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Evaluation of plasma activity level of anticoagulant proteins in patients with acute lymphoblastic leukemia in Shafa hospital Ahwaz 2010

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Abstract. – BACKGROUND AND OBJECTIVES, Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy as associated with various coagulation abnormalities such as hemorrhage and thrombosis. This study was designed to investigate the distribution pattern of plasma activity level of anticoagulant protein such as proteins C and S, antithrombin, activated protein C resistance (APCR-V) and D-dimer in patients with ALL.

PATIENTS AND METHODS, We studied thirty patients with confirmed ALL admitted in Shafa Hospital Hematology-Oncology and Thalassemia-Hemoglobinopathy Research Center and thirty normal (age and sex matched) subjects as control group. Proteins C and S, antithrombin, APCR-V were measured by coagulation analyzer and D-dimer analysed with Asserachrom D-Di enzyme immunoassay kit in patients and control group.

RESULTS, The mean activity levels of protein C (p = 0.017) and antithrombin (p = 0.014) were significantly lower in patient to group compared to the control group. However, the patient group had significantly elevated mean levels of protein S (p = 0.004) and D-dimer (p = 0.0001) compared to the control grup. About 3% of patients had APCR-V. There was no significant difference in APCR-V found between patient and control group (p = 0.674).

CONCLUSIONS, The hypercoagulability in ALL patients may attribute to the low levels of protein C and antithrombin and the high level of protein S and D-dimer. According to our findings, the use of suitable anticoagulant therapy as a prophylactic measure can be proposed.

Key Words:
Acute lymphoblastic leukemia, Thrombosis, Protein C, Protein S, Antithrombin.

Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disorder that originates in a single B- or T-lymphocyte progenitor. Proliferation and accumulation of blast cells in the marrow result in suppression of the hematopoiesis and thereafter the presence of anemia, thrombocytopenia, and neutropenia[1,2]. The occurrence of various coagulation abnormalities, i.e. an hypercoagulability state, in acute leukemia is a well established phenomenon with hemorrhage and thrombosis as the most common hemostatic disorders[3,4], that appear with different degree of blood clotting activation. *Protein C* previously described as the anticoagulant factor autoprothrombin II-A[5], is a plasma vitamin k-dependent protein, a plasma serine protease zymogene which can be converted to an active serine protease by thrombin[6]. This glycoprotein, activated by thrombin and thrombomodulin, acts as inhibitor of blood coagulation through a selective inactivation of factors Va and VIIIa. *Protein S* is a glycoprotein cofactor for protein C circulating as free (active) or inactive form as complex with C4b binding protein(C4BP)[7]. *Antithrombin,* synthesized in the liver, is a typical member of the serine protease inhibitor (SERPIN) superfamily and is denoted as SERPINC1[8,9]. Antithrombin, initially designated antithrombin III, is clinically the best known inhibitor of clotting factor proteases, that neutralizes thrombin and factors Xa, IXa, and XIa by irreversibly forming complexes in reactions accelerated by heparin or by heparan sulfate on endothelial surfaces[10]. *APCR (activated protein C resistance)* is defined as an abnormally reduced anticoagulant response of a plasma sample to APC aroused by many potential abnormalities in the protein C anticoagulant pathway and resulting in venous thrombosis[11,12]. The *D-dimer* is the ultimate degradation product of fibrin, the presence of which in plasma marks the coagulation activation process followed by a reactive

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thrombolysis[13,14]. Although for understanding of the underlying pathologies causing coagulation abnormalities, informations concerning the physiological elements involved in the coagulation process (endothelium, anticoagulants, platelets, fibrinolysis, genetics, homocysteinemia, lipoprotenia(a), interleukins) are essential, in this study we focused only on the evaluation of the distribution pattern of plasma proteins activity in the ALL group of patients in order to find a possible correlation between these parameters and the prevalent phenomenon of hypercoagulation.

Patients and Method

Thirty patients with ALL, were recruited from march to September 2010 in the Department of Hematology-Oncology Center of Ahwaz, Shafa Hospital, (Table I). A formal consent was obtained from all subjects. Subjects recently diagnosed, relapsed and in remission were included, while partially treated subjects and those with hepatic and/or renal dysfunction were excluded from this study. Thirty normal subjects (age and sex matched) as control group were also selected for coagulation analysis. Venous blood samples were collected by venopuncture in 1/10 volume of 3.8% buffered trisodium citrate in plastic tubes (9 part venous blood in 1 part sodium citrate solution 0.109 mol/L). Platelets poor plasma was prepared using citrated blood by centrifugation at 2000 rpm for 15 min and the supernatant plasma was aliquoted and frozen rapidly in well closed plastic tubes container at –70°C for subsequent analysis. Then, plasma aliquots were thawed in 37±0.5 water bath for 10 min. Activity levels of protein C, protein S, antithrombin, activated protein C receptor (APCR), D-dimer level, cells blood count (CBC), prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured using standard protocols. Activity levels of protein C, S, antithrombin and APCR were assayed by commercial kit (HemoIL, Milan, Italy) and 16 parameter ACL coagulation analyzer. D-dimer level was assayed by commercially available kit (Asserochrom® D-Di enzyme immunoassay) from Diagnostica Stago, Asenieres, France.

Statistical Analysis

Statistical analysis was done using SPSS (Statistical Package for Social Science) version 17: (Chicago, IL, USA); for windows statistical software package. Variables were normally distributed. The descriptive analysis used for continuous variables were mean ± SD and percentage. Student's *t*-test and One-way ANOVA, correlation with Pearson's test were used for all statistical analysis.

Results

Table II shows summary of results for patient and control groups. The mean levels of protein C and antithrombin plasma activity were lower in patient group compared with the control group ($p = 0.017$ and $p = 0.014$). However, the mean plasma activity levels of protein S and D-dimer were higher in patient group compared with the control group ($p = 0.004$ and $p < 0.0001$). The correlation between D-dimer plasma level and protein C, antithrombin and platelets count was found to be inverse ($r = –0.36$; $p = 0.004$), ($r = –0.13$; $p = 0.001$) ($r = –0.75$; $p < 0.0001$) (Figures 1-3). But, a positive correlation with Protein S was found ($r = 0.030$; $p = 0.016$) (Figure 4). No significant correlation was observed between protein C and protein S or antithrombin. Protein S plasma level showed an inverse correlation with platelet count ($r = –0.3$; $p = 0.020$) (Figure 5), while a positive correlation was found between platelets count and protein C and antithrombin ($r = 0.27$; $p = 0.03$) ($r = 0.33$; $p = 0.009$) (Figures 6 and 7) respectively.

Table I. Main characteristics of the study subjects.

Summary of study population	Patients	Control	ALL-L1	ALL-L2	ALL-L3
Number	30	30	24 (80%)	4 (13.4%)	2 (6.6%)
Age (mean years)	16.4 (2-16)	17.2 (2-17)			
Sex	17 M; 13 F	17 M; 13			
Total	Male 34 (56.7%)				
	Female 26 (43.3%)				

Table II. Mean values of plasma level anticoagulant factors in control and patients group with acute lymphoblastic leukemia.

	Patient group	Control group	*P* value
Protein C (%)	86.7 ± 24.6	98.7 ± 8.9	0.017
Protein S (%)	136.4 ± 57.5	102.9 ± 11.6	0.004
Antithrombin (%)	100.7 ± 17.2	110.3 ± 11.5	0.014
APCR (ratio)	2.7 ± 0.31	2.7 ± 0.23	0.674
D-dimer (ng/ml FEU)	759.5 ± 297.4	165.3 ± 103.6	0.0001
PT (sec)	13.9 ± 2.1	12.5 ± 0.34	0.002
aPTT (sec)	26.2 ± 3.55	29.5 ± 1.2	0.0001
WBC count (??/??)	12.8 ± 14.03	7.3 ± 0.98	0.039
Platelet count (??/??)	121.2 ± 87.8	256.1 ± 33.2	0.0001
Hb (gr/dL)	9.9 ± 1.8	13.4 ± 0.98	0.0001
Hct (%)	31.7 ± 6.1	41.8 ± 4.9	0.0001
MPV (FL)	11.8 ± 2.7	9.4 ± 0.49	0.0001

Values are expressed as mean ± SD. WBC, white blood cell; PT, prothrombin time; aPTT, activated partial thromboplastin time; AT, antithrombin; F, factor; PC, protein C; PS, protein S; D-Di, D-Dimers. APCR, Activated protein C receptor; Hb, Hemoglobin; Hct, Hematocrit; MPV, mean platelet volume.

Discussion

The observed high plasma activity levels of protein S and D-dimer concentration indicate the presence of an accelerated thrombin generation process. The occurrence of an impaired coagulative status is frequently observed in patients with cancer (both solid and hematological malignancies) which could be resulted from the effect of the tumor cell activity on the hemostatic system. The findings of a hypercoagulable state in this study agree with previous reports on ALL patients[15,16]. An increased amount of inflammatory cytokines, released in response to the tumor, play an important role in the hemostatic system activation in ALL[19]. These inflammatory mediators in association with low level of plasma activity of anticoagulant factors such as protein C and antithrombin and high levels of protein S can affect the hemostatic system[20]. In fact, in

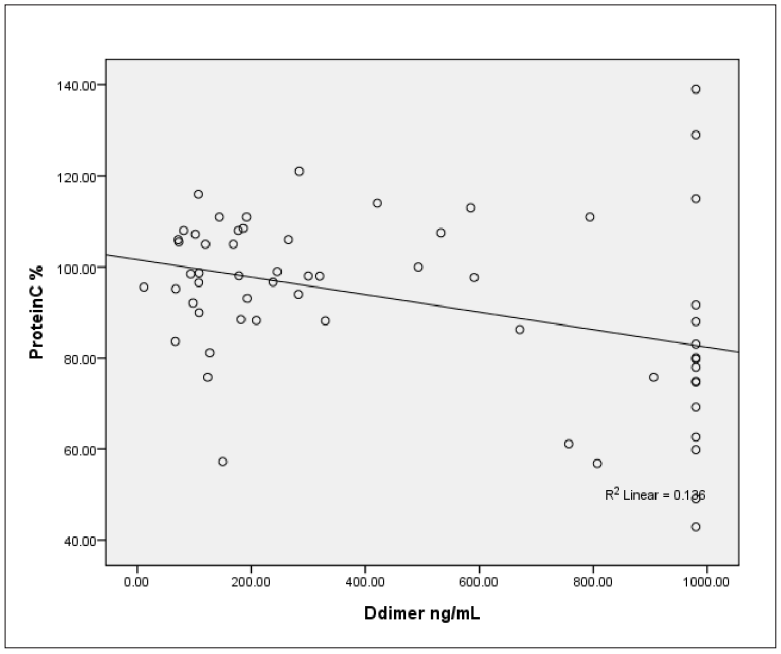

Figure 1. Scatterplot with correlation line of protein C to D-dimer. Pearson's correlation coefficient (r) (r = –0.36; $p = 0.004$).

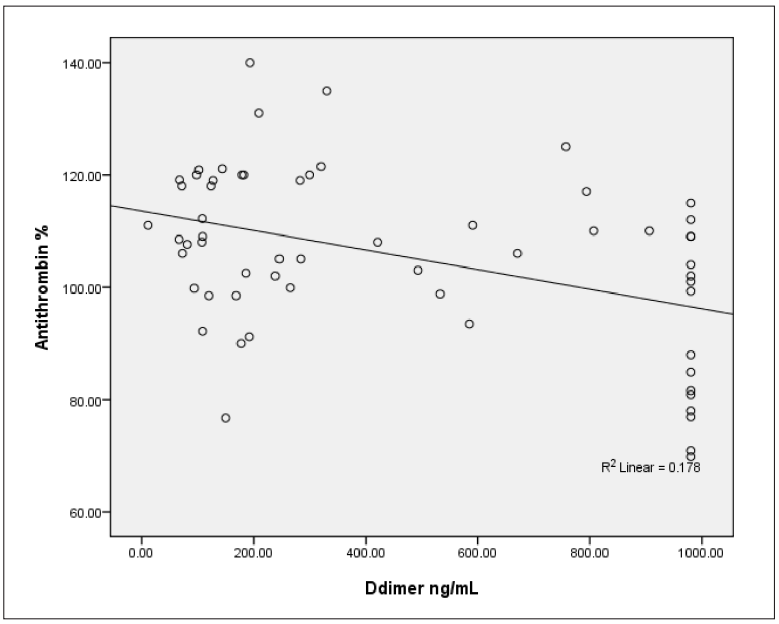

Figure 2. Scatterplot with correlation line of antithrombin to D-dimer. Pearson's correlation coefficient (r). (r = –0.13; $p = 0.001$).

ALL inflammatory mediators can prompt the coagulation by inducing the expression of intracellular tissue factors (TF) and the depression of the cell membrane glycoprotein, endothelial thrombomodulin[17]. Furthermore, cytokines support the cell adhesion molecules by endothelial cells and suppress the fibrinolytic system by inducing the expression of protease activator inhibitor-1 (PAI-1)[18]. All these effects might explain the findings of an augmented thrombin generation and an inhibition of fibrinolysis as expressed by the increase of the plasma level of protein S and D-dimer in our patients. Starting for a treatment during the induction, reduces the number of tumor cells and coincides with the significant decrease of protein C, antithrombin and cytokines plasma levels and the increase of protein S activity that occurs without a parallel modification of the hemostatic profile of these patients[21].

Particularly, while the plasma level of D-dimer and protein S increases without a significant modification in APCR the levels of both protein C and antithrombin decreased. This decrease as-

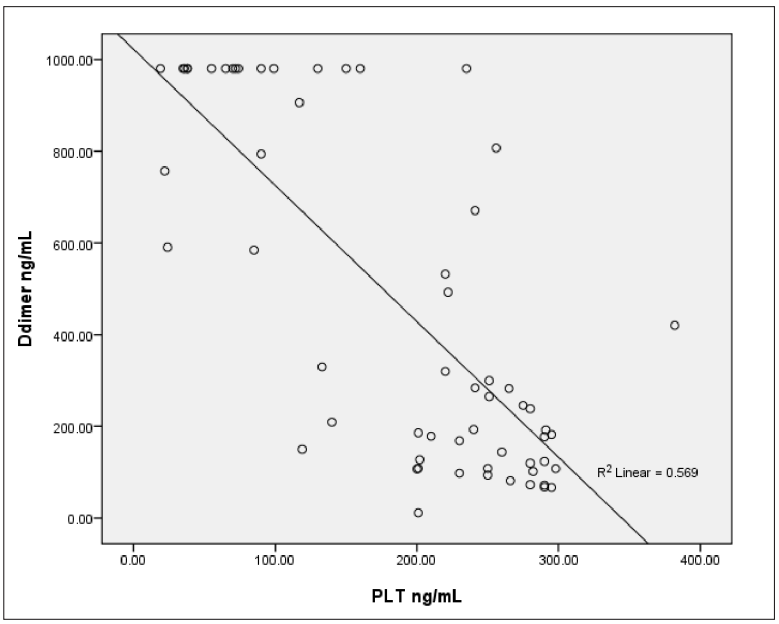

Figure 3. Scatterplot with correlation line of D-dimer to PLT count. Pearson's correlation coefficient (r). (r = –0.75; $p = 0.0001$).

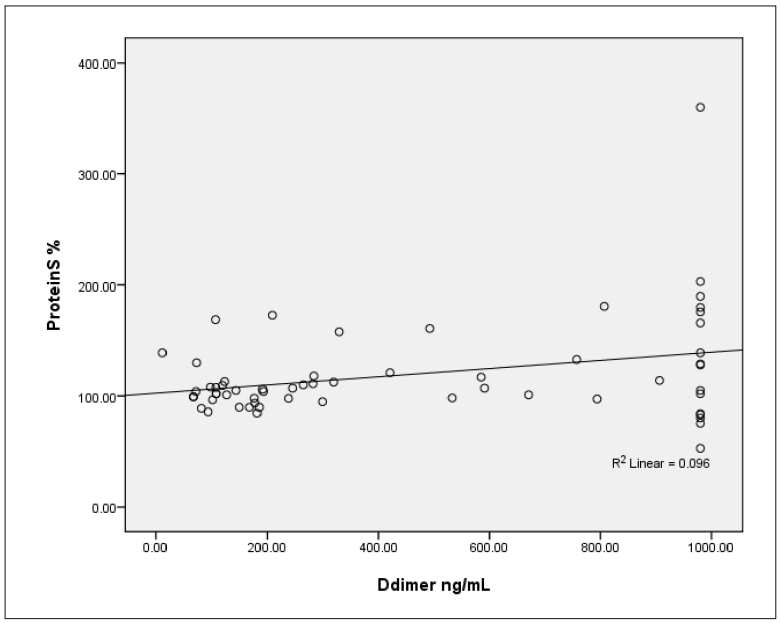

Figure 4. Scatterplot with correlation line of protein S to D-dimer. Pearson's correlation coefficient (r) (r = 0.030; $p = 0.016$).

sociated with the increased risk of thrombosis in ALL patients has been attributed to the corticosteroids and L-asparaginase which product such effect *in vitro*[22]. Likewise, several investigations report that more than half of ALL blasts have tissue factor-like and/or cancer procoagulant-like activities[23,24]. Ziegler et al[25] suggested that venous thromboembolism (VTE) is more frequent when L-asparaginase is given in conjunction with steroids. This was observed in this study in which thrombosis occurred in patients. Ten patients experienced thrombotic event during the remission induced by the therapy. Therefore, this phase in the ALL patients is associated with high risk of thrombosis. Protein S level can be increased in ALL patients because of the lack of binding of the protein S to complement inhibitors C4b binding protein (C4BP) which can inhibit the activity of protein S. Probably, the liver cells don't express C4BP and the liver synthesizes higher amount of protein S[26, 27]. Thus, the acute phase reaction commonly seen in ALL patients might induce a protein S synthesis in liver and secondly a (decreased) level of protein C and an-

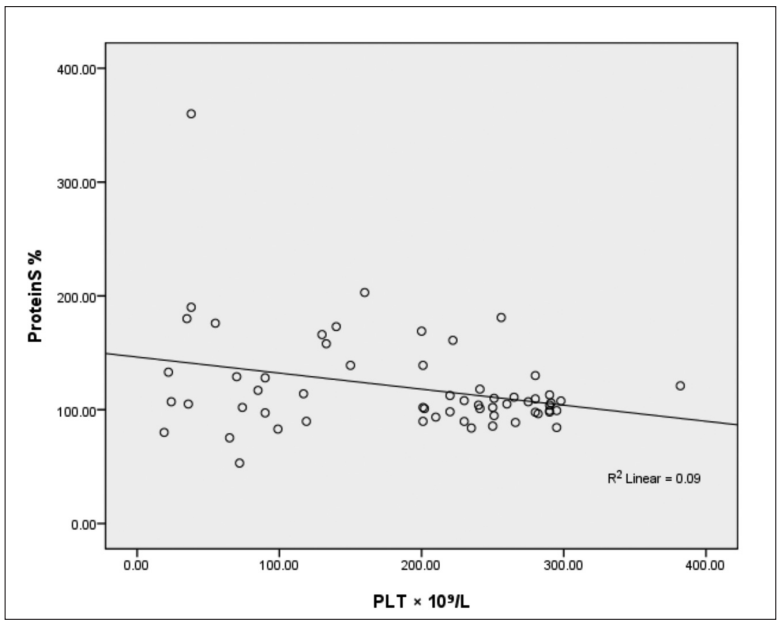

Figure 5. Scatterplot with correlation line of D-dimer to PLT count. Pearson's correlation coefficient (r). (r = –0.75; $p = 0.0001$).

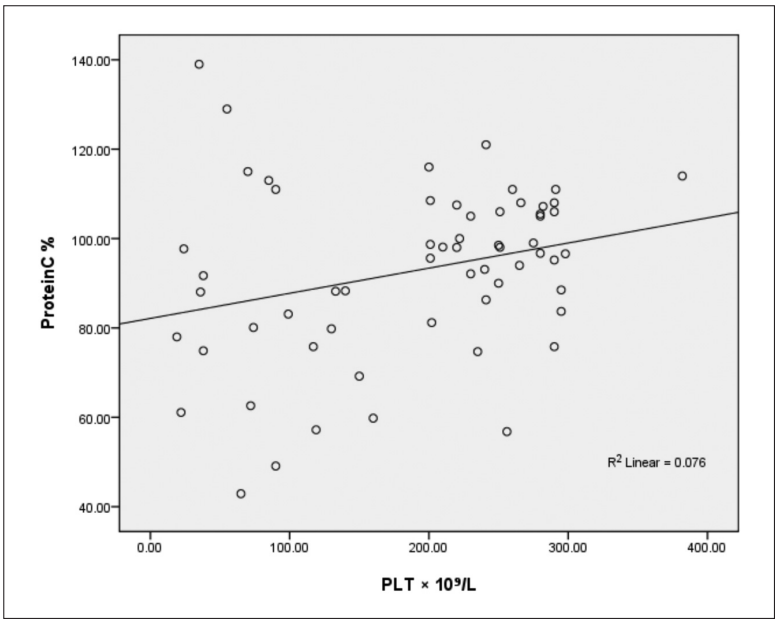

Figure 6. Scatterplot with correlation line of protein C to PLT count. Pearson's correlation coefficient (r) (r = 0.27; $p = 0.03$).

tithrombin. These findings suggest that ALL patients who have high WBC count, high D-dimer and protein S, low platelets count and/or low protein C and antithrombin level must be followed by attention for thrombosis and disseminated intravascular coagulation (DIC) after starting or during the chemotherapy. An effective prophylaxis and treatment of thrombin generation in ALL may reduce the mortality and morbidity due to thrombosis.

In conclusion, our data confirm that ALL is associated with hemostatic derangement due to an increase of the thrombin generation.

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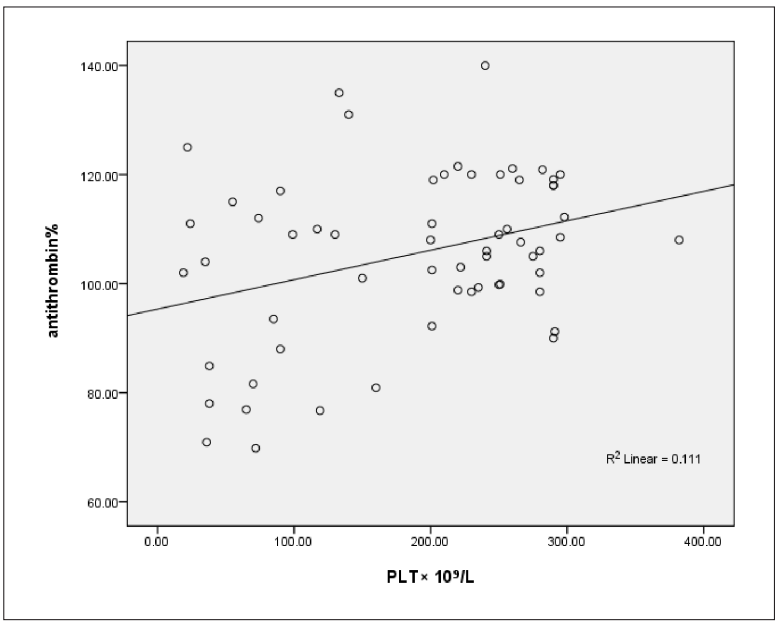

Figure 7. Scatterplot with correlation line of antithrombin to PLT count. Pearson's correlation coefficient (r) (r = 0.33; $p = 0.009$).

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