

**Original Article****Predictive Value of Platelet Aggregation Responses and Coagulation Parameters for Thromboembolic Risk in Children with Idiopathic Nephrotic Syndrome.****Mohammad Abdelmonaem Sharaf<sup>1</sup> and Soha Abuelela<sup>2</sup>**

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**Abstract**

**Introduction:** Idiopathic nephrotic syndrome (INS) is the most common renal disease among children. INS present with proteinuria, hyperlipidemia, edema, hypoalbuminemia, and hypercoagulability. A serious consequence of INS is arterial and/or venous thromboembolism e.g., deep vein thrombosis (DVT), pulmonary emboli and renal vein thrombosis. This hypercoagulability may be due to urinary loss of small proteins, specifically antithrombin (AT) III, protein C and S. INS children have been observed to develop thrombocytosis and platelet hyper aggregation leading to increased thromboembolic risk.

**Aim of the Study:** To evaluate the coagulation profile and results of platelet functions during active nephrosis in children with steroid sensitive and steroid resistant INS.

**Methods:** Thirty-six children with INS were studied. Twenty-four steroid sensitive and 12 steroid resistant patients were evaluated for platelet function and coagulation parameters during active nephrosis (either at presentation or relapse). The study also included 15 healthy age and sex matched children as control. Doppler imaging was done to confirm thromboembolic events.

**Results:** Thrombo-embolic complications were observed in 14/36 patients, diagnosed both clinically and/or by doppler. Prolonged PTT was observed during active nephrosis, compared to control. Hypoalbuminemia and hyperlipidemia contributed to hypercoagulability. Mean platelet aggregation to ADP and Ristocetin were significantly elevated among both groups (108.3±10.8% and 104.8±13.2% respectively). Levels of ATIII were significantly reduced, further adds to thrombotic risk.

**Conclusion:** Thromboembolic complications are not uncommon in nephrotic children. Platelet hyperaggregability positively correlated with VTE in INS children and can be a beneficial predictor of thrombosis during active nephrosis especially those past history of VTE.

**Keywords:** Coagulation profile, Nephrotic syndrome, Platelets, Thrombosis.

**Running title:** Predictive Value of Platelet Aggregation Responses and Coagulation Parameters for Thromboembolic Risk in Idiopathic Nephrotic Syndrome.

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**geget: The Journal of the Egyptian Society of Pediatric Nephrology and Transplantation (ESPNT)**

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## Introduction

Idiopathic Nephrotic syndrome (INS) is the most common renal disease among children. INS is characterized by glomerular membrane dysfunction leading to excess filtration of proteins of intermediate size (40-200 kDa) together with different macromolecules in the urine [1]. Examples of these substances that are lost in the urine are albumin, immunoglobulins, hormones, and proteins of the coagulation cascade [2]. INS is associated with massive proteinuria (urinary protein levels of  $> 40 \text{ mg/m}^2/\text{h}$ ), hyperlipidemia, edema, hypoalbuminemia and hypercoagulability [3].

One of the most serious, yet not well considered, consequences of INS is thromboembolism of the arterial or venous circulation such as deep vein thrombosis (DVT) with or without pulmonary emboli and renal vein thrombosis [4, 5]. The overall risk for thromboembolism in pediatric patients with INS has been estimated to be around 3%, mostly of venous thrombosis [6]. Thromboembolic events are rare but generally lethal for INS patients [7]. It occurs in 1–27 % of children. Variable incidence is attributed to etiology, degree of the INS and on imaging techniques used to diagnose thromboembolism [8]. A few studies have demonstrated elevated levels of prothrombotic factors, for example, factor V (FV), FVIII, von Willebrand factor (vWF), and fibrinogen [9]. This hypercoagulability may also be a result of small proteins lost in the urine because of INS, specifically antithrombin (AT) III, protein C and S [1, 9].

As coagulation abnormalities appear to play a significant role for venous thrombotic events, platelets are

considered a critical agent of thrombosis among INS children [10]. Frequently patients with INS have elevated platelet counts and platelet hyper-aggregation that led to increased risk of thromboembolism [5, 10]. Spontaneous platelet aggregation responses together with increased responses to some aggregating agents have been observed [11]. Hypoalbuminemia and hypercholesterolemia have been proposed to play an important role in the pathogenesis of increased platelet counts in INS [10]. Proteinuria and loss of proteins that enhance platelet function led to increased platelet aggregation. Changes in platelet surface markers in INS patients demonstrate increased platelet activation [12]. Besides patients with INS have demonstrated elevated levels of different active substances secreted by platelets such as  $\beta$  thromboglobulin, platelet-derived growth factor (PDGF) and interleukin-7 (IL-7) [13].

The objective of the present study was to evaluate the coagulation profile and the results of platelet function testing during the state of active nephrosis in children with steroid sensitive and steroid resistant NS and to assess the correlation between the hypercoagulable state and the occurrence of thromboembolic events in these patients. We also studied the correlations between the coagulation profile and altered platelet functions on one hand and the different clinical presentations and laboratory results (kidney function tests and level of proteinuria) of these patients on the other hand.

## Methods

This cross-sectional study was conducted at our university pediatric

hospital, over one-year period (February 2019-February 2020). Patients were chosen from the pediatric nephrology outpatient clinic and the inpatient wards. Patients were enrolled in the study after taking informed consent from their guardians and after the acceptance of the medical ethical committee of the Faculty of Medicine. We studied 36 patients with INS. The patients were divided into 2 groups; group A included 24 patients with steroid responsive NS and group B which included 12 patients with steroid resistant NS. The diagnosis of INS was done based on the following criteria:

- Heavy proteinuria  $\geq 40$  mg/ m<sup>2</sup>/h, or
- Serum albumin  $\leq 2.5$  mg/L and generalized edema.

Steroid-sensitive NS (SSNS) diagnosis was made when remission of NS was obtained within the period of 4 weeks of adequate treatment with prednisolone 60 mg/m<sup>2</sup>/day, maximum 80 mg/day.

Steroid-resistant NS (SRNS) diagnosis was made when patients had continuous proteinuria despite 4 weeks of adequate treatment with prednisolone 60 mg/m<sup>2</sup>/day [14].

Patients were selected randomly, with the following exclusion criteria:

- INS children with evidence of cardiac disease.
- INS children with any other concomitant risk factor that may increase thromboembolic risk e.g. patients with central venous catheters and/or positive family history of thromboembolic disease in absence of NS.
- INS patients with congenital or infantile onset nephrotic syndrome.

Venous blood samples were taken from all patients during the stage of active

nephrosis by sterile venipuncture in vacutainer tubes (BD Life Science, Le Pont de Claix, France). The first blood sample, filled to capacity containing buffered sodium citrate 0.105 M (approximately 3.2%) with a whole blood-to-anticoagulant ratio of 9:1 vacutainer tube, was used for platelet function testing, antithrombin III measurement and the coagulation profile (PT and PTT) on Sysmex CS-2000i coagulation analyzer (Sysmex Corporation, Japan). Another plain tube was used to separate sera samples for serum albumin, kidney function tests and lipid profile which was performed using Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany). 24-hour urine sample was collected into 1ml of 1% normal HCL from each of the participants for 24h urinary protein quantitation using Cobas Integra 800.

A Color Doppler ultrasound was done for the diagnosis of renal vein thrombosis, Inferior vena cava (IVC) thrombosis or lower limb DVT. It was done for all cases immediately after being enrolled in the study at the time of presentation with picture of nephrosis (edema and massive proteinuria). Cases with documented thrombosis were notified at our center for further management and follow up. No one of our patients suffered from neurologic symptoms during the period of the study that is why neuroimaging was not done.

Fifteen healthy age and sex matched children (who did not suffer from any renal or cardiac disease and had no past history of thromboembolic events and negative family history of thromboembolism) were evaluated as a control group to standardize the results of laboratory testing.

For platelet function and coagulation testing, platelet rich plasma (PRP) and platelet-poor plasma (PPP) were prepared and tested within 4 hours of samples collection according to the platelet physiology subcommittee of SSC/ISTH recommendations for the standardization of LTA [15]. Briefly, vacutainer tubes were gently mixed by inversion and centrifuged at 200 g for 10 min at room temperature (RT). The resultant PRP was collected into polypropylene tubes and kept at RT till testing. The primary tube was centrifuged again at 1500 g for 15 min at RT to produce PPP, which was collected as described for the PRP. Platelet counts were then performed on the PRP using a Sysmex XN analyzer (Sysmex, Kobe, Japan) to confirm that there were enough platelets to perform aggregometry ( $>150 \times 10^9/L$ ). Platelet aggregation was measured by monitoring the changes in light absorbance occurring in response to ADP 5  $\mu M$  and Ristocetin 1.25 mg/mL (all from HYPHEN BioMed, France). On the analyzer, 100% aggregation was defined by observing the absorbance of 140  $\mu L$  PPP to which 20  $\mu L$  normal saline had been added. For analysis of PRP, the analyzer pipetted 140  $\mu L$  PRP into a plastic stir-bar cuvette and then 20  $\mu L$  of agonist (7:1 PRP to agonist) was added, where 0% aggregation is defined by the instrument. Absorbance was monitored for 600 seconds while the contents of the cuvette were stirred at a constant speed of 800 rpm. Finally, the measuring traces were saved, and data was analyzed by the analyzer's PC software system. The results were compared with the baseline values and they were analyzed by using the SPSS, version 10 software. AT (III) level was assayed on PPP samples using

INNOVANCE Antithrombin reagent kit according to the manufacturers' specifications and standard laboratory methods.

## Results

Our study was carried on 36 patients with INS: 21 males and 15 females. The patients were divided into 2 groups according to their response to steroids. In Group A (SSNS) patients, their mean age was  $6.1 \pm 2.7$  years and in group B (SRNS) patients it was  $11.2 \pm 2.4$  years (Table 1). Hypertension was found in 17/36 patients; 8 SSNS patients had hypertension while 9 out of 12 children with SRNS had hypertension.

Thrombo-embolic complications were observed in 14/36 patients (38%); 9 were among group A, while the remaining 5 belonged to group B. Among group A, 7 patients were symptomatic and only 2 patients were diagnosed by doppler US. This shows that 30 % of our patients had symptomatic TE, while 8 % had subclinical TE. While in group B, 4 patients were symptomatic and only one patient was accidentally discovered by doppler. This shows that 30 % of our patients had symptomatic thrombo-embolic complications, while 8 % were subclinical. Patients of the SSNS group who presented with VTE included a patient with renal vein thrombosis who presented with loin pain and gross hematuria, 2 other patients with renal vein thrombosis who just complained of gross hematuria, 2 patients with IVC thrombosis both of them presented with progressive abdominal distension and extensive lower limb oedema and 4 patients with LL DVT. Patients who presented with LL DVT included 2 children aged 7 and 11 years

who complained of unilateral calf muscle pain and tenderness and 2 other children aged 9 and 11 years who were asymptomatic and DVT was accidentally diagnosed by Doppler US examination during relapse.

Patients of the SRNS group who presented with VTE included 2 children who were symptomatic for unilateral LL DVT, one child who was accidentally diagnosed to have unilateral renal vein thrombosis during screening by abdominal Doppler US examination and 2 children with renal vein thrombosis who complained of loin pain and gross hematuria.

There was no statistically significant difference between both groups regarding the incidence of current thromboembolic events. Our patients did not have any positive family history of thromboembolism (that may be suggestive of an underlying hereditary thrombophilia. Thirteen patients had history of previous thromboembolic events during the stage of active nephrosis and/or relapse of NS. Eight patients were among group A and the remaining 5 belonged to group B. There was no

statistically significant difference between the two groups as regards the history of previous thrombosis (**Table 2**).

The biochemical profile of the studied patients shown in (**Table 3**), 24h urinary protein excretion (**Figure 1**) showed a highly statistically significant difference between both groups (p value <0.001). SRNS children had higher levels of proteinuria in comparison with SSNS patients. Serum albumin levels were significantly lower in groups A and B in comparison with controls. Serum triglycerides and total cholesterol levels were significantly higher in patients as compared to the control group.

Regarding the coagulation profile in our patients, mean PTT values were significantly different among the studied patients in relation to control (mean PTT of group A was 44.6 seconds, and that of group B was 44.8 s, while in the control group was 38.6 s) with a p value of <0.001 (**Figure 2**). However mean PT values for the studied groups was 13.5 s in group A and 14.0 s in group B & it did not attain any statistically significant difference when compared to control values (**Table 4**).

**Table 1 :** Demographic characteristics of the studied subjects.

Parameter		Group A (SSNS) (24)	Group B (SRNS) (12)	Control (15)
Gender	Male (N) (%)	14 58.3	7 58.3	9 60
	Female (N) (%)	10 41.7	5 41.7	6 40
Age (years)	(Mean $\pm$ SD)	6.1 $\pm$ 2.7	11.2 $\pm$ 2.4	7.4 $\pm$ 2.8

**Table 2 :** Number of thromboembolic events in the studied patients.

		Group A (SSNS) (24)	Group B (SRNS) (12)	X <sup>2</sup>	P value*
Current thrombo-embolism	Negative	15	7	8.891	0.059
	Renal vein	3	3		
	IVC	2	0		
	LL DVT	4	2		
History of thrombo-embolism	Positive	8	5	0.178	0.673
	Negative	16	7		

\*Chi square test

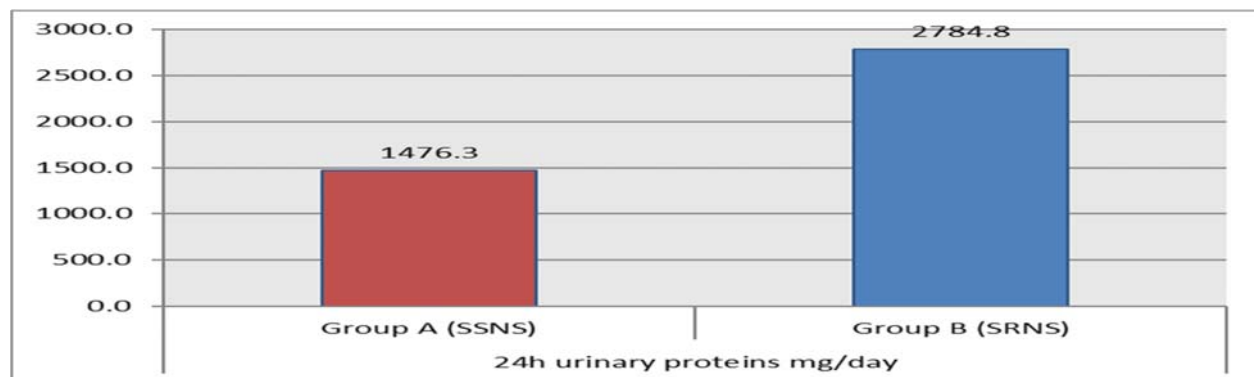


**Table 3 :** Comparison of biochemical profile between studied groups.

Parameter	Group A Mean ± SD	Group B Mean ± SD	F	P value*	Sig
24h urinary proteins (mg/day)	1476.3 ± 617.6	2784.8 ± 453.8	34.975	<0.001**	HS
Serum albumin (g/dL)	2.1 ± 0.4	2.4 ± 0.4	106.207	<0.001	HS
Total cholesterol (mg/dL)	229.1 ± 39.5	291.8 ± 35.8	58.349	<0.001**	HS
Triglycerides (mg/dL)	190.7 ± 46.7	223.3 ± 54.3	17.256	<0.001**	HS

\* One Way ANOVA test

\*\* Post Hoc test shows significant difference between group A and B



**Figure 1 :** 24h urinary protein excretion among the studied groups.

Platelet aggregation responses to both ADP and Ristocetin were significantly increased in both group A (mean  $108.3 \pm 10.8\%$  for ADP and  $104.8 \pm 13.2\%$  for Ristocetin) and group B patients (mean  $116.3 \pm 12.4\%$  for ADP and  $104.7 \pm 7.1\%$  for Ristocetin) in relation to the control group (p value <0.001). Moreover, there was also a significantly increased agreeability in response to ADP in Group B patients when compared with group A (p value <0.001) (Table 4). On the other hand, AT III levels were significantly decreased among the studied patients (mean was 72% for both groups A and B) when compared to control group with a p value <0.001 (Table 4, Figure 2).

Total serum cholesterol and triglycerides together with 24h urinary protein excretion in the studied patients were positively correlated with the presence of hypertension among them (r value of 0.462, 0.156 and 0.187 respectively). While there was no

significant correlation between hypertension and the coagulation profile or with platelet aggregation and ATIII in the studied patients (Table 4).

In order to obtain additional correlation between the coagulation abnormalities and platelet functions, we stratified our 36 patients according to the presence or absence of recent thrombo-embolic events into two groups: group I were nephrotic patients with current thrombo-embolic complications (N = 14); and group II comprised nephrotic patients without current thrombo-embolic complications (N = 22). Platelet aggregation responses to the studied agonists and AT III levels were assessed in each group to correlate the coagulation and platelet abnormalities with thrombotic complications in NS patients. The only significant difference concerns platelet aggregation to ADP which was increased to  $115.2 \pm 10.3\%$  in group I, while it was  $105.8 \pm 8.1\%$  in group II (p value 0.003).

Platelet aggregation to Ristocetin & AT III levels did not differ significantly in the two groups (Table 5).

As 13 of the studied patients had a previous past history of thromboembolism, we tried to further evaluate the correlation between platelet aggregation response to ADP in the patients who had current thromboembolic

event (N = 14) and whether they had previous past history of thrombosis or not. Platelet aggregation response was significantly correlated to the occurrence of thromboembolic events in NS patients who had previous history of thrombosis as shown in (Table 6).

**Table 4 :** Comparison between platelet function, ATIII & coagulation profile between studied groups.

Parameter	Group A Mean ± SD	Group B Mean ± SD	F	P value**	Sig
Platelet Aggregation (ADP %)	108.3 ± 10.8	116.3 ± 12.4	64.864	< 0.001 a,b	HS
Platelet Aggregation (Ristocetin)	104.8 ± 13.2	104.7 ± 7.1	12.550	< 0.001 a & b, c	HS
PT (s)	13.5 ± 1.2	14.0 ± 0.95	2.313	0.06 a & b, c	NS
PTT (s)	44.6 ± 5.0	44.8 ± 4.4	10.923	< 0.001 a & b, c	HS
Anti thrombin III (%)	72.8 ± 5.9	72.9 ± 3.5	29.736	< 0.001 a,b	HS

a ,b Post Hoc test shows significant difference between group A and B/ \*\*One Way ANOVA test  
a & b, c Post Hoc test shows significant difference between both group A and B patients with controls

**Table 5 :** Coagulation parameters in patients with thrombo-embolic events (group I N = 14) versus patients without thrombo-embolic events (group II N = 22).

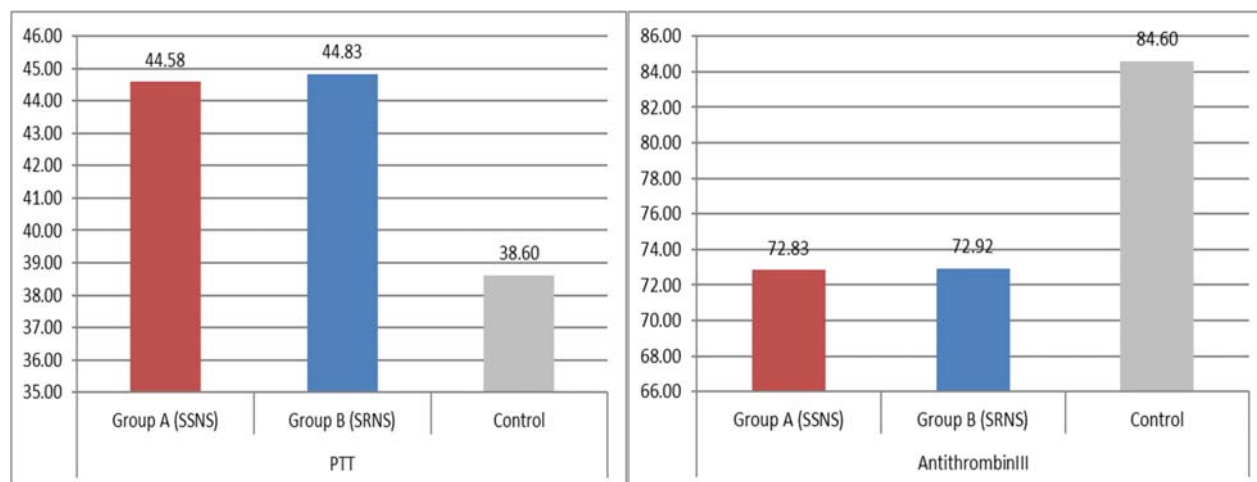
Parameter	Group I (Mean ± SD)	Group II (Mean ± SD)	P value*	Sig.
Platelet Aggregation (ADP %)	115.2 ± 10.3	105.8 ± 8.1	0.003	S
Platelet Aggregation (Ristocetin)	100.3 ± 8.2	101.7 ± 7.3	0.995	NS
Antithrombin III (%)	70.8 ± 4.9	71.2 ± 2.5	0.35	NS

\* One Way ANOVA test.

**Table 6 :** Correlation of Platelet aggregation response to ADP with past of history of thromboembolism in patients with thromboembolic events.

Platelet response to ADP (%)		History of Thrombo-embolism		P value
		Positive (n, %)	Negative (n, %)	
		Renal V thrombosis	2 (15.4)	
	114.2 ± 13.5	109.1 ± 10.4		
IVC thrombosis	2 (15.4)	0 (0.0)		
	107.1 ± 10.9	103.4 ± 11.4		
LL DVT	4 (30.8)	2 (8.7)		
	114.2 ± 9.3	104.8 ± 7.1		

\*: Significant



**Figure 2 :** PTT and Antithrombin III levels among the studied groups.

## Discussion

Thrombo-embolic complications represent a rare but devastating adverse effect of childhood INS. It occurs in almost 3% of NS cases in children [16]. Venous thromboembolism (VTE) is the predominant type of thromboembolic disease in INS children. Although most reported cases of VTE in NS children were clinically symptomatic and diagnosis was confirmed by imaging, it should be noted that subclinical VTE may still be more common in childhood NS than is previously thought. Childhood VTE in NS has significant long-term morbidity, with about 6-21% of children suffering from recurrent VTE [6].

The occurrence of nephrotic syndrome related VTE may be multifactorial in children, the disease related hypercoagulopathy being the most important factor besides therapy (Corticosteroids and Diuretics) related risk [17]. Platelet hyperaggregability, together with the loss of low molecular weight proteins and enzymes that are implicated in the procoagulant, anticoagulant, and fibrinolytic systems may potentiate the coagulopathy in pediatric patients with nephrotic syndrome [6]. Besides,

endothelial dysfunction can be regarded as a prothrombotic factor in adults; however, this relationship has not been fully studied in pediatric nephrotic syndrome [18].

The biochemical abnormalities characteristic of nephrotic syndrome such as severe proteinuria and marked hypoalbuminemia in addition to hypercholesterolemia and hypertriglyceridemia were again confirmed by the results of our study as well as previous reports [19, 20, 21, 22]. In addition to these findings, there was a significantly increased platelet aggregation response with ADP and Ristocetin which were used as agonists in our patients. The mean aggregation responses to ADP (108% in group A and 116% in group B patients) and to Ristocetin (104.8% in group A and 104.7% in group B) were significantly high when compared to control values. Mittal et al., [5] also revealed platelet hyperaggregability in response to ADP but that to Ristocetin was not increased. This platelet hyperaggregability can be attributed to the hypoalbuminemia found in these patients, because albumin is known to inhibit Arachidonic Acid induced platelet aggregation and its



conversion to Thromboxane A2 and other intermediates [23].

In NS abnormalities affecting the major proteins of the coagulation cascade occur. In our patients, mean PTT values were significantly prolonged among the studied patients in relation to the control group (mean PTT of group A was 44.6 seconds, and that of group B was 44.8s) with a p value of <0.001. The same results were found in the study carried out by Sujatha Rani A [23] which revealed that PTT was prolonged in patients with NS compared with healthy controls, while PT in patients was not different from that of healthy controls. This PTT prolongation may be explained by the relative deficiency of coagulation factors that are lost because of severe proteinuria. As none of our patients had a bleeding diathesis so this prolongation seemed to be non-significant clinically. This finding also comes partly in line with the previous work done by Ghanny et al., [24] and Taiwo et al., [22] who reported significantly higher both PT and PTT values among NS children as compared to the controls, but in our study, PT values did not differ significantly among patients. This was explained by the loss of significant amounts of clotting factors of the common coagulation pathway in urine without compensatory increase in the rate of synthesis of these clotting factors. However, the study performed by Farida et al., [25] did not find any statistically significant difference regarding PT and PTT values among NS patients.

In our study, AT III levels were significantly decreased among the studied patients (mean was 72% for both group A and B) when compared to control group with a p value <0.001. This comes in accordance with the results of Mittal et al.,

[5], al-Mugeiren et al., [26] who also found that AT III levels lie in the lower part of the reference interval, which was suggestive of a reduced AT III level attributed to urinary loss and/or consumption during intravascular coagulation.

Till now the following factors can be regarded as additional significant prothrombotic risk in NS, hypoalbuminemia, severe proteinuria, elevated fibrinogen levels, low AT III levels, and hypovolemia [27].

Albeit the incidence of TEM is higher among our patients than previously stated in literature, we can attribute this to the fact that all of our patients were screened for radiologically documented thromboembolic event by doppler imaging which is not done as a routine investigation for children with active nephrosis. Besides, DVT can be asymptomatic or present with non-specific symptoms e.g., leg pain that may pass unnoticed. Our patients may have several concomitant risk factors e.g. dehydration and deficient fluid intake, which could be a result of the hazardous use of diuretics, most commonly Furosemide, added to immobilization and concurrent infections. In addition, many of our patients came from either rural areas or towns far from Cairo, that may cause some delay till presenting to our center.

Since 14 of our patients had radiologic evidence of thromboembolic manifestations, and according to our results platelet aggregation response to ADP correlated significantly with occurrence of VTE, we can conclude that increased platelet aggregability in pediatric patients with NS contribute to the hypercoagulability. In addition, as platelet aggregation response to ADP was

significantly higher in patients with current thromboembolic events and in those who had a positive past history of thrombosis, the results of platelet function can be used as a predictor of occurrence of thromboembolism in NS patients with or without previous history of thrombosis.

Thus, these patients should be fully investigated and carefully examined for a clinical and radiologic evidence of VTE if a higher platelet aggregation response indicates a hypercoagulable state and consequently early intervention can be instituted.

Even though, there are no trials demonstrating the safety and efficacy of thromboprophylaxis for the prevention of NS-related VTE in adults or children, many authors recommended prophylactic therapies for children according to evident prothrombotic risk factors e.g., coagulation abnormalities in addition to regular ambulation, adequate hydration, avoidance of CVC whenever possible, and the use of graduated compression stockings and/or sequential compression devices for bedridden children [8, 28, 29]. However, due to the known overall low incidence of childhood NS-related VTE and that only 3 % of children with NS develop clinically significant VTE while

another 24–25 % are affected by subclinical VTE, identifying platelet hyperaggregability in these patients as a prothrombotic risk factor is helpful to define these high-risk patients [8, 13, 16]. We recommend that that patients with nephrotic syndrome during relapse must be carefully assessed clinically and by doppler US examination to rule out thromboembolic complications. Studies with larger scale should be performed for further evaluation of different platelet function tests and a wider panel of coagulation profile among a bigger number of this category of patients to establish a full panel of workup needed to assess the risk of development of thromboembolism in these patients.

## Conclusion

Thromboembolic complications are not uncommon in children with nephrotic syndrome whether clinical or subclinical. Platelet hyper aggregability was positively correlated with the occurrence of VTE in children with INS. This test can be a beneficial predictor of the risk of development of thromboembolic events in children during active nephrosis especially those with past history of VTE.

## Abbreviations

ADP	adenosine diphosphate	PRP	platelet rich plasma
AT III	antithrombin III	PT	prothrombin time
DVT	deep vein thrombosis	PTT	partial thromboplastin time
FV, FVIII	factor V and factor VIII	RT	room temperature
INS	idiopathic nephrotic syndrome	SRNS	steroid-resistant nephrotic syndrome
IVC	Inferior vena cava	SSNS	steroid-sensitive nephrotic syndrome
LTA	light transmission aggregometry	VTE	venous thromboembolism
PDGF	platelet-derived growth factor	vWF	von Willebrand factor
PPP	platelet poor plasma		

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## Statements

### Ethics approval and consent to participate

The local ethical committee permitted the study under the Helsinki declaration of Bioethics and its later amendments. Informed written consent was obtained from all participants caregivers.

### Consents for publication

The contents and material of the manuscript have not been previously reported at any length or being considered for publishing elsewhere.

### Availability of data and material

All data generated or analyzed during this study are included in this submitted article and its supplementary information files.

### Non-existing conflict of interest

The authors declare no conflict of interest.

### Funding

No funding support or financial disclosure.

**Acknowledgments** None to mention.

<b>Submission</b>	<b>01/09/2020</b>
<b>Acceptance</b>	<b>19/11/2020</b>
<b>Published online</b>	<b>31/12/2020</b>