PLASMA FIBRINOGEN, ANTITHROMBIN-III, α_2 - MACROGLOBULIN AND α_2 - ANTIPLASMIN LEVELS OF CHILDREN WITH NEPHROTIC SYNDROME

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SUMMARY : We studied the plasma fibrinogen, antithrombin III (AT III), α_2-macroglobulin (α_2-MG) and α_2-antiplasmin (α_2-AP) levels of 13 children with nephrotic syndrome (NS) in relapse and remission and compared them with healthy controls. In patients with relaps, the mean plasma levels of fibrinogen and α_2-MG were significantly increased when compared with patients in remission ($p<0.01$ and $p<0.01$) and control group ($p<0.01$ and $p<0.05$). The mean plasma level of AT III was significantly increased in remission ($p<0.01$) and the mean plasma level of α_2-AP was significantly decreased in relapse ($p<0.01$). A positive correlation of the serum albumin concentration with AT III and α_2-AP levels ($r=+0.53$, $p<0.01$ and $r=+0.49$, $p<0.05$) and a negative correlation with fibrinogen levels ($r=-0.40$, $p<0.05$) was found. A positive correlation of the serum cholesterol concentration with plasma fibrinogen levels ($r=+0.50$, $p<0.05$) and a negative correlation with AT III and α_2-AP ($r=-0.43$, $p<0.05$ and $r=-0.66$, $p<0.05$) was found. A positive correlation was found between proteinuria and plasma fibrinogen levels ($r=0.63$, $p<0.01$) and a negative correlation between proteinuria and AT III levels ($r=-0.63$, $p<0.01$).

It is considered that the increase of plasma fibrinogen and α_2-MG levels may contribute to the thrombotic diathesis in childhood nephrotic syndrome.

***Key Words :** Nephrotic Syndrome, Antithrombin III, α_2-Antiplasmin, α_2-Macroglobulin.*

INTRODUCTION

The increase in thrombotic diathesis in patients with nephrotic syndrome first came under notice in 1948, when Addis reported the deep vein thrombosis of a patient (27). Later on thromboembolic complications in arterial and venous systems were reported both in adults (19-70 %) and in children (1.8-5 %), though with a lower level of prevalence in the latter (7, 11, 13, 18, 20). Although it is well known that this complication develops along with hypercoagulability; there is no consensus in the literature about the responsible factors and the prophylaxis (19).

The several different factors involved in the process of coagulation are classified into these five systems : a) Zymogens (F II, V, VII, IX, X, XI, and XII) and cofactors (FV and VIII), b) Fibrinogen, c) Fibrinolytic system, d) Coagulation inhibitor, e)

Platelets (19). Various studies have reported different findings about these five systems in nephrotic patients. This study compares the fibrinogen and the plasma levels of AT-III, α_2-MG and α_2-AP in patients with childhood NS, with those of a control group and investigates their roles in hypercoagulability.

MATERIALS AND METHOD

Thiteen children (seven girls, six boys) between the ages of 2-13 years (mean 6.6 ± 3.4 years) were included in this study. The diagnosis was determined by kidney biopsy in eight children and by clinical and laboratory findings in the others. Six of these children had minimal change disease (MCD), four mesengioproliferative glomerulonephritis (Mes PGN) two mesengiocapillary glomerulonephritis (MCGN) and one rapidly progressive glomerulonephritis (RPGN). All of them, except for the patient with RPGN, displayed normal renal functions. The plasma fibrinogen, AT-III, α_2-MG and α_2-AP values-during relapse and remission (when proteinuria was negative)-were measured along with serum creatinin, BUN, total protein, albumin, total lipid, cholesterol and quantitative proteinuria (Table 1). During the relapse period in which these measurements were made, the patients were not receiving any medications that might influence haemostasis such as corticosteroids, immunsupressives, antiaggregant drugs or any others. But during the remission period in which these measurement were made, the patients were receiving steroids. The relapse and remission values were compared with those of a control group of ten children (six boys, four girls) of similar ages (5-14 years) who didn't have NS or any infections.

Free flowing venous blood samples were obtained by venipuncture. Chilled plastic syringes and tubes containing 3.8 % sodium citrate solution (blood/citrate : 9/1 volume) were immediately stored at -80°C until assayed.

AT-III, α_2-MG and α_2-AP were measured by means of radial immunodiffusion (Human anti AT-III antiserum binding site, human anti α_2-MG antiserum binding site and human anti α_2-AP antiserum binding site). Maximum diffusion was attained by the application of standard and samples onto separate plates containing certain levels of antibodi-

Patient	AGE	SEX	DIAG.	S CREATININ		PROTEINURIA		S.ALBUMIN		CHOLESTEROL	
				REL mg/dl	REM	REL mg/m^2/st	REM	REL g/dl	REM	REL mg/dl	REM
1	2	M	MCD	1.1	0.9	45	(-)	4.3	4.6	201	219
2	11	M	Mez.	1.1	0.8	148	(-)	1.6	3.2	488	211
3	5	M	Mez.	0.9	1.0	67	(-)	2.8	4.2	353	231
4	13	F	RPGN	9.6	1.2	98	37	1.9	3.2	520	302
5	12	M	MCGN	0.9	0.9	147	27	1.7	3.9	702	322
6	3	M	MCD	0.8	0.6	80	(-)	3.7	4.6	634	236
7	6	M	MCD	0.8	0.9	246	(-)	2.0	3.5	1193	116
8	9	F	MCD	0.5	0.5	90	(-)	2.1	4.5	542	125
9	5	M	Mez.	0.7	1.0	34	(-)	2.5	4.4	689	173
10	8	F	Mez	0.5	0.7	96	(-)	1.2	4.0	489	128
11	6	F	MCGN	1.0	0.6	195	(-)	1.7	3.6	459	180
12	3	F	MCD	0.8	0.9	120	5	1.2	3.9	584	169
13	4	F	MCD	0.8	0.9	67	2.9	1.2	4.2	479	205
MEAN				1.5	0.8	110.2	5.5	2.1	3.9	564.07	201
± SD				2.4	0.2	60.5	12	0.9	0.5	231.65	63.2

ABBREVIATIONS : REL : Relapse MCD : Minimal change disease
REM : Remission Mez : Mezengioproliferative glomerulonefritis
MCGN : Mezengiocapiller glomerulonefritis

Table 1 : Laboratory values of the patients with nephrotic syndrome in relapse and remission.

es. The diameters of the precipitation circles were measured and the plasma levels of AT-III, α_2-MG and α_2-AP were calculated in mg/lt. Student's t test was used in the statistical analysis.

RESULTS

The values of plasma fibrinogen, AT-III, α_2-MG and α_2-AP in the nephrotic syndrome (relapse and remission) and the control groups can be seen in Table 2.

	FIBRINOGEN	AT-III	α_2-MG	α_2-AP
RELAPSE	741.7 ± 239.3	294.2 ± 172.1[1]	13086.1 ± 5213.6	51.25 ± 33.46
REMİSSION	365.2 ± 202.7*	493.6 ± 141.0	7260.7 ± 870.6*	85.46 ± 18.76*
CONTROLS	369.5 ± 71.9*	384.0 ± 62.7[2]	3170.0 ± 512.0*	73.70 ± 18.50[3]

* p<0.01 vs relapse
1 p<0.01 vs remission
2 p< 0.5 vs remission
3 p<0.05 vs relapse

Table 2 : Plasma fibrinogen (mg/dl), AT-III (mg/lt), α_2-MG (mg/lt) and α_2-AP levels of the patients and the control group.

Plasma fibrinogen levels were found to be significantly higher in the relapse group than in either the remission or the control group (p<0.01 in both). There was no difference between the remission and the control groups in the levels of plasma fibrinogen.

The AT-III levels were lower in the relapse group than in the control group; however the difference was insignificant. On the other hand the increase in the remission values compared to both the relapse and control groups was significant (p<0.01 and p<0.05 respectively).

The α_2-MG levels were significantly higher during relapse when compared with remission and control groups (p<0.01 and p<0.01 respectively). The values of the remission group were still higher than of the control group (p<0.01).

The α_2-AP levels were found to be lower in the relapse group in comparison to both the remission and control groups (p<0.01 and p<0.05 respectively). There was no difference between the remission and control groups.

When serum albumin, cholesterol level and quantitative proteinuria were compared with the plasma fibrinogen, AT-III, α_2-MG and α_2-AP levels; according to the linear regression model; the following observations were found:

1) The correlation between the low levels of serum albumin and the increase in plasma fibrinogen levels along with the decrease in AT-III and α_2-AP was found to be significant (respectively r=-0.4, p<0.05, r=+0.53, p<0.01 and r=+0.49, p<0.05). The correlation with α_2-MG was insignificant.

2) The correlation between the increase in serum cholesterol and the increase in plasma fibrinogen along with the decrease in AT-III and α_2-MG was found to be significant (respectively r=+0.5, p<0.05, r=-0.43, p<0.05 and r=-0.66, p<0.01). The correlation with α_2-MG was found to be insignificant.

3) The correlation between the increase in 24 hour urine protein and the increase in plasma fibrinogen along with the decrease in AT-III was significant (r=+0.63, p<0.01 and r=-0.63, p<0.01). The correlation between α_2-AP and α_2-MG with proteinuria was found to be insignificant (Table 3).

When the values from the five patients with MCD are compared with the values from the other eight patients, the AT-III and α_2-AP averages of MCD patients in both relapse and remission were found to be lower but does not have statistical significance (Table 4).

DISCUSSION

Thromboembolism is one of the most serious complications of nephrotic syndrome. Its incidence has been reported as clinical episodes in 19-70 % of adults and 1.8-4 % of children. Hypothesizing that subclinical episodes may be of higher incidence, some investigators determined by radionucleid studies that pulmonary embolisms are found in 28 % of

	FIBRINOGEN	AT-III	α_2-MG	α_2-AP
ALBUMIN	r=-0.4	r= + 0.53		r= +0.49
	p<0.05	p<0.01	NS*	p<0.05
CHOLESTEROL	r=+0.5	r= -0.43		r= -0.66
	p<0.05	p<0.05	NS	p<0.05
PROTEINURIA	r= +0.63	r= -0.63		
	p<0.01	p<0.01	NS	NS

* : Not significant

Table 3 : Correlation coefficients of plasma fibrinogen, AT-III, α_2-MG and α_2-AP levels with serum albumin, cholesterol and proteinüria in relapse.

		MINIMAL CHANGE DISEASE	OTHERS
FIBRINOGEN	RELAPSE	810.5 ± 206.7*	682.8 ± 264.8
	REMISSION	398.5 ± 207.6*	336.7 ± 210.4
AT-III	RELAPSE	241.1 ± 200.8*	339.8 ± 142.8
	REMISSION	443.3 ± 123.2*	536.7 ± 149.7
α_2-MG	RELAPSE	15110 ± 4389.1*	11351 ± 5541.9
	REMISSION	7593.3 ± 865.9*	6975.7 ± 828.2
α_2-AP	RELAPSE	39.5 ± 43.18*	54.7 ± 26.5
	REMISSION	78.0 ± 10.7*	91.8 ± 22.4

* p>0.05 vs OTHERS

Table 4 : Plasma fibrinogen, AT-III, α_2-Macroglobulin and α_2-Antiplasmin levels of patients with minimal change disease and the other (MezPGN, MCGN, RPGN).

children with NS; and have emphasized that this complication is as frequent in childhood NS as in adulthood NS (13).

It is important, for prophylactic purposes, that the causes of thrombotic diathesis are understood. Numerous studies on this subject have been conducted since 1970 and several factors have been considered to be responsible. Changes in the systems involved in coagulation have been considered to be the contributing factors. These changes include changes in zymogens and cofactors (increases in F V, VII, VIII, and X decreases in F II, IX, XI, XII), increases in fibrinogen, changes in fibrinolytic system (decreases in plasminogen and increases in α_2-MG and α_2-AP), changes in the plasma inhibitors (decrease in AT-III and increase in α_2-MG) and defects in trombocytes (3). Apart from these systems it has been suggested that increased plasma lipoproteins may be atherogenic (21) and disturb the trombocyte function (2); or even that increases in erythrocyte aggregation and plasma viscosity may facilitate the process (5). However, it was supposed that protein C and protein S levels were low in patients with nephrotic syndrome (19). Vaziri et al (29) found high protein C and protein S levels in these patients.

Fibrinogen is a protein which weighs 330.000 Dalton moles and is mostly synthesized in liver. It is generally agreed that there is significant increase in the plasma fibrinogen levels of nephrotic patients (1, 15, 17, 27). This increase is related to increased hepatic synthesis in reaction to protein loss through the urine (25). This study has also found the plasma fibrinogen levels to be higher in the relapse group than those in the remission or control groups

($p<0.01$). The fact that there is a significant correlation between increase in plasma fibrinogen and serum albumin; cholesterol and proteinuria, supports the hypothesis that this increase is related to protein loss through the urine.

AT-III is a protein which weighs 64.000 Dalton moles. It inactivates thrombins along with α_2-MG, α_1-antitrypsin and C_1 inhibitor and is generally accepted to play the most important role among these coagulation inhibitors (19). AT-III also inactivates activated F XII, IX, XI and plasmin (22, 30). Several investigators have found plasma AT-III levels to be low in nephrotic patients (4, 16, 26) whereas the others have reported normal levels (14, 21, 23, 29). Jorgenson and Stofferson have found both high and low levels (14). This study has found plasma AT-III levels to be insignificantly lower in the relapse group than in the control group, but significantly increased in remission. This increase may be related to the use of steroids (12). Even though the decrease in plasma AT-III levels in relapse is insignificant; the fact that this decrease is correlated with lower levels of serum albumin, higher levels of cholesterol and amount of proteinuria suggests that the level of plasma AT-III in these patients is related to the balance between hepatic synthesis and its loss through the urine. There are reports that show AT-III loss through the urine (28).

Levels of plasma α_2-MG have been found to be high in nephrotic patients (4, 6, 24, 26, 27). α_2-MG is a protein which weighs 820.000 Dalton moles. Its increase is a result of increased synthesis and the fact that it cannot be eliminated through the urine due to its mole weight. It has been suggested that plasma α_2-MG levels increase in compensation for the loss of AT-III through urine. According to Cameron, the AT-III / α_2-MG ratio may be used as a determinant of thrombotic diathesis (7). In keeping with the literature, this study has found the plasma α_2-MG levels of nephrotic children to be significantly higher in the relapse group than in either the remission or the control group and to be still higher than the control group after treatment. α_2-MG may act as an inhibitor of coagulation along with AT-III as well as a facilitator of coagulation by increasing antiplasmin activity in the fibrinolytic system along with α_2-AP and α_1-antitrypsin.

α_2-AP is a protein which weighs 67.000 Dalton moles. It acts as a facilitator of coagulation by its role, primary plasmin inhibitor in the fibrinolytic system. Its deficiency may cause haemorrhagic episodes and its increase hypercoagulopathy. Several investigators have found plasma α_2-AP levels to be high in nephrotic patients and have suggested that it may be responsible for thrombotic phenomenon in these patients; and that is an important determinant of predisposition (1, 3, 19). On the other hand, Hoyer et al (13) have found the α_2-AP levels of patients in relapse below those of remission and control groups, and therefore it has been concluded that α_2-AP does not play a role in the coagulopathy seen in nephrotic patients.

In conclusion, the results of this study support the general consensus in the literature that plasma fibrinogen and α_2-MG increases in nephrotic patients. On the other hand we have not observed the deficiency of plasma AT-III and the increase in α_2-AP levels that some investigators have suggested as responsible factors for thrombotic diathesis. It was concluded that fibrinogen accelerates the formation of active fibrin and that the increase in α_2-MG levels may cause hypercoagulopathy in these patients. It should be taken into consideration that other factors that have not been studied here may also contribute to the thrombotic diathesis.

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