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EXPRESSION AND RELEASE OF LEPTIN AND PROINFLAMMATORY CYTOKINES IN PATIENTS WITH ULCERATIVE COLITIS AND INFECTIOUS DIARRHEA

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Leptin plays not only an important role in regulation of food intake, but also in the mechanism of inflammation. The universal presence of leptin in the cells of immune system and its secretion by these cells caused increasing interest in the role of this hormone in ulcerative colitis (UC). We determined the role of leptin in 80 patients, aged from 18 to 69 years, including 50 patients with active UC and 30 patients with infectious diarrhea. The tests were performed within 48 hours of the first symptoms, in the period of remission of UC and 8 weeks after resolution of infectious diarrhea. Endoscopy was performed in each patient, and the biopsy samples were taken for the assessments of expression of mRNA for leptin, IL-1β, IL-6 and TNF-α by RT-PCR and Western blot. Blood tests included concentrations of leptin, IL-1β, IL-6 and TNF-α. In addition, the plasma levels of leptin, IL-1β, IL-6 and TNF-α were assessed by ELISA. Serum concentrations of leptin was significantly increased in patients with exacerbation of UC over that in patients with UC in remission. The serum leptin concentration was significantly higher in patients with infectious diarrhea, than the patients that recovered from infectious diarrhea. The leptin protein was overexpressed in the biopsy samples of the mucosa of large intestine compared to those with exacerbation of UC, and in patients after successful recovery from infectious diarrhea. The leptin mRNA was overexpressed in patients with infectious diarrhea compared with that in the group of patients after successful recovery from this condition. Serum concentrations of leptin failed to correlate with severity of exacerbation of UC and with extent of intestinal inflammatory lesions in patients with UC. However, the correlation was observed between serum concentrations of leptin in patients with exacerbation of UC and serum concentrations of proinflammatory cytokines IL-1β and TNF-α. We conclude that 1) the increased leptin in exacerbated UC is related to the increased serum proinflammatory cytokines IL-1β, TNF-α and IL-6 levels; 2) In patients with infectious diarrhea, the concentrations of leptin in intestinal mucosa correlates with serum concentrations of cytokines IL-1β, IL-6 and TNF-α and with an increased expression of leptin mRNA in intestinal mucosa but not with alterations in serum levels of this hormone; 3) leptin may serve as useful predictive marker of inflammation in inflammatory bowel disease (IBD).

Key words: *inflammatory bowel disease, ulcerative colitis, leptin, infectious diarrhea, colonic mucosa, interleukin-1β, tumour necrosis factor-α, interleukin-6*

INTRODUCTION

Leptin is a 16 kDa nonglycosylated protein that is the member of the type I cytokine superfamily, and is mainly produced by adipocytes (1, 2). The primary role of leptin in the human body is linked to the regulation of food intake and lipid metabolism, but it also modulates various inflammatory responses. The role that leptin plays in immunology includes it activates cytokine production, mainly Th1 response, and it directly simulates the expression and release of IL-1α, IL-1β, IL-6 and TNF-α by T lymphocytes (3) The universal presence of leptin in the immune system and its secretion there caused an increase in interest to its function in the pathogenesis of intestinal bowel disease (IBD), mainly ulcerative colitis (UC) and Crohn disease (CD) (1-4). Alterations in the production of many cytokines have been reported in patients with UC but its importance to the pathogenesis of UC is not fully understood. In active IBD, the balance between the function of regulatory and effector cells is disturbed because the effector T cells (Th1, Th2) predominate over regulatory cells (Th3, Tr) (5).

The results of studies on leptin levels in patients with UC are conflicting. For example, Zumbach *et al*. (6) showed that administration of TNF-α induced an increase in leptin release from the fat tissue in patients with UC. Tuzun *et al*. (7) investigated patients with exacerbation of UC and found markedly higher serum concentrations of leptin in these patients as compared to the individuals who did not have the disease. Moreover, they documented a correlation between the extent of inflammatory intestinal lesions and the concentrations of leptin (7). Barbier *et al*. (1) observed increased expression of leptin mRNA in mesenteric fat tissue in patients with UC or Crohn's disease. Sitaraman *et al*. (8) have studied human intestinal

biopsies *in vitro* and concluded that leptin can be released by intestinal epithelial cells into the lumen of the intestine (8). The serum concentrations of leptin was reported to be either normal (2, 9) or decreased in patients diagnosed with UC (3). Karmiris *et al*. (3) compared patients with IBD to the control subjects by assessing serum concentrations of leptin, adiponectin and resistin and analyzing their correlations between the extent of intestinal inflammatory lesions and severity of IBD. They found that serum concentrations of leptin were diminished in patients with UC and revealed no correlations between CRP levels, severity of UC or extent of intestinal inflammatory lesions and concentrations of leptin (3). However, the study included patients receiving immunosuppressive treatment, so this may have influenced results of this study. Hoppin *et al*. (2) investigated serum concentrations of leptin in children with UC or Crohn's disease and found no significant differences in serum leptin levels in disease groups versus healthy control group. However, to the best of our knowledge, the leptin concentration in serum and tissue of human subjects with infectious diarrhea has not been investigated.

Therefore, the aim of our study was to assess the serum of concentrations of leptin and proinflammatory cytokines such as interleukin (IL)-1β, IL-6 and tumor necrosis factor-alpha (TNF-α) and their expression in intestinal mucosa of patients with UC in the phases of exacerbation and remission as well in patients with infectious diarrhea both in acute phase and after successful recovery from this condition.

MATERIAL AND METHODS

Patients

We studied prospectively 80 patients, aged from 18 to 69 years, hospitalized at the Gastroenterology Clinic of Jagiellonian University Medical College. The study group included 50 patients with active UC and 30 patients with infectious diarrhea. The study was conducted in accordance with the Declaration of Helsinki (1975) and approved by the Jagiellonian University Ethical Committee. All study participants signed informed consent form (KBET/81/B/2006).

The tests were performed within 48 hours of the first symptoms, in the period of remission of UC and 8 weeks after resolution of infectious diarrhea. The diagnosis of UC was based on clinical and laboratory criteria, which included history taking, physical examination, colorectal endoscopy and histology of biopsy samples of the large intestine mucosa. The activity of UC was assessed according to the Montreal Classification (10). The diagnosis of infectious diarrhea was based on patient history, epidemiology data, physical examination and laboratory tests, which included the microbiology of fecal samples. The resolution of infectious diarrhea was based on clinical features: normal stools, no clinical signs and normal body temperature (11). The exclusion criteria included: diseases of the endocrine system (particularly diabetes), hiperlipidemia, obesity (BMI>30 kg/m^2), malignancy, autoimmune diseases, ischemic heart disease, chronic obstructive pulmonary disease (COPD), and other serious systemic diseases, as well as use of immunosuppressive, hormonal or lipid-lowering drugs. The patients with UC received long-term mesalazine treatment (12). Corticosteroids were used in 24 patients with severe exacerbations of UC, in whom endoscopy was performed and histological samples were taken before such therapy. Patients with infectious diarrhea received supportive therapy, including hydration and electrolyte repletion (11). The clinical characteristics and distributions of disease diagnoses in the study within particular groups are presented in *Table 1*.

Laboratory tests

Blood samples were collected in the morning (from 7.00 a.m. to 8.00 a.m.) after an overnight fast, centrifuged (3500 g/15min) and stored at –80°C until assayed. Blood tests included complete blood count with differential leukocyte count, electrolytes, blood urea nitrogen, glucose, C-reactive protein (CRP), blood protein electrophoresis and blood lipids; all tests were performed according to generally standard methods. Serum concentrations of leptin were measured using commercially available sandwich enzyme-linked immunosorbent assay kits (R&D Systems, Abington, UK). Serum concentrations of cytokines were measured using highly sensitive ELISA assays (Quantikine HS, R&D Systems, Minneapolis, MN., USA). Microbiology of fecal samples was assessed in all patients and included bacteriology (culture), identification of *Clostridium difficile* antigen and toxins A and B using ELISA assay (Techlab, Blacksburg, VA, USA), search for parasites (microscopically) and identification of rotaviruses and adenoviruses using immunochromatography (Vikia Rota-Adeno, Biomerieux, France).

Table 1. Clinical characteristics and disease distributions of the study population.

	Ulcerative colits	Acute infectious diarrhea
Total Male n (%) Female n (%)	50 20 (40%) 30 (60%)	30 15 (50%) 15 (50%)
Age, yr (min-max) Mean age, yr	18-64 38	19-60 35
Mean BMI (kg/m^2)	22.3	22.9
Mean disease duration, week	153	
Disease localization: Proctitis/ileum Left-side colitis Extensive colitis	 14 (28%) 25 (50%) 11 (22%)	
Etiologic agent of infectious diarrhea: *Salmonella sp.* *Cl. difficile* Rota or adenoviruses Unknown		 7 (23.3%) 11 (36.7%) 6 (20%) 6 (20%)

Table 2. The nucleotide sequences of the primers used in the study.

	Size (bp)	Primers sense antisense
β-actin	307	5′-AGC GGG AAA TCG TGC GTG-3′ 5′-GGG TAC ATG GTG GTG CCG-3′
leptin (ob gen)	298	5′-CAC ACA CGC AGT CAG TCA GTC TCC-3′ 5′-ACC ACC TCT GTC GAC TAG CCA TAG-3′
IL-6	237	5′-CTT TTG GAG TTT GAG GTA TAC CTA G-3′ 5′-GCG CAG AAT GAG ATG AGT TGT C-3′
IL-1β	141	5′-ACA TCA GCA CCT CTC AAG-3′ 5′-AGT CCA CAT TCA GCA CAG-3′
TNF-α	440	5′-GAG TGA CAA GCC TGT AGC CCA TGT TGT AGC A-3′ 5′-GCA ATG ATC CCA AAG TAG ACC TGC CCA GAC T-3′

Endoscopy

Colorectal endoscopy was performed in each patient. Samples of inflammatory mucosa were taken at the level of 15 to 20 cm above the anus and were collected for histology assessment and fixed in 10% buffered formalin. The biopsy samples for the assessments of expression of leptin, IL-1β, IL-6 and TNF-α mRNA, were frozen in liquid nitrogen immediately after sampling and stored at –80° C until assayed.

The assessments of expression of leptin, IL-1β, IL-6 and TNF-α mRNA in the mucosa of the large intestine

Complementary DNA (cDNA) was synthesized using reverse transcriptase (RT) reaction. The elements necessary for the RT reaction included: template RNA molecule, template antisense primer, reverse transcriptase, nucleotide mix (dNTP) and buffer to maintain proper electrolyte environment. Single-strand cDNA was synthesized from 2 μg of complete RNA using the RT-PCR Kit (Promega, Madison, USA). 2 μg of RNA were suspended in appropriate volume of H_2O-DEPC and subsequently 2 μL (1 μg) of the oligo primer (dT) were added to each test tube and incubated for 5 minutes at 70°C to bind the primer. Then the RT reaction was conducted for 60 minutes at 42°C. This was done by adding 35 μL of initially prepared RT-MIX reaction mixture to each test tube (13). 2 μL of single-strand cDNA (template) were amplified in 50 μL of reaction mixture containing: 1 U Taq of DNA polymerase, 200 μM of dATP, 200 μM of dCTP, 200 μM of dGTP, 200 μM of dTTP, 1.5 mM of $MgCl_2$, 2 mM of Tris-HCl (pH 8.3), 10 mM of KCl, 10 μM of EDTA, 100 μM of DTT and the 5′ sense primer and 3′antisense primer, both in target concentration of 1 μM. The amplification was conducted in automatic Biometra T3 thermocycler. The primers for IL-1β, IL-6 and TNF-α and leptin were specifically synthesized (SIGMA-Genosys, UK) by the order based on the sequences published in the literature and the GenBank (*Table 2*).

The PCR products were isolated using electrophoresis on 1.5% agarose gel with ethidium bromide. Sizes of the products were assessed according to the 100 bp ladder standard (Promega) applied simultaneously. The band intensity was assessed by densitometry (Gel-Pro Analyzer, Fotodyne Incorporated, USA) and analyzed using internal software. The results were normalized as ratio of signal expression of the assessed gene and signal of the reference gene (so-called housekeeping gene)-β-actin gene extracted from the same amount of cDNA and showing stable expression in the investigated intestinal tissue (14).

Assessment of leptin concentration using Western blot assay

The tests were performed on the mucosal isolates sampled during endoscopy. The total cellular protein was isolated using standard methods (14). The concentration of the protein in the samples was assessed using NanoDrop spectrophotometer. The samples containing equal amounts of the protein were denaturized by boiling in the standard Western blot buffer solution and were subsequently placed on 12% polyacrylamide gel with sodium dodecyl sulfate (SDS) (Invitrogen, UK) (14). The separated proteins were transferred to nitrocellulose membrane (PVDF) (Invitrogen, UK) using wet electrotransfer procedure. The membranes were subsequently blocked in 5% PBS solution of skimmed milk for 1 hour in ambient temperature and the reactions with primary (leptin) and secondary antibodies (Dako Cytomation, Ely, UK) were performed in 1:1000 dilution. Between the exposures to the antibodies the membranes were washed three times for 15 minutes in TBST solution (TBS with 0.05% Tween 20) (0.1M Tris pH 8.0; 1.5M NaCl; 0.5%TritonX-100). The bound antibodies were identified by chemiluminescence using the Amersham kit (Little Chalfont, UK). The exposure results were obtained using Chemi Genius2 BioImaging System (Syngene, Cambridge, UK) and GeneTools image analysis software (14, 15).

Statistical analysis

Statistical analyses of the results were performed using the analysis of variance (ANOVA) for comparisons of more than 2 groups. In cases when ANOVA revealed differences between the groups, multiple-comparison of *post hoc* Duncan test was used to identify the groups for which the differences were statistically significant. The differences of quantitative parameters were assessed using Pearson's r linear correlation coefficient. If at least one parameter was a rank, Spearman's r rank correlation coefficient was calculated. The correlations between two rank and descriptive parameters were assessed using Chi^2 test. The initial analysis of the protein concentration (Kolmogorov-Smirnov test) revealed that in the study groups these concentrations had normal distribution. Single-sided variance analyses followed by Tukey's a posteriori test were performed. Statistical significance level was $p<0.05$.

Table 3. Serum levels of leptin, TNF-α, IL-1β and IL-6 in studied groups.

	Groups			
	Gr I	Gr II	Gr III	Gr IV
Leptin (pg/ml)	12133.84 ± 848.07*	6240.41 ± 215.77	9650.39 ± 287.15	6890.82 ± 951.59
TNF-α (pg/ml)	0.96 ± 0.06*$^{+}$	0.62 ± 0.04	1.04 ± 0.09*	0.57 ± 0.04
IL-1β (pg/ml)	3.06 ± 0.27*	1.47 ± 0.12	2.05 ± 0.26*$^{+}$	0.58 ± 0.06
IL-6 (pg/ml)	8.03 ± 0.7*$^{+}$	5.13 ± 0.4	4.92 ± 0.3	5.78 ± 0.4

Gr I – active UC, Gr II – UC in remission, Gr III – infectious diarrhea, Gr IV – after successful recovery from infectious diarrhea, * – p<0.001 *vs*. Groups II and IV, *$^{+}$ – p<0.01 *vs*. Groups II and IV.

RESULTS

Serum concentrations of leptin, IL-1β, IL-6 and TNF-α in patients with exacerbated UC and UC in remission

Serum concentrations of leptin in patients with exacerbation of UC were significantly higher than in patients with UC in remission (p<0.001) (*Table 3*). No statistically significant differences in serum leptin concentrations were found between the patients with infectious diarrhea and those who recovered from this condition. The serum concentrations of TNF-α reached significantly higher values in patients with exacerbation of UC than in those with UC in remission (p<0.01). In patients with infectious diarrhea serum concentrations of TNF-α were elevated comparing to patients after successful recovery from this diarrhea (p<0.001). Serum IL-1β concentrations in patients with exacerbation of UC were significantly higher than those in patients with UC in remission (p<0.01). Statistically significant differences were found between the serum concentrations of IL-1β in patients with infectious diarrhea and patients after successful recovery from this condition (p<0.001). IL-6 concentrations reached the highest concentrations in patients with UC exacerbated comparing to those at remission (p<0.01) (*Table 3*).

Serum concentrations of leptin in patients with exacerbation of UC showed direct correlation with TNF-α levels (r=0.52, p<0.001) and IL-1β levels (r=0.5, p<0.001). In patients with acute infectious diarrhea levels of leptin in the serum showed a direct correlation with serum TNF-α levels (r=0.25, p<0.03), IL-1β levels (r=0.65, p<0.001) and IL-6 levels (r=0.63, p<0.001) (*Figs*. *1* and *2*; *Table 4*).

Serum leptin and cytokines in patients with exacerbation of UC and at remission from UC

According to the Montreal Classification all UC patients were divided to three groups depending upon the mild, intermediate and severe exacerbation of UC (10). Serum concentrations of leptin in patients with mild exacerbation of UC were not significantly different to those or patients with intermediate exacerbation and in patients with severe UC exacerbation. The highest serum concentration of TNF-α was found in patients with severe UC. In other groups the levels of this cytokine were lower and the differences between the concentrations in the groups with moderate and severe UC were statistically significant (p<0.03). The highest serum concentrations of IL-6 were traced in patients with severe UC. The differences of IL-6 levels between the patients with mild and severe UC (p<0.04) and those with moderate and severe UC (p<0.01) were statistically significant. In patients with severe exacerbation of UC the statistically significant (p<0.001) correlations between serum concentrations of TNF-α and leptin and serum concentrations of IL-1β and leptin were found (*Figs*. *3* and *4*).

Table 4. Correlations between serum concentrations of leptin and IL-1β, IL-6 and TNF-α in patients with exacerbation of UC and acute infectious diarrhea; p<0.05.

Cytokine		Leptin Ulcerative colitis exacerbation	Leptin Acute infectious diarrhea
TNF-α (pg/ml)	r	0.52	0.25
	n	50	30
	p	p<0.001	p<0.03
IL-1β (pg/ml)	r	0.50	0.65
	n	50	30
	p	p<0.001	p<0.001
IL-6 (pg/ml)	r	0.042	0.63
	n	50	30
	p	p=0.76	p<0.001

Analysis of expression mRNA for leptin, TNF-α, IL-6 and IL-1β in patients with different stages of UC and those with infectious diarrhea

The results of assessments of expression of leptin, TNF-α, IL-6 and IL-1β mRNA in the biopsy samples of the large intestine mucosa in the study groups are presented in *Fig. 5-8*. As shown in *Fig*. *5*, in the group of patients with exacerbation of UC the expression of leptin mRNA was higher compared to those in patients with UC in remission (p<0.05) and in patients after successful recovery from infectious diarrhea (p<0.05). The expression of leptin mRNA in patients with infectious diarrhea reached statistical significance over that in the group of patients after successful recovery from this condition (p<0.05), though it was significantly lower than in patients with exacerbation of UC (p<0.05) (*Fig*. *5*).

Semi-quantitative assessment revealed that the expression of TNF-α and IL-6 mRNAs in active UC was significantly higher than at the patients with UC in remission (p<0.05) (*Figs*. *6* and *8*). In patients with exacerbation of UC the expression of IL-1β mRNA was significantly increased compared to the patients with UC in remission and the patients after successful recovery from infectious diarrhea (*Fig*. *7*). The expression of mRNA for IL-1β, TNF-α and IL-6 significantly was also increased in patients with infectious diarrhea as compared to those in patients after successful recovery from this condition. The comparison of the expression of mRNA of those cytokines in patients with active UC and with infectious diarrhea revealed that in the patients with exacerbation of UC the expression of TNF-α and IL-6 mRNA was significantly higher than in those with infectious diarrhea (p<0.05) (*Figs*. *6* and *8*).

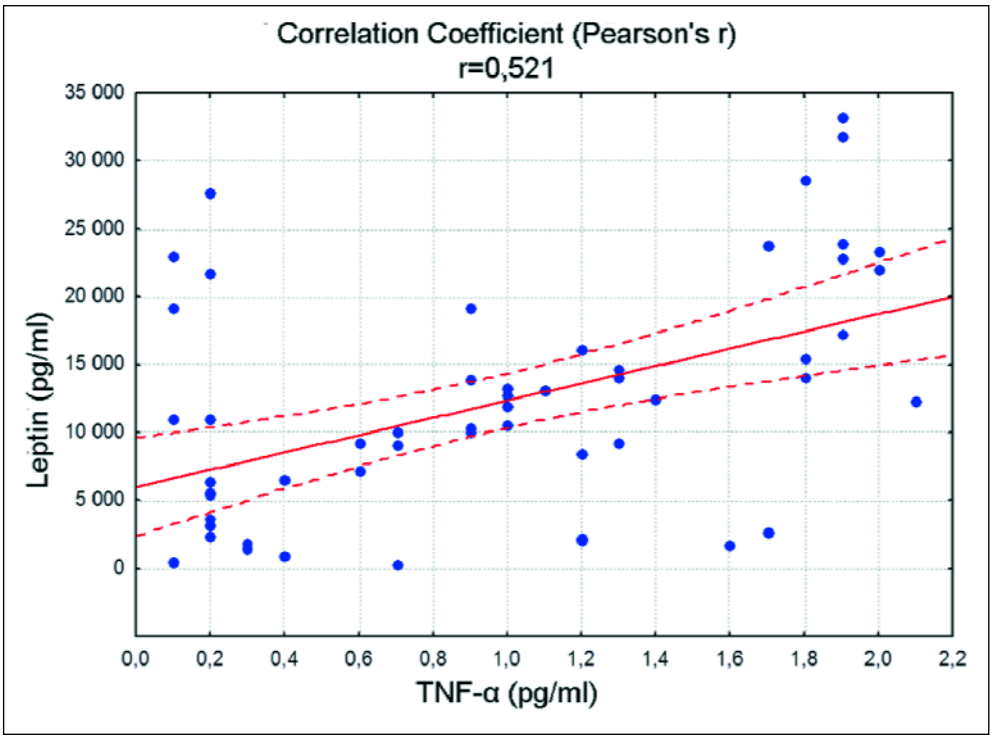

Fig. 1. Correlations between serum concentrations of leptin and TNF-α assessed using ELISA assay in patients with exacerbation of UC.

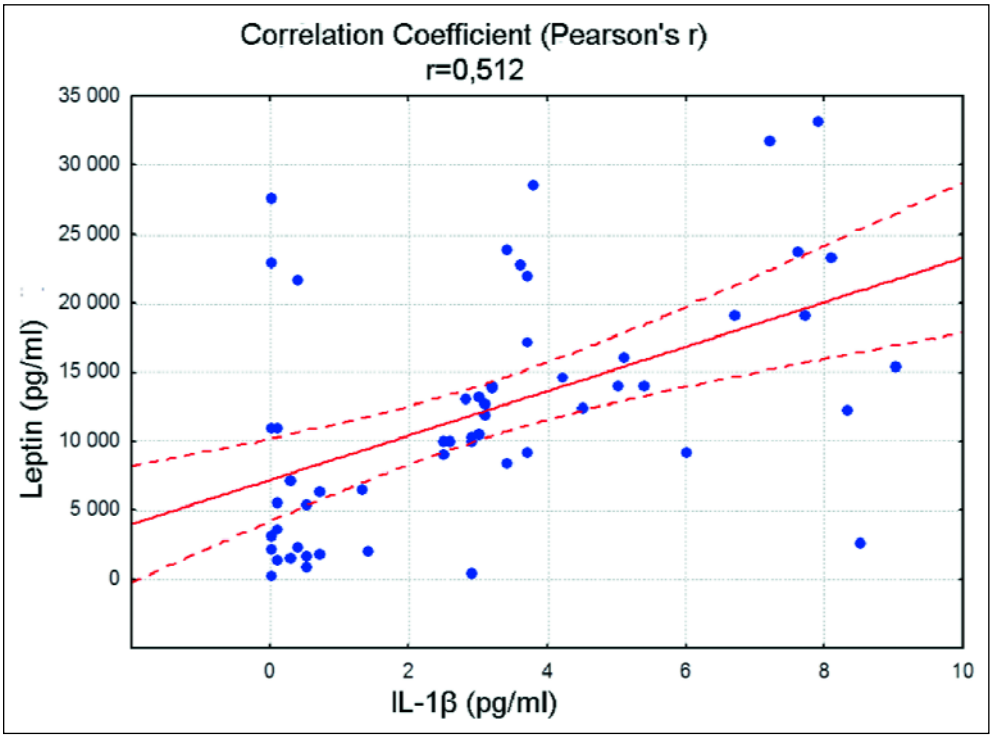

Fig. 2. Correlations between serum concentrations of leptin and IL-1β in patients with exacerbation of UC.

Assessments of leptin protein in biopsy samples of the mucosa at different stages of UC

The expression of leptin protein in homogenates of colonic mucosal cells from patients with exacerbation of UC were significantly lower than in samples from patients with UC in remission (*Fig. 9*). In patients with infectious diarrhea the expression of leptin protein were significantly lower than in patients after successful recovery from this condition. The differences between the groups were statistically significant ($p<0.05$), however, the levels of leptin in the biopsy samples of the large intestinal mucosa inversely correlated with the expression of leptin mRNA in the same biopsy samples and with the serum concentrations of leptin.

DISCUSSION

The present study demonstrated that leptin is closely related to the human intestinal metabolism and inflammation which both become altered during the course of UC. Initial studies on patterns of leptin involvement to the pathogenesis of IBD were

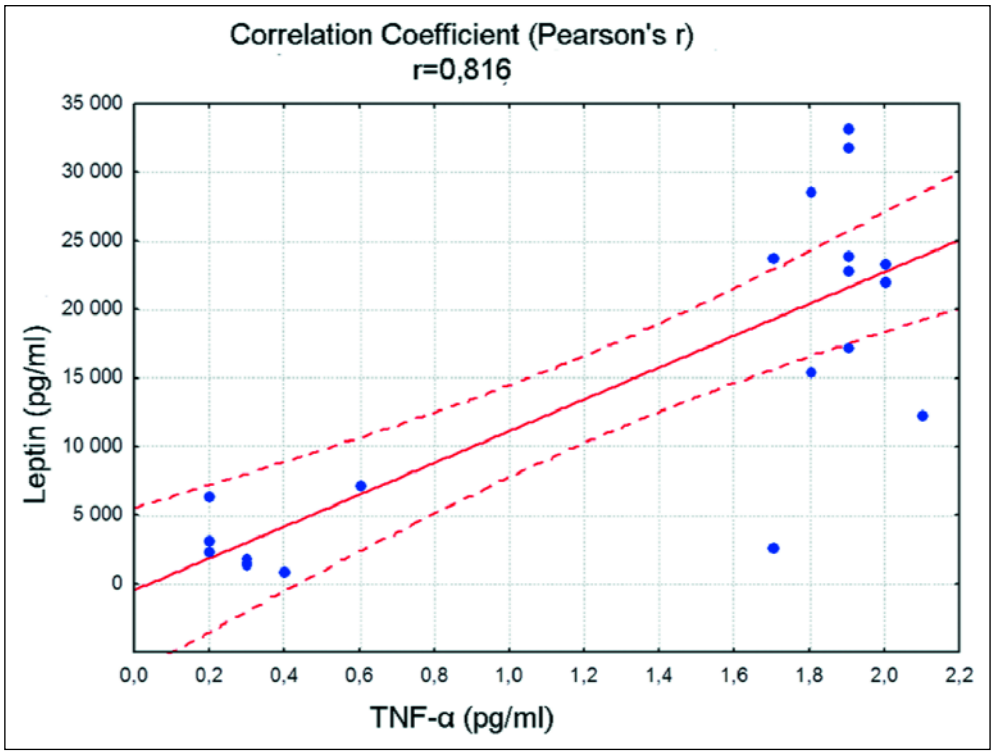

Fig. 3. The correlations between serum concentrations of leptin and TNF-α in the subgroup of patients with severe exacerbation of UC.

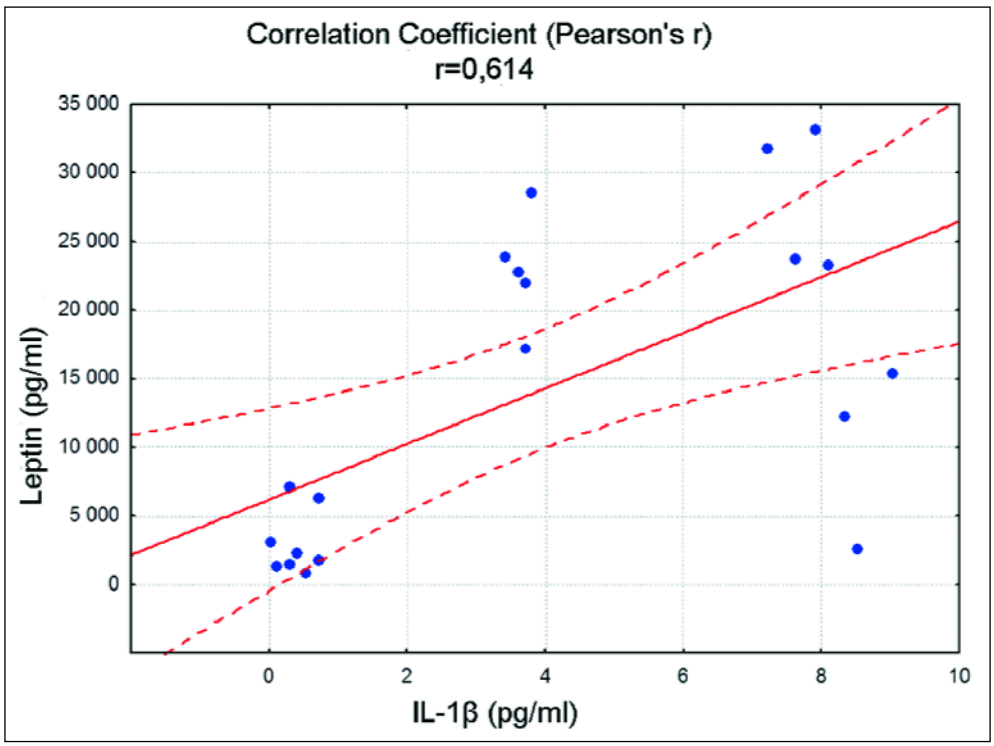

Fig. 4. The correlations between serum concentrations of leptin and IL-1β in the subgroup of patients with severe exacerbation of UC.

conducted in animal models. Grunfeld *et al*. revealed direct correlation between the concentrations of lipopolysaccharide (LPS), TNF-α and IL-1 and the increased expression of leptin mRNA in fat tissue and the decrease of body mass of hamsters with induced inflammation (16). Mosheydi *et al*. (17) found that both plasma levels of leptin and the expression of leptin mRNA were elevated in mice with peritonitis as compared to control animals. Parenteral administration of TNF-α receptor antagonist inhibited the serum leptin levels as well as downregulated mRNA for leptin in fat tissue, however, the apparent lack of correlation between the decrease of body mass and the rate of elevation of serum concentrations of leptin was observed in these mice (17). Siegmund *et al*. (18) reported the lower severity of intestinal inflammation and lower cytokine levels in ob/ob leptin-deficient mice as compared to the wild control mice, and that leptin supplementation of the ob/ob mice eliminated this effect.

Our present study revealed that concentrations of leptin in patients with exacerbation of UC were higher than in patients with UC in remission and correlated with serum concentrations of proinflammatory cytokines IL-1β and TNF-α, while showing no correlation with extent of inflammatory lesions of the large

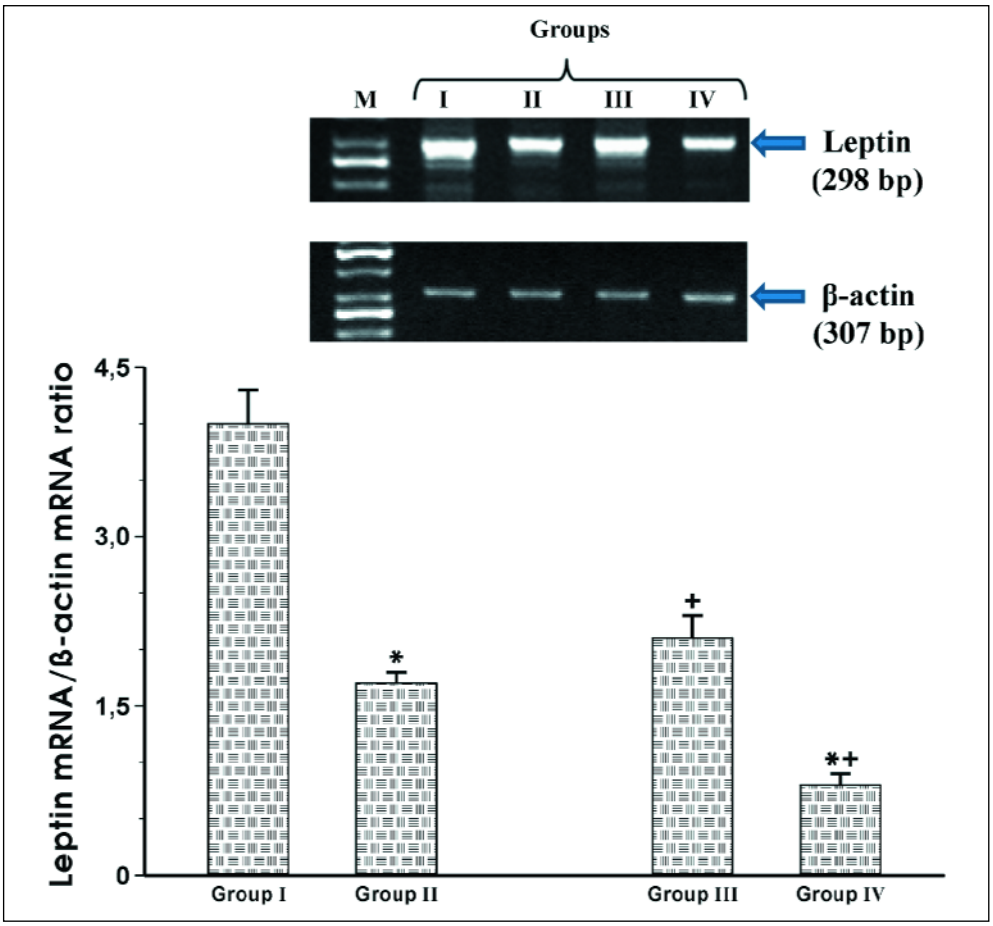

Fig. 5. The RT-PCR expression of leptin mRNA in the biopsy samples of intestinal mucosa of patients at different stages of UC and in those with infectious diarrhea. Results and mean ±S.E.M. of 4 determinations. Groups: I – active UC, II – UC in remission, III – infectious diarrhea, IV – after successful recovery from infectious diarrhea. *– $p<0.05$ *vs*. Group I; $^{+}$ – $p<0.05$ *vs*. Groups I and II, $^{*+}$ – $p<0.05$ *vs*. Group III.

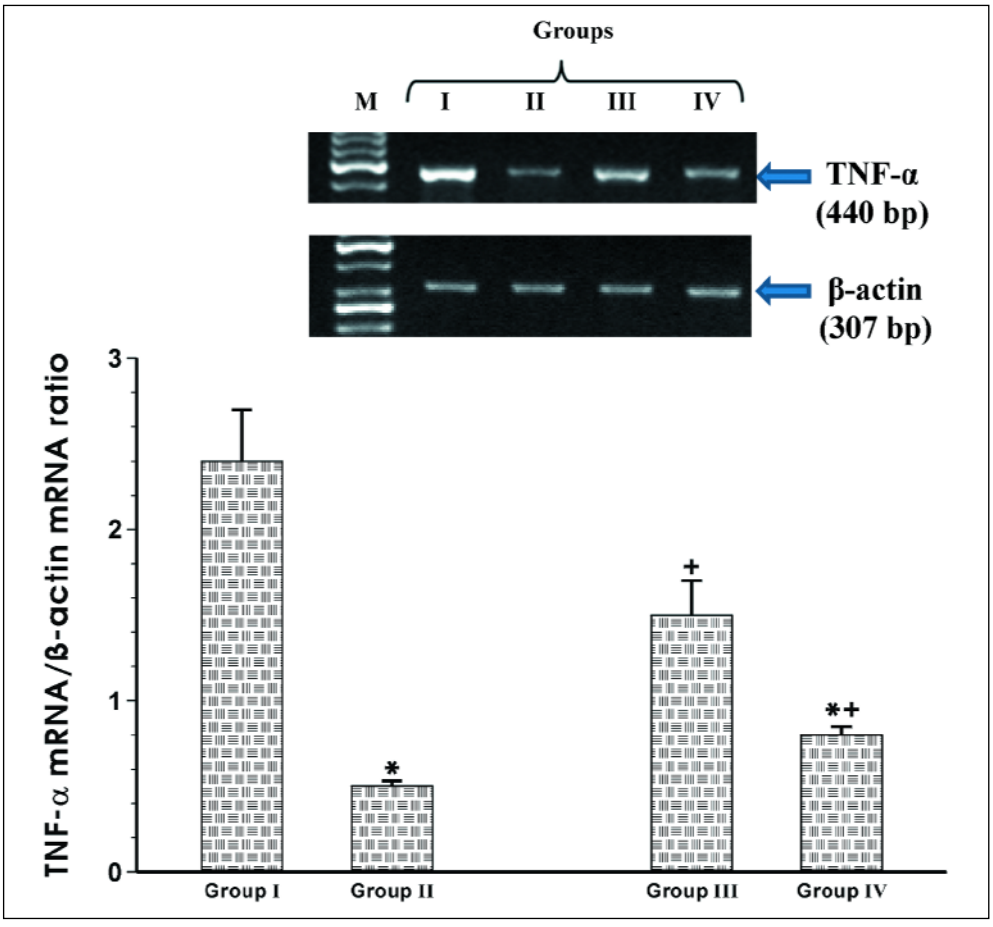

Fig. 6. The RT-PCR expression of TNF-α mRNA in the biopsy samples of intestinal mucosa of patients at different stages of UC and in those with infectious diarrhea. Results and mean ±S.E.M. of 4 determinations. Groups: I – active UC, II – UC in remission, III – infectious diarrhea, IV – after successful recovery from infectious diarrhea. *– $p<0.05$ *vs*. Group I; $^{+}$ – $p<0.05$ *vs*. Groups I and II, $^{*+}$ – $p<0.05$ *vs*. Group III.

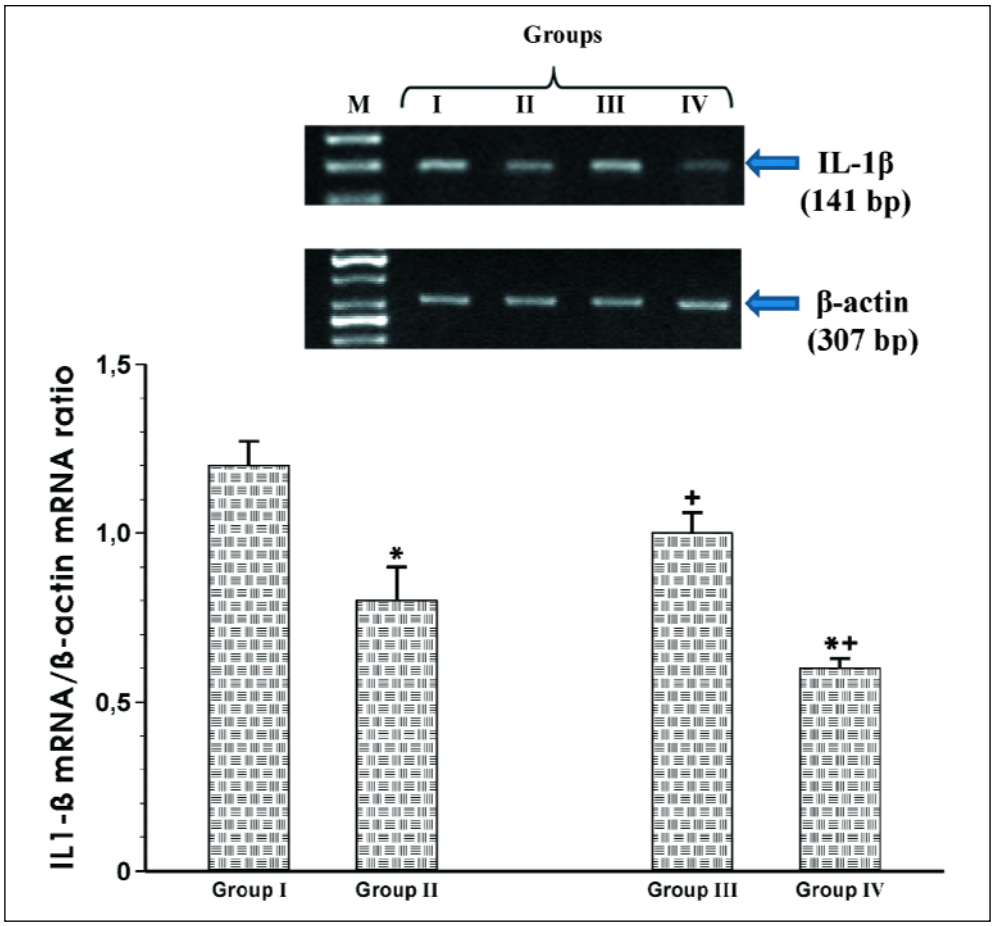

Fig. 7. The levels of expression of IL-1β mRNA (RT-PCR) in the biopsy samples of the mucosa of large intestine in the study groups. Results and mean ±S.E.M. of 4 determinations. Groups: I – active UC, II – UC in remission, III – infectious diarrhea, IV – after successful recovery from infectious diarrhea. *– $p<0.05$ *vs*. Group I; $^{+}$ – $p<0.05$ *vs*. Groups I and II, $^{*+}$ – $p<0.05$ *vs*. Group III.

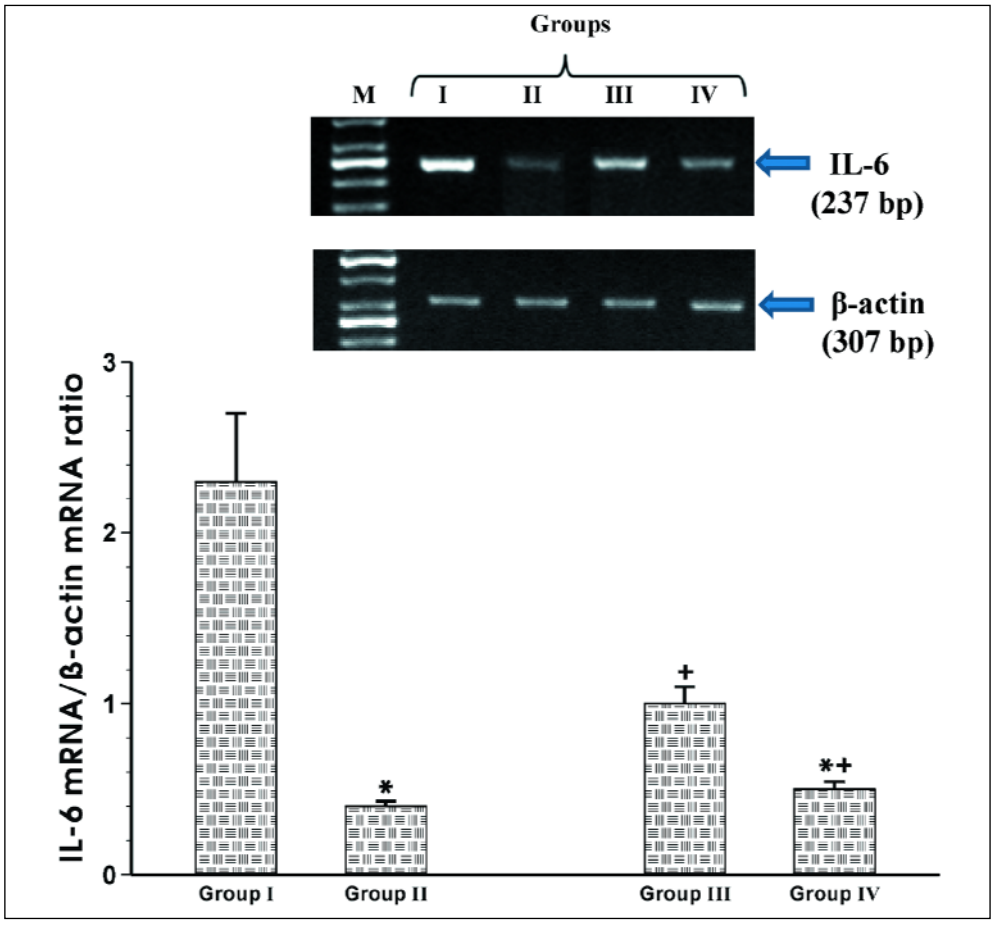

Fig. 8. The RT-PCR expression of IL-6 mRNA in the biopsy samples of intestinal mucosa in patients at different stages of UC and in those with infectious diarrhea. Results and mean ±S.E.M. of 4 determinations. Groups: I – active UC, II – UC in remission, III – infectious diarrhea, IV – after successful recovery from infectious diarrhea. *– $p<0.05$ *vs*. Group I; $^{+}$ – $p<0.05$ *vs*. Groups I and II, $^{*+}$ – $p<0.05$ *vs*. Group III.

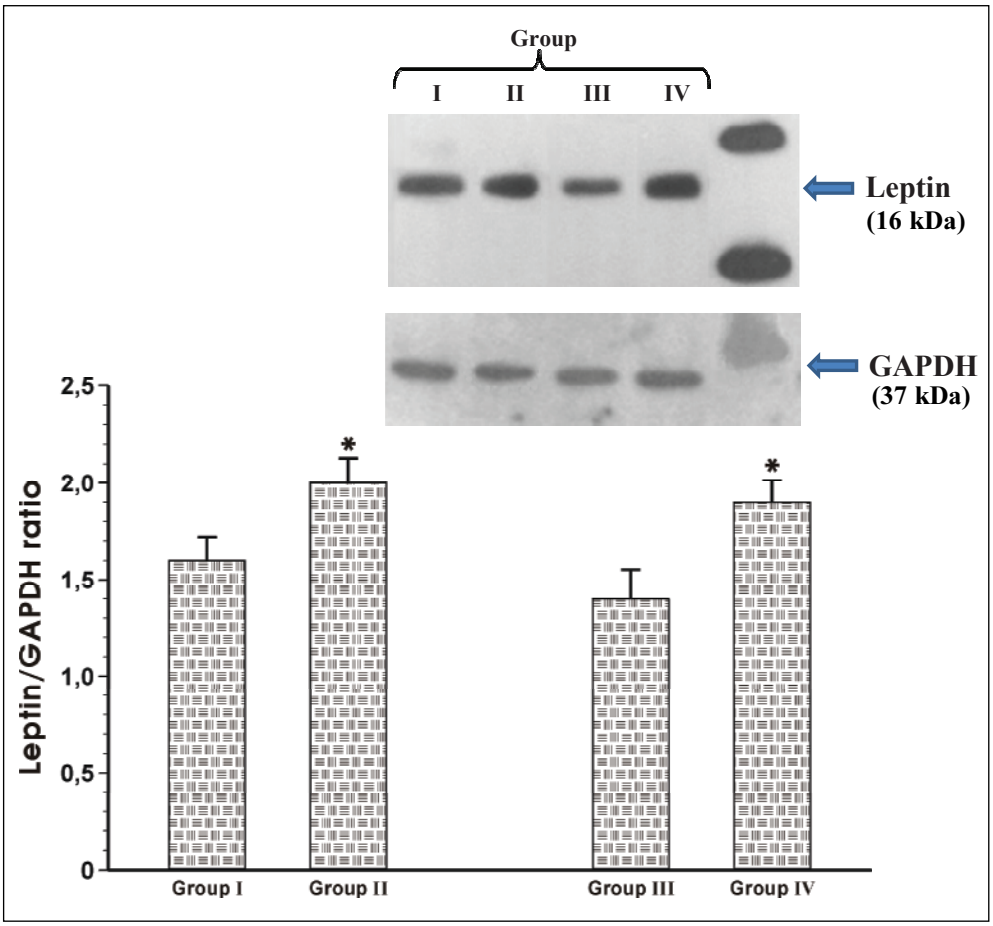

Fig. 9. The expression of leptin protein assessed by Western-blot assay in the sample biopsies of the intestinal mucosa of patients at different stages of UC and in those with infectious diarrhea. Results and mean ±S.E.M. of 4 determinations. Groups: I – active UC, II – UC in remission, III – infectious diarrhea, IV – after successful recovery from infectious diarrhea. *– $p<0.05$ *vs*. Group I.

intestine. Moreover, we found higher levels of serum leptin in patients with exacerbated UC comparing to those after successful recovery from infectious diarrhea, who became healthy individuals afterwards. Our results confirm the earlier findings of Tuzun *et al*. (7) and are consistent with results accumulated in animal model of experimentally induced IBD showing that leptin could be implicated in pathogenesis of UC. On the other hand, Karmiris *et al*. (3) found that in patients with exacerbation of UC serum concentrations of leptin were lower than those in periods of UC in remission. It needs to be emphasized, however, that the patients in their study (3) received corticosteroids or azathioprine, and one of them received biological therapy with anti-TNF-α antibody, infliximab. Undoubtedly, these drugs may have influenced the patterns of concentrations of inflammatory mediators, and indirectly also the serum level and the expression of leptin. Hoppin *et al*. (2) reported a comparable levels of leptin in patients with IBD and control group, though there were some objections because the control group included persons without features of IBD, but with other comorbidities such as peptic ulcer, gastritis, chronic diarrhea or unexplained rectal bleeding. Our study revealed that colonic inflammation contributes to exacerbation of UC and this is associated with an overexpression of leptin mRNA in the mucosa of large intestine. Interestingly, the protein levels of leptin in biopsy samples of the colonic mucosa were decreased during exacerbation of UC compared to those observed in UC patients in remission. Also in patients with infectious diarrhea the protein levels of leptin in biopsy samples of the mucosa of large intestine were decreased compared with that in patients after successful recovery from this condition. These results could be interpreted that the lower synthesis of protein of leptin in the colonic tissue was associated with higher expression of leptin mRNA in the colonic mucosa and higher serum leptin levels. Our results are in keeping with the hypothesis of Kirchgessner *et al*. (19) that the existing mucosal pre-leptin pool may be depleted in the course of inflammatory process and overexpression of proinflammatory cytokines such as IL-1β, TNF-α and IL-6 could interfere with proper leptin protein synthesis and the mucosal tissue leptin concentrations.

The mechanism by which cytokines could influence the expression and release of leptin remains unknown but several authors have previously investigated the relationships between leptin and proinflammatory cytokines. Grunfeld *et al*. (16) observed correlation between administration of TNF-α and increased levels of leptin in mesenteric fat tissue of hamsters. These results were confirmed by Mosheydi *et al*. (17) *w*ho revealed lower leptin levels in mice with inflammation receiving parenteral treatment antagonist of TNF-α as compared to control mice without treatment without this inhibitor. On the other hand, Mantzoros *et al.* (25) reported that the serum concentrations of leptin showed correlation with levels of soluble TNF-α receptor, a sensitive marker of TNF-α activation in human subjects. Zumbach *et al*. (6) stated that TNF-α undoubtedly influenced blood leptin levels in both humans and experimental animals. As mentioned, the mechanism by which cytokines may influence secretion of leptin in patients with inflammation is not fully understood. Kirchgessner *et al*. (19) found that the secretion of leptin is regulated by TNF-α at the post-translational level. They concluded that TNF-α exerts a direct effect on adipocytes resulting in release of leptin by these cells (19). It is not excluded that peripheral accumulation of inflammatory mediators including products of cyclooxygenase-2 (COX-2) and inducible

nitric oxide synthase (iNOS) such as prostaglandins (PGs) and NO during the inflammation (20, 21) might also stimulate the secretion of leptin into blood circulation and account for higher levels of this hormone observed in human subjects with exacerbation of UC. Recently, the enhanced phenotypic and functional maturation of monocyte-derived dendritic cells were reported in patients with UC (22). This suggests that local transformation of monocytes into macrophages could explain the source of COX-2 and iNOS in inflamed colonic mucosa and these effects can also alter the local expression and release of leptin expression and its release during the course of UC. The alternative explanation could be that stress which augments the colonic injury and enhances the local expression of inflammatory mediators including cytokines, COX-2 and iNOS (23), activated the hypothalamic-pituitary brain-gut axis triggering an increase in the corticosteroids levels. Recent evidence indicates that corticosteroids directly stimulate the expression of the *ob* gene resulting in enhancement of leptin release (19, 24). Interestingly, this inflammatory component of UC could be effectively ameliorated by melatonin, a potent reactive oxygen metabolite scavenger and antioxidant, employed as adjuvant to the treatment of human UC (26).

Herein, we present a statistically significant correlation between serum concentrations of leptin and pro-inflammatory cytokines (TNF-α, IL-1β) in patients with exacerbation of UC (*Table 2*) which confirms the pivotal role of leptin and these cytokines in pathogenesis of UC. In severe exacerbations of UC, in which TNF-α levels were markedly elevated, leptin levels also correlated with concentrations of TNF-α and IL-1β. However, the extent of intestinal lesions of the mucosa of large intestine in patients with exacerbation of UC remained without the correlation with the serum concentrations of leptin.

Patients with UC typically exhibit a significant weight loss but we were unable to demonstrate a correlation between loss of the weight and a decrease in serum leptin level in our patients with UC. Therefore, we conclude that the increased leptin contributes to the process of weight loss in UC, on contrary to the subjects during fasting periods in whom a decrease in serum level of leptin is observed. The leptin level in our patients with active UC was increased, which could be attributed to the activation of inflammatory response by this hormone. It seems that inflammation *per se* is a potent stimulant of leptin release than fasting and that is why serum leptin levels were not decreased regardless of a significant weight loss in patients with UC. Furthermore, in UC subjects with prior weight loss an increase in leptin additionally led to further decrease in weight indicating that the increase in leptin release exerts unfavorable effect in patients with exacerbation of UC.

Our study for the first time attempted to determine the serum leptin concentration and this hormone intestinal tissue levels in the course of infectious diarrhea. The patterns of leptin levels in serum or tissues of large intestine in patients suffering from infectious diarrhea have not been extensively investigated; otherwise these studies were performed only in animal models. For instance, Jenkins *et al*. (27) have investigated the serum concentrations of leptin in pigs with infectious diarrhea caused by *Salmonella enterica* and found no alterations of leptin levels in pigs with this infection. Mykoniatis *et al*. (28) investigated mice receiving *Clostridium difficile* toxin A and showed that leptin levels in wild mice were higher compared with the buffer-injected animals in control group and this effect was accompanied by increased expression of leptin mRNA in intestinal mucosa. Studies in ob/ob and db/db mice revealed that these genetically modified animals were partially protected from the effect of this toxin and had no intestinal symptoms, however, administration of leptin resulted in a development of diarrhea symptoms in the ob/ob mice (28).

In our study, lack of statistically significant differences between serum concentrations of leptin in patients with infectious diarrhea and patients after successful recovery from this condition were found. However, in patients with infectious diarrhea a significant correlation between concentrations of leptin and concentrations of TNF-α, IL-1β and IL-6 were observed. These results confirm a correlation between the inflammatory activity reflecting the concentrations of proinflammatory cytokines and serum leptin levels in this group of patients.

In our study the expression of leptin mRNA in the mucosa of large intestine in patients with infectious diarrhea was increased while no major alterations in serum leptin levels were observed in these patients as compared to the expression and serum concentration of this hormone after successful recovery from this condition. Conversely, the concentrations of leptin in the biopsies of the intestinal mucosa taken in the course of infectious diarrhea were lower than in the respective control samples collected in patients in remission. Based on these results, we conclude that short-term inflammatory reaction which takes place in infectious diarrhea is associated with increased levels of proinflammatory cytokines and enhanced expression of leptin mRNA in the intestinal mucosa but these effects are not associated with a significant alterations of serum concentrations of leptin.

We conclude that the development of active inflammatory process in the lower gastrointestinal tract leading to UC can stimulate the expression of leptin mRNA in the mucosa of large intestine. The increased leptin in exacerbated UC is related to the proinflammatory cytokines IL-1β, TNF-α and IL-6 and leptin may serve as useful predictive marker of inflammation in IBD. Serum concentrations of leptin do not correlate with extent of intestinal inflammatory lesions in patients with UC. The lower concentrations of leptin in the intestinal mucosa of patients with active inflammatory process as compared to the periods of remission from UC or after successful recovery from infectious diarrhea may suggest the presence of the pre-leptin pool in the mucosa of large intestine and its depletion in response to inflammation or immune activation *via* yet unknown mechanism. In patients with infectious diarrhea, the serum concentrations of leptin correlated with those of cytokines IL-1β, IL-6 and TNF-α. We therefore conclude that the short-term inflammatory reaction associated with the infectious diarrhea is accompanied by the rise in the serum levels of proinflammatory cytokines and the increased expression of leptin mRNA in the intestinal mucosa without significant alterations in serum leptin levels.

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