The Turkish Journal of Pediatrics 35: 45-51, 1993

PROTEIN C AND ANTITHROMBIN III IN CHILDREN WITH ACUTE LEUKEMIA*

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SUMMARY: Atlıhan F, Karakaş Z, Batun S. (Department of Pediatrics, Dicle University Faculty of Medicine, Diyarbakır, Turkey). Protein C and antithrombin III in children with acute leukemia. Turk J Pediatr 35: 45-51, 1993.

In this study Protein C (PC) and antithrombin III (AT III) levels in childhood acute leukemia were investigated. The mean PC activity levels in 19 newly diagnosed cases of acute leukemia were significantly lower as compared with the normal controls ($p < 0.05$). A significant increase was found ($p < 0.01$) in the patients in remission. Prior to treatment 78.8 percent of patients had decreased PC activity levels, but all patients had normal PC activity during remission.

Decreased PC activity levels were found to be independent of the leukocyte count and liver function.

No statistically significant difference was found in the AT III antigen levels between the untreated patients, the patients in remission and the control group. Our results indicate that apart from thrombocytopenia, low PC activity levels and alterations in fibrinolysis and coagulation may be responsible for the hemorrhagic manifestations observed in cases of acute leukemia. *Key words: protein C, antithrombin III, acute leukemia.*

Hemostasis is a complex mechanism involving local reactions in blood vessels, platelet activities and interactions of specific coagulation factors which circulate in the blood.

Four basic systems are involved in the regulation of coagulation: the fibrinolytic system, endothelial cells, protein C (PC) and antithrombin III (AT III)[1-3].

Protein C is a vitamin K dependent plasma glycoprotein that, when activated by thrombin and thrombomodulin, acts as a major inhibitor in blood coagulation. Activated PC has an anticoagulant effect by inactivating factors Va and VIIIa. It also facilitates fibrinolysis by neutralizing an inhibitor of the plasminogen activator (Fig. 1)[2-7].

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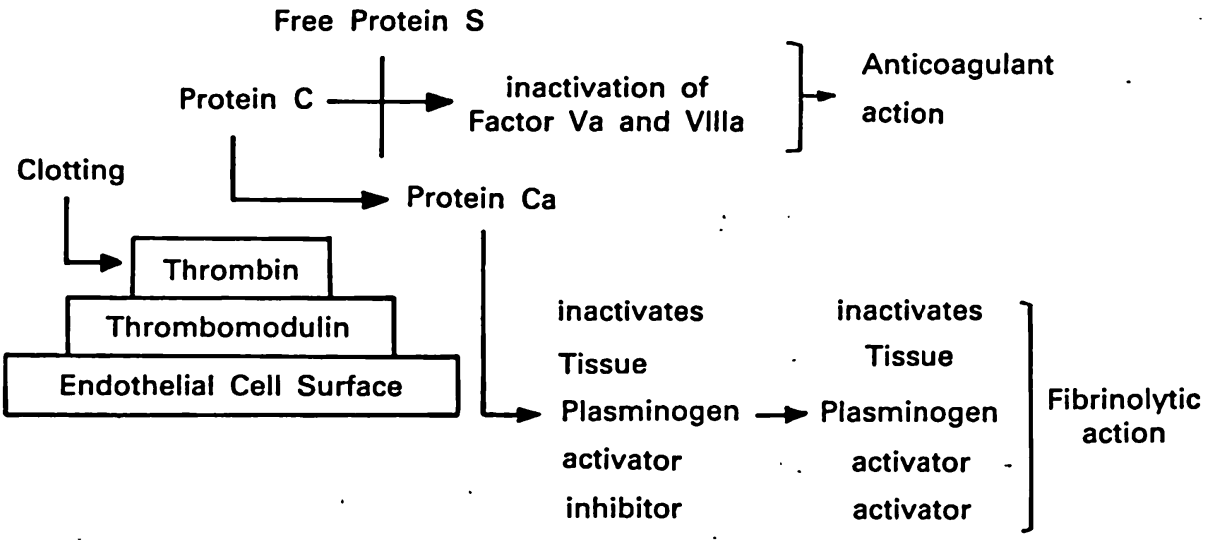

Fig. 1: Anticoagulant and fibrinolytic action of protein C.

Thrombin binds to thrombomodulin, a receptor protein on the surface of endothelial cells. The thrombin-thrombomodulin complex rapidly activates protein C.Protein Ca has an anticoagulant effect by inactivating factors Va and VIIIa. It also facilitates fibrinolysis by neutralizing an inhibitor of plasminogen activator.

AT III is a globulin which mainly inactivates thrombin, while factor Xa is a major inhibitor of blood coagulation[2, 8]. Hemostatic disorders occur very frequently in patients with acute leukemia.

Forty to sixty percent of patients demonstrate minor or major signs of bleeding at diagnosis. Thrombocytopenia cannot be considered the only cause of these hemorrhagic manifestations, but also changes in extravasal structures, the coagulation system, and fibrinolysis have been implied. In various studies, different data describing alterations in coagulation and fibrinolysis in acute leukemia have been reported. Therefore, it is difficult to evaluate the role of the deficiency in coagulation factors and alterations in fibrinolysis during hemorrhagic episodes in the acute leukemias[9-11].

The present study aims at investigating PC and AT III levels in childhood acute leukemia and to determine whether these two major coagulation inhibitors play a role in the hemorrhagic manifestations of this disease.

Material and Methods

The PC and AT III levels were studied in 19 children diagnosed as having either acute lymphoblastic leukemia (ALL) or acute non-lymphoblastic leukemia (ANLL). These levels were measured upon admission to the Department of Pediatrics of Dicle University Faculty of Medicine prior to initiation of treatment. The ages of

the patients ranged between 2.5 and 13 years. The control group consisted of 12 age-and-sex matched healthy children.

A diagnosis of acute leukemia was established after examining peripheral blood and bone marrow samples using conventional cytochemical stains. Acute leukemia was classified morphologically according to the criteria proposed by the FAB Group[12].

Plasma samples were analyzed in 19 children before treatment was initiated, and in 14 of these children, determinations were repeated after remission was achieved.

Blood samples were collected in sodium citrate (9 parts blood:1 part 0.11 mol/l anticoagulant) using a 5 ml syringe. Plasma was obtained by centrifugation at 1500 g for 10 minutes. Blood samples were frozen and stored at − 20 °C. PC activity was determined by the coagulometric method using a commercial kit (Behring Diagnostics). With the use of standard human plasma (Behringwerke Diagnostics), a reference curve was drawn on double logarithmic graph paper and the results were expressed as percentages. The normal range was 70-140 percent. An AT III assay was determined in plasma by the radial immunodiffusion technique using the Nor-Partigen AT III antigen kit (Behring Diagnostics). The results were expressed as a percentage of a normal pool. The normal range was 73-100 percent.

The diagnosis of disseminated intravascular coagulation (DIC) was confirmed by a decreased fibrinogen (< 300 mg/dl) and platelet count (< 100000/mm^3), prolonged partial thromboplastin time and an elevation of fibrin degradation products. Enzymatic synthesis function of the liver was evaluated by the following tests: SGPT, SGOT, prothrombin time and protein electrophoresis. The Student's *t* test was used for statistical analyses.

Results

Seventy-nine percent of the patients studied had ALL and 21.2 percent had ANLL. The mean age of the patients was 7.60 ± 3.59 years, and the mean age of the control group was 7.75 ± 3.49 years.

Mean PC concentrations were 52.15 ± 30.93 percent in the newly diagnosed cases of acute leukemia and 101.32 ± 16.46 percent in the control group. PC activity was significantly lower in the cases of acute leukemia as compared with the controls ($p < 0.01$). Mean PC activity levels in the 14 patients in remission were 94.5 ± 20.99 percent (Table I). A significant increase was found in the PC levels of the patients in remission as compared with the untreated patients ($p > 0.01$), but no statistical significant difference was found when comparing these values with those of the control group ($p > 0.05$). Of 19 patients, 14 (78.8 %) had decreased PC activity levels prior to treatment, but all patients in

Table I: Plasma Protein C Activity in Patients with Untreated Leukemia, in Patients in Remission and in the Control Group

	n	Protein C (%) (Range)	t	P
Control	12	101.32 ± 16.46 (85 - 140)	5.75	< 0.01
Leukemia	19	52.15 ± 30.93 (3 - 120)	4.68	< 0.01
Remission	14	94.5 ± 20.99 (74 - 140)		

remission had normal PC activity levels. The mean PC activity levels were 60.86 ± 26.07 percent in ALL patients and 19.5 ± 27.73 percent in ANLL patients. Mean PC activity was found to be significantly lower in the ANLL group. ($p < 0.05$).

The enzymatic synthesis function of the liver was evaluated. Mean PC activity levels were 42.62 ± 21.90 percent in eight patients with liver dysfunction and 56.3 ± 38.71 percent in ten patients with normal liver function. There was no statistical significant difference found between these groups ($p > 0.05$). PC activity was 50.4 ± 12.05 percent in patients with leukocyte counts over 50000/mm^3 and 52.42 ± 35.62 percent in patients with leukocyte counts below 50000/mm^3. There was no statistical significant difference found between these groups ($p > 0.05$).

Mean AT III antigen levels were 106.36 ± 23.03 percent in the cases of acute leukemia prior to treatment, 107.92 ± 19.83 percent in the cases in remission and

Table II: Plasma AT III Antigen Levels in Patients with Untreated Leukemia, in Patients in Remission and in the Control Group

	n	AT III (%) (Range)	t	P
Control	12	121.16 ± 17.42 (100 - 152)		
Leukemia	19	106.36 ± 23.03 (73 - 150)	2.02	> 0.05
Remission	14	107.92 ± 17.93 (77 - 141)	0.20	> 0.05

121.61 ± 17.42 percent in the control group. There was no significant difference statistically between these groups ($p > 0.05$). (Table II).

There was no significant statistical difference found between the AT III antigen levels of the patients with ALL and those with ANLL.

Discussion

Various coagulation and fibrinolysis disorders due to disease or to therapy are known to occur in children with acute leukemias. The two major inhibitors in coagulation, PC and AT III, have not been extensively investigated in pediatric cases of acute leukemia, and therefore, with the aim of determining whether they have a pathophysiological role in hemorrhagic manifestations, the PC and AT III levels were measured in 19 children newly diagnosed as having acute leukemia.

In our study, mean PC activity levels were significantly lower than in the normal controls, with 78.8 percent of the patients having PC levels below the normal range. Rodeghiero et al[13] in their study conducted in 58 patients with acute leukemia reported that the mean PC antigen concentrations were slightly lower than in the normal controls, with about one third of patients having PC values below the normal range. In our study, we evaluated the functional activity of PC. Marlar and co-workers[14] determined both the PC antigen and activity levels in DIC and showed that functional activity decreases more rapidly than antigen levels. Many studies have shown that PC activity decreases progressively during the initial stages of DIC, but that it gradually returns to normal in nonfatal cases[5, 14, 15]. In our study, only one patient had DIC, therefore, low PC activity levels cannot be explained by DIC.

Rodeghiero and co-workers[13] observed no decrease in PC antigen levels in patients with DIC resulting from leukemia and where there was no liver involvement. But they observed a decrease in PC antigen levels in patients with DIC who had liver disease as a consequence of the reduced synthesis by the liver. In the present study, we tried to determine whether a decrease in PC levels correlated with liver dysfunction by measuring the SGOT, SGPT, and PT levels and protein electrophoresis. No significant difference was found between PC activity levels of patients with liver dysfunction and those with normal liver function. PC concentrations were also low in 80 percent of the patients with normal synthesis function of the liver. Therefore, we cannot conclude that liver dysfunction alone accounts for low PC activity levels as Griffin et al[15] and Rodeghiero et al[13] have stated.

Some authors have suggested that the release of specific protease inhibitors derived from leukemic cells that possess fibrinolytic and procoagulant activity are responsible for the alterations in fibrinolysis in acute leukemias. In patients with untreated acute leukemia, widely varying changes in parameters of coagulation

and fibrinolysis have been reported. Enhanced fibrinolytic activity has been reported by Brakman et al[9] in contrast to the reports of Guarini et al[10], who explained the reduction of tPA (tissue plasminogen activator) activity by degradation of proteases from the leukemic cells or inactivation by some inhibitors. A new protease inhibitor of the hemostatic system that has been recently described is able to slowly inactivate PCa generated within the blood. The second new protease inhibitor reported can rapidly neutralize the action of urokinase or tissue type plasminogen activator[12]. In conclusion, the decreased levels of PC activity in untreated cases of leukemia can be explained by proteases and inhibitors released from the leukemic cells. Obtaining normal PC levels during remission also supports our hypothesis. We attempted to find a correlation between leukocyte counts and PC activity, but we found decreased PC activity levels independent of leukocyte counts. We also found PC activity to be significantly lower in ANLL cases in comparison with ALL cases, which is probably due to the proteases released from the primary granules of myeloid leukemic cells[9, 10, 16].

There was no statistical significant difference found between the AT III antigen levels of the untreated leukemic patients, the remission group and the control group. AT III antigen levels were within the normal range in all patients. There was also no difference found between the AT III antigen levels in the ANLL and ALL groups. There was no correlation found between AT III, the number of circulating blast cells and liver function. Rodeghiero et al[13] found normal AT III antigen and activity levels in cases of acute leukemia and reported that AT III activity was significantly lower only in the acute myeloblastic leukemia (AML) group when compared with the controls. Other studies on AT III indicate that this inhibitor is usually normal[13, 17]. In conclusion, we can say that there are varying changes in parameters in the coagulation of fibrinolysis in pediatric patients with acute leukemia. Our results indicate that besides thrombocytopenia, low levels of PC activity and alterations in fibrinolysis and coagulation may be responsible for the hemorrhagic manifestations observed in cases of acute leukemia. However further studies are needed to prove this point.

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