1. Introduction and literature review

1.1Introduction:

Hemostasis is one of the most significant maintenance systems of human body homeostasis that maintain the liquid state of circulating blood and prevent bleeding that result from blood vessel damage. Bleeding after injury is stopped in three stages: Vascular stage, Platelet stage and Blood coagulation (Baklaja *et al*., 2008) which divided into 3 pathways of intrinsic, extrinsic and common. (Abdulrahaman and Dallatu, 2012).

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are hematological indices that give an insight into the coagulation status of patients. The blood contains about a dozen clotting factors .These factors are proteins that exist in the blood in an inactive state, but can be called into action when tissues or blood vessels are damaged. Blood clotting is the transformation of liquid blood into a semisolid gel. Clots are made from fibers (polymers) of a protein called fibrin. Fibrin monomers come from an inactive precursor called fibrinogen. Also called Factor I, fibrinogen play critical role in blood viscosity(Abdulrahaman and Dallatu, 2012).

In the laboratory, measurement of PT, APTT, and fibrinogen concentration are the most commonly employed laboratory tests in patients with a suspected coagulopathy. Prothrombin time is a laboratory screening test used to detect disorders involving the activity of the factors I, II, V, VII, and X of the extrinsic and common pathways . Activated partial thromboplastin time is used as screening test to detect disorders involving the activity of the factors for I, II, V, VIII, IX–XI, and XII of the intrinsic and common pathways , and to monitor the anticoagulant effect of circulating heparin (Abdulrahaman and Dallatu, 2012).Increase or decrease in these proteins favour the development of hyper-

coagulable and pro-thrombotic state, which may in turn enhance cardiovascular risk by increasing the occlusive thrombus (Abdulrahaman and Dallatu, 2012).

Diabetes mellitus is a common endocrine disease of multiple etiologies. It is characterized by chronic hyperglycemia with subsequent disturbances of carbohydrates, fat and protein metabolism .Excessive blood glucose produces the classical symptoms such as polydipsia (increased thirst), polyuria (frequent urination) and polyphagia (increased hunger) (Abdulrahaman and Dallatu, 2012). Diabetes mellitus is commonly associated with both microvascular and macrovascular complications. Patients with type II diabetes mellitus have a two- to four fold increase in the risk of coronary artery disease which accounts for 60% of their deaths. Hyperglycemia, a well defined risk factor for accelerated atherosclerosis and vascular disease, may cause vessel damage (Dhule and Gawali, 2014).

Hypertension is a chronic elevation of blood pressure that, in the long-term, causes end organ damage and results in increased morbidity and mortality (Osman and Muddathir, 2013)**.** In Sudan, the prevalence of hypertension in an urban increased from 7.5% in 1985 to 18.2% in 2002 (Sherif1 *et al*., 2008). In about 90% of cases of hypertension has no known cause (primary or essential hypertension), the remainder are mostly secondary to renal disease or (less often) to renal artery stenosis (renovascular hypertension), endocrine abnormalities, vascular malformations, or neurogenic disorders (Mitchell *et al.,* 2006). Thrombosis often appears to complicate the course of patients with hypertension; thormbosis in some patients with hypertension could be developed to organ damage (Osman and Muddathir, 2013)**.**

Aim of this study was to detect the possibility of thrombosis in hypertensive patients and type II diabetic patients.

1.2 literature review

1.2.1 Hemostasis (Physiological):

1.2.1.1Definition:

Hemostasis is one of the most significant maintenance systems of human bodily homeostasis; hemostasis plays two roles in the organism, namely:

- To provide blood flow through blood vessels, i.e., to maintain the liquid state of circulating blood.
- To prevent bleeding that results from blood vessel damage

Hemostasis is a complex system that includes the participation of several factors:

– Blood vessel endothelium

– Platelets

– Blood coagulation

– Fibrinolytic process

– Coagulation inhibitors (Baklaja *et al.*, 2008).

The basic function of normal hemostasis is to prevent blood loss from undamaged blood vessels and to inhibit massive bleeding from damaged blood vessels. Blood loss from undamaged blood vessels is prevented by the normal blood vessel structure and normal platelet function. Bleeding after injury is stopped in three stages:

1. Vascular stage
2. Platelet stage
3. Blood coagulation (Baklaja *et al.*, 2008).

The significance of these mechanisms depends on the size and the type of the injury. Normal hemostasis starts with the vascular stage, is then followed by the platelet stage, which creates a platelet clot, and finally ends with the mutual action of coagulation factors, resulting in plug formation (Baklaja *et al.*, 2008).

1.2.1.2 Coagulation stage:

1.2.1.2.1 Vascular Stage – Endothelium:

The role of the blood vessel in hemostasis is the construction of an injured blood vessel – vasoconstriction. This lasts less than a minute and is a reflex action that is prolonged by serotonine from thromboxane A2 (TA2) and fibrinopeptide B, which, in turn, is created by the action of thrombin on fibrinogen. Blood vessel constriction prevents blood release and is sometimes sufficient for hemostasis maintenance (Baklaja *et al.,* 2008).

When a blood vessel is injured, endothelium cells are damaged and circulating blood is exposed to the effect of various endothelium structures, such as collagen, fibronectin and von Willebrand's factor (vWF); this results in platelet adhesion (Baklaja *et al.*, 2008).

The endothelium actively affects the function of all hemostasis components. The endothelium has two roles: activation and inhibition of hemostasis (Baklaja *et al.*, 2008).

Formation of vWF from Weibl-Palady's corpusculums from endothelium cells is the most important role of endothelium activation. It also plays a role in the adhesive capacity of platelets and synthesis of vasoactive enzymes. Coagulation factors, which activate thrombin formation, are placed on the endothelium surface, synthesis of t-PA (tissue plasminogen activator) and u-PA (urokinase plasminogen activator) (Baklaja *et al.*, 2008).

The endothelium plays an inhibitory role in hemostasis. It synthesizes prostacycline PGI2 and nitrogen oxide (NO), which have an inhibitory effect on platelet aggregation. The endothelium contains ATP-asis (adenosine triphosphate) that destroys platelet andenosine diphosphate (ADP) and restricts platelet activation. Thrombomodulin, which is present on the surface of endothelium cells of other organs, except the brain, is found on the membrane. Thrombomodulin is

bound to thrombin forming a complex that activates protein C (PC). Heparin sulfate and other glycosoamines are also present on the membrane as well as some other glycosoamines that participate in AT III activation. The endothelium takes part in fibrinolysis and excretes t-PA and PAI-1 (plasminogen activator inhibitor) (Baklaja *et al.*, 2008).

Table (1.1) the Role of Blood Vessel Endothelium (Baklaja et al., 2008).

	Activation	Inhibition
Vasoregulation	synthesis of vasoactive enzymes (TA2 and edothelin)	synthesis of vasodilatators (prostacycline and NO)
Platelets	stimulates adhesive capacity and aggregation (collagen, fibronectin and vWF)	inhibits aggregation (prostacycline and NO)
Coagulation	phospholypid and coagulation factors are activated on the membrane	thrombomoduln (activates PC), heparin sulphate (activ. AT III) TFPI (inhibits TK)
Fibrinolysis	synthesis of t-PA and u-PA	synthesis of PAI-1 and PAI-2

1.2.1.2.2 Platelet Stage:

Normal physiological response to vascular injury includes rapid platelet adhesion on the subendothelium and generation of thrombin, which induces platelet aggregation and fibrin formation. Platelet adhesion occurs when proteins (collagen and fibronectin) bind to specific glycoprotein (GP) receptors, also known as integrins, on platelet membranes. When the endothelium is injured, circulating vWF binds to collagen and undergoes a conformational change allowing it to bind to platelet receptor GPIb/IX. Patients lacking GPIb/IX (Bernard-Soulier Syndrome) or suffering from a deficiency of high molecular weight multimers of

vWF, as in von Willebrand's disease, show a prolonged bleeding time and hemorrhagic syndrome (Baklaja *et al.,* 2008).

During adherence and activation, platelets change from discs into spheres with projections on long pseudopods. The platelet plug is developed further on the initial layer of adherent platelets by aggregation of platelets recruited to the site of vessel injury (Baklaja *et al.,* 2008).

Platelets undergo aggregation and release the contents of their dense granules and α-granules when exposed to agonists such as ADP, epinephrine, thrombin or collagen (Baklaja *et al.,* 2008).

Dense granules release calcium, serotonin and ADP, the latter of which promotes continued aggregation. The secreted α-granule content includes platelet factor 3, β-thromboglobulin, platelet-derived growth factor, thrombospondin, factor V and plasma proteins such as fibrinogen and IgG (immunoglobulin G). ADP and epinephrine, otherwise weak agonists for platelet aggregation, require prostaglandin T A2 for secretion of granular contents. Thromboxane A2 is synthesized from arachidonic acid that is released from platelet phospholipids during the aggregation process under the influence of phospholipid (Baklaja *et al.,* 2008).

1.2.1.2.2.1 The Role of Platelets in Hemostasis:

Two potent platelet aggregating agents are thrombin, which binds to GPV as well as to GPIb (vWF receptor), and collagen, which binds to GPIa/IIa. Thrombin and collagen can induce aggregation of platelets and secretion of platelet granular contents even if prostaglandin synthesis is blocked. A bond forms between fibrinogen and adjacent platelets through the interaction with platelet receptor complex GPIIb/IIIa. Fibrinogen binding only occurs after the platelet-activation-induced conformational change of the complex. GPIIb/IIIa is a transmembrane complex associated with actin on the inner surface of the platelet. Actin is a major

component of platelet cytoskeleton. An actin-GPIIb/IIIa association is essential for clot retraction. Patients who show either the rare condition of afibrinogenemia or lack GPIIb/IIIa complex (Glanzmann thrombastenia) have poor clot retraction and hemorrhagic syndrome (Baklaja *et al.,* 2008).

1.2.1.2.3 Blood Coagulation:

Blood coagulation is a series of enzyme processes in which inactive coagulation factors gradually become active. Each activated factor activates the next factor in the series. Blood remains liquid even though all coagulation factors are present in circulation. Plasma coagulation factors (F), indicated with roman numerals I to XIII, participate in the process of blood coagulation (Baklaja *et al.,* 2008).

All coagulation factors are synthesized in the liver. Certain coagulation factors require the presence of vitamin K for their synthesis. Vitamin K-dependent procoagulant factors II, VII, IX and X are synthesized in the liver, circulate as zymogens, are activated on the phospholipids surface and are limited by proteolysis. These factors belong to the serine proteases. Serine is found on the active site of the molecule's carboxyterminal. On the molecule's amino-terminal, each factor has 9 to 12 γ-carboxyglutamine residues, which are known as G1a domains, and are significant for binding calcium. Vitamin K is necessary for the carboxylation of these proteins. Inhibition of carboxylation leads to formation of factors that are incapable of binding to phospholipids and, thus, cannot manifest their procoagulant activity (Baklaja *et al.,* 2008).

There is a permanent balance between coagulation activation and inhibition. If this balance is disturbed, thromboembolic complications or bleeding may occur. The hemostatic process (fibrin formation in blood vessel) is under continuous control of plasma inhibitors: antithrombin III, heparin, PC, PS, α2-macroglobulin (α2MG), C1esterase inhibitor, α1-antitripsin and α2- antiplasmin (α2AP) (Baklaja *et al.,* 2008).

There are three known coagulation pathways: intrinsic, extrinsic and common.

1.2.1.2.3.1 Intrinsic Pathway:

The intrinsic pathway consists of a cascade of protease reactions initiated by factors that are present within the blood. When in contact with a negatively charged surface such as glass or the membrane of an activated platelet, a plasma protein called FXII (Hageman factor) becomes FXIIa (the suffix "a" indicates that this is the activatedform of FXII). A molecule called high molecular weight kininogen (HMWK), a product of platelets that may in fact be attached to the platelet membrane, helps anchor FXII to the charged surface and thus serves as a cofactor. However, this HMWK-assisted conversion of FXII to FXIIa is limited in speed (Riddel *et al.,* 2007).

Once a small amount of FXIIa accumulates, this protease converts prekallikrein to kallikrein, with HMWK as an anchor. In turn, the newly produced kallikrein accelerates the conversion of FXII to FXIIa, an example of positive feedback. In addition to amplifying its own generation by forming kallikrein, FXIIa (together with HMWK) proteolytically cleaves FXI, forming FXIa. In turn, FXIa (also bound to the charged surface by HMWK) proteolytically cleaves FIX to FIXa, which is also a protease (Riddel *et al.*, 2007).

FIXa and 2 downstream products of the cascade, FXa and thrombin, proteolytically cleave FVIII, forming FVIIIa, a cofactor in the next reaction. Finally, FIXa and FVIIIa together with Ca^{2+} (which may come largely from activated platelets) and negatively charged phospholipids (the major constituents of cell membranes) form a trimolecular complex termed tenase. Tenase then converts FX to FXa, yet another protease (Riddel *et al.,* 2007).

In a parallel series of interactions, FXa binds to the cofactor FVa, itself a downstream factor that participates in positive feedback with the present reaction to generate a complex with enzymatic activity known as prothombinase. This

complex converts the proenzyme prothrombin to its enzyme form, thrombin. Thrombin acts on fibrinogen to generate the fibrin monomer, which rapidly polymerizes to form the fibrin clot. During clinical laboratory analysis of blood clotting, the intrinsic pathway of blood coagulation is evaluated using the activated partial thromboplastin time (APTT) (Riddel *et al.,* 2007).

1.2.1.2.3.2 Extrinsic Pathway:

The extrinsic pathway also includes protein cofactors and enzymes. This pathway is initiated by the formation of a complex between tissue factor (TF) on cell surfaces and FVIIa that is located outside the vascular system. Nonvascular cells constitutively express the integral membrane protein TF (variably known as FIII or tissue thromboplastin), which is a receptor for the plasma protein FVII (Riddel *et al.,* 2007).

When an injury to the endothelium allows FVII to come into contact with TF, the TF nonproteolytically activates FVII to FVIIa. The mechanism of the initial conversion of the zymogen FVII to FVIIa is still debated but is most likely due to autocatalytic activation and not a TF effect (Riddel *et al.,* 2007).

This binding of FVIIa to TF forms an enzyme complex that activates FX to FXa. The FVIIa/TF complex, similar in function to the tenase complex, converts FX to its active form (FXa), which binds to the cofactor FV and is bound on membrane surfaces in the presence of calcium ions to generate the prothrombinase complex. The prothrombinase complex converts prothrombin to thrombin, which converts fibrinogen to fibrin to generate the fibrin clot. During laboratory analysis of blood clotting, the extrinsic pathway of blood coagulation is evaluated using the prothombin time (PT). Regardless of whether FXa arises by the intrinsic or extrinsic pathway, the cascade then proceeds along the common pathway (Riddel *et al.,* 2007).

1.2.1.2.3.3 Common Pathway:

The common pathway begins with the activation of FX within the intrinsic pathway, the extrinsic pathway, or both. FXa from either the intrinsic or extrinsic pathway is the first protease of the common pathway. FXa, in the presence of FV, Ca^{2+}, and phospholipids, converts prothrombin to its active form; thrombin. The main action of thrombin is to catalyze the proteolysis of the soluble plasma protein fibrinogen to form fibrin monomers that remain soluble. Fibrin monomers then polymerize to form a gel of fibrin polymers that trap blood cells. Thrombin also activates FXIII, which is converted to FXIIIa and mediates the covalent cross-linking of the fibrin polymers to form a mesh termed stable fibrin, which is less soluble than fibrin polymers. Thrombin can catalyze the formation of new thrombin from prothrombin and can catalyze the formation of the cofactors FVa and FVIIIa, resulting in efficient amplification of coagulation. Because the common pathway contains the factors FX, FV, and FII (any deficiency in which may lead to a hemorrhagic disorder), these factors may be monitored by both the PT and the APTT. In recent years, deficiencies of this scheme have become apparent. First, no explanation existed for the absence of a clinical bleeding tendency in deficiencies of FXII, prekallikrein, or high molecular weight kininogen, even though deficiencies in any one of these factors markedly prolong surface-activated coagulation assays for hemostasis in vitro. Second, no explanation existed for why FVIII or FIX deficiency caused clinically severe bleeding, even though the extrinsic pathway would be expected to bypass the need for FVIII and FIX. These key observations led to a revision of earlier models of coagulation. A vital observation was that a complex of FVIIa and TF activated not only FX but also FIX. More recent observations have led to the conclusion that activity of the FVIIa/TF complex is the major initiating event in hemostasis in vivo (Riddel *et al.*, 2007).

1.2.1.2.4 Cell-Based Model of Coagulation:

A major development over the past 15 years was the discovery that exposure of blood to cells that express tissue factor (TF) on their surface is both necessary and sufficient to initiate blood coagulation in vivo. This finding led to the belief that the intrinsic pathway (the contact system) does not have a true physiological role in hemostasis. Very recent evidence suggests that although FXII deficiency does not result in bleeding problems, the absence of FXII does protect against pathological thrombosis (Riddel *et al.,* 2007).

In the cell-based model, hemostasis requires the formation of an impermeable platelet and fibrin plug at the site of vessel injury, but it also requires that the procoagulant substances activated in this process remain localized to the site of injury. The process of blood coagulation is initiated by the exposure of cells expressing TF to flowing blood. Tissue factor is expressed constitutively on cells such as smooth muscle cells and fibroblasts but not on resting endothelium. Tissue factor is present in the membranes of cells surrounding the vascular bed but is normally not in contact with blood. Mounting evidence suggests that TF is present in blood on cellular microparticles. These membrane fragments derive from various cell types: white blood cells, endothelium, and platelets, which may play a more important role in pathological hemostasis (thrombosis) as opposed to normal clotting (Riddel *et al.,* 2007).

It is best to consider the highly interwoven array of physical, cellular, and biochemical processes that contribute to hemostasis as a series of process stages (phases) rather than pathways. The phases of initiation, propagation, and termination illustrate the intricate processes involved in the maintenance of vascular integrity (Riddel *et al.,* 2007).

Initiation Phase:

The initiation phase is localized to the cells that express TF, which are found normally outside the vasculature. The FVIIa/TF complex activates small amounts of FIX and FX. FXa associates with its cofactor, FVa, and forms a prothrombinase complex on the surface of the TF-bearing cell. FV can be activated by FXa or by noncoagulation proteases to produce the FVa required for prothrombinase assembly (Riddel *et al.*, 2007).

Low-level activity of the TF pathway occurs at all times within the extravascular space. Coagulation proteins percolate through tissues, leaving the vasculature, and are found in the lymph roughly in proportion to their molecular size. It is likely that FVII is bound to extravascular TF even in the absence of an injury, and the extravascular FX and FIX can be activated as they pass through the tissues. The coagulation process proceeds to the amplification phase only when damage to the vasculature allows platelets and FVIII (bound to vWF) to spill out into the extravascular tissues and to adhere to TF-bearing cells at the site of injury. Extrinsic is an appropriate name for the TF pathway because it can be thought of as operating outside the vasculature (Riddel *et al.*, 2007).

Amplification Phase:

A small amount of thrombin generated on the TF bearing cell has several important functions. A major function is the activation of platelets, exposing receptors and binding sites for activated clotting factors. As a result of this activation, the platelets release partially activated forms of FV onto their surfaces. Another function of the thrombin formed during the initiation phase is the activation of the cofactors FV and FVIII on the activated platelet surface. In this process, the FVIII/VWF complex is dissociated, permitting vWF to mediate additional platelet adhesion and aggregation at the site of injury. In addition, small amounts of

thrombin activate FXI and FXIa on the platelet surface during this phase (Riddel et al., 2007).

Propagation Phase:

As large numbers of platelets are recruited to the site of injury, the propagation phase of clot formation occurs on the surface of activated platelets. First, FIXa activated during the initiation phase can now bind to FVIIIa on the platelet surface. Second, additional FIXa can be supplied by platelet-bound FXIa. Third, because FXa cannot move effectively from the TF-bearing cell to the activated platelet, FXa must be provided directly on the platelet surface by the FIXa/FVIIIa complex. Fourth, the FXa rapidly associates with FVa bound to the platelet during the amplification phase. Finally, the completion of this platelet prothrombinase assembly leads to a burst of thrombin generation of sufficient magnitude to clot fibrinogen (Riddel et al., 2007).

Termination Phase:

Once a fibrin platelet clot is formed over an area of injury, the clotting process must be limited to avoid thrombotic occlusion in surrounding normal areas of the vasculature. Unless controlled, clotting could propagate throughout the entire vascular tree (Riddel *et al.*, 2007).

1.2.1.2.5 Coagulation inhibitors:

Inhibitors prevent the coagulation process; i.e., they limit the coagulation process at the site of blood vessel injury (AT III, thrombomodulin, TFPI, PC).

antithrombin (ATIII) inhibits the activity of thrombin and other serine proteases, such as F IXa, F Xa, F XIa, and F XIIa. Binding to heparin-like molecules on endothelial cells activates antithrombin. AT III deficiency is either inherited or acquired and is associated with the predisposition to thromboembolic diseases

Proteins C and S are characterized by their ability to inactivate the procoagulant cofactors FVa and FVIIIa. Protein C is a vitamin K–dependent plasma

glycoprotein that, when activated, functions as an anticoagulant by inactivating FVa and FVIIIa. Protein C activity is enhanced by another vitamin K–dependent inhibitory cofactor, protein S. Protein S functions as a cofactor to protein C by enhancing its activity against FVa and FVIIIa . In human plasma, about 30% of protein S circulates as free protein; the remainder is bound to the complement regulatory protein C4b-binding protein. Only the free form of protein S functions as a cofactor to activated protein C (Riddel *et al.*, 2007).

Tissue factor pathway inhibitor (TFPI), a protein secreted by endothelium, complexes to FXa and to TF/FVIIa, inactivating them to rapidly limit coagulation (Riddel *et al.*, 2007).

Thrombomodulin has an anticoagulant effect by converting thrombin from coagulant to anticoagulant. Thrombin loses its ability to convert F I to fi brin. It does not activate platelets as well as F V and F VIII, but it activates protein C.In order to obtain the catalytic ability to activate PC into PCa, thrombin has to bind to the cofactor thrombomodulin (TM), which is synthesized by the vascular endothelium. In complex with thrombomodulin, thrombin loses its ability to bind to fibrinogen and to activate coagulation factors V and VIII and platelets. Thrombin only maintains the ability to activate PC (Baklaja *et al.*, 2008).

1.2.1.3 Fibrinolysis:

1.2.1.3.1 Definition:

The fibrinolytic system removes the blood clot after the blood vessel defect is closed. In addition to this basic function, the fibrinolytic system participates in tissue reparation, ovulation, embryo implantation, malignant transformation, inflammation, and macrophage functions. Plasminogen, plasmin, plasminogen activators, inhibitor activators and antiplasmins are active fibrinolysis factors (Baklaja et al., 2008).

Plasmin is the main fibrinolytic enzyme. In circulation it is found in its inactive form as plasminogen. Plasminogen is a glycoprotein and is synthesized in the liver. There are several types of plasminogen in plasma. Two of the most important are a "native"form that contains glutamine in the amino-terminal (glu- plasminogen) and a "modified"form that contains lysine in the amino-terminal (lys- plasminogen). Plasminogen is transformed into plasmin by an activator. The most important substance for plasmin activation is fibrin. As a non-specific proteolytic enzyme, however, fibrin degrades all peptides that contain arginyl-lysine residues. Activated plasmin degrades fibrinogen, FV, FVIII and vWF in the coagulation system, but it also activates HMWK and complementary elements (Baklaja *et al.,* 2008).

Plasmin cleaves both fibrinogen and fibrin into a family of fragments known as fibrinogen-fibrin degradation products or fragments X, Y, D and E. They can be found in the circulation in various degrees of degradation. Fragments D and E are terminal fragments resistant to further proteolytic plasmin activity. Intermediate fragments X and Y can be further degraded to fragments D and E. Fibrin and fibrinogen degradations are quite similar although, in several instances, they vary. Fragment E, resulting from fibrinogen degradation, contains fibrinopeptides A and B whereas fragment E, resulting from fibrin degradation, does not. Some fragments have biological activity. Fragments D and E, for instance, inhibit coagulation in the thrombin time test. Fragment X preserves the ability of intact fibrinogen molecules to support ADP-induced platelet aggregation and erythrocyte sedimentation. Furthermore, fragment X can be coagulated by thrombin. Terminal fragments D and E stimulate the synthesis and the release of fibrinogen from the liver. Fragments D and E influence electric heart activity; they increase capillary permeability and vasoactive effects of bradykinin and angiotensin II. (Baklaja *et al.,* 2008).

1.2.1.3.2 Activators of Fibrinolysis:

Numerous substances have the ability to change plasminogen into plasmin. The substances are known as plasminogen activators and are classified in endogenous and exogenous forms. Endogenous plasminogen activators are physiological constituents of hemostatic mechanisms and are divided into intrinsic and extrinsic activators. Exogenous plasminogen activators are non-physiological molecules, such as streptokinase. Intrinsic activators are plasma proteins that circulate as inactive precursors. Many of these substances act as mediators in coagulation and inflammatory processes. The intrinsic pathway of plasminogen activation requires FXIIa, which participates in the contact phase of the intrinsic coagulation pathway and induces fibrinolysis. Physiological F XII is activated by contact with a negatively charged surface. Several other coagulation factors can also activate F XII, including HMWK, prekallikrein and plasmin. Upon activation, FXII is cleaved into three peptides; the last of which contains an active part. FXIIa activates plasminogen, directly or indirectly, through prekallikrein and FXI activation (Baklaja *et al.,* 2008).

Extrinsic plasminogen can be activated by numerous tissues, including neoplasms. They are far more potent than intrinsic activators and form a heterogeneous group of molecules that can be divided into two main subgroups: t-PA and u-PA. Both directly activate plasminogen by cleaving an Arg-560-Val-561 bond (Baklaja *et al.,* 2008).

t-PA is produced in almost all tissues but mostly in the vascular endothelium. Tissue plasminogen activator is also found in body fluids such as tears, saliva, breast milk and sperm. Its enzyme activity is increased in the presence of fibrin. Over 95% of the circulating t-PA is present in complexes with plasminogen activator inhibitor type 1 (PAI-1) (Baklaja *et al.*, 2008).

u-PA, or UK, forms the second subgroup of extrinsic plasminogen activators. Urokinase is produced in the kidney parenchyma. It is excreted in small amounts with the urine. Receptors for u-PA can be found in many tissues, since u-PA has an important role in inflammation, tumor invasion, fertilization and embryogenesis (Baklaja *et al.*, 2008).

Streptokinase is the most important substance among extrinsic plasminogen activators. It was first isolated from β-hemolytic streptococcus group C. In order to develop catalytic activity, streptokinase forms complexes with plasminogen. There, an active part is exposed without any peptide bonds being cleaved. Streptokinase has antigenic properties and, thus, may cause formation of anti- streptokinase antibodies (Baklaja *et al.*, 2008).

1.2.1.3.3 Inhibitors of Fibrinolysis:

Inhibitors of fibrinolysis directly inhibit plasmin (antiplasmins) or plasminogen activators. Endogenous substances that inhibit plasmin are serine proteases. The most important physiological inhibitor of plasmin is α2- antiplasmin, a glycoprotein synthesized in the liver. The level of α2AP is decreased in liver diseases and in DIC. Inherited deficiency of α2AP, which is associated with bleeding, is very dangerous (Baklaja *et al.*, 2008).

α2-macroglobulin is the next important physiological plasmin inhibitor. It is a serine protease synthesized by endothelial cells, monocytes and macrophages in the liver. Increased levels are found during pregnancy, in nephritic syndrome, in lung and liver diseases, and during oral contraceptive drug use. It is an acute-phase protein. Decreased levels are found during thrombolytic therapy and in DIC. It inhibits plasmin, but it also degrades fibrin, fibrinogen and FVIII. It inhibits other components of the fibrinolytic system: t-PA, streptokinase-plasminogen complex and kallikrein (Baklaja *et al.*, 2008).

Other inhibitors of fibrinolysis are:

α1AP - Increased levels can be found during inflammation in neoplasms and in estrogen therapy.

C1inhibitor- It inhibits the first complement component, as well as plasmin, kallikrein, and factors XIa and XIIa. Deficiency of C1inhibitor is associated with hereditary angioedema without bleeding.

AT III- The primary inhibitor of thrombin and some coagulation factors; it also slowly and irreversibly inhibits plasmin. The contribution of AT III to plasmin inhibition is small since only 1% of plasmin is bound to AT III.

PAI - The most important inhibitors being PAI-1 and PAI-2 while less significant ones are protease nexin I and PAI-3, otherwise identical to protein C inhibitor (PCI) (Baklaja *et al.*,2008).

1.2.1.4 Laboratory diagnostics of hemorrhagic disorders:

Diagnosis of hemorrhagic disorders can be made based on:

− History of the disease

− Clinical check-up

− Laboratory tests:

1. Screening tests for vascular and platelet disorders.
2. Screening tests for coagulation disorders.
3. Specific tests (Baklaja *et al.*, 2008).

Laboratory Analysis:

In order to diagnose vascular and platelet disorders, bleeding time and platelet count should be determined. Determination of bleeding time is the most easily and most frequently performed test. Normal values range from 1 to 3 minutes (Duke). It is prolonged in blood vessel wall structure disorder, decreased platelet count, disorders of adhesion and aggregation, disturbance of platelet reaction release. Normal platelet count ranges from 150 to $450x10^9$/l.

Bleeding time can be prolonged due to:

− Abnormal vessel constriction.

− Low platelet count.

− Abnormal platelet adhesion.

− Abnormal platelet release of ADP or TA2.

− Abnormal platelet aggregation.

− vWD.

TT, PT and APTT are determined in order to diagnose coagulopathies. These tests include disorders of the intrinsic, extrinsic and common activation pathways, both individual and associated defects of several coagulation factors (Baklaja et al., 2008).

Thrombin Time (TT):

Measures the time of fibrinogen transformation into fibrin under the influence of thrombin. Thus, all other factors are avoided. TT is prolonged in hypo- and afibrinogenemias, in the presence of inhibitors, when fibrinogen changes into fibrin, such as heparin, and in the presence of FDP and soluble fibrin monomers. Normal time ranges from 16 to 20 seconds (Baklaja et al., 2008).

Prothrombin Time (PT):

Determines extrinsic and common coagulation pathways and, thus, is prolonged in deficient F II, FV, FVII, FX and fibrinogen. This test is necessary in oral anticoagulant therapy control, as well as in the investigation of liver function. (Baklaja et al., 2008). Normal values range from 11 to 16 seconds (Lewis et al., 2006).

Activated Partial Prothrombin Time (APTT):

Determines the internal mechanism of blood coagulation and is prolonged in deficiencies of all plasma coagulation factors: FI, FII, FV, F VIII, FIX, FX, FXI

and F XII, except in F VII and F XIII deficiency. It is prolonged in prekallikrein and in HMWK deficiency.

APTT is prolonged in the presence of heparin and inhibitors. It is particularly prolonged in hemophilia and in the presence of heparin. This test has completely replaced the coagulation time test from venous blood. A coagulation time test from capillary blood is not safe because of the presence of tissue thromboplastin; thus, it shows normal values in all coagulopathies, even in hemophilias (Baklaja et al., 2008). Normal time ranges from 26 to 40 seconds (Lewis *et al.,* 2006).

All tests are performed in plasma obtained by blood centrifugation, drawn in 3.8% sodium citrate in 1:9 ratio. The blood is drawn with plastic syringes into plastic or silicon tubes. Tests are performed right after blood has been drawn (Baklaja *et al.,* 2008).

Fibrinogen (factor I):

(factor I) is a soluble dimeric protein each half consisting of three polypeptide named Aα ,Bβ and γ held together by 12 disulphide bonds, synthesized by the liver, that is converted by thrombin into fibrin during blood coagulation. This is achieved through processes in the coagulation cascade that activate the zymogene prothrombin to the serine protease thrombin, which is responsible for converting fibrinogen into fibrin (Baklaja et al., 2008). The normal range is 180-360 milligrams per deciliter (mg/dl) (Lewis *et al.,* 2006).

Increased in Tissue inflammation or damage, acute infection, myocardial infarction, medications, oral contraceptives, pregnancy. Decreased in disseminated intravascular coagulation (DIC), primary or secondary fibrinolysis, liver disease, hereditary a fibrinogenemia or hypofibrinogenemia, cachexia (Baklaja *et al.,* 2008).

1.2.2Diabetes mellitus:

1.2.2.1 Definition:

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood glucose. Hyperglycemia, or raised blood glucose, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels (WHO, 2013).

1.2.2.2 Types of diabetes:

1.2.2.2.1 Type 1 diabetes:

- Formerly known as Insulin-Dependent Diabetes Mellitus (IDDM).
- Characterized by hyperglycemia due to an absolute deficiency of the insulin hormone produced by the pancreas.
- Patients require lifelong insulin injections for survival.
- Usually develops in children and adolescents (although can occur later in life).
- May present with severe symptoms such as coma or ketoacidosis.
- Patients are usually not obese with this type of diabetes, but obesity is not incompatible with the diagnosis.
- Patients are at increased risk of developing microvascular and macrovascular complications (WHO, 1999).
- Usually (but not always) caused by autoimmune destruction of the beta cells of the pancreas, with the presence of certain antibodies in blood.
- A complex disease caused by mutations in more than one gene, as well as by environmental factors (WHO).

Symptoms and diagnosis of type I diabetes:

- Increased urinary frequency (polyuria), thirst (polydipsia), hunger (polyphagia), and unexplained weight loss.
- Numbness in extremities, pain in feet (disesthesias), fatigue, and blurred vision.
- Recurrent or severe infections.
- Loss of consciousness or severe nausea/vomiting (ketoacidosis) or coma. Ketoacidosis more common in T1D than in T2D (WHO, 1999).
- Diagnosis is made by the presence of classic symptoms of hyperglycemia and an abnormal blood test.
- A plasma glucose concentration >=7 mmol/L (or 126 mg/dL) or >=11.1mmol/L (or 200mg/dL) 2 hours after a 75g glucose drink.
- In a patient without classic symptoms, diagnosis can also be made by two abnormal blood tests on separate days.
- In most settings (although not always available in resource-poor countries), another test called HbA1C is done to approximate metabolic control over previous 2-3 months and to guide treatment decisions (WHO, 1999).

1.2.2.2.2 Type 2 diabetes:

- Formerly named non-insulin-dependent diabetes mellitus (NIDDM).
- Characterized by hyperglycemia due to a defect in insulin secretion usually with a contribution from insulin resistance.
- Patients usually do not require lifelong insulin but can control blood glucose with diet and exercise alone, or in combination with oral medications, or with the addition of insulin.
- Usually (but not always) develops in adulthood (and is on the rise in children and adolescents).

- Is related to obesity, decreased physical activity and unhealthy diets.
- As in T1D, patients are at a higher risk of microvascular and macrovascular complications (WHO, 1999).
- Associated with obesity, decreased physical activity and unhealthy diets (and involves insulin resistance in nearly all cases).
- Occurs more frequently in individuals with hypertension, dyslipidemia (abnormal cholesterol profile), and central obesity, and is a component of "metabolic syndrome".
- Often runs in families but is a complex disease caused by mutations in more than one gene, as well as by environmental factors (WHO, 1999).

Symptoms and diagnosis:

- Patients may have no symptoms at all or minimal symptoms for years before being diagnosed.
- May have increased urinary frequency (polyuria), thirst (polydipsia), hunger (polyphagia), and unexplained weight loss.
- May also experience numbness in extremities, pain in feet (disesthesias), and blurred vision.
- May have recurrent or severe infections.
- Patients may present with loss of consciousness or coma but this is less common than in T1D (WHO, 1999).
- Diagnosis is made by the presence of classic symptoms of hyperglycemia and an abnormal blood test.
- A plasma glucose concentration >=7 mmol/L (or 126 mg/dL) or >=11.1mmol/L (or 200mg/dL) 2 hours after a 75g glucose drink.
- In a patient without classic symptoms, diagnosis can also be made by two abnormal blood tests on separate days.

- In most settings (although it may not be available in some resource-poor settings), another test called HbA1C is done to approximate metabolic control over previous 2-3 months and to guide treatment decisions. This test can also be used to diagnose type II (WHO, 1999).

1.2.2.2.3 Gestational diabetes (GDM)

- Characterized by hyperglycemia of varying severity diagnosed during pregnancy (without previously known diabetes) and usually (but not always) resolving within 6 weeks of delivery.
- Risks to the pregnancy itself include congenital malformations, increased birth weight and an elevated risk of perinatal mortality.
- Increased risk to woman of developing diabetes (T2D) later in life. (WHO).
- The mechanism is not completely well understood but hormones of pregnancy appear to interfere with insulin action (WHO, 1999).

Symptoms and Diagnosis:

- Increased thirst (polydipsia) and increased urination (polyuria) are more commonly noted (although other symptoms can be present).
- Because pregnancy itself causes increased urination, these symptoms are difficult to recognize as abnormal.
- A larger than normal baby during pregnancy (noted on routine prenatal exam) may prompt diabetic screening (WHO, 1999).
- Diagnosis : Standard OGTT is done at 24-28 weeks after an overnight fast (fasting plasma glucose and a plasma glucose 2 hours after 75g glucose drink is done).
- A 2 hour level >=7.8 mmol/L (or 140 mg/dL) is diagnostic of gestational diabetes.

- If fasting and postprandial blood sugars are elevated in the first trimester, this may indicate preexisting diabetes mellitus (which is considered a different condition, with different implications) (WHO, 1999).

1.2.2.2.4 Intermediate states of hyperglycemia

Description:

IFG, IGT, and diabetes mellitus are seen as progressive stages of the same disease process, and treatment at earlier stages has been shown to prevent progression to later stages (by diet, exercise and lifestyle management). Not all patients with IGT have IFG, so it is considered a separate category. As well, the implications of the two states are slightly different.

Impaired Fasting Hyperglycemia (IFG) is a state of higher than normal fasting blood (or plasma) glucose concentration, but lower than the diagnostic cut-off for diabetes.

Impaired Glucose Intolerance (IGT) is a state of higher than normal blood (or plasma) glucose concentration 2 hours after 75 gram oral glucose load but less than the diagnostic cut-off for diabetes (WHO, 1999).

Symptoms and diagnosis:

Patients usually have no symptoms and are diagnosed because a test is done upon patient request or because he/she falls into a high risk category (WHO).

- Diagnosis: IFG: fasting plasma glucose >=6.1 mmol/L (110 mg/dL) and <7 mmol/L (126 mg/dL) per WHO 1999 criteria.
- IGT: fasting plasma glucose (if available) <7.0 mmol/L (126 mg/dL) AND 2 hour post 75g glucose drink of >= 7.8 mmol/L (140 mg/dL) and <11.1 mmol/L (200 mg/dL) (WHO, 1999).

1.2.2.3 Complications of diabetes:

Diabetes complications are divided into microvascular (due to damage to small blood vessels) and macrovascular (due to damage to larger blood vessels).

Microvascular complications include damage to eyes (retinopathy) leading to blindness, to kidneys (nephropathy) leading to renal failure and to nerves (neuropathy) leading to impotence and diabetic foot disorders (which include severe infections leading to amputation).

Macrovascular complications include cardiovascular diseases such as heart attacks, strokes and insufficiency in blood flow to legs. There is evidence from large randomized-controlled trials that good metabolic control in both type I and II diabetes can delay the onset and progression of these complications (WHO, 1999).

1.2.3 Hypertension (HTN):

1.2.3.1 Definition:

Hypertension (HTN) or high blood pressure, sometimes called arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated. Blood pressure is summarized by two measurements, systolic and diastolic, which depend on whether the heart muscle is contracting (systole) or relaxed between beats (diastole). This equals the maximum and minimum pressure, respectively. Normal blood pressure at rest is within the range of 100-140mmHg systolic (top reading) and 60-90mmHg diastolic (bottom reading). High blood pressure is said to be present if it is often at or above 140/90 mmHg (Carretero and Oparil, 2000).

1.2.3.2 Classification:

The classification is based on the mean of two or more properly measured seated blood pressure readings on two or more office visits. Normal blood pressure is defined as levels <120/80 mmHg. Systolic blood pressure of 120–139 mmHg or diastolic blood pressure 80–89 mmHg is classified as prehypertension. These patients are at increased risk for progression to hypertension. Hypertension is

defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Hypertension is divided into two stages.

- Stage 1 includes patients with systolic blood pressure 140–159 mmHg or diastolic blood pressure 90–99 mmHg.
- Stage 2 includes patients with systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥100 mmHg. (Kaplan, 2002)

A more elaborate classification of blood pressure is provided by the European Society of Hypertension and the European Society of Cardiology (ESH/ESC) (Table 1) (European Society of Hypertension, 2003).

Table (1.2) Classification of blood pressure for adults.

Category	Systolic BP (mmHg)	Diastolic BP (mmHg)
Optimal	<120	<80
Normal	120–129	80–84
High normal	130–139	85–89
Grade 1 hypertension (mild)	140–159	90–99
Grade 2 hypertension (moderate)	160–179	100–109
Grade 3 hypertension (severe)	≥180	≥110
Isolated systolic hypertension	≥140	<90

BP: blood pressure (European Society of Hypertension, 2003).

1.2.3.3 Causes of hypertension:

The various causes of hypertension. Primary (essential or idiopathic) hypertension is systemic hypertension of unknown cause that results from dysregulation of normal homeostatic control mechanisms of blood pressure in the absence of detectable known secondary causes. Over 95% of all cases of hypertension are in this category. Secondary hypertension is systemic hypertension due to an underlying disorder. It accounts for <5% of cases of hypertension. (Kaplan, 2002)

1.2.3.3.1 Essential hypertension:

Essential hypertension is the most prevalent hypertension type, Although no direct cause has been identified, there are many factors such as sedentary life style, smoking, stress, visceral obesity, potassium deficiency (hypokalemia), (Kyrou *et al.*, 2006) obesity (more than 85% of cases occur in those with a body mass index greater than 25), salt (sodium) sensitivity (Lackland and Egan, 2007), alcohol intake and vitamin D deficiency that increase the risk of developing hypertension (Lee *et al.*, 2008). Risk also increases with aging, some inherited genetic mutations, and having a family history of hypertension (Luma and Spiotta 2006). An elevated level of renin, a hormone secreted by the kidney, is another risk factor, as is sympathetic nervous system over activity (Rahmouni *et al.*, 2005). Insulin resistance, which is a component of syndrome X (or the metabolic syndrome), is also thought to contribute to hypertension. Recent studies have implicated low birth weight as a risk factor for adult essential hypertension (Uchiyama, 2008).

1.2.3.3.2 Secondary hypertension:

Secondary hypertension by definition results from an identifiable cause. This type is important to recognize since it's treated differently to essential hypertension, by treating the underlying cause of the elevated blood pressure. Hypertension results in the compromise or imbalance of the path physiological mechanisms, such as the hormone-regulating endocrine system, that regulate blood plasma volume and heart function. Many conditions cause hypertension; some are common and well recognized secondary causes such as Cushing's syndrome, which is a condition where the adrenal glands overproduce the hormone cortisol (Dodt *et al.*, 2009). In addition, hypertension is caused by other conditions that cause hormone changes such as hyperthyroidism, hypothyroidism , and certain tumors of the adrenal medulla (e.g., pheochromocytoma). Other common causes of secondary hypertension include kidney disease, obesity/metabolic disorder, pre-eclampsia

during pregnancy, the congenital defect known as coarctation of the aorta, and certain prescription and illegal drugs.

1.2.3.4 Pathophysiology of hypertension:

Cardiac output is raised early in the disease course, with total peripheral resistance (TPR) normal; over time cardiac output drops to normal levels but TPR is increased. Three theories have been proposed to explain this:

- Inability of the kidneys to excrete sodium, resulting in natriuretic factors such as atrial natriuretic factor being secreted to promote salt excretion with the side effect of raising total peripheral resistance.
- An overactive renin-angiotensin system leads to vasoconstriction and retention of sodium and water. The increase in blood volume plus vasoconstriction leads to hypertension (Pimenta and Oparil, 2009)
- An overactive sympathetic nervous system, leading to increased stress responses (Takahashi, 2008).

It is also known that hypertension is highly heritable and polygenic (caused by more than one gene) and a few candidate genes have been postulated in the etiology of this condition (Sagnella and Swift, 2006).

1.2.3.5 Complications of hypertension:

Hypertension is the most important risk factor for death in industrialized countries And possible complications include:

- Aortic dissection.
- Blood vessel damage (arteriosclerosis).
- Brain damage.
- Congestive heart failure.
- Chronic kidney disease.
- Heart attack.

- Hypertensive heart disease.
- Peripheral artery disease.
- Pregnancy complications.
- Stroke.
- Vision loss.

1.2.3.6 Diagnosis of hypertension:

Hypertension is generally diagnosed on the basis of a persistently high blood pressure. Usually this requires three separate sphygmomanometer measurements at least one week apart. Often, this entails three separate visits to the physician's office. Initial assessment of the hypertensive patient should include a complete history and physical examination. Exceptionally, if the elevation is extreme, or if symptoms of organ damage are present then the diagnosis may be given and treatment started immediately. Once the diagnosis of hypertension has been made, physicians will attempt to identify the underlying cause based on risk factors and other symptoms, if present. Secondary hypertension is more common in preadolescent children, with most cases caused by renal disease. Primary or essential hypertension is more common in adolescents and has multiple risk factors (Luma and Spiotta, 2006). Laboratory tests can also be performed to identify possible causes of secondary hypertension, and determine if hypertension has caused damage to the heart, eyes, and kidneys. Additional tests for diabetes and high cholesterol levels are also usually performed because they are additional risk factors for the development of heart disease require treatment (Carretero and Oparil,2000).

Routine tests:

− Electrocardiogram (ECG).

− Plasma glucose (preferably fasting).

− Serum total cholesterol.

− Serum high-density (cholesterol) lipoprotein (HDL).

−Fasting serum triglycerides.

− Serum uric acid, creatinine and potassium.

− Haemoglobin and haematocrit.

− Urinalysis (dipstick test and urinary sediment examination).

- **Recommended tests**:

− Echocardiogram.

− Carotid (and femoral) ultrasound.

− C-reactive protein.

− Microalbuminuria (essential in diabetics).

− Quantitative proteinuria (if dipstick is positive).

− Funduscopy (in severe hypertension).

- **Extended evaluation (domain of the specialist):**

− Complicated hypertension: tests of cerebral, cardiac and renal function.

− Search for secondary hypertension: measurement of renin, aldosterone, corticosteroids, catecholamines, arteriography, renal and adrenal ultrasound, computed tomography (CT) and brain magnetic resonance imaging (MRI) (European Society of Hypertension, 2003, Chobanian et al., 2003).

1.2.4 Objective:

- **General Objective:**

To test the thrombophilia in diabetics type II and hypertensive Sudanese patients.

- **Specific Objective:**

- To measure Prothrombin Time (PT), Activated Partial Prothrombin Time (APTT) and fibrinogen level in diabetics type II and hypertensive Sudanese patients.
- To compare PT, APTT and fibrinogen level between diabetic type II patients, hypertensive patients, patients with diabetic type II pulse hypertensive and normal healthy individuals.
- To identify the effect of duration of the both diseases in PT, APTT and fibrinogen level.

1.2.5 Rationale:

Since arterial disease is the major underlying factor leading to the most clinically relevant cardiovascular events and these events are usually due to formation of thrombus at the site of an atherosclerotic plaque, in this research we want to study coagulation pathway to detect the possibility of thrombosis in hypertensive patients and type II diabetic patients.

Materials and Methods

2.1 Materials

2.1.1 Study design:

It was descriptive analytical cross-sectional study conducted In Khartoum state during the period from March 2014 to April 2014 to measure the fibrinogen level, PT and APTT in diabetic type II patients, hypertensive patients and patients had both diseases diabetes type II and hypertension.

2.1.2 Study population:

First 90 patients (case group) were enrolled in this study. 30 of them with diabetes type II. 30 were hypertensive and 30 patients had both diabetes mellitus type II and hypertension disease. Seconds 30 apparently healthy individuals with matched age (control group).

2.1.3 Inclusion criteria:

Patients with diabetic history of more than one year and stabilized with antidiabetic medicines, hypertensive patients with history of more than one year and stabilized with anti hypertensive medicines and patients have both diseases were included for the study.

2.1.4 Exclusion criteria:

Patients with other known causes of hyperfibrinogenaemia such as cardiovascular diseases, liver disease, kidney disease, under anticoagulant therapy, smoking, pregnancy, disorders associated with inflammation and other diseases such as DVT or treatments that may affect coagulation system were excluded; also secondary causes of hypertension and hypertensive patients with target organ damage have been excluded.

2.1.5 Sample collection:

Individual were already diagnosed as diabetics type II, hypertensive or have both diseases were selected and data collected using self administrated questionnaire which specifically designed to obtain information that helped in study.

2.1.6 Ethical consideration:

The study was conducted after permission from the institutional ethical committee. Written consent of cases and controls were taken.

2.1.7 Blood collection and preparation:

3.5 ml of citrated anticoagulated venous blood samples were collected (9 part blood to 1 part anticoagulant). The blood is thoroughly mixed with the anticoagulant. The samples were centrifuged at 2500 rpm for 15 minutes to obtain platelet-poor plasma (PPP).Plasma was separated from cells into plain container and tested.

2.2 Methodology:

- **Principle of coagulometer:**

The coagulometer clot analyzer has an optical measurement system which detects a sudden variation in optical density when a clot is formed. The chronometer and the stirring system are activated by a sudden change of the optical density. This permits the initiation of the time measurement when the sample is added to the reagent and stop the measurement time at the moment that the clot is formed. The system has a programmable security time during which variations in optical density, when the reagent and the plasma are still in the homogenization phase, cannot activate the detection cell.

❖ **Measurement of prothrombin time (PT):**

Principle:

This tests the extrinsic pathway. It involves the addition of brain thromboplastin and calcium chloride to platelet-poor plasma (PPP) and observes the formation of clot and record the time in seconds.

Procedure:

100 micro litre test plasma was warmed at 37°C for 5 minutes. At the same time the thromboplastin reagent simultaneously was incubated. Then 200 microlitre thromboplastin reagent was added to the warmed plasma and mixed and again incubated at 37°C. Then the analyzer read the clotting time of PT and displayed the result in seconds.

Normal range:

Normal range from 11 to 16 seconds (Lewis *et al.,* 2006).

❖ **Measurement of Activated partial thromboplastin time (APTT):**

Principle:

Measurement the intrinsic pathway. The essential reagent is phosphoplipid substitute for platelet factor III, and contact activation is achieved by adding an activator such as kaolin. These reagents added to platelet-poor plasma (PPP) and observe the formation of clot and record the time in seconds.

Procedure:

50 micro litre test plasma was warmed at 37°C for 5 minutes. At the same time the APTT reagent and $CaCl_2$were also simultaneously incubated. Then 50 microlitre APTT reagent was added to the warmed plasma and mixed and again incubated at 37°C for 3 minutes. After that 50 micro litre pre-warmed $CaCl_2$ was added. Then the analyzer read the clotting time of APTT and displayed the result in seconds.

Normal range:

Normal time ranges from 26 to 40 seconds (Lewis *et al.,* 2006).

❖ **Measurement of Fibrinogen:**

Principle:

Diluted sample is clotted with a strong thrombin solution; the plasma must be diluted to give a low level of any inhibitors (e.g. FDP and heparin). A strong thrombin solution (50 NIH) must be used so that the clotting time over a wide range is independent of the thrombin concentration.

Procedure:

Fibrinogen level was measured by Clauss method. A calibration curve was prepared by preparing serial dilutions of calibration plasma with Owren's veronal buffer (1 in 5, 1 in 10, 1 in 20, and 1 in 40). 0.2 ml of each dilution was warmed to 37°C for 3 minutes, then 0.1 ml of bovine thrombin solution was added, and the clotting time was measured by using coagulometer. Each test was performed in duplicate, the average was calculated and a calibration curve was constructed (The clotting time in seconds against the fibrinogen concentration in mg/dl) on log/log graph paper. The 1 in 10 dilution was considered to be 100%fibrinogen concentration. 1 in 10 dilution was made from each patient's plasma, thrombin time was measured as mentioned above, also in duplicate, and the fibrinogen level was determined in mg/dl from the calibration curve.

Normal range:

The normal range from 180 to 360 milligrams per deciliter (mg/dl) (Lewis *et al.*, 2006)

❖ **Quality control:**

1- Control with known factor activity should be run with each test simultaneously to validate test run.
2- Correct mixture of blood.
3- Sufficient pre warming of plasma and reagent.
4- Duplicate test for each sample.

2.2.1 Statistical analysis:

Data were processed and analyzed using Statistical Package for Social Sciences (SPSS) version 14. T-Test, ANOVA and correlations were used to calculate P value. Differences were considered statistically significant when P value ≤ 0.05.

3. Results

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Table (3.1) Baseline Characteristics of the Study Population

Study groups	Age / years	Gender		Duration of the diseases / years
		Male	Female	
diabetic type II patients (N=30)	53.3 ± 13.6	15 (50%)	15 (50%)	9.2 ± 7.3
Hypertensive patients (N=30)	58.3 ± 13.4	10 (33.3 %)	20 (66.7%)	8.5 ± 5.7
Patients with diabetic type II and Hypertension (N=30)	57.2 ± 11.0	16 (53.3%)	14 (46.7%)	11.5 ± 7.1
Control (N=30)	45.13 ±7.7	13 (43.3%)	17 (56.7%)	

Table (3.2) Comparison of PT, APTT and Fibrinogen level between diabetic type II patients and controls

Parameters	Mean ± SD		P value
	Diabetic patients (N=30)	Control(N=30)	
PT(sec)	14.103 ± 1.1	13.367±1.2	0.016
APTT(sec)	37.937± 6.2	29.867± 4.3	0.000
Fibrinogen mg\dl	314.67 ± 92.8	225.7 ± 34.1	0.000

* The mean difference is significant at the 0.05 level.

Table (3.3) Comparison of PT, APTT and Fibrinogen level between hypertensive patients and controls

Parameters	Mean ± SD		P value
	hypertensive patients (N=30)	Control(N=30)	
PT(sec)	14.5 ± 1.9	13.367±1.2	0.011
APTT(sec)	29.767± 5.2	29.867± 4.3	0.936
Fibrinogen mg\dl	240.67 ± 51.9	225.7 ± 34.1	0.192

* The mean difference is significant at the 0.05 level.

Table (3.4) Comparison of PT, APTT and Fibrinogen level between patients had diabetic type II & hypertensive and controls

Parameters	Mean ± SD		P value
	Diabetic & hypertensive (N=30)	Control (N=30)	
PT(sec)	13.283±1.5	13.367±1.2	0.813
APTT(sec)	38.423± 6.7	29.867± 4.3	0.000
Fibrinogen mg\dl	362.67 ± 115.4	225.7 ± 34.1	0.000

* The mean difference is significant at the 0.05 level.

Table (3.5) Comparison of PT, APTT and Fibrinogen level between study population groups:

Dependent Variable	Sample (I)	Sample (II)	Mean of (I)	Mean of (II)	P value
PT (sec)	DM	DM & HTN	14.103± 1.1	13.283±1.5	0.044
		HTN		14.5± 1.9	0.325
	DM & HTN	DM	13.283±1.5	14.103± 1.1	0.044
		HTN		14.5± 1.9	0.003
	HTN	DM	14.5± 1.1	14.103± 1.1	0.325
		DM & HTN		13.283± 1.1	0.003
APTT (sec)	DM	DM & HTN	37.937± 6.2	38.423± 6.7	0.757
		HTN		29.767± 5.2	0.000
	DM & HTN	DM	38.423± 6.7	37.937± 6.2	0.757
		HTN		29.767± 5.2	0.000
	HTN	DM	29.767± 5.2	37.937± 6.2	0.000
		DM & HTN		38.423± 6.7	0.000
Fibrinogen mg\dl	DM	DM & HTN	314.67± 92.8	362.67± 115.4	0.043
		HTN		240.67± 51.9	0.002
	DM & HTN	DM	362.67± 115.4	314.67± 92.8	0.043
		HTN		240.67± 51.9	0.000
	HTN	DM	240.67± 51.9	314.67± 92.8	0.002
		DM & HTN		362.67± 115.4	0.000

* The mean difference is significant at the 0.05 level.

- DM: diabetes, HTN: hypertension.

- Found significant correlation between PT and duration of diseased in patients with diabetes type II pulse hypertension (P value 0.001) and No found significant correlation between APTT, fibrinogen level and diseased in patients with diabetes type II pulse hypertension.
- No found significant correlation between PT, APTT and fibrinogen level and duration of diabetes type II and hypertension (p value >0.005).

4. Discussion, Conclusion, and Recommendations

4.1 Discussion:

This study conducted In Khartoum state during the period from March 2014 to April 2014 to measure the fibrinogen level, PT and APTT in diabetic type II patients, hypertensive patients and patients had both diseases diabetes and hypertension.

The result of prothrombin time (PT) showed significantly higher value in patients with diabetes type II than the control (p valve <0.05) (Alao et al.,2009) found same result while (Dhule and Gawali., 2014) and (Sauls et al., 2007) showed significantly a lower value in diabetics than controls (p<0.05).Although PT was significantly higher value in hypertensive patients than the controls (p valve <0.05). There was no significant difference in patients has both diseases diabetes type II and hypertension than control group (p valve >0.05) and no significant difference between diabetes type II patients and hypertensive patients (No published data was found). There was significantly higher value in patients had diabetes only than patient has both diseases (p valve <0.05) and significantly higher value in patients had hypertension only than patient has both diseases (p valve <0.05) (No published data was found). The results from this study showed PT to be significantly lower in patients who had both diseases than those who had only hypertension or only diabetes.

The result of activated partial thromboplastin time (APTT) showed significantly higher value in patients with diabetes type II than the control (p valve <0.05) (Alao et al., 2009) found same result while (Dhule and Gawali., 2014), (Sapkota et al., 2013),(Sauls DL et al., 2007) showed significantly a lower value in diabetics than controls (p<0.05). There was no significant difference in the hypertensive patients

and control group (p valve >0.05). Although APTT showed significantly higher value in patients has both diseases diabetes and hypertensive than the control group (p valve <0.05). There was significantly higher in diabetic type II patients than hypertensive patients (p valve <0.05) (No published data was found). No significant difference between patients has both diseases and patients had diabetes type II only (p valve <0.05) and significantly higher value in patient has both diseases than patients had hypertension only (p valve <0.05) (No published data was found). The result from this study showed APTT to be significantly higher in patients who had both diseases diabetes and hypertensive than those who had only hypertension or only diabetes type II.

This study showed fibrinogen to be significantly higher in patients with diabetic type II than the control (p valve <0.05) (Ceriello et al., 1994), (James et al., 2000) , (Alao et al.,2009) ,(Kafle et al., 2010), (Sapkota et al., 2013), found same result. The plasma fibrinogen level was statistically insignificant difference in the hypertensive patients and control group (p valve >0.05) while (Tabak et al., 2009) and (Osman& Muddathir., 2013) their studies demonstrated that the plasma fibrinogen level was significantly increased in hypertensive patients. Although fibrinogen to be significantly higher in patients who had both diseases diabetes and hypertensive than the controls (p valve <0.05) (Khan et al., 2005) found same result. This study also showed fibrinogen to be significantly higher in diabetic type II patients than hypertensive patients (p valve <0.05). Also showed fibrinogen level significantly higher in patients who had both diseases than patients had diabetes type II only (p valve <0.05) (Khan et al., 2005) found same result .There was significantly higher value in patient has both diseases than patients had hypertension only (p valve <0.05). Also showed fibrinogen to be significantly higher in patients who had both diseases diabetes and hypertensive than those who had only hypertension or only diabetes type II.

No significant association between duration of diabetes mellitus type II and fibrinogen level (p value > 0.05) (Sapkota et al., 2013) found same result. No significant association between duration of diabetes mellitus type II and PT (p value > 0.05) and no significant association between duration of diabetes mellitus type II and APTT (p value > 0.05) (Sapkota et al., 2013) found same result.

No significant association between duration of hypertension and fibrinogen level (p value > 0.05). No significant association between duration of hypertension and PT (p value > 0.05). No significant association between duration of hypertension and APTT (p value > 0.05) (No published data was found).

No significant association between duration of both diseases hypertension &diabetes and fibrinogen level (p value > 0.05). There was significant association between duration of both diseases hypertension & diabetes and PT (p value > 0.05) and no significant association between duration of both diseases hypertension & diabetes and APTT (p value > 0.05) (No published data was found).

4.2 Conclusion:

1. PT significantly decrease in patients with diabetes type II pulse hypertension compared to patients with diabetes type II, patients with hypertension and insignificant compared to control.
2. APTT significantly increase in patients with diabetes type II pulse hypertension compared to patients with hypertension and control and insignificant compared to patients with diabetes type II.
3. Fibrinogen significantly increase in patients with diabetes type II pulse hypertension compared to patients with diabetes type II , patients with hypertension and control.
4. This results indicator to hypercoagulability in these patients so measurement of fibrinogen level, PT and APTT may be predictor for thrombosis which appears to complicate the hypertension and type II diabetes mellitus.

4.3 Recommendations:

1. Give antithrombophilic treatment for patients with type II diabetes mellitus or patients with hypertension to avoid the impact of thrombosis.
2. Patients with type II diabetes mellitus or hypertension should check their coagulation profile for early detection of abnormality.
3. Sample size should be increased in further studies.
4. Thrombin generation and D-dimer should be performed in further studies.

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Appendix

Sudan University of Science and Technology

College of Graduate studies

Questionnaire: Coagulation profile in diabetics and hypertensive Sudanese patients

- ✓ Name: ……..
- ✓ Age: …….
- ✓ Residence: ……...
- ✓ Tele: …….
- ✓ If you have DM: Yes NO
- Duration of disease ……
- Treatment use …….
- Type of DM…….
- ✓ If you have hypertension: Yes ☐ NO ☐
- Duration of disease ……
- Treatment use ……
- ✓ Other disease …….

Results

1. PT: …….....seconds control: ……seconds
2. APTT: ……. seconds control: ..... seconds
3. Fibrinogen level: ……..... mg\dl

Signature: …… Date: …….....

اقر انا ..... بأنى اواقف على المشاركه في هذه الدراسة