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 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 hastalığındaki trombin ile aktive olan fibrinolizis inhibitör antijeninin plazma düzeyleri

Giriş: Inflamatuar barsak hastalığının (IBH) seyri, arteriye ve venöz sistemde olabilen tromboembolik olaylar ile sıklıkla komplike olmaktadır. Tümay olmasına da çalışmalara olduğu gibi IBH'da hastalarda koagülasyon ve fibrinoliz göstergelerindeki değişiklikleri ortaya koymaktadır.

Yöntem: Çalışmada IBH olan 45 hasta (31 UC, 14 CD) dahil edildi. Yaş ve cinsiyeti uyumlu 16 unveilvolu deney grubu oluşturuldu. TAFI antijeninin kantitatif olarak saptanması için VisuLiseTM ELISA kit kullanıldı.


Sonuç: IBH’de TAFI düzeylerini araştırık. Literatürdeki bilgilerle çelişeci şekilde TAFI düzeyleri IBH’in aktivasyon göstergesi olarak kullanılabılır gözümlemektedir. Hastalığı farklı aşamalarında ve aktivasyon düzeylerinde olan daha fazla hastayı kapsayan çalışmaların yapılması konunun daha iyı aydınlanmasına 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

<table>
<thead>
<tr>
<th>Active (n=23)</th>
<th>Inactive (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>43±17</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>14/9</td>
</tr>
<tr>
<td>Disease duration (month)</td>
<td>58±49 (1-168)</td>
</tr>
<tr>
<td>Crohn disease</td>
<td></td>
</tr>
<tr>
<td>Extent of disease</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>2</td>
</tr>
<tr>
<td>Ileum and colon</td>
<td>2</td>
</tr>
<tr>
<td>Colon</td>
<td>2</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td></td>
</tr>
<tr>
<td>Extent of disease</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>1</td>
</tr>
<tr>
<td>Rectum and sigmoid</td>
<td>5</td>
</tr>
<tr>
<td>Left side</td>
<td>5</td>
</tr>
<tr>
<td>Pancolitis</td>
<td>6</td>
</tr>
<tr>
<td>Severity of disease (UCDAI)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>9</td>
</tr>
<tr>
<td>Moderate</td>
<td>7</td>
</tr>
<tr>
<td>Severe</td>
<td>1</td>
</tr>
<tr>
<td>Smoking</td>
<td>7</td>
</tr>
<tr>
<td>Extraintestinal involvement</td>
<td>1 (uveitis)</td>
</tr>
</tbody>
</table>

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-
dominant colitis, inherited coagulation abnormal-
ity, 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 in-
formed consent before the study.

**Blood collection**

Blood samples were obtained from antecu-
bital vein into citrated tubes (trisodium-citrate
0.129 mol/L, whole blood ratio 1:9) and cen-
trifuged 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 anti-
body. After washing away unbound material, per-
oxidase-labeled detecting antibody was applied.
After washing, tetramethylbenzidine, which is
a peroxidase substrate, was added to the wells. Color
formed was then measured spectrophotome-
trically 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 us-
ing Asserachrom® PF4 kit (Diagnostica Stago,
France). All procedures were done according to
manufacturer’s instructions.

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**Table 2. Laboratory results of the patients**

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

**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 lev-
els 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 signifi-
cant difference between active and control, inac-
tive and control as well as active and inactive
groups (Table 3) (Figure 1).
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

| 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 |

of thrombosis. As shown in patients who experienced a deep vein thrombosis, 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.

Interestingly, we did not find elevated levels of \( \beta \)-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. \( \beta \)-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.

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


