SERUM FETUIN-A LEVELS IN PATIENTS WITH ULCERATIVE COLITIS

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ABSTRACT

Introduction: *Fetuin-A acts as a negative acute phase protein that may also be involved in the pathogenesis of Ulcerative colitis (UC).The aim of this study was to investigate the relationship between serum Fetuin-A levels and clinical and biochemical parameters in patients with UC.*

Materials and methods: *This study included 40 healthy subjects and 40 patients with UC. Fetuin levels were measured by using ELISA. Haemoglobin, platelet, platelet width distribution (PDW), leukocyte, C-reactive protein (CRP), procalcitonin, total protein, albumin, and sedimentation values were analysed.*

Results: *Haemoglobin, albumin, and fetuin levels of the control group increased compared to the patient group ($p<0.01$). Procalcitonin, C-reactive protein ($p <0.05$), leukocyte, and sedimentation values ($p <0.01$) were higher in the patient group. Fetuin-A had a negative correlation with sedimentation and leukocyte values ($p <0.05$).*

Conclusion: *Fetuin-A level was found to be significantly lower in patients with UC compared to control group. There was a significant negative correlation between leukocyte and sedimentation values and fetuin-A. Fetuin-A level was significantly lower in the left colon and the advanced involvement than the distal involvement. Fetuin-A may be a guide for the involvement site of disease and the inflammation level in UC.*

Key Words: *fetuin-A, inflammation, ulcerative colitis*

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Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are chronic relapsing diseases of the gastrointestinal tract with spontaneous exacerbations and healing periods. UC mainly affects the mucosa of the rectum due to increased infiltration of gut bacteria and formation of crypt abscess in colon[(1)].

Basic pathophysiological mechanism of inflammatory bowel diseases includes the imbalance of proinflammatory cytokines (TNF, IFN-γ, IL-6, IL-12, IL-21, IL-23, IL-17, etc.) and anti-inflammatory cytokines (IL-35, IL-4, IL-10, IL-11, IL-13,) as well as the disturbance in activation of Th1 and Th2 lymphocytes[(2-5)]. These changes cause apoptosis, cellular infiltration, and the loss of integrity and function of the gut.

In patients with UC, ulcers and inflammation of the inner lining of the colon lead to symptoms of abdominal pain, diarrhoea, and rectal bleeding[(6)]. Today, histopathological examination and endoscopic and radiological findings play a major role in the diagnosis and management of UC. However, specific biomarker profiles can be used in simple, economical and reliable test as an alternative to more complex procedures for development and treatment of UC[(7,8)].

Fetuin-A, a multifunctional glycoprotein known as alpha 2 glycoprotein, is secreted from hepatocytes. Fetuin-A acts as a negative acute phase reactant in acute and chronic inflammatory diseases[(3,9)]. The aim of this study was to investigate the effect of fetuin-A on clinical and biochemical parameters in patients with UC.

Materials and methods

Study design

This study was formally approved by the local Ethics Committee (approval date and number: 2016/491) and conducted according to the ethical standards of the Declaration of Helsinki. Written informed consent was obtained from all the patients. Forty healthy control subjects and Forty patients with UC who had treatment during activation and remission were included in the study. The demographic characteristics of the patients and the control group were recorded.

Body mass index (BMI) was calculated based on weight in kg divided by height in square meter (m^2). Patients with Crohn's disease, radiation colitis, infectious colitis, ischemic colitis, coronary artery disease, diabetes mellitus, chronic obstructive pulmonary disease, connective tissue disease, advanced renal and hepatic disease, any systemic disease, and pregnancy were excluded from this study.

Clinical and biochemical measurements

After 12 h of night fasting, venous blood samples were taken, centrifuged (3000 cycle/min) and then stored at -80°C in a freezer for the analysis.

Haemoglobin, platelet, PDW, leucocyte, and total protein were measured using the related kit in an autoanalyzer (Cobas autoanalyzer, DPC, Diagnostic products corporation, USA). The erythrocyte sedimentation rate was measured and evaluated based the method by Fahraeus and Westergren.

Serum fetuin-A levels were measured using ELISA kit (Elabscience, UK, cat no: E-EL-H0386) with ELX 800 UV model ELISA reader. The results were calculated in nanograms per millilitre.

Albumin, CRP, and procalcitonin levels were measured using an auto-analyzer (Roche/Hitachi Cobas C Systems, USA), according to the manufacturer's protocol.

Statistical analysis

All statistical analyses were performed using SPSS v.22.0. The data were first analysed by the Kolmogorov-Smirnov test to determine whether or not they had normal distribution and the unpaired Student t-test was used to compare means of two groups with normal distribution.

When appropriate, the Mann-Whitney test and Chi-square test were used to compare means of two groups showing no normal distribution (non-parametric data).

Pearson correlation analysis and Spearman's rank correlation analysis were used to determine the relationship between fetuin-A and other parameters. The level of significance was accepted at $p < 0.05$ in all analyses.

Results

This study included 40 healthy controls (19 female, 21 male) and 40 patients with UC (24 female, 16 male). There was no statistically significant difference between the groups in terms of age, body mass index, distribution of males and females, and smoking use ($p>0.05$). Table 1 shows demographic characteristics of the control and patient groups.

Variables		Mean±SD		Test values
		Control	Patient	
age	Male	40.52±13.56	48.25±16.25	t=1.23 p=0.22
	Female	33.26±9.39	37.63±12.90	
body mass index (kg/m^2)	Male	24.74±3.34	24.24±2.84	t=1.57 p=1.12
	Female	22.27±4.05	24.17±3.42	
		n(%)		Test values
		Control	Patient	
gender	Male	21(52.5%)	16(40)	X^2=1.25 p=0.26
	Female	19(47.5%)	24(60)	
smoking	No	25 (62.5)	24(60)	X^2=0.5 p=0.81
	Yes	15 (37.5)	15(40)	

Table 1: Demographic characteristics of control and patient groups.
SD: Standard Deviation, X^2: Chi-quare test t=student-t test.

There was no difference between the groups in terms of the levels of platelet, PDW and total protein. Fetuin-A, albumin, and haemoglobin levels significantly increased in the control group compared to the patient group ($p < 0.01$). There was increased procalcitonin, CRP, leucocyte, and sedimentation levels in the patient group ($p < 0.01$) (Table 2).

There was no statistically significant difference between the fetuin-A values of the patients in terms of the variables of gender and smoking use ($p>0.05$). Fetuin-A values were significantly lower in patient with extensive colitis compared to left sided colitis ($p<0,01$) (Table 3).

There was a statistically significant correlation between fetuin-A and sedimentation and leucocyte values of the patients ($p<0.05$). No correlation was found between fetuin A and other parameters ($p>0.05$) (Table 4).

Variable	Group	Mean	SD	Test values	p
haemoglobin (g/dl)	Control Patient	14.24 13.12	1.73 1.99	t=2.69	0.009**
platelet (mm^3)	Control Patient	272665 282547.5	70767 94583	t=0.52	0.59
leukocyte (10^3/ml)	Control Patient	7.34 8.63	1.51 1.51	t=1.73	0.001**
PDW (fl)	Control Patient	18.92 18.57	2.17 1.92	t=0.75	0.45
albumin (g/dl)	Control Patient	4.65 4.48	0.28 0.38	t=2.73	0.008**
procalcitonin (ng/ml)	Control Patient	0.033 0.043	0.013 0.026	t=1.94	0.04*
sedimentation (mm/hr)	Control Patient	8.67 24.53	5.22 17.12	Z=5.59	0.000**
total protein (g/dl)	Control Patient	7.4 7.34	0.31 0.53	t=0.63	0.52
CRP (mg/l)	Control Patient	1.89 6.02	1.42 11.75	Z=2.20	0.03*
fetuin-A (g/l)	Control Patient	0.306 0.158	0.043 0.06	t=12.51	0.000**

Table 2: The comparison of biochemical parameters in patient and control groups.
*SD:Standard deviation t= student t test, Z=Mann Whitney U test, *p<0.05, **p<0.01.*

Variable	Group	Fetuin-A		Test values	p
		Mean	SD		
gender	male (n=16)	0.17	0.05	t=1.76	0.08
	female (n=24)	0.14	0.06		
smoking	no (n=24)	0.15	0.06	t=0.83	0.4
	yes (n=16)	0.16	0.05		
extent of disease	left sided (n=20)	0.18	0.04	t=3.42	0.001**
	extensive (n=20)	0.13	0.04		

Table 3: Comparison of Fetuin-A levels of the patients according to gender, smoking status, and extent of disease.
*SD:Standard deviation, t= student t test, **p<0.01.*

	Fetuin A	
	r	p
age (Year)	0.01	0.9
body mass index (kg/m2)	-0.003	0.98
haemoglobin (g/dl)	0.26	0.09
PDW (fl)	0.21	0.18
platelet (mm^3)	0.06	0.68
leucocyte (10^3/ml)	-0.28	0.01*
sedimentation (mm/hr)	-0.32	0.03*
albumin (gr/dl)	0.05	0.75
C-reactive protein (mg/l)	-0.11	0.47
procalcitonin (ng/ml)	-0.11	0.48
total protein (g/dl)	0.1	0.53

Table 4: The correlation between the fetuin-A values and the variables in patients.
***r**: correlation coefficient. *p<0.05.*

Discussion

UC is characterised by inflammation affecting only the colon and is limited to the mucosa and superficial submucosa. The inflammation may affect small intestine and colon and is accompanied usually by diarrhoea and bleeding. Microulcers and ulcers bleed and produce pus and mucus[1,10].

The balance of pro- and anti-inflammatory molecules in the colonic mucosa is significant for normal gut homeostasis. A failure of the cytokine profile in favour of pro-inflammatory cytokine overproduction leads to tissue damage as in inflammatory bowel diseases[11]. Anti-inflammatory molecules decrease proinflammatory cytokine production in many different ways[12]. It is suggested that fetuin is a cofactor taking an important role in counter-regulating macrophage activation[13].

Fetuin-A inhibits the production of macrophage proinflammatory cytokine synthesis during inflammation. Moreover, the hepatic fetuin-A expression is negatively regulated by several proinflammatory cytokines such as TNF, IL-1, IL-6, and IFN-γ[14].

In vivo and in vitro studies have consistently approved that one function of fetuin-A is an essential inhibitor of pathological calcification on the molecular level. In addition, fetuin-A also acts as a carrier for lipids, which may influence calcification, inflammation, and apoptosis[15].

Fetuin acts as a negative acute-phase protein and its level reduces in acute and chronic inflammation[16]. In the present study, Fetuin-A levels significantly decreased in the patient group. Ma et al., showed that the active patients with Crohn's disease and patients with UC both had significantly lower serum fetuin-A levels compared with the inactive ones with Crohn's disease and UC[17]. In patients with pancreatitis and chronic kidney diseases, serum fetuin-A levels also decreased by 20-30%[18,19].

Also, a negative correlation was found between fetuin-A and sedimentation and leucocyte values in patients. There was a reverse correlation between fetuin-A levels and the levels of the proinflammatory cytokines such as IL-1β, IL-6, and TNF-α and a reverse correlation between low levels of fetuin-A and CRP, which is a marker of inflammation in patients undergoing haemodialysis[20].

Sato H. et al., showed that Serum Fetuin-A levels significantly decreased in patients with Rheumatoid Arthritis. In addition, it was found that while Serum C-reactive protein concentration and erythrocyte sedimentation rate were inversely cor-

related, albumin, haemoglobin and total cholesterol were positively correlated with Fetuin A[21]. On the contrary, it was suggested that fetuin-A was a positive acute phase protein in injury. Clinically, plasma fetuin-A levels significantly elevated in patients with cerebral ischemic injury[22]. In this study, fetuin-A values were significantly lower in patient with extensive colitis compared to left sided colitis. It is indicated that the fetuin A is more vulnerable to decline as the area of inflammation grows. The present study revealed the relationship between values of leucocyte and sedimentation and fetuin-A level in UC patients. Inflammatory markers may be an alternative to other complex procedures to detect UC. Biochemical markers are non-invasive, simple, economical, and reliable tests[17]. The present study supported fetuin-A concentration measurements as a negative acute phase protein of disease activity in patients with UC.

Ma et al. found that no significant correlation was found between 5-aminosalicylic acid, steroids, Immunosuppressants and disease activity but decreased serum fetuin-A levels were independently associated with disease activity in patients with UC[23]. Manolakis et al. was found that significantly lower fetuin-A levels in patients with UC the need for anti-TNFα therapy or surgery compared to other treatment groups[24]. The result of the present study should be validated in larger cohorts. One of the present study's limitations is the sample size. Another limitation is that liver Fetuin-A and serum cytokine levels might also be evaluated. It would be more meaningful to perform comparative studies by measuring repeated fetuin-A levels in prospective, longitudinal and more patients with and without treatment.

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