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Significance of Von Willebrand Factor-Cleaving Protease Activity in Patients with Chronic Kidney Diseases

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This study was carried out in nephrology unit of the pediatric department at Benha University Hospital and nephrology clinic, Benha University.

Abstract: ***Background: Chronic kidney disease (CKD) is a major health problem worldwide with increasing incidence and prevalence. ADAMTS13 is a proteolytic enzyme that is responsible for degradation of large multimers of VWF released in the plasma by endothelial cells and platelets. Aim of work: The aim of our study was to assess plasma concentration of von Willebrand factor cleaving protease activity in pediatric patient with chronic kidney disease. Subject and Methods: This case control study was conducted on patients attending Pediatric Nephrology unit and outpatient nephrology Clinic pediatric departmentfrom January 2020 to November 2020. They were divided as following: Patient group (Group I): twenty children previously diagnosed with chronic kidney disease on hemodialysis. Patient group (Group II): twenty children previously diagnosed with chronic kidney disease and on conservative therapy. Control group (Group III): twenty children apparently healthy from outpatient clinic.All patients were subjected to history (age, sex, residence, birth order, level of education, socioeconomic status), examination, laboratory investigations and Von Willebrand factor cleaving protease enzyme was assayed. Results: Lower levels of plasma concentration of von Willebrand factor cleaving protease activity was more among hemodialysis than chronic kidney disease on conservative therapy. A statistically significant positive correlation between Von Willebrand factor cleaving protease enzyme e and G.F.R. Conclusion: CKD patients have statistically significant lower levels of plasma concentration of von Willebrand factor cleaving protease activity when compared to healthy controls. Concentration of von Willebrand factor cleaving protease activity was significantly decreased in the studied HD patients with thrombosis compared with those without thrombosis.***

Keywords: CKD- hemodialysis- von Willebrand factor-cleaving protease activity-thrombosis

1. Introduction

Chronic kidney disease (CKD) is a major health problem worldwide with increasing incidence and prevalence that is threatening to bring on the onset of a real 'epidemic' [(1)].

Hemodialysis (HD) process is associated with increasing thrombotic trend especially owing to platelets and clotting factors activation [(2)].

ADAMTS13 is a proteolytic enzyme that is responsible for degradation of large multimers of VWF released in the plasma by endothelial cells and platelets [(3)].

Given the dependency of kidney function on the adequate blood flow to the glomerulus, the kidney is one of the most susceptible organs to thrombotic events in its microcirculation. The imbalance between VWF and ADAMTS13 may promote thrombosis in kidney vessels[(4)].

Aim of work
The aim of our study was to assess plasma concentration of von Willebrand factor cleaving protease activity in pediatric patient with chronic kidney disease

2. Methods

This case control study was conducted on patients attending Pediatric Nephrology unit and outpatient nephrology Clinic from January 2020 to November 2020. Apparently healthy control children were taken from outpatient clinic. Laboratory work was conducted in Clinical Pathology Department.

They were divided as following:

- Patient group (Group I): twenty children previously diagnosed with chronic kidney disease on hemodialysis.
- Patient group (Group II): twenty children previously diagnosed with chronic kidney disease and on conservative therapy according to Schwartz formula[(5)].
- Control group (Group III): twenty children apparently healthy from outpatient clinic.

Inclusion criteria: All children diagnosed with chronic kidney disease less than 18 years.Chronic kidney diseases patients on dialysis and conservative therapy. Both sexes were included.

Exclusion criteria: Multiple congenital anomalies. Metabolic disorders. Malignancy.Sepsis.

Methods
Children were subjected to history taking to fulfill needed data:

A. History taking:
- Full history taking including the onset of diagnosis of renal disease.
- Drugs used and their doses.
- History suggestive of acute metabolic complications, as (sweating, headache, blurring of vision, tremors, convulsions, and coma)
- Prenatal history, birth history and family and history of similar renal problems.
- Nutritional and vaccination history.

B. Clinical examination:
General examination:
- Vital signs: pulse, blood pressure, respiratory rate and body temperature.
- Anthropometric measures: weight, height and mid arm circumference.
- Upper limb and lower limb examination.

Local examination:
- Full examination of heart, chest and abdomen.
- Full neurological examination.

C. Laboratory investigations:
- Complete blood count.
- Blood Urea Nitrogen (BUN).
- Serum creatinine.
- Microalbumin in urine.
- Random blood glucose.
- Serum electrolytes (sodium, potassium, calcium, phosphorus).
- Arterial blood gases (ABG)
- Von Willebrand factor cleaving protease enzyme was assayed once by enzyme-linked immunosorbent assay (ELISA) technique in patients and control groups.

Blood sampling
Five of venous blood were collected from each participants and divided into two parts:
- First part: Two millimeters on EDTA, ethylene-diamine tetra-acetic salt (1.2mg / mL) as an anticoagulant, were used to evaluate CBC.
- Second part: Three millimeters were loaded into a serum separating tube. The tube was kept at room temperature for 30 minutes till coagulation, and then centrifuged (at 1500 rpm for 15 minutes). Serum was separated and the resultant serum was used for clinical chemistry tests.
- Urine sample: second voided morning urine sample was collected in a sterile container, ten millimeters were separated for immediate estimation of urine creatinine and albumin in all subjects
- A Heparinized syringe was used for ABG

Methods

- Blood Urea Nitrogen (BUN), serum creatinine, random blood sugar, serum calcium and phosphorus were performed by standard methods using Biosystem A15 (Barcelona, Spain) chemical auto analyzer by appropriate chemical principles.
- Estimated G.F.R was measured by using Schwartz formula which is:

  Estimated G.F.R ***(ml/min/1.73m2)*** $= \frac{(\mathbf{K} \times \mathbf{Height\ cm})}{\mathbf{s.creatinine(mg/dl)}}$.

  K=Constant ***(0.33 in preterm neonate,0.45 in infants and 0.55 in other children)***[5]**.**
- Serum electrolytes sodium and potassium were done using Ion selective electrode ST200plus Sensa core, India
- ABG was done using blood gases analyzer Cobas b 121-Roche Germany
- Urinary microalbumin was done using BTS-350 semiautomated analyzer using kits supplied by Biosystem (Barcelona, Spain)

The determination of Von Willebrand factor cleaving protease enzyme was performed by enzyme-linked immunosorbent assay, using the Von Willebrand factor cleaving protease enzyme Kit supplied by (Sun Red , China).Catalouge No. 201-12-5258-48T. Its kit was based on sandwich enzyme-linked immune-sorbent assay technology. Senstivity was 0.041ng/ml and detection range was 0.05- 10 ng/ml

Statistical analysis: The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.Mean, Standard deviation (± SD) for parametric numerical data, while Median and range for non-parametric numerical data.Shapiro test was done to test the normality of data distribution. Significant data was considered to be nonparametric.Student T Test was used to assess the statistical significance of the difference between two study group means. Chi-Square test was used to examine the relationship between two qualitative variables. Correlation analysis: To assess the strength of association between two quantitative variables.

3. Results

There was statistically significant difference between Group I, Group II and Group IIIregarding age and gender.Mean value of age was higher among group I than group II (14.18, 7.85**) p value = 0.000**Mean value of age was higher among group I than group III (14.18, 5.22) **p value = 0.32**Mean value of age was higher among group I than group III (7.85, 5.22) **p value = 0.000(Table 1).**

Table 1: Comparison between Group I, Group II and Group IIIregarding demographic data

			Group I	Group II	Group III	F.test	P. value
Age (year)	Range		2.0 - 18.0	1.0 - 15.0	1.0 - 12.0	29.511	0.000**
	Mean ± SD		14.18 ± 4.49	7.85 ± 3.79	5.22 ± 2.89		
Gender	Female	No.	12	4	7	X^2 6.910	0.032*
		%	60.0%	20.0%	35.0%		
	Male	No.	8	16	13		
		%	40.0%	80.0%	65.0%		

There was statistically significant difference between Group I, Group II and Group III regarding Von Willebrand factor cleaving protease enzyme. Mean value of **Von Willebrand factor cleaving protease enzyme** was lower among group I than group II (0.67, 2.43) **p value = 0.000**Mean value of **Von Willebrand factor cleaving protease enzyme** was lower among group I than group III (0.67, 4.28) **p value = 0.000**Mean value of **Von Willebrand factor cleaving protease enzyme** was lower among group II than group III (2.43, 4.28) **p value = 0.000(Table 2).**

Table 2: Comparison between Group I, Group II and Group III regarding Von Willebrand factor cleaving protease enzyme

		Group I	Group II	Group III	F.test	P. value	
Von Willebrand factor cleaving protease enzyme (ng/ml)	Range	0.06 - 1.50	0.95 - 4.10	2.00 - 6.50	58.562	0.000**	P1=0.000**
	Mean ± SD	0.67 ± 0.46	2.43 ± 1.08	4.28 ± 1.39			P2=0.000**
							P3=0.000**

** High significant

P1--→ between Group I and Group IIP2-→ between Group I and Group IIIP3-→ between Group II and Group III

There was statistically significant difference between cases had thrombosis among Group I and cases hadn't thrombosis among Group Iregarding Von Willebrand factor cleaving protease enzyme.

Mean value of **Von Willebrand factor cleaving protease enzyme** was lower among cases had thrombosis among Group I than cases hadn't thrombosis among Group I (0.44, 0.78) **p value = 0.000(Table 3).**

Table 3: Relation between thrombosis and Von Willebrand factor cleaving protease enzyme among Group I

		Cases had thrombosis among Group I (No.=7)	Cases hadn't thrombosis among Group I (No.=13)	t.test	P. value
Von Willebrand factor cleaving protease enzyme(ng/ml)	Range	0.06 - 0.90	0.4 - 1.50	73.19	0.000**
	Mean ± SD	0.44 ± 0.16	0.78 ± 0.45		

* Significant **High significant

Table (4) show there were statistically significant positive correlation between Von Willebrand factor cleaving protease enzyme and (DBP, RR, HB(gm/dl), TLC, GFR(ml/min/1.73 m2), PH, HCO3), while there were statistically significant negative correlation between Von Willebrand factor cleaving protease enzyme and (Age Years), Duration of renal failure , Random blood sugar (mg/dl), Urea (Mg/dl), Creatinine before hemodialysis session (mg/dl) or without hemodialysis, BUN (mg/dl), Serum K, Serum ph+(2.7-4..5 mg/dl)), while there was no statistically significant difference between Von Willebrand factor cleaving protease enzyme and other numerical data.

Table 4: Correlation between Von Willebrand factor cleaving protease enzyme and other numerical variables

Correlation	Pearson's correlation	
	r	p
Age (years) * Von Willebrand factor cleaving protease enzyme	-0.622-	0.000**
Duration of renal failure (years) * Von Willebrand factor cleaving protease enzyme	-0.181-	0.044*
Duration of hemodialysis (years) * Von Willebrand factor cleaving protease enzyme	-0.282-	0.228
duration of hemodialysis session (hours) * Von Willebrand factor cleaving protease enzyme	0	1
HR * Von Willebrand factor cleaving protease enzyme	0.212	0.104
SBP * Von Willebrand factor cleaving protease enzyme	-0.027-	0.840
DBP * Von Willebrand factor cleaving protease enzyme	0.275	0.033*
RR * Von Willebrand factor cleaving protease enzyme	0.404	0.001*
Temperature * Von Willebrand factor cleaving protease enzyme	0.249	0.055
HB (gm/dl) * Von Willebrand factor cleaving protease enzyme	0.299	0.020*
TLC * Von Willebrand factor cleaving protease enzyme	0.329	0.010*
Platelets * Von Willebrand factor cleaving protease enzyme	0.071	0.589
Random blood sugar (mg/dl) * Von Willebrand factor cleaving protease enzyme	-0.310-	0.016*
Urea (Mg/dl) * Von Willebrand factor cleaving protease enzyme	-0.707-	0.000**
Creatinine before hemodialysis session (mg/dl) or without hemodialysis * Von Willebrand factor cleaving protease enzyme	-0.729-	0.000**
creatinine after hemodialysis session (mg/dl) * Von Willebrand factor cleaving protease enzyme	.008	0.975
BUN (mg/dl) * Von Willebrand factor cleaving protease enzyme	-0.707-	0.000**
GFR (ml/min/1.73 m2) * Von Willebrand factor cleaving protease enzyme	0.778	0.000**

Serum Na * Von Willebrand factor cleaving protease enzyme	-0.163-	0.214
Serum K * Von Willebrand factor cleaving protease enzyme	-0.457-	0.000**
Serum ph.+(2.7-4.5 mg/dl) * Von Willebrand factor cleaving protease enzyme	-0.306-	0.017*
Serum ca++(mmol/L) (4-4.8) * Von Willebrand factor cleaving protease enzyme	0.156	0.023*
PH * Von Willebrand factor cleaving protease enzyme	0.496	0.000**
HCO3 * Von Willebrand factor cleaving protease enzyme	0.704	0.000**

* Significant ** High significant

4. Discussion

Our study showed that, a male predominance (80%) among group II, andthis agreeswith**Harambat et al.,** [6] who found the incidence and prevalence of CKD is greater in males than females because of the higher frequency of congenital abnormalities of the kidney and urinary tract (CAKUT) in males**.**

This study showed that, there was statistically significant difference between Group I, Group II and Group III regarding Von Willebrand factor cleaving protease enzyme. Mean value of Von Willebrand factor cleaving protease enzyme was lower among group I than group II **(0.67, 2.43) p value = 0.000.** Mean value of Von Willebrand factor cleaving protease enzyme was lower among group I than group III **(0.67, 4.28) p value = 0.000.** Mean value of Von Willebrand factor cleaving protease enzyme was lower among group II than group III **(2.43, 4.28) p value = 0.000**and this agrees with**El-Gamasy et al.,** [7] who found in their study, CKD patients have statistically significant lower levels of serum ADAMTS 13 when compared to healthy controls. This may explain the role of kidneys in ADAMTS13 synthesis, in its metabolism or its release into plasma by injured endothelial cells especially in patients with chronic kidney disease. Our result also agrees with**El Hady et al.,** [8] who aimed to evaluate ADAMTS13 in patients under maintenance hemodialysis (HD This case–control study was conducted on 60 patients on HD for more than 6 months. Moreover, 20 healthy individuals served as a control group. They found that, mean ADAMTS13 serum level was found to be lower in HD groups of patients compared with control group.But this results were in disagreement with other published papers e.g. **Scheiflinger et al.,** [9]as they stated that the marked elevation in plasma level of vWF leads to increased ADAMTS 13 synthesis as a compensatory mechanism. This compensatory elevation in ADAMTS13 Ag level to increase ADAMTS13 activity which may be responsible for keeping VWF/ADAMTS13 Ag and VWF/ADAMTS13 activity ratios unchanged.

On the other hand, this study agrees with **Taniguchi et al., [10],** who found about the association between reduced ADAMTS13 and diabetic nephropathy, who found lower ADAMTS13 levels in HD patients, suggesting a potential role of the kidneys function regarding ADAMTS13 synthesis or metabolism, regardless of other known sources of ADAMTS13. and this also agrees with the study done by **Rios *et al.*, [2]**, that showed ADAMTS13 plasma levels were reduced and VWF was increased in HD patients, as compared with healthy controls. Also, in **Elzorkany et al., [11]** study, there was a significant statistical decrease was observed in ADAMTS13 plasma levels in HD patients than in the control group.

Additionally, this study showed that mean value of Von Willebrand factor cleaving protease enzyme was lower among cases had thrombosis among Group I than cases hadn't thrombosis among Group I **(p value = 0.000)**.This agrees with **Cohen-Hagai*et al.*, [12]** who demonstrated that higher concentrations of VWF were associated with increased breakdown of ADAMTS13. This breakdown is performed by either protease present in plasma such as thrombin or secreted by granulocyte elastases. Higher thrombin levels might increase ADAMTS13 turnover in hypercoagulable states. Also, according to the study done by **Ruggeri, [13]**, the imbalance between ADAMTS13 and VWF levels does not explain the development of vascular access thrombosis (VAT) in HD patients by itself, although it should contribute for the hypercoagulability state. Therefore, additional studies to identify other risk factors are warranted and essential for better management of HD patients.

Consequently, this study showed there was statistically significant negative correlation between Von Willebrand factor cleaving protease enzyme and age.**(p value=0.000)**,and this agrees with **Elzorkany et al., [11]** who found that, there was statistically significant negative correlation between ADAMTS13 and age. This study also found that there was statistically significant negative correlation between Von Willebrand factor cleaving protease enzyme and duration of renal failure.**(p value=0.04)**, and this agrees with **Shen et al., [14]** Furthermore, this study revealed that there was statistically significant negative correlation between Von Willebrand factor cleaving protease enzyme and duration of CKD along with showing that there was statistically significant positive correlation between Von Willebrand factor cleaving protease enzyme and diastolic blood pressure (DBP). **(p value=0.03).** And these results are in agreement with**Abd-Allah Ebrahim, [15]** who showed that There was a significant positive correlation between plasma vWF-CP activity and both systolic and diastolic blood pressure.

This study shows there were statistically significant negative correlation between Von Willebrand factor cleaving protease enzyme and (Urea Creatinine, BUN).**(p value=0.000).** And these results are in agreement with **Shen et al., [14]**who aimed to assess the relationships between ADAMTS13 and CKD patients. showed that negatively correlated with kidney functions and ADAMTS13.

Moreover, the study showed there were statistically significant positive correlation between Von Willebrand factor cleaving protease enzyme and calcium, and This agrees with **Jakobi et al., [16]**who found that, there were statistically significant positive correlation between Von Willebrand factor cleaving protease enzyme and calcium**(p value=0.02)**. Thestudy also showedthat there was statistically significant negative correlation between Von

Willebrand factor cleaving protease enzyme and RBS.**(p value=0.01).**

It's important to mention that our study showed that there was no significant correlation between ADAMTS 13 and duration of dialysis of studied patients.**(p value=0.22)**In spite of previously published relation between dialysis duration and serum ADAMTS13 level which may indicate the role of uremic state in the pathogenesis of decreased level of ADAMTS 13 **(17).**

5. Conclusion

Based on the results of the current study, it can be concluded that CKD patients have statistically significant lower levels of plasma concentration of von Willebrand factor cleaving protease activity when compared to healthy controls. Lower levels of plasma concentration of von Willebrand factor cleaving protease activity was more among hemodialysis than chronic kidney disease on conservative therapy. A statistically significant positive correlation between Von Willebrand factor cleaving protease enzyme and GFR. Concentration of von Willebrand factor cleaving protease activity was significantly decreased in the studied HD patients with thrombosis compared with those without thrombosis.

6. Declaration

- **Ethical approval and consent to participate:** Ethical permission for the study was obtained from the parents who was fully informed about all study procedures and **their informed written consent was obtained prior to the children enrollment in the study**. This study was approved by the ethical committee of the faculty of Medicine, Benha University Hospitals.
- **Ethical committee reference number:** MS-35-12-2019
- **Funding**: The authors have no financial relationships relevant to this article to disclose and the study is self-funded by the author.
- **Availability of data and materials**:The corresponding author had full access to all of the data and takes full responsibility for the veracity of the data and statistical analysis**.**
- **Authors' contributions:**All authors reviewed the final version of the manuscript, Dr.Wessam El-Menshawy and Dr Amro Mahmoud analysed and interpreted the patient data regarding the progress of the renal disease. Dr Asmaa Adel performed the Von Willebrand factor cleaving protease enzyme assay Dr Abd-El hameed El-Hamshary was a major contributor in writing the manuscript.
- **Consent for publication**:Not applicable.
- **Competing interests :**There is no financial nor non-financial competing interests.
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