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RESEARCH ARTICLE

Role of circulating adipokines as a diagnostic tool in ulcerative colitis

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Abstract

Adipokines participate in the modulation of immune and inflammatory responses and their significance in inflammatory bowel disease is a subject of intensive research. Aim: to evaluate serum levels of leptin, resistin, and ghrelin in ulcerative colitis patients, and to correlate these results with the disease activity and the ordinary inflammatory markers. Subject & methods: Our study included 55 ulcerative colitis patients (32 patients with acute ulcerative colitis (AUC), and 33 patients with remission ulcerative colitis (RUC)). In addition, 50 healthy control subjects served as control group. Serum levels of adipokines were assessed by sandwich enzyme linked immunosorbent assay kits. Results: Mean serum levels of ESR, CRP, resistin and ghrelin were significantly increased while serum leptin was significantly decreased in AUC patients than the control group, while in the RUC group the mean values of these biochemical parameter were non significant with the healthy control group. Positive correlation between CRP and both resistin and ghrelin and negative correlation with leptin indicate that these adipokines correlated with severity of disease. Conclusion: Serum adipokines could play a significant role in the pathogenesis of inflammatory processes and progress of ulcerative colitis.

INTRODUCTION

Ulcerative colitis (UC) is an inflammatory intestine disease that causes long-lasting inflammation and ulcers in the most inner lining of our large intestine (colon) and rectum. Although the disease has a variable distribution, it is limited to the distal intestine (Kornbluth & Sachar, 2010 , Juckett & Trivedi, 2011). Patients with ulcerative colitis usually present with diarrhea which may be associated with blood. Bowel movements are frequent and small in volume as a result of rectal inflammation. Associated symptoms include colicky abdominal pain, urgency, tenesmus, and incontinence. Patients with mainly distal disease may have constipation accompanied by frequent discharge of blood and mucus (Silverberg etal., 2005).

Although numerous investigations were performed, the etiology and pathogenesis of human IBD remains unknown. Experimental and clinical studies suggested that the initiation and pathogenesis of IBD are multifactorial, involving the intervention of genetic, environmental, immunological and infectious factors. (Juckett & Trivedi, 2011).

The significance of adipokines for IBD pathophysiology is the subject of intensive research, as on one hand the adipokine levels might serve as an index of inflammatory activity and on the other hand, inhibition of specific adipokines could expand the spectrum of therapeutic interventions for this disease.

Leptin is a member of the type I cytokine superfamily and an adipokine, predominantly produced by adipocytes and to a lesser extent by the placenta, muscles, pituitary gland and gastric epithelium (sitaraman etal., 2004). Leptin is

considered a multi-task hormone, as well as an important regulator of inflammation (wozniak etal., 2009, lago et al., 2007).

Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor (GHS-R) and it has been identified in T cells (Dixit et al., 2004). ghrelin is produced primarily by gastric endocrine cells while small amounts are expressed in the hypothalamus, small intestine, and other tissues. Ghrelin is traditionally thought of as a meal-initiating hormone, given that levels increase just prior to meals and fall after meals (cummings et al., 2002). Ghrelin can inhibit cytokine activation including interleukins, TNF-a, and most interestingly leptin (Dixit et al., 2004). Resistin is a 108-amino acid peptide hormone with a molecular weight of 12.5 kDa (steppan et al., 2001).There is evidence that resistin is involved in inflammatory and metabolic pathways in humans and a possible role in IBD was recently postulated (paul et al., 2005).

Among the diverse metabolic manifestations of IBD are anorexia, malnutrition, altered body composition, and development of mesenteric white adipose tissue hypertrophy. Since all these phenomena are also associated with fluctuations in the production of adipokines and ghrelin (Karmiris et al., 2008).

The present study was designed to evaluate serum levels of leptin, resistin, and ghrelin in ulcerative colitis patients, to correlate these results with the disease activity and the ordinary inflammatory markers.

Subjects and methods

55 consecutive patients with ulcerative colitis were enrolled in the study between October 2014 and august 2015. They were subdivided into 2 subgroups, 32 patients with acute ulcerative colitis (AUC) (18 male and 14 female), and 33 patients with remission ulcerative colitis (RUC) (19 male and 14 female). In addition, 50 healthy control subjects served as control group.

The study was approved by Zagazig University Ethics Committee. All samples were obtained with written informed consent of the patients prior to their inclusion in the study.

Exclusion criteria were 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.

A diagnosis of UC is based on the generally accepted clinical, radiographic, endoscopic and histologic criteria (Forbes, 2002, Goh & O'morian, 2003).

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 of ulcers, 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.

Sampling:

2-mL blood samples were collected in the morning after an overnight fast and after centrifugation (3500g for 15 min) serum samples were stored at -80 ^{o}C until assayed.

Leptin, resistin, and ghrelin concentrations were measured using commercially available sandwich enzyme linked immunosorbent assay kits (R&D Systems, Abington,UK, for leptin, and resistin, and Peninsula Laboratories/Bachem, Torrance, Calif, for ghrelin).

Statistical Analysis

All results are expressed as mean ± SD. Comparisons among the 3 groups in terms of continuous measurements were made by the one way analysis of variance (ANOVA) and Post hoc multiple comparisons tests. Correlations between serum adipokines and indices of disease activity were analyzed with the Pearson's correlation method. All comparison was conducted using SPSS software version 13. Level of $P \leq 0.05$ was considered statistically significant.

Results

Demographic and clinical data of ulcerative colitis patients and control

A total of 32 AUC, 33 RUC, and 50 healthy controls were included in the study. There was no statistically significant difference between the AUC, RUC, and healthy control groups as regards to age, gender ($P \geq 0.05$), however there were significant decrease in the level of BMI in both AUC and RUC groups when compared to the control group (Table 1).

Serum adipokines levels in all studied groups

Mean serum levels of ESR, CRP, resistin and ghrelin were significantly higher in AUC patients than the control group, while in the RUC group the mean values of these biochemical parameter deceased significantly than the values at the active disease and were non significant with the healthy control group.

The mean level of serum leptin was significantly decreased in AUC patients when compared to the control group, while in RUC patients, the mean value increased significantly from their value in AUC group and became non significant to the control group (Table 2).

Correlation between studied adipokines and nutritional or inflammatory parameters in AUC patients (Table 3).

Serum resistin level were positivly correlated with ESR (r =0.32, P=0.02) and CRP (r=0.34, p=0.01) while there was no significant correlation between serum resistin level and BMI.

Serum leptin was positively correlated with BMI (r= 0.37, p=0.01) and negatively correlated with ESR (r=-0.34, p=0.01) and CRP (r= -0.32, p=0.02).

Serum ghrelin level was negatively correlated with BMI (r=-0.47, p= 0.01) and positively correlated with ESR (r =0.33, P=0.01) and CRP (r=0.35, p=0.01)

Table1: Demographic and clinical data of ulcerative colitis patients and control

	Control(50)	Active ulcerative colitis(32)	Remission ulcerative colitis(33)	p
Age(years)	31.5±8.2	30.6±9.5	33.2±7.6	0.3
Gender male female	 30(60%) 20(40%)	 18(56%) 14(44%)	 19(57.6%) 14(42.4%)	0.94
BMI(kg/m^2)	22.1±4.7	18.3±5.6	19.5±2.7	0. 001
Localization of disease recto-sigmoiditis left- sided colitis pancolitis		 10 10 12	 8 15 10	

Table 2: Serum adipokines level in the studied groups:

	Control (50)	Active ulcerative colitis (32)	Remission ulcerative colitis (33)
ESR (mm/h)	17.7±1.3	64.1±10.1*	19.2±9.5
CRP(mg/l)	3.7±0.6	20.8±5.6*	3.9±0.5
Resistin(ng/ml)	9.9±0.5	17.8±2.9*	10.1±0.4
Leptin(ng/ml)	9.8±0.6	4.3±0.7*	9.9±0.3
Ghrelin (ng/ml)	5.8±0.8	18.04±1.7*	6.1± 0.9

*significant P≤0.05 when compared with control group

Table 3: **Correlation between studied adipokines and nutritional or inflammatory parameters**

	BMI(kg/m^2)	ESR(mm/h)	CRP(mg/l)
Resistin(ng/ml)	r=-0.28 p=0.1	r=0.32 P=0.02	r=0.34 0.01
Leptin(ng/ml)	0.37 0.01	r=- 0.34 P=0.01	r=-0.32 P=0.02
Ghrelin(ng/ml)	-0.47 P=0.01	r=0.33 P=0.01	r=0.35 P=0.01

Discussion

Diagnosis of UC is based on clinical symptoms combined with radiological and endoscopic investigations. Tests for the diagnosis and care of patients with UC are usually invasive which may result in discomfort and potential complications. The employment of non-invasive biomarkers is needed. The ability to determine the type, severity, prognosis and response to therapy of UC, using biomarkers has long been a goal of clinical researchers (cioffi et al., 2015).

We studied serum levels of leptin, resistin, and ghrelin in patients with ulcerative colitis together with the ordinary inflammatory markers to evaluate their role in the pathogenesis and severity of the disease, to elucidate its role in the diagnosis and follow up of patients and decide which patient should undergo further assessment by colonoscopy.

Our study demonstrated that there was a significant decrease in the BMI in AUC groups when compared with the control group however in RUC group BMI was non significant with the control group. These results were in accordance with Vermiere et al., 2006 and Afify et al., 2010.

The laboratory tests most used to measure the acute-phase proteins in clinical practice are the serum concentration of C-reactive protein and the erythrocyte sedimentation rate.Our results showed that, serum ESR and CRP levels were significantly increased in the AUC group when compared with control group .while in the remission group these levels approximate normal levels and were nonsignificant with the control group. These results are in accordance with Vermeire et al. (2006) who demonstrated that, CRP is an objective marker of inflammation and correlates well with disease activity in Crohn's disease (CD). However, CRP correlates less well with disease activity in patients with ulcerative colitis as compared with CD.

ESR also gives reliable information on disease activity (Oruc et al., 2009). The ESR, compared with CRP, reaches the highest point less quickly, decreases more slowly and has a lesser degree of change making them less useful in clinical practice compared with CRP (cioffi et al., 2015).

Adipokines have both pro- and anti-inflammatory effects in inflammatory bowel syndrome. Leptin, is one of the most well studied adipokines, exerts many metabolic, endocrine, and immunologic effects (lord et al., 1998).

The mean level of serum leptin was significantly decreased in AUC patients as compared to the control group, while in patients achieved complete remission (RUC) the mean value increased significantly.

Low leptin concentrations in AUC subjects, which are consistent with previous studies [Bruun et al. 2002, **Karmiris** et al., 2006], may be the result of TNF-α hyperactivity. TNF-α stimulates the temporary release of substantial amounts of leptin in response to inflammation eventually leading to a decrease in leptin mediated chronic inflammation. Moreover, serum leptin levels increase in CD subjects treated with the TNF-α antagonist infliximab confirming the role of TNF-α in the regulation of leptin release by adipocytes (**Franchimont** etal.,2005). Similarly, the study of Waluga M et al 2014 showed that a 3-month treatment period with corticosteroids alone or with azathioprine leads to increased blood leptin concentrations, which is particularly noticeable in CD subjects.

Interestingly, while some studies report increased expression of leptin mRNA in the mesenteric adipose tissue or increased concentrations of leptin in large bowel lavage fluid from patients with mild to severe CD or UC (Sitaraman et al., 2004, Barbier et al., 2003), leptin is not detected in normal colonic epithelium. However, it is detected in the subapical region of the epithelial cells during conditions of inflammation (Sitaraman et al., 2004). This finding explains the differences between leptin concentrations measured in the serum, tissue, or gut lumen.
Resistin is a proinflammatory agent, as it stimulates the synthesis and secretion of TNF-α, IL-12, and adhesive factors upon nuclear factor kappa B activation (Silswal et al.,2005). In our study, the patients with active UC had significantly higher circulating levels of resistin when compared with control group and these results are consistent with previous studies (Waluga M et al 2014 , Valentini L,2009. Konrad A,2007).
In the remission group resistin levels were non significant with the control group. Moreover resistin level were positively correlated with CRP level and ESR level in the group of active ulcerative colitis. 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.

In our study, the patients with active UC had significantly higher circulating levels of ghrelin as compared with the control group. However in the remission group these levels were no significant with normal values as a response to therapy. 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.
Ghrelin can inhibit cytokine activation including interleukin, TNF-á, 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 control group.
We studied the correlation between the three studied adipokines and BMI as well as with level of ESR and CRP in the AUC group. The positive correlation between body mass index and leptin and negative correlation with ghrelin in AUC, indicate their role in aneroxia in these patients.
Positive correlation between CRP and both resistin and ghrelin and negative correlation with leptin indicate that these adipokines correlated with severity of disease.

Conclusion

Serum adipokines could play a significant role in the pathogenesis of inflammatory processes and progress of ulcerative colitis. We could consider serum adipokines as an alternative or additional diagnostic tool to CRP, ESR and other indicators of inflammatory disease, used in diagnosis and follow up of patients of ulcerative colitis.

Conflict of interest: None
Ethical statement : The study was approved by Zagazig University Ethics Committee. All samples were obtained with written informed consent of the patients prior to their inclusion in the study.

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