The Turkish Journal of Pediatrics 30:231 - 236, 1988

PROTEIN C AND ANTITHROMBIN III LEVELS IN CHILDREN WITH CYANOTIC CONGENITAL HEART DISEASE*

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Key words: protein C, antithrombin III, cyanotic congenital heart disease

Cyanotic congenital heart disease (CCHD) in children can be complicated with both thromboembolism and a bleeding disorder[1-8]. Patients with severe hypoxemia and polycytemia are at greater risk in developing venous thrombosis, especially in the central nervous system[3]. The mechanism of a tendency to thrombosis is not quite clear. Studies on the hemostatic mechanism have yielded evidence of the activation of the platelet and intrinsic coagulation systems and disseminated intravascular coagulation (DIC)[9].

Protein C (PC) and antithrombin III (AT III) inhibit the various activated components of coagulation and constitute a major defence mechanism against intravascular coagulation. Congenital deficiency of either protein results in thromboembolic diseases[10-15]. AT III has been reported to be slightly decreased in children with CCHD[9], but no information is available regarding PC. In an attempt to define their role in the pathogenesis of a tendency to thrombosis, we examined plasma levels and correlations of both PC and AT III in children with CCHD.

Material and Methods

Seventeen children with CCHD who ranged in age between one and 14 years and 18 age-matched children with acyanotic congenital heart disease (ACHD) were studied. The control group consisted of 15 children without evidence of systemic disease that would affect the hemostatic system.

In the cyanotic group, one patient had experienced cerebrovascular accident. Hematocrit levels were elevated in 15 patients, PT was prolonged in two patients, APTT was prolonged in one patient and transaminase levels were elevated in two patients. None of the patients with ACHD had abnormal coagulation or liver function tests.

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Plasma samples were prepared from citrated blood, stored at-70°C and tested within four weeks. PC antigen level was measured by an enzyme-linked immunosorbent assay, AT III antigen level by a synthetic chromogenic assay and fibrinogen-fibrin degradation products (FDP) by a latex agglutination method (Diagnostica Stago, France). Hematocrit value, prothrombin time (PT), activated thromboplastin time (APTT) and fibrinogen level were determined by standard methods. Serum albumin, bilirubin, SGOT and SGPT levels were measured in a 12-channel autoanalyser.

The results were expressed as mean ± the standard deviation. The Student's *t* test and Mann-Whitney test were performed to assess the significance of the differences between the groups. Linear correlations were calculated using standard procedures.

Results

PC antigen was significantly lower ($p < 0.001$) in the patients with CCHD (57 ± 30%) than the patients with ACHD (107 ± 48%) and controls (87 ± 12%). A decreased PC value (less than 65%) was observed in 12 of the 17 patients with CCHD and in only one of the 18 patients with ACHD (Fig. 1). Six patients with ACHD had elevated PC values.

AT III antigen was also significantly lower ($p < 0.001$) in the patients with CCHD (76 ± 29%) than in those with ACHD (92 ± 33%) and controls (113 ± 21%). Seven patients with CCHD and three patients with ACHD had low AT III values. Both PC and AT III were low in six patients with CCHD. There was no significant correlation between the PC and AT III levels ($p > 0.05$). Neither PC nor AT III showed a significant correlation with serum albumin and hematocrit ($p > 0.05$). Seven patients with CCHD had elevated FDP values and were diagnosed as having DIC. The mean values of both PC and AT III were significantly lower in the patients with DIC than those without DIC (Table I).

TABLE I: Protein C and Antithrombin III Levels in Patients with CCHD with and without DIC

	Patients with DIC (n: 7)	Patients without DIC (n: 10)	p value
PC (%)	51 ± 26	62 ± 34	< 0.05
AT III (%)	63 ± 31	87 ± 24	< 0.001
Fibrinogen (mg/dl)	280 ± 184	300 ± 104	> 0.05
PT (sec)	15.7 ± 1.4	12.6 ± 0.7	< 0.05

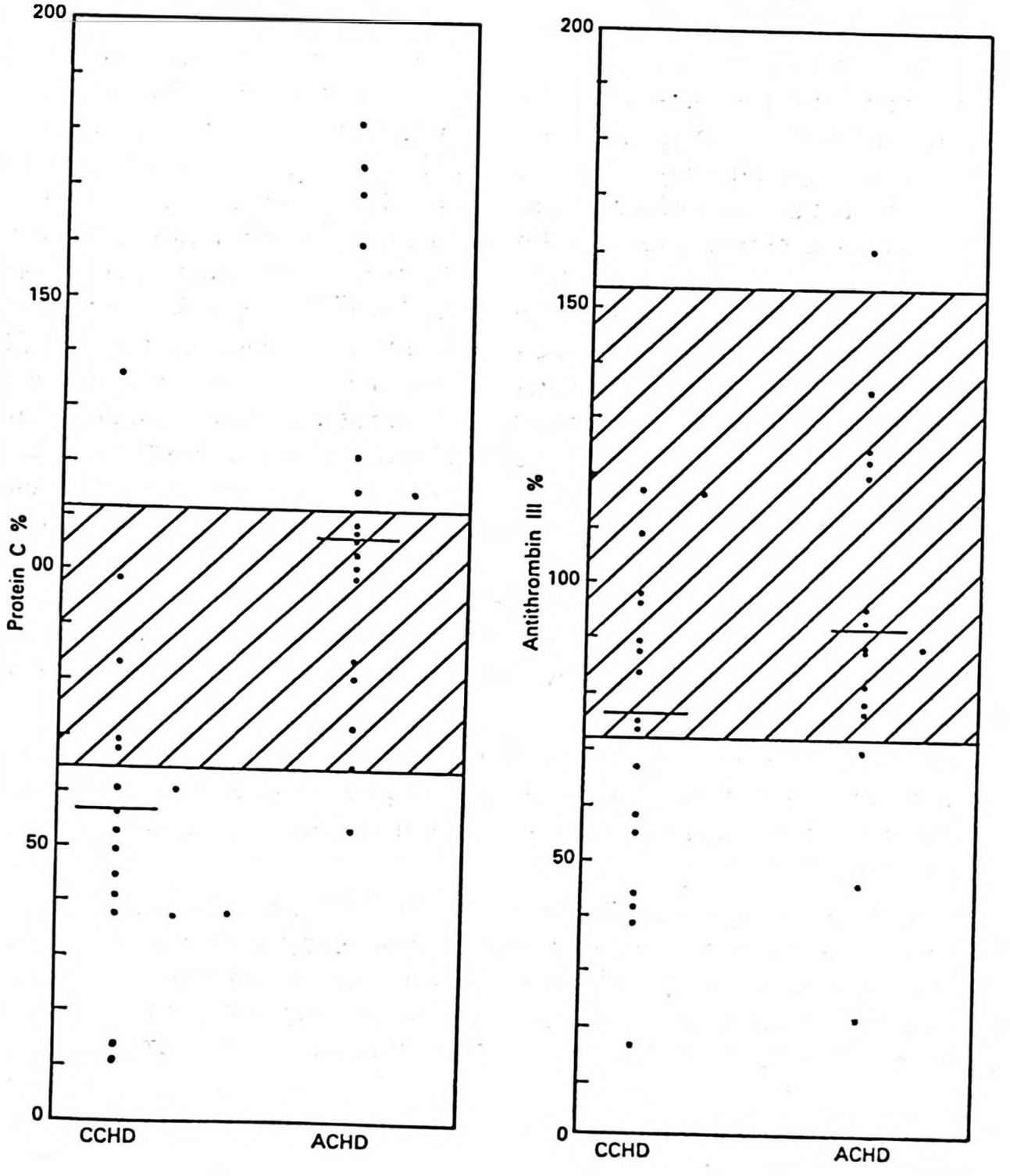

Fig. 1: Protein C and antithrombin III in children with cyanotic congenital heart disease (CCHD) and acyanotic congenital heart disease (ACHD). The shaded areas show the normal ranges of PC and AT III in the normal controls. The bars show the mean values.

Discussion

We found AT III levels to be decreased in children with CCHD which is in accord with a previous finding[9]. In addition, we demonstrated that PC levels were also decreased in these patients. In contrast, children with ACHD showed normal AT III levels and normal or increased PC levels. The decrease of PC and AT III in CCHD may be caused by either diminished synthesis or accelerated removal from the circulation[16]. Liver dysfunction was evident by elevated transaminase levels in two patients, but absence of a significant correlation between either inhibitor or serum albumin level which reflects the liver's capacity for synthesis, suggests that impaired hepatic synthesis is not likely to be present. Plasma levels of PC and AT III have been shown to be lowered during DIC[17-19]. It is believed that PC, activated by excess thrombin, is rapidly cleared from the circulation in the DIC process. The finding by Suarez et al[9] that fibrinopeptid A increased in children with CCHD is an indication of thrombin generation. In our study, we found that patients with evidence of DIC had lower PC and AT III levels than those without DIC. In fact the lowest PC and AT III levels were observed in a patient with decompensated DIC (hypofibrinogenemia). It seems therefore that DIC is the most likely cause of the decrease of PC and AT III in children with CCHD. DIC is assumed to be triggered by tissue hypoxemia induced by polycytemia. There was, however, no direct correlation found between either inhibitor or hematocrit levels. Other hemodynamic factors may play important roles in the pathogenesis of DIC in these patients.

The increase of PC in some patients with ACHD was not related with the type of cardiac lesion and remains to be explained. High PC levels were observed in patients with ischemic heart diseases and rheumatoid arthritis without an apparent cause[20,21].

Activated PC electively inhibits factor V_a and $VIII_a$[22,23]. The decrease of PC may explain the previously described findings of increased factor V and factor VIII levels in children with CCHD[1,3]. An increased incidence of thromboembolytic complications is observed when the plasma levels of either PC or AT III are below 50 percent[10,12]. About half of our patients with CCHD had a PC or AT III value less than this level. These defects in the inhibitory function may contribute to the tendency to thrombosis in these patients. Therefore, raising the plasma levels of these inhibitors may be recommended when plasma levels are decreased, especially in patients who have evidence of DIC in order to stop excess thrombin formation.

Summary

Plasma levels of protein C (PC) and antithrombin III (AT III) were measured in 17 children with cyanotic congenital heart disease (CCHD), 18 children with acyanotic

congenital heart disease (ACHD) and 15 healthy controls. The mean values of both PC and AT III were found to be significantly lower ($p < 0.001$) in the children with CCHD than in those with ACHD and the controls. There was no significant ($p > 0.05$) correlation found between either inhibitor or serum albumin. Both inhibitors were lower in the cyanotic patients with disseminated intravascular coagulation (DIC) than those without DIC. This data suggests that decrease of PC and AT III in children with CCHD is probably secondary to DIC rather than decreased hepatic synthesis. Decrease of these inhibitors of coagulation may enhance tendency to thrombosis in the patients with CCHD.

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