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Changes in Some Haemostatic Parameters Among Patients with Chronic Kidney Disease in Kano Metropolis.

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Abstract

It has long been accepted that Chronic Kidney Disease (CKD) causes alterations in haemostatic parameters. These changes have not been reported in our community. The aim of this study was to determine the pattern of some haemostatic parameters of patients with CKD in Kano metropolis. This cross-sectional study involved 115 participants grouped into 60 CKD subjects (26 of which were on dialysis treatment and 34 were not) and 55 apparently normal subjects without CKD who served as controls. Five milliliters of venous blood were collected from each participant and divided into three aliquots; one milliliter of blood in a plain non-anticoagulated tube that was used for urea and creatinine; two milliliters of blood in sodium citrate tube used for prothrombin time (PT) and activated partial thromboplastin time (APTT) and two milliliters of blood into Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulated tube used for platelet count. Urea and Creatinine were estimated by Urease Berthelot's Reaction and Alkaline Picrate methods respectively. Citrated plasma was used within one hour of sample collection for the determination of PT and APTT. Platelet count (PLC) was determined using haematology autoanalyser (Nortek Genesis Tm HA6000). Our results have shown extremely large alterations in PT and platelet counts with prolongation in PT and insignificantly higher platelet counts and lower APTT among the CKD patients compared to controls. This result indicates that patient with CKD have impaired haemostatic parameters and are therefore at risk of bleeding and require close monitoring.

Keywords: Chronic kidney disease, prothrombin time, activated partial thromboplastin time, Kano.

Introduction

Patients at various stages of Chronic Kidney Disease (CKD) display a wide range of derangement in all three aspects of haemostasis and they experience a wide spectrum of clinical manifestation that lead to considerable morbidity and mortality with some having prothrombotic tendency leading to excessive cardiovascular events, while some will have platelet dysfunction leading to increased bleeding tendency (Boccardo *et al.*, 2004).

Thrombosis and bleeding diathesis are some of the risks posed by renal disorder associated with a high morbidity (Jalal *et al.*, 2010). The bleeding disorders are the result of insufficient function of the platelets, the coagulation cascades and/or activation of the fibrinolytic system, while the hypercoagulability is rather as a result of disorders of the coagulation regulatory factors as well as platelet hyper reactivity (Boccardo *et al.*, 2004).

No much reason is known about why one patient develops bleeding problems, while other tends to have excessive thrombus formation but however both problems are of significant

clinical relevance as some patients can be endangered by fatal bleeding episodes such as prolonged bleeding from the dialysis fistula, gastrointestinal bleeding or cerebral haemorrhage, while other patients experience prothrombotic status associated with an increased number of cardiovascular events (Jalal *et al.*, 2010 and Boccado *et al.*, 2004). The reasons for this disorder are complex and involve the coagulation cascade, fibrinolytic system, the platelets, the endothelium, the vessel and its extracellular matrix. The relationship between these components is influenced by accumulation of uraemic toxins and metabolic compounds during renal insufficiency (Jalal *et al.*, 2010). Also, patients with renal insufficiency suffer from a varying degree of inflammation which also affects haemostasis (Molino *et al.*, 2006). Also, it was established that the generation or exposure of tissue factor at the wound site is the primary physiologic event that initiates clotting and patients with CKD mostly have structural changes in their vessel wall. Thus, patients with CKD have a complex disturbance of their coagulation system, which makes them prone to severe bleeding episodes and thromboembolic events (Rapaport *et al.*, 1995).

CKD not only multiplies the risk for cardiovascular disease which is a leading cause of morbidity and mortality worldwide but also leads to the development of end-stage renal disease (ESRD) (Shang *et al.*, 2010).

The aim of this study was to determine the changes in some haemostatic parameters among CKD patients attending renal clinics and dialysis centres of Aminu Kano Teaching Hospital (AKTH) and Muhammad Abdullahi Wase Specialist Hospital (MAWSH) Kano, Nigeria.

Methods

Study Area

This descriptive cross-sectional study was carried out at Aminu Kano Teaching Hospital (AKTH) and Muhammad Abdullahi Wase Specialist Hospital (MAWSH) over a period of

seven months (June through December, 2016). The two hospitals are located in Kano metropolis. AKTH was established in 1988. It has 500- beds which serve as a referral centre catering for the medical need of about ten million people spread across five states of Kano, Jigawa, Kaduna, Katsina, and Bauchi all located in the northern region of Nigeria (Ado-Kurawa, 2006). Both hospitals have haemodialysis centres for dialysis treatment among patients with chronic kidney disease. The metropolitan city of Kano lies from Latitudes $11^{\circ} 52'N$ to $12^{\circ} 7'N$ and Longitudes $8^{\circ} 22.5'E$ to $8^{\circ} 47'E$ and is 1549 ft, above sea level (Ahmed, 2010).

Study Population

The study population comprised of sixty (60) CKD patients attending renal clinics and dialysis centres of the two hospitals in Kano metropolis and fifty-five (55) apparently healthy blood donors were monitored as controls.

Inclusion Criteria

Only CKD patients who were confirmed by both clinical and laboratory investigations to have CKD were included in this study and apparently healthy individuals attending blood donor clinic of the two hospitals were included as control group.

Exclusion Criteria

Patients with the following conditions were excluded in this study: CKD patients with family history of hereditary bleeding disorder, patients who had present or past history of hepatic diseases, viral diseases, cancer, cigarette smokers those who take alcohol and patients on erythropoietin and oral anticoagulant therapy.

Ethical Consideration

Ethical approvals were obtained from the Ethical Committees of AKTH and Hospital Management Board, Kano State. Permission to carry out the study in the selected departments was also obtained from the respective Heads of Department. Verbal informed consent for inclusion was obtained from the selected participants.

Sample Collection and Processing



The samples were collected from dialysis patients on dialysis a day before commencement of the dialysis. A tourniquet was applied to the upper arm in order to obtain venous blood from the subjects through the antecubital vein, after which it is disinfected with 75% alcohol and allowed to air-dry. Sterile 5ml syringe was used to withdraw venous blood from each subject which was divided into three aliquots. One milliliter (1ml) of blood was dispensed into a plain test tube, allowed to clot and retract and centrifuged at 2500 rpm for 10 minutes to obtain a clear unhaemolysed serum for urea and creatinine estimations. Then, 2ml of the blood was put into a tube containing 0.2ml of tri sodium citrate (3.8%) anticoagulant and the plasma was obtained by centrifugation at 2500 rpm for 15minutes. The plasma was then stored at -20°C , while the remaining 2ml of blood were dispensed into an ethylene diamine tetraacetic acid (EDTA) container. The urea and creatinine were estimated by Urease Berthelot's Reaction and Alkaline Picrate Method respectively. The platelet was determined using a haematology autoanalyser (Nortek Genesis Tm HA6000, China). For PT and APTT analysis, the frozen plasma was allowed to thaw and then tested manually for prothrombin time and activated partial thromboplastin time according to the manufacturer's instructions.

Data Analysis

Data analysis was done using Graphpad Instat, software (version 6.0). Unpaired t-test was used for mean comparison between groups. All

statistical analysis was considered at 5% level of significance, $p \leq 0.05$ (95% confidence level).

Results

Out of total sixty (60) CKD patients recruited in this study, thirty-three (33) were males and twenty-seven (27) were females. The male to female ratio was 1.2:1. Twenty-six (26) were on dialysis and thirty-four (34) were not on dialysis. Among the total fifty-five (55) control group, forty-seven (47) were males, while eight (8) were females. The mean age \pm SD of test subjects was 43.75 ± 16.06 years while that of control subjects was 30.69 ± 7.57 years.

Table 1 compares the mean of some biochemical and haemostatic parameters in CKD subjects and controls. It shows a significantly higher ($p < 0.0001$) levels of serum urea and creatinine among CKD patients compared to controls. There was no statistically significant difference ($p > 0.05$) in Platelet count and APTT between the groups. There was a statistically significant increase ($p < 0.0001$) in PT among the subjects when compared with controls.

Table 2 compares the mean values of some Haemostatic parameter (PLC, PT and APTT) among CKD patients on dialysis and not on dialysis. The results showed no statistically significant differences in the platelet count, prothrombin time as well as the activated partial thromboplastin time among patients on dialysis and patients not on dialysis.

Table 1: Mean values of some biochemical and haemostatic parameters in CKD subjects and controls

Parameter	Study subjects		t-value	p-value
	CKD (n=60)	Non-CKD (n=55)		
Urea (mmol/L)	18.33±4.87	3.92±1.03	14.860	0.0001(s)
Creatinine (µmol/L)	849.78±501.48	59.96±20.65	8.004	0.0001(s)
Platelet count (x10 ⁹ /L)	277.47±91.86	263.67±60.42	0.9422	0.3481 (ns)
PT (seconds)	18.27±2.71	15.47±1.29	6.964	0.0001(s)
APTT (seconds)	59.68±15.13	59.82±8.73	0.05785	0.9540 (ns)

CKD= chronic kidney disease, n=number of subjects, PT=prothrombin time, APTT= activated partial thromboplastin time, s= significant, ns= not significant

Table 2: Mean values of some Haemostatic parameters among CKD patients separated by dialysis

Parameter	Chronic Kidney Disease		t-value	p-value
	Dialysis (n=26)	Non-dialysis (n=24)		
Platelet count (x10 ⁹ /L)	254.73±95.85	294.85±86.08	1.703	0.0939 (ns)
PT (seconds)	18.23±2.46	18.29±2.92	0.08913	0.9293 (ns)
APTT (seconds)	58.81±11.51	60.35±17.54	0.3893	0.6985 (ns)

n=number of subjects, PT= Prothrombin time, APTT= activated partial thromboplastin time, ns= not significant

Discussion

Chronic kidney disease is associated with inflammation and accumulation of uraemic toxins. These inflammations and uraemic toxins may affect the haemostatic parameters. The result of this study reveals that there was no statistically significant difference in the platelet count of CKD subjects and controls. This indicates that CKD may not likely be associated with changes in platelet count. The result of this study is at variance with finding in previous studies (Ramaprabha *et al.*,2014; Akbar *et al.*, 2013 and Abdullah *et al.*, 2012) which indicated lower mean platelet count compared to ours (187.32 ± 49.74 x 10⁹/L; 172.4 ± 90.44x 10⁹/L and 199.19±56.74x10⁹/L respectively). All these studies reported a statistical significant decreased in platelet count. The result of this study is in agreement with previous reports (Quentin, 2003; Rabelink *et al.*,1994) which indicated that platelet count in patients with

renal failure is normal when compared to normal individuals but they have diminished activity in terms of adhesion and aggregation which may be due to decrease release of vascular von-Willebrand factor or due to increase production of nitric oxide which can be reversed following dialysis. Also, the diminished activity can be due to anaemia which is a major contributor to the bleeding tendency as it decreases the availability of platelet near an injured vascular wall which can also be reversed following dialysis. One of the limitations of this study is our inability to evaluate platelet activity as studied by Quentin (2003) and Rabelink *et al.*, (1994).

In this study, we observed a marked increase in the PT of CKD subjects when compared to the control groups. Our observed mean PT value (18.27±2.71 seconds) is higher than that obtained by Isaac *et al.* (2014) in Sokoto



(17.7±3.9 seconds) ($p < 0.001$) and lower than that of Ramaprabha *et al.* (2014) (27.33±8.73 seconds) (p -value < 0.001). This finding can be explained by the regular heparin (anticoagulant) usage during conventional haemodialysis for extracorporeal procedures this is because these patients are more prone to anaemia and vessel wall thrombosis which decreases the efficiency of the circulatory system. Heparin binds to the enzyme inhibitor antithrombin III, which inactivates activation of thrombin and other factors involved in clotting (Inagaki *et al.*, 2001).

Prothrombin time (PT) determines the tissue factor pathway (extrinsic pathway). A prolongation in PT indicates that there is either deficiency in factor II, V, VII, and X, vitamin K deficiency and warfarin usage. This is because deficiency of vitamin K results into deficiency in vitamin K dependent factors (II, VII, IX and X). Vitamin K is necessary for the post transcriptional carboxylation of the glutamic acid residues near the terminal end of these proteins but the use of warfarin is very rare in patients with renal failure because it only increases the risk of haemorrhage in them (Bates *et al.*, 2006).

Also, another contributing factor that may likely lead to the prolongation of the PT is the use of nephrotoxic drugs like non-steroidal anti-inflammatory drugs (NSAIDS) for example aspirin, ibuprofen and diclofenac, aminoglycosides, beta-lactam antibiotics. These drugs have the ability to inhibit clotting factors. For example, aspirin also known as acetyl salicylic acid which is a medication that is used to treat pain, fever and inflammation has been shown to have antiplatelet effect by stopping the binding together of platelets (Choudhry *et al.*, 2006).

In this study, we observed that there was no statistically significant difference in the mean APTT value of renal failure subjects and controls. Our finding is in agreement with the observation in a previous report by Abdullah *et al.* (2012) which indicated that there was no significant difference in the values of CKD

subjects when compared to the control group. Our finding is however at variance with a previous report (Isaac *et al.*, 2014) which indicated a statistically significantly lower value among CKD subjects compared to control. Also, the value of this research is at variance with a previous report by Ramaprabha *et al.* (2014) in which the APTT was increased compared to the control groups.

The result of the present study shows that dialysis which is a procedure that involves the removal of urea and other toxic substances from the plasma as well as correction of electrolyte imbalance does not have a significant effect on the haemostatic parameters as it shows that there were no statistically significant differences in the PLC, PT, and APTT values of patients on dialysis and those not on dialysis.

Conclusion

This study indicates that patients with renal dysfunction are at risk of bleeding and however need close monitoring due to prolonged prothrombin time.

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