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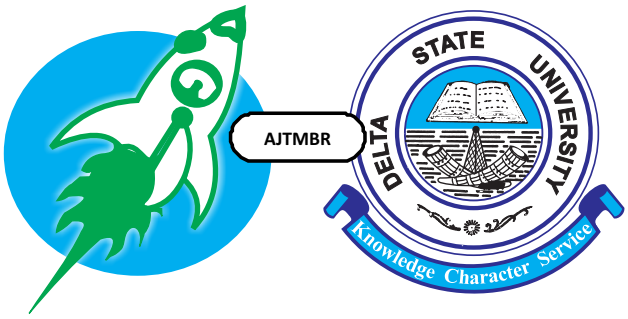

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Focus and Scope

The African Journal of Tropical Medicine and Biomedical Research is a multidisciplinary and international journal published by the College of Health Sciences, Delta State University of Abraka, Nigeria. It provides a forum for Authors working in Africa to share their research findings on all aspects of Tropical Medicine and Biomedical Sciences and to disseminate innovative, relevant and useful information on tropical medicine and biomedical sciences throughout the continent. The journal will publish original research articles, reviews, editorials, commentaries, short reports, case reports and letters to the editor. Articles are welcome in all branches of medicine and dentistry including basic sciences (Anatomy, Biochemistry, Physiology, Pharmacology, Psychology, Nursing etc) and clinical sciences (Internal Medicine, Surgery, Obstetrics and Gynaecology, Dental surgery, Child Health, Laboratory Sciences, Radiology, Community Medicine, etc). Articles are also welcome from social science researchers that document the intermediating and background social factors influencing health in countries of Africa. Priority will be given to publication of articles that describe the application of the principles of primary health care in the prevention and treatment of diseases.

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Plasma Fibrinogen Levels and Protein C Activity in Patients with Chronic Kidney Disease in a Nigerian Tertiary Hospital

Adeyemi O[1] and Awodu OA[2]

ABSTRACT

INTRODUCTION

Haemostatic abnormalities have been observed in patients with chronic kidney disease (CKD). Hypercoagulability and thrombotic complications may contribute to the increased morbidity and mortality associated with CKD. Thrombotic tendency has not been fully evaluated in CKD subjects in our environment. This research was to assess the plasma fibrinogen and protein C activity levels in patients with CKD in a Nigerian tertiary hospital, with the view of deducing their associations with thromboembolic manifestations and providing possible therapeutic interventions.

MATERIAL AND METHODS

A hospital based, cross-sectional survey conducted amongst CKD patients on renal replacement therapy (RRT), who were managed in the renal unit of the University of Benin Teaching Hospital (UBTH). Participants were appropriately matched with apparently healthy control subjects and these were interviewed with a structured questionnaire to obtain data on their clinical details as well as hospital records. Citrated plasma was obtained and analysed to determine the functional protein C activity as well as the fibrinogen levels of both subjects on RRT and apparently healthy control. Haematologic and biochemical parameters were also determined including complete blood count, urea and creatinine and liver function tests. Descriptive and Inferential statistics were performed using Statistical Package for Social Sciences (SPSS) version 20. Results were presented in tables and charts

RESULTS

A total of 222 subjects participated in this study, comprising 74 patients with CKD on conservative management, 74 subjects with CKD on RRT, and 74 apparently healthy control subjects.

The mean age across the groups including subjects on conservative management, RRT, and controls were 54.70±14.42, 49.5±15.73, and 49.3±12.5 years respectively; with the median age of 56-, 52-, and 48.5-years f across the groups respectively.

The mean plasma fibrinogen levels across the groups were 240.4±57.76, 241.8±54.87, 360.9±69.5mg/dl respectively. This was statistically significant. The mean protein C activity for the respective groups was 59.6 ± 24.20, 63.6 ± 28.89, 86.7.±2.6%. Protein C activity was significantly depleted in the CKD subjects (p = 0.002).

CONCLUSION

The study showed a low protein C activity amongst patients with CKD and those with cardiovascular or thromboembolic events, suggestive of thromboembolic risk in these individuals.

Contrary to existing literature, plasma fibrinogen levels were not significantly elevated in patients with CKD, and there was no association with thromboembolic events.

Keywords: Lichen Sclerosus, Paediatric Age, Elderly, Skin of Colour, Nigeria.

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***Corresponding author:** Protein C activity, plasma fibrinogen, chronic kidney disease, renal replacement therapy

INTRODUCTION

Chronic kidney disease (CKD) is increasingly recognized as a public health problem as its incidence and prevalence has increased significantly in recent years in both developed and developing nations and poses a huge economic burden on scarce health resources both in developed countries, while contributing significantly to morbidity, mortality, and decreased life expectancy in the developing ones. This has necessitated renewed interest in global CKD prevention.[1,2]

Data (meta-analysis) from around the world suggest that CKD prevalence is between 13.4% for CKD stages 1–5 and 10.6% for CKD stages 3–5 [3]; affecting an estimated number of over 843.6 million persons globally. [4] However, the situation in Nigeria from a few community-based studies represents a prevalence ranging between 2.5 to 26%. [5]

Patients with CKD exhibit a variety of haemostatic disorders, ranging from increased clotting tendency and reductions in the levels of natural inhibitors of coagulation to defective fibrinolysis [6-9] and bleeding diasthesis [8-11] Their occurrence and severity correlate with the progressive loss of renal function to end-stage renal disease (ESRD). Both bleeding diathesis and thromboembolism have been identified in renal disease. [9-11]

The principal cause of these abnormalities is the uraemic state. [8] The pathogenesis of uraemic bleeding is multifactorial. The most important pathogenetic determinants are increased levels of clotting factors, decreased levels of clotting inhibitors, diminished fibrinolytic activity and platelet hyperaggregability. [6,7] At present, the incidence of bleeding is declining, whereas thrombotic complications have become the predominant cause of mortality. [11,12]

Fibrinogen is an essential component of the haemostatic process, with key roles both in plasmatic clot formation and as a cofactor in the aggregation of platelets. [13] Fibrinogen is a major plasma protein (normal concentration: 1.5-4g/l) which is synthesized in hepatocytes predominantly. It comprises each of three polypeptide chains (Aα, Bβ and γ) linked by disulphide bridges. Thrombin cleaves Aα & Bβ chains to release fibrinopeptides A & B resulting in formation of fibrin monomers that polymerize to form insoluble fibrin clot. [13] Fibrinogen is also an acute phase reactant. [14,15]

Severe fibrinogen deficiency can occur as an inherited disorder, [15, 16] as a result of reduced synthesis secondary to liver failure, [17] and during consumptive coagulopathies,[18] and may result in a life-threatening haemorrhagic diathesis. [16] Similarly, congenital and acquired dysfibrinogenaemias have been described which may lead to a bleeding or thrombotic state. [16-18]

Protein C, a vitamin k- dependent plasma protein, is the key component in a physiologically important anticoagulant system. [19] After its activation on the surface of endothelial cells by a complex of thrombin with thrombomodulin, activated protein C (APC) inhibits coagulation by selectively degrading coagulation factors Va and VIIIa with protein S serving as a cofactor in these reactions. Thus, decreased levels of protein C zymogen may impair the natural inhibition of thrombin generation and contribute to hypercoagulability. [19, 20] Young and middle aged adults heterozygous for protein C deficiency have an increased risk of venous thrombosis. [21,22]

Plasma fibrinogen and protein C, a procoagulant and anti-coagulant respectively have been reported to be altered in CKD. [23, 24] However, the extent of derangement of these haemostatic

proteins may not have been fully determined locally, especially in our environment. Hence, the study was set at assessing the plasma fibrinogen and protein C activity levels in patients with CKD in a tertiary hospital in South-south Nigeria, with the view of deducing their associations with thromboembolic manifestations and providing possible therapeutic interventions.

METHODOLOGY

This is a hospital based cross sectional study conducted in University of Benin Teaching Hospital (UBTH), Benin City, Nigeria between September 2016 and June 2017. The study population comprised of participants with CKD managed in the Nephrology Department of the hospital both at the outpatient clinic and haemodialysis unit. These subjects were categorised into three groups comprising: 74 non-dialysis (conservative) patients, 74 patients on RRT-renal replacement therapy i.e. on haemodialysis or previous renal transplant, and 74 healthy subjects as control (appropriately matched based on age and sex with apparently healthy control subjects).

The sample size was determined using this formula for cross sectional study:
n= (z2pq)/d2.
Where n= minimum sample size
z= Standard normal deviate (1.96)

p= prevalence of CKD in Nigeria. 10% (0.10) [5,25]
The participants were recruited consecutively as they presented in the outpatient clinic and haemodialysis unit. Relevant bio-data and clinical details were collected using a structured interviewer questionnaire as well as from patients' case notes. Plasma fibrinogen was determined using Clauss method [26] (Technoclone Fibrinogen reagent, REF: 5138080); PC activity was assayed using standard chromogenic method (Technochrom Protein C, REF: 5341013). Haematologic parameters were determined using the Sysmex Haematology Autoanalyser, model KN21), serum creatinine was also determined in order to calculate their Estimated Glomerular Filtration Rate (eGFR- Croft Gault formula). Descriptive and inferential statistics were performed using SPSS version 20. Significance level was set at 0.05. Results were presented in tables and charts with percentages for categorical variables, means and standard deviations for numerical variables.

RESULTS

The mean age across the groups including subjects on conservative management (non-HD), RRT, and controls were 54.70±14.42, 49.5±15.73, and 49.3±12.5 years respectively as shown in table 1. The CKD subjects comprising those on conservative management and RRT were categorized according to their eGFR using Cockcroft-Gault formula as defined by the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines, which classified CKD into five stages. (27) Their frequencies are shown in table 2. Majority of the participants on conservative care were in stage IIIA to IV disease, 54(73.0%), while participants on RRT had stage V disease, 43 (58.1%).

TABLE 1: AGE DISTRIBUTION OF STUDY POPULATION

Subjects in groups	Mean± SD (Years)	Median (Years)	Range (Years)
CONSERVATIVE	54.70±14.42	56	23 – 88
RRT	49.5±15.73	52	19 -76
CONTROLS	49.3±12.5	48.5	24 – 82

SD: Standard deviation, RRT: Renal replacement therapy

TABLE 2: DISTRIBUTION OF CKD SUBJECTS ACCORDING TO CKD STAGE

CKD STAGE	CONSERVATIVE n (%)	RRT n (%)	Stat test	P value
I	2 (2.7)	3 (4.7)		
II	5 (6.8)	3 (4.7)		
IIIA	15 (20.3)	2 (2.3)	χ^2=227.4	0.000
IIIB	20 (27.0)	2 (2.3)		
IV	19 (25.7)	21 (27.9)		
V	13 (17.6)	43 (58.1)		

CKD: Chronic kidney disease, RRT: Renal replacement therap

Of the 74 subjects on RRT, 70 (94.6%) were on haemodialysis (HD), while 4 (5.4%) had renal transplant. The Mean duration of illness from time of diagnosis of CKD is 12.7 ± 2.1 months, while for those on RRT upon commencement of HD is 6.3 ± 1.1months.

For those subjects on HD, mean number of HD sessions undertaken is 17.8 ± 4.4. (Table 3)

TABLE 3: CLINICAL HISTORY OF CKD SUBJECTS

	n (%)	Mean ± S.D	Range
CKD (DOI) (Months)	148	12.7 ± 2.1	0.3 – 96.0
RRT (Duration on therapy in months)	74	6.3 ± 1.1	0.3 – 36.0
No on hemodialysis	70 (94.6)		
No of sessions of HD		17.8 ± 4.4	1.0 – 125.0
No transplanted	4 (5.4)		
Hypertensive (DOI in Months)	137 (92.3)	96.3 ± 9.97	
Diabetic (DOI in Months)	78 (53.0)	146.9 ± 14.2	

DOI: Duration of illness, CKD: Chronic kidney disease, RRT: Renal replacement therapy, HD:Haemodialysis

There were significant elevated levels of mean serum creatinine levels among the CKD group compared with the control subjects: 3.26 ± 2.46, 5.81 ± 2.52, 0.70 ± 0.21mg/dl for the subjects on conservative care, RRT, and control subjects respectively (Table 4).
The mean glomerular filtration rate across the groups was 35.4 ± 21.7, 21.5 ± 24.3, 130.0±44.2ml/min/1.73m2 respectively. The differences between the means are significant (p=0.000).

TABLE 4: CLINICAL PARAMETERS AND RENAL STATUS OF STUDY SUBJECTS

	Conservative (A) Mean±SD	**RRT (B) Mean± SD**	**Controls (C) Mean±SD**	**ANOVA F value**	*Overall P*	**AvsB** *p*	**AvsC** *P*	**B vs C** *p*
Weight (Kg)	69.1±11.0	67.4±13.4	71.6 ± 11.1	1.758	0.175	0.465	0.206	0.072
SBP (mmHg)	147.1± 25.49	154.1±24.1	115.9±12.4	52.976	0.000	0.088	0.000	0.000
DBP (mmHg)	87.0 ± 16.3	95.8 ± 16.6	72.2 ± 9.9	36.781	0.000	0.002	0.000	0.000
Creatinine (mg/dl)	3.26 ± 2.46	5.81 ± 2.52	0.70 ± 0.21	85.394	0.000	0.000	0.000	0.000
eGFR(mL/min per 1.73m^2)	35.4 ± 21.7	21.5 ± 24.3	130.0±44.2	203.326	0.000	0.025	0.000	0.000

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, eGFR: Estimated glomerular filtration rate

The mean plasma fibrinogen levels across the groups were 240.4±57.76, 241.8±54.87, 360.9±69.5mg/dl for the subjects on conservative management, RRT, and control subjects respectively. This was statistically significant. Although the mean levels of fibrinogen are within the reference range across the groups, the control subjects had higher levels compared to the subjects with CKD (non- HD and HD subjects).
The mean protein C activity for the respective groups were 59.6 ± 24.20, 63.6 ± 28.89, 86.7. ±2.6%. Protein C activity was significantly depleted in the CKD subjects (p = 0.002) as shown in table 5.

TABLE 5: COAGULATION PARAMETERS OF STUDY SUBJECTS

	Conservative (A) Mean±SD	**RRT (B) Mean± SD**	**Controls (C) Mean±SD**	**ANOVA F value**	*Overall P*	**AvsB** *p*	**AvsC** *P*	**B vs C** *p*
Fibrinogen (mg/dl)	240.4±57.76	241.8±54.87	360.9±69.5	75.779	0.000	0.907	0.000	0.000
PC Activity (%)	59.6 ± 24.20	63.6 ± 28.89	86.7±2.6	6.739	0.002	0.363	0.005	0.001

PC: Protein C. Reference range: Fibrinogen: 150 – 400g/l; Protein C activity: 70 – 140%

Fibrinogen results of four subjects on the conservative group and one on the RRT group were skewed and therefore removed from the analysis. Sixty-six (94.3%), 70 (95.9%) and 57 (77.0%), of the subjects on conservative care, RRT and controls had normal fibrinogen levels with respect to the reference range. However, 17 (23.0%) of the control subjects had elevated fibrinogen levels.

Sixty- five (87.8%) of subjects on conservative management and 53 (71.6%) of RRT subjects had low levels of protein c activity, while 72 (97.3) of the control subjects had normal protein C activity. These are shown in table 6. Plasma fibrinogen levels was significantly higher in control subjects than CKD subjects and was statistically significant ($\chi 2$= 38.561; p = 0.001). However, CKD is significantly associated with low Protein C activity ($\chi 2$= 121.8; p = 0.001)

TABLE 6: FREQUENCY OF PLASMA FIBRINOGEN LEVEL AND PROTEIN C ACTIVITY IN STUDY POPULATION

	Conservative n (%)	**RRT n (%)**	**Control n (%)**	**Total n (%)**	**Stat test**	**P value**
Fibrinogen Level						
Low	4 (5.7)	3 (4.1)	0 (0.0)	7 (3.2)	χ^2= 38.561	0.001
Normal	66 (94.3)	70 (95.9)	57 (77.0)	193 (89.0)		
Elevated	0 (0)	0 (0)	17 (23.0)	17 (7.8)		
Total	70 (100)	73 (100)	74 (100)	222 (100)		
PC Activity Level						
Low	65 (87.8)	53 (71.6)	2 (2.7)	120 (54.1)	χ^2=121.8	0.001
Normal	9 (12.2)	21 (28.4)	72 (97.3)	102 (45.9)		
Total	74 (100)	74 (100)	74 (100)	222 (100)		

RRT: Renal replacement therapy, PC: Protein C

COMPARISON OF MEANS BETWEEN PC ACTIVITY, FIBRINOGEN, PLATELET INDICES, AND CREATININE ACCORDING TO CKD STAGE.

Table 7 shows a comparison of PC activity, fibrinogen, platelet indices and creatinine levels in CKD subjects on conservative management. Apart from serum creatinine that showed a rising trend with declining eGFR which was statistically significant, there was no significant difference in other parameters across the several CKD stages. There seems to be a progressive decline in protein C activity with the exception of stage IIIB and IV and a progressive elevation in plasma fibrinogen levels except for stage 111A, although these were of no statistical significance ($p > 0.05$).

TABLE 7: MEANS OF PC ACTIVITY, FIBRINOGEN, PLATELET INDICES, AND CREATININE ACCORDING TO CKD STAGE IN CONSERVATIVE SUBJECTS

Parameter	I n = 2 mean±SD	II n = 5 mean±SD	IIIA n = 15 mean±SD	IIIB n = 20 mean±SD	IV n = 19 mean±SD	V n = 13 mean±SD	P value
PLT x $10^3/\mu L$	196.5±149.2	276.8±140.8	174.0±70.7	202.7±63.4	221.6±87.4	156.7±59.5	0.053
PCT (%)	0.19±0.11	0.21±0.07	0.16±0.07	0.18±0.05	0.18±0.78	0.143±0.563	0.379
MPV (fl)	10.8±2.40	8.8 ± 1.19	9.3 ± 1.21	9.0 ± 0.92	9.3 ± 1.03	18.2±31.80	0.442
PDW (%)	13.6 ±0.07	13.5 ± 1.90	13.4 ±31.47	13.5 ±1.73	13.1 ± 1.93	12.9 ± 3.28	0.986
Cr (mg/dl)	0.9±0.14	1.0 ± 0.15	1.7 ± 0.9	2.1 ±0.56	3.7± 1.15	7.5 ± 2.45	0.000
FIB (mg/dl)	201.2±0.0	236.1±93.6	207.1±55.5	246.5±58.8	254.5±44.6	258.8 ± 57.7	0.129
PC (%)	70.0±1.34	57.0±19.41	56.4 ±13.65	62.1±22.72	69.1±33.49	45.1 ±17.43	0.126

PLT: Platelet count, PCT: Plateletcrit, MPV: Mean platelet volume, PDW: Platelet distribution width, Creatinine, FIB: Fibrinogen, PC: Protein C activity.

For subjects on HD, there was only a statistical difference in serum creatinine levels with respect to falling eGFR. However, other parameters did not show any significant difference among the CKD stages, as shown in table 8.

TABLE 8: MEANS OF PC ACTIVITY, FIBRINOGEN, PLATELET INDICES, AND CREATININE ACCORDING TO CKD STAGES IN RRT SUBJECTS

Parameter	I mean±SD	II mean±SD	IIIA mean±SD	IIIB mean±SD	IV mean±SD	V mean±SD	P value
PLT x $10^3/\mu L$	188.5 ±6.36	144.5±13.44	112.5±0.71	350.0±4.2	249.6±74.12	265.3±109.3	0.254
PCT (%)	0.810±0.05	0.049±0.02	0.18±.01	0.28±0.01	0.24±0.66	0.24±0.13	0.078
MPV (fl)	9.2±0.28	13.2±2.90	9.70±0.07	9.50±0.14	9.1±1.175	9.1±1.84	0.084
PDW (%)	14.7±2.40	12.7±2.19	19.4±0.04	12.5±0.04	12.3±3.41	12.7±3.99	0.577
Cr (mg/dl)	3.8±4.31	1.3±0.14	1.7±0.01	1.8±0.07	4.8±1.7	7.2±1.76	0.000
FBG (mg/dl)	269.3±13.2	228.8±70.1	193.4±23.2	191.7±10.7	256.6±64.0	235.5±52.2	0.637
PC (%)	50.0±3.3	67.0±29.6	77.8±21.5	57.9±12.2	58.0±28.9	66.9±31.	0.918

PLT: Platelet count, PCT: Plateletcrit, MPV: Mean platelet volume, PDW: Platelet distribution width, Cr: Creatinine, FIB: Fibrinogen, PC: Protein C activity.

Association between Thrombotic Risk Factors/ events with the Levels of Plasma Fibrinogen and Protein C Activity.

In subjects with CKD (both on conservative care and RRT) the following cardiovascular and thrombotic events were observed: angina, 3 (2.2%), thrombotic CVA, 1(0.7%), DVT, 1 (0.7%), and thrombotic vascular access, 1 (0.7%). All of these subjects had normal plasma fibrinogen levels. Thromboembolic events were not associated with low fibrinogen levels as it was not statistically significant (p = 1.000), table 9.

TABLE 9: ASSOCIATION BETWEEN THROMBOTIC RISK FACTORS/ EVENTS WITH THE LEVELS OF PLASMA FIBRINOGEN

Thrombolic Events		Fibrinogen			Total	Test	P value
		Low	Normal	High			
Angina	Yes	0 (0.0)	3 (2.2)	0(0)	3 (2.1)	Fisher's	1.000
	No	7 (100.0)	133 (97.8)	0(0)	140(97.9)		
Thrombotic-CVA	Yes	0 (0.0)	1 (0.7)	0(0)	1 (0.7)	Fisher's	1.000
	No	7 (100.0)	135 (99.3)	0(0)	142 (99.3)		
DVT	Yes	0 (0.0)	1 (0.7)	0(0)	1 (0.7)	Fisher's	1.000
	No	7 (100.0)	135 (99.3)	0(0)	142 (99.3)		
Thrombotic Access	Yes	0 (0.0)	1 (0.7)	0(0)	1 (0.7)	Fisher's	1.000
	No	7 (100.0)	135 (99.3)	0(0)	142 (99.3)		

CVA: cerebro-vascular accident, DVT: Deep vein thrombosis

However, these same subjects had low levels of protein c activity: angina, 3 (2.5), DVT, 1 (0.8%), thrombotic CVA, 1 (0.8%), thrombotic vascular access, 1 (0.8%). However, there was no significant association between low protein C activity and thromembolic events in CKD subjects, table 10.

TABLE 10: ASSOCIATION BETWEEN THROMBOTIC RISK FACTORS/ EVENTS WITH PROTEIN C ACTIVITY

Thrombolic Events		PC Activity		Total	Test	P value
		Low n (%)	Normal n (%)			
Angina	Yes	3 (2.5)	0 (0)	3 (2.0)		
	No	115 (97.5)	30 (100)	145 (98.0)	Fisher's	1.000
DVT	Yes	1 (0.8)	0 (0.0)	1 (0.7)		
	No	117 (99.2)	30 (100)	147 (99.3)	Fisher's	1.000
Thrombotic-CVA	Yes	1 (0.8)	0 (0.0)	1 (0.7)		
	No	117 (99.2)	30 (100)	147 (99.3)	Fisher's	1.000
Thrombotic Access	Yes	1 (0.8)	0 (0)	1 (0.7)		
	No	117 (99.2)	30 (100)	147 (99.3)	Fisher's	1.000

CVA: cerebro-vascular accident, DVT: Deep vein thrombosis

CORRELATION BETWEEN PLATELET COUNT AND INDICES AMONG CKD SUBJECTS

Table 11 shows a strong and significant correlation between the platelet count and PCT in these subjects (r=0.784, p= 0.000), however, there was a weak negative correlation between platelet count and PDW, which was statistically significant (r= -0.205, p=0.027).

TABLE 11: CORRELATION BETWEEN PLATELET COUNT AND PLATELET INDICES AMONG CKD SUBJECTS

	Platelet count	
Platelet Indices	**R**	**p value**
PCT (%)	0.784	0.000
MPV (fl)	- 0.173	0.62
PDW (%)	- 0.205	0.027

PCT: Plateletcrit, PDW: Platelet distribution width, MPV: Mean platelet volume,

DISCUSSION

Chronic kidney disease is an emerging public health problem worldwide, [28, 29] with the increasing cost of care representing a substantial burden in third world nations like Nigeria. [30] End stage renal diseases poses a huge financial burden on family and health care sector and the outcome in most situations is associated with high morbidity and mortality.

Haemostatic abnormalities in patients CKD have been described extensively and these may be complicated by thromboembolic events thereby worsening the prognosis in this group of patients. The roles of these haemostatic alterations in triggering these events remain poorly characterized in our environment.

In this study, the median age of patients with CKD was 56 years (conservative subjects) and 52 years (RRT subjects), setting the middle age population as the most affected by this disease. The age distribution is similar with findings from previous studies which showed peak prevalence in Nigeria between the 3rd and 5th decade of life. [30,31] Diabetes and hypertension are the major co- morbidities seen in the study subjects and are the major risk factors leading to CKD, [31] hence, there is need for aggressive prevention and screening programs to identify individuals in this ageing population at risk of renal insufficiency and institute treatment protocol to slow down the progression to CKD in established cases of renal insufficiency.

Unfortunately, most patients in this part of the world present late and may be at either stage IV or V of the disease. Contributing factors for late presentation may include a poor attitude to health, as such individuals could live in denial of the existence of the disease, financial constraints to seeking treatment from the hospital, instead they consult trado-medical practitioners and there may be poor access to health care centre or specialist care. [32] It was observed from the study that most patients on HD were not regular with their maintenance dialysis sessions due to the financial implications of regular maintenance dialysis of at least 3 sessions per week. Thereby making sampling in most cases to be done at least one week after their last dialysis.

Contrary to findings from preexisting studies that showed elevated levels of fibrinogen in CKD

patients, [23,33] this study revealed normal levels of fibrinogen. The mean levels for the subjects on conservative care, RRT, and controls were 240.4±57.76, 241.8±54.87, and 360.9±69.5mg/dl respectively, reference range for normal individuals being 150 – 400mg/dl.

Martinez et al, from their study reported that the plasma fibrinogen level was increased in CKD patients. [34] This finding also corroborates results from the study done by Ramaprabha et al. [35] The reason behind the elevated level of plasma fibrinogen is due to inhibition of release of platelet derived growth factors and endothelial growth factors which increases the circulating plasma fibrinogen level . Rattan et al., in their study also observed significantly elevated fibrinogen levels. The mean levels of fibrinogen amongst control subjects, stage III and stage IV subjects were 152±6.3mg/dl, 268±5.4mg/dl and 374±5.6mg/dl respectively. [36]

Elevated levels of plasma fibrinogen have also been reported in predialysis as well as in dialysis patients and have been associated with an increased prevalence of coronary heart disease both in the normal situation as well as in dialysis patients. [37] The mechanisms responsible for the hyperfibrinogenemia are not fully understood in dialysis patients. Fibrinogen is an acute-phase protein and ESRD is associated with significant increases in the acute phase. [38,39] One possibility is that fibrinogen synthesis is increased as a component of the acute-phase response. [38,39]

The reason for the normal levels of plasma fibrinogen observed in this study is unclear. The plasma level of this protein is influenced by several conventional risk factors such as smoking, hypertension, and diabetes, [40] and by emerging risk factors such as inflammation. [41] Another possibility is the analytic method (Clauss method) [26] used in this study to assay for fibrinogen which may be less sensitive compared to those used in other studies example, magnetic bead assay. There is scarcity of studies with normal levels of fibrinogen in CKD patients.

It was observed that there was significant reduction in protein C (PC) activity in CKD subjects compared with the controls (p values = 0.005 and 0.001 respectively). This decrease in PC activity was observed to correlate with falling eGFR as the lower mean values were seen among those in the non-dialysis and RRT groups, although this did not show any statistical significance with respect to eGFR ($P > 0.05$). Lai et al. in their study assessed the effect of haemodialysis on natural coagulation inhibitors including protein C (PC), protein S (PS), and antithrombin (AT). Plasma AT, PS, and PC were measured in 20 uremic patients on maintenance haemodialysis immediately before, during, and after dialysis treatment. Their predialysis plasma PC antigen level and functional activity were not different from those of normal controls. A significant correlation between the antigen level and functional activity of PC, PS, and AT was demonstrated in healthy controls, but not in haemodialysis patients, [42] but a progressive increase of functional activity of PC was documented with haemodialysis. Another study observed lower level of natural anticoagulants (PC, PS and AT) in patients' groups than control and they were statistically significant. The levels were lower in group I than group II (20 of them had history of thrombotic events "group I", the remaining 22 patients showed no history of thrombosis" group II" and 20 apparently health control group) and the differences between patients group were statistically significant for protein C ($p < 0.001$). [33]

Faioni et al. investigated the possible changes of natural anticoagulant (PC, PS. And AT) and found reduced PC level in uremic patients. [43] Similar

finding was observed in an Egyptian HD unit. [44] Other studies reported that the effect of dialysis on PC activity are conflicting; partial normalization, reduction, and no change following dialysis treatment have been demonstrated Kant et al. [45] Huang et al., in their study observed PC was lower in patients with CKD stage 5 than that in the controls ($p = 0.024$). [33]

Contrary to existing literature findings, a study among Sudanese CKD patients showed the mean concentration of serum level of PC among CKD patient were significantly elevated when compared with control group ($p = 0.000$). In that study the serum level of PC was significant with age and number of dialysis ($p < 0.004, 0.000$ respectively). [46]

Although the finding of thromboembolic events in this study was low, which was obtained from clinical history, this study might undermine the true incidence of thromboembolic events among patients in this region. Subjects with CKD (both on conservative care and RRT) who had either of the cardiovascular or thrombotic events all had normal plasma fibrinogen levels. Hence thromboembolic events in them were not associated with low fibrinogen levels and it was not statistically significant ($p = 1.000$). Despite the lack of significant association, all subjects who had one thromboembolic event or the other had low protein C activity. A longitudinal study may help to clearly define the association between low PC activity and thrombotic complications in CKD. Although a prospective observational study done by Huang M-J et al. showed no case of thromboembolic event after follow up within the one-year study duration. [33.] Unlike a population-based study, which showed a low PC (but not AT) level was associated with an increased incidence of venous thromboembolism and accounting for ≈2.5% of venous thromboembolic events in that study population. [47]

Therefore, clinical suspicion assessed with the use of prediction scores coupled with tests such a D-dimer and appropriate clinical imaging techniques may enable one determine objectively the presence of a thromboembolic event in this group of patients.

CONCLUSION

A plasma fibrinogen level is significantly higher in control subjects than CKD subjects and was statistically significant. Chronic kidney disease is significantly associated with low Protein C activity. There was no significant association between plasma fibrinogen levels, protein C activity and thromboembolic events in CKD subjects.

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AUTHORS DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. George C, Mogueo A, Okpechi I, et al. chronic kidney disease in low-income to middle-income countries: the case for increased screening. *BMJ Glob Health* 2017;2: e000256. doi:10.1136/ bmjgh-2016-000256
2. Josef Coresh. Update on the Burden of CKD. *J Am Soc Nephrol* 2017; 28: 1020–1022: doi: 10.1681/ASN.2016121374

3. Kovesdy CP. Epidemiology of chronic kidney disease: an update 2022. *Kidney Int Suppl* (2011). 2022 Apr;12(1):7-11. doi: 10.1016/j.kisu.2021.11.003. Epub 2022 Mar 18. PMID: 35529086; PMCID: PMC9073222.
4. Jager K.J., Kovesdy C., Langham R., et al. A single number for advocacy and communication-worldwide more than 850 million individuals have kidney diseases. *Kidney Int.* 2019; 96:1048–1050.
5. Olanrewaju, T.O., Aderibigbe, A., Popoola, A. et al. Prevalence of chronic kidney disease and risk factors in North-Central Nigeria: a population-based survey. *BMC Nephrol* 2020; 21, 467.
6. Jalal, Diana & Chonchol, Michel & Targher, Giovanni. Disorders of Hemostasis Associated with Chronic Kidney Disease. *Seminars in thrombosis and hemostasis*. 2010. 36. 34-40. 10.1055/s-0030-1248722.
7. Adams MJ, Irish AB, Watts GF, Oostryck R, Dogra GK. Hypercoagulability in chronic kidney disease is associated with coagulation activation but not endothelial function. *Thromb Res.* 2008; 123(2):374–380.
8. Mohapatra A, Valson AT, Gopal B, Singh S, Nair SC, Viswabandya A, Varughese S, Tamilarasi V, John GT. Hemostatic Abnormalities in Severe Renal Failure: Do They Bark or Bite? *Indian J Nephrol.* 2018 Mar-Apr;28(2):135-142.
9. Suzuki, Hiromichi. Bleeding Diathesis in Hemodialysis Patients. *Haemodialysis*. 2013. 10.5772/45929(Chapter 4), –. doi:10.5772/52926
10. Ga¨ckler A, Rohn H, Lisman T, Benko¨ T, Witzke O, Kribben A, et al. Evaluation of hemostasis in patients with end-stage renal disease. *PLoS ONE* 2019; 14(2): e0212237.
11. Wu T, Tang LV, Hu Y. Venous Thromboembolism in Kidney Diseases and Genetic Predisposition. *Kidney Dis*. 2022; 8:181–189
12. Nikolova-Vlahova M, Baleva MP, Nikolov PK. Thromboembolism in Renal Diseases [Internet]. Embolic Diseases - Unusual Therapies and Challenges. InTech; 2017. Available from: http://dx.doi.org/10.5772/intechopen.68486
13. Kattula S, Byrnes JR, and Wolberg AS. Fibrinogen and Fibrin in Hemostasis and Thrombosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2017. Volume 37, Issue 3, Pages e13-e21
14. Luyendyk JP, Schoenecker JG, Flick MJ. The multifaceted role of fibrinogen in tissue injury and inflammation. *Blood* (2019) 133 (6): 511–520.
15. Tamayo-Velasco Á, Cebeira MJ, Bombín-Canal C, Acevedo-García RM, Peñarrubia-Ponce MJ. Fibrinogen Deficiency with Thrombotic Manifestations. *Eur J Case Rep Intern Med*. 2022 Jun 28;9(6):003400.
16. Rui Vilar, Richard J. Fish, Alessandro Casini, Marguerite Neerman-Arbez. Fibrin(ogen) in human disease: both friend and foe. *Haematologica* 2020;105(2):284-296
17. Simurda T, Asselta R, Zolkova J, Brunclikova M, Dobrotova M, Kolkova Z, Loderer D, Skornova I, Hudecek J, Lasabova Z, Stasko J, Kubisz P. Congenital Afibrinogenemia and Hypofibrinogenemia: Laboratory and Genetic Testing in Rare Bleeding Disorders with Life-Threatening Clinical Manifestations and Challenging Management. Diagnostics (Basel). 2021 Nov 19;11(11):2140. doi: 10.3390/diagnostics11112140. PMID: 34829490; PMCID: PMC8622093.
18. Susan E. Shapiro. Diagnosis and Management of Dysfibrinogenemia. *Clinical Advances in Hematology & Oncology*. September 2018 - Volume 16, Issue 9
19. Henri H. Versteeg, Johan W. M. Heemskerk, Marcel Levi, and Pieter H. Reitsma. *Physiol Rev*

*2*013 93:1, 327-358
20. Dahlbäck B and Villoutreix BO. Regulation of Blood Coagulation by the Protein C Anticoagulant Pathway: Novel Insights into Structure-Function Relationships and Molecular Recognition. Arteriosclerosis, Thrombosis, and Vascular Biology. 2005; 25:1311–1320
21. Dahbläck B, Stenflo J. The protein c anticoagulant system. In: Stamatoyannopoulos G, Nienbuis AW, Majerus PW, Varmus H (eds). The molecular basis of blood diseases. 2nd ed. Philadelphia: W. B. Saunders, 1994: 599 -627.
22. Broekmans AW, Conard J. Hereditary protein c deficiency. In: Bertina RM (ed). Protein C and related proteins. Harlow Essex, United Kingdom: Churchill Livingstone, Longman, 1988: 160 –168.
23. Nunns GR, Moore EE, Chapman MP, Moore HB, Stettler GR, Peltz E, Burlew CC, Silliman CC, Banerjee A, Sauaia A. The hypercoagulability paradox of chronic kidney disease: The role of fibrinogen. *Am J Surg*. 2017 Dec;214(6):1215-1218. doi: 10.1016/j.amjsurg.2017.08.039.
24. Khurshid S, Tariq R, Qureshi J, Mazher R. Prevalence of Abnormal Thrombophilia Profile in Chronic Kidney Disease. *Int.j. pathol*.2021;19(1): 2-5.
25. Odubanjo MO, Oluwasola AO, Kadiri S. The epidemiology of end-stage renal disease in Nigeria: the way forward. *Int Urol Nephrol*. 2011 Sep;43(3):785-92.
26. Monagle, Paul. Methods in Molecular Biology. Haemostasis. 2013. Volume 992 | | Fibrinogen., 10.1007/978-1-62703-339-8(Chapter 14), 181–192.
27. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N, Eknoyan G. Definition and classification of chronic kidney disease: a position statement from kidney disease: Improving Global Outcomes (KDIGO). *Kidney Int*. 2005 Jun;67(6):2089-100.
28. Wang V, Vilme H, Maciejewski ML, Boulware LE. The Economic Burden of Chronic Kidney Disease and End-Stage Renal Disease. *Semin Nephrol*. 2016 Jul;36(4):319-30.
29. Jha V, Al-Ghamdi SMG, Li G, Wu MS, Stafylas P, Retat L, Card-Gowers J, Barone S, Cabrera C, Garcia Sanchez JJ. Global Economic Burden Associated with Chronic Kidney Disease: A Pragmatic Review of Medical Costs for the Inside CKD Research Programme. *Adv Ther*. 2023 Jul 26. doi: 10.1007/s12325-023-02608-9.
30. Chukwuonye II, Oviasu E. The plight of chronic kidney disease patients in Nigeria. *J Dental Med Sci*. 2012;2(2):52–55.
31. Chukwuonye II, Ogah OS, Anyabolu EN, Ohagwu KA, Nwabuko OC, Onwuchekwa U, Chukwuonye ME, Obi EC, Oviasu E. Prevalence of chronic kidney disease in Nigeria: systematic review of population-based studies. *Int J Nephrol Renovasc Dis*. 2018 May 22; 11:165-172.
32. Ulasi, Ifeoma I.; Ijoma, Chinwuba K. The Enormity of Chronic Kidney Disease in Nigeria: The Situation in a Teaching Hospital in South-East Nigeria. *Journal of Tropical Medicine,* 2010, 1–6. doi:10.1155/2010/501957
33. Huang M, Wei R, Wang Y, et al. Blood coagulation system in patients with chronic kidney disease: a prospective observational study. *BMJ Open* 2017;7: e014294.
34. Martinez M, Vaya A, Alvarino J, Barbera JL, Ramos D, et al. Hemorheological alterations in patients with chronic renal failure. Effect of hemodialysis. *Clin. Hemorheol. Microcirc*. 1999; 21(1):1-6
35. Ramaprabha P, Bhuvaneswari T, Aravind KR. Coagulation profiles an indicator of vascular haemostatic function in chronic renal failure

patients who are on renal dialysis. *Sch. J. App. Med. Sci.* 2014; 2(2B):592-59.
36. Rattan R, Swain M, Srikrushnamahapatra. Plasma fibrinogen level, homeostatic alterations and nitric oxide levels in chronic renal disease. *IOSR-JDMS*. 2017; 16(4): 35-37.
37. A.G. Stack and others, Plasma fibrinogen associates independently with total and cardiovascular mortality among subjects with normal and reduced kidney function in the general population, *QJM: An International Journal of Medicine,* Volume 107, Issue 9, September 2014, Pages 701–713,
38. Irish A. Cardiovascular disease, fibrinogen and the acute phase response: associations with lipids and blood pressure in patients with chronic renal disease. *Atherosclerosis.* 1998; 137:133–139.
39. Stenvinkel P, Heimburger O, Paultre F, Diczfalusy U, Wang T, et al. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. *Kidney Int.*1999; 55(5):1899–1911.
40. Lee AJ, Lowe GD, Woodward M et al. Fibrinogen in relation to personal history of prevalent hypertension, diabetes, stroke, intermittent claudication, coronary heart disease, and family history: the Scottish Heart Health Study. *Br Heart J* .1993; 69: 338–342.
41. Ridker PM. Inflammation, atherosclerosis, and cardiovascular risk: an epidemiologic view. *Blood Coagul Fibrinolysis.* 1999; 10(Suppl 1): S9–S12.
42. Lai KN, Yin JA, Yuen PM, Li PK. Effect of hemodialysis on protein C, protein S, and antithrombin III levels. *Am J Kidney Dis.* 1991;17(1):38-42.
43. Faioni EM, Franchi F, Krachmalnicoff A, Valsecchi C, Vigano GL, et al. Low levels of the anticoagulant activity of protein C in patients with chronic renal insufficiency: an inhibitor of protein C is present in uremic plasma. *Thromb Haemost.* 1991; 66:420–425.
44. Nosseir, N. M., Tawfik, M. S., Bakheet, O., El-Maghraby, D. F. Procoagulant FVIII and Anticoagulant Protein C in Renal Failure Patients on Hemodialysis. *Egyptian Journal of Radiation Sciences and Applications,* 2022; 35(1): 13-18.
45. Kant KS, Glueck HI, Coots MC, Tonne VA, Brubaker R, et al. Protein S deficiency and skin necrosis associated with continuous ambulatory peritoneal dialysis. *Am J Kidney Dis.*, 1992;19(3):264-71
46. Mohamad NM, Alsiddig B. Estimation and assessment of protein c/s among Sudanese patients in Aljazeera state with chronic renal failure. *IJIRR.* 2015; 2(12):1447-1449.
47. Folsom, AR. Protein C, Antithrombin, and Venous Thromboembolism Incidence: A Prospective Population-Based Study. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2002; 22(6), 1018–1022

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