Published in final edited form as:
SAJCH. 2011 December ; 5(4): 107–111.

Tissue factor pathway inhibitor in paediatric patients with nephrotic syndrome

Farida Ahmed Farid, **Ahmed Abdullah Mohammed**, **Hanaa Mohammed Afifi**[1], and **Rania Saleh Beltagi**
Department of Paediatrics Ain Shams University, Cairo, Egypt

[1]Department of clinical pathology, Ain Shams University, Cairo, Egypt

Abstract

Background—Tissue factor pathway inhibitor 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 and its correlation with disease activity.

Subjects and Methods—Fifteen nephrotic patients in relapse (proteinuria>40mg/m2/hour, hypoalbuminemia, and edema) before initiating steroid therapy (Group I), and another15 nephrotic patients in remission after withdrawal of steroid therapy (Group II) were compared to 15 age- and sex-matched healthy children (Group III). Besides clinical evaluation and routine laboratory investigations of nephrotic syndrome, tissue factor pathway inhibitor levels in plasma were measured by ELISA.

Results—Plasma TFPI level was higher in nephrotic patients during relapse (Group I) and during remission (Group II) [102.53±14.23 and 82.93±3.83ng/ml respectively] compared to that in the control group (62.40 ± 7.53 ng/ml) ($p< 0.0001$). In children with nephrotic syndrome Plasma TFPI level was higher during relapse (Group I) compared to that in remission (Group II) ($p< 0.0001$). There was a negative correlation between plasma TFPI level and total protein and serum albumin, and there was a positive correlation between plasma TFPI level and urine protein /creatinine ratio with a statistically significant difference ($p< 0.05$).

Conclusion—Nephrotic syndrome was associated with increased level of plasma tissue factor pathway inhibitor in comparison to control group and the increase was more apparent in patients with active disease.

INTRODUCTION

Thromboembolic disease is an important complication in childhood nephrotic syndrome, affecting about 5 % of patients [1]. The pathophysiological mechanisms of thromboembolism in patients with NS include alterations in plasma levels of proteins involved in coagulation and fibrinolysis, enhanced platelet aggregation, low plasma albumin, hyperviscosity, and hyperlipidemia, 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 cofactor 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].

Europe PMC Funders Author Manuscripts

The source of TFPI is vascular endothelium, and, therefore, the observed elevated TFPI blood levels could 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 nephrotic syndrome [5].

AIM OF THE STUDY

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

SUBJECTS AND METHODS

Study population

This case-control study was conducted on 45 children, 30 of them were paediatric patients with nephrotic syndrome following up at the Paediatric Nephrology Clinic, Children's Hospital, Ain Shams University, Cairo, Egypt, and 15 age- and sex-matched healthy children served as a control group.

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

Informed consent was obtained from the parents or caregivers of each child before enrollment in the study and the study was approved by Ain Shams Medical Ethics Committee (FMASU REC).

Patients

Group I: This group included 15 nephrotic patients in relapse before initiating steroid therapy (9 males and 6 females); their ages ranged from 3-16 years with mean age of 7.77±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 4-18 years with mean age of 10.13 ± 3.85 years.

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

Clinical evaluation

Details of history and clinical examination were recorded including pointers to the underlying aetiology of nephrotic syndrome, response to steroid therapy, other medications (diuretics, antiplatelet drugs), and manifestations of thromboembolic disease

Laboratory investigations

Urine protein/ creatinine ratio on Synchrone Cx7 system employing a time end point colorimetric method.

Serum creatinine, total serum protein and serum albumin by using Hitachi automatic analyzer 917.

Investigations for thrombophilia included: Platelet count (Coulter Micro Diff 18, Fullerton CA, USA),, Prothrombin time (PT) and activated partial thromboplastin time (a PTT) by using Diagnostic a STAGO,

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 1000 x g was done within 30 minutes of collection. Samples were then aliquoted and stored at −20° C till time of analysis.

The TFPI assay employs the quantitative sandwich enzyme immunoassay technique by kit supplied by Uscn Life Science Inc., USA. The microtiter plate provided in this kit has been pre-coated with an antibody specific to TFPI. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for TFPI and 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 color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color 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 (O.D.) 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 numeric variables were expressed as mean ± standard deviation (SD). Comparisons of multiple subgroups were done using ANOVA and Kruskall Wallis tests for normal and nonparametric variables respectively. Multiple comparisons between pairs of groups were performed using LSD test (Post hoc range test). Pearson 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 nephrotic syndrome. 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 higher in Group I compared to group II with highly significant statistical difference ($p< 0.001$) (Table 1).

ROC (receiver operating characteristic curve) revealed that the area under the curve (AUC) with plasma TFPI was 0.94, i.e. plasma TFPI is a very good differentiating parameter between relapse and remission at the cutoff value of 89 with a sensitivity of 93%, and specificity of 93%.(Fig. 1).

Correlation between plasma TFPI and other clinical and laboratory parameters among patients (both group I and group II) revealed that there was a positive significant correlation between plasma TFPI and urine protein /creatinine ratio ($r = 0.59$, $p <0.05$), 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 2 and Fig. 2, 3).

Comparison of clinical findings between the three groups revealed that weight centiles were lower in proteinuria group than the control group with statistically significant difference ($p<0.05$), and the diastolic blood pressure was higher in proteinuria group than both remission and control groups with statistically significant difference ($p<0.05$). No statistically significant difference was found in other parameters (age, sex, height, BMI, systolic blood pressure) ($p>0.05$) (Tables 3,4).

Table 5 shows the comparison between the three groups in respect of the routine laboratory findings. The platelet count was higher in patients (both proteinuria and remission groups) than 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 proteinuria and remission groups) than the control group ($p<0.001$), but not different between proteinuria and remission groups ($p>0.05$). Total protein and serum albumin were lower in proteinuria group than both remission and control groups ($p<0.001$), but not different between remission and control groups ($p>0.05$). Urine protein /creatinine ratio was higher in the proteinuria group than both the remission and control groups ($p<0.001$) and also higher in the remission group than the control group ($p<0.001$). No statistically significant difference could be detected between the studied groups as regards PT and PTT ($p>0.05$) (Table 5).

DISCUSSION

In the present study, there was a highly significant difference in the plasma level of tissue factor pathway inhibitor (TFPI) between the studied groups where the plasma level of TFPI in children with nephrotic syndrome in relapse was markedly higher than that of patients in remission and healthy controls. This result agrees with Al-Mugeiren et al., (2006) [6] who had observed that plasma level of TFPI in relapsed nephrotic patients was significantly higher than 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. (2003) [8] reported higher levels of both basal and post-heparin TFPI activity (assayed by a chromogenic technique) in type I diabetes complicated with 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 are highest during the active (relapse) phase of the disease and disappear with the resolution of the proteinuria. Hypercholesterolaemia has also been mentioned as a causative factor for 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., (2006) [6] and Lizakowski et al.,(2007)[11] who also observed that plasma level of TFPI is positively correlated with the urine protein creatinine ratio.

An increased plasma level of TFPI in NS with active disease could be a compensatory mechanism against thromboembolism in these patients, as it is known from previous studies that nephrotic syndrome was associated with increase in tissue factor during activity and therapeutic intervention with low molecular weight heparin led to decrease in tissue factor and to significant clinical improvement in patients with nephrotic syndrome [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 in

uremia may reflect reduced kidney catabolism or endothelial cell injury due to haemodialysis (Malyszko et al., 2004) [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], though, others have observed that aPTT is prolonged in patients with NS in relapse compared with patients in remission and healthy controls, while prothrombin time (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 to those of the control group. This supports the hypothesis that platelets may play a significant role in generating hypercoagulability in nephrotic syndrome [20].

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

In conclusion, plasma TFPI was elevated in nephrotic syndrome patients compared to the healthy control group,, and the increase was more apparent in patients during relapse. Plasma TFPI was significantly negatively correlated with both total proteins and serum albumin, and positively correlated with the urine protein creatinine ratio.

REFERENCES

[1]. Ozkaya O, Bek K, Fişgin T, Aliyazicioğlu Y, Sultansuyu S, Açikgöz Y, et al. Low protein Z levels in children with nephrotic syndrome. Pediatr Nephrol. 2006; 21(8):1122–6. [PubMed: 16810511]

[2]. Mahmoodi, Bakhtawar K.; ten Kate, Min Ki; Waanders, Femke; Veeger, Nic J.G.M.; Brouwer, Jan-Leendert P.; Vogt, Liffert, et al. High Absolute Risks and Predictors of Venous and Arterial Thromboembolic Events in Patients With Nephrotic Syndrome. Circulationb. 2008; 117:224–30.

[3]. Lopes-Bezerra LM, Filler SG. Endothelial cells, tissue factor and infectious diseases. Braz J Med Biol Res. 2003; 36(8):987–91. [PubMed: 12886452]

[4]. Adams J, Oostryck R. Further investigations of lupus anticoagulant interference in a functional assay for tissue factor pathway inhibitor. Thrombosis Res. 1997; 87(2):245–9.

[5]. Warshawsky I, Bu G, Mast A, Saffitz JE, Broze GJ, Schwartz AL. The carboxy terminus of tissue factor pathway inhibitor is required for interacting with hepatoma cells in vitro and in vivo. J Clin Invest. 1995; 95:1773–81. [PubMed: 7706485]

[6]. Al- Mugeiren MM, GaderAM Al-RasheedSA, Abdel Galil M, Al-Salloum A. Coagulopathy of childhood nephrotic syndrome. Pediatric Nephrol. 2006; 21:771–7.

[7]. Maroney, Susan A.; Mast, Alan E. Mast Expression of Tissue Factor Pathway Inhibitor by Endothelial Cells and Platelets Transfus Apher Sci. 2008; 38(1):9–14.

[8]. Leurs PB, van Oerle R, Hamulyak K, Wolffenbuttel BH. Tissue factor pathway inhibitor (TFPI) release after heparin stimulation is increased in Type 1 diabetic patients with albuminuria. Diabet Med. 2003; 20(1):16–22. [PubMed: 12519315]

[9]. Merouani A, Levy E, Mongeau JG, Robitaille P, Lambert M, Delvin EE. Hyperlipidemic profiles during remission in childhood idiopathic nephrotic syndrome. Clin Biochem. 2003; 36:571–4. [PubMed: 14563452]

[10]. Morishita E, Asakura H, Saito M, Yamazaki M, Ontachi Y, Mizutani T, et al. Elevated plasma levels of free-form of TFPI antigen in hypercholesterolemic patients. Atherosclerosis. 2001; 154(1):203–12. [PubMed: 11137101]

[11]. Lizakowski, Slawomir; Zdrojewski, Zbigniew; Jagodzinski, Piotr; Rutkowski, Boleslaw. Plasma tissue factor and tissue factor pathway inhibitor in patients with primary glomerulonephritis. Scandinavian journal of urology and nephrology. 2007; 41(3):237–242. [PubMed: 17469034]

[12]. Farid FA, Reda SM, El-Awady HM, El-Said HM, Yousef FM. new concept in management of childhood nephrotic syndrome through inhibition of hypercoagulation. Egyptian J Pediatr. 2005; 22(1):33–50.

[13]. Lwaleed BA, Bass PS, Cooper AJ. The biology and tumour related properties of monocyte tissue factor. J Pathol. 2001; 193:3–12. [PubMed: 11169509]

[14]. Malyszko J, Suchowierska E, Ma.yszko JS, Mysliwiec M. A comprehensive study on hemostasis in CAPD patients treated with erythropoietin. Kidney Blood Press Res. 2004; 27:71–7. [PubMed: 14691349]

[15]. Malyszko J, Ma.yszko JS, Mysliwiec M. Comparison of hemostatic disturbances between patients on CAPD and HD. Perit Dial Int. 2001; 21:158–65. [PubMed: 11330560]

[16]. Yalçinkaya F, Tümer N, Gorgani AN, Ekim M, Cakar N. Haemostatic parameters in childhood nephrotic syndrome. Int Urol Nephrol. 1995; 27(5):643–7. Is there any difference in protein C levels between steroid sensitive and resistant groups? [PubMed: 8775051]

[17]. Al- Mugeiren MM, Gader AM, Al-Rasheed SA, Bahakim HM, Al-Momen AK, Al-Salloum A. Coagulopathy of childhood nephrotic syndrome -areappraisial of the role of natural anti coagulants and fibrinolysis. Haemostasis. 1996; 26:304–10. [PubMed: 8979144]

[18]. Ueda N, Kawaguchi S, Niinomi Y, Nonoda T, Matsumoto J, Ohnishi M, Yasaki T. Effect of corticosteroids on coagulation factors in children with nephrotic syndrome. Pediatr Nephrol. 1987; 1(3):286–9. [PubMed: 3153290]

[19]. Anand NK, Chand G, Talib VH, Chellani H, Pande J. Hemostatic profile in nephrotic syndrome. Indian Pediatr. 1996; 33(12):1005–12. [PubMed: 9141800]

[20]. Sirolli V, Ballone E, Garofalo D, Merciaro G, Settefrati N, Di Mascio R, et al. Platelet activation marker in patients with nephrotic syndrome. Nephron. 2002; 91:424–30. [PubMed: 12119472]

Europe PMC Funders Author Manuscripts

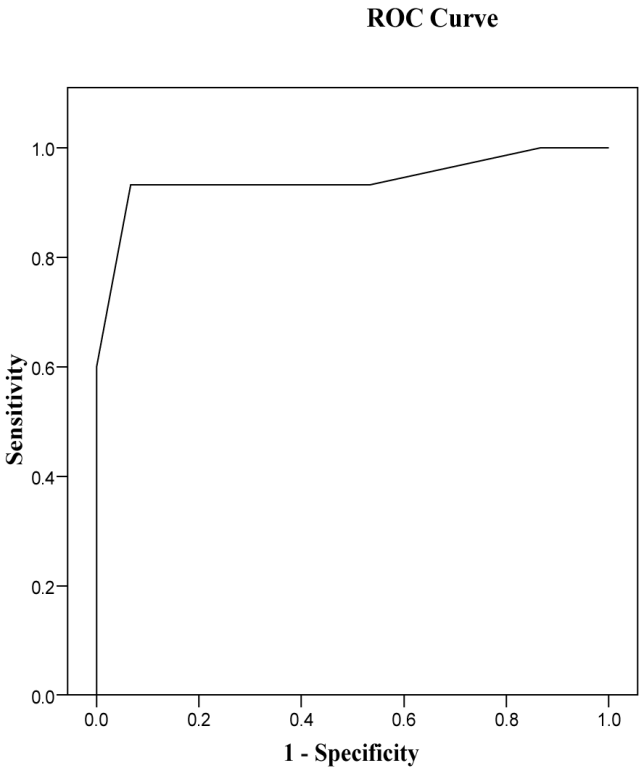

Figure (1).
ROCcurve differentiating proteinuria and remission groups

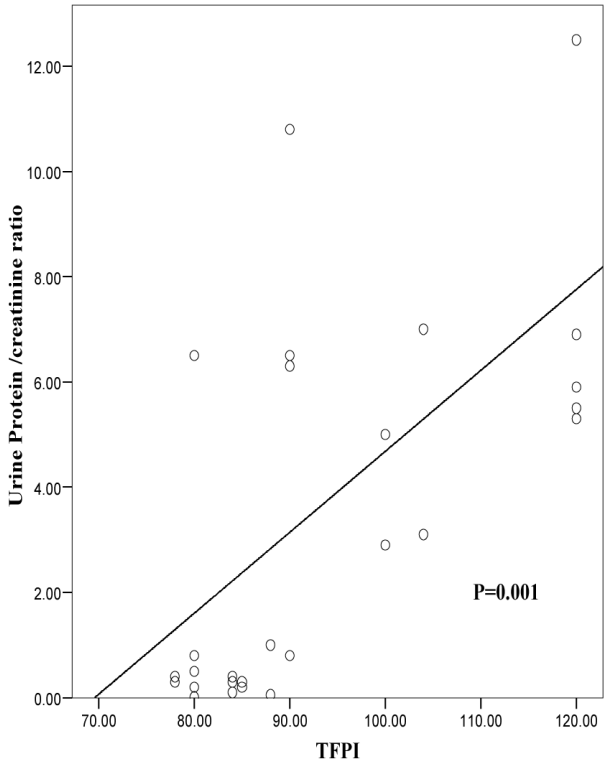

Figure (2).
Correlation between TFPI and urine protein /creatinine ratio

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

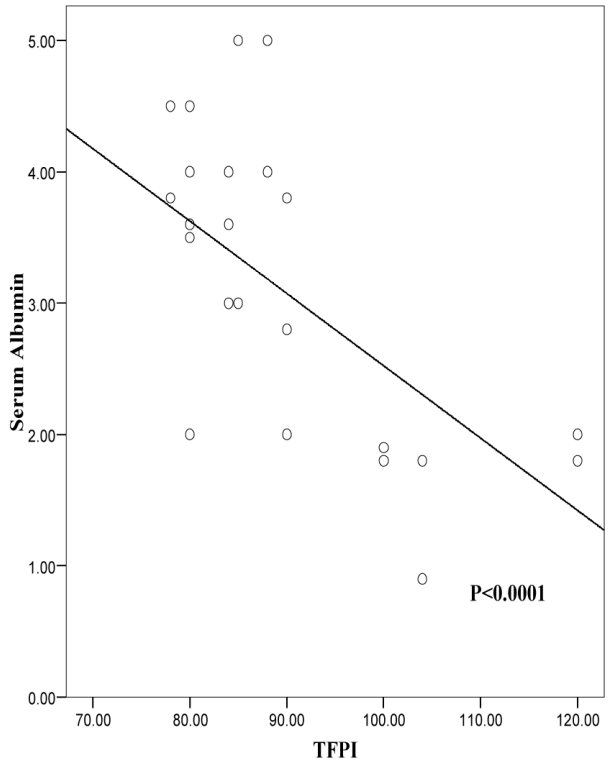

Figure (3).
Correlation between TFPI and serum albumin

Europe PMC Funders Author Manuscripts

Table (1)

Comparison between the three groups as regards plasma tissue factor pathway inhibitor:

	Group I Mean ±SD	Group II Mean ± SD	Group III Mean ± SD	pI≠II	pI≠III	pII≠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

Europe PMC Funders Author Manuscripts

Table (2)

Correlation between TFPI and other measured parameters

	TFPI		
	r	**P**	**Sig.**
Age/year	−0.36	0.05	S
Wt/kg	−0.26	0.16	NS
Ht/cm	−0.40	0.03	S
B.M.I(kg/m^2)	−0.12	0.51	NS
Sys. B.P	0.16	0.40	NS
Dias.B.P	0.25	0.19	NS
PLT109/L	−0.03	0.86	NS
PT/sec	0.12	0.51	NS
PTT/sec	−0.12	0.54	NS
Creatinine/mg/dl	0.22	0.24	NS
Total Protein/gm/dl	−0.60	<0.0001	S
Serum Albumin/gm/dl	−0.68	<0.0001	S
Urine Protein /creatinine ratio	0.59	0.001	S

Wt =Weight, Ht =Height, B.M.I =Body mass index , PLT =Platelet count, PT =Prothrombin time, PTT =Partial thromboplastin time, TFPI =Tissue factor pathway inhibitor

Pearson correlation coefficient: r

Europe PMC Funders Author Manuscripts

Table (3)

Comparison between the three groups as regards mean age, weight, height, BMI and blood pressure

	Group I Mean ±SD	Group II Mean ± SD	Group III Mean ± SD	pI≠II	pI≠III	PII≠III
Age/year	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
Weight percentiles	25±<5-50	25±25-75	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
Height percentiles	10±5-25	10 ±10-50	25±10-50	0.17	0.09	0.68
B.M.I(kg/m^2)	17.57±4.91	21.22 ±14.08	18.08 ±1.83	0.25	0.87	0.33
Sys. B.P/mmHg	107.33±12.80	100.67±10.33	99.33± 12.23	0.13	0.07	0.76
Dias.B.P/mmHg	70.33 ±10.43	62.86 ± 6.11	61.00 ±8.49	0.02	**0.005**	0.56

Wt =Weight, Ht =Height,,B.M.I =Body mass index , Sys B.P =Systolic blood pressure, Dias B.P = Diastolic blood pressure

Europe PMC Funders Author Manuscripts

Table (4)

Comparison between the studied groups as regard sex:

		Group I		Group II		Group III				
		N	%	N	%	N	%	χ^2	P	Sig.
Sex	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

Europe PMC Funders Author Manuscripts

Table (5)

Comparison between the three groups as regards PLT, PT, PTT, creatinine, total protein, serum albumin, and urine protein creatinine ratio

	Group I Mean ±SD	Group II Mean ± SD	Group III Mean ± SD	pI≠II	pI≠III	pII≠III
PLT10^9/L	406.20±94.78	385.73±116.85	243.67±35.13	0.533	**<0.0001**	**<0.0001**
PT/sec	12.00±0.85	11.87±0.74	11.80±0.86	0.66	0.51	0.82
PTT/sec	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/gm/dl	4.34±0.46	6.65±0.58	6.94±0.54	**<0.0001**	**<0.0001**	0.14
Serum Albumin/gm/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**

PTT =Partial thromboplastin time

PT =Prothrombin time

PLT =Platelet count

SAJCH. Author manuscript; available in PMC 2012 February 05.