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Original Article

Evaluation of Protein-Z and Antithrombin III Plasma Levels in Children with Minimal Change Nephrotic Syndrome

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ABSTRACT

Background: In children with the nephrotic syndrome, acquired deficiency of anticoagulant proteins due to loss in the urine has been proposed as one of the major thrombogenic alterations. Low levels of protein Z, which is a single chain vitamin K-dependant glycoprotein synthesized by the liver, were reported to be a risk factor for increased incidence of thrombosis. Another biologic antagonist of coagulation is antithrombin III which has a relatively low molecular weight and expected to be lost in urine in patients with nephritic syndrome.

Objectives: Evaluation of both plasma protein Z levels, and antithrombin III activity as markers of thrombogenic disorders in children with minimal change nephrotic syndrome, and to elicit the relationship between their plasma levels in nephrotic children and different stages of the disease.

Methods: This study was carried out on 30 children with minimal change nephrotic syndrome with no thromboembolic manifestations. They were classified into 2 groups; group (I) included 15 children in the acute stage of nephrotic syndrome, and group (II) included 15 children in the remission stage of nephrotic syndrome under treatment with corticosteroids only. Fifteen healthy age and sex matched children were chosen as a control group; group III.

Results: The mean plasma levels of both protein Z and of antithrombin III activity (%) in children with acute stage (group I) were significantly lower than those of children in remission (group II) and control group (group III) while there was no significant difference in these levels when group II was compared to group III. In group I, there was a significant positive correlation between protein Z and antithrombin III activity (%) (r-value = 0.83, p-value = 0.0001). Also, there were significant positive correlations between both parameters and total serum protein, serum albumin while there were significant negative correlations of both protein Z and antithrombin III to 24 hours protein in urine.

Conclusion: Even though there were no clinically detectable thromboembolic complications in the studied children with nephrotic syndrome, the plasma levels of protein Z and antithrombin III activity (%) were significantly decreased in children in the acute stage of the nephrotic syndrome, while those in the remission stage, plasma levels of protein Z and antithrombin III returned to normal values. Thus, children in the acute stage of nephrotic syndrome are still susceptible to thromboembolic complications and need close observation.

INTRODUCTION

The association between the nephrotic syndrome and intravascular coagulation has been known for more than a century, but it was not until 1948 that the concept of a thrombotic diathesis in nephrotic patients was proposed[1]. Thromboembolic disease in nephrotic syndrome is an important

complication in both children and adults, where thrombotic episodes are seen in about 5% of children and 10% of adults with nephrotic syndrome. Although these complications are less common in children, the consequences are more severe[2]. Acquired deficiency of anti coagulant proteins, due to their loss in the urine, has proposed as one of the major thromboembolic alterations in nephrotic syndrome[3]. One of the most important inhibitors of coagulation is protein Z which plays an important role in coagulation regulation, because protein Z functions as cofactor to enhance the inhibition of factor Xa by a serpine termed protein Z dependant protease inhibitor (PZI) in a process that appears to involve a calcium—ion — dependant tertiary complex containing factor Xa, protein Z, and phospholipid surfaces[4]. Protein Z is one of the anticoagulant proteins which has low molecular weight 62 kilodalton, so low levels of protein Z in the plasma of nephrotic patients are expected to be a risk factor for thromboembolic complications in these patients[5,6]. In contrast to other vitamin K-dependent coagulation factors, protein Z is not a serine protease because a lack of an active center in its amino acid sequence. Its structure is very similar to vitamin K-dependent coagulation factors such as FXII, FIX, FX and protein C[5,7,9]. Most well studied biologic antagonist of coagulation is antithrombin ППР [0], which also has a relatively low molecular weight. Because hereditary antithrombin III deficiency is associated with frequent thrombosis, it was hypothesized that, the low plasma antithrombin III levels were insufficient to inactivate procoagulant factors and were the major cause for the hypercoagulable state and the development of thrombosis in nephrotic syndrome[11]

AIM OF THE WORK

The aim of this study was to evaluate both plasma protein Z levels, and antithrombin III activity as markers of thrombogenic disorders in children with minimal change nephrotic syndrome, and to elicit the relationship between their plasma levels in nephrotic children and different stages of the disease.

SUBJECTS AND METHODS

This study was carried out on 30 children suffering from the nephrotic syndrome selected from the Pediatric Department and Pediatric Nephrology Outpatient Clinic of Minia University Hospital (patient groups I and II), and 15 apparently healthy children taken as a control group, during the period from March 2008 till October 2008. Patients were subdivided into 3 groups:

Group I: Included 15 children "10 males and 5 females" fulfilling the criteria of the acute stage of minimal change nephrotic syndrome, including puffiness of eyelids, lower limb edema, ascites and generalized edema., with proteinuria (> 40 mg/rr^2/hour), hypoalbuminemia (< 3 g/dl), hypercholesterolemia (total cholesterol > 250 mg/dl) and edema[12]. Their ages ranged from 24-144 months, with a mean f SD 76 Ë 35.23.

Group II: Included 15 children "8 males and 7 females" fulfilling the criteria of the emission stage of minimal change nephrotic syndrome with urinary protein excretion (< 4 mg/m^2/hour) for three consecutive days

associated with disappearance of edema[12]. Patients were under treatment with corticosteroids only for 3-4 weeks. Their ages ranged from 24-148 months, with mean SD 76 37.2. We excluded patients with relapsing, steroid resistant or dependant nephrotic syndrome, ,nephritic-nephrotic syndrome, and those clinically diagnosed as having thromboembolic complications of nephrotic syndrome.

Group III: Included 15 apparently healthy children "9 males and 6 females" as a control group. Their ages ranged from 30 — 132 months with mean SD 68.8f 33.7.

All cases and control groups were subjected to careful history taking. For all cases, thorough clinical history concerning nephrotic syndrome, its onset, course, and duration was taken. Clinical examination was performed including general examination particularly, the vital signs "temperature, respiratory rate, heart rate, and arterial blood pressure" were measured. Criteria of generalized edema including eye lid and/or lower limb edema, ascites and the presence of scrotal edema, in males, were recorded. Weight, height and body surface area were measured. Body surface area was estimated by using Bayed Nomogram[13]. All patients and controls were subjected to investigations including; complete urine analysis, 24 hours urinary protein, renal function tests (blood urea and serum creatinine), total serum protein, serum albumin, total serum cholesterol, bleeding time, coagulation time, prothrombin time and partial thromboplastin time, complement-3 by radial immunodiffusion[14], antithrombin III by enzyme immune-assay[15], and protein Z by enzyme immunoassay[16]. Sampling was done under complete aseptic conditions, about 5 ml blood was taken from fasting children for 12 hours and divided into 3.0 ml of blood on a plain plastic tube left to be clotted in the incubator and centrifuged to be separated for estimation of renal function test, total serum protein, serum albumin and total serum cholesterol, and the rest of the serum was separated till the time of assessment of complement 3, and 1.8 ml of blood on 0.20 mg of trisodium citrate were rapidly centrifuged at 5000 rpm for 10 minutes for detection of prothrombin time and partial thromboplastin time and the rest of the plasma is kept in two Eppendorf tubes and kept in the refrigerator in - 20°C till the time of estimation of antithrombin III and protein Z. Another 1 ml of blood was added to EDTA tube for CBC.

Statistical Analysis:

All data were tabulated on Excel Microsoft 11 program using PC computer. SPSS® statistical software was used for statistical analysis. Categorical data were expressed as number and percent (%), while numerical data were expressed as mean and standard deviation (SD). Chi-square test was used to compare categorical data, and t-student test was used to compare numerical data. P-value was calculated and considered to be significant if < 0.05.

RESULTS

As shown in Table 1 there was no significant difference between the studied groups as regarding anthropometric and vital signs, age, sex distribution, weight, height, body surface area, temperature, respiratory rate (RR), heart rate (HR), systolic blood pressure (SBP),

and diastolic blood pressure (DBP).

As shown in Table 2, daily urine output, serum urea, creatinine and complement-3 levels showed no significant difference between the studied groups. There was a statistically significant difference between 24 hour urine albumin content in group I, and group II (p value < 0.0001), group I, and group III (p value < 0.0001), and there was no significant difference between group II and group III. Total serum protein and serum albumin were significantly decreased in group I when compared to group II and group III. These levels were also significantly decreased in group II when compared to group III. Serum cholesterol levels were significantly increased in group I when compared to group II and group III, and were significantly increased also in group II when compared to group III as shown in the same table. The same table shows no significant difference in coagulation parameters measured in the three groups; namely bleeding time, prothrombin time and partial thromboplastin time.

The comparative study of mean plasma. protein Z levels in the 3 studied groups, as shown in Table 3, revealed that the mean levels were statistically significantly lower in group I when compared to group II and III (p = 0.01 and 0.03 respectively), while there was no significant difference in these levels when group II was compared to group III. The same results were obtained as regarding antithrombin III activity (%) in the three studied groups (p = 0.01 and 0.02 respectively).

As shown in Table 4 in group I there was a significant positive correlation of plasma protein Z levels to antithrombin III activity (%) (r-value = 0.83, p-value = 0.0001), total serum protein (r-value = 0.88, p-value = 0.0001), and serum albumin (r-value = 0.90, p-value = 0.0001). Meanwhile there was a significant negative correlation of plasma protein Z to 24 hours protein in urine (r-value = - 0.70, p-value = 0.003). Also, protein Z was negatively correlated to serum cholesterol levels but with no significant difference (r-value = - 0.48, p-value = 0.06). In both group II and group III, plasma protein Z levels were not significantly correlated to total serum protein, serum albumin, 24 hours protein in urine, and serum cholesterol.

In group I, there were significant positive correlations between antithrombin III activity % and both total serum protein and serum albumin in group I patients, while there was a significant negative correlation between antithrombin III activity % and 24 urine protein. Other correlations :were not statistically insignificant as sho '.vn in Table 5.

Table 1: Anthropometric and vital signs data of the three groups.

Variables		Group I	Group II	Group III	p-value		
		(No. = 15)	(No. = 15)	(No. = 15)	I vs II	I vs III	II vs III
Age (years)		6.36 ± 2.93	6.23 ± 3.1	5.37 ± 2.82	1	0.55	0.56
Sex	Male, no (%)	10 (67%)	8 (53%)	9 (60%)	0.45	0.70	0.71
	Female, no (%)	5 (33%)	7 (47%)	6 (40%)			
Weight (Kg)		23.16 ± 8.31	21.6 ± 8.39	18.7 ± 5.87	0.62	0.10	0.27
Height (cm)		110.3 ± 17.7	111.8 ± 17.4	107.6 ± 14.9	0.81	0.66	0.48
Temperature		36.92 ± 0.13	36.8 ± 0.21	36.8 ± 0.19	0.11	0.39	0.48
RR (/min.)		29.93 ± 3.15	29.6 ± 3.43	31.73 ± 3.76	0.78	0.16	0.11
HR (/min.)		92.53 ± 4.70	91.8 ± 5.46	93.8 ± 3.23	0.69	0.39	0.23
Systolic BP (mmHg)		98 ± 8.61	98 ± 8.61	96.6 ± 7.23	1	0.65	0.65
Body surface area (m^2)		0.83 ± 0.21	0.84 ± 0.31	0.72 ± 0.17	0.95	0.13	0.23

Table 2: Comparison of chemical and hematological parameters in the 3 studied groups.

Variables	Group I	Group II	Group III	p-value		
	(No. = 15)	(No. = 15)	(No. = 15)	I vs II	I vs III	II vs III
Amount of urine (ml)	728 ± 233.24	872.6 ± 293.6	772.6 ± 274.6	0.14	0.63	0.34
Albuminuria (g/m^2/ 24 hr)	2.44 ± 0.51	0.09 ± 0.04	0.08 ± 0.2	0.0001*	0.0001*	0.39
Serum creatinine (mg/dl)	0.98 ± 0.15	0.93 ± 0.18	0.92 ± 0.10	0.45	0.20	0.85
Complement 3 (mg/dl)	91.8 ± 7.7	92.5 ± 11.7	100.1 ± 18.1	0.86	0.11	0.18
Total serum protein (g/dl)	4.96 ± 0.53	6.78 ± 0.41	7.12 ± 0.32	0.0001*	0.0001*	0.01*
Serum albumin (g/dl)	1.76 ± 0.15	3.85 ± 0.23	4.01 ± 0.15	0.0001*	0.0001*	0.03*
Serum cholesterol (mg/dl)	342.8 ± 75.68	198.3 ± 8.43	180 ± 8.29	0.0001*	0.0001*	0.0001*
Platelets count	345 ± 62	300 ± 50.31	293.5 ± 60.59	0.082	0.088	0.31
Bleeding time (min.)	4.23 ± 0.75	3.83 ± 0.72	3.86 ± 0 63	0.14	0.16	0.89
Prothrombin time (sec.)	13.86 ± 1.12	13.8 ± 1.08	14.26 ± 0.88	0.87	0.28	0.20
Partial thromboplastin time (sec.)	30 ± 1.96	31.3 ± 3.26	30.6 ± 1.18	0.18	0.32	0.42

*: significant difference.

Table 3: Comparison of the mean levels of both protein Z and antithrombin III activity (%) in the studied groups.

Variable	Group I	Group II	Group III	p-value		
	(No. = 15)	(No. = 15)	(No. = 15)	I vs II	I vs III	II vs III
Protein Z (ng/ml)	37.73 ± 34.68	66.6 ± 22.4	62.53 ± 25.78	0.01*	0.03*	0.64
Antithrombin III activity (%)	79.8 ± 31.4	105.8 ± 19.1	100.8 ± 14.2	0.01*	0.02*	0.42

*: significant difference.

Table 4: Correlation of protein Z levels to antithrombin III activity (%), total serum protein, serum albumin, proteinuria, and serum cholesterol levels in the studied groups.

Correlation of Protein-Z to:	Group-I (No. = 15)		Group-II (No. = 15)		Group-III (No. = 15)	
	r-value	p-value	r-value	p-value	r-value	p-value
Anti-thrombin III	0.83	0.0001*	0.48	0.06	0.23	0.39
Total serum protein (g/dl)	0.881	0.0001*	0.44	0.09	0.36	0.17
Serum albumin (g/dl)	0.928	0.0001*	0.38	0.15	-0.04	0.88
Proteinuria (g/m^2/24 hr)	-0.707	0.003*	-0.09	0.74	-0.01	0.82
Serum cholesterol (mg/dl)	-0.48	0.06	-0.09	0.72	0.32	0.23

*: significant difference.

Table 5: Correlation between antithrombin III activity % and total serum protein, serum albumin, proteinuria and serum cholesterol.

Correlation of antithrombin-III to:	Group-I (No. = 15)		Group-II (No. = 15)		Group-III (No. = 15)	
	r-value	p-value	r-value	p-value	r-value	p-value
Total serum protein (g/dl)	0.852	0.0001*	0.42	0.091	0.37	0.18
Serum albumin (g/dl)	0.931	0.0001*	0.37	0.14	-0.035	0.86
Proteinuria (g/m^2/24 hr)	-0.714	0.003*	-0.092	0.74	-0.01	0.83
Serum cholesterol (mg/dl)	-0.462	0.06	-0.094	0.73	0.34	0.25

*: significant difference.

DISCUSSION

The nephrotic syndrome is associated with an increased tendency for thrombo-embolism in both children and adults[17]. The reported incidence of thromboembolic complications is about 1.5°/r6.6% among children[18]. Thrombosis in nephrotic syndrome may arise from the loss of proteins involved in the inhibition of systemic hemostasis (low antithrombin III and protein Z levels), the increased synthesis of factors promoting thrombosis (high factors I, V, VIII and vonWillebrand factor), or by the local activation of the glomerular hemostasis systems "intraglomerular fibrin deposition"[3]. Low protein-Z plasma levels have been reported to be associated with a prothrombotic state. Several reports

suggested that low protein-Z plasma levels increase the frequency of ischemic stroke[19-23].

In the present study, there was no significant difference between the studied groups as regards comparison of bleeding time, prothrombin time (PT) partial thromboplastin time (PTT) and complement-3. We studied coagulation parameters to prove that our patients in the acute stage were clinically and laboratory free of any thromboembolic manifestations. Similarly, Al-Mugeiren et al.[24] showed that prothrombin time, and partial thromboplastin time levels during the attack periods in patients with nephrotic syndrome were normal. Also, in the study by Ozkaya et al., the mean prothrombin and thrombin times were within normal range in the study children[23].

In the present study, the mean level of serum protein-Z of children with acute stage was lower than that of children in remission and control group, and this difference was statistically significant ($p = 0.01$, 0.02 respectively). Similar to our findings, Ozkaya et al.[23] found a significant decrease in plasma protein-Z levels in proteinuric patients when compared with patients in remission or healthy controls[23]. Also, Malyszko et al.[25] demonstrated, low protein Z levels in 22 patients with nephrotic syndrome in the acute stage than its levels in the remission stage.

Along with the other coagulation abnormalities, decreased levels of protein Z in nephrotic syndrome patients with active disease may contribute to thromboembolism in these patients, where protein-Z decreases in the plasma of nephrotic patients in the acute stage due to its loss in their urine, while in the remission stage of nephrotic patient with normalization of total serum protein and albumin, protein-Z becomes within normal values[23,25].

In the present study, the mean level of antithrombin III activity (%) of children with acute stage was lower than that of children in remission and control group, and this difference was statistically significant when acute stage was compared to remission and control.

Similarly, Ozkaya et al. observed decreased levels of antithrombin III during the attack period. Five of their patients had antithrombin III levels below 75%, which considered previously as a predictor of increased risk of thromboembolic complications with nephrotic syndrome[23]. Also, Obach et al.[21] found that antithrombin III level was significantly lower ($p < 0.001$) in patients with active disease as compared to controls. The levels became normal with remission of the disease.

In the study by Citak et al.[2], antithrombin III levels in nephrotic patients were significantly lower than in the control group and increased after corticosteroid therapy. In steroid-responsive patients in remission, antithrombin III levels were not significantly different from those of the control group. In steroid-resistant patients, antithrombin III levels also increased but were still lower than the control group[2]. Also, in the same study there was a significant correlation between antithrombin III levels and serum albumin levels, but there was no significant correlation with proteinuria.

In the study by Wygledowska et al.[26],

at the onset of the disease, activity of antithrombin **III was** found to be significantly decreased as compared with the control group. Activity of antithrombin III lowered at the onset of the disease, was significantly higher during both the improvement and the remission stage. Those authors concluded that sufficient rise of antithrombin III at the early stage of treatment may limit the risk of thromboembolic complications in children with nephrotic syndrome.

In the present study, protein Z had a significant positive correlation to total serum protein, serum albumin, and antithrombin III activity (%), and it had a significant negative correlation to proteinuria, in patients with acute stage of nephrotic syndrome. However, in remission and control groups, protein Z was not significantly correlated to total serum protein, serum albumin, serum cholesterol, and antithrombin III activity (%).

Similar findings were obtained by Ozkaya et al.[23] who found that, protein Z levels was positively correlated with serum total protein and albumin levels ($p = 0.003$, $p = 0.003$, respectively) and negatively with the degree of proteinuria ($p = 0.0001$). Protein Z levels were positively correlated with antithrombin III ($r = 0.037$, $p = 0.04$).

The negative correlation between proteinuria and protein Z levels and the positive correlation with serum albumin levels and protein Z levels suggest the possibility of renal protein Z loss. This relationship might be explained by the molecular weight and charge of protein Z, which are quite similar to those of albumin[231]. The return in levels of both protein Z and antithrombin III in remission group to normal levels may be attributed to the healing effect of corticosteroid on glomerular structure[27].

Conclusion

From this study it was concluded that, though there were no clinically detectable thromboembolic complications in the studied children with nephrotic syndrome, the plasma levels of protein Z and antithrombin III were significantly decreased in children in the acute stage of the nephrotic syndrome, while in those patients in the remission stage, plasma levels of protein Z and antithrombin **III** returned to normal values. Thus children in the acute stage of nephrotic syndrome are still susceptible to thromboembolic complications and need close observation. The mechanism by which both protein Z and antithrombin III activity were reduced in the acute stage nephritic syndrome children is still not clear. So, we recommend further studies for the detection of both parameters in the urine of those patients to clarify this mechanism.

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