

# Chapter one

## Introduction and literature review

### 1.1 Introduction:

The hemostatic mechanisms have several important functions like maintain blood in fluid state, arrest bleeding at the site of injury or blood loss by formation of hemostatic plug and removal of the plug when healing is complete. Normal physiology thus constitutes a delicate balance between these conflicting tendencies and a deficiency of any one may lead to either thrombosis or hemorrhage (John, 1995).

Normal coagulation has classically been conceptualized as a Y-shaped pathway, with distinct “intrinsic” and “extrinsic” components initiated by factor XII or factor VIIa tissue factor, respectively, and converging in a “common” pathway at the level of the FXa/FVa(prothrombinase)complex (Michael, 2006). Until recently, the lack of an established alternative concept of hemostasis has mean that most physicians view the “cascade” as a model of physiology. This view has been reinforced by the fact that screening coagulation tests (APTT, PT and INR) are often used as though they are generally predictive of clinical bleeding (Michael, 2006). The hemostatic system is in a delicate balance between prothrombotic and antithrombotic processes aiming to prevent excessive blood loss from injured vessels and to prevent spontaneous thrombosis.

Obesity is associated with significant morbidity and mortality including an increased incidence and prevalence of arterial and venous thrombotic events. The various mechanisms by which obesity may cause thrombosis include increased activity of the coagulation cascade and decreased activity of the fibrinolytic cascade, the actions of so-called adipocytokines from adipose

tissue like leptin and adiponectin, increased inflammation, increased oxidative stress and endothelial dysfunction and disturbances of lipids and glucose tolerance in association with the metabolic syndrome (Darvall *et al.*, 2007). Abnormalities in coagulation and haemostasis represent a well-known link between obesity and thrombosis (both arterial and venous). Several studies have shown that obese patients have higher plasma concentrations of all pro-thrombotic factors (fibrinogen, vonWillebrand factor (vWF), and factor VII), as compared to non-obese controls. Similarly, plasma concentrations of plasminogen activator inhibitor-1 (PAI-1) have been shown to be higher in obese patients as compared to non-obese controls (De pergola *et al.*, 2002).

## **1.2 Literature Review:**

### **1.2.1 Hemostasis:**

Is a Process by which blood is maintained in a fluid state and confined to the circulatory system, act to stop bleeding and to do so only at the site of injury (Marlies, 2006). The normal hemostatic response to vascular damage depends on a closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors. An efficient and rapid mechanism for stopping bleeding from sites of blood vessel injury is clearly essential for survival. Nevertheless, such a response needs to be tightly controlled to prevent extensive clot developing and to break down such clot once damage is repaired. The hemostatic system thus represents delicate balance between anticoagulant and procoagulant mechanisms allied to the process for fibrinolysis (Hoffbrand *et al.*, 2010). There are five major component involved in hemostasis include blood vessels, platelets, coagulation factors, natural inhibitors of the coagulation cascade and fibrinolysis (Hoffbrand *et al.*, 2010).

### **1.2.1.1 Primary hemostasis:**

Primary hemostasis primarily involves platelets and vWF and results in the formation of a platelet plug. If the endothelial injury is small, this may be adequate to stop bleeding. However, if the injury is greater, participation by the coagulation cascade is required. Platelets and blood vessels play key role in primary hemostasis (Kern, 2002).

#### **1.2.1.1.1 Blood vessels structure:**

The basic structure of blood vessels can be broken down into three layers the intima, the media and the adventitia. It is the materials that make up these layers and the size of these layers themselves that differentiate arteries from veins, and indeed one artery or one vein from another artery or vein. The endothelium functions in a multitude of physiological processes including the control of cellular trafficking, the regulation of vasomotor tone and maintenance of blood fluidity. Endothelial cell system (ECs) possesses surface receptors for a variety of physiological substances, for example thrombin and angiotensin II, which may influence vascular tone directly or indirectly. Once activated, ECs express at their surface, and in some cases release into the plasma, a variety of intracellular adhesion molecules which modulate platelet adhesion and vascular permeability (Hoffbrand *et al.*, 2005).

#### **1.2.1.1.2 Platelets:**

##### **1.2.1.1.2.1 Platelet production:**

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. The precursor of the megakaryocyte the megakaryoblast arises by a process of differentiation from the hemopoietic stem cell (Hoffbrand *et al.*, 2010). The

megakaryocyte matures by endomitotic synchronous replication (that is DNA replication in the absence of nuclear or cytoplasmic division) enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two. Very early on invaginations of plasma membrane are seen, called the demarcation membrane, which evolves through the development of the megakaryocyte into a highly branched network. Mature megakaryocytes are extremely large, Platelets form by fragmentation of megakaryocyte cytoplasm, approximately each megakaryocyte giving rise to 1000-5000 platelets. The time interval from differentiation of the human stem cell to the production of platelets averages approximately 10 days (Hoffbrand *et al.*, 2010). Thrombopoietin is the major regulator of platelet production and is constitutively produced by the liver and kidneys. The normal platelet count is approximate  $250 \times 10^9/L$  (range  $150-400 \times 10^9/L$ ) and the normal platelet life span is 7-10 days. Up to one-third of the marrow output of platelets may be trapped in spleen but this rises to 90% in cases of massive splenomegaly (Hoffbrand *et al.*, 2010).

#### **1.2.1.1.2.2 Platelet structure and function:**

Platelets are extremely small and discoid,  $3.0 \times 0.5 \mu m$  in diameter, with a mean volume 7-11 fL. The glycoproteins of the surface coat are particularly important in the platelet reactions of adhesion and aggregation which are the initial events leading to platelet plug formation during hemostasis. Adhesion to collagen is facilitated by glycoprotein Ia (GpIa). Glycoproteins Ib and IIb/IIIa are important in the attachment of platelets to von Willebrand factor (VWF) and hence to vascular subendothelium where metabolic interactions occur. The binding site for IIb /IIIa is also the receptor for fibrinogen which is important in platelet-platelet aggregation (Hoffbrand *et al.*, 2010). The plasma membrane invaginates into the platelet interior to form an open

membrane (canalicular) system which provides a large reactive surface to which the plasma coagulation proteins may be selectively absorbed. The membrane phospholipids (previously known as platelet factor 3) are of particular importance in the conversion of coagulation factor X to Xa and prothrombin (factor II) to thrombin (factor IIa) (Hoffbrand *et al.*, 2010). The platelet contains three types of storage granules: dense, alpha and lysosomes. The more frequent specific granules contain a heparin antagonist (PF4), platelet-derived growth factor (PDGF), thromboglobulin, fibrinogen, VWF and other clotting factors. Dense granules are less common and contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), 5-hydroxytryptamine (5-HT) and calcium. Lysosomes contain hydrolytic enzymes and peroxisomes contain catalase. The contents of the granules are discharged into the open canalicular system. Platelets are also rich in signaling and cytoskeletal proteins which support the rapid switch from quiescent to activation that follows vessel damage (Hoffbrand *et al.*, 2010). The main function of platelet is the formation of mechanical plugs during the normal hemostatic response to vascular injury. In the absence of platelets, spontaneous leakage of blood through small vessels may occur. Platelet function falls into three adhesion, aggregation and release reaction. There is also amplification. The immobilization of platelet at sites of vascular injury requires specific platelet-vessel wall (adhesion) and platelet – platelet interactions, both mediated through VWF (Hoffbrand *et al.*, 2010).

#### **1.2.1.1.3 Mechanism of primary hemostasis:**

##### **1.2.1.1.3.1 Vasoconstriction:**

An immediate vasoconstriction of the injured vessel and reflex constriction of adjacent small arteries and arterioles is responsible for an initial slowing of blood flow to the area of injury. The reduced blood flow allows contact

activation of platelets and coagulation factors. The vasoactive amines and thromboxane A<sub>2</sub> liberated from platelets and the fibrinopeptides liberated during fibrin formation, also have vasoconstrictive activity (Hoffbrand *et al.*, 2010).

#### **1.2.1.1.3.2 Platelet reactions and primary hemostatic plug formation:**

Following a break in the endothelial lining there is an initial adherence of platelets to exposed connective tissue potentiated by VWF. Collagen exposure and thrombin produced at the site of injury cause the adherent platelets to release their granule contents and also activate platelet prostaglandin synthesis leading to the formation of thromboxaneA<sub>2</sub>. Released ADP causes platelets to swell and aggregate (Hoffbrand *et al.*, 2010). Additional platelets from the circulating blood are drawn to the area of injury. This continuing platelet aggregation promotes the growth of the hemostatic plug which soon covers the exposed connective tissue. The unstable primary hemostatic plug produced by these platelet reactions in the first minute or so following injury is usually sufficient to provide temporary control of bleeding. It seems likely that prostacyclin, produced by endothelial and smooth muscle cells in the vessel wall adjacent to the area of damage, is important in limiting the extent of the initial platelet plug (Hoffbrand *et al.*, 2010).

#### **1.2.1.1.4 Causes of primary hemostasis deficiencies:**

Causes of primary hemostasis deficiencies may include Thrombocytopenia, inherited disorders of platelet function (Bernard-Soulier syndrome, Glanzmann's thrombasthenia and storage pool deficiency), and Also medications: aspirin, ticlopidine, etc. And defects in the blood vessel wall which include hereditary hemorrhagic telangiectasia (HHT; Osler Weber-

Rendu syndrome), Scurvy (vitamin C deficiency), Vasculitis, Amyloidosis and Senile purpura (Kern, 2002).

#### **1.2.1.2 Secondary hemostasis:**

Secondary hemostasis primarily involves the coagulation cascade proteins, which ultimately results in the conversion of fibrinogen to fibrin; fibrin polymerizes to form a clot. The fibrin clot is cross-linked and stabilized by factor XIIIa (Kern, 2002).

##### **1.2.1.2.1 Coagulation Factors:**

The clotting factors are designated by Roman numerals. The inactive precursor is designated by the plain Roman numeral, and the active form is designated by the suffix “a” (for example, factor X is the inactive precursor, factor Xa is the active enzyme form). The clotting factors were also given common names, but most of these are no longer used. A few other proteins involved in laboratory tests of coagulation were not given Roman numerals examples include high-molecular-weight kininogen (HMWK) and prekallikrein (PK) (Kern, 2002).

**Table (1.1) the coagulation factors (Hoffbrand *et al.*, 2010).**

Factor number	Descriptive name	Active form
I	Fibrinogen	Fibrin subunit
II	Prothrombin	Serine protease
III	Tissue factor	Receptor/ cofactor
V	Labile factor	Cofactor
VII	Proconvertin	Serine protease
VIII	Antihaemophilic factor	Cofactor
IX	Christmas factor	Serine protease
X	Stuart-Prower factor	Serine protease
XI	Plasma thromboplastin antecedent	Serine protease
XII	Hageman (contact) factor	Serine protease
XIII	Fibrin-stabilizing factor    Prekallikrein	Transglutaminas
	(Fletcher factor)    HMWK (Fitzgerald factor)	Serine        protease Cofactor

#### **1.2.1.2.2 Mechanism of secondary hemostasis:**

The term coagulation cascade refers to the sequential activation of coagulation factors, resulting in the conversion of fibrinogen to fibrin and the subsequent polymerization of fibrin into a fibrin clot. Most of the coagulation factors are serine proteases. They circulate in the plasma as inactive precursors (zymogens), which are converted to the active enzyme by protease cleavage. One coagulation factor cleaves and activates the next factor along the line and so on. Since each active enzyme can activate many molecules of the subsequent factor, there is a geometric increase in the number of molecules activated. The end result of the coagulation cascade is an avalanche of activated clotting factors (Kern, 2002).

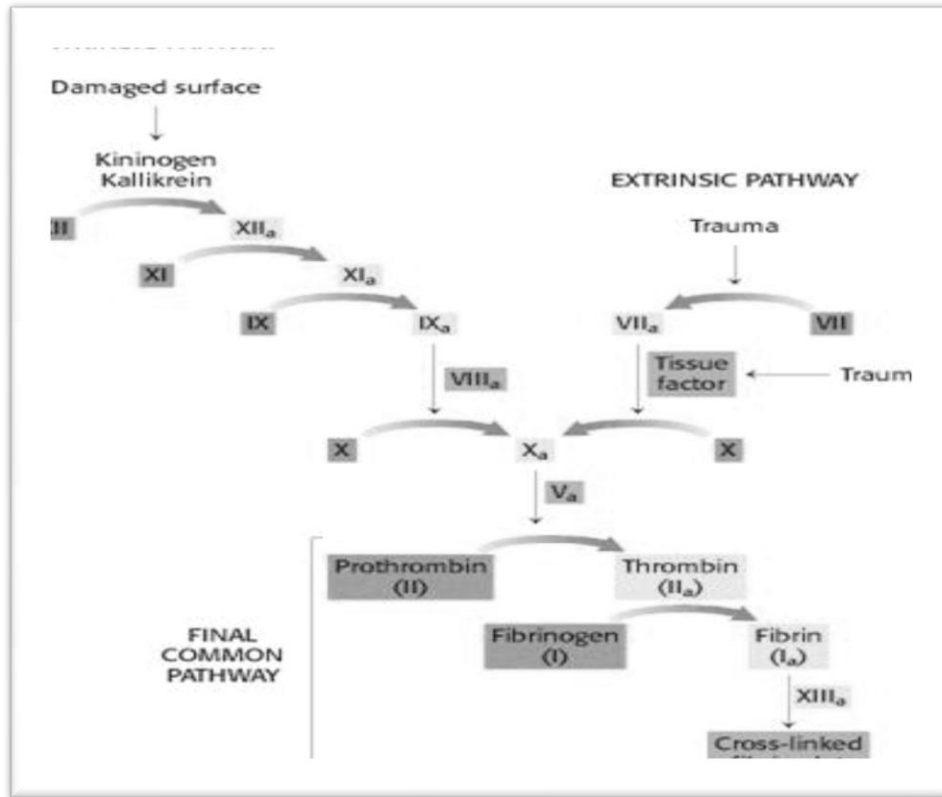


#### **1.2.1.2.2.1 Classic Concept of the Coagulation Cascade:**

The classic concept of the coagulation cascade featured two separate and independent pathways: the intrinsic pathway measured by the partial thromboplastin time (PTT), and the extrinsic pathway measured by the prothrombin time (PT). The two pathways came together at the activation stage of factor X to Xa, and hence the pathway from factor X down to fibrin was called the common pathway. Now it is known that there is really only one pathway; the intrinsic pathway is largely a laboratory artifact. However, because still the same two main tests are used to investigate the status of the coagulation cascade, must be understood both pathways in order to interpret the results of laboratory tests of coagulation. The intrinsic pathway (also called the contact activation pathway) starts with factor XII coming in contact with a negatively charged surface and being activated to XIIa. High-molecular-weight kininogen and PK are required. Factor XIIa then activates XI to XIa, XIa activates IX to IXa, IXa activates X to Xa (in the presence of factor VIIIa, calcium, and phospholipid), and so on. Deficiencies in any factors involved in the intrinsic pathway result in prolongation of the PTT: factors XII, XI, IX, VIII, and HMWK and PK. Deficiencies of factors in the common pathway also result in prolongation of the PTT, but the PT is also prolonged. The extrinsic pathway (also called the tissue factor pathway) starts with exposure of tissue factor (TF) to blood. Tissue factor is a transmembrane protein that is highly expressed in the adventitia of blood vessels, the brain, glomeruli, and other tissues. It is not normally present on endothelial surfaces or blood cells. Exposed tissue factor reacts with trace amounts of factor VIIa, which are normally present in the circulation. The TF-factor VIIa complex then activates factor X to Xa, starting the common pathway (Kern, 2002).

#### **1.2.1.2.2.2 Current Concept of the Coagulation Cascade:**

In the current concept of the coagulation, the key initiating step is the exposure of TF to the circulation and reaction of TF with factor VIIa. The TF-factor VIIa complex can enzymatically activate factor X to Xa, factor IX to IXa, and factor XI to XIa. The initial activation of factor X to Xa may be important in getting the coagulation cascade started; however, a specific inhibitor produced by endothelium called tissue factor pathway inhibitor (TFPI) rapidly inactivates the TF-VIIa-Xa complex. Therefore, the major action of the TF-VIIa complex in vivo is the activation of factor IX to IXa, which then activates factor X to Xa. Activation of factor XI to XIa by the TF-VIIa complex appears to play a relatively minor role in the coagulation cascade, activation of factor X to Xa and prothrombin (II) to thrombin (IIa) are key steps in the coagulation cascade since both Xa and thrombin have positive feedback activity on earlier steps of the cascade. Factor Xa activates VII to VIIa, increasing the amount of VIIa available to complex with TF. Thrombin converts factor V to Va and factor VIII to VIIIa. It also activates factor XI to XIa and XIII to XIIIa. Thrombin is also a potent platelet agonist. Factor X is activated by a complex of factor IXa, VIIIa, phospholipid, and calcium. Prothrombin is activated by a complex of factor Xa, Va, phospholipid, and calcium. Thrombin cleaves off two small peptides from fibrinogen (fibrinopeptides A and B), converting fibrinogen to fibrin monomer. Fibrin monomer spontaneously polymerizes to form soluble fibrin polymer, which is then covalently cross-linked by factor XIIIa, converting it to a stable fibrin clot (Kern, 2002).



**Figure (1.1) Coagulation Cascade** (Miracles, 2009).

#### 1.2.1.2.3 Natural Inhibitors of the Coagulation Cascade:

The rampant amplification of the coagulation cascade must be checked and controlled in order to limit clotting to the area where it is needed. Factors that inhibit coagulation include the following Blood flow and hepatic degradation of clotting factors. Normal blood flow dilutes the activated clotting factors below the level required to propagate the cascade. Hepatocytes in the liver digest and destroy the activated clotting factors washed away from the site of clot formation (Kern, 2002). Antithrombin (AT; previously called antithrombin III [AT III]) is the most important physiologic inhibitor of activated coagulation factors. Antithrombin is synthesized in the liver and endothelial cells. It irreversibly binds to and inhibits thrombin, factor Xa, and other activated clotting factors. Heparin (or heparansulfate on endothelial cells) binds to and activates AT, AT has a low

affinity for thrombin. However, heparin AT complex increases the activity of AT approximately 1000-fold. (Kern, 2002). Proteins C and S are vitamin K-dependent inhibitors of the coagulation cascade that control coagulation by inactivating factors Va and VIIIa (Kern, 2002).

#### **1.2.1.2.4 Causes of secondary hemostasis disorders:**

Causes include Hemophilia's which is inherited decrease in clotting factor levels or production of abnormal clotting factors, type of hemophilia include Haemophilia A is a recessive X-linked genetic disorder involving a lack of functional clotting Factor VIII and represents 80% of haemophilia cases. Haemophilia B is a recessive X-linked genetic disorder involving a lack of functional clotting Factor IX. It comprises approximately 20% of haemophilia cases. Haemophilia C is an autosomal genetic disorder involving a lack of functional clotting Factor XI. Haemophilia C is not completely recessive, as heterozygous individuals also show increased bleeding (Kern, 2002). Other disorder affect in secondary hemostasis include: decreased fibrinogen, Liver disease, Warfarin drugs and Fibrin degradation products also interfere with platelet function (Kern, 2002).

#### **1.2.1.3 Fibrinolytic system:**

Fibrinolysis (clot dissolving) is as important in the global processes of hemostasis as the coagulation cascade. The important players in fibrinolysis are plasminogen/plasmin and tissue Plasminogen Activator (t-PA). There are also plasmin inhibitors, the most important of which is alpha 2 antiplasmin. ( $\alpha$ 2-AP). As well as inhibitors of plasminogen activation (Kern, 2002).

##### **1.2.1.3.1 Plasminogen/Plasmin:**

Plasmin is the enzyme that digests fibrin and thus dissolves clots. Plasmin circulates as an inactive precursor plasminogen. Plasminogen is activated to plasmin primarily by t-PA, which is secreted by endothelial cells.

Plasminogen can also be activated by the contact activation pathway (factor XII, HMWK, and PK). Plasminogen can also be activated by urokinase-type plasminogen activator (u-PA), streptokinase, and a variety of reptile (snake) venoms. Recombinant t-PA, u-PA, and streptokinase are used therapeutically to dissolve clots (deep venous thrombi, pulmonary emboli, and coronary artery thrombosis) (Kern, 2002). The results of fibrin degradation by plasmin are a variety of fibrin degradation products (FDPs; sometimes called fibrin split products or FSPs). Fibrin degradation products inhibit coagulation by inserting into the fibrin clot in place of fibrinogen. They also inhibit platelet aggregation. A variety of assays for FDPs are available; most of these detect a miscellaneous mixture of FDPs. Fibrin degradation product assays are not actually specific for fibrin degradation plasmin can also digest fibrinogen, and fibrinogen degradation products will also result in a positive test for FDPs. A specific fibrin degradation product is the D-dimer (Kern, 2002). Plasmin can degrade fibrinogen and other clotting factors as well as fibrin clots, and excessive activity of the fibrinolytic system can result in severe bleeding. Therefore, the fibrinolytic system also needs to be controlled. One important control mechanism is localization of plasmin activity to the surface of fibrin clots. Plasminogen is bound into fibrin clots as they are formed. Tissue plasminogen activator has a much higher affinity for plasminogen that is localized on the surface of a fibrin clot than it does for free plasminogen, and this helps to specifically localize fibrinolysis to the clot (Kern, 2002). There is also a circulating inhibitor of plasmin, alpha 2 antiplasmin which inactivates any plasmin that is free in circulation. Plasmin bound to fibrin is protected from inhibition by  $\alpha$ 2-AP (Kern, 2002).

#### **1.2.1.3.2 Inhibition of Plasminogen Activation:**

The primary inhibitor of t-PA is plasminogen activator inhibitor-1 (PAI-1). A second inhibitor of plasminogen activation is called plasminogen activator inhibitor-2 (PAI-2). The concentration of PAI-2 is high during pregnancy and is present in high concentration in placental circulation. Otherwise, it plays a relatively minor role (kern, 2002).

#### **1.2.1.4 Evaluation of primary hemostasis:**

##### **1.2.1.4.1 Bleeding time test:**

The bleeding time is a useful test for abnormal platelet function including the diagnosis of VWF deficiency. It has largely been replaced by the platelet function analysis (PFA- test). It will be prolonged in thrombocytopenia but is normal in vascular causes of abnormal bleeding. The test involves the application of pressure to the upper arm with a blood pressure cuff, after which small incisions are made in the flexor surface forearm skin. Bleeding stops normally in 3-8 min (Hoffbrand *et al.*, 2010).

##### **1.2.1.4.2 Platelet count:**

A platelets count may be requested to investigate abnormal skin and mucosal bleeding which can occur when the platelet count is very low (usually below  $20 \times 10^9/L$ ). Blood is diluted 1:20 in a filtered solution of ammonium oxalate reagent which lyses the red cells. Platelets are counted microscopically using an improved Neubauer ruled counting chamber and the number of platelets per liter of blood calculated (Cheesbrough, 2006).

##### **1.2.1.4.3 Platelet Aggregation test:**

Measure ability of platelets to aggregate, in vitro, when subjected to various stimulators (agonists) predominantly assesses function of platelet glycoprotein IIb/IIIa receptor (Marlies, 2006).

### **1.2.1.5 Evaluation of secondary haemostasis:**

#### **1.2.1.5.1 Prothrombin Time:**

The PT incorporates a source of tissue thromboplastin (TF) such as rabbit brain, which also includes the phospholipid required for clotting. Pre warmed PT reagent suspended in calcium chloride (CaCl<sub>2</sub>) is added to the test plasma, which is anticoagulated with citrate. The CaCl<sub>2</sub> neutralizes the effect of the citrate and initiates clotting. Clotting (fibrin polymerization) can be detected by either photo-optical or mechanical methods. (Kern, 2002). The PT tests the extrinsic and common pathways; it is prolonged with deficiencies of factor VII and factors in the common pathway (X, V, prothrombin, fibrinogen). It is also prolonged in liver disease, vitamin K deficiency, therapeutic warfarin and heparin anticoagulation, disseminated intravascular coagulation (DIC), with high levels of FDPs, and occasionally by lupus anticoagulants (Kern, 2002). The PT may be shortened during acute inflammatory conditions, which are accompanied by increase in Fibrinogen levels and also by agents such as antihistamines, butabarbital, Phenobarbital, caffeine, oral contraceptives and vitamin K (Biggs and Macfarlane, 1962). The PT is traditionally used to follow anticoagulant therapy with vitamin K antagonists such as warfarin. For monitoring warfarin therapy, the PT is usually reported in terms of the International Normalized Ratio (INR). The INR is the PT adjusted for the sensitivity of the specific thromboplastin reagent to the effect of warfarin, normalized to 1. The  $INR = (\text{patient PT} / \text{mean normal PT})^{ISI}$ , where the ISI (International Sensitivity Index) depends on the sensitivity of the specific thromboplastin to warfarin. The desired INR value depends on the process being treated (Kern, 2002).

#### **1.2.1.5.2 Partial Thromboplastin Time:**

The PTT uses phospholipid usually derived from an extract of rabbit or bovine brain tissue (the partial thromboplastin reagent) and an activator of the contact activation system such as silica particles (Kern, 2002). The anticoagulated test plasma is added to the partial thromboplastin/activator mix and incubated briefly. CaCl<sub>2</sub> is added to initiate clotting and clot formation is detected as described above. The PTT is prolonged with deficiencies of the contact activation pathway (factors XII, XI, IX, VIII; also HMWK and PK) and the common pathway (X, V, prothrombin, and fibrinogen). It is prolonged in liver disease, vitamin K deficiency, therapeutic warfarin and heparin anticoagulation, DIC, and with high levels of FDPs. Lupus anticoagulants usually prolong the PTT. A typical reference range for PTT is ~23 to 35 seconds (Kern, 2002). Recent studies have also shown that shortened APTTs may also reflect procoagulant imbalances with increased levels of coagulation factors like in obesity (Tripodi *et al.*, 2004).

#### **1.2.1.5.3 Thrombin Time (Sometimes Called Thrombin Clotting Time):**

The thrombin time (TT) uses exogenous thrombin to convert fibrinogen to fibrin. It is prolonged with decreased fibrinogen, elevated levels of FDPs, dysfibrinogenemias, and heparin. It is not affected by deficiencies in the intrinsic or extrinsic pathways. The TT can be used to monitor heparin therapy, although the PTT is more often used (Kern, 2002). The thrombin clotting time is inversely proportional to the fibrinogen concentration of the plasma (i.e. a high fibrinogen concentration = a short thrombin clotting time) (Cheryl and John, 1998).



#### **1.2.1.5.4 Second line investigation:**

Relevant second line investigation is discussed with each of the possible patterns of abnormalities detected by the first line test (John, 1995).

#### **1.2.2 Hypercoagulability state (thrombophilia):**

The concept of a state of hypercoagulability dates back to 1854, when German pathologist Rudolph Virchow postulated that thrombosis resulted from, and in turn precipitated, three interrelated factors References. Decreased blood flow (venous stasis), inflammation of or near the blood vessels (vascular endothelial injury), and intrinsic alterations in the nature of the blood itself. These “blood changes” alluded to in Virchow's triad have become what are now known as hypercoagulable states, or thrombophilias (Kitchens, 1985). Hypercoagulable states can be defined as a group of inherited or acquired conditions associated with a predisposition to venous thrombosis (including upper and lower extremity deep venous thrombosis with or without pulmonary embolism, cerebral venous thrombosis, and intra-abdominal venous thrombosis), arterial thrombosis (including myocardial infarction, stroke, acute limb ischemia, and splanchnic ischemia), or both. Venous thromboembolic disease is the most common clinical manifestation resulting from hypercoagulable states. Although most inherited conditions appear to increase only the risk of venous thromboembolic events (VTEs), some of the acquired conditions have been associated with both VTEs and arterial thrombosis. These include cancer, myeloproliferative syndromes, antiphospholipid antibodies (APAs), hyperhomocysteinemia, and heparin-induced thrombocytopenia. Most hypercoagulable states alter the blood itself, whereas others affect the vasculature directly. Although patients with hypercoagulable states are at greater risk for developing a thrombotic event than those without such disorders, not all persons with a well-defined

hypercoagulable state will develop an overt thrombosis and not all persons with thrombosis have an identifiable hypercoagulable state. In fact, in 2003, testing for an inherited hypercoagulable state was likely to uncover an abnormality in more than 60% of patients presenting with idiopathic (i.e., spontaneous or unprovoked) VTEs (Deitcher *et al.*, 2000) Although the remaining 30% to 40% would have unremarkable test results, this does not imply a true absence of a hypercoagulable state. Some of these individuals may have an acquired condition such as cancer or APA, and others may have a disorder or defect that has not yet been discovered or characterized. This can be illustrated by the fact that before 1993—before the discoveries of factor V Leiden and the prothrombin G20210A mutation—an inherited predisposition to hypercoagulability was identified in only 15% to 20% of patients presenting with idiopathic VTEs (Deitcher *et al.*, 2000)

#### **1.2.2.1 Causes of hypercoagulable states:**

Hypercoagulable states are usually genetic inherited or acquired conditions. The genetic form of this disorder means a person is born with the tendency to form blood clots. Acquired conditions are usually a result of surgery, trauma, medications or a medical condition that increases the risk of hypercoagulable states (Deitcher *et al.*, 2000)

##### **1.2.2.1.1 Inherited hypercoagulable conditions include:**

- Factor V Leiden (the most common)
- Prothrombin gene mutation
- Deficiencies of natural proteins that prevent clotting (such as antithrombin, protein C and protein S)
- Elevated levels of homocysteine

- Elevated levels of fibrinogen or dysfunctional fibrinogen (dysfibrinogenemia)
- Elevated levels of factor VIII (still being investigated as an inherited condition) and other factors including factor IX and XI
- Abnormal fibrinolytic system, including hypoplasminogenemia, dysplasminogenemia and elevation in levels of plasminogen activator inhibitor PAI-1

(Deitcher *et al.*, 2000)

#### **1.2.2.1.2 Acquired hypercoagulable conditions include:**

- Cancer
- Some medications used to treat cancer, such as tamoxifen, bevacizumab, thalidomide and lenalidomide
- Recent trauma or surgery
- Central venous catheter placement
- Obesity
- Pregnancy
- Supplemental estrogen use, including oral contraceptive pills (birth control pills)
- Hormone replacement therapy
- Prolonged bed rest or immobility
- Heart attack, congestive heart failure, stroke and other illnesses that lead to decreased activity
- Heparin-induced thrombocytopenia (decreased platelets in the blood due to heparin or low molecular weight heparin preparations)
- Lengthy airplane travel, also known as "economy class syndrome"
- Antiphospholipid antibody syndrome

- Previous history of deep vein thrombosis or pulmonary embolism
- Myeloproliferative disorders such as polycythemia vera or essential thrombocytosis
- Paroxysmal nocturnal hemoglobinuria
- Inflammatory bowel syndrome
- HIV/AIDS
- Nephrotic syndrome

(Deitcher *et al.*, 2000)

#### **1.2.2.2 Diagnosis of hypercoagulable state:**

- Careful Medical History
- Certain conditions increase a person's risk for developing blood clots, but do not necessarily indicate a genetic hypercoagulable state. Therefore, a careful evaluation of the patient's personal and family medical history is needed.

Patients may be candidates for screening for hypercoagulable states if they have:

- A family history of abnormal blood clotting
- Abnormal blood clotting at a young age (less than age 50)
- Thrombosis in unusual locations or sites, such as veins in the arms, liver (portal), intestines (mesenteric), kidney (renal) or brain (cerebral)
- Blood clots that occur without a clear cause (idiopathic)
- Blood clots that recur
- A history of frequent miscarriages
- Stroke at a young age

(Deitcher *et al.*, 2000)

#### **1.2.2.2.1 Laboratory Testing:**

If you have one of the previously listed conditions, your doctor may recommend blood tests to further evaluate your condition. These tests should be performed at a specialized coagulation laboratory and interpreted by a pathologist or clinician with expertise in coagulation, vascular medicine or hematology. Ideally, the tests should be done when you are not having an acute clotting event. The most common lab tests include:

- PT-INR: The prothrombin time (PT or protime) test is used to calculate your International Normalized Ratio (INR). The information is used to monitor your condition if you are taking warfarin (Coumadin). Your INR will help your healthcare provider determine how fast your blood is clotting and whether your warfarin (Coumadin) dose needs to be changed.
- Activated partial thromboplastin time (APTT): Measures the time it takes blood to clot. This test is used to monitor your condition if you are taking heparin.
- Fibrinogen level
- Complete blood count (CBC)

Tests used to help diagnose inherited hypercoagulable states include:

Genetic tests, including factor V Leiden (Activated protein C resistance) and prothrombin gene mutation (G20210A), Antithrombin activity, Protein C activity, Protein S activity, Fasting plasma homocysteine levels and Factor V Leiden and prothrombin gene mutation (G20210A) are the most commonly identified genetic defects that increase a person's risk for blood clotting.

Tests used to help diagnose acquired hypercoagulable states include:

Tests for Anti-cardiolipin antibodies (ACA) or beta-2 glycoproteins, which are part of the antiphospholipid antibody syndrome, Lupus anticoagulants (LA), part of the antiphospholipid antibody syndrome (Deitcher *et al.*, 2000)

### **1.2.3 Obesity:**

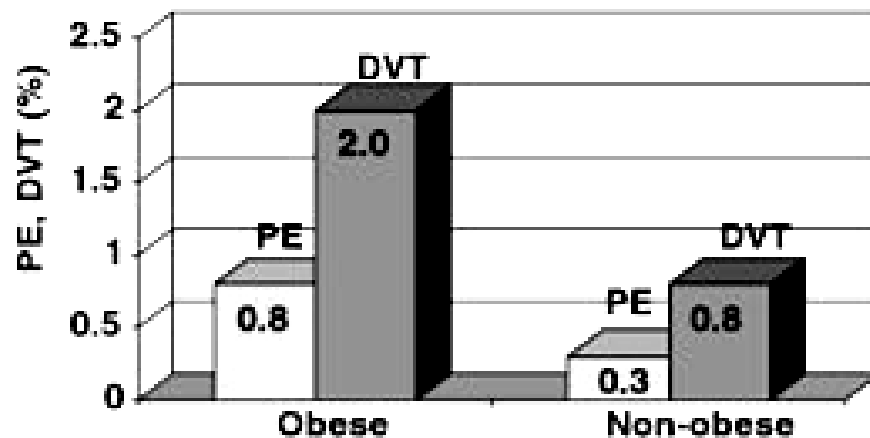
Is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health, leading to reduced life expectancy and/or increased health problems (Haslam *et al.*, 2005). People are considered obese when their body mass index (BMI), a measurement obtained by dividing a person's weight by the square of the person's height, exceeds 30 kg/m<sup>2</sup> (World Health Organization, 2000). Obesity is characterized by multiple hemostatic disturbances in blood coagulation, including enhanced platelet activation increased concentrations and enhanced activities of plasma coagulation factors as well as impaired fibrinolysis in form of increased production of plasminogen activator inhibitor-1 (PAI-1) Several other mechanisms, such as systemic inflammation, endothelial dysfunction, disturbances of lipid and glucose metabolism and insulin resistance also contribute to the hypercoagulable state in obesity, Both environmental and genetic factors account for the altered coagulation profile in obese individuals. Genetic factors contribute to the inter individual variation of blood coagulation protein levels Inherited genetic polymorphisms influence the hemostatic profile and increased risk of vascular events pleiotropically acting genes may contribute to the clustering of procoagulant and metabolic risk factors in obese individuals (Sanna *et al.*, 2012). Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer, and osteoarthritis (Haslam *et al.*, 2005). Obesity is most commonly caused by a combination of excessive food energy intake, lack of physical activity and

genetic susceptibility, although a few cases are caused primarily by genes, endocrine disorders, medications or psychiatric illness. Evidence to support the view that some obese people eat little yet gain weight due to a slow metabolism is limited. On average obese people have greater energy expenditure than their thin counterparts due to the energy required to maintain an increased body mass (Robert, 2007). Dieting and physical exercise are the mainstays of treatment for obesity. Diet quality can be improved by reducing the consumption of energy-dense foods such as those high in fat and sugars, and by increasing the intake of dietary fiber. Anti-obesity drugs may be taken to reduce appetite or decrease fat absorption when used together with a suitable diet. If diet, exercise and medication are not effective, a gastric balloon may assist with weight loss, or surgery may be performed to reduce stomach volume and/or bowel length, leading to feeling full earlier and a reduced ability to absorb nutrients from food (Imaz *et al.*, 2008). Obesity is a leading preventable cause of death worldwide, with increasing rates in adults and children. Many researchers view it as one of the most serious public health problems of the 21st century (Barness *et al.*, 2007). Obesity is stigmatized in much of the modern world (particularly in the Western world), though it was widely seen as a symbol of wealth and fertility at other times in history, and still is in some parts of the world (Haslam *et al.*, 2005). In 2013, the American Medical Association classified obesity as a disease. Obesity is an important and growing public health issue that is estimated to affect more than half of the UK adult population. Obesity is associated with significant and largely preventable morbidity and mortality including an increased incidence and prevalence of arterial and venous thrombotic events. The various mechanisms by which obesity may cause thrombosis include the actions of so-called adipocytokines from

adipose tissue, e.g. leptin and adiponectin, increased activity of the coagulation cascade and decreased activity of the fibrinolytic cascade, increased inflammation, increased oxidative stress and endothelial dysfunction and disturbances of lipids and glucose tolerance in association with the metabolic syndrome (Darvall *et al.*, 2007). Several studies have shown that obese patients have higher plasma concentrations of all pro-thrombotic factors (fibrinogen, vonWillebrand factor (vWF) and factor VII), as compared to non-obese controls. Similarly, plasma concentrations of plasminogen activator inhibitor-1 (PAI-1) have been shown to be higher in obese patients as compared to non-obese controls. The issue of whether adipose tissue contributes directly to plasma PAI-1, its products stimulating other cells to produce PAI-1, or whether it primarily contributes indirectly has not yet been resolved. It has been proposed that the secretion of interleukin-6 (IL-6) by adipose tissue, combined with the actions of adipose tissue-expressed TNF-alpha in obesity, could underlie the association of insulin resistance with endothelial dysfunction, coagulopathy and coronary heart disease. The role of leptin in impairing haemostasis and promoting thrombosis has been recently reported. Finally, some hormonal abnormalities (androgen, catecholamines) associated with the accumulation of body fat may contribute to the impairment of coagulative pathway in obesity. As to intervention strategies, dietary (i.e., low-fat high-fiber diet) and lifestyle (i.e., physical activity) measures have been demonstrated to be effective in improving the obesity-associated pro-thrombotic risk profile (De pergola *et al.*, 2002). Obesity leads to a 2 to 3-fold higher risk of venous thrombosis in men and women (Tsai *et al.*, 2002). The risk associated with severe obesity (BMI above 40 kg/M<sup>2</sup>) is even higher. The risk of venous thrombosis increases with obesity, so that the incidence of this pathology is



also expected to rise significantly. There is remarkable and consistent evidence from a systematic review, as well as cohort and case-control studies that obesity might predispose to venous thromboembolism (VTE). The risk appears to be at least double that for normal weight subjects (BMI 20 to 24.9 kg/m). Plausible mechanisms exist to explain this relationship including the physical effects of body fat limiting venous return and a proinflammatory, prothrombotic, and hypofibrinolytic. Loss of body weight has been shown to reduce the concentrations of coagulation factors and plasminogen activator inhibitor-1 toward the normal range (Margaret *et al.*, 2011). The obese have a further increase in thrombosis risk when they are exposed to other thrombosis risk factors, such as exogenous contraceptive or postmenopausal hormones (Abdollahi *et al.*, 2003). One study estimated that the absolute risk of thrombosis for five years of estrogen plus progestin use in women aged 50–59 years with obesity was 1.5%, compared to 0.5% in normal weight women (Cushman *et al.*, 2004). There are conflicting findings from different studies of the larger studies that have been performed; one found that obese people were 2.4 times as likely as non obese people to develop DVT. Another study, in women, found that those who were obese were 2.9 times as likely as non obese women to develop PE, the most serious complication of DVT (Alyaseen *et al.*, 2004). The relative risk of deep venous thrombosis, comparing obese patients with non-obese patients, was 2.50 (95% confidence interval [CI] = 2.49-2.51). The relative risk of pulmonary embolism was 2.21 (95% CI = 2.20-2.23). Obesity had the greatest impact on both men and women; the data indicate that obesity is a risk factor for venous thromboembolic disease in men as well as women (Stein *et al.*, 2005).



**Figure (1.2) pulmonary embolism (PE) and deep venous thrombosis (DVT) in hospitalized patients from 1979 to 1999 showing the prevalence in obese and non obese patients (Stein *et al.*, 2005).**

#### **1.2.3.1 Classification of obesity according to body mass index:**

BMI is defined as the subject's weight divided by the square of their height and is calculated as follows.

$$\text{BMI} = m/h^2$$

Where m and h are the subject's weight in kilograms and height in meters respectively. BMI is usually expressed in kilograms per square meter. To convert from pounds per square inch multiply by 703 (kg/m<sup>2</sup>)/ (lb/sq). The most commonly used definitions, established by the World Health Organization in 1997 and published in 2000), provide the values listed in the table below:

**Table (1.2) classification of obesity by World Health Organization**

<b>Class of body mass index*</b>	<b>BMI (kg/m<sup>2</sup>)</b>
Underweight	18.50 >
normal weight	24.99–18.50
Overweight	29.99–25.00
class I obesity	34.99–30.00
class II obesity	39.99–35.00
class III obesity	40.00 ≤

\*(World Health Organization, 2000).

### **1.2.3.2 Effects of obesity on health:**

#### **1.2.3.2.1 Mortality:**

Obesity is one of the leading preventable causes of death worldwide (Barness *et al.*, 2007). Large-scale American and European studies have found that mortality risk is lowest at a BMI of 20–25 kg/m<sup>2</sup> (Berrington, 2010). In non-smokers and at 24–27 kg/m<sup>2</sup> in current smokers, with risk increasing along with changes in either direction (Calle *et al.*, 1999). A BMI above 32 kg/m<sup>2</sup> has been associated with a doubled mortality rate among women over a 16-year period (Manson *et al.*, 1995). In the United States obesity is estimated to cause 111,909 to 365,000 deaths per year (Haslam *et al.*, 2005). While 1 million (7.7%) of deaths in Europe are attributed to excess weight (Tsigosa *et al.*, 2008). On average, obesity reduces life expectancy by six to seven years (Haslam *et al.*, 2005). A BMI of 30–35 kg/m<sup>2</sup> reduces life expectancy by two to four years. (Whitlock *et al.*, 2009). While severe obesity (BMI > 40 kg/m<sup>2</sup>) reduces life expectancy by ten years.

#### **1.2.3.2.2 Morbidity:**

Obesity increases the risk of many physical and mental conditions. These comorbidities are most commonly shown in metabolic syndrome (Haslam *et al.*, 2005). A combination of medical disorders which includes diabetes mellitus type 2, high blood pressure, high blood cholesterol and high triglyceride levels (Grundy, 2004). Complications are either directly caused by obesity or indirectly related through mechanisms sharing a common cause such as a poor diet or a sedentary lifestyle. The strength of the link between obesity and specific conditions varies. One of the strongest is the link with type 2 diabetes. Excess body fat underlies 64% of cases of diabetes in men and 77% of cases in women (Seidell, 2005). Health consequences fall into two broad categories: those attributable to the effects of increased fat mass (such as osteoarthritis, obstructive sleep apnea, and social stigmatization) and those due to the increased number of fat cells diabetes, cancer, cardiovascular disease, non-alcoholic fatty liver disease (Haslam *et al.*, 2005). Increases in body fat alter the body's response to insulin, potentially leading to insulin resistance. Increased fat also creates a proinflammatory state (Shoelson *et al.*, 2007).

#### **1.2.3.3 Relation between obesity, thrombosis and inflammation:**

Clinical and epidemiological studies support a connection between obesity and thrombosis, involving elevated expression of the prothrombotic molecules, plasminogen activator inhibitor-1 and tissue factor (TF) and increased platelet activation. Cardiovascular diseases and metabolic syndrome-associated disorders, including obesity, insulin resistance, type2 diabetes, and hepatic steatosis, involve inflammation elicited by infiltration and activation of immune cells, particularly macrophages, into adipose tissue. Although TF has been clearly linked to a procoagulant state in

obesity, emerging genetic and pharmacologic evidence indicate that TF signaling via G protein-coupled protease-activated receptors (PAR2, PAR1) additionally drives multiple aspects of the metabolic syndrome. TF–PAR2 signaling in adipocytes contributes to diet-induced obesity by decreasing metabolism and energy expenditure, whereas TF–PAR2 signaling in hematopoietic and myeloid cells drives adipose tissue inflammation, hepatic steatosis, and insulin resistance. TF-initiated coagulation leading to thrombin–PAR1 signaling also contributes to diet-induced hepatic steatosis and inflammation in certain models. Thus, in obese patients, clinical markers of a prothrombotic state may indicate a risk for the development of complications of the metabolic syndrome (Samad *et al.*, 2013).

### **1.3 Rationale:**

Obesity is a leading preventable cause of death worldwide, with increasing rates in adults and children. Many researchers view it as one of the most serious public health problems of the 21st century. In the United States obesity is estimated to cause 111,909 to 365,000 deaths per year. While 1 million (7.7%) of deaths in Europe. On average, obesity reduces life expectancy by six to seven years. A body mass index (BMI of 30–35 kg/m<sup>2</sup>) reduces life expectancy by two to four year. While severe obesity (BMI > 40 kg/m<sup>2</sup>) reduces life expectancy by ten year. Obesity is an important and growing public health issue, is associated with significant, and largely preventable, morbidity and mortality including an increased incidence and prevalence of arterial and venous thrombotic events, heart disease, type2 diabetes, certain type of cancer.

## **1.4 Objectives:**

### **1.4.1 General objectives:**

- Measurement the Effect of Obesity on Some Coagulation Parameter in Obese Sudanese Subjects in Khartoum State

### **1.4.2 Specific objectives:**

- To Measure PT, APTT and INR in obese subjects and control groups.
- To correlate the finding of PT, APTT activity and INR with age groups, gender, and obesity class.

## **2. Materials and Method**

### **2.1 Study design:**

This is analytical case control study which was conducted in Khartoum State to measure the effect of obesity on some coagulation profile: Prothrombin Time (PT), Partial Thromboplastin Time (APTT) and International Normalised Ratio (INR) in obese subjects who attended Al Arabie Fitting Center in khartoum in the period from March to May 2014.

### **2.2 Study population:**

The study covered one hundred individuals which include seventy cases and thirty controls. The cases including Forty six obese females and Twenty four obese males. The case divided in to three obesity class according to WHO classification 33 individuals were obesity class I the BMI (30.00–34.99 kg/m<sup>2</sup>), 29 individuals were obesity classII the BMI (35.00–39.99 kg/m<sup>2</sup>), and 8 individuals were obesity classIII the BMI  $\geq 40.00$  kg/m<sup>2</sup>.

### **2.3 Inclusion criteria:**

Healthy Sudanese subjects who were obese and resident in khartoum State with no history of any medical condition and not on anticoagulant drugs. Obese subjects matched to the control by age and history of any medical condition and anticoagulant drugs.

### **2.4 Exclusion criteria:**

Non obese subjects and obese subjects with history of medical condition or on anticoagulant drugs.

### **2.5 Data collection:**

Data were collected using self-administered pre-coded questionnaire which was specifically designed to obtain information including age, sex, body mass index, medical history and history of medication and other information.



## **2.6 Sample collection:**

Two milliliters of citrated, platelet-poor plasma is prepared from peripheral venous blood collected by clean, nontraumatic venipuncture directly into a plastic tube containing (3.2%) trisodium citrate at a ratio of 9:1. The blood transferred from the syringe to a plastic tube containing the proper amount of anticoagulant within one minute after collection. The platelet poor plasma prepared from the whole blood specimen by centrifuging the capped specimen tube at an appropriate speed for an appropriate time. Centrifugation at 1500 g for 15 minutes at room temperature. A centrifuge with a swing-out bucket rotor used to avoid remixing of platelets and plasma during plasma removal (Roger S, 2005).

## **2.7 Ethical consideration:**

Ethical clearance from the institution and permission of AL Arabia Fitting Center the fitting center were taken. It was considered that all information obtained participants was kept as highly confidential data and specimens results were not permitted. The participants were provided with information about the study and any risk which may be arisen especially when the collection technique was applied and the consent of study subjects was taken after being informed with all detailed objectives of the study and its health benefit future.

## **2.8 Data analysis:**

Data were analyzed by independent t test using the SPSS computer programme version 11.5 with confidence level 95% and significant Level < 0.05.

## **2.9 Methods:**

### **2.9.1 Prothrombin Time (PT) using coagulometer (Biobas):**

#### **2.9.1.1 Principle of PT by automated method:**

The coagulometer (Biobas) has an optical measurement system which detects a sudden variation in optical density when clot is formed. The chronometer and the stirring system are activated by 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 continuous mixing guarantees a perfect homogenization and make the measurement possible of low concentration of fibrinogen by grouping the fibrin filament in the center of the optical pass. Tissue Thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulation mechanism. When liquiplastin reagent is added to normal citrated plasma, the clotting Mechanism is initiated, forming a solid gel clot within a specified period of time.

(Biggs *et al.*, 1962).

#### **2.9.1.2 Reagents:**

liquid calcified liquiplastin Reagent, which is derived from rabbit brain.

(Biggs *et al.*, 1962).

#### **2.9.1.3 Prothrombin Time Test procedure:**

1. First of all, the cuvette was placed and a magnetic stirrer was inserted in every cuvette and waited for instrument to reach 37°C.
2. After that into the cuvette 200 ml of reagent were introduced.
3. When the incubation time is finished, the cuvette was placed on the reading well the chronometer was remained inactive for some second and then it was showed 000:0. At this moment 100 ml of plasma was added and the result appeared on the display.

$$\text{INR} = (\text{prothrombin}_{\text{test}} / \text{prothrombin}_{\text{control}})^{\text{ISI}}$$

(Tripodi A, 2004).

Reference PT, INR range:

Normal values of PT using liquiplastin Reagent are between 10-15 seconds and typically INR of 0.8 – 1.1 (Biggs *et al.*, 1962).

Quality control was done followed Biggs *et al.*, 1962

## **2.9.2 Activated partial thromboplastin time (APTT) using coagulometer (Biobas)**

### **2.9.2.1 Principle of APTT automated method:**

The coagulometer 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 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 continuous mixing guarantees a perfect homogenization and make the measurement possible of low concentration of fibrinogen by grouping the fibrin filament in the center of the optical pass. Cephaloplastin activates the coagulation factors of the intrinsic pathway of the coagulation mechanism in the presence of calcium ions (Biggs *et al.*, 1962).

### **2.9.2.2 Reagents:**

Liquocelin is a phospholipids preparation derived from rabbit brain with ellagic acid as an activator, calcium chloride :0.025mol/l (Biggs *et al.*, 1962).

### **2.9.2.3 Activated partial thromboplastin time Test Procedure:**

1. First of all, the cuvette was placed and a magnetic stirrer was inserted in every cuvette and waited for instrument to reach 37°C.

2. After that into the cuvette 100ml of reagent+100ml of plasma were introduced and incubated for 3-5min.
3. When the incubation time is finished, the cuvette was placed on the reading well the chronometer was remained inactive for some second and then it was showed 000:0. At this moment 100ml of CaCl was added and the result appeared on the display.

Reference APTT range:

Normal values using liquicelin reagent are between 28-40seconds.

(Biggs *et al.*, 1962).

Quality control was done followed Biggs R *et al.*, 1962

# Chapter three

## 3. Results

Figure (3.1) shows the distribution of the study population according to gender, twenty four male and forty six female in case group and nineteen male and eleven female in control group.

Figure (3.2) shows the mean age of the cases in (thirty two years) and the control group in (thirty one years).

Comparison of mean of PT, APTT and INR in the cases and control group is presented in Table (3.1). The mean of PT, APTT and INR ( $12.2 \pm 0.7$ sec), ( $30.3 \pm 2.0$ sec) and ( $0.97 \pm 0.06$ ) respectively in the cases and ( $13.0 \pm 0.8$ sec), ( $32.6 \pm 2.1$ sec) and ( $1.03 \pm 0.07$ ) respectively in the control group (P. value 000). The number and percentage of different obesity class are obesity class1 33 (52%), obesity class 2 29(12%), obesity class3 8(14%), as in Table (3.2).

According to age groups, the mean of PT is ( $12.4 \pm 0.7$ sec) in age group < 30 years and ( $12.1 \pm 0.6$ sec) in Age group > 30 years with asinificant difference between two groups (P. value 0.03). The mean of APTT was ( $30.5 \pm 2.1$ sec) in age group < 30years and ( $30.2 \pm 1.9$ sec) in age group > 30 years (P.value0.7). The mean of INR ( $0.99 \pm 0.07$ ) in age group < 30, and ( $0.97 \pm 0.06$ ) in Age group > 30 years (P. value 0.03), as in Table (3.3).

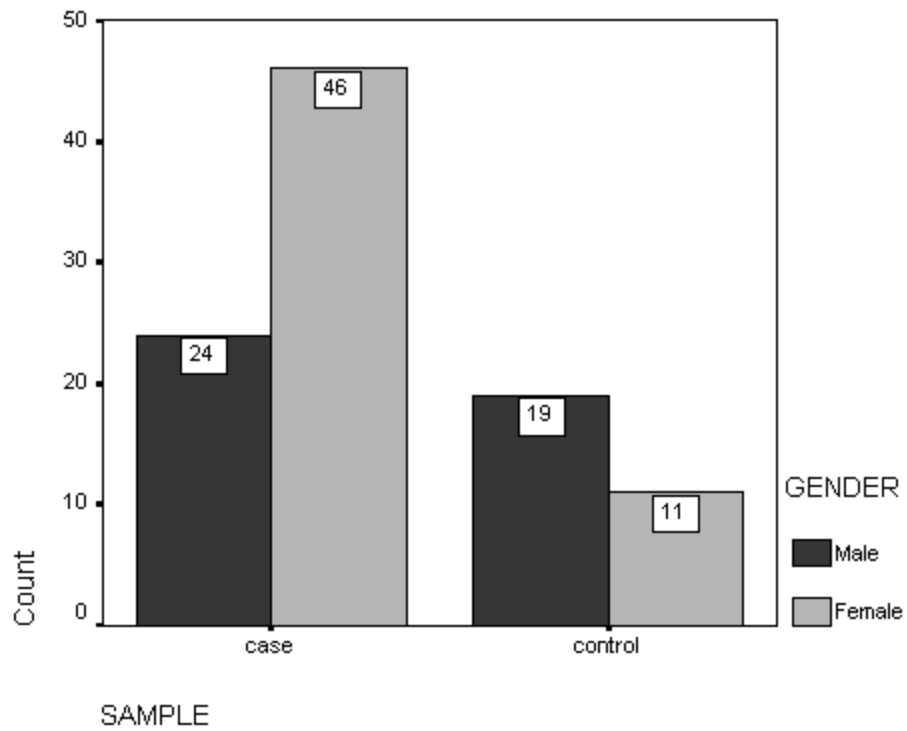
Table (3.4) shows the mean of PT ( $12.1 \pm 0.6$ sec) in male group and ( $12.3 \pm 0.7$ sec) in female group (P. value 0.3). The mean APTT ( $30.3 \pm 1.9$ sec) in male group, and ( $30.4 \pm 2.1$ sec) in female group (P. value 0.8). The mean of INR ( $0.96 \pm 0.05$ ) in male group, and ( $0.98 \pm 0.06$ ) in female group (P. value 0.3).

Table (3.5) shows the mean values of PT, APTT and INR among case group according to different obesity class. The mean of PT in obesity class1 ( $12.4 \pm 0.7$ sec), in obesity class2 ( $12.1 \pm 0.7$ sec), in obesity class3 ( $12.0 \pm 0.5$ sec), (P value 0.1). The mean of APTT in obesity class1 ( $30.7 \pm 2.2$ sec), in obesity class2 ( $30.2 \pm 1.9$ sec), in obesity class3 ( $29.5 \pm 1.9$ sec), (P value 0.3). The mean of INR in obesity class1 ( $1.0 \pm 0.06$ ), in obesity class2 ( $0.97 \pm 0.05$ ), in obesity class3 ( $0.96 \pm 0.04$ ), (P value 0.1).

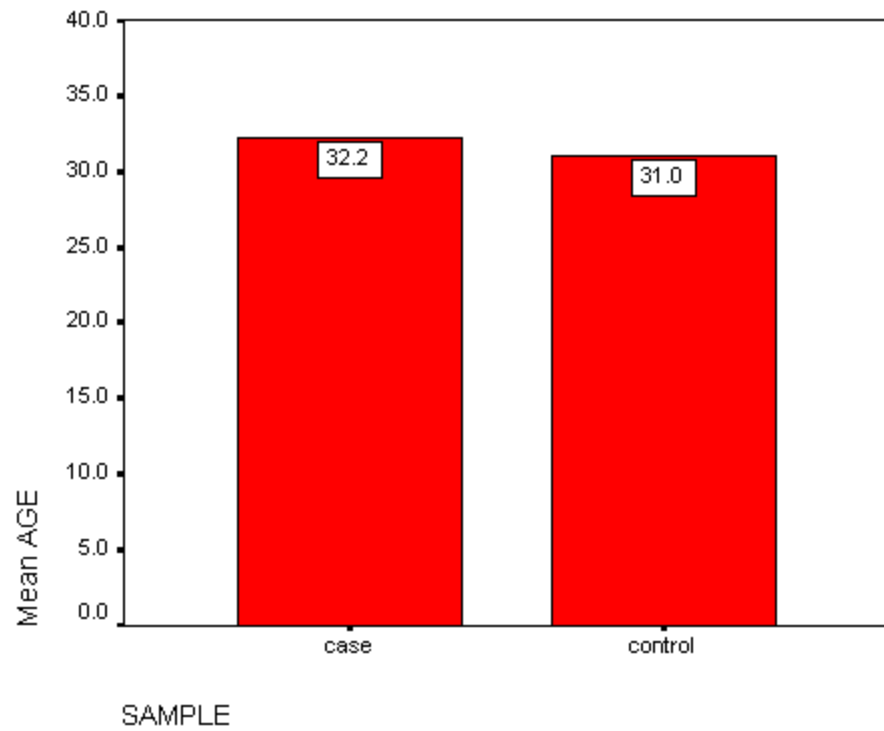
Figure (3.4) Scatter plot shows insignificant weak negative correlation between body mass index and PT/sec ( $r$  0.193 P. value 0.109).

Figure (3.5) Scatter plot shows significant weak positive correlation between body mass index and APTT/sec ( $r$  0.243 P. value 0.042).

Figure (3.6) Scatter plot shows insignificant weak negative correlation between body mass index and INR ( $r$  0.201 P. value 0.096).



**Figure (3.1) Distribution of study population according to gender**



**Figure (3.2) The Mean of age among case and control group**



**Table (3.1) Comparison of mean PT/sec, PTT/sec, INR in case and control groups.**

<b>Parameter</b>	<b>Sample</b>	<b>Mean</b>	<b>SD</b>	<b>P. value</b>
PT/sec	Control	13	0.8	0.00
	Case	12.2	0.7	
PTT/sec	Control	32.6	2.1	0.00
	Case	30.3	2.0	
INR	Control	1.03	0.07	0.00
	Case	0.97	0.06	

**Table (3.2) Number and percentage of the cases according to the obesity class.**

<b>Parameters</b>	<b>Number</b>	<b>Percentage</b>
Obesity class1	33	47.1
Obesity class2	29	41.4
Obesity class3	8	11.4
<b>Total</b>	<b>70</b>	<b>100</b>

**Table (3.3) Effect of age on PT/sec, PTT/sec, INR in the cases.**

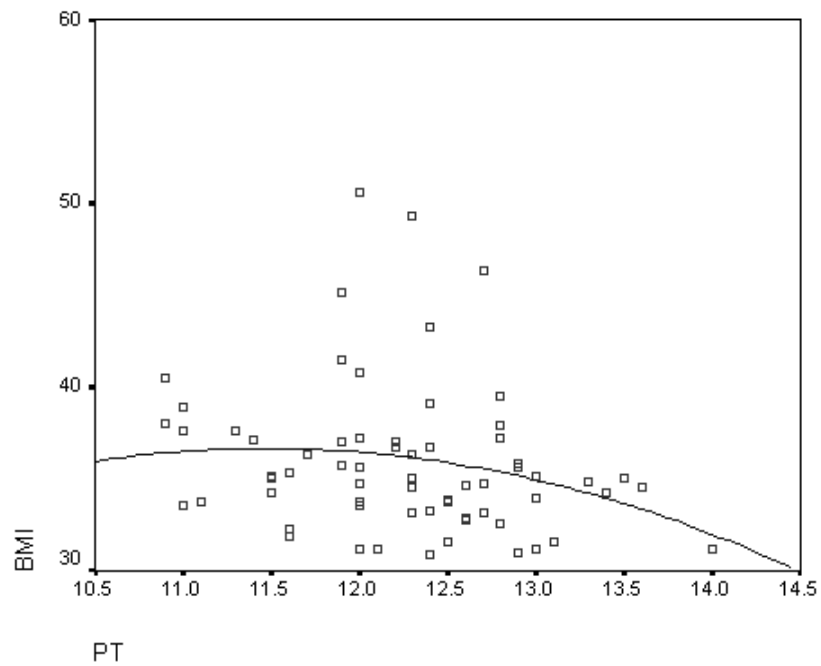
<b>Parameter</b>	<b>Age/years</b>	<b>Number</b>	<b>Mean</b>	<b>SD</b>	<b>P. value</b>
PT/sec	<30	33	12.4	0.7	0.03
	>30	37	12.1	0.6	
PTT/sec	<30	33	30.5	2.1	0.7
	>30	37	30.2	1.9	
INR	<30	33	0.99	0.07	0.03
	>30	37	0.97	0.06	

**Table (3.4) Effect of gender on PT/sec, PTT/sec, INR in the cases.**

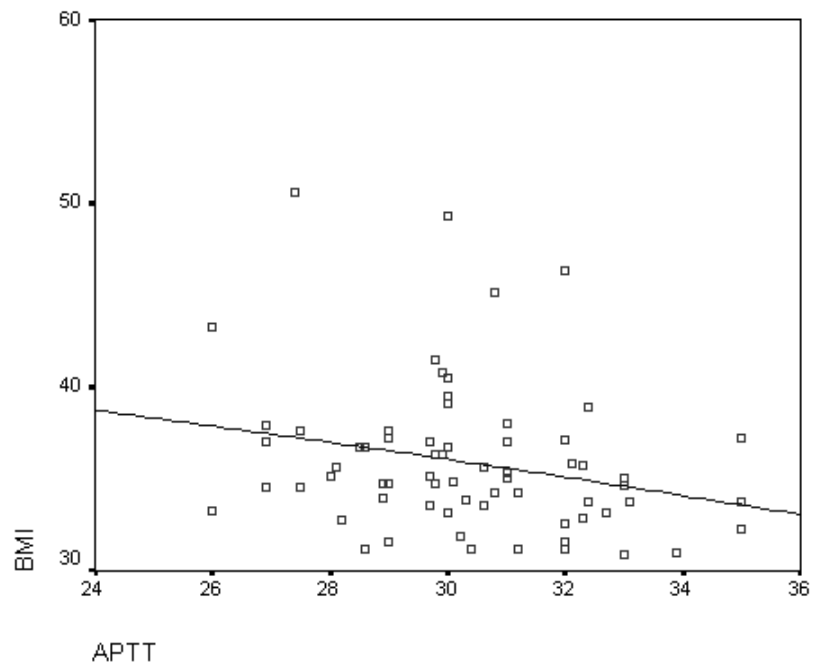
<b>Parameter</b>	<b>Gender</b>	<b>NO</b>	<b>Mean</b>	<b>SD</b>	<b>P. value</b>
PT/sec	Male	24	12.1	0.6	0.3
	Female	46	12.3	0.7	
PTT/sec	Male	24	30.3	1.9	0.8
	Female	46	30.4	2.1	
INR	Male	24	0.96	0.05	0.3
	Female	46	0.98	0.06	

**Table (3.5) Effect of obesity class on PT/sec, PTT/sec and INR results.**

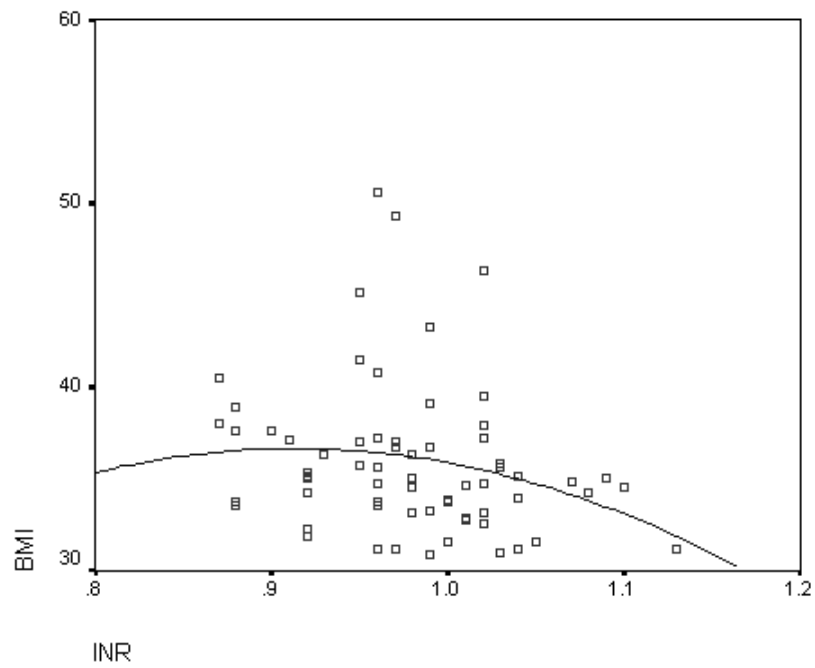
<b>Parameter</b>	<b>Obesity class</b>	<b>Mean</b>	<b>SD</b>	<b>P. value</b>
PT/sec	Obesity class 1	12.4	0.7	0.1
	Obesity class 2	12.1	0.7	
	Obesity class 3	12.0	0.5	
PTT/sec	Obesity class 1	31	2.2	0.3
	Obesity class 2	30	1.9	
	Obesity class 3	29	1.9	
INR	Obesity class 1	1.0	0.06	0.1
	Obesity class 2	0.97	0.05	
	Obesity class 3	0.96	0.04	



**Figure (3.3) Correlation between the body mass index and PT/sec among the cases**



**Figure (3.4) Correlation between the body mass index and APTT/sec among the cases**



**Figure (3.5) Correlation between the body mass index and INR among the cases**



# Chapter Four

## Discussion, Conclusion and Recommendation

### 4.1 Discussion:

Abnormalities in coagulation and haemostasis represent a well-known link between obesity and thrombosis (both arterial and venous). Obesity is characterized by multiple hemostatic disturbances in blood coagulation, including enhanced platelet activation increased concentrations and enhanced activities of plasma coagulation factors as well as impaired fibrinolysis in form of increased production of plasminogen activator inhibitor-1 (PAI-1). Several other mechanisms, such as systemic inflammation, endothelial dysfunction, disturbances of lipid and glucose metabolism and insulin resistance also contribute to the hypercoagulable state in obesity. Several studies have shown that obese patients have higher plasma concentrations of all pro-thrombotic factors, compared to non-obese controls. There is remarkable and consistent evidence from cohort and case-control studies that obesity might predispose to venous thromboembolism (VTE). The risk appears to be at least double that for normal weight subjects.

The study revealed the following: The mean PT result of the study group was significantly shortened than that of the control group (p value 0.00), as well as the mean INR result of study group was significantly decreased than that of control group (P value 0.00). These findings are agreed with a study of Chan, (1995), who demonstrated that obese patients have decreased PT and INR values. These findings also agreed with study of Sophie, (2012), who found that obese patients have a short of PT and INR. These findings are also agreed with study of (De pergola, 2002), who concluded that obese

patients have higher plasma concentrations of factors (I, VII) that lead to increase activities of extrinsic coagulation pathway and subsequently decrease PT and INR. This study showed that mean APTT result of study group was significantly shortened than that of control group (P value 0.00). These finding is agreed with study of (Neil, 2008), who concluded that obese patients have decreased activated partial thromboplastin times. The finding is also agreed with study of (Sanna M, 2012) who found that obese patients have increased activities of fibrinogen and FIX, FXI, and FXII that lead to increase activities of intrinsic coagulation pathway and subsequently decrease APTT predisposing obese subjects to thrombosis. These finding agreed with study of (Chan, 1995), who demonstrated that obese patients have decrease in APTT. And agreed with study of (Sophie, 2012), who concluded that obese patients have decrease of APTT due to increase in fibrinogen and FVII. Overall, the mean of PT/sec, APTT/sec and INR results were within normal ranges among the study group. The present study revealed that a significant difference in PT/sec and INR results in obese according to age in which there was increase in PT/sec and INR results in age < 30 years when compared to age group > 30 years. The finding is agreed with study of (Song, 2006), in which there study concluded that the obesity with an increasing age are identified as statistically significant risk factors for DVT. According to obesity class there was no significant difference in mean level of PT, APTT and INR results among the study group. The finding is disagreed with the study of (Margaret, 2011), who found the association of obesity with venous thrombosis becomes stronger as the BMI increases. Morbidly obese (i.e., those with BMI over 40 kg/m<sup>2</sup>) are at an even higher risk than those with BMI 30 to 40 kg/m<sup>2</sup>. The study explained that there is no significant change in PT, APTT and INR results in

obese according to gender. The finding is disagreed with study of (Margaret, 2011), in which there study concluded that the Obese females had a greater relative risk for deep venous thrombosis than obese males.

## **4.2 Conclusion:**

The study concluded that:

- There was significant shortening of PT/sec, APTT/sec and INR in obese subjects in compare with control group.
- A significantly decreased in PT/sec and INR in obese subjects who in age group of > 30 year than that of age group of < 30 years while APTT was not affected by age that found.
- There were no significant changes in coagulation parameters related to gender and obesity class.
- The mean of PT/sec, APTT/sec and INR results were within normal ranges among the study group.

### **4.3 Recommendations:**

The study recommended that:

1. The concentration of coagulation factors should be done to identify the factors which were affected by obesity.
2. Study of other coagulation profile like fibrinogen, thrombin time (TT) as well as the mechanisms that altered them in obese subjects
3. Health program should be implemented through multimedia (Radio, Television) to explain Mortality and Morbidity due to obesity.

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## Appendices

### **Appendix (1): Questionnaire.**

**Sudan University of Science and Technology**

**Collage of Graduate Studies and Scientific Research**

#### **General Information:**

1- Name

2- No

3- Age

4- Weight (kg)

5- Height(m)

6- Sex

7- Medical condition: Heart disease (   )   Diabetes (   )   Liver  
disease (   )   Hypertension (   )   Others

#### **Investigation:**

PT

INR

APTT

BMI

Signature

Date