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## Assessment of some Haemostatic and Inflammatory Markers in Renal Disease Patients in Specialist Hospital, Sokoto

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### Abstract

There is a continuous increase in the prevalence of renal disease worldwide as well as in Nigeria and the patient with severe renal failure are at risk of bleeding diathesis. This study was designed to investigate the status of some haemostatic and inflammatory parameters in patients with kidney disease and to compare their effect on different types of kidney disease. A total number of 104 participants (52 with kidney disease and 52 without kidney disease) were recruited. Data on socio-demographic was collected using questionnaire while laboratory result was generated using appropriate laboratory methods. The result obtained showed PT and APTT was significantly higher in renal disease subject compared to the control subjects ( $39.42 \pm 33.68$  and  $13.46 \pm 1.24$  seconds) and ( $72.34 \pm 35.52$  and  $43.42 \pm 9.36$  seconds) respectively. There was no significant difference in Platelet count of renal disease patient and the control subject. There was a significant increase in the ESR and TLC of the subjects compared with the control. There was no significance difference in PT, APTT, platelet count, ESR and TLC between male and female subject. There was no significance difference in PT, activated partial thromboplastin time APTT, platelet count, ESR and TLC in age of renal disease subjects. P-value of  $<0.05$  was considered significant in all the analysis. There is need to routinely monitor the haemostatic parameters among patient with renal diseases. In conclusion, this study has shown that kidney failure leads to increased level of Prothrombin Time, Partial Thromboplastin Time with Kaolin.

**Keywords:** *haemostasis; inflammatory markers; inflammation; renal disease*

### Introduction

The kidneys are two bean-shaped organs found in vertebrates. They are located on the left and right in the retroperitoneal space, and in adult humans are about 12 centimeters ( $4\frac{1}{2}$  inches) in length [1]. They receive blood from the paired renal arteries; blood exits into the paired renal veins. Each kidney is attached to a ureter, a tube that carries excreted urine to the bladder [2]. Acute renal failure (ARF) is an abrupt or rapid loss of renal function due to damage to the kidneys. It results

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in retention of nitrogenous (urea and creatinine) and non-nitrogenous waste products that are normally excreted by the kidney [3]. Acute kidney failure (ARF) or injury and chronic kidney disease (CKD) together with a number of other diseases may cause either form of renal failure. The World Health Report 2002 and Global Burden of Disease project reports show that kidney disease contribute to the global burden of diseases— with approximately 850,000 deaths every year and 15,010,167 disability-adjusted life years. Globally, it represents the 12th cause of death and 17th cause of disability [4]. In Nigeria, 27million people had CKD as of 2011, with the prevalence of 15,000 new cases occurring every year and 45,000 persons living with kidney failure annually according to the National kidney foundation.

Patients with chronic kidney disease 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 chronic kidney disease [5]. An increased bleeding risk has been described for patients with end-stage renal disease. Bleeding Occurs in about 50% of patients with end-stage renal disease [6-7], reaching from minor events such as bruises and bleeding at venipuncture sites to menorrhagia, gastrointestinal blood loss, severe perioperative bleeding and retroperitoneal as well as intracranial hemorrhage. Bleeding can significantly contribute to mortality and morbidity and blood transfusions can lead to alloimmunization and thereby limit options for transplantation [8].

The role of inflammation in CKD pathogenesis and progression has been recognized since the late 1990s, when the first provocative theory was launched, in which inflammation, via monocyte release of interleukin-1 (IL-1), the master cytokine of inflammation, was the starting point concerning the major complications and the increasing rate of mortality in patients undergoing chronic dialysis. Factors contributing to chronic inflammation include associated pro-inflammatory comorbidities and recurrent infections. Nevertheless, a significant proportion of patients with ESKD have elevated inflammatory markers in the absence of an active inflammatory pathology or clinical evidence of infection. Despite the elimination of many dialysis-related factors, a persistent inflammatory state remains prevalent in ESKD. Increased gastrointestinal tract inflammation and permeability have been proposed as significant contributors to inflammation [9-10]

The aim of this study is to determine some of the haemostatic parameters and inflammation markers in renal disease patients and non-renal disease patients attending Specialist Hospital, Sokoto.

## **Materials and Methods**

### **Study Area**

The study was conducted in the Specialist Hospital Sokoto, in collaboration with the Department of Haematology School of Medical Laboratory Science of Usmanu Danfodiyo University Sokoto.

### **Study Design**

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This was cross sectional study designed among renal disease patients to assess some of the coagulation parameters and inflammation markers of the renal disease patients attending Specialist Hospital, Sokoto.

#### **Sample Size Determination**

The sample size, N for the study was calculated using formula below [11]

$$N = Z^2pq/d^2$$

Where n= minimum required sample size

Z= standard normal deviation set at 95% (1.96)

P= prevalence which is 3.5% [12]

Q= complement of p (1-p)

D= precision 5% (0.05)

$$N = (1.96)^2 \times 0.035 \times (1-0.035) / (0.05)^2$$

$$N = 3.84 \times 0.035 \times 0.965 / 0.0025$$

$$N = 52$$

#### **Study Population**

A total of fifty-two (52) subjects were selected for the study among patient with all types of kidney diseases who are on admission, or were for medical checkup in Specialist Hospital, Sokoto.

#### **Inclusion Criteria**

The inclusion criteria for patient selection for the research were as follows

- I. patient with AKD and CKD
- II. Patient who are not currently undergoing any anticoagulation and inflammation medications
- III. Patient that wilily to give their consent to participate in the study

#### **Exclusion Criteria**

The patient who does not meet the following criteria will be excluded from participating in the study:

- I. Patient on anticoagulation and inflammation medications
- II. Patient with congenital coagulation disorders.

#### **Ethical Clearance**

The Ethical clearance for the study was obtained from the Ethics and Research committee of Specialist Hospital Sokoto.

#### **Informed Consent**

Informed consent was sought from the study participant using the written and oral consent form.

#### **Sampling Method**

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The study was carried out on blood samples collected from the nephrology units of Specialist Hospital Sokoto. All subjects who met the inclusion criteria and were given a written informed consent was consecutively recruited for this study.

### **Sample Collection**

Six millilitres (6.0ml) of venous blood was collected from the subjects of which 2.25mls was dispensed into 0.25ml of tri-sodium citrate anticoagulant container for the estimation of PT and APTT, and the other 2.25mls will be dispensed into ethylene diamine tetra acetic acid (EDTA) container containing 0.5ml of anticoagulant for the estimation of platelet count and total leucocyte count. 1.6ml will be dispensed into 0.4ml of sodium citrate for the estimation of erythrocyte sedimentation rate. And the sample collected for PT and APTT was centrifuged at 3000rpm for ten minutes to obtain the poor platelet plasma. The plasma will be stored at -20°C until required for analysis.

### **Analytical Methods**

#### **Prothrombin Time Test**

##### **Procedure**

0.1ml of plasma was delivered into a glass tube placed in a water bath and 0.1ml of thromboplastin was added. The mixture was allowed to warm for 1-3minutes. 0.1ml of warmed calcium chloride will be added and the stopwatch will be started. The contents of the tube were mixed and the endpoint will be recorded. The test was carried out in duplicate on the patient's plasma and the control plasma.

Normal values: 11-16 seconds for most rabbit thromboplastins and 10-12 seconds for recombinant human thromboplastin.

#### **Activated Partial Thromboplastin Time**

##### **Procedure**

Equal volumes of the phospholipid reagent and the kaolin suspension was mixed and leave in glass tube in a water bath 37°C. 0.1ml of plasma was placed into a second glass tube. 0.2ml of the kaolin-phospholipid solution to the plasma, contents were mixed and the stopwatch was started simultaneously. It was left at 37°C for 10minutes with occasional shaking. At exactly 10minutes, 0.1ml of pre warned calcium chloride was added and then a second stopwatch will be started. The time taken for the mixture to clot was recorded

Normal range: 26-46 seconds.

##### **Procedure**

1:20 dilution of the blood was prepared using the diluting fluid in a thoma pipette and altered negatively. 0.02ml of blood was added to 3.98ml of diluting fluid. Neubauer counting chamber

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was charged with well mixed diluted blood. The platelet was allowed to settle in a moist chamber for 3 to 5 minutes. The ruled area of the counting chamber was located under 10× objective. The illumination was reduced by closing the iris diaphragm. Platelet appeared as high retractile particle. The total number of platelets was counted using a high power (40×) objective in four large corner squares (4mm<sup>2</sup>)

### **Total Leucocyte Count**

#### **Procedure**

1:20 dilution of the blood was prepared using the diluting fluid in a thoma pipette and altered negatively. 0.02ml of blood was added to 3.98ml of diluting fluid (Turk's solution). Neubauer counting chamber was charged with well mixed diluted blood. The white blood will be allowed to settle in a moist chamber for 3 to 5 minutes. The ruled area of the counting chamber was located under 10× objective. The illumination was reduced by closing the iris diaphragm. The total number of white blood cells was counted using a high power (40×) objective in four large corner squares (4mm<sup>2</sup>)

### **Erythrocyte Sedimentation Rate**

#### **Procedure**

0.4ml of sodium citrate was mixed in 1.6ml of anticoagulated blood in a caped filling tube. The filling tube was recapped after filling. Then Westergren tube was inserted into the recapped tube. Then the Westergren tube was allowed to filled to the mark 0 by capillary action. Then it was allowed to stand vertically undisturbed in standing rack for 1hour

### **Statistical Analysis**

The data analysis was performed using statistical package of social sciences (SPSS) version 22.0. Data was presented as mean ± standard deviation (SD) and percentage. Student t-test for mean comparison between two groups was used. Test for association (Chi-square) between categorical variables was used. One-way analysis of variance (ANOVA) with Least Significant Difference (LSD) was employed for mean comparison between the types of PT and APTT. Correlation between the patterns of PT, APTT and TWBC count and platelet count was done using Pearson's linear correlation tool. A p-value of less than 0.05 (p<0.05) was considered as statistically significant in all statistical analysis.

### **Results**

The results of the analysis of PT, APTT, Platelet count, total leucocyte count and erythrocyte sedimentation rate of 52 patient with kidney disease and 52 individuals without kidney disease as controls were as shown below in tables 4.1 to 4.5. They are presented as mean standard deviation.

**Table 1: mean distribution of haemostatic variables of the subjects and control**

PARAMETERS	SUBJECTS (n=52)	CONTROL (n=52)	t-value	P-value
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PT (Seconds)	39.42±33.68	13.46±1.24	3.916	0.000
APTT (Seconds)	72.34±35.52	43.42±9.36	4.066	0.000
Platelet count ( $\times 10^9/l$ )	303.80±99.28	202.80±80.80	4.470	0.486

**KEY:** **PT:** Prothrombin time; **APTT:** Activated Partial Thromboplastin Time platelet count; **n=**number; Significant difference =  $p < 0.05$

Table 1 shows the results of the haemostatic parameters of the subjects and control. The results obtained showed that there was a significant increase ( $P < 0.05$ ) in the PT and APTT of the subjects compared with the control but there was no significant increase ( $p < 0.05$ ) in the platelet count of the subjects compared with the control.

**Table 2: mean distribution of inflammatory markers of the subjects and control**

PARAMETERS	SUBJECTS (n=52)	CONTROL (n=52)	t-value	P-value
ESR (mm/h)	76.26±43.32	26.27±23.82	5.460	0.000
TLC ( $\times 10^9/l$ )	8.32 ±3.11	6.81 ±1.79	2.284	0.007

**Key:** **ESR;** Erythrocyte sedimentation rate **TLC;** Total leucocyte count **n=**number; Significant difference =  $p < 0.05$

Table 2 shows the results of inflammatory markers of the subjects and control. The results obtained showed that there was a significant increase ( $P < 0.05$ ) in the ESR and TLC of the subjects compared with the control.

**Table 3: gender distribution of the haemostatic parameters and inflammatory markers of renal disease subjects**

Parameters	males ( n=68)	Female ( n=36)	t-value	P- Value
PT (seconds)	30.26±31.21	33.55±30.09	0.462	0.895
APTT (seconds)	63.15±34.58	60.83±29.35	0.313	0.87
Platelet count( $\times 10^9/l$ )	271.15±108.47	259.28±108.38	0.64	0.661
ESR( mm/h)	55.98±45.25	64.31±43.68	-0.797	0.782
TLC ( $\times 10^9/l$ )	7.75±2.79	7.96±2.89	-0.303	0.637

**Key:** **PT:** Prothrombin time; **APTT:** Activated Partial Thromboplastin Time; platelet count; **ESR:** erythrocyte sedimentation rate, **TLC:** Total leucocyte count **n=** number  
Significant difference =  $p < 0.05$

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Table 3 shows the gender distribution of the haemostatic parameters and inflammatory markers in the subjects studied. The results showed no significance difference in PT, APTT, platelet count, ESR and TLC in male subjects compared with the female subjects studied ( $p>0.05$ ). The mean PT, APTT and platelet count of male and female subjects were ( $30.26\pm31.21$  and  $33.55\pm30.09$  seconds), ( $63.15\pm34.58$  and  $60.83\pm29.35$  seconds) and ( $271.15\pm108.47$  and  $259.28\pm108.383$ ). While the mean ESR and TLC of the male and female subjects were ( $55.98\pm45.25$  and  $64.31\pm43.68$  mm/h) and ( $7.75\pm2.79$  and  $7.96\pm2.89$ ) respectively.

**Table 4: shows the haemostatic parameters and inflammatory markers in patients with renal diseases according to age**

Parameters	19-29yrs	30-40yrs	41-50yrs	51-60yrs	61-70yrs	p-value
Number	10	24	35	25	10	
PT(s)	$13.40\pm1.14$	$34.65\pm37.64$	$29.0\pm19.30$	$37.25\pm40.17$	$44.10\pm37.25$	0.694
APTT(s)	$46.30\pm13.12$	$72.00\pm36.51$	$61.32\pm32.02$	$57.58\pm29.54$	$67.22\pm40.60$	0.556
Plc( $\times 10^9/l$ )	$218.0\pm102.4$	$250.80\pm74.60$	$267.56\pm106.77$	$259.0\pm92.54$	$363.33\pm54.39$	0.97
ESR(mm/h)	$35.60\pm36.25$	$53.25\pm47.64$	$66.60\pm43.19$	$45.42\pm35.79$	$96.11\pm40.14$	0.08
TLC( $\times 10^9/l$ )	$7.31\pm1.49$	$8.04\pm3.10$	$8.10\pm3.32$	$7.51\pm2.07$	$7.60\pm2.97$	0.938

**Key: PT:** Prothrombin time; **APTT:** Activated Partial Thromboplastin Time; **PLC** platelet count; **ESR:** erythrocyte sedimentation rate, **TLC:** Total leucocyte count

Significant difference =  $p<0.05$

Table 4 shows age distribution of the haemostatic parameters and inflammatory markers in the subjects studied. The results showed no significance difference in PT, activated partial thromboplastin time APTT, platelet count, ESR and TLC in age of renal disease subjects ( $P>0.05$ ). The mean values for PT, APTT and platelet count of  $13.40\pm1.14$  seconds,  $46.30\pm13.12$  seconds and  $218.0\pm102.94\times 10^9/l$ , respectively for age group of 19-29 years;  $34.65\pm37.64$  seconds,  $72.00\pm36.51$  seconds and  $250.80\pm74.60\times 10^9/l$ , respectively for 30--40 years;  $29.0\pm19.30$  seconds,  $61.32\pm32.02$  seconds and  $267.56\pm106.77\times 10^9/l$ , respectively for 41-50 years;  $37.25\pm40.17$  seconds,  $57.58\pm29.54$  seconds  $259.0\pm29.54\times 10^9/l$ , respectively for 51-60 years; and  $44.10\pm37.25$  seconds,  $67.22\pm40.60$  seconds and  $363.33\pm154.39\times 10^9/l$  respectively, for 61-70 years. The mean value for ESR and TLC of  $35.60\pm36.25$ mm/h and  $7.31\pm1.49\times 10^9/l$  respectively for 19-29years;  $53.25\pm47.64$  mm/h and  $8.04\pm3.10\times 10^9/l$  respectively for 30-40years;  $66.60\pm43.19$  mm/h and  $8.10\pm3.32\times 10^9/l$  respectively for 41-50 years;  $45.42\pm35.79$  mm/h and  $7.51\pm2.07\times 10^9/l$  respectively for 51-60 years;  $96.11\pm40.14$  mm/h and  $7.60\pm2.97\times 10^9/l$  respectively for 61-70years.

**Table 5: comparison the effect of the different types of kidney disease with the haemostatic and inflammatory markers**

Parameters	Type	Mean $\pm$ SD	f-value	p-value
PT (seconds)	AKD	$37.94 \pm 32.03$	0.956	0.640

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	CKD	42.59 ±35.80		
APTT (seconds)	AKD	69.11 ±37.73	0.087	0.674
	CKD	73.72 ±35.27		
Platelet count( $\times 10^9/l$ )	AKD	328.56 ±147.32	8.379	0.237
	CKD	283.63 ±71.01		
ESR( mm/h)	AKD	8.74 ±3.17	0.672	0.936
	CKD	8.10 ±3.10		
TLC ( $\times 10^9/l$ )	AKD	75.61 ±40.79	0.680	0.493
	CKD	76.63 ±45.31		

**Key:** **PT:** Prothrombin time; **APTT:** Activated Partial Thromboplastin Time; **PLC** platelet count; **ESR:** erythrocyte sedimentation rate, **TLC:** Total leucocyte count  
Significant difference =  $p < 0.05$ , **CKD:** chronic kidney disease, **AKD:** Acute kidney disease.

Table 5 shows the comparison of different types of kidney disease with the Haemostatic and inflammatory markers. The result shows no significant difference in PT prothrobim time, APTT activated partial thromboplastin, PLC platelet count, TLC total leucocyte count and ESR erythrocyte sedimentation rate on AKD and CKD patients. ( $p < 0.05$ ). The mean PT of AKD and CKD ( $37.94 \pm 32.03$ ) and ( $42.59 \pm 35.80$ ), APTT of AKD and CKD ( $69.11 \pm 37.73$ ) and ( $73.72 \pm 35.27$ ), PLC of AKI and CKD ( $328.56 \pm 147.32$ ) and ( $283.63 \pm 71.01$ ), ESR of AKD and CKD ( $8.74 \pm 3.17$ ) and ( $8.10 \pm 3.10$ ) and the mean TLC of AKD and CKD ( $75.61 \pm 40.75$ ) and ( $76.63 \pm 45.31$ ) respectively.

## Discussion

Chronic kidney disease is associated with inflammation and accumulation of uraemic toxins. These inflammations and uraemic toxins may affect the haemostatic parameters which is associated with multiple complex alterations in coagulation, although, excessive bleeding following trauma and during surgical procedures in patients with chronic renal failure continues to be a problem. However, an increased incidence of thrombotic complications has also been reported [13-14]. This study has shown significantly higher values of PT and APTT in Chronic kidney patients. This is in agreement with some of the previous reports [15-16] but in contrary with the findings of other researchers who showed no significant differences when PT and APTT were compared to healthy individuals [17]. However, variation of sample sizes, methods of analysis and peculiarities of different stages of CKD could contribute to the divergent opinions expressed by the authors [15-16]. Prolongation of PT and APTT in Chronic kidney disease patients could be associated with bleeding and this may probably be due to deficiencies of blood coagulation factors that are linked to extrinsic and intrinsic blood coagulation pathways.

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

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changes in platelet count. Finding from this study indicates a significant difference between Erythrocyte sedimentation of renal disease subject compared to control ( $p < 0.05$ ). This is in agreement with some of the previous reports [18]. These may suggest that factors associated with renal failure itself, rather than dialysis, are responsible for ESR elevation since most of the subjects are not in dialysis. Finding from this study indicates a significant difference between Total leucocyte count of renal disease subject compared to control ( $p < 0.05$ ). The study has revealed that gender had no significant influence on haemostatic factors PT, APTT and platelet count values and inflammatory markers ESR and TLC and this observation is similar to the previous study [19-27]. Also, this finding from this study indicates that age has no significant influence on haemostatic factors PT, APTT and platelet count values and inflammatory markers ESR and TLC.

### Conclusion

In conclusion, this study has shown that kidney failure leads to increased level of Prothrombin Time, Partial Thromboplastin Time with Kaolin. This abnormality could contribute to bleeding diathesis in patients with kidney failure. It is advocated that renal failure patients should eat a balanced diet to keep their vitamin K intake consistent from day-to-day needs. Regular monitoring of coagulation profile of renal failure patients is also recommended.

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