بسم الله الرحمن الرحيم

**Sudan University of Science and Technology**

**College of Graduate Studies**

# Determination of ProthrombinTime, Activated Partial Thromboplastin Time and D-dimer among Sudanese Patients with Chronic Renal Failure

**تحديد زمن البروثرومبين،زمن الثرمبوبلاستين الجزئي المنشط و دي دايمر**

**عند المرضى السودانيين المصابين بالفشل الكلوي المزمن**

A dissertation submitted in partial fulfillment for the degree of M.Sc. In Hematology and Immunohematology

**Submitted by:**

**Ola Mohamed Ahmed Altayeb Mohamed**

**B.Sc. Sudan University of Science and Technology, 2008**

**Supervisor:**

**Prof. Babiker Ahmed Mohamed Ahmed**

**Karary University**

2017

# الآيـــة

قال تعالى:

**إِنّ رَبّكُمُ اللّــهُ الّذي خَلَقَ السّماواتِ وَالأَرضَ في سِتّةِ أَيّامٍ ثُمّ استَوى عَلَى العَرشِ يُغشِي اللّيلَ النّهارَ يَطلُبُهُ حَثيثًا وَالشّمسَ وَالقَمَرَ وَالنُّجومَ مُسَخّراتٍ بِأَمرِهِ أَلا لَهُ الخَلقُ وَالأَمرُ تَبارَكَ اللّــهُ رَبُّ العالَمينَ ﴿٥٤﴾**

صدق الله العظيم

سورة الأعراف الآية 54

## Dedication

To my beloved and pleased parents whom every things for me.

To my husband and my kids.

To my wonderful supervisor;

Prof. Babiker Ahmed Mohamed who was with me when need.

To my special friends and collegues who were integral pats of support group .

I dedicate this work

# Acknowledgement

The greatest thank to Allah . I would like to express my deep graduate and thanks to every one who help me throughout this work at any step of it .

Firstly , Most grateful to my supervisor prof . Babiker Ahmed Mohamed for this expertise support and gaudiance

I would like to thanks the staff of haematology department for their valuable assistance and encouragement ,throughtout the research.

Finally , thanks extend to all people whom the blood sample has been collected from.

To all them very thanks with regards**.**

# Contents

## List of tables

# List of abbreviation

**ACEI** :angiostensin converting enzyme inhibitor

**ADP** : adenosine diphosphate

**AKI** :acute kidney injury

**ARF** : acute renal failure

**ARB** :angiostensin receptor inhibitor

**APC**: activating protein C

**APTT**: activated partial thrmboplastin time

**CACL2** : calcium chloride

**CKD** : chronic kidney disease

**COX** :cyclo_ oxegenese

**CRF**: chronic renal failure

**DIC**: disaminated intravascular coagulopathy

**DVT**: deep venous thrombosis

**ESRD** : end stage chromic renal failure

**GFR** :glomular filtration rate

**IV** : intravenous

**LMWH**: low molecular weight heparin

**LOH** : loop of henle

**MBD** : mineral bone disorder

**NND** : non dialysis- depandant chronic kidney disease

**PAR**: protease activated receptor

**PE**: pulmonary embolism

**PCT** : proximal convolant tubules

**PPP**: platelets poor plasma

**PT** :prothrombin time

**RAAS**: rennin angiostinsin –aldesteron enzyme

**TF** : tissue factor

**tPA**: tissue plasminogen activater

**uPA** :urokinase  plasminogen activater

**UFH**: unfractionated heparin

**VWD** : von willibrand disease

**VWF**: Von willibrand factor

# Abstract

This is case control study carried out in Khartoum State in IbnSina hospital, and haematolog department , in the period from December 2016 to march 2017 to evaluate some coagulation parameters Sudanese patient with chronic renal failure Specialy PT , APTT, D-dimer in Khartoum state . Hundred diagnosed chronic renal failure patients were selected 58 (58%) male and 42(42%) female and hundred healthy individuals were selected as control group, 4.5 ml of venous blood was with drown from each patient, placed in tri sodium citrate container , then centrifuge to get platelet poor plasma (PPP) and that to measure PT , APTT and D-dimer , The result was analyzed by SPSS version 15 , and express as meams , the results obtained from patients showed that the means of Prothrombin time, Activated partial thromboplastin time (APPT) and D- dimer were 14 sec ,33 sec and 1423 ng /ml respectively , the means of PT , APTT and D-dimer in control group ,13, 32 and 53 ng / ml respectively . The result showed no significant differences in PT, APTT in patients in regards to control and there is no significant differences according to gender (p. value> 0.05) . While there was signidicant in mean of D-dimer differences in patient group compared to control group( p.value< 0.05) . According to the duration of disease there was significant increase in D-dimer (p. value < 0.05), and there was no significant increase in PT and APTT and according to the duration of dialysis there was significant increase in D dimer ( p. value < 0.05), and there is no significant increase in PT and APTT.

The result of study raised that measurement of D-dimer could be use in identifying risk group of chronic renal failure who were likely to develop thrombotic events.

# ملخص الدراسة

هذه دراسة تحليلية حالة وحالة ضابطة في ولاية الخرطوم في مستشفى ابن سينا في الفترة من ديسمبر٢٠١٦ الى مارس٢٠١٧ لتحديد تأثير الفشل الكلوي المزمن على بعض معاملات تخثر الدم . تم اختيار مائة شخص مشخصين كمرضى الفشل الكلوي المزمن منهم ثمانية وخمسون من الذكور واثنان واربعون من الاناث كما تم أخذ مائة عينة من الاصحاء كمجموعة ضبط . تم اخذ ٤.٥ مليلتر من الدم الوريدي من كل مريض وتم وضعه في وعاء يحتوي على مانع تجلط ثلاثي سترات الصوديوم واستخلص المصل الدموي لقياس زمن البروثرومبين ، زمن الثرمبوبلاستين الجزئي المنشط و دي دايمر وتم تحليل النتائج بواسطة برنامج الحزم الاحصائية للعلوم الاجتماعية اصداره ١٥، تم حساب متوسط وكان كالاتي : متوسط زمن الثرومبين (١٤ ثانية ) ، متوسط زمن الثرمبوبلاستين الجزئي المنشط (٣٣ ثانية) ومتوسط دي دايمر (٤٢٣انانوجرام/مليلتر) وهذا بالنسبة للمرضى ، بينما كان متوسط زمن البرثرومبين ، زمن البروثرومبين الجزئيالمنشط و دي دايمر ( ١٣ ثانية )،(٣٢ ثانية) و(٥٣ نانوجرام/مليلتر) على التوالي ، اظهرت النتائج عدم وجود فروقات ذات دلالة احصائية بين المرضى والمجموعة الضابطة في زمن البروثرومبين وزمن الثرومبوبلاستين الجزئي المنشط ( القيمة المعنوية اكبر من ٠،٠٥) كما لا توجد فروقات ذات دلالة احصائية بين الجنسين (القيمة المعنوية اكبر من ٠،٠٥) بينما اظهرت فروقات ذات دلالة احصائية في متوسط دي دايمر ( القيمة المعنوية اقل من ٠،٠٥)مقارنة بعينات المعادلين الطبيعين، كما اظهرت الدراسة وجود فروقات ذات دلالة احصائية حسب مدة المرض في دي دايمر (القيمة المعنوية اقل من ٠،٠٥) ، وكذلك وجود فروقات ذات دلالة احصائية حسب مدة الغسيل الدموي الكلوي في دي دايمر( القيمة المعنوية اقل من ٠،٠٥ .

اشارت المحصلة على ان قياس مستويات دي دايمر يمكن ان يكون مفيد في تحديد مجموعة مرضى الفشلالكلوي المزمن الذين يحتمل تعرضهم لمخاطر التجلط .

# Chapter one

## Introduction and literature Review

# Chapter One

## Introduction and Literature Review

### 1.1 General Introduction:

Chronic kidney disease (CKD) is a growing global health problem CKD is typically associated with a prothrombic tendency in the early stages of the disease where as in its more advanced stages that is end stage renal disease patients suffer from a prothrombic tendency and in many cases a bleeding diathesis.(Jalal *et al* ,2010) Platelet dysfunction is observed mainly in advanced uraemia and is probably due to uraemic toxin present in circulation. Urea alone however is not responsible for platelet dysfunction and there is no correlation between blood urea nitrogen and bleedingtime in chronic renal failure. Other potential toxins include guadinosuccsinic acid and phenolic acid.

Coagulation abnormalities associated with renal disease are seen in chronic renal failure, acute renal failure, nephritic syndrome, glomerulonephritis, neoplasm and renal transplantation. Abnormal platelet function occurs due to accumulation of toxic metabolites. hypercoagulopathy with predisposition to thrombosis can also occur, Fibrinolytic activity, anti-thrombin III and protein C are all reduced and factors V,VII,VIII and X are increased (Mehta *et al*, 2005).

Renal diseases may be also complicated by thromboembolic phenomenon. These are related to vascular access for dialysis. Upto 60% of patients with central venous catheter develop thrombosis.(Hunt *etal*.,2005)

Patientswith chronic renal failure traditionally have been recognized as being at risk for perioperative bleeding and data suggest a hypercoagulable state in chronic renal failure, 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 uraemic state the pathogenesis of uraemic bleeding is multifactorial. The most important determinants of pathogenesis is increased levels of clotting factors, decreased levels of clotting inhibitors, diminishedfibrinolytic activity and platelet hyperaggregability. At present the incidence of bleeding declining, where thrombotic complications have become the predominant cause of mortality.(Malyszko*et al*, 2007)

## 1.2 Literature Review:

### 1.2.1Normal haemostasis :

Hemostasis or haemostasis is a process which causes bleeding to stop, meaning to keep blood within a damaged blood vessel (the opposite of hemostasis is hemorrhage). It is the first stage of wound healing. This involves coagulation, blood changing from a liquid to a gel. Intact blood vessels are central to moderating blood's tendency to form clots. The endothelial cells of intact vessels prevent blood clotting with a heparin-like molecule and thrombomodulin and prevent platelet aggregation with nitric oxide and prostacyclin. When endothelial injury occurs, the endothelial cells stop secretion of coagulation and aggregation inhibitors and instead secrete von Willebrand factor which initiate the maintenance of hemostasis after injury.

Hemostasis has three major steps: 1) vasoconstriction, 2) temporary blockage of a break by a platelet plug, and 3) blood coagulation, or formation of a fibrin clot. These processes seal the hole until tissues are repaired.

### 1.2.2 Historical back ground of haemostasis:

The process of preventing blood loss from a vessel or organ of the body is referred to as hemostasis. The term comes from the Ancient Greek roots "heme" meaning blood, and "stasis" meaning halting; Put together means the "halting of the blood" (Maieb*et al* ,2010) .The origin of hemostasis dates back as far as ancientGreece; first referenced to being used in the Battle of Troy. It started with the realization that excessive bleeding inevitably equaled death. Vegetable and mineral styptics were used on large wounds by the Greeks and Romans until the takeover of Egypt around 332BC by Greece. At this time many more advances in the general medical field were developed based off the study of Egyptian

mummification practice, which led to greater knowledge of the hemostatic process. It was during this time that many of the veins and arteries running throughout the human body were found and the directions in which they traveled. Doctors of this time realized if these were plugged, blood could not continue to flow out of the body. Nevertheless, it took until the invention of the printing press during the fifteenth century for medical notes and ideas to travel westward, allowing for the idea and practice of hemostasis to be expanded.(Wies*et al* ,1929)

### 1.2.3 Historical development of clinical haemostasis

It was until 1913 that a laboratory test to evaluate the clotting mechanism was described in literature . this test was the lee – white whole blood coagulations " clotting" time .(Lee *et al*, 1913)

As recently as the 1940s , there were only a few routine tests for evaluating the haemostatic mechanism : the platelet count , bleeding time, and the prothrombin time (PT), the PT was developed Quick in the 1930s , Even though it is some what misnamed , being dependent on more than prothrombin , slightly modified vessions are still in use today .( Bloom *et al* ,1994)

### 1.2.4 Normal haemostasis balance :

Prior to the many advance in biomedical research and laboratory techniques of recent decades , haemostasis was understood simple as normal process by which bleeding from injured blood vessels was stopped through blood coagulation today haemostasis is more toughly understood as complex interaction between blood vessels ,platelets and coagulation action factors in the plasma as in figure (1-1)

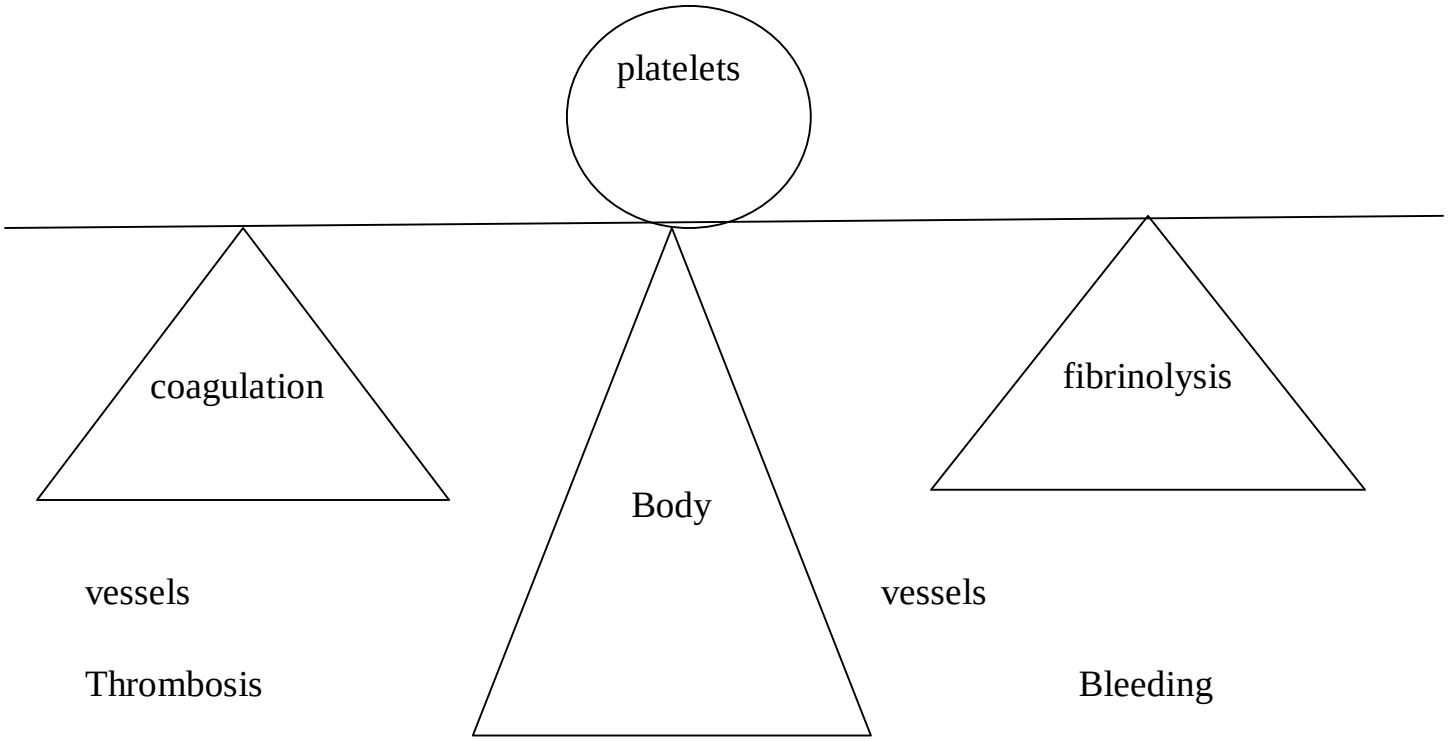



Figure (1-1): balance of haemostasis ,vessels, coagulation and fibrinolytic proteins and platelets, all together toward thrombous formation in well balanced

Under normal condition , the formation and dissolution of thrombus is maintained in a delicate balance (see fig 1-1) without this balance , an individual my experience either excessive bleeding (as a result of poor clot formation or excessive biolysis) or thrombosis uncontrolled formation of thrombi in the vascular system that black resells and deprives organs of blood ) the condition associated with excessive bleeding are referred to as hypocoagulable stat .Also the conciliations in which there is uncontrolled thrombus formation is calledhypercoagulable state . and both abnormal states can be fetal if not controlled promptly.

### 1.2.5Primary Haemostasis

#### 1.2.5.1 Platelets production and function

Platelets are small anuclear cell fragments that bud off from megakaryocytes, specialized large polyploid blood cells that originate in the bone marrow (Schulze *et al.,* 2005). Platelets are present at 150 to 400 million per milliliter of blood and circulate for about ten days (Zucker-Franklin, 2000).

The main function of platelet is the formation of mechanical plug during the normal haemostatic response to vascular injury .in the absence of platelets spontaneous leakage of blood through small vessels may occur .Central of their function are the platelet reaction of adhesion , secretion aggregation and fusion as well as their procoagulant activity

#### 1.2.5.2 Platelet adhesion and aggregation:

In a healthy blood vessel, and under normal blood flow, platelets :do not adhere to surfaces or aggregate with each other. However, in the event of injury platelets are exposed to subendothelial matrix, and adhesion and activation of platelets begins Multiple receptors on the surface of platelets are involved in these adhesive interactions, and these receptors are targeted by multiple adhesive proteins. Detailed descriptions are available in these recent reviews (Jackson, 2007), The key for all of these receptors is that the adhesive interaction only takes place in the event of an injury to the blood vessel. This restriction is maintained in several different ways.(Varga-Szabo *et al.,* 2008).

Receptor GPIb-IX-V binds to immobilized von Willebrand factor (VWF) specifically through an interaction between GPIbα and the A1 domain of VWF. VWF is a large multimeric protein secreted from endothelial cells and

megakaryocytes that is always present in the soluble state in the plasma as well as in the immobilized state in subendothelial matrix (Ruggeri, 2007). However, soluble VWF in the circulation does not bind with high affinity to GPIbα (Yago*etal.,* 2008). The high affinity interaction may be dependent upon high sheer stress exerted by flowing blood on immobilized VWF, whether that VWF is immobilized on subendothelial matrix or other activated platelets((Yago *et al*., 2008) .

Receptor GPVI is constitutively active but its ligand is collagen, which is present in the subendothelial matrix and thus is only exposed to the blood in the event of injury. GPVI and GPIb-IX-V are critical for adhesion of platelets to subendothelial matrix at the site of injury and for their subsequent activation ( (Niesmandt *etal* , 2001)

**1.2.5.3 Platelet activation and aggregation**:

Activation of platelets is critical for aggregation. In particular the integrins, αIIbβ3, α2β1 and αvβ3 are normally present on the platelet surface in an inactive form, but platelet activation induces a conformational transition in these receptors that exposes ligand binding sites (Xiao *et al.,* 2004).αIIbβ3 is arguably the most important of these receptors as it is present at the highest density on the platelet surface. In addition, αIIbβ3 binds to multiple ligands that promote platelet-platelet aggregation. These include fibrinogen, VWF, collagen, fibronectin and vitronectin (Varga-Szabo*et al*., 2008). α2β1, αvβ3, α5β1 and α6β1 play smaller roles, binding primarily to collagen, vitronectin, fibronectin or laminin, respectively (Emsley*et al*., 2000), though other ligands have also been identified for each of these. All of the integrins are maintained in an inactive state on quiescent platelets.

Feedback activation of nearby platelets surrounding a new site of injury is critical for further aggregation and propagation of the platelet plug. This activation is mainly mediated by agonists released by activated platelets themselves acting on G protein-coupled receptors. ADP is released from platelet dense granules and binds to receptors P2Y1 and P2Y12 Thromboxane A2 is synthesized de novo by activated platelets and binds to the thromboxane receptor primarily, and other prostanoid receptors to a lesser degree, locally on platelets Serotonin is also secreted from dense granules and contributes to platelet activation.

Another critical mechanism of platelet activation that links secondary hemostasis to platelet function is activation by thrombin. Thrombin is the terminal serine protease of the coagulation cascade . Thrombin cleaves 2 protease activated receptors (PARs) on human platelets, PAR1 and PAR4. These are also G protein-coupled receptors, and cleavage by thrombin exposes a new N-terminus that serves as a tethered ligand to activate the receptor ,All of these receptors initiate cell-signaling pathways when they are ligated, which result in platelet granule secretion, integrin activation and platelet cytoskeleton remodeling (Brass, 2000).

Thus, in summary, platelet adhesion is initiated by GPIbα binding to immobilized VWF and GPVI binding to collagen, which is exposed to the blood due to endothelial injury. These platelets and other local platelets are then activated, and adhesion and aggregation is strengthened and expanded via platelet-platelet connections between αIIbβ3 bound to fibrinogen, VWF, fibronectin or vitronectin as well as between αvβ3 bound to vitronectin or thrombospondin, with α5β1-fibronectin and α6β1-laminin interactions perhaps also playing a role. In addition, adherence to subendothelial collagen is strengthened via interaction of integrin α2β1 and collagen. This platelet plug is also stabilized by deposition of insoluble fibrin generated by the coagulation cascade( Brass, 2000).

### 1.2.6 Secondary Hemostasis:

Secondary hemostasis consists of the cascade of coagulation serine proteases that culminates in cleavage of soluble fibrinogen by thrombin ,Thrombin cleavage generates insoluble fibrin that forms a crosslinked fibrin mesh at the site of an injury. Fibrin generation occurs simultaneously to platelet aggregation (Furie, 2009). In intact and healthy blood vessels this cascade is not activated and several anticoagulant mechanisms prevent its activation. These include the presence of thrombomodulin and heparan sulfate proteoglycans on vascular endothelium. Thrombomodulin is a cofactor for thrombin that converts it from a procoagulant to an anticoagulant by stimulating activation of the anticoagulant serine protease protein C Heparan sulfate proteoglycans stimulate the activation of the serine protease inhibitor (or serpin) antithrombin, which inactivates thrombin and factor Xa

When the vascular system is injured, blood is exposed to extravascular tissues, which are rich in tissue factor (TF), a cofactor for the serine protease factor VIIa The complex of TF and factor VIIa activates factor X and factor IX. This activation pathway is historically termed the extrinsic pathway of coagulation. Factor IXa also activates factor X, in the presence of its cofactor factor VIIIa. Factor Xa, also in the presence of its cofactor factor Va, then activates prothrombin to generate thrombin (Dahlback, 2000).

Thrombin is the central serine protease in the coagulation cascade, and it executes several critical reactions (Lane *et al.*, 2005). Thrombin critically cleaves fibrinogen to generate insoluble fibrin. Thrombin activates platelets via cleavage of PAR1 and PAR4 ,Thrombin is also responsible for positive feedback activation of coagulation that is critical for clot propagation. Thrombin activates factor XI, which then activates factor IX and thrombin activates cofactors VIII and V (Lane *et al.*, 2005).

This has historically been called the intrinsic pathway of coagulation, but it is more appropriate to think of it as a positive feedback loop (Bouma*et al*., 1998).

The updated cell-based model of hemostasis focuses on the important fact that these reactions are controlled by their localization on different cellular surfaces. Coagulation is initiated by the cofactor TF (the extrinsic pathway), which is a transmembrane protein present on fibroblasts and other extravascular tissues. The factor Xa generated here forms prothrombinase complex on these surfaces sufficient to generate only a small amount of thrombin. Then amplification and propagation of coagulation via the positive feedback loop occurs on the surface of platelets, which are activated near the site of injury by that trace thrombin and by adherence to extracellular matrix. Thus, the active coagulation complexes of this positive feedback loop form on the surface of activated platelets (Hoffman *et al*., 2001).

Ultimately thrombin also plays an important role in down regulation of the coagulation cascade by binding to thrombomodulin on endothelial cells and then activating protein C (APC) ,The activated protein C anticoagulant system is important for the down regulation of the coagulation cascade. APC cleaves and inactivates the procoagulant cofactors VIIIa and Va,This reaction also requires a cofactor, protein S; in addition, factor V provides anticoagulant function as a cofactor for APC/protein S in the inactivation of factor VIIIa and factor Va ,These complexes between proteases and cofactors (procoagulant and anticoagulant) form on negatively charged membrane surfaces that are provided by activated platelets . This localization of the coagulation cascade reactions is critical to restrict coagulation to the site of injury.

The coagulation cascade is also down-regulated by inactivation of all the serine proteases by serine protease inhibitors. Most of these inhibitors are in the serpin family of inhibitors (Rau *et al*., 2007). Antithrombin is arguably the most important of these .Antithrombin inhibits thrombin and factor Xa, as well as factor IXa and factor XIa in the presence of heparin or heparan sulfate (Quinsey*et al*., 2004). Other serpins that play roles in coagulation include heparin cofactor II (thrombin inhibitor), protein Z-dependent protease inhibitor (factor Xa inhibitor), protein C inhibitor (APC inhibitor) and C1-inhibitor (factor XIainhibitor) ,Two non-serpin inhibitors, tissue factor pathway inhibitor and alpha-2-macroglobulin, also play a significant role, inhibiting factor Xa and thrombin, respectively (Cramer *et al*, 2010)

### 1.2.7 Fibrinolysis:

The role of the fibrinolytic system is to dissolve blood clots during the process of wound healing and to prevent blood clots in healthy blood vessels. The fibrinolytic system is composed primarily of three serine proteases that are present as zymogens (i.e., proenzymes) in the blood. Plasmin cleaves and breaks down fibrin. Plasmin is generated from the zymogen plasminogen by the proteases tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). tPA and plasminogen come together on the surface of a fibrin clot, to which they both bind. tPA then activates plasminogen, which subsequently cleaves fibrin. UPA activates plasminogen in the presence of the uPA receptor, which is found on various cell types (Lijnen*et al*., 2000). All three of these serine proteases are down-regulated by serpins that are present in blood. Alpha-2-antiplasmin inhibits plasmin, and plasminogen activator inhibitors 1 and 2 inhibit tPA and uPA (Rau *et al.*, 2007).

### 1.2.8Pathology of haemostasis:

Proper hemostasis is a function of balance between procoagulant systems (platelets, coagulation cascade) and anticoagulant systems (APC/protein S, fibrinolysis, serpins). If hemostasis is out of balance due to a defect in one of these systems, then either thrombosis (too much clotting) or bleeding (not enough clotting) may be the result.

#### 1.2.8.1Thrombosis:

Arterial thrombi are composed largely of aggregated platelets, whereas venous thrombi are composed more of fibrin with red blood cells enmeshed. The composition of these different thrombi is dictated by the different conditions in the arterial circulation and the venous circulation. One important aspect of this is blood flow, with higher flow rates and therefore higher sheer forces in the arterial circulation Classically, arterial thrombosis and venous thrombosis are thought to have different risk factors. However, recent studies have suggested that some of the classic risk factors for arterial thromboses, such as obesity and high cholesterol, are also risk factors for venous thrombosis The classic risk factors for venous thrombosis cause a hypercoagulable state and result in an increased tendency for activation of the coagulation cascade. These include acquired risk factors such as cancer, surgery, immobilization, fractures and pregnancy. Genetic risk factors include multiple variants in the coagulation cascade. The most common are factor VLeiden and prothrombin G20210A. Others are protein C or protein S heterozygosity and mutations in antithrombin (Bauer, 2000).

Arterial thrombosis is generally treated with drugs that inhibit platelet aggregation. Acetylsalicylic acid (aspirin) is a cyclo-oxygenase (COX) inhibitor and irreversibly inhibits COX-1 in the thromboxane A2 synthesis pathway

Clopidogrel (trade name Plavix) blocks adenosine diphosphate (ADP) activation of platelets by inhibiting the ADP receptor P2Y12(Sugidachi *etal*, 2001) Prasugrel (trade name Effient) is a new P2Y12 inhibitor that was recently approved in the United States for certain indications , Several new P2Y12 receptor antagonists are in clinical trials, but none of them has been approved as of yet. Unlike clopidogrel, some of these are reversible inhibitors and thus might only temporarily inhibit platelet aggregation (Giossi*et al*., 2010). These might be more appropriate for acute short-term treatment rather than for long-term prophylaxis. There are also several other drugs that inhibit platelet aggregation whose use is less widespread. There are three αIIbβ3 integrin inhibitors, abciximab, eptifibatide and tirofiban. These are all intravenous (IV) drugs that are used primarily to treat or prevent acute coronary events in hospital (Cohen, 2009). Dipyridamole (Persantine) is an oral drug that inhibits adenosine reuptake and thromboxane synthesis and is used for secondary prevention of stroke and transient ischaemic attack (Weber *et al*., 2009).

Pharmaceutical agents currently used to treat hemostatic disorders Venous thrombosis is treated with drugs that inhibit the coagulation cascade. Historically this has included unfractionated heparin (UFH), which stimulates inhibition of coagulation serine proteases by antithrombin,UFH is only bioavailable via IV injection, which limits its utility to hospital care. Long-term prophylaxis has typically been maintained with one of several coumarine derivatives, which are vitamin K antagonists (e.g. warfarin, [trade name Coumadin], acenocoumarol [trade name Sintrom]). Vitamin K antagonists inhibit post-translational processing of the vitamin K-dependent coagulation serine proteases and therefore down-regulate the coagulation cascade overall

A new class of anticoagulant drugs is small molecules that directly inhibit factor Xa or thrombin. Many of these are being developed as oral drugs, which could be a significant advantage over LMWH or fondaparinux. Rivaroxaban is the only direct factor Xa inhibitor that is currently approved, but it is still not yet approved in the United States. Apixaban, betrixaban and edoxiban are similar drugs that are still in clinical trials. Initially these are in trials for relatively short-term prophylaxis during/after orthopedic surgery, but the hope is that they would ultimately prove safe for long-term prophylaxis(laux *et al*, 2009).

### 1.2.8.2 Bleeding:

The main bleeding disorders are genetically inherited. Hemophilia results from defects in secondary hemostasis. Hemophilia A is due to deficiency of factor VIII, and hemophilia B is due to deficiency of factor IX. Activated factor VIII and activated factor IX together form the intrinsic factor Xase complex on activated membrane surfaces that is critical in the positive feedback loop of blood coagulation (Dahlback, 2000). Therefore, deficiency in either of these proteins causes a very similar bleeding phenotype characterized by excess bruising, spontaneous bleeding into joints, muscles, internal organs and the brain. Factor VIII and factor IX are both X-linked genes. Thus hemophilia is primarily expressed in males, with hemophilia A present in about 1 in 5000 males and hemophilia B present in about 1 in 20,000 males. However, multiple mutations in either factor VIII or factor IX have been identified, and not all of them cause complete loss of protein or protein function. In fact, almost half of hemophilia A sufferers have de novo mutations that were not inherited from their parents. Depending on the mutation, hemophilia can be severe (<1% function), moderate (1–5%) or mild (5–20%) (Mannucci*et al.*, 2001).

Hemophilia A and hemophilia B are treated mainly by infusion of recombinant factor VIII or factor IX, respectively. Before factor VIII and factor IX were available for infusion, hemophiliacs had a greatly reduced life expectancy and quality of life.

Von Willebrand disease (VWD) is a bleeding disorder caused by deficiency or defect in von Willebrand factor (VWF). VWF is involved in platelet aggregation and is also a carrier for factor VIII. Thus deficiency of VWF causes defects in platelet aggregation but also causes a deficiency of factor VIII ,VWD can result from multiple different mutations in VWF. These different mutations cause various different forms of VWD. These have been grouped into three overall categories. Type 1 is a partial quantitative defect, while type 3 results from a complete absence of VWF. In type 2 VWD, a normal amount of VWF is present but it has functional defects. Type 2 VWD is broken down into several subcategories ,VWF is an autosomal gene, so the disease is present equally in men and women. The severity of the different types of VWD varies, and various therapies are available and preferred for different forms of the disease.(Ruggeri, 2007)

Hemostasis has now been widely studied for more than a century. In that time we have generated a very detailed picture of the molecular and cellular events that play roles in normal and pathological hemostasis. But there are still many interesting questions that remain to be answered. This research has also led to the development of numerous drugs that impact many different mechanisms of thrombosis or bleeding. Moving forward, drug development for treatment of hemostatic disorders is still a significant area of interest in the biotechnology and pharmaceutical industries. Therefore, the prospect for continued improvements in patient health and quality of life is great.

### 1.2.9 Blood count and coagulation :

Screening tests provide and assessment of the extrinsic and intrinsic system blood coagulation and also the control conversion of fibrinogen to fibrin, The prothrombin time (PT) measure fibers VII ,V , X , prothrombin and fibrinogen . tissuethromboplastin (a brain extract ) and calcium are added to citrated plasma . the normal time for clotting is 11-16sec.

The activated partial thromboplastin time (APPT) measure factors VIII , Id X, XI and XII in addition to factors X, V prothrombin and fibrinogen . three substances – phospholipids , surface activator e.g kaolin and calcium are added to citrated plasma , the normal times for clotting is about 28-42 sec.

Prolonged clotting times in the PT and APTT because of factor deficiency are corrected by the addition of normal plasma to the best plasma , if there is no correction or in complete correction with normal plasma the presence of an inhibitor of coagulation is suspected .

D-dimer, a degradation product of cross-linked fibrin formed during activation of the coagulation system, is commonly used to exclude thromboembolic disease in outpatients suspected of having deep venous thrombosis (DVT) and pulmonaryembolism (PE). DVT and PE is relatively common and can cause sudden, fatal embolic events in the pulmonary arteries and other regions. Measurement of the D-Dimer level in plasma has been used as a screening strategy for subclinical DVT. A systematic review reported that a normal range of a highly sensitive D-dimer level accurately ruled out DVT in patients classified as having a low or moderate clinical probability of DVT. The DVT is a high-risk factor for the stroke because of advanced age, hemiplegia, and coagulation disorders, and DVT can cause paradoxical embolic stroke via a right-toleft shunt. Thus, it is important

to monitor the level of D-Dimer the incidence and characteristics of DVT in acute stroke patients. The Plasma D-dimer level has proven to be useful for DVT screening in chronic stroke patients undergoing rehabilitation. National and international scientific organizations have suggested the use of these markers when implementing new diagnostic strategies in patients with coronary syndrome. Since D-Dimer is well known to be an important prognostic indicator of heart diseases, its most definitive role is on monitoring post-treatment clinical status and the post therapeutic evaluation of patients.

### 1.3Chronic kidney disease :

#### 1.3.1 The kidneys:

THE kidneys are paired shaped organs located retroperitoneally on either side of spinal column (Micheal *et al* , 2005). Eack kidney of adult human weight about 150 grams ( Guyton and Hall , 2006 )

#### 1.3.2Renal anatomy:

Grossly, the kidneys are bean-shaped structures and weigh about 150 g in the male and about 135 g in the female. They are typically 10-12 cm in length, 5-7 cm in width, and 2-3 cm in thickness. (Wein*et al*, 2007)

The relationship of neighboring organs to the kidneys is important, as described below: Superiorly, the suprarenal (adrenal) glands sit adjacent to the upper pole of each kidney, On the right side, the second part of the duodenum (descending portion) abuts the medial aspect of the kidney,On the left side, the greater curvature of the stomach can drape over the superomedial aspect of the kidney, and the tail of the pancreas may extend to overlie the renal hilum.

### 1.3.2.1 Microscopic Anatomy:

The kidney is divided into the cortex and medulla. Renal pyramids in the medullary areas are separated by the cortical tissue called renal columns (of Bertin).

Histologically the functional renal unit is the nephron, which is composed of the following: The renal corpuscle glomerulus and Bowman capsule,Proximal convoluted tubules (PCT, located in the renal cortex),Descending loop of Henle (LOH),Ascending limb (which resides in the renal medulla, leading to the thick ascending limb),Thick ascending limb,Distal convoluted tubule and Collecting duct (which opens into the renal papilla).

### 1.3.2.2The glomerular:

Blood from the afferent glomerular arteriole passes through the juxtamedullary apparatus to the glomerulus. The glomerulus is a network of capillaries that filters blood across glomerulus contains podocytes and a basement membrane allowing water and certain solutes to be filtered across. This filtrate then reaches the PCT, which reabsorbs glucose and various electrolytes along with water as the filtrate passes through. Meanwhile, after being filtered at the glomerulus, the blood passes into the efferent glomerular arteriole and then descends into the renal pyramid.

### 1.3.3 Renal failure:

Failure of renal function may occur rapidly , producing the syndrome of acute renal failure (ARF), or develops inisidiously , often over many years producing Chronic renal failure (CRF) (William and Stevean, 2004).

### 1.3.3.1Chronic renal failure /chroic kidney disease :

Chronic kidney disease (CKD), also known as chronic renal disease, is progressive loss in kidney function over a period of months or years. The symptoms of worsening kidney function are not specific, and might include feeling generally unwell and experiencing a reduced appetite. Often, chronic kidney disease is diagnosed as a result of screening of people known to be at risk of kidney problems, such as those with high blood pressure or diabetes and those with a bloodline relative with CKD. This disease may also be identified when it leads to one of its recognized complications, such as cardiovascular disease, anemia, pericarditis or renal osteodystrophy (the latter included in the novel term CKD-MBD).(National kidney foundation2002),) CKD is a long-term form of kidney disease; thus, it is differentiated from acute kidney disease (acute kidney injury) in that the reduction in kidney function must be present for over 3 months. CKD is an internationally recognized public health problem affecting 5–10% of the world population (Martinez – Castelao*et al*, 2014) .

Chronic kidney disease is identified by a blood test for creatinine, which is a breakdown product of muscle metabolism. Higher levels of creatinine indicate a lower glomerular filtration rate and as a result a decreased capability of the kidneys to excrete waste products. Creatinine levels may be normal in the early stages of CKD, and the condition is discovered if urinalysis (testing of a urine sample) shows the kidney is allowing the loss of protein or red blood cells into the urine. To fully investigate the underlying cause of kidney damage, various forms of medical imaging, blood tests, and sometimes a kidney biopsy (removing a small sample of kidney tissue) are employed to find out if a reversible cause for the kidney malfunction is present.(National kidney foundation 2002).

### 1.3.3.2Sign and symptoms:

CKD is initially without specific symptoms and is generally only detected as an increase in serum creatinine or protein in the urine. As the kidney function decreases:

- Blood pressure is increased due to fluid overload and production of vasoactive hormones created by the kidney via the renin-angiotensin system, increasing one's risk of developing hypertension and/or suffering from congestive heart failure.

- Urea accumulates, leading to azotemia and ultimately uremia (symptoms ranging from lethargy to pericarditis and encephalopathy). Due to its high systemic circulation, urea is excreted in eccrine sweat at high concentrations and crystallizes on skin as the sweat evaporates ("uremic frost").

- Potassium accumulates in the blood (hyperkalemia with a range of symptoms including malaise and potentially fatal cardiac arrhythmias). Hyperkalemia usually does not develop until the glomerular filtration rate falls to less than 20-25 ml/min/1.73 m2, at which point the kidneys have decreased ability to excrete potassium. Hyperkalemia in CKD can be exacerbated by acidemia (which leads to extracellular shift of potassium) and from lack of insulin .

- Erythropoietin synthesis is decreased causing anemia. Fluid volume overload symptoms may range from mild edema to life-threatening pulmonary edema.

- Hyperphosphatemia, due to reduced phosphate excretion, follows the decrease in glomerular filtration. Hyperphosphatemia is associated with increased cardiovascular risk, being a direct stimulus to vascular calcification (Huska*et al*, 2008) , Moreover, circulating concentrations of fibroblast growth factor-23 (FGF-23) increase progressively as the renal capacity for phosphate excretion declines,

but this adaptative response may also contribute to left ventricular hypertrophy and increased mortality in CKD patients .(Faul*et al* ,2011)

- Hypocalcemia, due to 1,25 dihydroxyvitamin D3 deficiency (caused by stimulation of FGF-23 and reduction of renal mass), and resistance to the calcemic action of parathyroid hormone. Osteocytes are responsible for the increased production of FGF-23, which is a potent inhibitor of the enzyme 1-alpha-hydroxylase (responsible for the conversion of 25-hydroxycholecalciferol into 1,25dihydroxyvitamin D3). Later, this progresses to secondary hyperparathyroidism, renal osteodystrophy, and vascular calcification that further impairs cardiac function. An extreme consequence is the occurrence of the rare condition namedcalciphylaxis.(Brandenburg *et al,* 2011)

Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) represents one of the many complications of CKD. Notice the link between kidney, bone and heart in the CKD-MBD European-Dialysis-and-Transplantation (EDTA) Working Group logo

The concept of chronic kidney disease-mineral bone disorder (CKD-MBD),( Moe *et al*, 2006); currently describes a broader clinical syndrome that develops as a systemic disorder of mineral and bone metabolism due to CKD manifested by either one or a combination of: 1) abnormalities of calcium, phosphorus (phosphate), parathyroid hormone, or vitamin D metabolism; 2) abnormalities in bone turnover, mineralization, volume, linear growth, or strength (renal osteodystrophy); and 3) vascular or other soft-tissue calcification.CKD-MBD has been associated to poor hard outcomes.

- Metabolic acidosis (due to accumulation of sulfates, phosphates, uric acid etc.) may cause altered enzyme activity by excess acid acting on enzymes; and also

increased excitability of cardiac and neuronal membranes by the promotion of hyperkalemia due to excess acid (acidemia). Acidosis is also due to decreased capacity to generate enough ammonia from the cells of the proximal tubule.

Iron deficiency anemia, which increases in prevalence as kidney function decreases, is especially prevalent in those requiring haemodialysis. It is multifactoral in cause, but includes increased inflammation, reduction in erythropoietin, and hyperuricemia leading to bone marrow suppression.

People with CKD suffer from accelerated atherosclerosis and are more likely to develop cardiovascular disease than the general population. Patients afflicted with CKD and cardiovascular disease tend to have significantly worse prognoses than those suffering only from the latter.(Damman, *et al* , 2014).

Sexual dysfunction is very common in both men and women with CKD. A majority of men have a reduced sex drive, difficulty obtaining an erection, and reaching orgasm, and the problems get worse with age. A majority of women have trouble with sexual arousal, and painful menstruation and problems with performing and enjoying sex are common .(Vecchio*et al* , 2010)

**1.3.3.3 Causes**

The most common recognized cause of CKD is diabetes mellitus. High blood pressure is also a very common cause of chronic kidney disease. Other causes of CKD include idiopathic (i.e. unknown cause, often associated with small kidneys on renal ultrasound) and glomerulonephritis.(United State Renal Data System USRDS); Together, these cause about 75% of all adult cases.

Historically, kidney disease has been classified according to the part of the kidney anatomy involved.

-Vascular disease includes large vessel disease such as bilateral renal artery stenosis and small vessel disease such as ischemic nephropathy, hemolytic-uremic syndrome, and vasculitis.

- Glomerular disease comprises a diverse group and is classified into:

- Primary glomerular disease such as focal segmental glomerulosclerosis and IgA nephropathy (or nephritis).

- Secondary glomerular disease such as diabetic nephropathy and lupus nephritis.

- Congenital disease such as polycystic kidney disease.

-Tubulointerstitial disease includes drug- and toxin-induced chronic tubulointerstitial nephritis, and reflux nephropathy.

- Obstructive nephropathy is exemplified by bilateral kidney stones and diseases of the prostate such as benign prostatic hyperplasia.

- On rare cases, pinworms infecting the kidney can also cause nephropathy.

-Nontraditional causes of CKD (CKDu) are denoted if the common causes of CKD are not present ;

- CKD of unknown etiology is the subject of a major study by the Sri Lanka Ministry of Health and the World Health Organization 2009–2012.(Redmon*et al*, 2014).

- Mesoamerican nephropathy, a form of CKDu, is "a new form of kidney disease that could be called agricultural nephropathy".(Oranteset *al* ,2014)

### 1.3.3.4 Etiology

In many CKD patients, previous kidney disease or other underlying diseases are already known. A significant number present with CKD of unknown cause. In these patients, a cause is occasionally identified retrospectively.

### 1.3.3.5 Diagnosis:

Diagnosis of CKD is largely based on the clinical picture combined with the measurement of the serum creatininelevel .It is important to differentiate CKD from acute kidney injury (AKI) because AKI can be reversible. Abdominal ultrasound, in which the size of the kidneys is measured, is commonly performed. Kidneys with CKD are usually smaller (≤ 9 cm) than normal kidneys, with notable exceptions such as in early diabetic nephropathy and polycystic kidney disease. Another diagnostic clue that helps differentiate CKD from AKI is a gradual rise in serum creatinine (over several months or years) as opposed to a sudden increase in the serum creatinine (several days to weeks). If these levels are unavailable (because the patient has been well and has had no blood tests), it is occasionally necessary to treat a patient briefly as having AKI until the kidney impairment hasbeen established to be irreversible.

### 1.3.3.6 Stages:

All individuals with a glomerular filtration rate (GFR) <60 ml/min/1.73 m2 for 3 months are classified as having chronic kidney disease, irrespective of the presence or absence of kidney damage. The rationale for including these individuals is that reduction in kidney function to this level or lower represents loss of half or more of the adult level of normal kidney function, which may be associated with a number of complications such as the development of cardiovascular disease.(National kidney foundation, 2002)

Protein in the urine is regarded as an independent marker for worsening of kidney function and cardiovascular disease. Hence, British guidelines append the letter "P" to the stage of chronic kidney disease if protein loss is significant.(National Institute for health and clinical excellence , 2008).

Stage 1:

Slightly diminished function; kidney damage with normal or relatively high GFR (≥90 ml/min/1.73 m2). Kidney damage is defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies. (National kidney foundation ,2002)

Stage 2

Mild reduction in GFR (60–89 ml/min/1.73 m2) with kidney damage. Kidney damage is defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies. (National kidney foundation ,2002)

Stage 3

Moderate reduction in GFR (30–59 ml/min/1.73 m2(National kidney foundation ,2002) British guidelines distinguish between stage 3A (GFR 45–59) and stage 3B (GFR 30–44) for purposes of screening and referral. (National Institute for health and clinical excellence , 2008)

Stage 4

Severe reduction in GFR (15–29 ml/min/1.73 m2)( Preparation for renal replacement therapy. (National kidney foundation, 2002)

Stage 5

Established kidney failure (GFR <15 ml/min/1.73 m2), permanent renal replacement therapy,(National kidney foundation,2002) ,or end-stage kidney disease.

### 1.3.3.7 NDD-CKD vs. ESRD:

The term "non-dialysis-dependent chronic kidney disease" (NDD-CKD) is a designation used to encompass the status of those persons with an established CKD who do not yet require the life-supporting treatments for kidney failure known as renal replacement therapy (RRT, including maintenance dialysis or kidney transplantation). The condition of individuals with CKD, who require either of the two types of renal replacement therapy (dialysis or transplant), is referred to as the end-stage kidney disease (ESKD). Hence, the start of the ESKD is practically the irreversible conclusion of the NDD-CKD. Even though the NDD-CKD status refers to the status of persons with earlier stages of CKD (stages 1 to 4), patients with advanced stage of CKD (stage 5), who have not yet started renal replacement therapy, are also referred to as NDD-CKD .

### 1.3.3.8 Screening:

Screening those who have neither symptoms nor risk factors for CKD is not recommended.(Qassem*et al* ,2013) Those who should be screened include: those with hypertension or history of cardiovascular disease, those with diabetes or marked obesity, those aged > 60 years, subjects with indigenous racial origin, those with a history of kidney disease in the past and subjects who have relatives who had kidney disease requiring dialysis. Screening should include calculation of estimated GFR from the serum creatinine level, and measurement of urine albumin-to-creatinine ratio (ACR) in a first-morning urine specimen (this reflects

the amount of a protein called albumin in the urine), as well as a urine dipstick screen for hematuria. (Johnson, 2011) The GFR (glomerular filtration rate) is derived from the serum creatinine and is proportional to 1/creatinine, i.e. it is a reciprocal relationship (the higher the creatinine, the lower the GFR). It reflects one aspect of kidney function: how efficiently the glomeruli (filtering units) work. But as they make up <5% of the mass of the kidney, the GFR does not tell you about all aspects of kidney health and function. This can be done by combining the GFR level with the clinical assessment of the patint (especially fluid state) and measuring the levels of hemoglobin, potassium, phosphate and parathyroid hormone (PTH). Normal GFR is 90-120 mLs/min. The units of creatinine vary from country to country.

**1.3.3.9 Treatment:**

The presence of CKD confers a markedly increased risk of cardiovascular disease, and people with CKD often have other risk factors for heart disease, such as high blood lipids. The most common cause of death in people with CKD is cardiovascular disease rather than kidney failure. Aggressive treatment of hyperlipidemia is warranted.(Chauhan*et al*, 2009)

Apart from controlling other risk factors, the goal of therapy is to slow down or halt the progression of CKD to stage 5. Control of blood pressure and treatment of the original disease, whenever feasible, are the broad principles of management. Generally, angiotensin converting enzyme inhibitors (ACEIs) or angiotensin II receptor antagonists (ARBs) are used, as they have been found to slow the progression of CKD to kidney failure. They have also been found to reduce the risk of major cardiovascular events such as myocardial infarction, stroke, heart failure, and death from cardiovascular disease when compared to placebo in individuals

with CKD ,further more, ACEIs may be superior to ARBs for protection against progression to kidney failure and death from any cause in those with CKD.( Xie, *etal*, 2015)

Replacement of erythropoietin and calcitriol, two hormones processed by the kidney, is often necessary in people with advanced disease. Guidelines recommend treatment with parenteral iron prior to treatment with erythropoietin. A target hemoglobin level of 9–12 g/dL is recommended.

At stage 5 CKD, renal replacement therapy is usually required, in the form of either dialysis or a transplant.

**1.3.3.10 Prognosis:**

The prognosis of patients with chronic kidney disease is guarded as epidemiological data have shown that all cause mortality (the overall death rate) increases as kidney function decreases , The leading cause of death in patients with chronic kidney disease is cardiovascular disease, regardless of whether there is progression to stage 5(Perazella*etal* , 2006)

While renal replacement therapies can maintain patients indefinitely and prolong life, the quality of life is severely affected. Kidney transplantation increases the survival of patients with stage 5 CKD significantly when compared to other therapeutic options.;however, it is associated with an increased short-term mortality due to complications of the surgery. Transplantation aside, high-intensity home hemodialysis appears to be associated with improved survival and a greater quality of life, when compared to the conventional three-times-a-week hemodialysis and peritoneal dialysis.(Pierratos*et al*, 2005).

### 1.3.3.11Cancer risk:

Patients with ESKD are at increased overall risk for cancer. This risk is particularly high in younger patients and gradually diminishes with age. (Maisonneuve*et al* ,1999)

### 1.3.4 Chronic kidney disease as procoagulant state:

is a growing global health problem, and although end-stage renal disease (ESRD) is a prominent and much feared complication of the disease, the high mortality rate associated with CKD is mainly due to increased incidence of cardiovascular disease.(levey*et al* ,2007) This is not surprising because CKD patients have a greater prevalence of traditional cardiovascular risk factors such as older age, smoking, hypertension, type 2 diabetes, and obesity (all considered prothrombotic conditions) than the general population. Several hemostatic abnormalities have been described in patients with even mild CKD in addition to platelet hyperactivity , One report documented impaired release of tPA from the endothelium in patients with CKD despite intact endothelium-dependent vasodilatation.Because acute release of tPA by the endothelium is important in modulating the thrombotic process, the impairment ofits release likely affects timely thrombolysis in patients with CKD and may contribute to the hypofibrinolytic state and the increased risk of atherothrombotic events in this patient population. Elevated plasma fibrinogen concentrations are associated with increased cardiovascular risk in the general population and may contribute to atherosclerotic plaque growth by increasing plasma viscosity, promoting platelet aggregation, and inducing regional fibrin deposition in the injured endothelium. In CKD patients, plasma fibrinogen concentration is reportedly increased and correlates with systemic markers of inflammation such as C-reactive protein (CRP) and interleukin-6. Increased plasma TF levels have also been reported in patients with CKD.(Cetin *et al* ,2006). In

addition to its role in platelet activation, TF has been proposed to be an inflammatory mediator because it ultimately activates protease-activated receptor-1 and induces intracellular inflammatory signaling cascades, such as those dependent on nuclear factor k B, and thus may contribute to the development of atherosclerosis in CKD patients. Activation of the renin-angiotensin-aldosteronesystem.(Chu ,2005) (RAAS) has been linked to the procoagulant state in patients with hypertension,( and is well documented in patients with CKD because its pharmacological inhibition has been associated with a reduced risk of cardiovascular morbidity and mortality and with a slower progression of the underlying kidney disease (Brenner *et al*,2001)in these patients. In particular, activation of RAAS has been associated with increased plasma fibrinogen, D-dimer, and PAI-1 concentrations in hypertensive patients, and fibrinogen and PAI-1 specifically have been associated with evidence of end-organ damage including cardiac and renal disease., Plasma PAI-1 levels are elevated in patients with diabetic kidney disease in association with endothelial dysfunction and inflammation. Experimentally, PAI-1 inhibits plasmin-dependent extracellular matrix turnover, stimulates infiltration of macrophages and myofibroblasts, and regulates transforming growth factor b1 expression, and hence may play a pathogenetic role in the progression of kidney diseaseand atherosclerosis. Other reported hemostatic abnormalities mayinclude elevated plasma levels of vWF and thrombomodulin in the context of endothelial dysfunction, increased FXIIa and FVIIa activities, andincreased activated protein C complex levels, as well as increased levels of thrombin-antithrombin complex levels and reduced antithrombin activity.(Malyskzo *et al*, 2004)

Although patients with CKD express hemostaticabnormalities that suggest impaired fibrinolysis and enhanced prothrombosis, it remains unclear whether

these markers of procoagulation such as increased fibrinogen, PAI-1, or TF may play a direct role in the development of atherothrombotic complications or whether they interplay with other traditional and nontraditional risk factors for cardiovascular disease in this set of patients. Similarly, their impact on the progression of underlying CKD is unknown.

### 1.3.5 End stage of CKD and increase risk of bleeding:

As CKD advances, the procoagulant abnormalities such as impaired release of tPA, increased PAI-1,(Segarra*et al* ,2001) elevated fibrinogen and D-dimer and increased TF/FVIIa persist, but in addition, patients start to exhibit platelet dysfunction that typically manifests with an increased risk of cutaneous, mucosal, or serosal bleeding. Several factors are thought to contribute to platelet dysfunction in patients with advanced CKD, such as impaired function of platelet glycoproteins like GPIIb/IIIa, altered release of ADP and serotonin from platelet granules, and faulty arachidonic acid and prostaglandin metabolism,which all lead to impaired platelet adhesion and aggregation. Certain uremic toxins such as guanidinosuccinic acid and methyl guanidine may contribute to platelet dysfunction by stimulating NO release. Anemia may also play a pathogenetic role in the increased risk of bleeding in patients with advanced CKD because correcting it results in improved platelet function in this patient population.(Hedges *et al*,2007) The modern hemodialysis procedure itself may also directly activate tPA, but it is unknown whether this activation contributes to an increased bleeding tendency in patients receiving it.(Sabovic*et al* ,2005).

### 1.3.6 Clinical manifestations of hemostatic abnormalities in patients with chronic kidney disease:

As mentioned previously, patients with CKD suffer considerably from major cardiovascular events such as myocardial infarction, stroke, and peripheral vascular disease. It remains unknown whether some of the abnormalities just cited, such as increased plasma TF, fibrinogen, or PAI-1 concentrations, are independent risk factors of cardiovascular events and mortality. The clinical manifestations of platelet dysfunctionin patients with ERSD are better described and primarily include mucocutaneous bleeding, such as epistaxis, and easy bruising of the skin. Patients with CKD also have a higher risk of gastrointestinal bleedingand of intracranial bleeding that might be partially explained by the associated platelet dysfunction.(Pierratos *et al* ,2005) .

## 1.4 Rationale:

Chronic kidney disease is associated with several haemostatic abnormalities, ranging from bleeding to thrombosis ,indicated by platelets dysfunction and prolongation in coagulation profile. Haemostatic abnormalities are relatively common in general practice medicine .In order to assist in clarifying the cause of these abnormalities and help in some instances with diagnosis , in this study we sought to assess the impact of CKD onPT, APTT and D dimer, and then may help to establish secondary prevention medication in individual patients.

## 1-5Objectives

### 1.5.1 General objective:

To determine some haemostatic parameters in patients with chronic renal failure in sudan.

### 1.5.2 Specific objectives:

1- To measureprothrombin time (PT) ,Activated partial thromboplastin time (APTT) and D-dimer in patients with chronic renal failure.
2- To correlate tests to duration of disease .
3- To explore the effects of haemodylsis on the tests .

# Chapter two

## Materials and methods

# Chapter two

# Material and method

## 2.1 Study design:

This is analytical case control study conducted from December 2016 _march 2017.Aimed to measured prothrombintime,activated partial thromboplastin time, D dimer in chronic renal failure (case) and non chronic kidney disease individual (control) .

## 2.2 Study area and population:

This study was conducted in IbnSina Hospital in haematologydepartment , sample size of 100 venous blood samples was collected from diagnosed CKD patients and 100 samples were collected from healthy individuals as control

## 2.3 sampling:

Individual whom diagnosed as chronic renal failure were selected and data collected using self –administrated per-coded questionnaire which was specifically designed to obtain information that helped in study

## 2.4 sample:

Venous blood collected using sterile disposable plastic syringe after cleaning the veinpuncture area with 70% ethanol ,the blood was add to the anticoagulant at ratio of 4.5 to 5.0of citrate (3.2%(0.109M) bufeered sodium citrate and gently mixed.

The sample was centrifuged at 1300 rpm for for 15min to obtain platelet poor plasma (ppp) .thepppplaced into plastic tubes , capped and frozen at -70 used for D dimer .

## 2.5 Inclusion criteria:

- Diagnosed chronic renal failure patients .
- Confirmed cases of chronic renal failure patients , were on haemodialysis .
- All Samples were collected before haemodailysis session .
- Non chronic renal failure individual as control group for comparing .

## 2.6 exclusion criteria:

- Patients on CKD had recent blood loss and transfusion .
- Patient had recent thrombosis .
- Patients had recent infection that are known to affect the parameters we investigate.
- Patients non consent for investigation also exclude.

## 2.7 Data analysis:

The collected data proceed for analysis using SPSS version 15 computerized program and the data presented in form of tables

## 2.8Methods

### 2.8.1Prothrombin time(PT)(automated using Bio Bas coagulant)

#### 2.8.1.1 Principle of coagulometer:

The coagulometer clot has an optical measurement system which detect a sudden variation in optical densitywhen aclot is formed . the chronometer and the stirring system are activated by sudden change of the optical density , this permits the intiationof the time measurement when the reagent and plasma are in contact a O.D. variation is produced, that automatically activates the digital chronometer and the magnetic mixer . the clotting time appears on the display.

### 2.8.1.2 Principle of PT:

The PT was performed by automated testing measure the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin )with calcium chloride (cacl2) which indicates over all the efficiency of the extrinsic clotting system.

### 2.8.1.3 Reagent and materials:

1. pooled normal plasma control.

2. prothrombin time kits.

Kit contents : PT reagent contains an extract of rabbit brain with buffer, stabilizer and calcium chloride .

3. coagulation analyzer

4. small cuvettes

5.magnetic.

6.Pipette tips.

7.calibrated pipettes.

### 2.8.1.4 Assay procedure :

-Cuvettes were placed in incubation area for prewarming at 37c for at least 3 minutes

- A magnetic was dispensed to each Cuvette , in the incubation area 100ul of ppp of patient(which were thawing in water bath at 37c ) or control was dispensed in each Cuvette

- After warming the Cuvette transferred to test column area 100ul of the mixureCa+ /thromboplastin was dispensed into Cuvette with the test column area

- The time was started immediately pipette key, Then after clot produced the instrument automatically stopped the timer and the result of PT appear at the display of the instrument per seconds

**2.8.1.5 Normal value :**

11-17 seconds ( depend on PT reagent)

**2.8.1.6 Interpretation:**

The common cause of prolonged PTincludes :

1.Administration of oral anticoagulant drugs (vitamin k).

2. liver disease, partially obstructive.

3. vaitamin k deficiency.

4. Disseminated intravascular coagulation (DIC).

**2.8.2 Activated Partial Thromboplastin time (APTT) (using Automated Bio Bas Coagulometer)**

**2.8.2.1 Principle of APTT**:

The APTT was performed by automated testing in the batch or state mode. inthe APTT an aliquot of undiluted, platelet poor plasma was incubated at 37c with a particulate factor XII activator( i.e. ,silica, celite, kaolin ,ellagicacid,etc).A reagent containing phospholipid (partial thromboplastin) was added,followed by CaCl2 .the time required for clot formation after the addition ofCaCl2 .it measure over all activity of in intrinsic pathway.

### 2.8.2.2 Reagent and Materials:

-Pooled normal plasma control.

-Activated partial thromboplastin time kits. Kit contents: APTT reagent contains kaolin cephalin with phospholipid, buffer and preservatives. CaCl2 (.025M) contain sodium azide. Coagulation analyzer Small cuvettes Magnetic. Pipette tips. Calibrated pipettes.

### 2.8.2.3 Assay procedure:

- Cuvettes were placed in incubation area for prewarming at 37 for at least 3 minutes.

- A magnetic was dispensed to each cuvette - In the incubation area 100 of PPP of patient (which were thawing in water bath at 37or control was dispensed in each cuvette.

- 100 of cephalin /kaolin mixture was add to each cuvette

- After incubation for 3 minutes the cuvette transform to test column area - Then 100

column area.

- The timer was started immediately by pressing the pipette key - Then after clot produce the instrument automatically stopped the timer and the result of APTT appear at the display of the instrument per seconds.

### 2.8.2.4 Normal value:

27 – 42 seconds (depend on APTT reagent).

### 2.8.3 D dimer using ichroma™ Reader

#### 2.8.3.1Priniciple of ichroma™ D-Dimer:

The test uses the sandwich immunodetection method, such that the detection antibody in buffer binds to D-Dimer in the plasma sample and antigen-antibody complexes are captured by antibodies that have been immobilized on the test strip as sample mixture migrates through nitrocelluose matrix. The more D-Dimer antigen in the plasma, the more antigen-antibody complexes are accumulated on test strip. Signal intensity of fluorescence on detection antibody reflects amount of antigen captured and is processed by ichroma™ Reader to show D-Dimer concentration in

the specimen. The working range of ichroma™ D-Dimer test is 50 – 10,000 ng/ml.
* Reference Value: 500 ng/mL (FEU: Fibrinogen equivalent units)

#### 2.8.3.2Components and Reagents

Ichroma™ D-Dimer consists of Cartridge, an ID Chip, and Detection Buffers. - The test cartridge contains a test strip; on the membrane of which, antibodies against D-Dimer and streptavidin have been immobilized at the test line and the control line respectively.

- Each test cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed test cartridges are packed in a box which also contains an ID chip.

- The detection buffer pre-dispensed in a tube contains fluorochrome-labeled anti-D-Dimer antibodies, fluorescentlabeled biotin-BSA, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.

- The detection buffer is dispensed in each detection buffer tube. 25 detection buffer tubes are packed in a separate pouch which is further packed in a Styrofoam box provided with ice packs for the purpose of shipment.

**2.8.3.3 Test procedure:**

contactBoditech Med Inc.'s Technical Services for assistance. ichroma™-Dimer test has a built-in internal control that satisfies the routine quality control requirements. This internal control test is performed automatically each time a clinical sample is tested. An invalid result from the internal control leads to display an error message on the ichroma™ Reader indicating that the test should be repeated.

Transfer 10 µL of serum/plasma/control sample using a transfer pipette to a tube containing the detection buffer.

2. Close the lid of the detection buffer tube and mix the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately.).

3. Pipette out 75 µL of a sample mixture and dispense it into the sample well on the test cartridge.

4.Leave the sample-loaded test cartridge at room temperature for 12 minutes.

5.For scanning, insert it into the test cartridge holder of the ichroma™ Reader. Ensure proper orientation of the test cartridge before pushing it all the way inside the test cartridge holder. An arrow has been marked on the test cartridge especially for this purpose.

6.Press 'Select' button on the ichroma™ Reader to start the scanning process.

7.ichroma™ Reader will start scanning the sample-loaded test cartridge immediately.

8.Read the test result on the display screen of the ichroma™ Reader.

**2.8.3.4Interpretation of the result**

- Ichroma™ Reader calculates the test result automatically and displays D-Dimer concentration of the test sample as ng/mL.

- Working range of ichroma™ D-Dimer is 50-10,000 ng/mL.

- Reference value of ichroma™ D-Dimer is 500 ng/ml. (FEU: Fibrinogen equival units).

# Chapter three

## Results

## Chapter Three

## 3.Results

**Table (3.1) Demographic characteristic of study participants :**

A Case control study 1oo sample collected from chronic renal failure patients and 100 samples collected as control from healthy (non chronic renal failure individuals) include frequency of sex was 58 male (58%) and 42 female(42%) ,frequency of age group <40 years 26 (26%), 40-55 years 62(62%) and > 55years 12(12%), frequency of CRF duration <3 years 25 (25%), 3-5years 47 (47%) and >5 years 28 (28%),frequency of HD duration <3 years 63(63%), 3-6 years 34(34%) and >6 years 3 (3%).

| **Characteristic** | | **Frequency** | **Percent%** |
|---|---|---|---|
| **Sample** | Case | 100 | 50 |
| | Control | 100 | 50 |
| **Sex** | Male | 58 | 58 |
| | Female | 42 | 42 |
| **Age** | <40 | 26 | 26 |
| | 40-55 | 62 | 62 |
| | >55 | 12 | 12 |
| **CRF duration** | <3 years | 25 | 25 |
| | 3-5 years | 47 | 47 |
| | >5 years | 28 | 28 |
| **Haemodialysis duration** | < 3 years | 63 | 63 |
| | 3-6 years | 34 | 34 |
| | >6 years | 3 | 3 |

**Table (3.2): Comparison between control and case in study group of chronicrenal failure :**

Table ( 3.2) show significant increase in mean of D- dimer when compared with control (p. value < 0.05), and there is no significant differences in mean of PT and APTT(p. value >0.05) .

| Coagulation parameters | Sample | Number | Mean +/- SD | P. value |
|---|---|---|---|---|
| **PT** | Case | 100 | 14 +/- 2.0 | 0.578 |
| | Control | 100 | 13 +/- 1.5 | |
| **APTT** | Case | 100 | 33+/- 9.6 | 0.819 |
| | Control | 100 | 32 +/-8.5 | |
| **D dimer** | Case | 100 | 1423 +/- 586 | 0.000 |
| | Control | 100 | 53 +/- 81 | |

**Table (3.3): Mean of PT, APTTand D dimer among sex group of chronic kidney disease:**

Table(3.3) show means of PT, APTT and D dimer among sex group of chronic renal failure , there is no significant differences between means of males and females ( p. value > 0.05)

**Table (3.3): Mean of PT, APTTand D dimer among sex group of chronic kidney disease:**

| Coagulation parameters | Sex | Number | Mean+/-SD | P.value |
|---|---|---|---|---|
| **PT** | Male | 58 | 13.4+/-2.1 | 0.549 |
| | Female | 42 | 13.6+/-1.7 | |
| **APTT** | Male | 58 | 33.1 +/- 10.0 | 0.245 |
| | Female | 42 | 31.2 +/- 5.7 | |
| **D dimer** | Male | 58 | 1664 +/- 1363 | 0.864 |
| | Female | 42 | 1738 +/- 2900 | |

**Table (3.4) Effects of chronic renal failure duration**

Table ( 3.4) show significant increase in D dimer according to duration in haemodialysis (p. value <0.05) and there is no significant increase in PT and APTT ( p .value > 0.05) .

| **Duration group** | **Less than 3 years** | **3-5 years** | **More than 5 years** | **P.value** |
|---|---|---|---|---|
| **PT** | 13 | 13 | 14 | 0.923 |
| **APTT** | 30 | 32 | 33 | 0.366 |
| **D dimer** | 924 | 1626 | 2497 | 0.025 |

**Table 3.5 Effect of of haemodialysis duration on study group**:
Table( 3.5)show significant increase on D dimer according to increase the duration ( p.value <0.05 ) , and there is no significant change on PTand APTT.

| **Dialysis group** | **Less than 3 years** | **3-6 years** | **More than 6** | **P.value** |
|---|---|---|---|---|
| **PT** | 13 | 13 | 14 | 0.976 |
| **APTT** | 30 | 31 | 31 | 0.771 |
| **D dimer** | 1035 | 1779 | 2686 | 0.041 |

# Chapter four

## Discussion,Conclusion and Recommendations

# Chapter four

## Discussion , Conclusion and Recommendations

### 4.1Discussion :

This is case control study was conducted in ibn sina hospital during the period of December 2016_ march 2017 .the study included 100 patients male and female with chronic renal failure comparing with 100 healthy individuals . the age was range from between (20-75 years ) and the duration of disease and duration of haemodialysis to measure the prothrombin time and activated partial thromboplastin time and the concentration of D dimer.

The study showed that the mean of D dimer level in patient with CRF was significantly increase when compared with d dimer of the control group ( p. value < 0.05) and there is no significant different in PT and APTT which is agree with (Gordge . *et al , 1989*) in which D dimer increased in patient with CRF (244+\- 31 ng/ml) and diabetic nephrophathy (308 +/- 74 ng/ml)when compared to control group (96 +/- 13 ng/ml)

The study showed that there was significant increase of D- dimer according to increase of duration (p.value <0.05), and there is no significant change on PT and APTT. A similar study has been carried on 49 patients of CKD, showed significantly elevated levels of D.dimer according to increase of duration of the disease .(Bollow *et al* ,2007) .

The study showed that there was significant increase in D dimer but insignificant in PTand APTT . This agreed with the report of ( Ali*et al.*, 2008)and further agreement in the duration of dialysis significantly increased with the increase of the duration of dialysis.

On other hand, elevation of procoagulant markers measured by D dimer was similar to the results reported by (Shibata *etal*. 2000).

This result was also in agreement with the results of (Shibata *et al*.,2000) who have identified renal dysfunction as a state of activated coagulation due to the elevated D-dimer levels, which are not cleared by the kidney. Further, more agreement with (Lane *et al*,2004)who found the metabolism and elimination of fibrinogen was decreased in renal insufficiency and ESRD. The role of the kidney in the elimination of these biomarkers has not been established, nor is it known whether mild to moderate renal insufficiency has an impact on their clearance.

Renal insufficiency was associated with increased levels of inflammatory and procoagulant biomarkers as reported by( Shlipak *et al*,2003) their D-dimer results are in agreement with my Ddimer results. Also, (Oda *et al*, 2000)in Japan, reported abnormalities of coagulation and fibrinolysis in patients with end stage renal disease as their results of D-dimer levels were significantly higher in the dialysis groups than in control the group.

The risk of venous thromboembolism (VTE) is increased across the spectrum of CKD, including mild and more advanced CKD, nephrotic syndrome, ESRD and after kidney transplant. This increased risk may be due to underlying hemostatic derangements, including activation of procoagulants, decreased endogenous anticoagulants, enhanced platelet activation and aggregation, and decreased fibrinolytic activity (Keattiyoat and Mary, 2009) . It is reasonable to assume that the higher levels of D.dimer are primarily as a result of increased fibrin clot formation and breakdown. The increased thrombogenic state may be related to increased susceptibility to vascular disease in these patients( Coasta *et al* ,2007).

## 4.2 Conclusion:

- Prothrombin time and Activated partial thromboplastin time was normal in patient with chronic renal failure but D-dimer was increase , so patients with chronic renal failure at risk to thromboembolic phenomenon.

## 4.3 Recommendations :

- D-dimer should be considered for patients with chronic renal failure to fellow up and management to avoid risk of thrombosis .

# References and Appendixes

## References

**Ali M, Babiker M, Merghani L, Ali F, Abdulmajeed M.,(**2008). Hematological change post-hemo and potential dialysis among renal failure patients in Sudan. Sudia J kidney Dis and transpl; 19(2):274-279.

**Bauer KA**(2000).Hypercoagulable States. In: **Hoffman R, Benz EJ, Shattil SJ, Furie B, Silberstein LE, McGlave P**, editors. Hematology Basic Principles and Practice. 3. Churchill Livingstone; New York: . pp. **2009–2039.**

**Bauer KA**(2001). Fondaparinux sodium: a selective inhibitor of factor Xa. Am J Health Syst Pharm. ;58(Suppl 2):**S14–S17**.

**Bennett JS**(2000). Hereditary disorders of platelet function. In**: Hoffman R, Benz EJ, Shattil SJ, Furie B, Silberstein LE, McGlave P**, editors. Hematology Basic Principles and Practice. 3. pp. **2154–2172.**

**Bloom .A.L.,Forbes C.D., Thomas D.P. and Tuddenham E,G.D**(1994)(eds) Haemostasis ,and thrombosis .**3rd** edn.

**Bollow A, Gadar AMA. Hurab S, Mitwali A, Alwakeel** J (2007). Successful Kidney Trensplantation does not reverse coagulopathy in patients with CRF on either heamodialysis or peritoneal dialysis. Saudi J Kid. Dis Transplant ; 18: 177-85.

**Bouma BN, von dem Borne PA, Meijers JC (**1998). Factor XI and protection of the fibrin clot against lysis--a role for the intrinsic pathway of coagulation in fibrinolysis. Thromb Haemost.;80:**24–27**.

**Brandenburg VM, Cozzolino M, Ketteler M** (2011). "Calciphylaxis: a still unmet challenge". J Nephrol. 24 (2): 142–8.

**Brass LF**(2000) , The molecular basis for platelet activation. In: **Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, McGlave P**, editors. Hematology Basic Principles and Practice. 3. pp. **1753–1770**

**Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S** (2001). "Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy". N Engl J Med. 345 (12): 861–9.

**Cohen M (**2009). Antiplatelet therapy in percutaneous coronary intervention: a critical review of the 2007 AHA/ACC/SCAI guidelines and beyond.;74:**579–597**.

**Costa E, Rocha S, Rocha P, Castro E, Miranda M. Farrias, Loureiro A, Belo L**(2007). Fibrinolytic activity in chronic renal failure patients under Haemodialysis.Haematologica ; 92: 429.

**Chauhan V, Vaid M** (2009). "Dyslipidemia in chronic kidney disease: managing a high-risk combination". Postgrad Med. 121 (6): **54–61.**

**Chertow GM, Paltiel AD, Owen WF, Lazarus JM** (1996). "Cost-effectiveness of Cancer Screening in End-Stage Renal Disease".Archives of Internal Medicine. 156.

**Cramer TJ, Griffin JH, Gale AJ**(2010**)**. Factor V Is an Anticoagulant Cofactor for Activated Protein C during Inactivation of Factor Va. Pathophysiol HaemostThromb.;37:17–23.

**Dahlback B(** 2000).Blood coagulation.Lancet.;355:**1627–1632**.

**Damman, Kevin; Valente, Mattia A. E.; Voors, Adriaan A.; O'Connor, Christopher M.; Veldhuisen, Dirk J. van; Hillege, Hans L**. (2014). "Renal

impairment, worsening renal function, and outcome in patients with heart failure: an updated meta-analysis". European Heart Journal. 35 (7): **455–469.**

**Furie B**( 2009).Pathogenesis of thrombosis. Hematology Am Soc Hematol Educ Program. 2009:**255–258**.

**Giossi A, Pezzini A, Del ZE, Volonghi I, Costa P, Ferrari D, Padovani A** (2010). Advances in antiplatelet therapy for stroke prevention: the new P2Y12 antagonists. Curr Drug Targets.;11:380–391.

**Guyton and Hall**(2006). **(**. Functional organization of the human body and control of internal environment. Text book of medical physiology .**11th** Edition .Jakson .mississii.**p 3-9** .

**Harenberg J**(2009).Development of idraparinux and idrabiotaparinux for anticoagulant therapy. Thromb Haemost. ;102:**811–815**.

**Hedges SJ, Dehoney SB, Hooper JS, Amanzadeh J, Busti AJ**(2007).Evidence-based treatment recommendations for uremic bleeding. Nat Clin Pract Nephrol ;3(3):**138–153**

**Hoffman M**, **Monroe DM**(2001)., III A cell-based model of hemostasis. Thromb Haemost. ;85:958–965.

**Hruska KA, Mathew S, Lund R, Qiu P, Pratt R** (2008)."Hyperphosphatemia of chronic kidney disease". Kidney Int. 74 (2): 148–57.

**Jackson SP**(2007).The growing complexity of platelet aggregation.Blood.;109:5087–5095.

**Johnson, David** (2011). "Chapter 4: CKD Screening and Management: Overview". In Daugirdas, John.Handbook of Chronic Kidney Disease Management.Lippincott Williams and Wilkins. pp. **32–43**.

**Keattiyoat Wattanakita and Mary Cushmanb**( 2009). Chronic kidney disease and venous thromboembolism: epidemiology and mechanisms, Department of Medicine, University of Texas Southwestern Medical Center, Dallas, Texas. Curr Opin Pulm Med.; 15(5): 408–412.

**Lane DA, Philippou H, Huntington JA**(2005).Directing thrombin. Blood**.**;106:2605–2612.

**Lane DA, Ireland H, Knight I**(2004).Thesignificance of fibrinogen derivatives in plasma in human renal failure. Br J Haematol; 56: 251–260.

**Laux V, Perzborn E, Heitmeier S, von DG, Dittrich-Wengenroth E, Buchmuller A, Gerdes C, Misselwitz F**(2009).Direct inhibitors of coagulation proteins - the end of the heparin and low-molecular-weight heparin era for anticoagulant therapy? Thromb Haemost. ;102:892–899.

**Lee R.I., white PD**(1913) ,A clinical study of coagulation time of blood . Am J .Med sci .145 :495.

**Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer M, Raij L, Spinosa DJ, Wilson PW** ( 2003). "Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention". Circulation. 108 (17): 2154–69.

**Lijnen HR, Collen D. Molecular and cellular basis of fibrinolysis. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Silberstein LE, McGlave P (**2000 ), editors. Hematology Basic Principles and Practice.3.. pp. **1804–1814**.

**Marieb, Elaine Nicpon**; Hoehn, Katja (2010). Human Anatomy & Physiology (**8th**ed.). San Francisco: Benjamin Cummings. pp. **649–650.**

**Mannucci PM**(2008). Back to the future: a recent history of haemophilia treatment. Haemophilia.;14(Suppl 3):10–18.

**Mannucci PM, Tuddenham EG (**2001). The hemophilias--from royal genes to gene therapy. N Engl J Med. 2001;344:1773–1779.

**Maisonneuve P, Agodoa L, Gellert R, Stewart JH, Buccianti G, Lowenfels AB, Wolfe RA, Jones E, Disney AP, Briggs D, McCredie M, Boyle P** (1999). "Cancer in patients on dialysis for end-stage renal disease: An international collaborative study". Lancet. 354 (9173): 93–99.

**Małyszko J, Małyszko JS, Myliwiec M**(2004).Endothelial cell injury markers in chronic renal failure on conservative treatment and continuous ambulatory peritoneal dialysis. Kidney Blood Press Res ;27(2):**71–77**

**Martínez-Castelao; JL.Górriz; J Bover; et al**. (2014)."Consensus document for the detection and management of chronic kidney disease".Nefrologia. 34 (2): 243–62.

**Micheal L**., **Bishope** (2005), clinical chemistry principles, procedure,correlation., **6th**. Edition.

**Moe S, Drüeke T, Cunningham J, Goodman W, Martin K, Olgaard K, Ott S, Sprague S, Lameire N, Eknoyan G** (2006). "Definition, evaluation, and

classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO)". Kidney Int. 69 (11): 1945–53.

**Nieswandt B, Brakebusch C, Bergmeier W, Schulte V, Bouvard D, Mokhtari-Nejad R, Lindhout T, Heemskerk JW, Zirngibl H, Fassler R** (2001). Glycoprotein VI but not alpha2beta1 integrin is essential for platelet interaction with collagen. EMBO J. ;20:2120–2130.

**National Kidney Foundation (2002).**"K/DOQI clinical practice guidelines for chronic kidney disease".Retrieved 2008-06-29.

**National Institute for Health and Clinical Excellence**(2008). Clinical guideline 73: Chronic kidney disease. London, 2008

**Oda H, Ohno M, Ohashi H**., (2000).Coagulation and fibrinolysis factors in dialysis patients with and without ischemic heart disease. PubMed.; 16:152-5

**Orantes CM, Herrera R, Almaguer M, Brizuela EG, Núñez L, Alvarado NP, Fuentes EJ, Bayarre HD, Amaya JC, Calero DJ, Vela XF, Zelaya SM, Granados DV, Orellana P** (2014 ). "Epidemiology of chronic kidney disease in adults of Salvadoran agricultural communities". MEDICC Rev. 16 (2): 23–30.

**Perazella MA, Khan S**( 2006). "Increased mortality in chronic kidney disease: a call to action". Am. J. Med. Sci. 331 (3): 150–3.

**Pierratos A, McFarlane P, Chan CT**( 2005). "Quotidian dialysis–update 2005".Curr.Opin.Nephrol.Hypertens. 14 (2): 119–24.

**Quinsey NS, Greedy AL, Bottomley SP, Whisstock JC, Pike RN**(2004). Antithrombin: in control of coagulation. Int J Biochem Cell Biol. ;336-386

**Qaseem A, Hopkins RH, Sweet DE, Starkey M, Shekelle P** (2013). "Screening, Monitoring, and Treatment of Stage 1 to 4 Chronic Kidney Disease: A Clinical Practice Guideline From the Clinical Guidelines Committee of the American College of Physicians.".Annals of Internal Medicine. 159 (12): 835–47.

**Rau JC, Beaulieu LM, Huntington JA, Church FC**(2007).Serpins in thrombosis, hemostasis and fibrinolysis.J Thromb Haemost. 2007;5(Suppl 1):102–115.

**Rahman, Mahboob; Smith, Redmon JH, Elledge MF, Womack DS, Wickremashinghe R, Wanigasuriya KP, Peiris-John RJ, Lunyera J, Smith K, Raymer JH, Levine KE** (2014). "Additional perspectives on chronic kidney disease of unknown aetiology (CKDu) in Sri Lanka – lessons learned from the WHO CKDu population prevalence study". BMC Nephrology. 15 (1): 125.

**Redmon JH, Elledge MF, Womack DS, Wickremashinghe R, Wanigasuriya KP, Peiris-John RJ, Lunyera J, Smith K, Raymer JH, Levine KE** (2014). "Additional perspectives on chronic kidney disease of unknown aetiology (CKDu) in Sri Lanka – lessons learned from the WHO CKDu population prevalence study". BMC Nephrology. 15 (1): 125.

**Rodeghiero F, Castaman G, Tosetto A**(2009). How I treat von Willebrand disease. Blood. 2009;114:1158–1165.

**Ruggeri ZM**(2007).The role of von Willebrand factor in thrombus formation. Thromb Res. ;120(Suppl 1):S5–S9.

**Ruggeri ZM, Mendolicchio GL**(2007). Adhesion mechanisms in platelet function. Circ Res.;100:1673–1685.

**Sabovic M, Salobir B, Preloznik Zupan I, Bratina P**, **Bojec V, Buturovic Ponikvar J**.(2005) The influence of the haemodialysisprocedure on platelets, coagulation and fibrinolysis. Pathophysiol Haemost Thromb ;34(6):274–278

**Segarra A, Chaco´n P, Martinez-Eyarre C**, et al(2001). Circulating levels of plasminogen activator inhibitor type-1, tissue plasminogen activator, and thrombomodulin in hemodialysis patients: biochemical correlations and role as independent predictors of coronary artery stenosis. J Am Soc Nephrol ;12(6):**1255–1263**.

**Schmaier, Alvin H., Lazarus Hillard M.,** (2012). Concise Guide to Hematology, **1st** Edition, Pp.: 91 – 92.

**Schulze H, Shivdasani RA**.( 2005) Mechanisms of thrombopoiesis. Thromb Haemostasis .;3:1717–1724.

**Shibata T, Magari Y, Kamberi P, et al.,** (2000). Significance of urinary fibrin/fibrinogen degradation products (FDP) D-dimer measured by a highly sensitive ELISA method with a new monoclonal antibody (D-D E72) in various renal diseases. Clin Nephrol ; 44: 91– 95

**Shlipak M, Fried L, Crump C, et al.,** (2003). Elevations of Inflammatory and Procoagulant Biomarkers in Elderly Persons with Renal Insufficiency. Circulation; 107:87-92.

**Sugidachi A, Asai F, Yoneda K, Iwamura R, Ogawa T, Otsuguro K, Koike H** (2001). Antiplatelet action of R-99224, an active metabolite of a novel thienopyridine-type G(i)-linked P2T antagonist, CS-747. Br J Pharmacol.;132:47–54.

**"United States Renal Data System (USRDS)".**

**Varga-Szabo D, Pleines I**, **Nieswandt B**(2008).Cell adhesion mechanisms in platelets. Arterioscler Thromb Vasc Biol.;28:403–412.

**Vecchio M, Navaneethan SD, Johnson DW, Lucisano G, Graziano G, Saglimbene V, Ruospo M, Querques M, Jannini EA, Strippoli GF** (2010). "Interventions for treating sexual dysfunction in patients with chronic kidney disease". Cochrane Database Syst Rev (12): CD007747.

**Weber R, Weimar C, Diener HC**(2009).Medical prevention of stroke and stroke recurrence in patients with TIA and minor stroke.Expert Opin Pharmacother.;10:1883–1894.

"**Wies, C. H**(1929). "The History of Hemostasis." Yale Journal of Biology and Medicine 2".: 167–68.

**Wein AJ, Kavoussi LR, Novick AC, Partin AW ,Peters CA**(2007). Campell-Walsh Urology .**9th**ED. Saunders; .Vol1: **p 24.**

**Wein AJ, Kavoussi LR, Novick AC, Partin AW ,Peters CA**(2007). Campell-Walsh Urology .**9th** ED. Saunders; .Vol1: **pp 30-1.**

**Xiao T, Takagi J, Coller BS, Wang JH, Springer TA**(2004).Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. Nature.;432:59–67.

**Xie, X; Liu, Y; Perkovic, V; Li, X; Ninomiya, T; Hou, W; Zhao, N; Liu, L; Lv, J; Zhang, H; Wang, H** ( 2015). "Renin-Angiotensin System Inhibitors and Kidney and Cardiovascular Outcomes in Patients With CKD: A Bayesian Network

Meta-analysis of Randomized Clinical Trials.".American Journal of Kidney Diseases (Systematic Review & Meta-Analysis). S0272-6386 (15): 01312–8.

**Yago T, Lou J, Wu T, Yang J, Miner JJ, Coburn L, Lopez JA, Cruz MA, Dong JF, McIntire LV, McEver RP, Zhu C** (2008). Platelet glycoprotein Ibalpha forms catch bonds with human WT vWF but not with type 2B von Willebrand disease vWF. J Clin Invest. ;118:3195–3207.

**Zucker-Franklin D. Megakaryocyte and platelet structure**(2000). **In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Silberstein LE, McGlave P**, editors. Hematology Basic Principles and Practice. 3.pp. **1730–1740** .

**Sudan university of science & Technology**

**Collage of medical laboratories science**

# Measurement of prothrombin time and activated partial thromboplastic time and d dimer in CKD Patients

---

## Questionnaire

**Name :……**

**Age :……**

**Gender :** **Male( )** **Female ( )**

**Duration of chronic kideney disease :……..**

**Duration of haemodialysis ?.....**

**Do you have previous thrombosis?.....**

**Do you have previous bleeding**?**.....**

## Investigation :

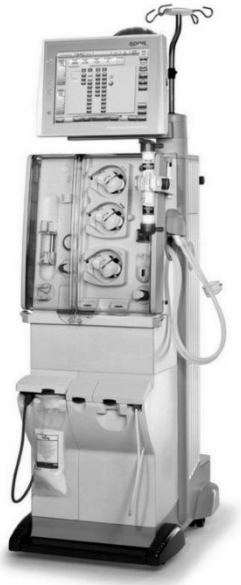
**PT**……

**APTT**……

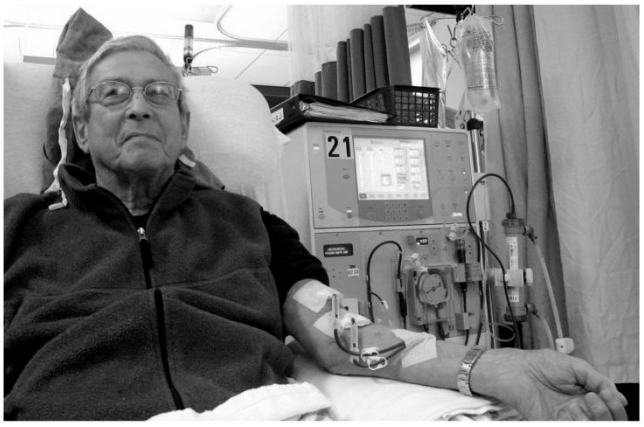
**D dimer**……

**After understanding the contentsof this questionnaire and the aim of research I agree …… ……. To collect the sample**.

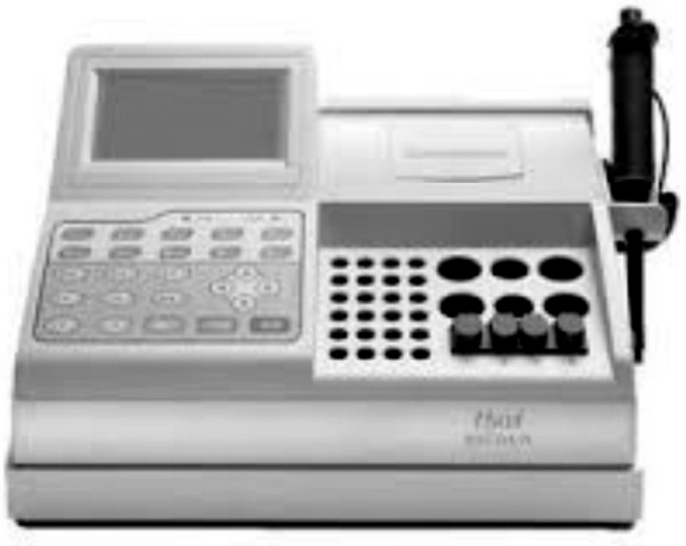
**Signature**:**…….Date**……..



**Haemodialysis machine**




**Patient during haemodialysis**



**Automated Bio Bas Coagulometer**