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Adel N. Hanna
25 Wadi El-Nil St. 12411,
Giza, Egypt

Tel: +20123440310

Study of Tissue Factor and Factor VIIa in Children with Nephrotic Syndrome

¹Kh. Saber, ²Z. El-Khayat, ²G.S. Hussein and ¹A.N. Hanna

The present study was planned to evaluate the serum levels of tissue factor and factor VIIa of nephrotic children and to investigate the possible alteration in their levels in relation to heparin treatment and disease activity. The study included 40 patients with nephrotic syndrome, further divided into 2 groups and 15 controls. Group IA included 20 nephrotic patients and received low molecular weight heparin beside steroid therapy. Group IB included 20 nephrotic patients and received steroid therapy only. The patients were evaluated initially, one week and one month from the commencement of therapy. At the start of study, tissue factor and factor VIIa levels showed no significant difference between group IA and group IB, while both patients' groups showed significantly higher tissue factor and factor VIIa as compared to the control group. At day 7, the study revealed obvious clinical improvement accompanied by significant decrease in tissue factor and factor VIIa in group IA as compared to group IB. Moreover, the laboratory improvement was more marked after 30 days of follow up. Also, steroid dependent and resistant nephrotic patients of group IA had comparable serum tissue factor and factor VIIa with control group at day 30 of the study. Time to achieve remission was significantly shorter in steroid resistant patients of group IA as compared to those of group IB. There was no significant difference in time to remission in both steroid dependent subgroups. Therapy with low molecular weight heparin led to decrease in serum tissue factor and factor VIIa and led to significant clinical improvement in the course of nephrotic syndrome in the form of shorter time to induce remission.

Key words: Serum tissue factor, factor VIIa, nephrotic syndrome

INTRODUCTION

Nephrotic syndrome is a primarily pediatric disorder with an incidence of 2-3/100 children per year. The characteristic features of nephrotic syndrome are heavy proteinuria ($> 40 \text{ mg m}^{-2} \text{ h}^{-1}$), hypoalbuminemia ($< 2.5 \text{ g dL}^{-1}$), edema and hyperlipidemia (Vogt and Avner, 2004).

A hypercoagulable state and the risk of thromboembolism in both arterial and venous circulation are relatively frequent and serious features of nephrotic syndrome in children (Citak *et al.*, 2000; Gangakhedkar *et al.*, 2005).

Recently Tissue Factor (TF) and its inhibitor (TFPI) had been reported to participate in the process of coagulation in various diseases such as angina pectoris, end stage renal disease and insulin dependent diabetes mellitus (Prandoni, 2005).

Proinflammatory effects of TF, which appear to be independent of its procoagulant function, have been demonstrated in crescentic glomerulonephritis. Moreover, the binding of factor VIIa (FVIIa) to TF may be an important intracellular signaling mechanism that increases reactive oxygen species production by macrophages (Cunningham *et al.*, 1995).

Recombinant TFPI has been shown to attenuate the lethal inflammatory and coagulation responses (Al-Mugeiren *et al.*, 2006). Several studies have demonstrated high serum concentration of TF and TFPI in nephrotic patients. They suggested that, the increase in TFPI might attenuate but not abolish the action of TF on the vascular endothelium (Malyszko *et al.*, 1999).

Heparin is known to exert its antithrombotic effect by accelerating the effect of antithrombin (AT) and by mobilizing TFPI into the circulation from vascular endothelium. Recently, heparin has been reported to decrease FVIIa, this was suggested as a new antithrombotic action of heparin and that FVIIa assay is more sensitive than TFPI and AT assays during heparin treatment (Hansen *et al.*, 2000).

The aim of this study was to evaluate the degree of the hypercoagulation in children with nephrotic syndrome by measuring serum TF and FVIIa and to study the role of heparin in inhibiting the coagulation action of TF either directly or indirectly via inhibition of FVIIa and its effect on the clinical condition of the patients.

MATERIALS AND METHODS

This follow up study comprised 40 children with primary nephrotic syndrome and 15 controls. They were classified into two groups.

Patients (group I): Included 40 nephrotic children all were in activity at the start of the study. Group I was further subdivided according to the treatment protocol into two groups:

Group IA: This group received low molecular weight heparin (LMWH) (50 unit kg^{-1} body weight single daily dose subcutaneously for six days) beside steroid therapy as prednisone 60 mg m^{-2} orally daily. This group included 20 nephrotic patients (13 males and 7 females); their ages ranged from 3.5-15.0 years (mean \pm SD = 9.0 ± 3.9). Twelve of them were steroid dependent and eight were steroid resistant. Duration of illness ranged from 2-120 months (mean \pm SD = 45.7 ± 40.2).

Group IB: This group received steroid therapy only. It included 20 nephrotic patients (12 males and 8 females); their ages ranged from 2.5-12.0 years (mean \pm SD = 7.8 ± 3.2). Twelve of them were steroid dependent and eight were steroid resistant. Duration of illness ranged from 5-108 months (mean \pm SD = 41.6 ± 36.0).

Exclusion criteria for the patients: Patients with abnormal kidney function (creatinine clearance $< 80 \text{ mL}^{-1} \text{ min}$), secondary nephrotic syndrome, chronic liver disease, prolonged PT or PTT, platelet dysfunction, history of active bleeding or thrombosis, severe hypertension and recipients of cytotoxic drugs were excluded from the study.

Controls (group II): It included 15 apparently healthy age and sex matched children. They were 9 males and 6 females. Their ages ranged from 4.0-11.0 years (mean \pm SD = 8.3 ± 2.4).

All patients were evaluated as follows

- Full history taking.
- Thorough clinical examination.
- Laboratory investigations: Complete urine analysis, 24 h urinary proteins, Complete blood count, ESR, Total serum protein and serum albumin, serum cholesterol, serum creatinine and creatinine clearance, blood urea nitrogen, Prothrombin Time (PT) and partial thromboplastin time (PTT), serum Tissue Factor (TF) and serum FVIIa by ELISA.

The clinical assessment and laboratory investigations were done at day 1, 7 and 30.

Statistical methods: Descriptive and analytical statistics were performed on IBM-compatible computer by using SPSS 11.5 software package. Continuous data were

presented as mean±standard deviation (mean±SD), median and range. For continuous non-parametric data, Mann-Whitney (U) test was used for inter-group analysis and Spearman correlation coefficient (r) tests were used for intra-group analysis. Inter-group comparisons of categorical data were performed by using chi square test (χ^2 value). p-values < 0.05 were considered significant and p value <0.01 and <0.001 were considered highly significant.

RESULTS

Table (1 and 2) and Fig. (1 and 2) show the levels of serum TF and FVIIa among the different studied groups. At day 1, both serum TF and FVIIa levels showed no significant difference between GIA and GIB patients (p>0.05). Both groups showed significantly higher serum TF and FVIIa levels as compared to the control group (p<0.01). At day 7, GIA had significant decrease in serum TF and FVIIa levels as compared to GIB (p<0.01 and p<0.05, respectively). However, serum TF and FVIIa in GIA was still higher than that of the control group

(p<0.01). Further decrease in serum TF and FVIIa levels in GIA as compared to GIB patients (p<0.01) was observed at day 30 and was comparable with the values of the control group (p>0.05). Meanwhile, in GIB patients, the values of serum TF at day 30 were still significantly higher than the control group (p>0.01).

At the start of the study, serum TF and FVIIa levels were comparable between steroid resistant and steroid dependent nephrotic patients (p>0.05 in each comparison). At day 1, there was no significant difference in serum TF and FVIIa results between group IA and group IB in both steroid dependant and steroid resistant patients (p>0.05 in each comparison). At day 1 and 7, steroid dependent and steroid resistant nephrotic patients of groups IA and IB showed significantly higher serum TF and FVIIa levels as compared to the control group (p<0.01) in each comparison except in comparison of serum FVIIa in group IA steroid resistant patients at day 7 versus control group, p<0.05). At day 30, steroid dependent and steroid resistant nephrotic patients of group IA had comparable serum TF and FVIIa levels with the control group (p>0.05 in each comparison). On the contrary, steroid dependent and steroid resistant

Table 1: Serum tissue factor in the studied groups during the follow up period

Group	Day 1		Day 7		Day 30		
	Control	IA	IB	IA	IB	IA	IB
Mean	93.33	400.00	370.00	167.50	304.17	101.67	268.33
SD	30.33	250.96	130.52	80.01	110.65	66.58	115.98
Comparison between group IA versus group IB:							
Z-value	0.523		2.901		3.541		
p-value	>0.05*		<0.01**		<0.01**		
Comparison between control group and both group IA and group IB							
Group	Group IA			Group IB			
	Day 1	Day 7	Day 30	Day 1	Day 7	Day 30	
Z-value	4.118	2.848	0.410	3.852	4.168	4.058	
p-value	<0.01**	<0.01**	>0.05*	<0.01**	<0.01**	<0.01**	

* = Non significant ** = Highly significant

Table 2: Serum FVIIa in the studied groups during the follow up period

Group	Day 1		Day 7		Day 30		
	Control	IA	IB	IA	IB	IA	IB
Mean	5.13	26.38	25.33	11.21	18.00	7.58	15.50
SD	1.25	10.05	15.07	4.98	9.17	3.28	7.78
Comparison between group IA versus group IB:							
Z-value	0.665		2.435		3.246		
p-value	>0.05		<0.05*		<0.01**		
Comparison between control group and both group IA and group IB							
Group	Group IA			Group IB			
	Day 1	Day 7	Day 30	Day 1	Day 7	Day 30	
Z-value	4.168	4.005	-1.529	4.166	4.171	4.170	
p-value	<0.01**	<0.01**	>0.05	<0.01**	<0.01**	<0.01**	

* = Significant, ** = Highly significant

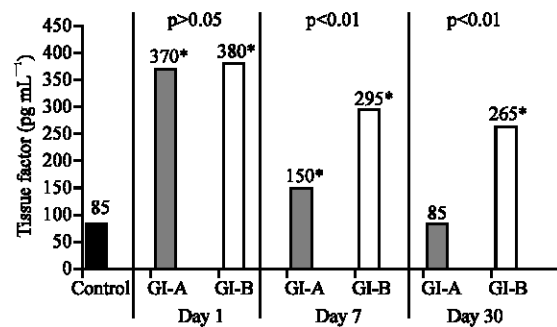


Fig. 1: Serum tissue factor in both patients' groups during follow up period [* = highly significant increase versus control group (p<0.01)]

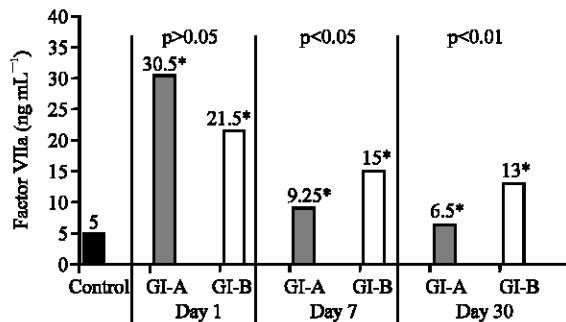


Fig. 2: Serum factor VIIa in both patients' groups during follow up period [* = highly significant increase versus control group (p<0.01)]

nephrotic patients of group IB had still significantly higher levels of both serum TF and FVIIa as compared to the control group ($p < 0.01$ in each comparison).

At the start of the study, serum albumin results were comparable in both group IA and group IB patients ($p > 0.05$). In addition, significant negative correlation was found between serum albumin and both serum TF and serum FVIIa in all nephrotic patients at the start of the study ($p < 0.05$ and $p < 0.001$, respectively). At day 7 and 30, serum albumin was significantly higher in group IA as compared to group IB ($p < 0.01$ in each comparison).

As regards 24 h urinary proteins results at day 1, there was no significant difference between both groups ($p > 0.05$). At day 7, 30, 24 h urinary proteins were significantly lower in group IA as compared to group IB ($p < 0.01$ in each comparison). At day 1, 7 and 30, there was no significant difference between both patients' groups in the serum cholesterol levels ($p > 0.05$). However, significant positive correlation was found between serum cholesterol level and both serum TF and FVIIa levels in all nephrotic patients at the start of the study ($p < 0.05$ in each correlation).

At the end of the study period (30 days), 35 of our patients were in clinical remission as evidenced by disappearance of both edema and proteinuria. Group IA patients showed significantly shorter time to remission compared with group IB ($p < 0.05$). Time to remission was significantly reduced in steroid resistant patients who received LMWH as compared to steroid resistant patients who received steroids only ($p < 0.05$).

DISCUSSION

The proposed role of TF and FVIIa in the pathogenesis of glomerular diseases has urged us to evaluate their levels in the serum nephrotic children and to investigate the possible alteration in their levels in relation to heparin treatment and disease activity.

Our results revealed that, in all nephrotic patients before starting heparin therapy, serum levels of TF and FVIIa were significantly higher as compared to the control group ($p < 0.01$). This finding denotes increased activity of the coagulation pathway during the stage of active nephrosis. The elevated levels of serum TF and FVIIa in our series are in accordance with those observed by Malyszko *et al.* (1999) on nephrotic syndrome.

On the first day of the current study, serum TF levels and FVIIa were positively correlated with the values of 24 h urinary proteins and were negatively correlated with serum albumin levels in all nephrotic children. This finding raises in mind the well established relationship between the severity of albuminuria and progressive renal disease

(Assadi, 2005; de Jong and Brenner, 2004) and the possible link between elevated serum TF level and FVIIa and the severity of glomerular injury.

A positive correlation between cholesterol and both TF and FVIIa was observed in all nephrotic patients at the start of our study. This is in accordance with other studies (Puccetti *et al.*, 2000; Camera *et al.*, 2002) that concluded that, the prothrombotic phenotype of arterial wall associated with elevated serum cholesterol levels, is mediated by TF over expression and they demonstrated that, prothrombotic tendency was reduced through inhibition of TF synthesis. Moreover, Jeanpierre *et al.* (2003) analyzed the effect of dietary lipid lowering on TF expression. They showed that, dietary lipid lowering decreases the thrombotic potential of ruptured atherosclerotic plaques through TF decrease. In addition, Chi *et al.* (2004) concluded that, thrombus formation is initiated by the activation of TF/FVIIa pathway, which is attributed to TF expression in the atherosclerotic plaque and enhanced plasma FVIIa coagulant activity.

Follow up of the clinical parameters and the laboratory results after 7 days from the start of the study revealed obvious clinical improvement in group IA as compared to group IB in the form of significant weight reduction, fading of edema, significant increase in serum albumin and decrease in urinary proteins. This was also accompanied by significant decrease in serum TF ($p < 0.01$) and FVIIa ($p < 0.05$). Therefore, LMWH therapy induced a more rapid improvement in both clinical and laboratory markers of remission and this improvement was parallel with the reduction of serum TF and FVIIa.

Moreover, the laboratory improvement was more marked after 30 days of follow up since serum albumin was significantly higher and the 24 h urinary proteins were significantly lower in group IA as compared to group IB. Furthermore, significant reduction in serum TF and FVIIa was observed in group IA as compared to group IB ($p < 0.01$) to the extent that serum TF and FVIIa levels reached the control baseline ($p > 0.05$). Therefore, the beneficial effects of LMWH extended beyond the period of its administration since the improvement in the laboratory parameters was maintained until the end of the study.

Steroid resistant nephrotic syndrome (SRNS) causes morbidity and mortality due to persistent edema, hypertension and progression to renal failure (Crew *et al.*, 2004).

In the present study, the beneficial effect of LMWH in SRNS was evident. Steroid resistant patients of group IA had serum TF and FVIIa levels comparable with control values at day 30 of the study. Meanwhile, the corresponding values of steroid resistant patients in

group IB remained significantly higher than the controls at that time. Also, a significant reduction in time to induce remission among patients with SRNS who received heparin was observed. This finding highlights the value of heparin therapy in SRNS and supports the finding of Mori *et al.* (2004) who demonstrated in their study that methyl prednisolone therapy with heparin can induce remission in children with SRNS, even when the patient is resistant to cyclophosphamide or cyclosporine A.

Several studies have been conducted on adults demonstrating the efficiency of heparin as a useful agent in reducing the duration of proteinuria and edema (Crew *et al.*, 2004; Appel, 2006) based on the fact that, heparins exert an anti-inflammatory action through stopping adhesion of leucocytes to endothelial surfaces, they are anticomplementary, they modulate the activities of phagocytes and they stop the proliferation of mesangial cells on vascular smooth muscle cells. Heparin prevents the release of endothelin-1 and potentiates the action of constitutive nitric oxide (Dos Santos *et al.*, 2006).

Although none of the children in our study had evidence of thromboembolism, yet they showed significant elevation of serum TF and FVIIa levels, such disturbance predispose them to the risk of thrombotic complications.

In conclusion, therapy with LMWH led to decrease in serum TF and FVIIa. The beneficial effects of LMWH extended beyond the period of its administration since the improvement in the laboratory parameters was maintained until the end of the study. It produced significant reduction in proteinuria and elevation of serum albumin. Moreover, LMWH led to significant clinical improvement in the course of nephrotic syndrome in the form of shorter time to induce remission, thus having steroid sparing effect without bleeding complications or rebound thrombosis.

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