIS suggesting at least the mild stage for UC, and at least score 1 of the SES-CD and B2 of the Montreal classification for CD. The size of mucosal ulcers, ulcerated surface, endoscopic extension and stenosis features were evaluated.

Marker quantification. The levels of 10 biomarkers, C-peptide, ghrelin, gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP), glucagon (GCG), insulin (INS), leptin (LEP), total plasminogen activator inhibitor-1 (PAI-1), resistin (RETN) and visfatin, were analyzed using the Bio-Plex Pro Human Diabetes 10-Plex Panel (Bio-Rad Laboratories, Hercules, CA, USA). Serum quantification was performed as follows: Samples (aliquots of 200 μl) were centrifuged at 30,000 x g for

5 min at RT to remove any precipitate. The appropriate analyte standards and samples were diluted in standard diluent and sample diluent, respectively. A standard curve composed of eight points was prepared from the recombinant analyte standard. Standards, blanks and samples were added to a 96-well plate containing antibodies that were chemically attached to fluorescent-labeled microbeads. The samples were incubated in the dark at room temperature in constant motion for 1 h. The plate was washed three times, a detection antibody was added to each well, and the plate was incubated in the dark for 30 min at RT with agitation, followed by three washes. Streptavidin-phycoerythrin was added to each well and the plate was incubated in the dark for 10 min at RT with agitation. The beads were re-suspended in 125 μl buffer, and the reaction was quantified using the BioPlex®200 Multiplex System platform (Bio-Rad Laboratories). Each sample was analyzed in duplicate and the data were automatically analyzed and processed using Bio-Plex Manager 6.1 software (Bio-Rad Laboratories).

Statistical analysis. Risk factors and clinical and personal characteristics were compared using a Chi-square or Fisher's exact test for categorical variables, and a Student's t-test, Mann-Whitney U test or analysis of variance (ANOVA) as appropriate, for numerical variables. ANOVA was coupled to Holm-Sidak or Dunn's Method as appropriate for the multiple comparison procedure. The usefulness of serum leptin levels to correctly classify the study groups according to their disease status was evaluated using a receiver operating characteristic curve (ROC) analysis. In this analysis, leptin sensitivity and specificity values were used to calculate the related area under the curve and the positive and negative predictive values at fixed protein concentration cutoffs. Each cutoff value for serum leptin level was obtained considering the ROC curve in which the value of the sum of sensitivity and specificity was maximal (sensitivity + specificity closest to two). The odds ratios with Yates continuity correction were calculated for significant comparisons. To evaluate the correlation between two variables, a Spearman Rank Order Correlation test was performed. $P<0.05$ was considered to indicate a statistically significant difference. Data analysis was performed using Sigma Plot v.11 (Systat Software Inc., San Jose, CA, USA) and GraphPad Prism v.5.03 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Patient characteristics. A total of 53 participants were enrolled in the present study, including 34 patients diagnosed with IBD (UC, 23; CD, 11) and 19 healthy controls. General data and clinical characteristics of the study population are listed in Tables I and II. The median of age was 59 years (range, 26-78) for the cases and 54 (range, 31-49) years for the controls ($P=0.312$). There were no differences between the study groups in terms of risk factors and/or clinical variables, including gender, family history of IBD, smoking, BMI, hemoglobin, glucose, triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very LDL (VLDL) or systolic/diastolic blood pressure ($P>0.05$).

Table I. Classification of patients with inflammatory bowel disease (n=34).

Item	N (%)
Diagnosis	
UC	23 (67.6)
CD	11 (32.4)
Mayo endoscopic activity	
Remission	8 (23.5)
Mild	14 (41.2)
Moderate	1 (2.9)
Severe	0 (0)
Montreal UC	
E1	7 (20.6)
E2	8 (23.5)
E3	8 (23.5)
UCEIS	
Remission	8 (23.5)
Mild	14 (41.2)
Moderate	1 (2.9)
Severe	0 (0)
Truelove-witts clinical	
Remission	14 (41.2)
Mild	6 (17.6)
Moderate	3 (8.8)
Severe	0 (0)
CDAI	
<150	11 (32.4)
150-220	0 (0)
220-450	0 (0)
>450	0 (0)
Montreal CD	
Location	
SB	3 (8.8)
Colon	4 (11.8)
SB-colon	4 (11.8)
Upper GI	0 (0)
Behavior	
Inflammatory	6 (17.6)
Stricturing	2 (5.9)
Fistulizing	3 (8.8)
Endoscopy simple CD score	
0	5 (14.7)
1	5 (14.7)
2	1 (2.9)
3	0 (0)

UC, ulcerative colitis; CD, Crohn's disease; E, extension; UCEIS, ulcerative colitis endoscopic index of severity; CDAI, Crohn's disease activity index; GI, gastro-intestinal; SB, small bowel.

IBD and endoscopic activity. In the case group, the clinical activity in UC patients according to the Truelove-Witts scale was as follows: 14 patients were in remission, 6 had mild activity and 3 had moderate activity. In the group of CD patients, the

Table II. Comparison of clinical parameters between the study groups.

Characteristic	IBD (n=34)	Control (n=19)	P-value
Age (years)	54.8±15.1	53.2±9.6	0.312
Gender (female/male ratio)	1.43	5.3	0.111
Family history of IBD	1 (2.9%)	1 (5.2%)	1.000
HDL (mg/dl)	45.2±12.3	45.7±12.5	0.905
LDL (mg/dl)	110.5±27.6	123.2±32.7	0.155
VLDL (mg/dl)	25.7±10.8	23.2±6.5	0.37
Triglycerides (mg/dl)	131.7±51.5	120.9±38.3	0.446
Cholesterol (mg/dl)	181.0±28.6	191.6±41.3	0.288
TIA (mg/dl)	4.0±1.1	4.3±1.1	0.374
Glucose (mg/dl)	90.2±20.2	87.7±7.7	0.953
Hemoglobin (g/dl)	14.6±1.9	14.1±1.8	0.293
Hematocrit (%)	44.2±9.5	41.0±4.3	0.108
BMI (kg/m^2)	27.6±5.9	28.4±6.5	0.711
SBP (mm/Hg)	114.1±9.8	110.0±5.8	0.228
DBP (mm/Hg)	77.2±5.8	75.4±6.6	0.315
ESR (mm/h)	1.5±0.51	1.3±0.49	0.322
CRP (mg/l)	0.51±0.63	0.35±0.29	0.888

Values are expressed as the mean ± standard deviation, n (%) or ratio. HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; TIA, transient ischemic attack; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IBD, inflammatory bowel disease.

Table III. Serum concentrations of the ten proteins evaluated in the IBD and control groups.

Analyte	IBD (n=34)	Control (n=19)	P-value
C-peptide (pg/ml)	972.3±512.2	975.4±281.0	0.414
Ghrelin (pg/ml)	3,781.5±1,498.4	3,471.4±878.9	0.781
GIP (pg/ml)	524.9±381.9	426.4±197.6	0.549
GLP-1 (pg/ml)	834.9±126.9	817.5±46.7	0.838
Glucagon (pg/ml)	1,155.4±118.9	1,119.4±64.9	0.182
Insulin (pg/ml)	961.8±306.6	911.8±236.1	0.656
Leptin (pg/ml)	5,039.2±5,219.8	8,847.6±4,044.2	$4.9x10^{-4a}$
PAI-1 (pg/ml)	78,984.5±58,986.9	82,870.7±38,624.1	0.541
Resistin (pg/ml)	4,491.8±1,984.1	5,131.0±3,061.5	0.738
Visfatin (pg/ml)	11,622.4±3,6291.3	4,494.1±883.1	0.656

[a]P<0.05. Serum levels of the proteins were determined at baseline. Values are expressed as the mean ± standard deviation. IBD, inflammatory bowel disease; GIP, gastric inhibitory polypeptide; GLP, glucagon-like peptide-1, PAI-1, total plasminogen activator inhibitor-1.

clinical activity was determined as <150 points on the CDAI scale. In the UC group, maximal endoscopic involvement (E1) was observed in 7 cases, E2 was observed in 8 subjects and the E3 stage was observed in the remaining 8 UC cases. The location of CD according to the Montreal classification was in the small bowel for 3 participants, in the colon for 4 cases and in the colonic ileum for the remaining 4 CD cases. Evaluation of the endoscopic activity of UC according to the UC-MES indicated that 8 cases were in remission, 14 had mild activity and one had moderate endoscopic activity. In CD patients, no endoscopic activity (SES-CD score=0) was observed in 5 patients, while 5 and 1 patients displayed activity with score of 1 and 2, respectively (Table I). Extraintestinal manifestations were observed in 4 UC cases (27.3%) and in 3 CD cases (17.4%), respectively.

Leptin levels are associated with IBD. Table III displays the results of the adipokine quantification determined at baseline in the serum of IBD patients and control subjects. Of note, the serum levels of leptin in the IBD group were significantly lower than those in the controls (5,039.2±5,219.8 vs. 8,847.6±4,044.2 pg/ml; $P=4.9x10^{-4}$). However, there

Table IV. Comparison of serum adipokine levels between the study groups.

Analyte	Control (n=19)	CD (n=11)	UC (n=23)	P-value
C peptide (pg/ml)	975.4±281.0	1,112.6±468.2	905.19±528.5	0.208
Ghrelin (pg/ml)	3,471.4±878.9	4,192.5±1,929.8	3,584.94±1,245.1	0.745
GIP (pg/ml)	426.4±197.6	635.3±506.2	469.7±301.1	0.150
GLP-1 (pg/ml)	817.5±46.7	860.4±133.0	822.71±125.0	0.292
Glucagon (pg/ml)	1,119.4±64.9	1,184.7±163.7	1,141.4±91.5	0.251
Insulin (pg/ml)	911.8±236.1	1,052.0±291.4	918.68±310.5	0.176
Leptin (pg/ml)	8,847.6±4,044.22	7,257.4±8,051.3	3,978.26±2,787.0	0.001[a]
PAI-1 (pg/ml)	82,870.7±38,624.1	109,456.2±93,303.5	64,411±23,996.3	0.090
Resistin (pg/ml)	5,131.0±3,061.5	5,189.8±2,282.6	41,58.01±1,782.3	0.457
Visfatin (pg/ml)	4,494.1±883.2	6,230.6±6,232.3	14,201.0±44,005	0.745

[a]P<0.05 Control vs. UC vs. CD (analysis of variance test). Serum levels of the proteins were determined at baseline. Values are expressed as the mean ± standard deviation. IBD, inflammatory bowel disease; GIP, gastric inhibitory polypeptide; GLP, glucagon-like peptide-1; PAI-1, total plasminogen activator inhibitor-1; UC, ulcerative colitis; CD, Crohn's disease.

were no significant differences in the serum levels of the 9 remaining adipokines (P>0.05).

When the IBD participants were stratified into UC and CD groups (Table IV), the serum levels of leptin in UC patients were significantly lower than those in CD patients (P=0.001). Leptin serum levels did not significantly differ between UC and CD groups (P>0.05). No differences in the serum levels of the other adipokines were identified between the UC, CD and healthy controls groups.

Leptin levels are associated with endoscopic activity in IBD patients. Leptin serum concentrations were determined at baseline and as presented in Fig. 1, the leptin levels were compared between patient groups stratified by the presence/absence of disease activity on endoscopy. Compared with the control group, IBD patients with and without disease activity on endoscopy had lower serum leptin levels (P<0.001). In CD (Fig. 1B) and UC patients (Fig. 1C), significant differences in serum leptin levels were observed between controls and patients with and without disease activity on endoscopy (P=0.001). Compared with those in the controls and IBD without disease activity on endoscopy, the serum leptin levels in IBD patients with positive endoscopic activity was significantly lower (P<0.001; data not shown).

To determine whether the circulating leptin concentration was affected by IBD treatment, IBD cases were stratified according to their pharmacological therapy, and the leptin levels were compared between the groups (Fig. 2A; Table V). Compared with the controls, serum leptin levels were reduced in patients treated with 5-aminosalicylic acid (5-ASA) monotherapy (P=0.008), 5-ASA + azathioprine (P=0.002) and 5-ASA + adalimumab (P=0.036). IBD participants then evaluated separately as CD (Fig. 2B) or UC groups (Fig. 2C). Compared with the controls, the serum leptin levels were significantly lower in UC patients with 5-ASA monotherapy (P=0.0015) and 5-ASA + azathioprine (P=0.002), but not in those with 5-ASA + adalimumab (P=0.165). Leptin levels in CD patients receiving various treatments were not significantly different from those in healthy controls (P>0.05). In the same sense, additional differences between pairs of treatments were not found with or without stratification of the IBD participants (P>0.05).

Table V. General description of the treatment of patients with inflammatory bowel disease.

Treatment/item	Ulcerative colitis	Crohn's disease
Current treatment		
5-ASA	15	2
5-ASA + Azathioprine	5	7
5-ASA + Adalimumab	3	2
Use of anti-TNF-α in the course of the disease		
Yes	5	2
No	15	7
Change in anti-TNF-α regimen		
Maintenance treatment	3	2
Failure	2	1
Allergic reaction	1	0
Restart treatment due to relapse	2	0
Azathioprine intolerance	0	1

5-ASA, 5-aminosalicylic acid; TNF, tumor necrosis factor.

Association of leptin with the location of IBD. Regarding the location of IBD, leptin levels were significantly decreased in UC patients with right colitis (P=4.95x10^{-7}) and pancolitis (P=0.001) compared with those in the controls. In the CD group, a significant reduction of the leptin concentration was observed in patients with disease located in the colonic ileum compared with that in the control group (P=0.001; Fig. 3). Significant differences in leptin levels between right colitis

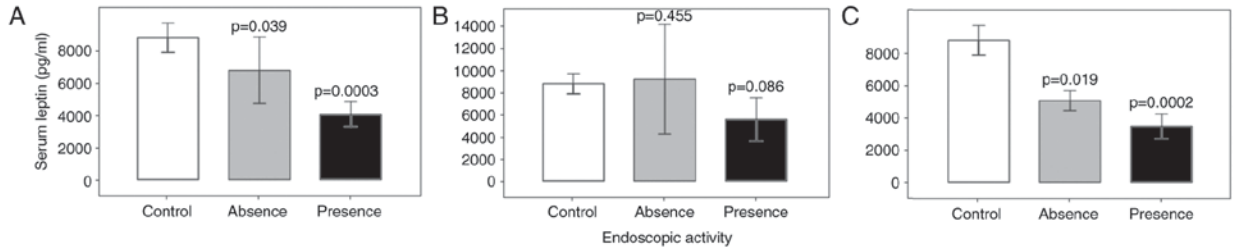

Figure 1. Serum concentrations of leptin and disease activity in groups with and without endoscopic activity. Leptin levels were determined at baseline and using the healthy control group as a reference, the serum leptin concentration (pg/ml) was compared between the patients with and without endoscopic activity in the (A) inflammatory bowel disease (B) Crohn's disease and (C) ulcerative colitis groups. Values are expressed as the mean ± standard deviation from duplicate readings.

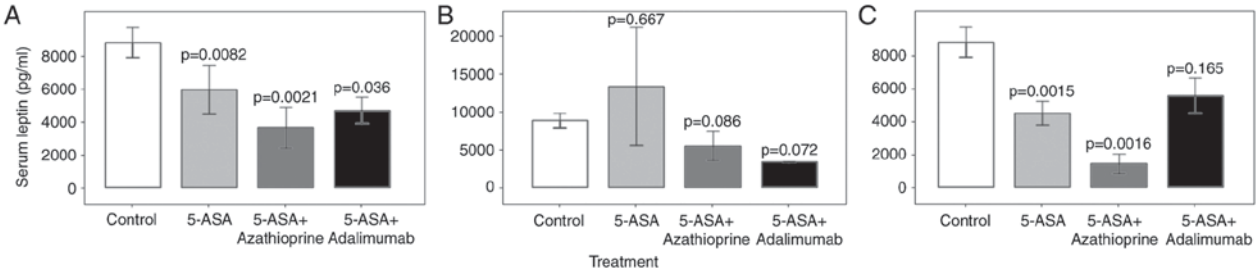

Figure 2. Modulation of the serum concentration of leptin with treatment. The IBD patients were stratified into 5-ASA, 5-ASA+azathioprine and 5-ASA+adalimumab groups according to their pharmacological therapy. The serum levels of leptin (pg/ml) in the (A) IBD, (B) Crohn's disease and (C) ulcerative colitis patients were compared with those in the control group as a reference. Values are expressed as the mean ± standard deviation from duplicate readings. IBD, inflammatory bowel disease; ASA, 5-aminosalicylic acid.

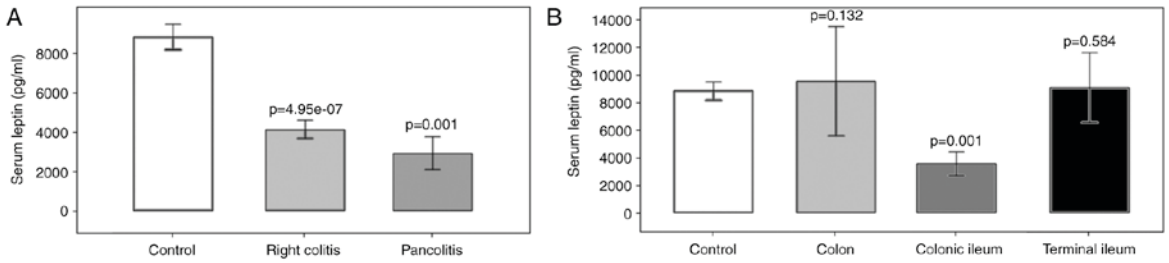

Figure 3. Association of serum leptin concentration with the extent of the disease. (A) Comparison of serum leptin concentration (pg/ml) with extent of disease in UC patients. UC patients were classified into right colitis and pancolitis groups and compared with the healthy control group. (B) Comparison of serum leptin concentration (pg/ml) with extent of disease in CD patients. CD patients were classified into colon, colonic ileum and terminal ileum and compared with the healthy control group. Values are expressed as the mean ± standard deviation from duplicate readings. UC, ulcerative colitis; CD, Crohn's disease.

and pancolitis, or colon, colonic ileum or terminal ileum were not identified (P>0.05).

Regarding the presence/absence of an extraintestinal manifestation of IBD as a classifier and using the control group as a reference, circulating leptin was lower in patients without extraintestinal manifestations (6,404.4±5,133.4 pg/ml; P=0.002). No significant differences were observed in serum leptin levels between controls and IBD cases with extraintestinal manifestations (P=0.053), or between IBD cases with or without extraintestinal manifestations (P=0.848). The serum levels of the 9 remaining markers evaluated were not affected by the presence of extraintestinal manifestations (data not shown).

Correlation of leptin with clinical parameters. To evaluate the correlation between leptin levels and clinical features of the study population, a correlation analysis was performed. A positive correlation was identified between serum leptin levels and BMI (r=+0.35, P=0.017), while a negative correlation between serum leptin levels and hemoglobin was observed (r=-0.31, P=0.026). No significant correlation between leptin levels and other clinical parameters, including C-reactive protein (CRP) or the erythrocyte sedimentation rate (ESR), were identified (P>0.05).

Circulating leptin as a marker for IBD and endoscopic activity. To evaluate the usefulness of serum leptin levels to correctly classify the study groups according to their disease status, a receiver operating characteristic (ROC) analysis was performed (Table VI). Regarding the use of serum leptin for the diagnosis of IBD with a cutoff value of 5,494 pg/ml, the sensitivity and specificity values were calculated as 71 and 84%, respectively. Serum leptin levels <5,494 pg/ml significantly increased the odds of IBD by 12.8-fold among the study population [odds ratio (OR)=12.8, 95% confidence interval (CI)=3.04-53.9,

Table VI. ROC analysis of serum leptin in IBD patients.

Parameter	IBD vs. controls	Endoscopic activity (presence vs. absence)
Area under ROC curve	0.791	0.651
Leptin cutoff (pg/ml)	5,494	2,498
Sensitivity (%)	71	45
Specificity (%)	84	88
Predictive positive value (%)	89	87
Predictive negative value (%)	38	1
Odds ratio	12.8	5.8
95% Confidence interval	3.04-53.9	1.52-22.4
P-value[a]	<0.001	0.013

[a]P-value refers to the odds ratio. ROC, receiver operating characteristic; IBD, inflammatory bowel disease.

P=0.001]. The odds of disease activity on endoscopy in patients with serum leptin levels of <2,498 pg/ml was increased 5.8-fold among the IBD patients (OR=5.8, 95% CI=1.52-22.4, P=0.013).

Discussion

The traditional assessment of IBD patients is somewhat complicated by the necessary, but rather invasive nature of evaluation, including endoscopic procedures with biopsies (27). To date, no ideal biomarker has been identified for the assessment and management of IBD. The aim of the present study was to evaluate the serum levels of C-peptide, ghrelin, GIP, GLP, glucagon, insulin, leptin, PAI-1, resistin and visfatin and their association with endoscopic activity in IBD. These proteins are produced by white adipose tissue (WAT), which functions not only as a reservoir of free fatty acids (energy source) but also as an endocrine organ, sending out and responding to signals that modulate appetite, energy expenditure, insulin sensitivity, the endocrine and reproductive systems, bone metabolism and immunity. Accordingly, WAT and its signaling molecules provide an important link between obesity, insulin resistance and inflammatory disorders (28,29). In the present study, among the 10 adipokines evaluated, leptin levels were significantly lower in patients with IBD compared with those in healthy controls. Leptin, a 16-kDa polypeptide encoded by the *ob* gene, is mainly produced by adipocytes in adipose tissue, and at lower levels by fundic epithelium of peripheral tissues including that of the gastric mucosa, skeletal muscle, lymph node, liver, thyroid, placenta and spinal cord (30). The most important functions of leptin are inhibition of appetite and modulation of immune and inflammatory reactions. The results of the present study are in agreement with those reported by Waluga *et al* (21) from 2014 and Karmiris *et al* (31) from 2006, whose case-control studies indicated decreased serum leptin levels in subjects with the two types of IBD compared with those in healthy controls. However, in other previous studies, serum leptin levels were reported to increase (32) or remain unchanged (33,34) in IBD patients compared with those in healthy controls. Despite the discrepancy in serum leptin levels, an increase in leptin in close proximity to the site of inflammation has been consistently reported, with a significant increase in mRNA expression and secretion of leptin from mesenteric adipose tissue of CD and UC patients compared with that in controls (35,36). At the tissue level, where protein production reflects local cellular behavior, the circulating concentrations of molecules represent the total contribution of the body tissues and therefore, they are useful to identify additional changes associated with the general health state of the patient or with other external variables. As observed in the present study, leptin levels were associated with the BMI, the extent of the affected area, disease activity and/or treatment. Accordingly, the discrepancies observed in serum leptin concentrations between studies may be explained in part by the differences in those features between the populations evaluated.

In the present study, when the participants were stratified according to the presence/absence of endoscopic activity, the serum leptin levels in the group with disease activity on endoscopy were significantly decreased relative to those in the controls, as well as in patients with vs. without disease activity on endoscopy, suggesting the involvement of a defective regulation of the leptin pathway in the pathogenesis of IBD. When the IBD participants were stratified into CD and UC groups and compared with the controls, differences in serum leptin levels were observed for the UC group but not for the CD group, reflecting differences in the molecular mechanisms for the two types of IBD. Although these results should be validated in other IBD cohorts with large patient numbers, the present study obtained a 12.8-fold increased odds of IBD among the study population when a cutoff for serum leptin levels <5,494 pg/ml was chosen. In addition, regarding the presence or absence of disease activity on endoscopy, serum leptin levels with cutoff <2,498 pg/ml provided a 5.8-fold increased odds of disease activity on endoscopy among the IBD patients, suggesting that the leptin concentration may represent an attractive marker to consider in IBD risk determination. It is important to note that in the study population, the known non-invasive biomarkers of IBD (CRP and ESR) had normal values in most of the participants, and therefore, its classifier value for disease activity on endoscopy was not comparable with that of leptin. Additional studies are therefore required to evaluate this comparison.

In the present study, a significant decrease in leptin levels was identified between the control and different treatment groups (5-ASA, 5-ASA + azathioprine, 5-ASA + adalimumab). Of note, when IBD patients were evaluated separately as CD or UC groups, differences in serum leptin levels compared

with those in the control group were only observed in the UC group for the 5-ASA and 5-ASA + azathioprine, but not for the 5-ASA + adalimumab treatment, suggesting that the treatment with 5-ASA + adalimumab may partially restore the leptin levels in UC but no in CD patients. As the present study was a transversal case-control study and considering that the cohort did not include any IBD patients without treatment, additional studies are required to determine whether the downregulation of leptin in IBD patients compared with that in healthy controls was independent of the prescribed treatments. In this sense, Waluga *et al* (21) postulated that low leptin levels may be a result of TNF-α hyperactivity. As TNF-α stimulates the temporary release of substantial amounts of leptin in response to inflammation, a decrease in leptin-mediated chronic inflammation may eventually be expected. It has been demonstrated that serum leptin levels increased in CD subjects treated with the TNF-α antagonist infliximab, confirming the role of TNF-α in the regulation of leptin release by adipocytes (37). In addition, in the study by Waluga *et al* (21) reported that a 3-month treatment period with corticosteroids alone or with azathioprine lead to an increased plasma concentration of leptin in CD patients (21). Is important to mention that large errors values in the leptin serum levels were observed, mainly on the CD subjects, this may be due to the sample size and/or the differences in pharmacologic treatment initiation between patients. The large data dispersion may have an impact on the statistical analysis, and may therefore be considered a limitation of the present study.

Finally, the present study identified a significant positive correlation between leptin and BMI and a negative correlation between leptin and hemoglobin levels. A previous study indicated that leptin and erythropoietin acted synergistically to increase erythroid development *in vitro* (38). Although the effect of leptin on hematopoiesis may be modest, the present results are in accordance with that reported previously by Togo *et al* (39), whose identified a negative correlation between the levels of leptin and those of hemoglobin in Japanese men, suggesting that leptin may have a role in hematopoiesis in humans.

Although CD and UC have certain clinical and pathological features in common, they may be distinguished based on their localization, endoscopic appearance, histology and behavior, which suggests differences in the underlying pathophysiology. In accordance with the present results, these differences are also reflected in the circulating leptin levels, supporting the involvement of leptin in the pathogenesis of IBD, suggesting the suitability of leptin as a non-invasive marker to determine the risk of disease activity on endoscopy and its potential utility as a marker to optimize the treatment of UC.

In conclusion, the present study indicated that serum leptin is decreased in IBD. Low serum leptin levels were associated with an increased risk of IBD and disease activity on endoscopy among the study population. Additional studies are required to validate these results in populations with a greater number of IBD patients.

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