Tissue factor pathway inhibitor in paediatric patients with nephrotic syndrome

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Background. Tissue factor pathway inhibitor (TFPI) is an endogenous protease inhibitor that regulates the initiation of the extrinsic coagulation pathway by producing factor Xa-mediated feedback inhibition of the tissue factor/factor VIIa (TF/VIIA) catalytic complex.

Objectives. To evaluate plasma TFPI levels in paediatric patients with nephrotic syndrome (NS) and its correlation with disease activity.

Subjects and methods. Fifteen nephrotic patients in relapse (proteinuria >40 mg/m²/h, hypo-albuminaemia and oedema) before initiating steroid therapy (group I) and another 15 nephrotic patients in remission after withdrawal of steroid therapy (group II) were compared with 15 age- and sex-matched healthy children (group III). Besides clinical evaluation and routine laboratory investigations of NS, tissue factor pathway inhibitor levels in plasma were measured by enzyme-linked immunosorbent assay (ELISA).

Results. The plasma TFPI level was higher in nephrotic patients during relapse (group I) and during remission (group II) (mean 102.53 (standard deviation (SD) 14.23) and 82.93 (SD 3.83) ng/ml, respectively) compared with that in the control group (62.40 (SD 7.53) ng/ml) ($p<0.0001$). In children with NS the plasma TFPI level was higher during relapse (group I) compared with the level in remission (group II) ($p<0.0001$). There was a negative correlation between the plasma TFPI level and total protein and serum albumin, and a positive correlation between the plasma TFPI level and the urinary protein/creatinine ratio ($p<0.05$).

Conclusion. NS was associated with increased level of plasma TFPI in comparison with the control group, and the increase was more apparent in patients with active disease.

Thrombo-embolic disease is an important complication in childhood nephrotic syndrome (NS), affecting about 5% of patients.[1] The pathophysiological mechanisms of thrombo-embolism in patients with NS include alterations in plasma levels of proteins involved in coagulation and fibrinolysis, enhanced platelet aggregation, low plasma albumin, hyperviscosity and hyperlipidaemia, as well as treatment with corticosteroids and diuretics.[2] Tissue factor (TF) is a transmembrane procoagulant glycoprotein and a member of the cytokine receptor superfamily. TF functions as a protein co-factor for factor VIIa (FVIIa). The TF-FVIIa complex then activates both factor IX and X, leading to thrombin generation and fibrin formation.[3] Tissue factor pathway inhibitor (TFPI) is a natural inhibitor that regulates the initiation of coagulation by inhibiting tissue factor-activated factor VII (TF-FVIIa) in the presence of activated factor X (FXa).[4]

The source of TFPI is vascular endothelium, and the observed elevated TFPI blood levels could therefore be accounted for by excessive endothelial release of this inhibitor. TFPI is cleared from the circulation primarily by the liver and kidney. It is possible that clearance and catabolism of TFPI may play a role in the fluctuations of TFPI in childhood NS.[5]

Aim of the study

The aim of this study was to evaluate plasma TFPI levels in paediatric patients with NS and its correlation with disease activity and the degree of hypoproteinaemia and proteinuria.

Subjects and methods

Study population

This case-control study was conducted on 45 children, of whom 30 were paediatric patients with NS being followed up at the Pediatric Nephrology Clinic, Children's Hospital, Ain Shams University, Cairo, Egypt, and 15 age- and sex- matched healthy children who served as a control group.

The following exclusion criteria were applied: any degree of renal impairment, steroid-dependent cases, and patients on anticoagulant drugs.

Informed consent was obtained from the parents or caregivers of each child before enrolment in the study, and the study was approved by Ain Shams Medical Ethics Committee.

Patients

Group I. This group included 15 nephrotic patients in relapse before initiating steroid therapy (9 males and 6 females); their ages ranged from 3 to 16 years, with a mean of 7.77 (standard deviation (SD) 4.14) years.

Group II. This group included 15 nephrotic patients in remission after withdrawal of steroid therapy (6 males and 9 females); their ages ranged from 4 to 18 years, with mean of 10.13 (SD 3.85) years.

Controls (group III). The control group comprised 15 apparently healthy, age- and sex-matched children (9 males and 6 females). Their ages ranged from 4 to 13 years with a mean of 8.67 (SD 2.89) years.

Clinical evaluation

Details of history and clinical examination were recorded, including pointers to the underlying cause of NS, response to steroid therapy, other medications (diuretics, antiplatelet drugs), and manifestations of thrombo-embolic disease.

Laboratory investigations

The urinary protein/creatinine ratio on was measured on a Synchrone Cx7 system employing a time end-point colorimetric method. Serum creatinine, total serum protein and serum albumin were measured using a Hitachi automatic analyser 917. Investigations for thrombophilia included the platelet count, the prothrombin time (PT) and the activated partial thromboplastin time (aPTT).

Plasma TFPI assay

A venous sample of 5 ml whole blood was withdrawn from each subject using citrate as an anticoagulant. Centrifugation for 15 minutes at 1 000 g was done within 30 minutes of collection. Samples were then aliquoted and stored at -20˚C until the time of analysis.

The TFPI assay employs the quantitative sandwich enzyme immunoassay technique using a kit supplied by Uscn Life Science Inc., USA. The microtitre plate provided in this kit has been pre-coated with an antibody specific to TFPI. Standards or samples are then added to the appropriate microtitre plate wells with a biotin-conjugated polyclonal antibody preparation specific for TFPI. Avidin conjugated to horseradish peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain TFPI, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of TFPI in the samples is then determined by comparing the optical density of the samples to the standard curve.

Statistical analysis

A standard computer program, SPSS for Windows, release 13.0 (SPSS Inc, USA), was used for data entry and analysis. All numerical variables were expressed as mean (SD). Comparisons of multiple subgroups were done using ANOVA and Kruskal-Wallis tests for normal and non-parametric variables, respectively. Multiple comparisons between pairs of groups were performed using the LSD test (*post hoc* range test). Pearson's correlation was used to test the strength of association between variables. A receiver-operator characteristic (ROC) curve was drawn to detect diagnostic reliability of plasma TFPI in NS. For all tests a probability (p) less than 0.05 was considered significant.

Results

Plasma TFPI levels were higher in patients during relapse (group I) and during remission (group II) than in the control group, with a statistically highly significant difference ($p<0.001$). Plasma TFPI levels were also higher in group I compared with group II, with a highly significant statistical difference ($p<0.001$) (Table I).

The ROC revealed that the area under the curve (AUC) with plasma TFPI was 0.94, i.e. plasma TFPI is a very good parameter to differentiate between relapse and remission at the cut-off value of 89 ng/ml, with a sensitivity of 93% and a specificity of 93% (Fig. 1). Correlations between plasma TFPI and other clinical and laboratory parameters among patients in both group I and group II revealed a positive significant correlation between plasma TFPI and the urinary protein/creatinine ratio ($r=0.59$, $p<0.05$) and a negative significant correlation between plasma TFPI and serum albumin ($r=-0.68$, $p<0.05$), total protein ($r=-0.60$, $p<0.05$), age ($r=-0.36$, $p<0.05$), and height ($r=-0.40$, $p<0.05$) (Table II and Figs 2 and 3).

Comparison of clinical findings between the three groups revealed that weight centiles were lower in the proteinuria group than the control group, with a statistically significant difference ($p<0.05$), and that diastolic blood pressure was higher in the proteinuria group than in either the remission or the control groups, with statistically significant difference ($p<0.05$). No statistically significant difference
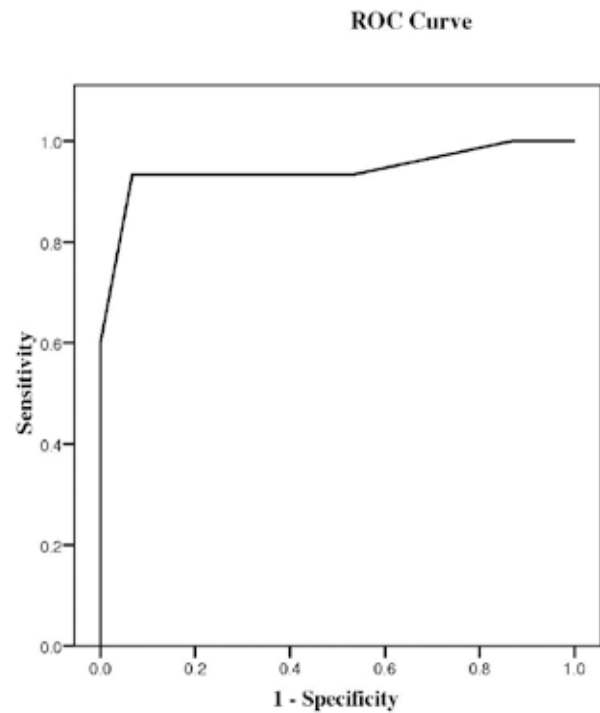

Fig. 1. ROC curve differentiating proteinuria and remission groups.

TABLE I. PLASMA TFPI LEVELS IN THE THREE GROUPS

	Group I (mean (SD))	Group II (mean (SD))	Group III (mean (SD))	p I/II*	p I/III	p II/III
TFPI/ng/ml	102.53 (14.23)	82.93 (3.83)	62.40 (7.53)	<0.0001	<0.0001	<0.0001

TFPI = tissue factor pathway inhibitor.
*p-values of the three groups compared.

TABLE II. CORRELATION BETWEEN TFPI AND OTHER PARAMETERS MEASURED			
	TFPI		
	r	*p*	**Sig.**
Age (yrs)	-0.36	0.05	S
Wt (kg)	-0.26	0.16	NS
Ht (cm)	-0.40	0.03	S
BMI (kg/m^2)	-0.12	0.51	NS
Systolic BP	0.16	0.40	NS
Diastolic BP	0.25	0.19	NS
PLT (×10^9/l)	-0.03	0.86	NS
PT (s)	0.12	0.51	NS
PTT (s)	-0.12	0.54	NS
Creatinine (mg/dl)	0.22	0.24	NS
Total protein (g/dl)	-0.60	<0.0001	S
Serum albumin (g/dl)	-0.68	<0.0001	S
Urinary protein/creatinine ratio	0.59	0.001	S

Pearson correlation coefficient: *r*.
Wt = weight; Ht = height; BMI = body mass index; BP = blood pressure; PLT = platelet count; PT = prothrombin time; PTT = partial thromboplastin time; TFPI = tissue factor pathway inhibitor; Sig. = significance; S = significant; NS = not significant..
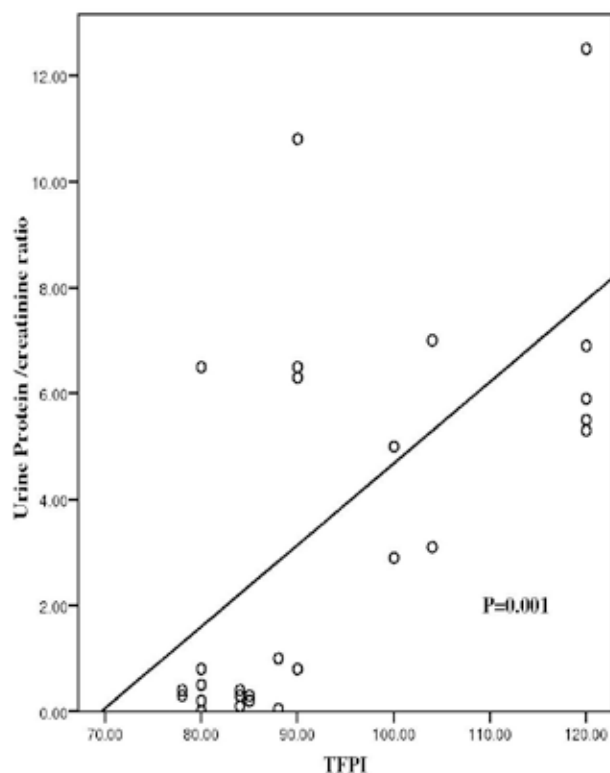

Fig. 3. Correlation between TFPI and serum albumin.

Fig. 2. Correlation between TFPI and urine protein/creatinine ratio.

was found in other parameters (age, sex, height, body mass index, systolic blood pressure) ($p>0.05$) (Tables III and IV).

Table V compares the three groups in respect of routine laboratory findings. The platelet count was higher in patients (both the proteinuria and remission groups) than in the controls, with statistically highly significant differences ($p<0.001$), but there was no difference in platelet count between the proteinuria and remission
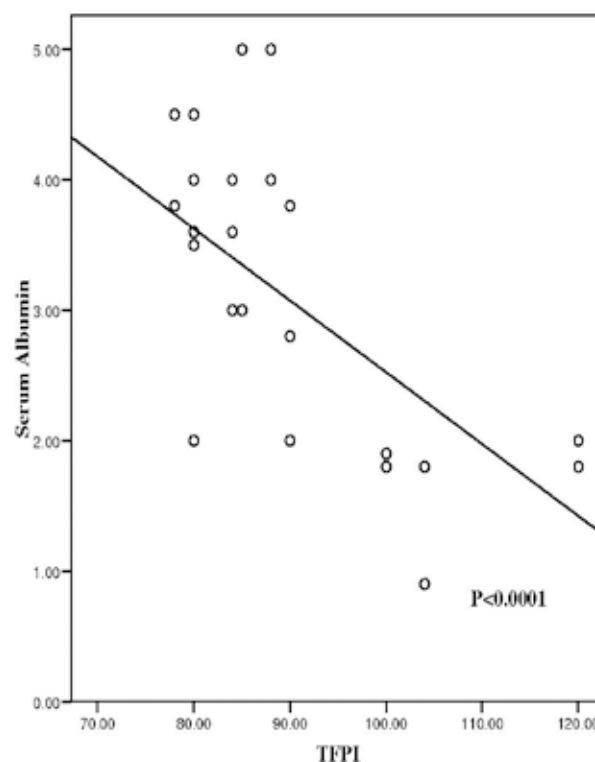
groups ($p>0.05$). Serum creatinine was lower in patients (both the proteinuria and remission groups) than in the control group ($p<0.001$), but did not differ between the proteinuria and remission groups ($p>0.05$). Total protein and serum albumin were lower in the proteinuria group than either the remission or the control group ($p<0.001$), but did not differ between the remission and control groups ($p>0.05$). The urinary protein/creatinine ratio was higher in the proteinuria group than either the remission or the control groups ($p<0.001$), and was also higher in the remission group than the control group ($p<0.001$). No statistically significant difference could be detected between the studied groups with regard to PT and PTT ($p>0.05$) (Table V).

Discussion

We found a highly significant difference in the plasma level of TFPI between the groups studied, with plasma levels of TFPI in children with NS in relapse being markedly higher than those of patients in remission and healthy controls. This is in accordance with the results of Al-Mugeiren *et al.*,[6] who observed that plasma levels of TFPI in relapsed nephrotic patients were significantly higher than those of patients in remission and healthy controls. This result can be explained by the excessive endothelial release of this inhibitor.[7] Albuminuria has also been invoked as a causal factor in the release of TFPI from vascular endothelium. Leurs *et al.*[8] reported higher levels of both basal and post-heparin TFPI activity (assayed by a chromogenic technique) in type I diabetes complicated by albuminuria, compared with patients with uncomplicated diabetes or those with retinopathy without albuminuria.

Elevated cholesterol levels are one of the features that define NS,[9] and levels are highest during the active (relapse) phase of the disease and disappear with resolution of the proteinuria. Hypercholesterolaemia has also been suggested as a factor causing elevated TFPI levels.[10]

In the present study, TFPI in plasma of nephrotic patients was significantly negatively correlated with serum albumin and total proteins, and positively correlated with the urine protein/creatinine ratio, confirming the findings of Al-Mugeiren *et al.*[6] and Lizakowski *et al.*,[11] who also observed that plasma level of TFPI is positively correlated with the urinary protein/creatinine ratio.

TABLE III. COMPARISON BETWEEN THE GROUPS WITH REGARD TO MEAN AGE, WEIGHT, HEIGHT, BMI AND BLOOD PRESSURE

	Group I (mean (SD))	Group II (mean (SD))	Group III (mean (SD))	p I/II*	p I/III	p II/III
Age (yrs)	7.77 (4.14)	10.13 (3.85)	8.67 (2.89)	0.08	0.51	0.28
Wt (kg)	27.27 (16.74)	35.73 (18.75)	30.87 (10.16)	0.15	0.53	0.40
Wt percentiles	25 (<5 - 50)	25 (25-750	50 (25 - 75)	0.44	0.03	0.10
Ht (cm)	119.00 (23.63)	134.93 (22.08)	128.87 (16.63)	0.04	0.21	0.43
Ht percentiles	10 (5 - 25)	10 (10 - 50)	25 (10 - 50)	0.17	0.09	0.68
BMI (kg/m^2)	17.57 (4.91)	21.22 (14.08)	18.08 (1.83)	0.25	0.87	0.33
Systolic BP (mmHg)	107.33 (12.80)	100.67 (10.33)	99.33 (12.23)	0.13	0.07	0.76
Diastolic PB (mmHg)	70.33 (10.43)	62.86 (6.11)	61.00 (8.49)	0.02	**0.005**	0.56

*p-values of the three groups compared.
Wt = weight; Ht = height; BMI = body mass index; BP = blood pressure. The p-value in bold is significant.

TABLE IV. COMPARISON BETWEEN THE GROUPS WITH REGARD TO GENDER

	Group I		Group II		Group III				
Gender	N	%	N	%	N	%	x^2	p	Sig.
Male	9	60.0	6	40.0	9	60.0	1.61	0.45	NS
Female	6	40.0	9	60.0	6	40.0			

Chi-square test.
Sig. = significance.

An increased plasma level of TFPI in NS with active disease could be a compensatory mechanism protecting against thrombo-embolism in these patients, as it is known from previous studies that NS is associated with an increase in TF during activity and that therapeutic intervention with low-molecular-weight heparin led to a decrease in TF and significant clinical improvement in patients with NS.[12]

Our results are in accordance with those observed in other renal diseases such as glomerulonephritis, where fibrin deposition might be a key mediator of injury, probably through TF-mediated coagulation activation.[13] In chronic renal failure a high TFPI level in uraemia may reflect reduced kidney catabolism or endothelial cell injury due to haemodialysis (Malyszko *et al.*[14]). In CAPD patients with no systemic anticoagulation, TFPI is elevated.[15]

In our study other haemostatic parameters such as PT and aPTT in children with NS in relapse were not different from those of patients in remission or healthy controls. This agrees with previous studies,[16,17] although others have observed that aPTT is prolonged in patients with NS in relapse compared with patients in remission and healthy controls, while PT in relapsed patients is not different from that of patients in remission or healthy controls.[18,19]

We found a significant increase in platelet counts in nephrotic patients, both in relapse and in remission, compared with the control group. This supports the hypothesis that platelets may play a significant role in generating hypercoagulability in NS.[20]

The above findings have informed the present policy in the Pediatric Nephrology Clinic, Children's Hospital, Ain Shams University, of using antiplatelet drugs and low-molecular-weight heparin in patients with NS.[12] The indications for anticoagulants include significant thrombocytosis, resistant oedema, severe ascites with dilated veins around the umbilicus, renal biopsy findings of fibrin deposition inside the glomeruli and in between the tubules, and glomerulosclerosis. Low-molecular-weight heparin is given at a dose of 50 U/kg subcutaneously once a day for a month, then every other day for another month. An antiplatelet drug such as low-dose aspirin 75 mg is given once daily for 3 months. The mechanism of action of anticoagulants in NS includes decreasing blood viscosity with subsequently increased blood flow in the glomeruli leading to increased diuresis and decreased oedema. Low-molecular-weight heparin also has an anti-inflammatory effect and promotes healing.

TABLE V. COMPARISON BETWEEN THE GROUPS WITH REGARD TO PLT, PT, PTT, CREATININE, TOTAL PROTEIN, SERUM ALBUMIN, AND URINARY PROTEIN-CREATININE RATIO

	Group I (mean (SD))	Group II (mean (SD))	Group III (mean (SD))	p I/II*	p I/III	p II/III
PLT (×10^9/l)	406.20 (94.78)	385.73 (116.85)	243.67 (35.13)	0.533	<0.0001	<0.0001
PT (s)	12.00 (0.85)	11.87 (0.74)	11.80 (0.86)	0.66	0.51	0.82
PTT (s)	34.93 (1.67)	35.00 (1.46)	35.47 (1.60)	0.91	0.36	0.42
Creatinine (mg/dl)	0.20 (0.10)	0.20 (0.10)	0.50 (0.65)	0.09	**<0.0001**	**<0.0001**
Total protein (g/dl)	4.34 (0.46)	6.65 (0.58)	6.94 (0.54)	**<0.0001**	**<0.0001**	0.14
Serum albumin (g/dl)	1.89 (0.37)	3.95 (0.60)	4.32 (0.69)	**<0.0001**	**<0.0001**	.086
Urine prot./creat. ratio	6.30 (5.30)	0.30 (0.20)	0.07 (0.02)	**<0.0001**	**<0.0001**	**<0.0001**

* p-values of the three groups compared.
PLT = platelet count; PT = prothrombin time; PTT = partial thromboplastin time. The p-values in bold are significant.

In conclusion, plasma TFPI was elevated in NS patients compared with the healthy control group, and the increase was most apparent in patients during relapse. Plasma TFPI was significantly negatively correlated with both total protein and serum albumin, and positively correlated with the urinary protein/creatinine ratio.

Thrombo-embolic disease is an important complication in childhood nephrotic syndrome.
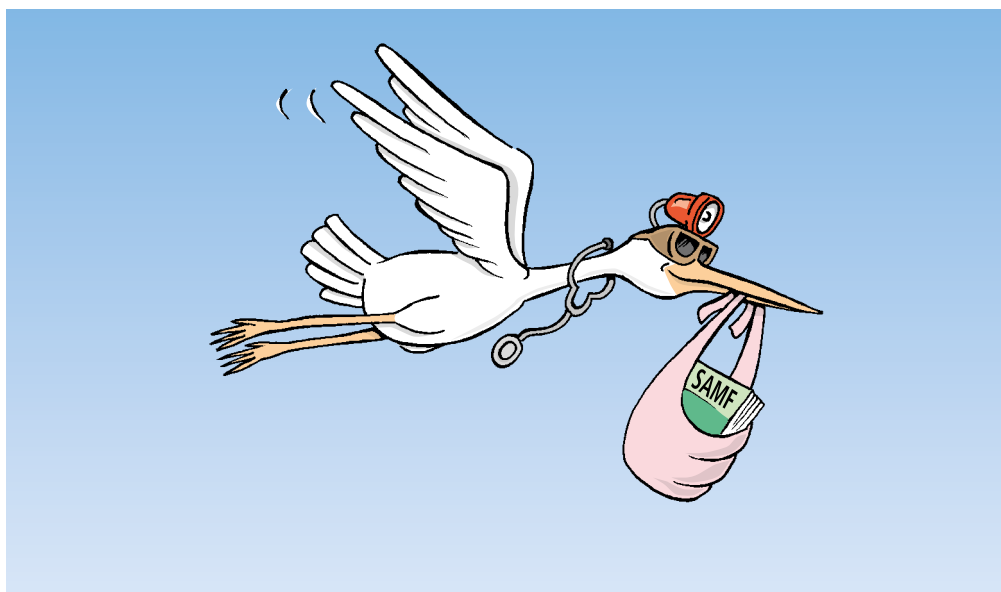
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