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Soluble CD40 ligand, high sensitive C-reactive protein and fetuin-A levels in patients with essential thrombocythemia

Ferda Bilgir [a], Oktay Bilgir [b,*], Levent Kebapcilar [c], Mehmet Calan [d], Fusun Ozdemirkiran [e], Turker Cinali [b], Giray Bozkaya [f]

[a] Izmir Buca State Hospital, Department of Internal Medicine, Buca, Izmir, Turkey
[b] Izmir Bozyaka Training and Research Hospital, Department of Hematology, 35380 Bozyaka, Izmir, Turkey
[c] Selcuk University Medical School Department of Endocrinology and Metabolism, Konya, Turkey
[d] Dokuz Eylul University Medical School Department of Internal Medicine, Izmir, Turkey
[e] Ege University Medical School, Department of Hematology, Izmir, Turkey
[f] Izmir Bozyaka Training and Research Hospital, Department of Biochemistry, 35380 Bozyaka, Izmir, Turkey

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ABSTRACT

Background: CD40 ligand (CD40L) is expressed on the surface of activated platelets and activated T lymphocytes. Circulating soluble CD40 ligand (sCD40L) is formed from these molecules proteolytically. Fetuin-A is a potent antiinflammatory cytokine.
Aim of the study: In this study, we aim to investigate sCD40L levels to determine whether there is platelet activation and to measure high sensitive C-reactive protein (hs-CRP) levels to demonstrate if this leads to an inflammatory process and also to study fetuin-A levels to see if there is any concomitant antiinflammatory event in patients with essential thrombocythemia (ET).
Methods: We compared 30 patients with essential thrombocythemia with 30 control subjects and in these patients we measured levels of sCD40L, hs-CRP and fetuin-A.
Results: sCD40L levels were significantly higher in the ET group compared to the control group (30.6 ± 14.4 vs. 18.5 ± 8.9, $p = 0.001$). Although fetuin-A levels showed a slight trend to be increased in ET patients, the difference did not reach significance (4.5 ± 4.2 vs. 3.2 ± 2.1, $p = 0.158$). There were no statistically significant differences in hs-CRP levels (24.6 ± 4.9 vs. 25.0 ± 5.2, $p = 0.750$).
Conclusion: sCD40L was significantly higher in patients with an ET without any association with an inflammatory process and we believe this may be a marker of platelet regeneration.

1. Introduction

Essential thrombocythemia (ET) is a myeloid disorder that is characterized by an increase in the peripheral blood platelet count that is associated with bone marrow megakaryocyte hyperplasia, without associated erythrocytosis or leukoerythroblastosis [1]. The cause of increased thrombotic risk and the mechanism underlying the hypercoagulable state in patients with ET is still unknown. Several studies have been conducted to elucidate these. Several investigators demonstrated an association between platelet turnover, Factor V, acquired activated protein C resistance and JAK2 V617F allele load and showed that decreases in free protein S and increases in tissue factor (TF) lead to platelet and endothelium activation [2,3]. The purpose of the present study was to evaluate the level of sCD40L, predictors of platelet activation, in patients with ET. Additionally, mean sCD40L levels were found to be statistically significantly higher in JAK2-positive patients which show that JAK2 is a factor in platelet activation.

* Corresponding author.
E-mail address: obilgir2001@yahoo.com (O. Bilgir).

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As is known, progressive thrombotic complications occur in patients with ET at the clinical, venous, arterial and microcirculatory levels [1]. Although advanced age and history of thrombosis are established independent markers, the cause of hypercoagulability in these patients is still unclear. Several mechanisms have been suggested, including the risk factors blood hyperviscosity, qualitative and quantitative changes in blood cells and leucocytosis [4,5]. An increased risk of thrombosis was reported in patients with ET with the V617F mutation in the JAK2 gene, since neutrophils and platelets are present in the circulation in the active form in these patients [6]. Also, activated neutrophils and platelets were shown to affect hemostatic balance. Activated neutrophils were shown to regulate coagulation by degrading coagulation factors by the same mechanisms as various inhibitors of coagulation (i.e., protein S, protein C, tissue factor pathway inhibitor, etc.) by introducing intragranular proteases of active neutrophils (i.e., elastase and cathepsin G) [7]. Acquired thrombophilia may be observed in patients with ET as a result of increased plasma markers of hypercoagulation which are secondary to *in vivo* activation in blood cells [2,8].

Fetuin-A is a glycoprotein which is synthesized in the liver and was formerly known as alpha-2 Heremans-Schmidglycoprotein (AHSG) [9]. Fetuin-A, a potent antiinflammatory cytokine, is involved in macrophage deactivation as a mediator, has antifibrotic activity and inhibits apoptosis in vascular smooth muscle cells [10,11]. A decrease in its levels in coronary artery disease was associated with disrupted antiinflammatory balance in its etiopathogenesis [12]. Our search of the literature for studies regarding the antiinflammatory status in essential thrombocythemia did not yield any results. Fetuin-A is associated with cardiovascular disease. Results from patients with metabolic syndrome have shown that fetuin-A levels are positively correlated with CRP, and higher levels of the molecule are associated with an increased risk of myocardial infarction and ischemic stroke [13]. In contrast, studies in patients with chronic kidney disease revealed lower circulating values of fetuin-A [14]. Merx et al. recently reported promotion of cardiac fibrosis, calcification, notably impaired diastolic function, and tolerance to ischemia as well as catecholamine resistance in the hearts of fetuin-A knockout mice [15].

CD40L is expressed in activated CD4 T lymphocytes and platelets and is converted to sCD40L via a proteolytic process [16]. Previously, increased plasma sCD40L levels were described in systemic lupus erythematosus and unstable angina, and associated with disease activity [17]. Although it is known that CD40L is expressed over the surface of activated platelets and that this is followed by a subsequent release of sCD40L from platelets, sCD40L levels may vary according to platelet count and/or the disease state. However, there may be an increase in sCD40L levels also as a result of platelet regeneration secondary to increased megakaryopoiesis as in myeloproliferative patients. In 2002, it was concluded in a study by Viallard et al. including 200 patients with ET, that platelet-associated CD40L may be a potential marker of platelet regeneration [18]. In our study we aimed to investigate whether inflammation exerts an effect on sCD40L levels in ET, therefore in addition to soluble CD40 ligand, we also measured hs-CRP since it is a marker of inflammation and fetuin-A since it is a potent anti-inflammatory cytokine.

2. Methods

2.1. Study population

Our study group consisted of 30 (15 male/15 female) essential thrombocythemia patients and 30 (14 male/16 female) healthy control subjects with a mean age of 59.5 ± 9.9 and 56.2 ± 6.1, respectively (Table 1). Age and gender were similar among the groups. ET diagnosis was based on the diagnostic criteria suggested by the Polycythemia Vera Study Group [19]. None of the patients with ET or healthy subjects had symptoms of an acute infection or inflammatory diseases. None of the subjects were on oral contraceptives or hormone replacement treatment at the time when blood samples were drawn. All subjects were receiving treatment for ET and their platelet counts were within normal limits as a result of their treatment (16 patients were on hydroxyurea and acetyl salicylic acid and 14 patients were on anagrelide). All investigations were approved by the local ethics committee (Izmir Bozyaka Training and Research Hospital). The procedure applied was in accordance with the Helsinki Declaration as amended in 2000. Blood samples were collected after informed consents were signed by the subjects.

2.2. Laboratory analyses

Venous fasting blood samples were collected from an antecubital vein into 8 mL evacuated tubes without anticoagulant (Vacuette, Greiner Bio-One, Austria). Blood samples were allowed to clot for 30 min and were centrifuged at 3000 rpm for 10 min at room temperature. After centrifugation, the serum samples were aliquated and stored at −80 °C for sCD40L, hs-CRP and fetuin-A analysis. Repeated freezing and thawing process was avoided.

Serum CD40L (Bender MedSystems Inc., Vienna, Austria), fetuin-A (Bio-Vendor, Modrice, Chech Republic) and high sensitive C-reactive protein (hs-CRP) (DRG Instruments GmbH, Marburg, Germany) levels were determined by ELISA method according to manufacturer's instructions. The sensitivities of the assays were determined to be 0.06 ng/mL, 0.35 ng/mL, 0.1 mg/L, respectively. The intra-assay coefficients of variation (CV) of sCD40L was 4% and inter-assay coefficients of variation was 6.8%. The

Table 1
Demographic and clinical parameters in patients with ET and controls (mean ± SD).

	Patient group	Control group	*p*
n	30	30	
Age (years)	59.5 ± 9.9	56.2 ± 6.1	0.121
Gender (male/female)	15/15	14/16	0.299
sCD40L (ng/mL)	30.6 ± 14.4	18.5 ± 8.9	0.001
Fetuin-A (ng/mL)	4.5 ± 4.2	3.2 ± 2.1	0.158
hs-CRP (mg/L)	24.6 ± 4.9	25.0 ± 5.2	0.750