Estimation of PT and PTT in CKD Patients: Comparison Study between ESRD and Other Stages of CKD in Sudanese Patients: January -2016

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Abstract:

   Background: *Chronic kidney disease is a common cause of death and disability in many countries throughout the world, chronic kidney disease can be classified into*

   Five disease stages depending upon the glomerular filtration rate, bleeding disorder are common complications of chronic kidney disease. Bleeding has been reported in 40-50 %of patients with chronic renal failure or on hemodialysis (HD), another study reported bleeding events in 24% in patients on HD.

Objective: *This study was examined PT and PTT in Sudanese patients with different stages of chronic kidney diseases, and compare*

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their significant values between end stage renal disease and the others stages of chronic kidney disease.

Method: Totally 100 subjects were selected for the study, among them 50wereESRD (pre dialysis sample) chronic renal failure patients (Group A), and 50subjectswere other stage of CKD as (Group B). The study was conducted in Soba University hospital, in Khartoum, Sudan, 5mlbloodsample was collected from chronic renal failure Sudanese patients by clean vein puncture, 1.8ml blood delivered into plastic tube containing 200µl of 3.2%sodium citrate and Stago semi auto analyzer was used in the estimation of PT& PTT. The APTT and PT parameters were calculated by system II software and all the significant P. value were calculated by (SPSS) program.

Results: the study include 100 samples from CKD Sudanese patients, among them 50 samples from group A (ESRD), and 50 samples from group B (other stages) CKD, two groups were compared for PT&PTT results ,the mean of PT is (16.18±3.62),(14.13±3.22) respectively, P. value <0.000, the mean of PTT (49.84±20.65) (40.57± 9.44) respectively P. value <0.000, we observe that the mean and P. value of PT& PTT for two groups were significant.

Key words: Chronic kidney disease, bleeding, PT, PTT, stages of CKD

1-INTRODUCTION & LITERATURE REVIEW

Chronic kidney disease is growing global health problem; CKD is typically associated with prothrombatic tendency in the early stages of the disease, whereas in its more advanced stage, that is, end stage renal disease, patient suffers from aprothrombotic tendency, and in many cases, bleeding diathesis. The exact etiology behind the co exiting of this conflicting haemostatic disorder is poorly understood [1].

Chronic kidney disease is condition in which the kidneys lose the ability to remove waste and excess water from the body stream ,as waste and fluids accumulate ,other body systems

affected, potentially lead to complications .The most causes of chronic kidney disease is diabetes and high blood pressure , in the early stages of chronic kidney disease there are no obvious symptoms ,the disease can progress to complete kidney Failure (ESRD) ,this occur when kidney function has worsened to the point that dialysis or kidney transplantation is required [22].

Chronic kidney disease can be classified into five stages depending upon the glomular filtration rate, in stage 1&2: in the both stages the person has kidneys damage with aglomerular filtration rate GFR of (60-90) ml\min. There are usually no symptoms to indicate the kidneys are damage , there is blood or protein in urine and family history of Polycystic kidney disease, in stage 3 the person has moderate kidney damage this stage is broken up in to two; 3A decrease in GFR is (54-59)ml\min 3B; the GFR is(30-44) ml\min, in stage 4 the person has advanced kidney damage with sever decrease in the glomerular filtration rate GFR to (15-30)ml\min. it is likely person with stage 4 need dialysis or kidney transplant in the near future. [3].

The stage:5 means that the patient in the end stage of renal disease, in this stage kidneys function is below 10 percent of their normal function, this may mean that the kidneys are barely functioning or not function at all. kidney disease is usually progressive ,does not reach the end stage until 10 to 20 years after diagnosis, the common symptoms include; general ill feeling and fatigue ,itching ,headaches weight loss and nausea ,patient with stage 5 treated by kidney transplant or hem dialysis[2].

Renal Dialysis involves the removal of urea and other toxic substances from the plasma as well as the correction of electrolyte imbalance. Two methods of dialysis, hemodialysis (HD) is the most commonly used method in which, blood is passed through an extra corporeal circuit and pumped across an artificial semi permeable membrane to bring the blood into

contact with the dialysate The second method is the intermittent and continuous ambulatory peritoneal dialysis (PD). This method utilizes the peritoneal membrane, as the semi permeable membrane, with capillaries on one side and high osmotic fluid infused into the peritoneal cavity on the other side. The peritoneal cavity is drained and the cycle is repeated after a suitable time to allow the equilibration of diffusible substances [5, 6].

Homeostasis is interaction between some components to keep the blood in fluidity state and to form clot after injury also to maintain the blood in the blood vessels. The mean component of homeostasis mechanism is extra vascular component, inter vesicular component like coagulation system and inhibiter system [1].The normally clotting process depend on healthy communication between endothelial cells and platelets it is also depend on Healthy balance between the pathways leading to thrombin- stimulated fibrin clot formation – and those of plasmin – induced clot lysis[1].

In the normal condition once vascular injury ensues the sub endothelial elements of vascular such as collagen and laminin are exposed. Platelets possess several integrin glycoprotein (GP) receptors including GP-VI that bind collagen and mediates both platelets adhesion and activation at the site of the injury, and GPlB-V-1X that inter act with collagen – bound VWF and is also required for platelets Adhesion. In addition to collagen – mediated platelets activation. Tissue factor (TF) trigger another distinct and independent pathway for platelets activation where it complex with the active factor V11(FV11a) forming TF/ FV11a complex and initiating proteolyses cascade by activating factor X interacting with several enzymes within haemostatic pathways and generating thrombin. Thrombin in its turn binds to its receptor (protease activated receptor -1) on platelets and result in release of (ADP), serotonin, and thromboxance A2, this platelets agonist

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activate other platelets recruiting them to site of clot formation
platelets activation also involve conformation change in
GPllb/llla that increase it affinity for fibrinogen and vWf
(enhance platelet – platelet affinity) fibronictin is also release
from Platelets and stabilized platelets aggregation.[1]Clot
formation also involves multiple factors like anti- thrombin AT,
tissue factor pathway inhibiter TFPI and protein C, protein S
system. In addition prostacyclin and Nitric Oxide NO temper
platelets activity, anti- thrombin neutralizes most of enzymes
in the coagulation cascade including factors Xa, lXa , Xlla and
thrombin , TFPI forms complex with factor Xa . Leading to its
inhibition and that of TF/FVlla. Protein C activate by thrombin
– thrombomodulin complex that form as clot progress its active
form complex with protein C to clearage and in active factor Va
and Vllla .Clot organization and removal is conducted by the
proteolysis' enzyme plasmin, it claves fibrin- releasing fibrin
degradation products (FDPs). [1].

Disturbances in haemostasis are common complications
of kidney disease. Both bleeding diathesis and
thromboembolism have been identified. The principle cause of
these abnormalities is the uremic state, the pathogenesis of
uremic bleeding is multifactorial. The most important
determinants of pathogenesis is increased levels of clotting
factors, decreased levels of clotting inhibitors, and diminished
fibrinolytic activity and platelet hyper aggregability. At present
the incidence of bleeding declining, where thrombotic
complications have become the predominant cause of mortality
[7].

The clinical manifestations of platelet dysfunction in
patients with ESRD are better described and primarily include
mucus coetaneous bleeding, such as epistaxis, and easy
bruising of the skin. Patients with CKD also have a higher risk
of gastrointestinal bleeding [8] and of intracranial bleeding that

might be partially explained by the associated platelet dysfunction [9].

Platelet dysfunction is observed mainly in advanced uremia and is probably due to uremic toxin present in circulation. Urea alone however is not responsible for platelet dysfunction and there is no correlation between blood urea nitrogen and bleeding time in chronic renal failure [10].

Unfractionated heparin (heparin) is the most commonly used anticoagulant for hemodialysis (HD). It is well-known that heparin can cause immune-mediated thrombocytopenia due to immunoglobulin antibody formation against the complex of platelet factor 4 (PF4) and heparin (HIT antibodies). Heparin may also contribute to HD associated platelet activation, thrombocytopenia, and increased PF4 release from platelets during a heparin dialytic session [11].

Patients with CKD have increased levels of fibrinogen that directly contribute to a hypercoagulable state. This is associated with increased levels of pro- in flammatory markers such as C-reactive protein and interleukin-6 [12, 13].Haemodialysis Results in a turbulent blood Flow resulting into platelet aggregation and leukocyte activation. Neutrophils attach to the dialysis membrane and release their granular content and coenocytes express tissue factor (TF), the natural initiator of the coagulation process [14].Endothelial injury frequently occurs in patients with chronic renal disease, probably resulting from uremia, dys lipidemia, hypertension, hyperparathyroidism, high levels of plasma interleukin-1 (IL-1) and tumor necrosis factor (TNF)[15].

Platelet or coagulation cascade disorder can lead to bleeding, while platelet hyper reactivity and abnormalities in the regulatory mechanisms may result in excessive thrombin formation and pathological thrombosis. Thrombotic complications and bleeding diathesis are some of the risks posed by renal disorder which in turn is associated with a high

morbidity. Laboratory tests such as PT and PTTK and platelet count are useful for assessing the etiology of kidney failure as well as establishing the level of injury insult thereby helping in the proper management and prompt treatment of the patient [16-17].

Bleeding has been reported in 40-50 %of patients with chronic renal failure or on hemodialysis (HD) , another study reported bleeding events in 24% in patients on HD, a hospital – based study showed that the risk of bleeding episodes is increased 2-fold in patients with renal failure ,clinically, an increased bleeding tendency ,gastrointestinal bleeding ,bleeding from canola site, retinal hemorrhage ,subdural hemorrhage, epistaxis, haematuria, prupura, gingival bleeding, haemarthrosis and petechiae[26].

Previous study was found in Pakistan by Subhan-ud-Din and shahida .A.R .Shah. Department of hematology. Sheikh Zayedhospital, Lahore. 2013.(haemostatic defect in CKD stage 3&4)result ; both PT&PTT in stage 3&4 CKD were within the normal range).

A study conducted in Cokato State, Nigeria found the mean PTTK and PT values of kidney failure patient and control groups were 33.7 ± 8.0 and 17.70 ± 3.9 seconds and 36.3 ± 3.5 and 15.7 ± 1.6 respectively. Statistical significant difference between PTTK and PT values of kidney failure patients and controls is found ($p < 0.05$) as the Mean ± Standard Deviation of PT of subjects males and females was compared with control males and females (16.3 ± 2.3 and 17.1 ± 3.8; 15.4 ± 1.1 versus $15.4 \pm 1.116.0 \pm 1.9$ respectively). Similarly, the PTTK was compared among the subject and control based on gender (35.0 ± 6.2 and 35.0 ± 6.4 versus 37.0 ± 3.9 and 35.8 ± 1.3 respectively) and was found to have no significant difference in PT and PTTK ($p> 0.05$) across the gender[20].

Other study found in Sudan medical laboratory journal by Abdulla et al; 2014 result an increased in PT&PTT in ESRD[18.].

Study found in Saudi journal kidney disease by Mohammed Ali et al ;2008 ,Concluded that increased in PT&PTT post dialysis, after collection sample pre and post proteinialor hemodialysis [23].

4- MATERIAL AND METHODOLOGY:

This study is cross sectional study design, was conducted in suba university hospital, Khartoum state, Sudan .Including Sudanese patients with CKD, excluding other patients with known haemostatic disorder.

Totally 100 subjects were selected for the study. Among them 50 were ESRD chronic renal failure patients (Group A) and 50 members were other stage of CKD as (Group B).5ml sample blood was collected from chronic renal failure Sudanese patients by clean vein puncture, 1.8ml blood delivered into plastic tube containing 200µl of 3.2% sodium citrate, the citrated blood was centrifuged at 3000 rpm for 15 minutes. The separated plasma was used for estimation prothombin time and activated Partial thrombo plastintime, for PT the samples storage at Room temperature, for PT at Room temperature, for PTT at 4 c .however test for PT and PTT preferable carried out on fresh samples as soon as possible .Prothrombin time (PT) measures the time taken by plasma to clot in the presence of an optimal concentration of tissue extract(Thromboplastin) using Spectrum reagent then Stago instrument was prep aired for analysis PT,   (PPP) was prepare, 50 μL of PPP sample was added in to Stago analyzer cuvate,100 μL of thromboplastin reagent was added, then started stop watch and Clot time was read ,the test was done in duplicate.

APTT was done by kaolin cephaline clotting method by using Spectrum reagent

(PPP) was prepared by centrifugation the sample at 3000g for 15 minute, stago Instrument was prepared for

analysis PTT,50μL of PPP was added into stagoInstrument cuvate,50 μL of kaolin Cephalin was added into cuvate, then incubate for 3min. ,50μLof calcium chloride reagent was added. then watch stop and the clot time was read.the test was done in duplicate.

RESULTS:

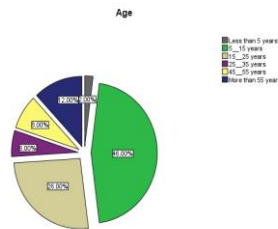**Figure -1: age distribution for group A(ESRD):**
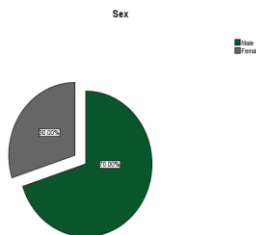

Figure-2 Sex distribution for group A (ESRD):

Table -1: means of PT, INR, and PTT of group A (ESRD)

Variable	Group1((ESRD) N=50
PT	16.18± 3.62 (10.8 − 26)
INR	1.68± 1.78 (.80 − 3.2)
PTT	49.84± 20.65 (26.5 − 151)

The table shows the mean ± SD (mini - max)

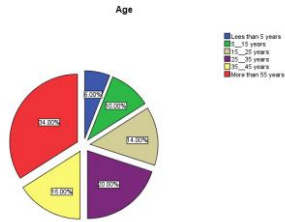## Figure -3;age distribution for group B(other stages)
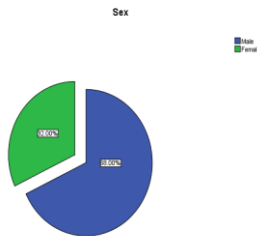

Figure 4; sex distribution for group B

Table (2): means of Age, PT, INR, and PTT of group B (Other stage)

Variable	Group2(Other stage) N=50
PT	14.13± 3.22 (1.3 – 26.7)
INR	1.19± .36 (0.8 – 2.8)
PTT	40.57± 9.44 (27.2 – 77.7)

The table shows the mean ± SD (mini - max)

Table (3):Comparison between the means of, PT, INR, PTT of group A (ESRD) and the group B (Other stage).

Variable	Group1(ESRD) N=50	Group2(Other stage) N=50	P value
PT	16.18± 3.62 (10.8 – 26)	14.13± 3.22 (1.3 – 26.7)	< 0.000
INR	1.68± 1.78 (.80 – 3.2)	1.19± .36 (0.8 – 2.8)	< 0.000

PTT	49.84± 20.65 (26.5 – 151)	40.57± 9.44 (27.2 – 77.7)	< 0.000

The table shows the mean ± SD (mini - max) and probability (P)

T-test was used for comparison.

P value ≤ 0.05 was considered significant.

ASSESSMENT OF THE RESULT:

Figure 1 and 2: show the frequency of age and sex in group A (ESRD) , the frequency of sex in this group is 30% female and 70% male ,the mean of age of this group is(23.65± 21.38), from table -1 the mean of PT&PTT is (16.18±3.62) , (49.84± 20.65) respectively ,Figer3 and 4 show the frequency of age and sex of group B (others stages)CKD ,the frequency of sex is 68% male and 32% female and the mean of age of this group is (38.3±22.7) , from table 2 the mean of PT&PTT in group B is (14.13± 3.22) , (40.57± 9.44) respectively, table 3 show the comparison of PT,INR,PTT and P.value in group A and group B, we observe that the parameters in group A is significantly raised than group B.

DISCUSSION:

In our study we found that both PT&PTT were significantly raised in both groups this agree with another study found in Sudan medical laboratory journal by Abdulla et al ;2014)concluded ,an increased in PT&PTT in ESRD [18].,also agree with another study found in Saudi journal kidney disease by Mohammed Ali et al ;2008 Concluded that increased in PT&PTT post dialysis ,after collection sample pre and post proteinialor hemodialysis[23], also another study made by Ramaprabha and et al;2014 concluded that an increased in PT&PTT in chronic kidney disease patients[27]. When comparing the means of PT,PTT of the two groups P.value was found to be less than 0.05 which is highly significant indicating that PT

and PTT showed prolongation with the disease progression (table3)

CONCLUSION:

From our study we found that coagulation profile level has a variation among chronic renal failure patients, we observe that the parameters in group A ESRD is significantly raised than group B other stages CKD and so all stages of CKD needs frequent monitoring of the PT and PTT to detect any abnormality very early and to treat it to avoid bleeding and to improve quality of life.

RECOMMENDATIONS:

Regular monitoring of coagulation profile in CKD patients improves the quality of life among CKD patients, monitoring the heparin dosage level and frequency of dialysis maintains the proper circulatory mechanism in CKD patients, further studies recommended to confirm this study, may provide best care for the patients with chronic renal failure.

Table-4-Abbreviations:

No	Abbreviation	Description
1	PT	Prothrombin time
2	APTT	Activated partial thromboplastin time
3	Sec	Second
4	MPs	Micro particles
5	CKD	Chronic kidney disease
6	HD	Hemodialysis
7	ESRD	End stage renal disease
8	GFR	Glomerular filtration rate
9	PPP	Fresh frozen plasma

ACKNOWLEDGEMENT:

REFERENCES:-

1- Jalal D. Conchol, M, Tougher G. Disorder of Homeostasis associated with chronic kidney disease. 2010: 36 L 34 – 40.
2- Christine Dimaria and Marijane Leonard; 2012 End stage renal disease .
3- Da Vita; Chronic kidney disease smart class ;2013 .CKD over view.
4- Bishop ML, Janet D, Edward PF. Clinical Chemistry: 4th edn. 1996:440-61.
5- Livolsi VA, Merino MJ, Books JJ. The National Medical Series of Independent Study. 3 ed. Pathology 1994:305-28.
6- Mayne PD, Mayne ZP. Clinical chemistry in diagnosis and treatment. 6 thedn, 1994:20-1.
7- Malyszko J, Malszko JS, Mysliwies M, Buczko W. Homeostasis in chronic renal failure. Annal Academiae Medical Bialostocensis 2007; 50: 127-130.
8- Wasse H, Gillen DL, Ball AM, et al. Risk factors for upper gastrointestinal bleeding among end-stage renal disease patients. Kidney Int 2003;64(4):1455–1461
9- Kawamura M, Fijimoto S, Hisanaga S, Yamamoto Y, Eto T. Incidence, outcome, and risk factors of cerebrovascular events in patients undergoing maintenance hemodialysis. Am J Kidney Dis 1998;31(6):991–996
10- Steiner RW, Coggin C, Carvalha AC, Bleeding time in uremia A useful test to assess clinical bleeding AMJ Hematol 1979; 7: 107-17.

11- T, Nakao K, Yamada T, Matsuo O (1986) Effect of a new anticoagulant (MD805) on platelet activation in the hemodialysis circuit. Thromb Res 41: 33-41.

12- Shlipak MG, Fried LF, Crump C et al. Elevations of inflammatoryand procoagulant biomarkers in elderly persons with renalinsufficiency. Circulation 2003; 107: 87–92

13- de la Serna G. Fibrinogen: a new major risk factor for cardiovascular disease. A review of the literature. J FamPract 1994; 39:468–477

14- Fischer KG. Essentials of anticoagulation in hemodialysis. HemodialInt2007;11:178–89.

15- Malyszko J, Malyszko JS, Mysliwiec M. Comparison of homeostatic disturbancesbetween patients on CAPD and patients on hemodialysis. Perit Dial Intern2001;21:158–65.

16- Jang, I.K. and Bursting, M.J. (2005). When heparins promote thrombosis: review of heparin-induced thrombocytopenia. Circulation 111(20): 2671-2683.

17- Rice, L. Nguyen, P.H. Vann, A.R. (2002). Preventing complications in heparin-induced thrombocytopenia. Alternative anticoagulants are improving patient outcomes. Postgraduate Medicine 112(3): 85-89.

18- Abdulla et al 2014 (The effect of heamodilysis in PT, PTT & D dimmer in patient with ESRD).Sudan medical laboratory journal.

19- Clair Bob Cock MPH PA ; 2009 . Coagulopathy in patient with renal disease

20- Isaac Zama Abdurrahman, Yakubu, Erhabo Osaro, Sadiya Usman, Liman Hamidu Mohammed, Aghedo Festus, Uko Emmanuel Kufre, Some coagulation profile among patients with renal failure International Journal of Clinical Medicine Research 2014; 1(4): 161-165.

21- Subhan-ud-Din and Shahida A.R Shah; 2005. Haemostatic defect in chronic kidney disease; Stage 3&4.

22- Wolter Kluwer; 2015.Chronic kidney disease up to date.

23- Mohammed ALI et al; 2008. Saudi journal kidney disease transplant.(Hematological change post heam and protienialdialysis).

24- Matsuo. T, Koide M, Kario K: et al Extrinsic coagulation factors and tissue factor pathway inhibitor in end stage chronic renal failure, Homeostasis 1997: 27: 163-167.

25- MalyszKo. J et al; 2004haemostatic in chronic renal failure; comparison between HD &GAPD.

26- Jens Lutz. Julia, Menke, Daniel, Sollinger, Helmut Schinzel; Klaaus Turmel. Nephrol Dial Transplant, 2014. Haemostasis in chronic kidney disease

27- Ramaprabha and et al (Coagulation Profiles an Indicator of Vascular Haemostatic Function in ChronicRenal Failure Patients Who Are on Renal Dialysis.)