Turk J Hematol 2006; 23:94-99

RESEARCH ARTICLE

Plasma levels of thrombin activatable fibrinolysis inhibitor antigen in active and inactive inflammatory bowel disease

Mehmet Ali Özcan[1], Mesut Akarsu[2], Fatih Demirkan[1], Hale Akpınar[2], Faize Yüksel[1], Güner Hayri Özsan[1], Bülent Ündar[1], Özden Pişkin[1], İnci Alacacıoğlu[1]

[1]Department of Hematology-Oncology, Dokuz Eylül University School of Medicine, İzmir, Turkey
✉ *mehmet.ozcan@deu.edu.tr*
[2]Department of Gastroenterology, Dokuz Eylül University School of Medicine, İzmir, Turkey

Received: *Nov 08, 2005* • ***Accepted:*** *Dec 15, 2005*

ABSTRACT

Background: The clinical course of patients with inflammatory bowel disease (IBD) is frequently complicated by thromboembolic events and may involve the arterial and venous systems. Although not uniformly documented, several studies document substantial alterations in markers of coagulation and fibrinolysis in patients with IBD. **Methods:** 45 patients with IBD (31 UC,14 CD) were included in the study. Age and sex matched 16 volunteers were used as a control group. TAFI antigen was determined using an ELISA kit VisuLiseTM for quantitative measurement. **Results:** Inflammatory parameters such as white blood cell, platelet levels, erythrocyte sedimentation rate, C-reactive protein were found to be significantly higher in active disease group compared to inactive patients. Coagulation parameters of prothrombin time, activated partial thromboplastin time and d-dimer levels showed no significant difference between active and inactive IBD. Fibrinogen levels were significantly higher in clinically active IBD patients. Plasma TAFI levels demonstrated no significant difference between active and control, inactive and control as well as active and inactive groups. We observed no significant changes in levels of β-TG and PF-4 between active and inactive disease group. **Conclusions:** We studied plasma TAFI levels in IBD. In conclusion, plasma TAFI levels does not appear to represent to be a marker of activation in IBD in contrast to literature. So further studies covering more patients with different clinic and disease activity status might improve the perspective on this issue.

Key words: Crohn's disease, TAFI, ulcerative colitis

ÖZET

Aktif ve aktif olmayan inflamatuar barsak hastalığında trombin ile aktive olan fibrinolizis inhibitör antijeninin plazma düzeyleri

Giriş: İnflamatuar barsak hastalığının (IBH) seyri, arteriyel ya da venöz sistemde olabilen tromboembolik olaylar ile sık olarak komplike olmaktadır. Tümü olmasa da çalışmaların çoğu IBH olan hastalarda koagülasyon ve fibrinoliz göstergelerindeki değişiklikleri ortaya koymaktadır.
Yöntem: Çalışmaya IBH olan 45 hasta (31 UC, 14 CD) dahil edildi. Yaşı ve cinsiyeti uyumlu 16 gönüllü kontrol grubu olarak alındı. TAFI antijeninin kantitatif olarak saptanması için VisuLiseTM ELISA kiti kullanıldı.
Sonuçlar: Beyaz küre sayısı, trombosit sayısı, eritrosit sedimentasyon hızı ve C-reaktif protein gibi inflamasyon belirteçleri aktif hastalığı olanlarda belirgin yüksek bulundu. Aktif ya da inaktif IBH olanların protrombin zamanı, aktive parsiyel tromboplastin zamanı ve d-dimer düzeyleri gibi koagülasyon ölçekleri arasında anlamlı fark bulunmadı. Hastalığı aktif olanların fibrinojen düzeyleri belirgin olarak daha yüksekti. Aktif ya da inaktif hastalığı olanlar ile sağlıklı kontrollerin plazma TAFI düzeyleri arasında anlamlı bir fark gösterilemedi. Aktif ve inaktif hastalığı olanların β-TG ve PF-4 düzeyleri arasında da anlamlı bir değişiklik gözlenmedi.
Sonuç: IBH'da TAFI düzeylerini araştırdık. Literatürdeki bilgilerle çelişecek şekilde TAFI düzeyleri IBH'nın aktivasyon göstergesi olarak kullanılabilir gözükmemektedir. Hastalığın farklı aşamalarında ve aktivasyon düzeylerinde olan daha fazla hastayı kapsayan çalışmaların yapılması konunun daha iyi aydınlamasına yardımcı olacaktır.

Anahtar sözcükler: Crohn hastalığı, TAFI, ülseratif kolit

INTRODUCTION

The clinical course of patients with inflammatory bowel disease (IBD) is frequently complicated by thromboembolic events and may involve the arterial and venous systems [1,2]. Previous clinical studies have found an incidence of thrombosis in IBD patients between 1.2 and 7.1%, although autopsy studies demonstrate an incidence up to 40% [3-6]. There is also histological evidence that small vessel occlusion may be important in the pathogenesis of ulcerative colitis (UC) [7,8]. As has been proposed in the past, endothelial lesion with sustained coagulation activation could be responsible for the generation of capillary microthrombi and subsequent ischemia [1]. Persistent activation of coagulation in patients with IBD has been shown. Although not uniformly documented, several studies document substantial alterations in markers of coagulation and fibrinolysis in patients with IBD [9-14]. Nevertheless, enhanced platelet activation and aggregation have been recognized in both Crohn's disease (CD) and UC [15].

Thrombin activatable fibrinolysis inhibitor (TAFI) is a recently described glycoprotein that is synthesized in the liver. TAFI circulates as an inactive proenzyme in the blood stream, and becomes activated during blood clotting by thrombin. The active form, TAFIa, inhibits fibrinolysis [16]. TAFI can be expected to play a role in thrombotic tendency associated with various clinical conditions. Increased plasma levels of TAFI were found in obese type II diabetic women [17], an increase in pro-TAFI has been observed in patients with symptomatic coronary artery disease [18], and an increase in TAFI antigen has been described as a mild risk factor for deep vein thrombosis [19]. Decreased levels of TAFI were found in patients with chronic liver disease [20].

The aim of this study was to investigate the role of TAFI β-Thromboglobulin and platelet factor as a marker of defective fibrinolysis in the pathogenesis of the thrombotic process in IBD patients.

MATERIALS and METHODS

Patients

Forty-five patients (28 male, 17 female) with IBD (31 UC, 14 CD) were included in the study. The median age of the patients was 44±15; 23 patients had active disease and 22 patients were inactive. None of the patients had thrombosis. Sixteen age- and sex- matched volunteers were used as a control group. Patient characteristics are summarized in Table 1. Disease activity was determined using Crohn's Disease Activity Index (CDAI) [21] and Ulcerative Colitis Disease Activity Index (UCDAI) (22). Active disease was accepted if CDAI was over 150, and the activity levels of UC was determined as mild 1-3, moderate 4-6, and severe 7-9 for UCDAI. The endoscopic and histological characteristics of patients were evaluated in the active disease.

Table 1. Patient characteristics

	Active (n=23)	*Inactive (n=22)*
Age	43±17	46±12
Gender (m/f)	14/9	9/12
Disease duration (month)	58±49 (1-168)	69±60 (1-204)
Crohn disease		
Extent of disease		
Ileum	2	3
Ileum and colon	2	3
Colon	2	2
Ulcerative colitis		
Extent of disease		
Rectum	1	2
Rectum and sigmoid	5	2
Left side	5	5
Pancolitis	6	5
Severity of disease (UCDAI)		
Mild	9	
Moderate	7	
Severe	1	
Smoking	7	7
Extraintestinal involvement	1 (uveitis)	0

None of the patients had history of thrombosis, diabetes mellitus, congestive heart failure, malignancy, pregnancy, severe systemic infection, recent transfusions (<2 weeks), recent surgical procedure (<3 months), liver insufficiency, ful-

minant colitis, inherited coagulation abnormality, malnutrition, family history of thrombosis or consumption of oral contraceptives or any other medication that affects the coagulation system.

The institutional ethical committee approved the study and all patients provided written informed consent before the study.

Blood collection

Blood samples were obtained from antecubital vein into citrated tubes (trisodium-citrate 0.129 mol/L, whole blood ratio 1:9) and centrifuged 2000xg for 15 minutes. Then, all plasma samples were divided into aliquots and frozen and preserved at -70°C until test time. At the time blood samples were taken, whole blood count, liver function tests, renal function tests, and coagulation parameters (PT, INR, aPTT, D-dimer) were also studied.

TAFI antigen assay

TAFI antigen was determined using an ELISA kit VisuLiseTM for quantitative measurement (Affinity Biologicals Inc, Ontario, Canada). Strip wells were pre-coated with polyclonal antibody to human TAFI. Plasma samples were thawed and diluted at a ratio of 1/200 and applied to the wells. The TAFI present binds to the coated antibody. After washing away unbound material, peroxidase-labeled detecting antibody was applied. After washing, tetramethylbenzidine, which is a peroxidase substrate, was added to the wells. Color formed was then measured spectrophotometrically in a microplate reader at 450 nm. The assay was calibrated using the reference plasma provided from the manufacturer. All necessary buffers and reagents were prepared according to manufacturer's instructions. All results were given as μg/ml.

β-Thromboglobulin (β-TG) and platelet factor-4 (PF-4) assay

Beta thromboglobulin levels were determined by sandwich ELISA method using Asserachrom® β-TG kit (Diagnostica Stago, France). PF-4 levels were also detected by a similar method by using Asserachrom® PF4 kit (Diagnostica Stago, France). All procedures were done according to manufacturer's instructions.

Table 2. Laboratory results of the patients

	Active Disease Patients	*Inactive Disease Patients*	*P value*
WBC (x109/L)	10.54±3.67	8.28±3.26	<0.003
Platelet (x109/L)	410±151	307±126	<0.008
ESR (mm/h)	22±17	13±8	<0.02
CRP (mg/L)	14.2±18.9	2.8±2.3	<0.001
PT (sn)	13.0±2.5	12.3±0.8	<0.22
aPTT (sn)	30.0±3.5	29.4±2.6	<0.91
Fibrinogen (g/L)	4.56±1.27	3.44±1.20	<0.004
D-dimer	0.45±0.24	0.34±0.18	<0.11
Triglyceride (mg/dl)	113±49	98±35	<0.27
Cholesterol (C) (mg/dl)	168±42	171±38	<0.74
HDL-C (mg/dl)	40±11	43±8	<0.09
LDL-C (mg/dl)	98±32	105±27	<0.57

Statistical analysis

Mann-Whitney U test was used to compare values between groups. P<0.05 was accepted as statistically significant. SPSS 10.0 for Windows was used for statistical analysis.

RESULTS

Inflammatory parameters such as white blood cell, platelet levels, erythrocyte sedimentation rate, and C-reactive protein were found to be significantly higher in the active disease group compared to inactive patients (Table 2).

Coagulation parameters of prothrombin time, activated partial thromboplastin time and D-dimer levels showed no significant difference between active and inactive IBD. Fibrinogen levels were significantly higher in active patients (Table 2) as expected. Triglyceride, cholesterol, HDL-cholesterol and LDL-cholesterol levels were found to be similar between active and inactive groups.

Plasma TAFI levels demonstrated no significant difference between active and control, inactive and control as well as active and inactive groups (Table 3) (Figure 1).

Table 3. Plasma levels of platelet activation markers and thrombin activatable fibrinolysis inhibitor

	Active	*Inactive*	*Control*
TAFI (μg/ml)	6.83±1.58	6.62±2.41	6.38±0.91
BTG (IU/ml)	173±7	168±3	178±4
PF-4 (IU/ml)	76±6	76±8	81±4

TAFI: Thrombin activatable fibrinolysis inhibitor.
BTG: β-thromboglobulin. **PF-4:** Platelet factor-4.

There were no significant correlations between plasma TAFI levels and acute phase reactants.

Platelet activation markers, β-TG and PF-4, were both significantly lower in the active disease group compared to control group. Although β-TG levels were also significantly lower in the inactive disease group than in the control group, this was not the case with PF-4. We observed no significant changes in levels of β-TG and PF-4 between active and inactive disease groups (Table 3).

DISCUSSION

Vascular thromboembolic complications can be seen in the course of both CD and UC. Furthermore, the microvascular thrombotic occlusions that have been documented histopathologically may play a role in the pathogenesis of IBD [1,2]. But the principal underlying pathogenetic mechanisms causing these events are still not understood.

Coagulation activation has been proposed in various studies to explain the pathogenesis by alterations in markers of coagulation and fibrinolysis [9-14]. At the same time, these markers have been studied in order to measure disease activity in IBD.

Coagulation system activation markers of prothrombin F1+2 [9,23], D-dimer [1], and thrombin-antithrombin (TAT) complex levels have been found to be increased [1] in several studies, and a relationship with disease activity has been suggested in some. Decreased levels of some coagulation inhibitors such as AT-III [1], protein C [1,24] and protein S [25] have been reported occasionally. The role of the fibrinolytic system has also been investigated, and elevated levels of plasminogen activator inhibitor (PAI)-I [23,26] and decreased levels of t-PA [26] have been found. Nevertheless, the role of the fibrinolytic system in IBD is controversial, as both hyperfibrinolysis [9,12,27] and hypofibrinolysis [23,26,28] have been described in patients with CD and UC.

TAFI is a recently described fibrinolysis inhibitor that is synthesized as a zymogen in the liver and can be activated by thrombin/thrombomodulin complex catalyzed proteolysis [29]. After activation, TAFI suppresses fibrinolysis through the removal of carboxy-terminal lysine residues on the fibrin surface [19]. Excessive activation of TAFI may constitute an additional contributing factor to thrombosis.

In the literature, plasma TAFI levels in IBD patients were studied for the first time and found significantly higher in IBD patients than in healthy controls [30]. So this may support the existence of hypofibrinolysis in IBD. We also studied plasma TAFI levels in IBD. We enrolled 45 IBD patients (31 UC, 14 CD), and 23 of them had active disease (17 UC, 6 CD). Saibeni et al. [30] enrolled 81 IBD patients (34 UC, 47 CD) and 35 of them had active disease (16 UC, 19 CD). Our healthy control group consisted of 16 sex- and age-matched volunteers, while Saibeni et al.'s enrolled 81 age- and sex-matched volunteers. They also studied 30 inflammatory controls, which we did not, and showed that median TAFI plasma levels were significantly higher in IBD patients than in healthy controls and significantly higher in inflammatory controls with respect to both IBD patients and healthy controls. However, we could not detect a significant difference between active and inactive disease and the control group. This might be attributable to the size of the study populations, as well as to the fact that our study group mostly contained mild [9] and moderate [7] active UC patients. Because of the small size of each group (according to severity) we could not mention them statistically. The literature did not mention severity of the disease for UC patients, and this might also have affected the result [30]. They also found a significant correlation between acute phase reactants and TAFI plasma levels, but we could not. This is interesting since in the literatures, associations between TAFI levels and acute phase response have been reported [31]. In our study group, we did not have any patient with clinical thrombosis at the time of sampling nor any with a history

of thrombosis. As shown in patients who experienced a deep vein thrombosis [19], a study group carrying these features might be more informative about the role of TAFI in IBD. TAFI is activated by thrombin at the site of the thrombus, hence it plays an important role in connecting the coagulation and fibrinolytic cascades [29].

Interestingly, we did not find elevated levels of β-TG and PF-4, as markers of platelet activation, and they were even significantly depressed compared to controls. In the literature, although elevated levels have been found in most studies, only some showed correlation with disease activity [3-5,32]. β-TG and PF-4 plasma assays may indirectly determine that a clinical condition activates platelets, but cannot measure changes in platelet reactivity associated with the condition [33].

In conclusion, pathogenesis of thromboembolic complication is probably multifactorial, and a single laboratory marker such as plasma TAFI level may not appear be a marker of activation in IBD. Further studies covering more patients with different clinic and disease activity status might improve the perspective on this issue.

REFERENCES

1. Souto JC, Martinez E, Roca M, Mateo J, Pujol J, Gonzalez D, Fontcuberta J. Prothrombotic state and signs of endothelial lesion in plasma of patients with inflammatory bowel disease. Dig Dis Sci 1995;40:1883-9.
2. Lam A, Borda IT, Inwood MJ, Thomson S. Coagulatio studies in ulcerative colitis and Crohn's disease. Gastroenterology 1975;68:245-51.
3. Stadnicki A, Gonciarz M, Niewiarowski TJ, Hartleb J. Rudnicki M, Merrell NB, Dela Cadena RA, Colman RW. Activation of plasma contact and coagulation systems and neutrophils in the active phase of ulcerative colitis. Dig Dis Sci 1997;42:2356-66.
4. Webberley MJ, Hart MT, Melikian V. Thromboembolism in inflammatory bowel disease: role of platelets. Gut 1993;34:247-51.
5. Collins CE, Cahill MR, Newland AC, Rampton DS. Platelets circulate in an activated state in inflammatory bowel disease. Gastroenterology 1994;106:840-5.
6. Talbot RW, Heppell J, Dozois RR, Beart RW Jr. Vascular complications of inflammatory bowel disease. Mayo Clin Proc 1986;61:140-5.
7. Thompson NP, Wakefield AJ, Pounder RE. Inherited disorders of coagulation appear to protect against inflammatory bowel disease. Gastroenterology 1995;108:1011-5.
8. Dhillon AP, Anthony A, Sim R, Wakefield AJ, Sankey EA, Hudson M, Allison MC, Pounder RE. Mucosal capillary thrombi in rectal biopsies. Histopathology 1992;21:127-33.
9. Kjeldsen J, Lassen JF, Brandslund I, Schaffalitzky de Muckadell OB. Markers of coagulation and fibrinolysis as measures of disease activity in inflammatory bowel disease. Scand J Gastroenterol 1998;33:637-43.
10. Edwards RL, Levine JB, Green R, Duffy M, Mathews E, Brande W, Rickles FR. Activation of blood coagulation in Crohn's disease. Increased plasma fibrinopeptide A levels and enhanced generation of monocyte tissue factor activity. Gastroenterology 1987;92:329-37.
11. Hudson M, Hutton RA, Wakefield AJ, Sawyerr AM, Pounder RE. Evidence for activation of coagulation in Crohn's disease. Blood Coagul Fibrinolysis 1992;3:773-8.
12. van Wersch JW, Houben P, Rijken J. Platelet count, platelet function, coagulation activity and fibrinolysis in the acute phase of inflammatory bowel disease. J Clin Chem Clin Biochem 1990;28:513-7.
13. Van Bodegraven AA, Tuynman HA, Schoorl M, Kruishoop AM, Bartels PC. Fibrinolytic split products, fibrinolysis, and factor XIII activity in inflammatory bowel disease. Scand J Gastroenterol 1995;30:580-5.
14. Novacek G, Kapiotis S, Moser G, Speiser W, Gangl A, Vogelsang H. No evidence of activated blood coagulation in Crohn's disease. Eur J Gastroenterol Hepatol 1997;9:963-7.
15. Fairburn RA. On the aetiology of ulcerative colitis. A vascular hypothesis. Lancet 1973;1:697-9.
16. Mosnier LO, von dem Borne PA, Meijers JC, Bouma BN. Plasma TAFI levels influence the clot lysis time in healthy individuals in the presence of an intact intrinsic pathway of coagulation. Thromb Haemost 1998;80:829-35.
17. Hori Y, Gabazza EC, Yano Y, Katsuki A, Suzuki K, Adachi Y, Sumida Y. Insulin resistance is associated with increased circulating level of thrombin-activatable fibrinolysis inhibitor in type 2 diabetic patients. J Clin Endocrinol Metab 2002;87:660-5.

18. Silveira A, Schatteman K, Goossens F, Moor E, Scharpe S, Stromqvist M, Hendriks D, Hamsten A. Plasma procarboxypeptidase U in men with symptomatic coronary artery disease. Thromb Haemost 2000;84:364-8.
19. van Tilburg NH, Rosendaal FR, Bertina RM. Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. Blood 2000;95:2855-9.
20. Van Thiel DH, George M, Fareed J. Low levels of thrombin activatable fibrinolysis inhibitor (TAFI) in patients with chronic liver disease. Thromb Haemost 2001;85:667-70.
21. Best WR, Becktel JM, Singleton JW. Rederived values of the eight coefficients of the Crohn's Disease Activity Index (CDAI). Gastroenterology 1979;77:843-6.
22. Truelove SC, Witts LJ. Cortisone in ulcerative colitis;final report on a therapeutic trial. Br Med J 1955;(4947):1041-8.
23. Weber P, Husemann S, Vielhaber H, Zimmer KP, Nowak-Gottl U. Coagulation and fibrinolysis in children, adolescents, and young adults with inflammatory bowel disease. J Pediatr Gastroenterol Nutr 1999;28:418-22.
24. Jorens PG, Hermans CR, Haber I, Kockx MM, Vermylen J, Parizel GA. Acquired protein C and S deficiency, inflammatory bowel disease and cerebral arterial thrombosis. Blut 1990;61:307-10.
25. Aadland E, Odegaard OR, Roseth A, Try K. Free protein S deficiency in patients with Crohn's disease. Scand J Gastroenterol 1994;29:333-5.
26. de Jong E, Porte RJ, Knot EA, Verheijen JH, Dees J. Disturbed fibrinolysis in patients with inflammatory bowel disease. A study in blood plasma, colon mucosa, and faeces. Gut.1989;30:188-94.
27. van Bodegraven AA, Schoorl M, Baak JPA, Linskens RK, Bartels PC, Tuynman HA. Hemostatic imbalance in active and quiescent ulcerative colitis. Am J Gastroenterol 2001;96:487-93.
28. Gris JC, Schved JF, Raffanel C, Dubois A, Aguilar-Martinez P, Arnaud A, Sanchez N, Sarlat C, Balmes JL. Impaired fibrinolytic capacity in patients with inflammatory bowel disease. Thromb Haemost 1990;63:472-5.
29. Chetaille P, Alessi MC, Kouassi D, Morange PE, Juhan-Vague I. Plasma TAFI antigen variations in healthy subjects. Thromb Haemost 2000;83:902-5.
30. Saibeni S, Bottasso B, Spina L, Bajetta M, Danese S, Gasbarrini A, de Franchis R, Vecchi M. Assessment of thrombin-activatable fibrinolysis inhibitor (TAFI) plasma levels in inflammatory bowel diseases. Am J Gastroenterol 2004;99:1966-70.
31. Sato T, Miwa T, Akatsu H, Matsukawa N, Obata K, Okada N, Campbell W, Okada H. Pro-carboxypeptidase R is an acute phase protein in the mouse, whereas carboxypeptidase N is not. J Immunol 2000;165:1053-8.
32. Vrij AA, Rijken J, Van Wersch JW, Stockbrugger RW. Platelet factor 4 and beta-thromboglobulin in inflammatory bowel disease and giant cell arteritis. Eur J Clin Invest 2000;30:188-94.
33. Michelson AD. Flow cytometry: a clinical test of platelet function. Blood 1996;87:4925-36.