Levels of TAFI, TFPI and ADAMTS-13 in inflammatory bowel disease

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ABSTRACT

Background/Aims: There is an increased tendency for thrombosis and thromboembolic complications in patients with inflammatory bowel disease (IBD). The aim of the present study was to determine the serum concentrations of thrombin-activatable fibrinolysis inhibitor (TAFI), tissue factor pathway inhibitor (TFPI) and a disintegrin and metalloproteinase with thrombospondin motif-13 (ADAMTS-13) in patients with IBD and to assess their possible role in the etiopathogenesis of the disease.

Materials and Methods: Thirty-four patients with IBD (23 ulcerative colitis and 11 Crohn's disease) and 20 healthy controls were included in the present study. TAFI, TFPI, and ADAMTS-13 concentrations were determined by enzyme-linked immunosorbent assay.

Results: Mean TAFI, TFPI, and ADAMTS-13 concentrations in the patient group were 17.75 ng/ml, 72.10 ng/ml, and 14.90 U/l, respectively. In the control group, these values were 117.10 ng/ml, 300 ng/ml, and 191.55 U/l, respectively. TAFI, TFPI, and ADAMTS-13 values were significantly lower in the patient group than in the control group (all $p<0.01$).

Conclusion: TAFI, TFPI, and ADAMTS-13 levels were significantly lower in the patient group. These findings indicate the presence of a clear, multifactorial imbalance in the coagulation–fibrinolytic system in the patient group. It is also possible that this imbalance in the coagulation and fibrinolytic system may play a role in the still unclear etiopathogenesis of the disease.

Keywords: Crohn's disease, thrombophilia, ulcerative colitis

INTRODUCTION

There is an increased tendency for thrombosis and also a greater risk of thromboembolic complications in patients with inflammatory bowel disease (IBD) (1-3). Thromboembolic events may develop in association with hypercoagulopathy and increased platelet activation (4). Although clinical studies and research have shown a hypercoagulable and prothrombotic state in both forms of IBD, details regarding the coagulation abnormalities are unclear and still has not yet been identified (2).

The complex of tissue factor (TF) and factor VIIa is the most important initiator of coagulation. The TF–factor VIIa complex is inhibited by tissue factor pathway inhibitor (TFPI), a natural anticoagulant (5,6). Inhibition of the TF–factor VIIa complex results in a decrease in thrombin generation and conversion of fibrinogen to fibrin in later stages (7-9). Studies determined significantly lower TFPI levels in the IBD patient groups than those in the control group. Thrombin formation increased with a decrease in TFPI (1,10).

Thrombin-activatable fibrinolysis inhibitor (TAFI) is a precursor of the carboxypeptidase TAFIa. It possesses powerful anti-fibrinolytic and anti-inflammatory activities (11). Fibrinolysis is regulated by some important factors, such as TAFI, which is activated by thrombin to TAFIa. Thrombomodulin causes increment in this activation. Lysine residues are removed from fibrin by this active form of TAFI (12). The clinical implications of TAFI have not yet been understood.

The powerfully prothrombotic large multiforms of von Willebrand factor (vWF) present in free form in plasma are converted into less active multiforms by a disintegrin and metalloproteinase with thrombospondin motif-13 (ADAMTS-13). If the enzyme is dysfunctional or below the measurable levels, circulating large vWF multimers

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can lead to massive intravascular platelet aggregation and thrombotic thrombocytopenic purpura (13-15). If very large vWF multimers are present in the microcirculation, this results in uncontrolled platelet aggregation and intravascular thrombus formation (16,17). Imbalance between vWF and ADAMTS-13 has been identified in patients with arterial thrombosis (18). One study measured ADAMTS-13 activity in various disease groups and identified pathological results in the cirrhosis and IBD patient groups (13).

Although clinical studies have shown a hypercoagulable and prothrombotic state in both forms of IBD, details regarding the coagulation abnormalities are unclear (2). Studies have sought to elucidate the relationship between IBD and hypercoagulability by investigating changes in the coagulation and fibrinolytic system in the disease (19). Changes in the fibrinolytic system in IBD have been intensively investigated. Most data describe an imbalance in the fibrinolytic system tending to a hypofibrinolytic state (20). Several studies have also examined genetic thrombophilic risk factors in IBD and the association between these and macrovascular thrombosis. The results of previous studies show the need for the investigation of the presence of thrombophilic markers commonly encountered in the IBD population (2).

Thromboembolic diseases are one of the three most common causes of death in ulcerative colitis (UC). Most authors think that hypercoagulable state, microthrombosis formation, and platelet activation play a significant role in the pathogenesis of the disease (21).

The purpose of the present study was to determine the plasma levels of TFPI, TAFI, and ADAMTS-13 in patients with IBD and to assess the relationship between these factors and the etiopathogenesis of the IBD–thrombosis association.

MATERIALS AND METHODS

In a total of 54 volunteers, 34 patients who were diagnosed with IBD (23 with UC and 11 with Crohn's disease (CD)) and 20 healthy controls were included in the study. Inclusion and exclusion criteria are listed in Tables 1 and 2, respectively. The study was approved by the ethics committee of Ümraniye Training and Research Hospital and conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all volunteers before participation in the study.

Table 1. Inclusion criteria.

- Aged ≥18 years
- Patients diagnosed with IBD (UC or CD) in lower gastrointestinal endoscopy

The extent of the disease was determined in patients diagnosed with IBD. Serum specimens were collected from the patient and control groups to determine TAFI, TFPI, and ADAMTS-13 concentrations. These specimens were stored at -20 °C until the study. Concentrations of TAFI, TFPI, and ADAMTS-13 were investigated on a Grifols Triturus device using enzyme-linked immunosorbent assay with Eastbiopharm ready-made kits.

Statistical analysis

NCSS (Number Cruncher Statistical System) 2007&PASS (Power Analysis and Sample Size) 2008 statistical software (UT, USA) was used for statistical analysis when evaluating the study findings. In addition to descriptive statistical methods (mean, standard deviation, median, frequency, and level) in data analysis, the Mann–Whitney U test was used for comparison of quantitative data. The Yates corrected chi-square test was used for comparison of qualitative data. Results were analyzed at a 95% confidence interval. A p value <0.05 was accepted as statistically significant.

RESULTS

The patient group consisted of 22 male and 12 female subjects. The mean age of the patient group was 32 years. The control group consisted of 10 male and 10 female healthy individuals. The mean age of the control

Table 2. Exclusion and termination criteria.

- Aged <18 years
- Having a chronic systemic disease (e.g., diabetes mellitus, hypertension, coronary artery disease, chronic obstructive pulmonary disease, cerebrovascular disease, chronic renal failure, and liver diseases)
- Taking drugs, such as oral contraceptive, aspirin, oral anticoagulants, and heparin
- Cancer
- Smoking
- Pregnancy
- Patients without informed consents

Table 3. Comparison of TAFI, TFPI, and ADAMTS-13 levels between the IBD patient (UC and CD) and control groups.

	n	TAFI Med.±SD	TFPI Med.±SD	ADAMTS-13 Med.±SD
Patient group	34	30.23±31.21 (17.75)[a]	74.52±82.08 (72.10)[a]	29.98±41.25 (14.90)[a]
UC	23	26.15±25.47 (17.40)[a,b]	61.75±62.04 (39.30)[a,c]	25.80±35.97 (14.70)[a,c]
CD	11	38.75±40.86 (21.80)[a,b]	101.29±112.27 (48.80)[a,c]	38.72±51.43 (17.60)[a,c]
Control group	20	100.81±50.66 (117.10)[a]	249.77±122.77 (300)[a]	153.67±92.41 (191.55)[a]

[a]p<0.01 vs. the control group, [b]p<0.05 between the UC group, [c]p>0.05 between the CD group.

group was 29 years. In the patient group, there were 23 cases diagnosed with UC and 11 with CD. Extensive disease was present in 6 of the cases diagnosed with UC and left-sided disease in 15 (involvement: distal to the splenic flexure in 5 and rectosigmoiditis in 10), whereas proctitis was diagnosed in 2 patients. Of the 11 patients with CD, 8 had ileal, and 3 had ileocolonic CD.

TAFI, TFPI, and ADAMTS-13 levels in the patient group were significantly lower than those in the control group (p<0.01, Table 3). TAFI levels in the UC patient group were significantly lower than those in the control group and also significantly lower in patients with CD than in controls (p<0.01, Table 3). TAFI levels were also significantly lower in the UC group than in the CD group (Table 3). No significant difference was observed between TFPI and ADAMTS-13 levels in the UC and CD patient groups (p>0.05).

DISCUSSION

The risk of thrombotic events increases in IBD (22-24). The prevalence has been reported to be 3.4% in various previous studies (3,22,25). A 1.5-3.5-fold higher risk of thrombosis has been reported in patients with IBD compared with the normal population (23). However, these coagulation abnormalities are not yet fully understood (2).

The relationship between IBD and thrombosis is thought to be multifactorial. The risk of thrombosis in patients with IBD is multifactorial, such as inflammation, steroid therapy, and vitamin deficiency (26). While IBD is a disease characterized by chronic inflammation accompanying a procoagulant state, the contribution of inflammatory response to the thrombotic complications that are frequently observed is unclear. While thrombotic events are more common in active disease, they may also occur in patients in remission (2). A correlation has been shown between the prevalence of thrombotic events in IBD and disease activity and extent of the disease (3,23,27). The effects of hereditary or acquired thrombophilia in the pathogenesis of thrombotic events in IBD and the contribution to these to inflammatory response are still unclear.

UC is a chronic inflammatory disorder. It is characterized by healing and recurring colonic lesions. In recent studies, microvascular dysfunction and endothelial barrier defect had been shown to be the cause of poor healing and chronic inflammation of the colon in UC, resulting in sustained tissue hypoperfusion and ischemia in the colonic mucosa (28). In light of this information, we think that coagulation, activated in different steps, may be involved in the etiopathogenesis of the disease by causing continuous, low-level microvascular thrombosis concluding with colonic ischemia in IBD.

The level of TFPI, one of the most important coagulation cascade inhibitors synthesized from endothelial cells, being significantly lower in all IBD patient groups than in the control group is a significant finding with respect to the IBD–thrombosis association. These findings suggest that TFPI plays an important role in the tendency for thrombosis and thrombotic complications in IBD. Thrombin formation increases as TFPI decreases, resulting in a tendency for thrombosis. In the literature, the levels of TFPI results are different. In a study conducted by Reichman-Warmusz et al., the levels of TFPI in mucosal biopsies were lower in IBD than in normal mucosa (10). Bernhard et al. observed that the level of the mean TFPI values in patients in the active and inactive states of CD is lower than that in the control group (1). Thrombin formation increases as TFPI decreases (2). The results of another study showed higher levels of TFPI in UC tissue than those in normal colon tissue (29). In our study, serum TFPI values were significantly lower in the IBD patient groups than in the control group (p<0.01). The findings show a similar low TFPI levels in both IBD patient groups,

suggesting that TFPI may be a marker of a tendency for thrombosis in all patients with IBD. In addition, low TFPI supports the idea of a potential tendency for thrombosis in the patient group. More controversially, TFPI may be an important factor in the still imperfectly understood pathogenesis of IBD.

TAFI is activated by thrombin. The product of that activation is TAFIa, which removes lysine residues from fibrin. These residues are essential to plasmin binding to fibrin. One study examined the levels of the fibrinolysis inhibitors TAFI and plasminogen activator inhibitor-1 (PAI-1) and interpreted low TAFI levels and high PAI-1 levels as an imbalance in the fibrinolytic system (30). In contrast, another study reported higher levels of TAFI and suggested that TAFI might be responsible for the thrombotic process in IBD (31). High TAFIa levels are a significant risk factor for thrombotic diseases (32,33). In the present study, TAFI levels in the patient group were significantly lower than those in the control group ($p<0.01$). When the patient groups were analyzed separately, TAFI levels were significantly lower in the UC and also CD patient groups than in the control group ($p<0.01$). Comparing the two patient groups, TAFI measurements were significantly lower in the UC group than in the CD group ($p<0.05$). In the present study, TAFIa levels were not investigated, but those of its inactive precursor. The low levels of TAFI, the precursor of TAFIa, in our study may be interpreted as being due to this molecule being intensively converted into its active form, TAFIa (and possibly in association with an increase in thrombin formation due to the effect of low TFPI). Low TAFI levels, which may be interpreted as pro-TAFI in this patient group, may have been due to this intense activation. If the high TAFIa levels in this patient group are regarded as an indirect marker, this may also be considered as a cause of the tendency for thrombosis in IBD. To summarize, these findings may be interpreted as imbalance in the coagulation–fibrinolytic system in all patients with IBD. This imbalance may also have acquired a greater disposition to thrombosis under the influence of other factors involved in this patient group.

The powerfully prothrombotic large multiforms of vWF present in free form in plasma are converted into less active multiforms by the metalloproteinase ADAMTS-13 (14,15,33). An imbalance has been identified between vWF and ADAMTS-13 in patients with arterial thrombosis. Both high vWF and low ADAMTS-13 plasma levels have been shown to be potential risk factors in the development of ischemic stroke and myocardial infarction. Feys et al. reported ADAMTS-13 antigen and activity in physiological and pathological conditions. One of the pathological conditions is IBD, and they reported that there is no difference between UC and CD (13). Additionally, a significantly increased risk of thrombotic events has been reported in the case of high vWF and low ADAMTS-13 levels in plasma (18). Moreover, mice deficient in ADAMTS-13 have increased inflammatory and thrombotic responses (34). In our study, ADAMTS-13 levels were significantly lower in the IBD patient group than in the control group ($p<0.01$). However, no statistically significant difference was found between the UC and CD groups ($p>0.05$). The results again show the presence of a tendency for thrombosis in the IBD patient group. All these findings indicate a tendency for thrombosis in the IBD population and a pronounced imbalance in the coagulation–fibrinolytic system in this patient group. We think that this imbalance may be involved in the etiopathogenesis of IBDs, and that intestinal microvascular thrombosis developing secondary to this imbalance may also play a role in the development, activation, and severity of the disease.

The present study reveals changes likely to cause a tendency for thrombophilia in all three parameters, all of which exhibit their effects through different pathways. TAFI is involved in the fibrinolysis stage and TFPI in the coagulation step, whereas ADAMTS-13 is involved in a different way to these two mechanisms. We believe that the measurement of TAFI, TFPI, and ADAMTS-13 in blood and the colonic mucosa together may be regarded as beneficial biochemical indicators in the understanding of IBD etiopathogenesis.

Ethics Committee Approval: Ethics committee approval for this study was received from the Ethics Committee of Ümraniye Training and Research Hospital.

Informed Consent: Written informed consent was obtained from all patients who participated in this study.

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