

Clinical utility of biochemical markers in ulcerative colitis among Egyptian patients

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Abstract

Biochemical markers are a non-invasive way of objectively measuring inflammation in ulcerative colitis and can play an adjunctive or primary role in the assessment of disease activity. **Aim** of this study was to A) evaluate serum levels of some biomarkers “leptin, adiponectin, resistin, and ghrelin” in ulcerative colitis (UC) patients, besides the ordinary inflammatory markers, B) to correlate the results with the disease activity, with the clinical characteristics of the disease C) and to examine the possible interaction between the estimated parameters values. Study was conducted on 56 UC patients from the Clinic of Internal Medicine Department and Endoscopy Unit of Alzahraa Hospital, Alazhar University, besides 30 healthy subjects served as control group. **Results:** Mean levels of ESR, CRP, TNF- α , resistin and ghrelin were significantly higher in active UC patients than the control group, while after the courses of treatment 47 patients achieved complete remission (inactive UC) mean values of these biochemical parameter decreased significantly than the original values at the active disease and the values reached nearly the normal ranges. While in patients (9 patients) who did not achieved complete remission, there were moderate decreased serum levels of these biochemical markers but still higher values than the control group and they still have manifestations of active UC. The mean level of leptin was significantly decreased in active UC patients compared to the control group, while after the course of treatment in patients achieved complete remission (inactive UC) the mean value increased significantly (with mean value 10.1 ng/ml). **Conclusion:** Our data indicate that, the increased plasma resistin, TNF- α and ghrelin levels correlated with activity of ulcerative colitis and so they could predict the response to therapy and possibly reflect an acute-phase response due to inflammation more than the ordinary inflammatory markers. Resistin, TNF- α and ghrelin levels could be considered as an independent predictor of disease activity in patients with UC and may represent link between inflammation and UC. [Journal of American Science 2010; 6(6):146-155]. (ISSN: 1545-1003).

Key words: ulcerative colitis, inflammatory markers, leptin, resistin, ghrelin, Tumor Necrosis factor alpha.

1. Introduction

Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD) usually affects only the innermost lining of your large intestine (colon) and rectum, with characteristic ulcers, or open sores, in the colon. Ulcerative colitis occurs in all age groups, with the most common age of diagnosis being 15–35 years of age with a second, lesser peak between 55 and 65 years. Men and women are affected equally. Whites are more frequently affected than other racial groups, and people of Jewish origin have 3–6 times greater likelihood of suffering from any IBD (Baumgart and Carding 2007).

Ulcerative colitis typically begins gradually, with abdominal pain and diarrhea that is sometimes bloody. In more serious cases, diarrhea is severe and frequent. Fever, loss of appetite, and weight loss occur. The severity of the disease depends on how much of the colon is involved. (Kasper DL et al. 2005).

Many aspects of the IBDs, Crohn's disease (CD) and UC, still present challenges for physicians treating this disorder: diagnosis, prognosis, assessment

of disease activity and severity, as well as outcome of therapy. For each of these aspects, there is no single “gold standard” test or examination. Instead, physicians apply a combination of symptoms, clinical examination, laboratory indices, radiology, and endoscopy with histology to make the diagnosis, to assess severity, and to predict the outcome of disease. There are several reasons why laboratory markers have been studied in IBD in the past decades: firstly, to gain an objective measurement of disease activity as symptoms are often subjective; and secondly, to avoid invasive (endoscopic) procedures which are often a burden to the patient (Vermeire et al. 2006).

Some studies suggest that white adipose tissue (WAT), besides its ability to respond to afferent signals from traditional hormone systems and the central nervous system also expresses and secretes factors with important functions, collectively called adipocytokines. There is evidence that adipocytokines are involved in inflammatory and metabolic pathways in humans. Among the adipocytokines, leptin, adiponectin, and

resistin appear to play an important role (Konstantinos et al. 2005).

Human resistin is a 108-amino acid peptide hormone with a molecular weight of 12.5 kDa. Resistin was extensively studied in patients with both rheumatoid arthritis and osteoarthritis and significant correlation with inflammation and elevated C-reactive protein (CRP) was reported (Schaffler et al. 2003). There is evidence that resistin is involved in the inflammatory and metabolic pathways in humans and a possible role in IBD was recently postulated in certain IBD patients (Paul et al. 2005).

Leptin is a 16-kDa non-glycosylated protein, adipocytes secrete leptin in direct proportion to WAT mass and this secretion is greater from subcutaneous compared to visceral WAT. Leptin possesses a proinflammatory as well as anti-inflammatory properties according to the experimental conditions. Its role in IBD has been studied, but the results are conflicting, therefore further investigation is required (Otero et al. 2005).

Ghrelin is a recently discovered hormone, with a crucial role in the regulation of food intake and energy homeostasis. It is mainly produced at the stomach but is also expressed in WAT, albeit in trace amounts. Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor (GHS-R) and it has been identified in T cells. Ghrelin can inhibit cytokine activation including interleukins, TNF- α , and most interestingly leptin. Recently, high levels of serum ghrelin were found in patients with celiac disease (Peracchi et al. 2003).

This study designed to study the utility of biomarkers (leptin, adiponectin, resistin, and ghrelin) besides the ordinary inflammatory markers in measuring disease activity and/or severity and predicting the response to therapy in patients with UC, and to examine the possible interaction between the estimated biomarkers values.

2. Subjects and methods

The present study was conducted on 56 patients (35 males and 21 females). They were selected from Inpatient and Outpatient Clinic of Internal Medicine Department and Endoscopy Unit of Alzahraa Hospital, Alazhar University. The Patients were newly diagnosed with acute active ulcerative colitis (AUC), besides 30 healthy subjects (18 males and 12 females) to serve as a control group. Patients with coexisting conditions that may influence the result of serum biomarkers, such as recurrent infections, malignancy, recent surgery, major systemic illnesses, and inflammatory arthritis were excluded from analysis. All

patients and control subjects were non-smoker and gave their informed consent to participate in the study, which was approved by the Hospital's Scientific Committee.

2.1 The diagnosis of UC disease was based on:

(i) - History taking:

Include a history of diarrhoea and/or rectal bleeding for 6 weeks or more.

(ii) - Complete clinical examination:

During baseline evaluation, disease severity in patients with UC (active or inactive) was assessed clinically according to the practice guidelines of the American College of Gastroenterology. Mild UC was defined as having 3 stools daily with or without blood and with no systemic toxicity. Moderate UC was defined as having 4–6 stools daily with or without blood or with minimal signs of toxicity. Severe UC was defined as having 7 stools daily with or without blood, and with moderate to severe systemic toxicity (fever, 10% weight loss, orthostasis, haemoglobin 10 g/dL), or required hospitalization.

(iii) - Endoscopic findings:

- Colonoscopy: baseline colonoscopy with biopsy sampling performed in all patients with UC, in order to assess the endoscopic severity and extent of disease. Endoscopic severity measured by a modified endoscopic score with an 18-point scale involving nine parameters: erythema, vascular pattern, friability, granularity, spontaneous bleeding, occurrence and severity of ulcers, extent of ulcerated surface, and presence of mucopurulent exudates. All parameters were scored from 0 to 2 points. Four grades of activity were considered according to the sum of all parameters: inactive disease (0-3), mild disease (4-7), moderate disease (8-12), and severe disease (13-18). Grading of endoscopic severity was done from the most inflamed part of the bowel. The extent of disease was recorded as recto-sigmoiditis, left-sided colitis, and pancolitis.

- Characteristic microscopic changes of biopsy specimens reveal abnormal mucosal architecture and lamina propria cellularity, neutrophil polymorph infiltration and epithelial cell abnormality.

2.2 Treatment protocol

Patients with active UC were treated for attenuation of disease activity with high-dose corticosteroids (prednisone 40 mg/day) and mesalazine (3-4gm/day) orally and rectally. Patients were set into a follow-up program with regular visits every 2nd wk for 12 wks. Corticosteroids were tapered off with a weekly based schedule throughout the study period. At the end of the study (12th wk), complete clinical, endoscopic and laboratory evaluation, similar to baseline week, was performed in all patients with active colitis. Complete response to therapy (remission) was considered, if a SCCAI score (Simple Clinical Colitis Activity Index) of

2 and endoscopic remission was achieved after 12 wks of therapy. Partial response was considered if a 50% reduction of SCCAI score was noted together with a reduction of endoscopic activity by at least one grade.

2.3 Sampling:

Peripheral blood samples were drawn from the patients at diagnosis (acute active group) and after treatment (inactive group). Five ml of fasting venous blood samples were collected from each subject. The blood was left to clot at room temperature to separate sera after centrifuging for 10 minutes at 3000 r. p. m. Sera were divided into several aliquots and stored at -70°C until assay.

2.4 Laboratory tests

- Hemogram: included hemoglobin concentration, total leucocytic count, platelet count using Coulter counter and examination of Lishman or Wright-stained peripheral blood smears.

- Body mass index (BMI) was calculated as body weight in kilogram divided by the square of height in meter (kg/m^2).

- ESR was measured by standard laboratory technique (normal values <20 mm/h) according to Westergren (Westergren, 1921).

- Tumor Necrosis factor alpha (TNF- α) was determined by immunoenzymometric assay using kit supplied by Bio-Sourse Europe S.A. (Rue de Industries 8-B-1400 Nivelles, Belgium) (Bienvenu A, 1993).

- Serum leptin concentration was determined using a direct enzyme-linked immunosorbent assay (ELISA) kit (Cat. No.DSL-10-23100, Med Diagn. Comp, Germany) (Agata et al, 1997).

- Serum resistin levels were measured by sandwich enzyme-linked immunosorbent assay (BioVendor Laboratory Medicine, Inc., Plackeho, Czech Republic) (Youn et al. 2004).

- Serum ghrelin levels were measured using DSL-10-33700 active total ghrelin ELISA kits, USA, using one-step sandwich-type immunoassay (Grochi et al. 2002).

- C reactive protein (CRP), C reactive protein (CRP), was determined by a high sensitive immunoassay for measuring human CRP which is a two step sandwich ELISA technique using kit supplied by diagnostic system laboratories (DSL-10-42100) Webster, Texas, USA (Rifai et al. 1999).

- Total cholesterol was determined by colorimetric method using Bio-Merieux test kit (Richmond W. 1973).

- High density lipoprotein cholesterol was measured after precipitation of LDL and VDL using phosphotungstate according to Henary et al. (1974).

- Low density lipoprotein cholesterol was measured by Friedwald method (Friedwald et al. 1972).

- Triglyceride level was determined by enzymatic colorimetric test with lipid clearing factor (Fossati and Prencipe, 1982).

2.5 Statistical analysis

Statistical analysis was performed using the SPSS for Windows package (version 11.0, SPSS, Chicago, IL, USA). Data were presented as range, mean \pm SD and number (%). Comparisons between the 3 groups (healthy controls, patients with active UC (before treatment) and patients with inactive UC after treatment) was performed using one way ANOVA as a parametric test for continuous variables as the biochemical markers, also, comparisons were performed between different subgroups of patients with active UC according to disease activity were made by the Kruskal-Wallis test (nonparametric ANOVA). Post hoc multiple comparisons tests were made by Dunn's test. Chi square test was used to compare between patients at diagnosis and after treatment regarding the clinical and endoscopic severity. Correlations between serum biomarkers and indices of disease activity were analyzed with the Pearson's correlation method. *P*-value of ≤ 0.05 was considered statistically significant.

3. Results

The study was conducted on 56 patients with active UC (35 males and 21 females, age range 26 - 45 years with a mean of 35.3 ± 2.1 years). besides 30 healthy subjects (18 males and 12 females, age range 25 - 46 years and mean of 36.05 ± 2.14 years) to serve as a control group. Colonoscopies were performed to all the patients; the active inflammation was confirmed by histological assessment. Among the 56 patients with acute colitis, there were 48 patients with non-extensive colitis and 8 patients with extensive colitis. The demographic, biochemical and clinical characteristic of the patients and the control groups are summarized in table (1).

All the patients and the control subjects were non-smokers, the patients received the treatment protocol for 12 weeks and then evaluated after therapy, 47 patients achieved a complete remission which is approved by the clinical, laboratory and endoscopic data. While, nine patients did not response well to the therapy as they did not achieve a complete remission (partial remission). So they received another therapy and excluded from the study.

Mean levels of ESR, CRP, TNF- α , resistin and ghrelin were significantly higher in active UC (56 patients (with mean values of 60 mm/l, 16.7 mg/l, 14.48 pg/ml, 18.86 ng/ml and 20 ng/ml respectively) than the

control group, while after the course of treatment 47 patients achieved complete remission (inactive UC) the mean values of these biochemical parameter decreased significantly (with mean values of 21.3 mm/l, 4.1 mg/l, 6.53 pg/ml, 10.93 ng/ml and 5.97 ng/ml respectively) than the original values at the active disease and the values reached nearly the normal ranges. While in patients (9 patients) who did not achieved complete remission there were moderate decreased (non-significant) serum levels of these biochemical markers (with mean values of 51 mm/l, 13.3 mg/l, 14.5 pg/ml, 16.3 ng/ml, and 8.9 ng/ml respectively) but still higher values than the control group because they still have manifestations of active UC (Table 2, figure 1).

The mean level of leptin was significantly decreased in active UC patients (with mean value 3.77 ng/ml) as compared to the control group, while after the course of treatment in patients achieved complete remission (inactive UC) the mean value increased significantly (with mean value 10.1 ng/ml). While in patients (9 patients) who did not achieved complete remission there were decreased serum level with mean

value 5.8 ng/ml but still lower values than the control group because they have still manifestations of active UC (Table 2).

There were no significant differences in the levels of fasting blood glucose, total cholesterol, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol and triglyceride between control group, patients with active UC, patients with complete remission (inactive UC), and patients with partial remission (Table 2).

The correlation of serum biomarkers with clinical severity of UC is shown in Table 3. This demonstrates that most the commonly used biomarkers (ESR, CRP, TNF- α , leptin, resistin and ghrelin) were significantly associated with clinically severe disease. But there were no significant correlation in the levels of fasting blood glucose, total cholesterol, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol and triglyceride with the severity of UC, as well as with patient's personal characteristics such as age and gender.

Table 1: Demographic and clinical data of ulcerative colitis patients (mean and range)

	Control	Active UC At diagnosis	Inactive UC After treatment	Partial remission During treatment
number	30	56	47	9
Age (years)	36.05(25 – 46)	35.3(26 – 45)	37.6 (26 – 40)	40.6 (36 – 45)
Male	18 (60%)	31(55.36 %)	26 (55.3 %)	5 (55.5%)
female	12 (40%)	25 (44.64%)	21(44.7 %)	4 (44.5%)
BMI (kg/m²)	22.5 (20 – 25)	19.3 (17 – 22) *	18.9(17.5 – 21.1)*	19.5(16.9 – 21.9)*
Leuko.count (×10⁹/L)	8.9 (4 – 10.5)	14.8 (6.9 – 24.5)*	9.1 (5.6 – 11.4) ¶	12.3(6.5 – 18.7) *¶
Hemoglobin (g/dL)	13.7 (12 – 14.3)	10.8 (7.5 -11.7)*	12.6 (8.9 – 13.5) ¶	10.5 (7.5 – 11.7)*
Plalelet (×10⁹/L)	224 (150-285)	375 (160 – 688)*	232 (165 – 311) ¶	345 (125 – 564) *
<u>Clinical severity</u>				
Mild	-	24 (42.9%)	4 (8.5%)	0
Moderate	-	26 (46.4%)	0	7 (77.7%)
Severe	-	6 (10.7%)	0	2 (22.3%)
SCCAI Score	-	7 ± 3 (4 -11)	0 ± 1 (0-2)	4 ± 2 (2-7)
<u>Endoscopic severity</u>				
Non extensive colitis	-	48 (85.8%)	45 (95.7 %)	3 (33.3%)
extensive colitis	-	8 (14.2%)	2 (4.3%)	6 (66.7%)

Leuko.count: leukocytic count SCCAI: simple clinical colitis activity index.

*Significant as compared to control group

¶ Significant as compared to active UC

SCCAI Score (Simple Clinical Colitis Activity Index) score (Walmsey et al. 1998)

Table 2: Biochemical parameters in patients with UC at diagnosis and after the treatment

Parameters	Control group (30)	At diagnosis	After treatment	
		Active UC (56)	Inactive UC (47)	Partial remission (9)
• ESR (mm/h)				
Range	16-19 mm/h	40-97	19 – 30	36 – 82
Mean \pm SD	17 \pm 1.2	60 \pm 21*	21.3 \pm 12	51 \pm 17*
• CRP (mg/l)				
Range	3- 4.5 mg/L	9.1- 35.5	3.1- 7.3	11 – 29.2
Mean \pm SD	4 \pm 0.8	16.7 \pm 2.2*	4.1 \pm 1.7	13.3 \pm 1.5*
• TNF- (pg/ml)				
Range	5 – 6.88	12.13 – 17.5	5.23 – 7.5	11 – 15.5
Mean \pm SD	6.06 \pm 0.28	14.486 \pm 1.75*	6.53 \pm 0.37	13.5 \pm 1.8*
• Resistin (ng/ml)				
Range	9.5 -10.81	15.99 – 26.12	10.51-13.39	15.2 – 24.6
Mean \pm SD	10.1 \pm 0.32	18.86 \pm 3.14*	10.93 \pm 0.699	16.3 \pm 2.5*
• Leptin (ng/ml)				
Range	9.59 – 10.92	3 – 4.5	8.98-11.39	3.4 – 4.5
Mean \pm SD	10.24 \pm 0.42	3.77 \pm 0.45*	10.147 \pm 0.68	5.8 \pm 0.51*
• Ghrelin (ng/ml)				
Range	5.21- 7.23	18.9- 21.22	5.13- 6.53	6.75- 11.96
Mean \pm SD	6.19 \pm 0.49	20 \pm 0.73*	5.97 \pm 0.48	8.9 \pm 0.52*
• FBS (mg/dl)				
Range	85 – 125	92 -132	95 – 135	95 – 125
Mean \pm SD	109.5 \pm 9.09	111.2 \pm 10.45	116.6 \pm 9.9334	117.5 \pm 8.6
• T-chol.(mg/dl)				
Range	140 -165	145 – 168	150 – 167	146 – 165
Mean \pm SD	152.85 \pm 7.85	153.5 \pm 5.24	155.05 \pm 5.15	151.2 \pm 4.7
• LDL-chol. (mg/dl)				
Range	68 – 85	68 -75	66.5 – 77.5	69 – 78
Mean \pm SD	73.6 \pm 5.99	70.2 \pm 3.59	71.35 \pm 3.66	72.2 \pm 3.1
• HDL-chol. (mg/dl)				
Range	41 – 59	44.5 – 58	45.8 – 59.4	44 – 58
Mean \pm SD	47.95 \pm 4.850	51.97 \pm 4.60	49.99 \pm 11.39	52.1 \pm 3.9
• Triglyceride (mg/dl)				
Range	88 -110	85 – 115	83.5 – 109.5	88 – 112
Mean \pm SD	94.6 \pm 5.77	95.5 \pm 7.61	93.96 \pm 2.25	93.3 \pm 8.21

* Significant P<0.05 compared to control group.

ESR: Erythrocytic sedimentation rate.

TNF- : tumor necrosis factor alpha

T-chol.: total cholesterol

LDL-chol: low density lipoprotein cholesterol

Data are expressed as range (mean \pm DS).

CRP: C-reactive protein.

FBS: fasting blood sugar

DHL-chol: high density lipoprotein cholesterol

Table (3): Correlation of serum biomarkers with clinical severity of ulcerative colitis

	Mild (24)	Moderate (26)	Sever (6)	p- value
Age	34 (31 – 40)	36 (26 – 41)	38 (30 – 45)	0.213§
Male	13 (54.2%)	14 (53.8%)	4 (66.7%)	0.126§
Female	11 (45.8%)	12 (46.2%)	2 (33.3%)	
ESR (mm/h)	42 (40- 50)	51 (41-80)	59 (45-97)	0.031 ¶*
CRP (mg/L)	16.5 (9.1- 38)	20 (9.5 – 52)	34.7 (10 – 65.5)	0.0044 ¶*
TNF- (pg/ml)	13 (12.1 – 14)	14.1 (14.5 – 16)2	16.7 (12.5 – 17.5)	0.0443 ¶*
Resistin (ng/ml)	15.8 (15.9 – 19)	17.8 (16.6 – 23.5)	21.8(15.9 – 26.1)	0.034 ¶*
Leptin (ng/ml)	4.2 (3 – 4.5)	3.7 (3.1 – 4.1)	3.5 (3.2 – 3.9)	0.054 ¶
Ghrelin (ng/ml)	16 (18.2 – 19.5)	16.5 (18.9-20.7)	17.4 (18.5- 21.22)	0.044 ¶*
FBS (mg/dl)	101.5 (98 -120)	110.2 (100 -129)	111.2 (115 -132)	0.065
T-chol.(mg/dl)	148.1 (145 – 151)	153.5 (155 – 160)	162.5 (162 – 168)	0.121
HDL-chol.(mg/dl)	49.9 (44.5 – 49)	51.3 (45 – 53)	55.2 (46 – 58)	0.078
LDL-chol. (mg/dl)	69.2 (68 -70)	70.9 (69.2 -75)	72.7 (70 -75)	0.098
Triglyceride (mg/dl)	90.6 (85 - 96)	92.5 (88 - 110)	93 (90 - 115)	0.059

Data are expressed as median (range).
 ESR: erythrocytic sedimentation rate.
 TNF- , tumor necrosis factor alpha
 T-chol.: total cholesterol
 LHL-chol: low density lipoprotein cholesterol

§ Chi-squared test; ¶ Kruskal–Wallis test.
 CRP: C-reactive protein.
 FBS: fasting blood sugar
 DHL-chol.: high density lipoprotein cholesterol
 * significant p<0.05

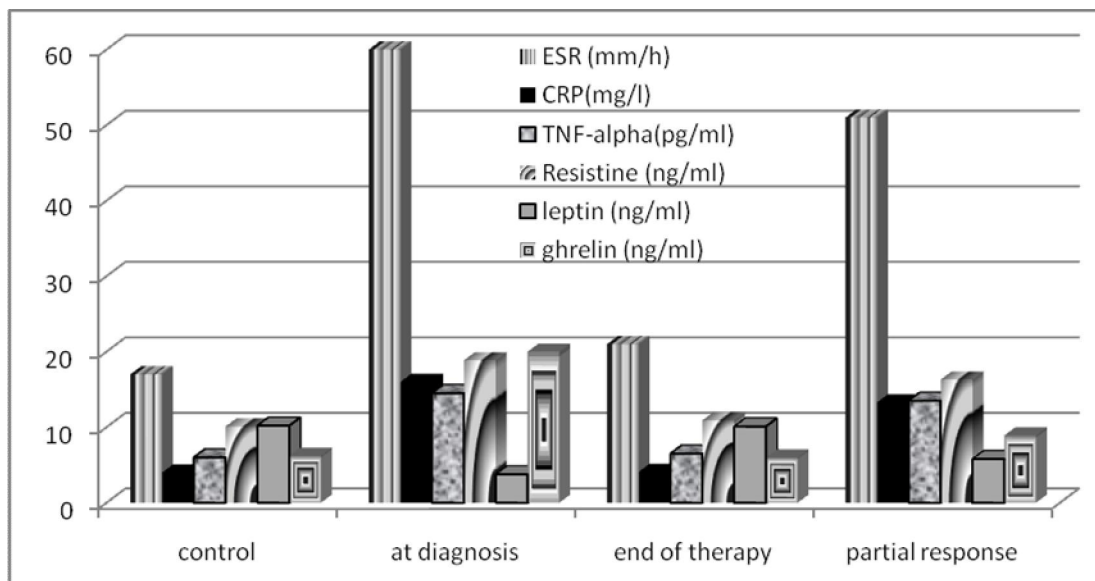


Figure (1): changes in serum values of biochemical markers in UC patients during therapy

4. Discussion

There are several reasons why laboratory markers have been studied in IBD in the past decades: firstly, to gain an objective measurement of disease activity as symptoms are often subjective; and secondly, to avoid invasive (endoscopic) procedures which are often a burden to the patient (Vermeire et al. 2006).

We studied serum levels of leptin, TNF- α , resistin, and ghrelin in patients with ulcerative colitis (UC) beside the traditional inflammatory markers to evaluate possible associations with the course and characteristics of the disease. Also, in order to provide objective assessment of the disease activity and decide which patient should undergo further assessment by colonoscopy.

The results of our study showed that there were significant decrement in the level of BMI and Hb%, while there were significant increase in the level of total leucocytic count and platelet count as compared to the normal control group. These levels were improved on the therapeutic protocol and reach nearly normal values after treatment, except in 9 patients who showed no improvement clinically and laboratory on therapy and we change the protocol of therapy to them and excluded from the study. Our results were in accordance with Vermeire et al. (2006) who stated that, more generally used laboratory markers include white blood cell count, platelets, and albumin. White blood cell count will increase as part of the acute phase response. Increased leucocytosis is therefore not a specific feature of IBD and may be seen in other inflammatory conditions and stressful events. Platelet count will also increase without being a specific marker of inflammation, given the wide range of normal values for platelet count, it has been less useful.

During an acute inflammatory episode, the leukocyte count and platelet count increase whereas the hemoglobin and albumin level decrease. Leucocytosis is not a useful marker of disease activity in clinical practice as there are many factors besides disease activity (systemic glucocorticosteroids, immunosuppressant, presence of abscess) that affect it. Platelet count correlates with disease activity in IBD but it is not used in clinical practice in IBD as there are other factors such as haemorrhage from other sites and iron deficiency anaemia which can cause elevation of platelet count. The role of these serum markers and their correlation with endoscopic and histological inflammation is not well established in UC patients (Desai et al. 2007).

Our results showed that, serum ESR and CRP levels were significantly increased in the acute active stage and these levels decreased following the therapy to nearly normal levels except in the 9 patients who did not respond to therapy. These results favour the results of Vermeire et al. (2006) who demonstrated that, established common serum biomarkers included CRP, ESR, hemoglobin, leukocyte count, platelet count and albumin level. The development of biological treatments has renewed interest in these biomarkers, given their potential to select responders to this therapy. CRP is found to be the most useful one in this respect. Whereas other acute phase reactants and markers of inflammation such as ESR also give reliable information on disease activity, their longer half life and interference with other factors make them less useful in clinical practice compared with CRP.

Lok et al. (2008) reported that Abnormal CRP, ESR, white cell count, haemoglobin, platelet count and albumin occurred in 42.3%, 55.1%, 23.1%, 21.8%, 32.1% and 25.6% of these mucosal inflammatory episodes, respectively. For the severity of the clinical disease, all serum biomarkers demonstrated a good correlation with the severity grading. On the other hand, the serum biomarkers correlated well with endoscopic extensive colitis but not with proctitis or left-sided colitis. Oruc et al. (2009) found that Ulcerative colitis patients had slightly higher procalcitonin levels and significantly higher C-reactive protein levels than controls and they conclude that Serum C-reactive protein is a reliable marker for disease activity in inflammatory bowel disease.

The results of our study showed the TNF- α level was significantly increased in the active UC patients and this levels decreased gradually in response to therapy in 47 patients who showed inactive disease (complete remission) while in 9 patients the level of TNF- α , did not return to nearly normal values in response to therapy which called (partial remission). The results of our study enforced the results of Komatsu et al. (2001) who reported that the median serum concentration of TNF- α , in IBD patients overall was 1.7-fold higher in the active stage of UC than in the inactive stage ($P < 0.05$), and this difference could be detected in individual patients.

Inflammation is undoubtedly a key component in the pathogenesis of ulcerative colitis (Podolsky 2002), and proinflammatory cytokines (IL-1, IL-6, IL-8, TNF- α , and IFN- γ) operate as a cascade and network in stimulating the production of acute-phase proteins and induction of acute-phase manifestations (Gabay, 1999). On the other hand, Umehara (2006) found that serum levels of TNF- α ,

were within the normal range in most of cases despite being in the active phase. Tumor necrosis factor- is produced by activated macrophages and monocytes. Although the serum concentration of TNF- is often increased in patients with active IBD, serum concentrations of TNF- have not been consistently elevated and are thus of limited utility as markers of disease activity in these patients (Desai et al. 2007).

In our study, we measured the circulating levels of 4 hormones (adipocytokines) in patients with IBD. These hormones are produced by WAT either in large amounts (leptin, adiponectin, and resistin) or in trace amounts (ghrelin), and all of them are closely related to human metabolism and inflammation. Human metabolism dramatically changes in IBD, and chronic inflammation is the hallmark of the disease. Our results demonstrated that serum leptin levels are significantly decreased in patients during the active stage (acute) of UC than in those of healthy subjects and these levels return to nearly normal values in response to therapy except in 9 patients who showed partial improvement.

These results were in accordance to Kirchgessner et al. (1997) and Bruun et al. (2002) who found that UC patients had significantly lower leptin levels than control group. Leptin levels were significantly lower in IBD patients, mainly in UC, compared with control, suggesting that chronic intestinal inflammation may decrease circulating leptin levels. TNF- α is possibly the major cytokine involved in intestinal inflammation in IBD patients. Recently, a number of studies indicated that whereas TNF- α , transiently induces acute release of intracellular pools of leptin, it decreases leptin synthesis during chronic inflammation. On the other hand, although inflammatory gut diseases, through their associated cytokines, mediate energy loss and weight loss, it is not clear how the mechanisms work. It has been proposed by the investigators that proinflammatory cytokines release leptin from adipose tissue, which leads to increased plasma concentrations and this increase is inappropriately high for the percentage of fat mass (Ballinger 1999).

Barbier et al. (1998) reported that increased secretion of plasma leptin concentrations has been observed in the early stages of experimental intestinal inflammation in rats. Tuzun et al. (2004) reported that patients enrolled in their study were in the acute stage of UC. High leptin levels in these patients suggest that the acute inflammation associated with UC increases circulating leptin levels. Yet, the mechanism of the induction of leptin secretion in intestinal inflammation remains obscure. Recently, inflamed colonic epithelial cells were found to express and release leptin into the intestinal lumen, and the product appears to induce epithelial wall

damage and neutrophil infiltration, a characteristic histological finding in IBD (Sitaraman et al. 2004).

The main production of resistin and ghrelin occurs in different sites, and in addition to their participation in the mechanisms of energy homeostasis, they seem to have in common a close association with inflammation, a fact that may implicate them in the pathogenesis of IBD (Konstantinos et al. 2006). In our study, the patients with active UC had significantly higher circulating levels of resistin and ghrelin as compared with healthy control. And these levels were decreased to nearly normal values as a response to therapy except the 9 patients who showed partial remission. Also, the results showed a significant increase of their levels with the progress of the disease.

The results of our study enforced the results of Ates et al. (2008) where serum ghrelin levels were significantly higher in patients with active UC than in those in remission. Their study demonstrates that patients with active IBD have higher serum ghrelin levels than patients in remission and high levels of circulating ghrelin correlate with the severity of disease and the activity markers. Finally, they arrived at the conclusion that ghrelin level may be important in determination of the activity in UC patients and evaluation of nutritional status. Also, Konrad et al. (2007) found that patients with IBD showed significantly higher resistin levels compared with controls. In patients with UC, resistin concentrations were significantly associated with elevated white blood cell count, C-reactive protein (CRP) and disease activity.

Ghrelin can inhibit cytokine activation including interleukin, TNF- α , and most interestingly leptin (Dixit et al. 2004). Our results for leptin levels agree with this observation because higher ghrelin levels were correlated with lower leptin levels in IBD patients compared with HC. Patients with celiac disease have higher ghrelin levels than BMI-matched controls and ghrelin decreases on a gluten-free diet. A correlation between the severity of the inflammation and ghrelin levels has been suggested (Peracchi et al. 2003).

5. Conclusion

Our data indicated that, the increased plasma resistin, TNF- α and ghrelin levels were positively correlated with activity of ulcerative colitis more than the ordinary inflammatory markers, so they could predict the response to therapy and early relapse of the disease, and possibly reflect an acute-phase response due to inflammation. The resistin, TNF- α and ghrelin levels could be considered as independent predictor of disease activity in patients with UC and may represent link between inflammation and UC.

Detection of adipocytokines level might decrease the need of repeated endoscopy to know the severity of the disease and to follow the response of therapy.

Further studies are needed to elucidate the role of adipocytokines in UC.

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