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EVALUATION OF THE HYPERCOAGULABLE STATE BY MEASURING PROTEIN C AND ANTITHROMBIN III LEVELS IN NEPHROTIC SYNDROME AND IN FAMILIAL MEDITERRANEAN FEVER-RELATED AMYLOIDOSIS*

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SUMMARY: Topaloğlu R, Saatçi Ü, Bakkaloğlu A, Beşbaş N, Başsoy Y. (Pediatric Nephrology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Evaluation of the hypercoagulable state by measuring protein C and antithrombin III levels in nephrotic syndrome and in Familial Mediterranean fever-related amyloidosis. Turk J Pediatr 34:15-20, 1992.

The levels of protein C (PC) and antithrombin III (AT III) antigens (ag) were measured in the plasma of 39 patients with various histologic types of primary nephrotic syndrome (NS) and in 12 patients with amyloidosis secondary to familial Mediterranean fever (FMF). The controls comprised 15 healthy children. Normal or elevated PC levels were observed in primary NS patients (mean 64%, range 36-98%) and in amyloidosis patients (mean 58%, range 48-70%).

There was no difference found between PC ag levels in primary NS and in amyloidosis patients. In addition, no correlation existed between protein selectivity and the PC ag levels in the primary NS patients. Normal and decreased levels of AT III were observed (mean 29 mg/dl, range 11.1-39 mg/dl) in the patients with primary NS and amyloidosis (mean 31 mg/dl range, 21-39 mg/dl). The AT III ag levels of these two groups did not differ and no correlation was found between protein selectivity and AT III levels in primary NS patients.

These results suggest that in patients with primary NS, or amyloidosis secondary to FMF, hypercoagulability is not related to a deficiency in PC ag levels due to a dynamic balance between urinary losses, increased rate of hepatic synthesis, catabolism and the distribution of PC in the body compartments. Patients with low AT III levels may be more susceptible to thromboembolic complications than patients with normal levels. *Key words: primary nephrotic syndrome, amyloidosis, protein C, antithrombin III.*

Protein C (PC) is a vitamin K dependent major anticoagulant which upon activation in vivo by thrombin binds to endothelial thrombomodulin and rapidly cleaves and destroys the procoagulant functions of activated factor V and factor VIII and also stimulates fibrinolysis[1,2]. Antithrombin III (AT III), an alpha$_2$ globulin synthesized in the liver, is the major inhibitor of thrombin and several other serine proteases such

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as activated factor IX and X. Both inherited and acquired deficiencies of AT III or PC are the major causes of recurrent venous or arterial thrombosis[1,3].

Patients with nephrotic syndrome (NS) are at high risk of venous thromboembolism. This fact has been attributed to abnormalities in the concentration of almost every coagulation factor and inhibitor as well as the platelets and fibrinolytic enzyme system[4].

Activity and antigen levels of PC and AT III have also been investigated as one of the possible causes of thromboembolic tendencies in nephrotic patients. But conflicting data have been published on changes in AT III and PC levels of NS patients[5-11].

In view of this controversy, the present study was undertaken to examine PC and AT III antigen levels in primary NS patients and also in a group of FMF-related amyloidosis patients. This is a preliminary report about PC and AT III antigen levels in patients with amyloidosis secondary to FMF.

Material and Methods

The study population consisted of 39 patients with primary NS (18 males and 21 females) aged between 2 to 17 years, and 12 patients with amyloidosis secondary to FMF (8 males and 4 females) aged between 6 and 19 years. The controls comprised 15 healthy children.

Proteinuria exceeding 2 g/m^2/day and hypoalbuminemia of a value of 2.5 g/dl or lower were considered the criteria for NS.

Protein selectivity was determined by the ratio of IgG clearance to albumin clearance. A ratio of < 0.1 indicated selective proteinuria while a ratio of > 0.1 indicated non-selective proteinuria.

In FMF patients, the diagnosis of amyloidosis was confirmed by renal biopsy.

All patients had normal creatinine clearance, SGOT, SGPT and PTT levels.

For PC and AT III antigen levels, blood samples were added to one ninth volume of 3.8% trisodium citrate anticoagulation solution. The plasma was separated by centrifugation for 10 min at 3000 rpm. Plasma samples were frozen immediately and stored at −20 °C. The AT III ag concentrations were measured using the Behring nor-partigen AT III kit which is based on the radial immunodiffusion method. Normal values were expressed as a percentage or g/dl of normal (normal 73-130% or 0.22-0.39 g/dl). PC concentrations were measured using the Asserachrom Protein C kit and the ELISA method. All values were expressed as a percentage of a normal plasma pool. Our laboratory normal ranged between 25-57% ($\bar{x} = 41\% \pm 16$). The Fischer's exact probability test and the Chi-square test were used for statistical analysis.

Results

No reduction was observed in the PC ag levels in the plasma of the primary NS patients and the amyloidosis patients when compared with the controls. The mean PC level was 64 percent (range 36-98%) in primary NS patients and 58 percent (range 48-70%) in amyloidosis patients (Fig. 1). This result was statistically insignificant ($p > 0.05$; Fig. 1).

Both normal and elevated PC ag levels were noted in primary NS patients and their relation to protein selectivity was studied. No correlation was found between PC levels and protein selectivity ($p > 0.05$; Table I).

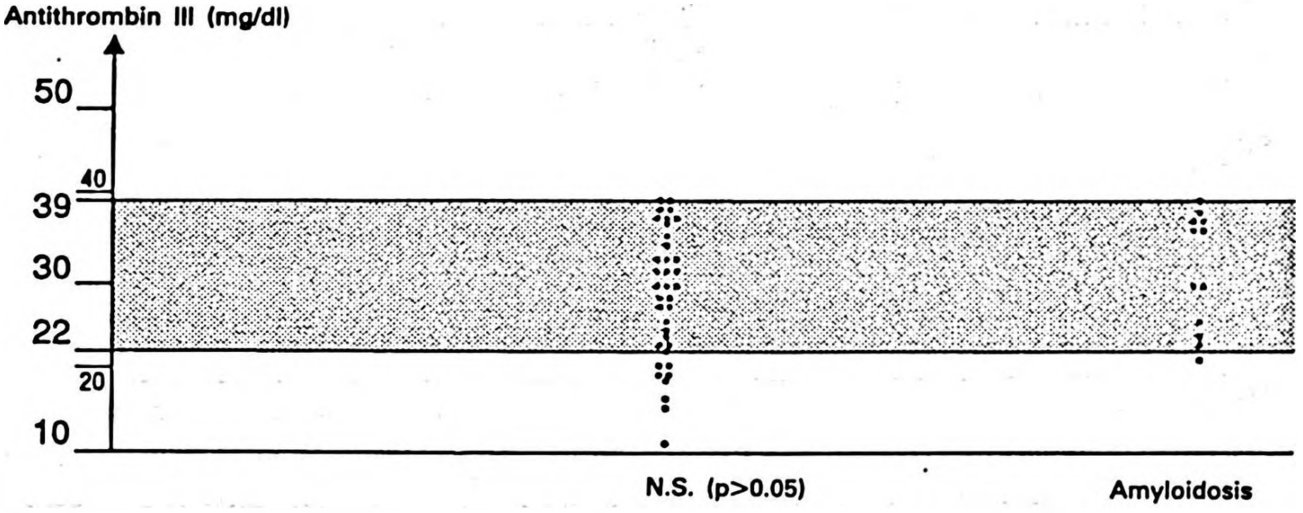

Fig. 1: Antithrombin III antigens levels in nephrotic syndrome and amyloidosis.

TABLE I: Protein C Antigen Levels and Protein Selectivity in Nephrotic Syndrome

	Protein C	
	Normal	Elevated
Nephrotic syndrome with selective proteinuria	11	13
Nephrotic syndrome with nonselective proteinuria	5	10

$p > 0.05$

AT III ag levels were either decreased or normal in primary NS patients (mean 29 mg/dl, range 11.1-39 mg/dl) while these levels were normal in all amyloidosis patients (mean 31 mg/dl, range 21-39 mg/dl) except for one. AT III levels were not significantly different in primary NS patients and amyloidosis patients ($p > 0.05$; Fig. 2). The AT III ag level was not correlated with protein selectivity ($p > 0.05$; Table II).

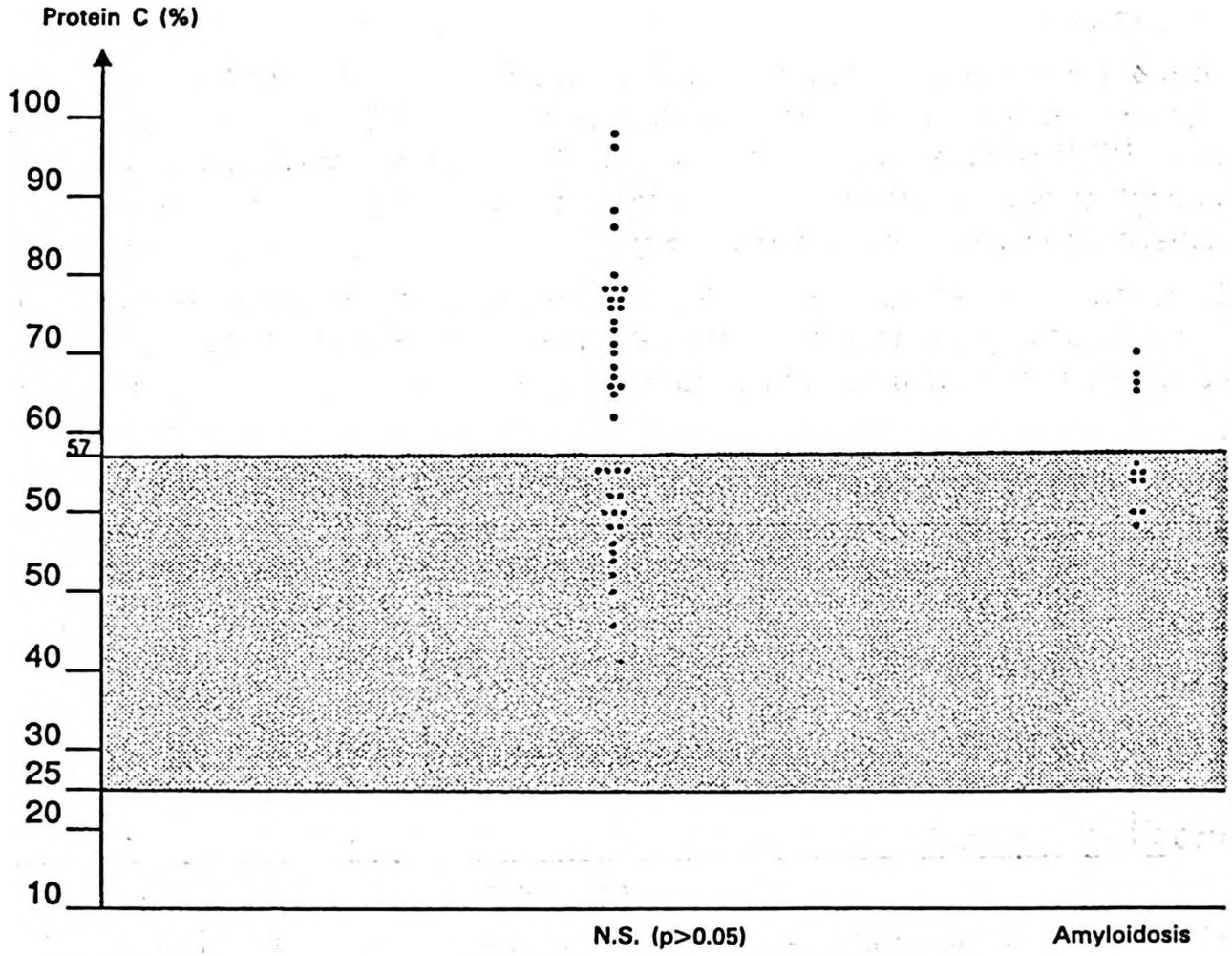

Fig. 2: Protein C antigen levels in nephrotic syndrome and amyloidosis.

TABLE II: Antithrombin III Antigen Levels and Protein Selectivity in Nephrotic Syndrome

	Antithrombin III	
	Normal	Low
Nephrotic syndrome with selective proteinuria	17	5
Nephrotic syndrome with nonselective proteinuria	12	3

$p > 0.05$

Discussion

PC ag levels were found to be high and/or normal in primary NS patients with normal renal function, PT and PTT levels and the absence of thrombotic symptoms. Pabinger-Fasching[5], Soff[6], and Mannucci[7] reported high and/or normal plasma PC ag levels in idiopathic NS. These investigators demonstrated

that PC ag is detectable in the urine of primary NS. It is concluded that patients compensate for PC losses in the urine by increased synthesis or by some other mechanism[6,7]. We have not studied urinary PC loss but PC has a very similar molecular weight to albumin (~ 66 000 daltons), and the negative charge is presumed to be lost in the urine. Due to conflicting data in the literature, we decided to study PC ag levels in patients with primary NS having selective or non-selective proteinuria and in patients with amyloidosis secondary to FMF. No statistically significant difference was found between the PC ag levels of NS patients with selective or non-selective proteinuria.

FMF induced amyloidosis is one of the major causes of secondary nephrotic syndrome in the Mediterranean region. There is an accumulation of amyloid fibrils in various tissues as well as in the kidneys[12], and bleeding, fibrinolysis, intravascular coagulation and vitamin K deficiency have also been reported[11-14]. We studied PC and AT III ag levels in patients with amyloidosis secondary to FMF to see if the levels of these two potent anticoagulants differed from patients with primary NS. No difference was observed between these two groups. AT III ag levels studied in patients with primary amyloidosis have been found to be low in reports in the literature. However, with the exception of one case, we found these levels to be normal. As to our knowledge, reports describing PC and AT III levels in patients with amyloidosis secondary to FMF have appeared only once in the literature.

In primary NS, the data for AT III ag varies. Some authors have observed decreased levels, while others have reported normal levels which are consistent with our findings[5-8,10,15,16]. In our study, conflicting AT III levels were not found to be related to protein selectivity.

In conclusion, PC and AT III ag levels are not only altered by urinary loss but are also affected by the synthesis, degradation and distribution rate as well. PC ag levels seem not to be related to hypercoagulability but low AT III levels may be related to it. In short, the AT III level should be determined in every nephrotic patient.

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