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Study of A Disintegrin and Metalloproteinase with ThromboSpondin Type 1 Repeats 13 (ADAMTS 13) in Children with Idiopathic Nephrotic Syndrome

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Abstract

Background: Nephrotic syndrome (NS) is a well-known risk factor for arterial or venous thromboembolism (TE). There is higher risk of TE in steroid-resistant NS (SRNS) than in steroid-sensitive NS (SSNS). Objective: The aim of this study was to investigate serum level of von Willbrand factor cleaving protease activity which is known as ADAMTS in children with idiopathic nephrotic Syndrome(INS) and its relation to clinical and laboratory parameters.

Patients and methods: This study was conducted on 120 children with INS including 40 SSNS, 40 SRNS, 40 healthy controls. All subjects are investigated by CBC, 24 hours collected urine analysis for urine volume, urinary proteins, total Serum protein and serum albumin,total serum cholesterol,prothrombi time (PT), partial thromboblastin time(PTT) and Serum ADAMTS 13 activity.

Results: There was highly significant decrease in serum ADAMTS 13 activity in SSNS and SRNS groups when compared to control group while there was no significant difference in serum ADAMTS 13 activity between SSNS and SRNS groups.

Conclusion: Serum ADAMTS 13 activity is a biomarker for endothelial dysfunction and hypercoagulable state. The decreased ADAMTS13 activity in different extent of nephrotic patients (SSNS and SRNS) may be one of the pathogenesis of thrombosis as a common complication of NS.

Recommendation: Regular follow-up of nephrotic patients and estimation of serum ADAMTS 13 level as its decresed level is a risk factors of thrombosis.

Keywords: ADAMTS; Children; Idiopathic; Nephrotic

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Introduction

Many predisposing factors for TE were reported in nephrotic patients, abnormalities in platelet activation and aggregation, activation of prothrombotic factors of coagulation system e.g. factors V,VII, VIII, X, von Willebrand factor (vWF), fibrinogen, and α 2-macroglobulin, decreased activity of fibrinolytic system such as plasminogen [1] and decreased endogenous anticoagulants,antithrombin III, protein C, protein S, and tissue factor pathway inhibitor resulting in local activation of the glomerular hemostasis system [2]. vWF mediates platelet adhesion and aggregation at sites of vascular injury [3]. It is released from the stimulated endothelium as unusually large (UL) multimer [4]. ULvWF favor platelet aggregation and formation of microvascular thrombi [5]. A disintegrin and metalloprotease with thrombospondin type-1 motif 13 (ADAMTS-13) can cleaves and thus converses ULvWF into a less active form [6]. Reduced ADAMTS-13 activity due to gene mutation or presence of autoimmune IgM and IgG inhibitors [7] results in deficient proteolysis of ULvWF with formation of disseminated platelet-rich thrombi in the microcirculation seen in thrombotic microangiopathies (TMA) [8,9].

The aim of this study was to investigate ADAMTS 13 Activity levels in children with idiopathic nephrotic Syndrome and its relation to clinical and laboratory parameters.

Patients and Methods

This study was conducted on 120 children aged from two to seventeen years of both sexes selected from Pediatric Nephrology Units of Pediatric Departments of Tanta University Hospital (TUH) and Fayum University Hospital (FUH), The study was done from June 2016 till June 2017 after approval from Research Ethical Committees of TUH and FUH. A formal written consent of each child' parents was taken separately after explanation and assurance of them. All participants' names were hidden and replaced by code number to maintain privacy of the participant.

The children were divided in three groups Group (1) Forty patients with steroid sensitive nephrotic syndrome, Group (SSNS) (2) Forty patients with steroid resistant nephrotic syndrome (SRNS) and Group (3) Forty healthy controls matched age and sex.

Inclusion criteria

Steroid sensitive nephrotic syndrome (SSNS) and steroid resistant nephrotic syndrome (SRNS) groups were diagnosed according to definitions of Bagga and Strivastava [10].

Exclusion criteria

Other causes of generalized edema as renal, hepatic and heart failure, nutritional and allergic causes, congenital anomalies of the kidney, other causes of thrombo embolic disorders and auto immune diseases.

All children included in this study were subjected to the following:

1. Complete history taking: concerning past history of recurrence, responseveness to steroid therapy, thrombo embolism,
2. Full clinical examination: with special emphasis on Hypertension, peritonitis, thrombosis as a complication of NS.
3. Laborayory investigation including: Complete blood count (CBC), 24 hours collected Urine analysis for urine volume, urinary proteins,Total Serum protein and serum albumin and total serum cholesterol, PT, PTT and Serum ADAMTS 13 activity.

Specimen collection and handling

A 6 ml morning venous blood sample was collected under complete aseptic conditions for assessment of the level of serum ADAMTS 13. Morning urine samples were taken from the 60 children for complete urine analysis. We put 2 ml of the blood in EDITA tube for CBC and the remaining blood allowed for clotting, and the serum separated by centrifugation at room temperature then divided in two Eppendorf tubes. One for the routine examination which was done immediately and the other tube preserved and froze at -20°C prior to the assay.

Serum ADAMTS 13 activity levels

Measured by Human ADAMTS 13 ELISA Microplate Kit which based on sandwich enzyme-linked immune-sorbent assay (ELISA) technology in (ng/ml). Anti-human ADAMTS 13 antibody was pre-coated onto 96-well plates, the Biotin conjugated anti-human ADAMTS 13 antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer.

Results

Demographic data of studied groups are summarized in **Table 1**. There was highly significant decrease in serum ADAMTS 13 activity in SSNS and SRNS groups when compared to control group while there was no significant difference in serum ADAMTS 13 activity between SSNS and SRNS groups (**Table 2**). There was no significant difference in serum ADAMTS 13 activity between males and females in studied patients (**Table 3**). There was significant positive correlation between plasma ADAMTS 13 activity and total serum protien in patients with SSNS (**Table 4**). There was significant positive correlation between plasma ADAMTS 13 activities of patients with SSNS with serum albumin (**Table 4**). There was significant negative correlation between plasma ADAMTS 13 activity and 24 hours urine protein in patients with SSNS (**Table 4**). There was significant negative correlation

Table 1 Demographic data of studied groups.

		Groups			ANOVA or Chi-Square	
		Steroid Senstive Nephrotic Syndrome (SSNS)	Steroid Resistant Nephrotic Syndrome (SRNS)	Control	F or X^2	P-value
Age (Years)	Range	3.5-13	2.5 - 16	3 - 17	X^2 =2.710	0.075
	Mean ± SD	8.03±2.59	7.53 - 3.45	10 ± 4.4		
Weight (kg)	Range	14 - 35	15 - 54	12 - 59	X^2=2.823	0.068
	Mean ± SD	26.3 ± 6.127	25.73 ± 9.54	32.85 ± 14.28		
Sex	Male	22%	32 - 80 %	30 - 75 %	F=3.333	0.189
	Female	18- 45 %	8 - 20 %	10 - 25 %		
SBP	Range	95- 140	90 - 120 %	90 - 125	X^2=0.896	0.414
	Mean ± SD	107.75 ± 9.66	104.25 ± 7.48	107.25 ± 9.53		
DBP	Range	55 - 90	45 - 80	50- 80	X^2=1.127	0.331
	Mean ± SD	65.5 ± 8.75	63.5 - 9.05	67.75 ± 9.24		

Table 2 Routine laboratory data of the studied groups.

		GROUPS			ANOVA		TUKEY 'S Test		
		Steroid sensitive Nephrotic Syndrome	Steroid Resistant Nephrotic Syndrome	Control	F	P-Value	I & II	I & III	II & III
HB % (g/dl)	Range	10.1-12.4	8.9-13.9	9.5-13.2	0.482	0.620			
	Mean ±SD	11.230 ± 0 .264	11.060 ± 1.114	10.920 ± 1.170					
WBc x/cmm	Range	5-14	4-10	5.9-9.9	2.839	0.067			
	Mean ±SD	8.110 ± 2.191	6.770 ± 1.921	7.715 ± 1.237					
Platelet (/cmm)		110-379	112-355	150-350	1.581	0.215			
		219.250 ± 65.952	232.800 ± 57.948	254.500 ± 65.492					
TSP (G/dl)	Range	4-6	4-6.5	6-8	23.379	0.001*	0.398	0.001*	0.001*
	Mean ±SD	4.990 ± 0.829	5.315 ± 0.777	6.600 ± 0.754					
Serum albumin (g/dl)	Range	2.1-3.5	2.1-3.5	3.5-5	77.742	0.001*	0.665	0.001*	0.001*
	Mean ±SD	2.720 ± 0.385	2.840 ± 0.402	4.275 ± 0.518					
24 hr Unire PTN (mg/24hr)	Range	206-2120	266-3162	100-200	31.932	0.001*	0.822	0.001*	0.001*
	Mean ±SD	1200.900 ± 503.304	1113.980 ± 617.465	153.000 ± 29.576					
Creatinine (mg/dl)	Range	0.3-1	0.5-2.5	0.2-1	5.923	0.005*	0.392	0.100	0.003*
	Mean ±SD	0.710 ± 0.215	0.845 ± 0.429	0.495 ± 0.293					
Urea (mg/dl)	Range	21-42	15-41	15-25	21.916	0.001*	0.020*	0.001*	0.001*
	Mean ±SD	31.350 ± 6.302	26.050 ± 7.749	18.800 ± 2.984					
GFR (ml/min/1.73 m²)	Range	100-165	18-165	100-165	2.998	0.058			
	Mean ±SD	136.000 ± 20.199	123.70 ± 35.861	143.20015.867					
PTT (sec)	Range	26-45	25-43	25-45	0.742	0.481			
	Mean ±SD	340650 ± 5.743	35.000 ± 6.164	32.700 ± 7.299					
PT (sec)	Range	11-16	11-15	11-16	0.526	0.594			
	Mean ±SD	12.465 ± 1.399	12.395 ± 1.033	12.795 ± 1.476					
Serum cholesterol(mg/dl)	Range	200-384	266-510	140-200	74.983	0.001*			
	Mean ±SD	321.200 ± 57.726	363.250 ± 67.942	169.500 ± 19.050					

Table 3 Comparison between studied groups as regards to ADAMTS 13.

Groups	ADAM TS 13 (ng/ml)		ANOVA	
	Range	Mean ± SD	F	P-value
Group(1)Steroid Sensitive Nephrotic Syndrome	0.07-4.62	1.03 ± 1.02	34.6	<0.001*
Group(2)Steroid Resistant Nephrotic Syndrome	0.06-1.79	0.95 ± 0.55		
Control Group(3)	2.78-20.3	7.52 ± 4.83		
TUKEY'S Test				
Resistant and Sensitive	Sensitive and Control	Resistant and Control		
0.995	<0.001*	<0.001*		

between plasma ADAMTS 13 activities of patients with SRNS with serum cholesterol level (**Table 4**).

In our study, there was no patients with thrombotic episodes in different studied patient groups. There was significant positive correlation between plasma ADAMTS 13 activity and both systolic and diastolic blood pressure in patients with SRNS with **Figures 1 and 2.**

Discussion

Elevated plasma vWF antigen (vWF: Ag) and/or decreased ADAMTS 13 activity are associated with negative outcomes of several disorders [11]. An imbalance between the circulating levels of vWF and ADAMTS 13 has been reported in a number of acquired diseases in adults such as coronary artery disease and myocardial infarction, peripheral arterial disease, ischemic stroke, preeclampsia, inflammatory bowel disease, and liver cirrhosis [12,13] and these findings have been reported also in different diseases of pediatric age such as type 1 diabetes mellitus [14] and ESRD [15].

Our results showed that there is no significant difference between studied groups regarding platelet count. Eneman B. et al found that NS is associated with a significantly increased risk of thrombotic events. Alterations in plasma levels of pro- and anti-coagulant factors are involved in the pathophysiology of venous thrombosis in NS. However, the fact that the risk of both venous and arterial thrombosis is elevated in NS points to an additional role for blood platelets. Increased platelet counts and platelet hyperactivity have been observed in nephrotic children. Platelet hyperaggregability, increased release of active substances, and elevated surface expression of activation-dependent platelet markers have been documented. The mechanisms underlying platelet alterations are probably due to changes in plasma levels of platelet-interfering proteins and lipid changes, as a consequence of nephrosis [16].

Table 4 Correlations between ADAMTS 13 activity and laboratory data and of studied Patients.

Correlations				
	ADAM TS 13(ng/ml)			
	Steroid Senstive Nephrotic Syndrome (SSNS)		Steroid Resistant Nephrotic Syndrome (SRNS)	
	r	P- value	r	P- Value
HB % (g/dl)	-0.032	0.892	0.224	0.342
WBc (x/cmm)	0.204	0.388	0.198	0.403
Platelet (/cmm)	-0.314	0.177	-0.228	0.334
Total serum protein (g/dl)	0.618	0.044*	-0.062	0.796
Serum albumin (g/dl)	0.607	0.005*	-0.082	0.73
24 hr Unire PTN (mg/24hr)	-0.638	0.002*	0.077	0.746
Serum Creatinine (mg/dl)	0.032	0.894	0.307	0.187
Serum Urea (mg/dl)	-0.199	0.399	-0.097	0.685
GFR (ml/min/1.73 m²)	0.242	0.304	-0.086	0.718
PTT (sec)	-0.062	0.794	-0.042	0.86
PT (sec)	-0.105	0.659	-0.21	0.374
Serum colesterol (mg/dl)	-0.272	0.246	-0.498	0.025*
Age (Years)	-0.309	0.184	0.303	0.194
Weight (kg)	-0.331	0.154	0.188	0.426
SBP	-0.141	0.553	0.46	0.041*
DBP	-0.3	0.2	0.485	0.030*

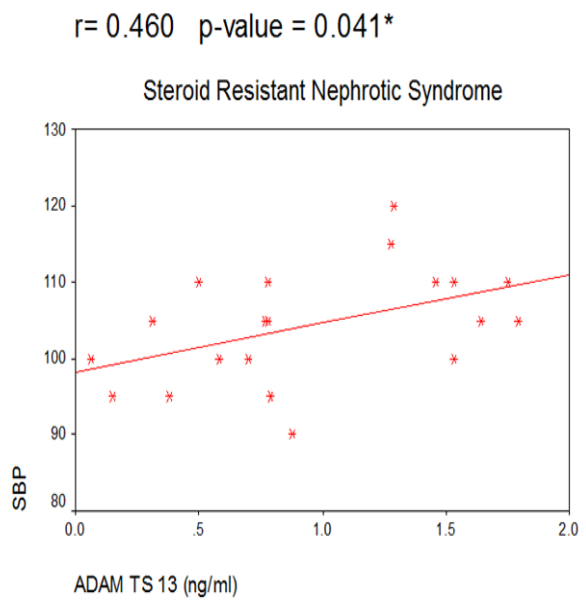

Figure 1 Correlation between plasma ADAMTS 13 levels and SBP of patients with SRNS.

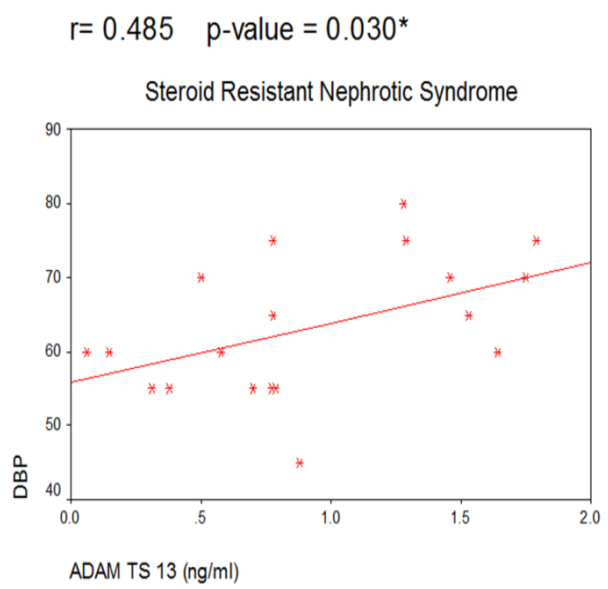

Figure 2 Correlations between plasma ADAMTS 13 levels and DBP of patients with SRNS.

Anand et al. [17] clarifies the importance of coagulation profile in nephrotic syndrome as a high index of suspicion for thromboembolic complications especially in patients with thrombocytosis.

Our results showed significant decrease in total serum protein and serum albumin in both steroid sensitive, steroid resistant NS when compared to control group. This is in agreement with U.S. National library of medicine that defined nephrotic syndrome [18] and Mulukala et al. [19] who stated that NS is manifested by hyperproteinuria, low total serum protein, hypoalbuminemia and edema. While there is no difference in serum albumin and total serum protein between SSNS and SRNS groups.

In our study, there is no significant difference in prothrombin time (PT) and partial thromboplastin time (PTT) between studied groups. This is in agreement with Yalçinkaya et al. [20] who stated that PT, PTT as well as platelet count and mean plasma Protein C activity were similar in the NS group when compared with the control group and in addition no remarkable difference was found in the mean plasma Protein C activity between the steroid sensitive and resistant NS groups. In contrast, the mean

plasma Anti thrombin III (AT III) activity was significantly reduced in patients with NS when compared to controls correspondingly, it was directly correlated with serum albumin and inversely correlated with proteinuria.

But our findings regarding PT and PTT are not in agreement with Anand NK, et al. [17] who stated that thromboembolic complications of NS should be suspected in patients with thrombocytosis, hyper fibrinogenemia, prolonged APTT and in children with low levels of AntithrombinT-III, protein C and protein S.

Limited studies of ADAMTS 13 have been done on pediatric nephritic patients. Our results showed that there was highly significant decrease in serum ADAMTS 13 activity in SSNS and SRNS studied groups when compared to control group. This is in agreement with JiangLiQion, who stated that ADAMTS 13 activities of both steroid sensitive group and steroid resistant group are decreased when compared with normal control group [21].

In this work, there was no significant difference in serum ADAMTS 13 activity between studied SSNS and SRNS groups. This is in agreement with JiangLiQion, stated that no differences are observed in ADAMTS 13 activity among Steroid Resistant nephrotic Syndrome group when compared to Steroid Sensitive nephrotic Syndrome group [21].

Our results showed that there was no significant difference between studied males and females as regard ADAMTS 13 activity in studied patients.

Correlation analysis of our study showed that there that there is a significant positive correlation between plasma ADAMTS13 activity of patients and total serum protien and serum albumin in patients with SSNS while there is a significant negative correlation between plasma ADAMTS 13 activity of patients and serum cholesterol level in SRNS group. This correlation results are in agreement with JiangLiQion, who stated that plasma ADAMTS 13 antigen of patients with NS is positively correlated with serum albumin ($r=0.385$, $P<0.01$) and negatively correlated with total blood cholesterol ($r=-0.317$, $P<0.01$) [21].

Correlation analysis of our study showed that there is significant negative correlation between plasma ADAMTS 13 activity and 24 hours urine protien of patients with SSNS. This is in agreement with LiQion J [21], who stated that ADAMTS 13 activity of patients with nephrotic syndrome is negatively correlated with the quantitative measurement of 24 hours urinary protein ($r=-0.242$, $P<0.05$).

Conclusion

It is recommended for regular follow-up of children with nephrotic syndrome and early estimation of reduced serum ADAMTS 13 level to control this risk factor of thrombosis. It may be possible to re-engineer ADAMTS 13 protease to improve specific activity, which may offer preventive and therapeutic benefits to nephrotic patients in pediatric age with thromboembolic complications.

Recommendations

Regular follow-up of nephrotic patients and estimation of serum ADAMTS 13 level as its decresed level is a risk factors of thrombosis.

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