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Elevated Levels of Fecal Calprotectin in Cirrhotic Patients and Spontaneous Bacterial Peritonitis

Sirotik Hastalarda ve Spontan Bakteriyel Peritonitte Fekal Calprotectin Düzeylerinde Artış

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ABSTRACT

Aim: The aim of this study is to investigate the relationship between fecal calprotectin (FC) which is a marker for intestinal inflammation and complications of cirrhosis which are due to increased bacterial translocation and intestinal inflammation.

Material and Methods: Out of 156 cirrhotic patients aged between 18-80 years who are admitted to our hospital, 64 were excluded according to exclusion criteria and a total of 92 patients, and 20 volunteers with similar age and sex as a control group were included in this study. Serum samples were taken at admission to measure erythrocyte sedimentation rate (ESR), c-reactive protein (CRP) and white blood cell count (WBC). All patients and the control group provided a single stool sample within 24 hours after admission. The study group divided into five subgroups (Child-Pugh Grade A, Grade-B, Grade-C, spontaneous bacterial peritonitis and hepatic encephalopathy) to investigate whether FC levels change as the disease progress or complications occur.

Results: Median FC levels were 168.8 mg/kg for cirrhotic patients and 9.8 mg/kg for control group, and the difference between the groups was statistically significant (p=0.039). In the subgroup analysis, the differences between spontaneous bacterial peritonitis and all other subgroups were statistically significant (p=0.002). In cirrhotic patients, FC levels were not correlated either with ESR (r=0.439, p=0.545) or CRP (r=0.403, p=0.321) or WBC count (r=0.061, p=0.645).

Conclusion: FC levels are increased in cirrhotic patients and early increase in FC levels before the rise of systemic inflammation markers can be used as a diagnostic marker for spontaneous bacterial peritonitis.

Keywords: Calprotectin; cirrhosis; secondary peritonitis.

ÖZ

Amaç: Bu çalışmanın amacı, intestinal inflamasyonun göstergesi olan fekal calprotectin (FC) ile artmış intestinal inflamasyon ve buna bağlı artan bakteriyel translokasyon sonucu meydana gelen sirozun komplikasyonları arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntemler: Hastanemize başvuran 18 ve 80 yaş arası 156 sirotik hastadan, dışlama kriterlerine göre 64'ü çıkartıldı ve toplam 92 hasta ve benzer yaş ve cinsiyette 20 gönüllü kontrol grubu olarak çalışmaya dahil edildi. Başvuru sırasında alınan kan örneklerinden eritrosit sedimantasyon değeri (ESR), c-reaktif protein (CRP) ve beyaz küre sayımı (WBC) çalışıldı. Her hastadan ve kontrol grubundan başvurudan sonraki 24 saat içinde bir adet spot gaita örneği alındı. Çalışma grubu, sirozun evresi ilerledikçe veya komplikasyonlar meydana geldiğinde FC değerlerinin değişip değişmediğini incelemek için beş alt gruba (Child-Pugh Evre-A, Evre-B, Evre-C, hepatiks ensefalopati ve spontan bakteriyel peritonit) ayrıldı.

Bulgular: Ortanca FC değerleri sirotik hastalarda 168,8 mg/kg ve kontrol grubunda 9,8 mg/kg idi ve gruplar arasındaki farklılık istatistiksel olarak anlamlıydı (p=0,039). Alt grup incelemesinde, spontan bakteriyel peritonit grubu ile diğer tüm alt gruplar arasındaki farklılıklar istatistiksel olarak anlamlıydı (p=0,002). Sirotik hastalarda FC ile ESR (r=0.439, p=0.545) veya CRP (r=0.403, p=0.321) ya da WBC sayımı (r=0.061, p=0.645) arasında korelasyon saptanmadı.

Sonuç: Sirotik hastalarda FC değerleri yükselmektedir ve sistemik inflamasyon belirteçlerinden önce FC değerlerinin erken yükselmesi sayesinde, spontan bakteriyel peritonitte tanısal bir test olarak kullanılabilir.

Anahtar kelimeler: Calprotectin; siroz; sekonder peritonit.

INTRODUCTION

Calprotectin was first described as an anti-microbial protein, which resides in the cytoplasm of granulocytes (1). It works as a pleiotropic molecule by activating endothelial cells and levels of calprotectin increase during active inflammatory processes. The soluble form of calprotectin can be found in blood, urine, and feces during inflammatory reactions because it is secreted from stimulated neutrophils and monocytes (2).

Fecal calprotectin (FC) levels increase in patients with inflammatory bowel diseases (IBD) and FC levels correlates with disease activity because inflammatory cytokines upregulate neutrophil migration to intestinal mucosa which causes high neutrophil turnover (3). FC levels also correlate with intestinal permeability (4).

Structural changes happen in the intestinal mucosa of the cirrhotic patients, such as vascular congestion, edema, fibromuscular proliferation, reduced villi to crypt ratio and thickening of muscularis mucosa. These changes increase intestinal permeability and facilitate bacterial translocation which is the driving factor for spontaneous bacterial peritonitis (SBP) and hepatic encephalopathy (HE). Increased bacterial activity causes the release of chemokines and triggers the inflammatory response. This defense mechanism paradoxically increases bacterial translocation because of the changes in tight junctions. Cirrhotic patients also have reduced chemotactic, opsonic and phagocytic activity that would cause systemic response and the degree of bacterial translocation increase as the disease progress (5).

In this study, we investigated the relationship between FC which is a marker for intestinal inflammation and complications of cirrhosis which are due to increased bacterial translocation and intestinal inflammation.

MATERIALS AND METHODS

Patients

One hundred fifty-six consecutive patients with cirrhosis, aged between 18 and 80 who were admitted to our hospital enrolled in this study after obtaining written consent. Demographic data, drug history and the cause of cirrhosis were recorded. Patients which had known causes for abnormal FC levels such as IBD, gastroenteritis, malignancies, drugs (proton pump inhibitors, non-steroidal anti-inflammatory drugs), gastro-esophageal reflux disease and Celiac disease (6) were excluded. Twenty volunteers with a similar age and sex distribution participated as a control group.

This study was performed in accordance with the principles of Good Clinical Practice, the principles of the Declaration of Helsinki and national laws. The study protocol was approved by the local ethics committee. (Eskişehir Osmangazi University, Non-drug Clinical Research Ethics Committee, dated 14.05.2013 and numbered 06).

Study Design

Diagnosis of cirrhosis was made by histopathologic assessment directly or indirectly with findings related to cirrhosis that indicate portal hypertension and impaired hepatic function. Child-Pugh classification (CP) was used to establish the severity of the disease. West-Haven criteria were used to determine HE and SBP were established as polymorphonuclear (PMN) leukocyte count was >250 cell/mm3.

We divided the study group into five groups to investigate whether FC levels change as the disease progress or complications occur:

i. 20 patients with CP Grade-A
ii. 21 patients with CP Grade-B
iii. 18 patients with CP Grade-C
iv. 17 patients with HE
v. 16 patients with SBP

Serum samples were taken at admission to measure erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and white blood cell count (WBC). All patients and the control group provided a single stool sample within 24 hours after admission. Stool samples stored properly at -80°C. Fecal calprotectin was assayed by an enzyme-linked assay (Phi-Cal Calprotectin ELISA Kit; Immundiagnostik AG, Bensheim, Germany) and FC values above 50 mg/kg were regarded as positive according to the manufacturer's instructions.

Statistical Analysis

The statistical analysis was performed with IBM SPSS Statistics for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA). Shapiro-Wilk's test was used to determine the normality of the data. Descriptive statistics are given by median and interquartile range (IQR) or mean and standard deviation, depending on the distribution of data. Student's t-test was used as a parametric test, Mann-Whitney U test was used to compare two groups and Kruskal-Wallis test was used to compare more than two groups as nonparametric tests. Spearman's correlation coefficient was used to examine the relationship between levels of FC, ESR, CRP, and WBC.

RESULTS

Baseline characteristics of the study and control groups are presented in Table 1. Sixteen patients were lost to follow-up and forty-eight patients had known factors that cause abnormal FC levels, therefore 92 patients (57 male, 35 female, mean age 60.5±11.9 years) and 20 healthy volunteers (12 male, 8 female, mean age 61.6±11.0 years) enrolled in this study. There was no statistically significant differences between patient and control groups in terms of sex (p=0.870) and age (p=0.430).

Median FC levels were 168.8 mg/kg (IQR 73.1-315.6 mg/kg) for cirrhotic patients vs. 9.8 mg/kg (IQR 6.8-13.8 mg/kg) for control group and the difference were statistically significant (p=0.039).

The etiology of cirrhosis was hepatitis C in 25,0% (n=23), hepatitis B in 19,6% (n=18), non-alcoholic steatohepatitis (NASH) in 21,7% (n=20), cryptogenic in 18,5% (n=17), alcohol in 8,7% (n=8), autoimmune hepatitis (AIH) in 4,3% (n=4) and primary biliary cholangitis (PBC) in 2,2% (n=2) of the patients.

FC levels in subgroups, as CP-A, CP-B, CP-C, HE and SBP are presented in Table 2. There was a significant difference in terms of FC levels between the subgroups (p=0.002). In the subgroup analysis, the difference between SPB and other groups was statistically significant (p=0.016, 0.011, 0.039 and 0.043, respectively). FC levels were higher in the CP Grade-C group but the difference was not statistically significant (Table 2).

In cirrhotic patients, FC levels were not correlated either with ESR (r=0.439, p=0.545) or CRP (r=0.403, p=0.321) or WBC count (r=0.061, p=0.645).

Table 1. Characteristics of the study group

	Cirrhotic Patients (n=92)	Control Group (n=20)	p
Sex, n (%)			
Male	57 (62.0)	12 (60.0)	0.870
Female	35 (38.0)	8 (40.0)	
Age, years, mean±SD	60.5±11.9	61.6±11.0	0.430
FC, mg/kg, median (IQR)	168.8 (73.1-315.6)	9.8 (6.8-13.8)	**0.039**
Etiology, n (%)			
Hepatitis-B	18 (19.6)		
Hepatitis-C	23 (25.0)		
NASH	20 (21.7)	-	-
AIH	4 (4.3)		
PBC	2 (2.2)		
Alcohol	8 (8.7)		
Cryptogenic	17 (18.5)		

SD: Standard Deviation, IQR: Interquartile Range, FC: Fecal Calprotectin, NASH: Non-alcoholic Steatohepatitis, AIH: Autoimmune Hepatitis, PBC: Primary Biliary Cholangitis

Table 2. Subgroup analysis

	FC, mg/kg, median (IQR)	p
CP Grade-A	135.0[a] (32.5-215.6)	
CP Grade-B	130.0[a] (59.4-365.0)	
CP Grade-C	152.5[a] (54.4-395.0)	**0.002**
HE	145.0[a] (78.8-292.5)	
SBP	363.8[b] (296.9-550.0)	

FC: Fecal Calprotectin, IQR: Interquartile Range, CP: Child-Pugh classification, HE: Hepatic Encephalopathy, SBP: Spontaneous Bacterial Peritonitis, a,b: According to the pairwise comparison results, FC levels in SPB subgroup was significantly higher than all other subgroups while the other four subgroups were similar each other

DISCUSSION

In this study, we found that FC levels in cirrhotic patients are significantly increased compared to healthy subjects and based on this data, FC can be considered as a valid marker for intestinal inflammation in cirrhotic patients. We also found that systemic markers of inflammation such as ESR, CRP and WBC count did not elevate despite a significant increase in FC levels. This finding also supports that FC is a sensitive and specific marker for intestinal inflammation in cirrhotic patients. Therefore FC can be a marker to diagnose the onset and severity of complications in cirrhotic patients.

The first study to investigate the prognostic value of calprotectin in cirrhotic patients was performed by Homann et al. (7). They showed that high levels of plasma calprotectin was related to poor survival in alcohol-related cirrhosis. They also described a subgroup of patients with recurrent bacterial infections which had higher levels of plasma calprotectin (8).

FC levels in IBD patients were investigated thoroughly in the literature (9-11) but we found only three studies investigating the relationship between FC and complications of cirrhosis. The first study by Yagmur et al. (12) found that FC levels in cirrhotic patients were significantly higher in cirrhotic patients. Other studies by Gundling et al. (13) and by Ibrahim et al. (14) had similar results. We also found that FC levels were significantly higher in cirrhotic patients 168.8 mg/kg (IQR 73.1-315.6 mg/kg) for cirrhotic patients vs. 9.8 mg/kg (IQR 6.8-13.8 mg/kg) for control group).

Contrary to our findings, both studies showed that FC levels also increase as the disease progress assessed by CP score and FC levels in HE patients were significantly higher. One explanation for this difference may be the routine use of prophylactic treatments in our clinic such as lactulose and rifaximin. Lactulose is degraded by colonic bacteria and the resultant acidic environment reduces the bacteria that produce ammonia and the risk of HE decrease in cirrhotic patients (15). Rifaximin also modulates gut microbiota and significantly decrease HE episodes and hospitalizations (16). Another explanation may be the timing of stool sampling. Especially in HE patients, most of the stool samples were obtained after the start of treatment that may have caused a reduction in FC levels. It should also be noted that FC has a biologic variability as day-to-day and even in spot one time only sampling (17).

Yagmur et al. (12) and Gundling et al. (13) also investigated the relationship between FC and markers of systemic inflammation such as CRP, WBC, interleukin-6, interleukin-8 and interleukin-10 and they did not find any significant influence of those laboratory parameters to FC levels. We also did not find any correlation between FC levels with either CRP, ESR or WBC count. The increase in FC levels before the rise of systemic inflammation markers also strengthen the rationale for the use of FC to diagnose the onset and severity of complications in cirrhotic patients.

FC levels in SBP patients were increased in all three studies mentioned above and Yagmur et al. (12) reported that the highest FC levels were determined in SBP group. We also found that FC levels were significantly higher in the SBP group (363.8 mg/kg) and the highest FC level (2108 mg/kg) was determined in this group.

CONCLUSION

The main conclusions of this study are the following: (i) FC levels are increased in cirrhotic patients and (ii) early increase in FC levels before the rise of systemic inflammation markers can be used as a diagnostic marker for SBP. Further comprehensive studies involving a larger number of patients are needed to confirm these suggestions and to determine whether FC can be used as a screening test to predict the complications of cirrhosis.

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