HEMOSTATIC PROFILE IN NEPHROTIC SYNDROME

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Objective: To evaluate the coagulation profile and its relation to steroid therapy, and the frequency of thromboembolic complications and its correlation with coagulation parameters in nephrotic syndrome (NS). Setting: Hospital based. Subjects and Methods: Forty children xvith NS were subdivided into four groups, namely, fresh cases, steroid dependent, remission after therapy and steroid resistant. An equal number of age and sex matched children served as controls. In all the study and control subjects, detailed clinical examination, liver function tests, renal function tests and detailed coagulation profile were done. Evaluation of renal veins and inferior vena cava for the presence of thrombosis was also done by abdominal ultrasonography. Results: Thrombocytosis was detected in 57.5% and the degree of thrombocytosis was directly related to the amount of proteinuria. The mean prothrombin and thrombin times were within normal range in the study children. The activated partial thromboplastine time (APTT) was prolonged in six cases (15%) and three out of these six children had thromboembolic complications. Antithrombin-III level was significantly lower (p <0.001) whereas protein C and S were significantly elevated (p <0.001) as compared to controls. The levels became normal with remission of the disease. Steroid therapy significantly increased the levels of proteins C, protein S, AT-III and fibrinogen as compared to controls. Thromboembolic complications were seen in 3. . . cases (7.6%) and were associated with very low levels of AT-111 and protein C and all three had ,; . serum albumin below 2 g/dl. Conclusions: The importance of coagulation profile in nephrotic syndrome is highlighted and a high index of suspicion for thromboembolic complications is warranted in patients xvith thrombocytosis, hyper fibrinogenemia, prolonged APTT and in children xvith low levels of AT-III, protein C and protein S.

Key words: *Nephrotic syndrome, Coagulation profile, Steroid therapy.*

NEPHROTIC syndrome (NS) has been considered a hypercoagulable state which may be complicated by thrombotic episodes of the venous or arterial circulation^). Thromboemboli.c episodes have involved pulmonary vessels, inferior vena cava, renal vein, mesenteric artery with small bowel necrosis, femoral artery, retinal and coronary artery. This hypercoagulable state probably has a multifactorial etiology. It is influenced by an increase in platelet aggregability and the loss of low molecular weight regulator proteins in the urine and may be made worse by an increase in viscosity resulting from the use of diuretics.

A variety of coagulation abnormalities have been described in NS including thrombocytosis. (60% cases) and low levels of antithrombin(2,3). Beta-thromboglobulin is a specific protien released by platelets on aggregation. It is elevated signifi-

cantly in patients with NS during relapse and returns to normal with remission(4). However, the role of other coagulation inhibitors such as proteins C or S is still unknown in NS.

The frequency of renal vein thrombosis is 5 to 62% with an overall figure up to 35%(3). The frequency of thromboembolic complications (TEC), other than renal vein thrombosis is 20%. Pulmonary embolism is the most frequent and serious complications, accounting for 8% of the known renal vein TEC(l). However, little data is available regarding coagulation defects in NS, its relationship to thromboembolic episodes and response to steroid therapy in children. If this correlation can be identified earlier, a rapid therapeutic intervention may be feasible.

Subjects **and Methods**

A total of 40 children (31 boys and 9 girls) with NS were investigated. Their ages ranged from 1 to 12 years. The diagnosis of nephrotic syndrome was made if the following criteria were fulfilled: *(a)* Serum albumin <2.5 g/dl; and *(b)* Heavy proteinuria >40 mg/h/m^2. The study children were divided into four groups as follows: *(i)* Fresh cases; *(ii)* Steroid dependent; *(Hi)* Remission on steroid therapy (protein free urine for three consecutive days); and *(iv)* Steroid resistant. Forty age and sex matched children served as controls. They were grouped as follows: *(i)* 20 healthy children; *(ii)* 10 non-nephrotic children on steroids; and *(Hi)* 10 cases with renal defects other than NS who were not on steroids.

All patients having abnormal liver function and bleeding disorder were excluded from the study. The nature and purpose of the study was explained to parents of all the subjects and informed consent was taken prior to inclusion. In all the study and control children, detailed clinical examination, liver function tests, renal function tests (blood urea, serum creatinine, urine microscopic and protein examinations, urine culture, 24 hour urinary protein excretion) and coagulation profile tests were done. Evaluation of renal veins and inferior vena cava for the presence of thrombosis was done by abdominal ultrasonography.

The coagulation profile tests performed included prothrombin time, activated partial thromboplastin time (APTT), thrombin time, fibrinogen level (heat precipitation method), fibrin degradation products, antithrombin (AT-III, modified micromethod) protein C (ELISA method) and protein S (ELISA method). Student's Y test was used to analyze the significance of differences.

Results

The mean age of NS subjects was 5.8 ± 3.1 year. Hypertension was seen in 37.5% of cases. Three children (7.5%) had thromboembolic complications. Two cases had deep vein thrombosis and one child had cerebral stroke during the course of the disease.

The mean hematocrit values were similar in children with NS and the controls. The mean platelet count was significantly higher in NS when compared to the controls ($p < 0.001$). Thrombocytosis was documented in 23 subjects (57.5%) and was present in all the cases of steroid resistant group and children with thromboembolic phenomenon. The mean prothrombin and thrombin times showed no significant differences between the study and control groups *(Table I).* The activated partial thromboplastin time was prolonged in 6 cases (1.5%). Three out of the six had thromboembolic complications. The mean fibrinogen level was significantly elevated in steroid dependent cases when compared to controls ($p < 0.001$). Fibrin degradation

products were not elevated in any of the study subjects.

Table II presents the relationship between serum albumin, proteinuria and platelet count. The mean level of serum albumin was lowest in fresh cases and steroid resistant children which returned to normal on remission. The level of serum albumin was below 2 g/dl in all the three children who had thromboembolic complications. Proteinuria was maximum in steroid resistant cases. The degree of proteinuria was directly related to platelet count. The mean level of antithrombin III was significantly low in fresh cases of NS ($p < 0.001$) and the level returned to within or above the control range, once remission had been achieved. Sferoid reistant and dependent cases had significantly higher levels as compared to controls *(Table III).* Proteins C and S levels were significantly elevated in fresh cases and steroid resistant and dependent subjects ($p < 0.001$) and returned to normal with onset of remission.

The coagulation profile in fresh cases of NS, when compared with controls on

TABLE I–*Coagulation Profile in Study and Control Children (Mean ± SD)*

Group	Platelet count (thousands mm^3)	Prothrombin time (sec)	Thrombin time (sec)	Activated partial thromboplastin time (sec)	Fibrinogen level (mg/dl)
Fresh cases	341.30* ±53.82	12.07 ±1.27	7.30 ±0.82	30.58 ±1.85	235.70 ±134.72
Steroid dependent	447.60* ±187.46	13.23 ±1.34	7.25 ±0.46	35.10 ±5.63	507.60 ±189.37*
Remission	439.40* ±95.60	12.70 ±2.00	7.68 ±0.92	35.70+ ±4.90	257.40+ ±91.04
Resistant	522.40* ±61.86	13.15 ±0.86	7.11 ±0.69	35.74++ ±3.93	200.00 ±85.80
Control	117.50 ±37.40	11.13 ±0.89	7.00 ±0.69	32.20 ±2.17	178.50 ±45.70

In comparison to controls: *$p < 0.001$; + $p < 0.01$; ++ $p < 0.05$.

TABLE II–*Relationship between Proteinuria, Serum Albumin and Platelet Count (Mean ± SD)*

Groups	Serum albumin (g/dl)	Platelet count (thousands/mm^3)	Proteinuria (g/l)
Fresh cases	2.15 ± 0.29	341.3 ± 53.82	2.58 ± 1.01
Steroid dependent	1.93 ± 0.33	447.6 ± 187.46	3.73 ± 0.89
Remission	2.15 ± 0.26	439.4 ± 95.65	2.15 ± 0.67
Steroid resistant	1.81 ± 0.43	522.4 ± 61.86	6.50 ± 3.84
Control (n=20)	3.24 ± 0.29*	177.5 ± 37.40*	–

$p < 0.001$ for all four groups versus controls.

steroids, did not show any significant differences in PT, TT, APTT, or fibrinogen levels. Anti-thrombin-III levels were significantly lower in fresh cases of NS as compared to controls on steroids (71 ± 8.43% and 117 ± 16.47%; p <0.001). Protein C was within normal range in both the groups. Protein S and platelet count were significantly higher in NS children.

The coagulation profile of 3 children with thromboembolic complications is depicted in *Table IV*. Serum albumin was <2 g/dl with very high fibrinogen levels and low values of proteins C and S.

Kidney biopsy was done in 12 out of 40 children. Minimal change and membranous glomerulopathy were seen in four cases each and focal glomerulosclerosis and membrano proliferative glomerulonephritis were diagnosed in two subjects each. Of the three cases with thromboembolic complications, one had minimal change glomerulonephritis (steroid dependent), another had membrano proliferative type I glomerulonephritis (child with cerebral stroke in remission) and the third case had focal glomerulosclerosis (steroid resistant).

Discussion

High urinary loss of protein in NS is associated with increased synthesis of several proteins including coagulant factors as a compensatory response. The hypercoagulable state may result as a consequences of these changes. Various studies have shown an increase in levels of fibrinogen, platelets, factor V, VII, VIII and X and increased platelet aggregation in NS(1,2). Impairment of the fibrinolytic system due to loss of plasminogen in the urine is postulated to contribute to the thrombotic tendency in these patients(5). The other contributing factor may be the common use of diuretics, such as thiazides or furosemide. These agents with their volume depleting effects lead to hemoconcentration which favors the development of thrombotic complications^).

Various abnormalities in platelet functions, as well as in the release of different products (ADP, thrombin, collagen, arachidonic acid, immune complex) by the platelets have been linked to increased thromboembolic phenomenon(5). The present study showed an increase in platelet counts, hypercholesteremia, hyperfibrinogenemia associated with hypoalbuminemia as compared to corresponding levels in normal controls. Thrombocytosis was present in 57.5% and was seen in all cases with steroid resistance. Similar observations have been made earlier(6). An increase in ADP and collagen induced plate-

TABLE III–*Correlation of Vitamin K Dependant Anticoagulant Levels in Study and Control Children (Mean ± SD)*

Groups	AT-III (%)	Protein C (%)	Protein S (%)
Fresh cases	71.0 ± 8.43	144.3 ± 13.47	169.7 ± 24.26
Steroid dependent	130.7 ± 18.97	149.3 ± 32.56	163.7 ± 30.19
Remission	120.0 ± 21.7	116.3 ± 32.55*	85.50 ± 14.42
Steroid resistant	131.5 ± 13.55	142.20 ± 34.26	158.6 ± 40.73
Control	96.05 ± 11.79	88.6 ± 9.42	106.9 ± 18.13

TABLE IV—*Coagulation Profile in Children with Thromboembolic Complications.*

Coagulation tests	Patients			Controls (Mean ± SD)
	A	B	C	
Prothrombin time (sec)	14	12	14	11.13 ± 0.89
Activated partial thromboplastin time (sec)	47	48	40	32.2 ± 2.17
Thrombin time (sec)	7	9	8	7 ± 0.70
Fibrinogen level (mg/dl)	600	350	460	178.5 ± 5.74
FDP	Nil	Nil	Nil	Nil
Anti-thrombin-III (%)	80	60	140	96 ± 11.79
Protein-C(%)	70	25	60	88.6 ± 9.42
Protein-S(%)	92	70	60	106.9 ± 18.13
Platelet counts (thousand/cu mm)	347	460	550	177.5 ± 37.40
Serum albumin (g/dl)	1.5	1.7	1.1	3.24 ± 0.29
Proteinuria (g/L)	4.5	3.5	7.0	–
Histology	MCNS	MPGN (type I)	FSGS	–
Thrombo-embolic complications	DVT	Cerebral stroke	DVT	

A - steroid dependent, B - Remission, C - Steroid resistant, DVT - Deep vein thrombosis, FSGS - Focal glomerulosclerosis, MCNS - Minimal change nephrotic syndrome, MPGN - Membrano proleferative glomerulonephritis.

let aggregation has been observed *in vitro* in 11 of 14 children with NS(7) and the hyperaggregability could be reversed if the urinary protein was added suggesting that urinary loss of some factor inhibited platelet aggregation. Hyperlipidemia is known to affect platelet function and may increase platelet aggregation in the nephrotic patients^). The increased level of cholesterol together with hypertriglyceridemia in the present study might be a factor responsible for hypercoagulability. It has been shown that the release of arachidonic acid from platelets is metabolized into platelet aggregating substances such as endoperoxides and thromboxane A2 and this conversion is regulated by albumin(9,10). In a prelimi-. nary report on renal thromboxane A2 synthesis, it has been observed that urinary thromboxane B2 (marker of the renal production of T x A2) excretion was significantly higher in children with MCNS than in the healthy controls and reached its maximum at the time of peak proteinuria(ll).

The present study demonstrated that the mean thrombin and prothrombin times were comparable in the study group and the controls. We observed significant prolongation of APTT in steroid resistant cases and in children who were in remission, when compared to controls. In an earlier series of 39 children, similar observations were documented. PTT showed significant prolongation only in patients having relapse without treatment(12). Prolonged APTT could be due to increased antithrombin-III activity, increased fibrin-

olytic state or loss of coagulant factors(2,6,13). APTT was prolonged in 6 of our patients (15%) and 3 of these had thromboernboiic complications. Serum fibrinogen levels were markedly elevated in steroid dependent cases when compared to controls. Even in remission, these levels were above the controls. Serum fibrinogen levels are elevated in response to hypoalbuminemia and proteinuria and are known to cause sludging and thrombosis(6,14,15). We also observed inverse relationship between serum fibrinogen and albumin levels in all the study cases except the resistant group. The serum fibrinogen levels were very high in all the three children with thromboembolic complications.

The mean AT-III level was low in fresh cases of nephrotic syndrome and levels returned to normal values with remission. The levels were significantly increased in the steroid dependent and resistant children. Similar observations have been made earlier(12). The key role in the genesis of thrombosis in nephrotic syndrome has been ascribed to low AT-III levels. Low AT-III levels, however, had no predictive value for the occurence of thrombotic episodes, perhaps, because of concomitant elevation of vitamin K dependent protein C, which is known to antagonize prothrombinase activation and to stimulate fibrinolysis. Two of three children with thrombotic episodes had significant low levels of AT-III. The reduction in the level of plasma AT-III is not affected by the underlying renal pathology, as observed in the present study.

The mean levels of proteins C and S in the present study were significantly elevated in all active stages of nephrosis as compared to controls and the levels of these proteins returned to normal with remission. However, the levels did not correlate with either serum albumin or the degree of proteinuria. An earlier series of 23 patients(16) documented reduced functional levels of protein S (69% ± 27%), despite having elevated levels of total protein S antigen (139% ± 42%). Decreased protein S and elevation of C-binding protein levels (170 ± 52%) favors complex formation and this might be a risk factor for the development of thromboembolic complications(16). Similar changes in proteins C and S have been documented. The mean levels of three protein (C, S, AT-III) in that study(17) did not differ significantly between nephrotic and non-nephrotic adult patients. However, in contrast to this, we observed significantly lower levels of AT-III ($p < 0.001$) and significantly higher levels of proteins C and S in nephrotic subjects. All the three children with TEC in the present study had reduced levels of proteins C and S.

It has been observed that steroid therapy raises the concentration of some zymogens, particularly factor VIII. Prothrombin time and APTT may be shortened with steroid therapy(18) and the concentration of AT-III may rise(19). Steroids might improve the procoagulant abnormalities but the exact mechanism of action is still unknown. The improvement may or may not paralleled by the improvement of proteinuria(2,20). The present study also showed the levels of fibrinogen and AT-III to be significantly elevated in children on steroid therapy ($p < 0.001$) in comparison to the fresh cases of NS. All ten steroid resistant children in our study had significantly high AT-III levels despite heavy proteinuria. Similar observations have been made earlier(12) and it was concluded that fluctuations in AT-III levels in childhood NS are determined by the response to steroids and not by renal histology *per se.* The effect on the PT, TT, APTT, platelet count, proteins C and S, however, were not significant in the present study.

The frequency of thromboembolic complications in our stud/ was 7.5% as compared to 19% to 70% in adults(lO). In most of the earlier studies, thrombosis coincided with the period of diuretic therapy but none of our patients with thromboembolic complications was on diuretics. With the same degree of hypercoagulability in children and adults with NS, the lower frequency of TEC reported in children was surprising and suggests that this entity may be clinically overlooked or misinterpreted. In a series of 26 children with the aid of pulmonary ventilation and perfusion scanning, the frequency of thromboembolic complications was documented to be as high as that reported in adults(21). It is possible that the use of angiography, doppler ultrasonography or pulmonary perfusion/ ventilation isotope scanning might have proved more useful in detecting TEC in our patients.

It is clear from the current study that no single factor can explain hypercoagulability state in patients with nephrosis. The predisposition to thrombosis is probably dependent on the balance between procoagulant and anticoagulant factors. It seems likely that the balance is variable in individual patients during different phases of their disease. However, one should entertain a high index of suspicion for TEC in children with serum albumin <2 g/dl, thrombocytosis, hyperfibrinogenemia and in subjects who are on diuretics. If facilities are available, one should perform detailed coagulogram including proteins C and S and AT-III levels.

REFERENCES

1. Llach F. Hypercoagulability, renal vein thrombosis and other thrombotic compli cations of nephrotic syndrome. Kidney Intern 1985, 28: 429-439.
2. Vaziri N, Nostratola D. Nephrotic syndrome and coagulation and fibrinolytic abnormalities. Am J Nephrol 1983, 3:1-6.
3. Kauffmann RH, Veltkamp JJ, VanTilburg NH. Acquired antithrombin III deficiency and thrombosis in nephrotic syndrome. AmJ Med 1978, 65: 607-617.
4. Kuhlmann J, Steurer K, Rhyner A. Platelet aggregation and P-thromboglobulin lev els in nephrotic patients with and without thrombosis. Clin Nephrol 1981, 15: 229-235.
5. Thompson C, Forbes CD, Prentice CRM, Kennedy AC. Changes in blood coagula tion and fibrinolysis in the nephrotic syn drome. J Med 1974,43: 399-407.
6. Kendal AF, Lohmsnn RC, Dossetor JB. Nephrotic syndrome: A hypercoagulable state. Arch Int Med 1971,127:1021-1027.
7. Bang N, Tygstad C, Schroeder J, *et al.* Ehnanced platelet functions in glomerular renal disease. J Lab Clin Med 1973, 81: 651-660.
8. Colman RW. Platelet function in hyperbetalipoproteinemia. Thrombosis Hemostat 19 78,39: 284-287.
9. Kaj Anker J, Stolfersen E. The inhibitory effect of albumin on platelet aggregation. Thrombosis Res 1980,17:13-18.
0. Yoshida A, Aoki N. Release of arachidonic acid from human platelets: A key role for the pdtentiation of platelet aggregability in normal subjects as well as in those with the nephrotic syndrome. Blood 1978,52: 969-977.
1. Benigni A, Rizzoni G, Antilini A, Piccinelli A. Preliminary report: Renal thromboxane A2 synthesis in children with frequent relapsing nephrotic syn drome. Lancet 1990,336: 533-536.
2. Elidreissy AT H, Abdurrahman MB, Bahakim HM, Jones MD. Hemostatic measurement in childhood nephrotic syn drome. Eur J Paediatr 1991,150: 374-378.
3. Mehis O, Andrassy K, Eberhand R. Hemostasis and thrombo-embolism in

children with nephrotic syndrome. Difference from adults. J Pediatr 1987, 110: 862-867.

14. Alkjarsig N, Fletcher AP, Narayanan M, Robson AM. Course and resolution of the coagulopathy in nephrotic children. Kid ney Int 1987, 31: 772-780.

15. Stewart J, Camenon L. The nephrotic syn drome and its complications. Am J Kidn Dis 1987,10:157-171.

16. Vigano D, D'Angelo S, Kaufmann CE, *et al*. Protein S deficiency occurs in the nephrotic syndrome. Ann Int Med 1987, 107: 42-47.

17. Allon M, Soffer O, Evatt BL, *et al*. Protein S and C antigen levels in proteinuric patients: Dependance on type of glomerular pathology. Am J Hemat 1989, 31: 96-101.

18. Veda N. Effect of corticostroids on coagu lation factors in children with nephrotic syndrome. Pediatr Nephrol 1987, 1: 286-289.

19. Thaler ET Blasar E, Kdpsa H, Pinggera W. Acquired antithrombin III deficiency in patients with glomerular proteinuria. Hemostasis 1978, 7: 257-272.

20. Alder AJ, Laudin AP, Friedman AE. Beta-thromboglobulin levels in the nephrotic syndrome. Am J Med 1980, 69: 551-554.

21. Hoyer PF, Gonda S, Barthels M, Krohn HP. Thromboembolic complications in children with nephrotic syndrome. Acta Pediatr Scand 1986, 75: 804-810.