Egypt. J. Rad. Sci. Applic. **Vol. 35,** No. 1-2, pp. 13-18 (2022)

Egyptian Journal of Radiation Sciences and Applications
http://ejrsa.journals.ekb.eg/

EGYPTIAN ATOMIC ENERGY AUTHORITY

Procoagulant FVIII and Anticoagulant Protein C in Renal Failure Patients on Hemodialysis

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THE PREVALENCE of kidney diseases is increasing. Several population-based studies have shown that chronic kidney disease (CKD) increases the risk of venous thrombosis. Unfortunately, studies that describe this association are limited in providing explanatory information. The aim of the present study is to estimate the procoagulant Factor 8 (FVIII) and anticoagulant Protein C (PC) in patients on hemodialysis. Thirty patients (21 males, 9 females) diagnosed with end stage renal disease (ESRD) from those attending the hemodialysis unit of Aswan Armed Forces Hospital and 30 healthy controls were included in the current study. Assessment of glomerular filtration rate (GFR) was performed using ^{18}F-fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT). Prothrombin time (PT) and partial thromboplastin time (PTT) were measured using a coagulation analysis apparatus. Plasma activities of PC and FVIII were measured automatically on IL coagulation system kit instrumentation. A significantly increased plasma level of FVIII and a significantly decreased plasma level of PC was found in patients with CKD on hemodialysis when compared to the control group (P= *0.0226 & 0.000103 respectively)*. Levels of FVIII were inversely associated with kidney function while there was a significant positive correlation between renal function and plasma PC levels in the group of patients. *(*r = *- 0.27 & 0.53 respectively)*. It could be concluded that Patients with ESRD on hemodialysis were shown to have an increased coagulation tendency, especially with high FVIII and decreased protein C activities. The abnormal hemostatic profiles may contribute to the elevated risk of thrombotic events.

Keywords: Chronic kidney disease (CKD), End stage renal disease (ESRD), Factor 8 (FVIII), Protein C (PC).

Introduction

Chronic kidney disease (CKD) inflicts between 8% and 16% of the current world's population and is often underdiagnosed by physicians. It is described as a glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m^2, albuminuria of at least 30 mg/day, or biomarkers of kidney injury (such as hematuria or histopathological abnormalities such as polycystic or dysplastic kidneys) lasting for more than 3 months. Internationally, CKD is most commonly related to diabetes or hypertension, but other etiologies such as infection, glomerulonephritis, and environmental factors (such as air pollution, herbs, and insecticides) are common in many developing countries in Asia or Africa. Genetic risk factors may also contribute to CKD risk. For example, sickle cell trait and the presence of 2 *APOL1* risk alleles, both prevalent in the dark race people of African descent, but not European descent, may double the risk of CKD (Jha et al., 2013).

The normal blood coagulation process involves the participation of the platelets, vascular

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Received 12/03/2022; Accepted 21/06/2022
DOI: 10.21608/EJRSA.2022.126870.1132

endothelium, coagulation system, anticoagulant system and fibrinolytic system. The standard coagulation pathway represents a balance between the pro-coagulant pathway that is responsible for clot formation and the mechanisms that inhibit the same pathway beyond the injury site. Imbalance of the coagulation system may occur in the perioperative period or during critical illness, which may be secondary to numerous factors leading to a tendency of either thrombosis or bleeding (Palta et al., 2014).

Coagulation Factor VIII is a non-covalent heterodimer containing a heavy chain (A1-A2-B domains) and a light chain (A3-C1-C2 domains) that circulates as an inactive procofactor in complex with von Willebrand factor. Metal anions are essential to the integrity of factor VIII, with Cu and Ca ions stabilizing the heterodimer and creating the active configuration, respectively. Activation of factor VIII is catalyzed by thrombin and appears dependent on interactions with both anion-binding sites I and II, and the conversion of the heterodimer to the active cofactor, factor VIIIa. This protein, comprised of A1, A2, and A3-C1-C2 subunits, is labile due to the poor affinity of the A2 subunit. Association of factor VIIIa with factor IXa and the formation of the intrinsic factor Xase complex are membrane-dependent and involve multiple inter-protein contacts that remain poorly understood. This complex catalyzes the conversion of factor X to factor Xa, a reaction that is essential for the propagation stage of coagulation. The role of factor VIIIa in this complex is to increase the catalytic efficiency for factor Xa generation by several orders of magnitude. Mechanisms for the down-regulation of factor Xase focus upon inactivation of the cofactor and include dissociation of the A2 subunit as well as activated protein C-catalyzed proteolysis (Fay, 2006).

The protein C system provides an important way of control of blood coagulation by regulating the activities of factor VIIIa (FVIIIa) and factor Va (FVa), cofactors in the activation of factor X and prothrombin, respectively. The system comprises either multi-molecular complex already bound to cell membrane surfaces or circulating proteins that eventually assemble into protein complexes on cell surfaces. Vitamin K-dependent protein C, the key component of the system, circulates in blood as zymogen to an anticoagulant serine protease. It is efficiently activated on the surface of endothelial cells by thrombin bound to the membrane protein thrombomodulin. The endothelial protein C receptor (EPCR) further stimulates the protein C activation. Activated protein C (APC) together with its cofactor protein S inhibits coagulation by degrading FVIIIa and FVa on the surface of negatively charged phospholipid membranes. Efficient FVIIIa degradation by APC requires not only protein S, but also intact FV, which like thrombin is a Janus-faced protein with both procoagulant and anticoagulant potentials. The protein C system is physiologically important, and genetic defects affecting the system are the most common risk factors of venous thrombosis (Dahlbäck & Villoutreix, 2005).

Most coagulation test methods mirror changes in a particular blood coagulation step, but they have difficulty completely verifying the entire coagulation process in patients with CKD.

Patients with CKD commonly have blood coagulation disorders. The resulting thrombotic complications have become the most common cause of death and one of the difficulties in renal replacement therapy among patients with CKD (Wattanakit et al., 2008).

The aim of the current work is to study the correlation between the coagulation factor VIII together with its regulating factor protein C and the renal function status of patients on renal replacement therapy as well as the possibility of using any of these factors as prognostic biomarkers for the follow up of this condition.

Experimental

The present study was performed on a selected group of 30 patients (21 males, 9 females) with diagnosed end stage renal disease from those attending the hemodialysis unit of Aswan Armed Forces Hospital in the period from June 2020 to September 2020. Their ages ranged between 34-66 years. All underwent dialysis three times weekly. All patients had either hypertension, diabetes mellitus (DM), (or both) or systemic lupus erythematosus (SLE) or glomerulonephritis (GN). The study included 30 patients. 10 patients with hypertension, 15 patients with DM, 17 patients with both DM and HT, 3 patients with SLE and 2 patients with GN. Exclusion criteria were as follows: pregnant women, patients with coagulation disorders such as hemophilia,

Von Willebrand disease, clotting factor deficiencies, hypercoagulable states, deep venous thrombosis, thrombosis of the visceral, cerebral, or pulmonary venous beds, antiphospholipid syndrome, patients with active cancer or a history of cancer in the previous year. The control group comprised another group of 30 ages and gender matched apparently healthy subjects who attended their routine checkup visits to the outpatient clinic. They were considered as healthy volunteers with normal vital signs, complete blood count, fasting and postprandial blood glucose, liver and kidney functions.

Measurement of glomerular filtration rate (GFR) was conducted using ^{18} F-fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT). The direct measurement of GFR (mGFR) was preferred to be used in the current study as opposed to estimated GFR (eGFR) using various commonly used formulas. In previous cross-sectional studies, the average error of eGFR was ± 30% of mGFR. Thus, in a patient with mGFR of 60 mL/min, eGFR could range between 42 to 78 mL/min. Furthermore, many patients were misclassified according to CKD stages using eGFR. Thus, eGFR formulas are not reliable indicators of true GFR in patients (Luis-Lima & Porrini, 2017).

Blood samples were collected from all participants in suitable vacutainers. Sera for urea and creatinine and citrated blood for prothrombin time (PT), partial thromboplastin time (PTT), protein C (PC), factor 8 (FVIII) assays were obtained.

Urea and creatinine were measured using a routine laboratory method on Hitachi 971 instrument (Roche Diagnostics, GmbH, D,68298 Mannheim).
- Prothrombin time (PT) and partial thromboplastin time (PTT) were measured by a suitable coagulation analyzer apparatus (Wondfo Biotech, China).
- Plasma PC and FVIII activity were measured automatically on an IL coagulation system (Kit instrumentation laboratory company - Bedford MA 01730-2443, USA) according to the method described by Roshan et al. (2019).

Results

The current study comprised 30 cases (21 males, 9 females) with end stage renal failure undergoing hemodialysis of different etiologies such as hypertension, diabetes mellitus type 2 (or a combination of both), systemic lupus erythematosus, glomerulonephritis as shown in Table 1. Their ages ranged between 34 and 66 (mean age was 53± 14.5 years). The control group comprised 30 healthy age and sex matched subjects (mean age was 51± 12.5 years) with normal laboratory and radiological investigations.

TABLE 1. General criteria of the subjects belonging to the studied groups

Parameter	Control group (n= 30)	Cases on dialysis group (n= 30)
Age (mean ± SD)	53± 14.5	51 ± 12.5
M/F ratio	21:9	20:10
Hypertension cases	---	n=10
Diabetes Mellitus Type II cases	---	n=15
Diabetes Mellitus Type II with Hypertension cases	---	n=17
Systemic Lupus Erythematosus cases	---	n= 3
Glomerulonephritis cases	---	n= 2

In the current study, GFR values were significantly lower in hemodialysis patients compared to those of the control group while the reverse was true for serum urea and creatinine levels. Values of PT and PTT were noted to be significantly higher in hemodialysis patients compared to those of the controls. Plasma level of factor VIII was found to be significantly higher in hemodialysis patients compared to that of the control group. On the other hand, plasma level of PC was observed to be significantly lower in hemodialysis patients compared to that of the control group. The various parameters measured in the present study are illustrated in Table 2.

In the present study, plasma level of factor VIII plasma level correlated negatively with GFR and positively with serum creatinine level and both correlations were found to be significant. Moreover, plasma level of protein C correlated positively with GFR and negatively with serum creatinine level and both correlations were found to be highly significant. However, the correlation between plasma factor VIII and PC was not found to be significant. The various correlations established in the current study are shown in Table 3.

TABLE 2. Comparison between the means of different parameters belonging to studied groups (using student's t test)

Parameter	Control group (n=30)	Cases group (n= 30)	T value	P value	Significance
Urea (mg/dl)	13.53 ± 4.34	102.5 ± 27.23	17.67	<0.00001	HS
Creatinine (mg/dl)	0.87 ± 0.18	8.63 ± 2.05	20.65	<0.00001	HS
GFR (mL/min/1.73 m^2)	6.13 ± 1.77	99.36 ± 19.13	26.57	<0.00001	HS
PT (seconds)	11.93 ± 0.37	14.08 ± 0.91	11.72	<0.00001	HS
PTT (seconds)	29.76 ± 3.33	33.91 ± 4.07	4.24	0.000041	HS
Factor VIII (IU/dl)	94.8 ± 18.42	106.72 ± 25.39	- 2.04	0.0226	S
Protein C(IU/dl)	104.13 ± 12.67	65.88 ± 39.8	3.96	0.000103	HS

N.B. S= Significant, HS= Highly significant.

TABLE 3. Pearson's correlation between various parameters in the study

Parameters	R value	R^2 Value	P value	Significance
Factor VIII vs. Creatinine	0.27	0.07	0.032	S
PC vs. Creatinine	- 0.57	0.32	< 0.00001	HS
Factor VIII vs. GFR	- 0.27	0.06	0.048	S
PC vs. GFR	0.53	0.28	0.000013	HS
Factor VIII vs. PC	0.11	0.01	0.41	NS

N.B. S= Significant, HS= Highly significant, NS= Non-significant.

Discussion

Thrombotic complications have become the most common cause of death and one of the difficulties in renal replacement therapy among patients with CKD (Huang et al., 2017). FVIII is one of the important coagulation factors in the coagulation pathway that has been shown to be associated with an increased prevalence of thromboembolic events (Bash et al., 2009). In the current study, elevated plasma FVIII levels were observed in patients with CKD on hemodialysis. Patients with CKD commonly present with changes in the levels of various inflammatory cytokines (Kaysen, 2001). Proinflammatory substances can activate procoagulant factors and result in elevated levels of particular hemostatic factors (Margetic, 2012).

In previous studies, patients with end-stage renal disease (defined as proteinuria >3 g/24 h) were shown to have elevated levels of factor VIII, and von Willebrand factor (Ocak et al., 2014). Because von Willebrand factor and factor VIII are markers of endothelial damage, it might be that endothelial damage, which is associated with chronic kidney disease, leadng to increased factor VIII and von Willebrand factor levels and eventually to venous thrombosis (Dmitrieva & Burg, 2014), (Kamphuisen et al., 2001).

Plasma level of PC was observed to be significantly lower in hemodialysis patients compared to that of the control group. This could be explained by the interplay between blood and dialyzer membrane surfaces and the use of anticoagulants in the hemodialysis circuit. The latter factors induce acute and chronic activation of platelets, resulting in platelet exhaustion along with consumption of protein C (PC) and protein S (PS) (Ichinose et al., 2019). One study has pointed out to the recovery of PC activity occurring after renal transplantation (Ghisdal et al., 2011).

Other studies showed a significantly greater prevalence of anti-protein C antibodies and anti-protein S antibodies in uremic patients on maintenance hemodialysis with thrombosis of vascular access, indicating that in uremia, in addition to endothelial damage, other pathogenetic mechanisms may cause a state of hypercoagulability (Molino et al., 2005; Nojima et al., 2002).

In the correlation analysis, FVIII levels were inversely associated with GFR and the correlation was found to be statistically significant. This was similar to the result of a study by Huang et al. (2017) who reported a significant inverse correlation between FVIII and GFR. This could be explained by the loss of the normal excretory function

and a reduction in the removal of procoagulant substances with the progressive renal impairment and damage of a large number of renal units (Mihai et al., 2019).

In the current study, there was a highly significant positive correlation between GFR and plasma PC level in the group of dialysis patients. A previous study of plasma PC in hemodialysis patients revealed a similar correlation with decreased level of activity of this important factor (Storozhuk et al., 2020). Additionally, functional impairment of PC activation during hemodialysis has also been reported in past studies thus suggesting a possible new mechanism of extracorporeal thrombogenesis (Voigt et al., 2019). Inversely, a study by Takagi et al. (1999) found elevated levels of activated protein C in patients with chronic renal failure including those receiving hemodialysis.

There was a significant positive correlation between creatinine and factor VIII and a highly significant negative correlation between creatinine and PC in the current study revealing the role of uremic toxins accumulation in ESRD and coagulation disorders. The prolonged PT and PTT increase the risk of bleeding in these patients before dialysis which may be related to platelets abnormalities. Although hemodialysis is the main replacement therapy for elimination of toxic products, it is believed to increase the risk of thromboembolism (Pavlou et al., 2021).

Conclusion

Patients with ESRD on hemodialysis are shown to have increased coagulation, especially FVIII and decreased protein C activities. The abnormal hemostatic profiles may contribute to the elevated risk of thrombotic events in these patients. Further studies with more samples and more coagulation factors abnormalities are still required to determine the relationship between the procoagulant factors and fibrinolytic system and the role of hemodialysis in the various clinical outcomes.

Conflict of interest declaration: The authors of the current work state that they do not have any conflicts of interest.

Funding: The current work has not received any particular funding.

Patient consent: The current research was carried out in line with the ethical criteria given in the Declaration of Helsinki 1964, as reviewed in 2013. A written informed agreement of participation in the study was signed by all of the participants.

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