Chapter one

1-Introduction and Literature Review

1.1 Introduction:

The hemostatic mechanisms have several important functions. First to maintain blood in fluid state.Second to arrest bleeding at the site of injury or blood loss by formation of hemostatic plug. Third they must insure the eventual removal of the plug when healing is complete. Normal physiology thus constitutes delicate balance between these conflicting tendencies and a deficiency of any one may lead to either thrombosis or hemorrhage (Sir john *et al*., 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 and Nathanson, 2006) Until recently, the lack of an established alternative concept of hemostasis has meant 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. The shortcomings of this older model of normal coagulation are nowhere more apparent than in its clinical application to the complex coagulation disorders of acute and chronic liver disease. (Michael H and Nathanson;2006)

The liver plays several key roles in blood coagulation being involved in both primary and secondary hemostasis.It is the site of synthesis of most coagulation factors and their inhibitors.Liver damage is commonly associated with impairment ofcoagulation, when liver reserve is poor. The hemostatic system is in a delicate balance between prothrombotic and antithrombotic processes, aiming to prevent

excessiveblood loss from injured vessels and to prevent spontaneous thrombosis. Liver failure is accompanied by multiple changes in the hemostatic system, because of reduced plasma levels of procoagulative and anticoagulative clotting factors synthesizedby hepatocytes and sinusoidal cells. Vitamin K deficiency may coexist, so that abnormal clotting factors are produced due to lack of gammacarboxylation. Moreover during liver failure, there is a reduced capacity to clear activated hemostatic proteins and protein inhibitor complexes from the circulation. Thus the global effect of liver disease with regard to hemostasis is complex, so that patients with advanced liver disease can experience severe bleeding or even thrombotic complication. (Tripodi*et al.*, 2005).

1.2 Literature Review:

1.2.1 Hemostasis:

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. Thehemostaticsystem thus represent delicate balance between anticoagulant and procoagulantmechanisms allied to the process for fibrinolysis.(Hoffbrand, 2010)

There are five major component involved in hemostasis include: blood vessels, platelets, coagulation factors, natural Inhibitors of the coagulation cascade, and Fibrinolysis. (Hoffbrand, 2010)

1.2.1.1 Primary hemostasis:

Primary hemostasis primarily involves platelets andvWF and results in theformation 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 processesincluding the control of cellular trafficking, the regulation of vasomotor tone and maintenance of blood fluidity. ECs possesssurface 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 ofintracellular adhesion molecules whichmodulate platelet adhesionand vascular permeability.(Hoffbrand, 2005)

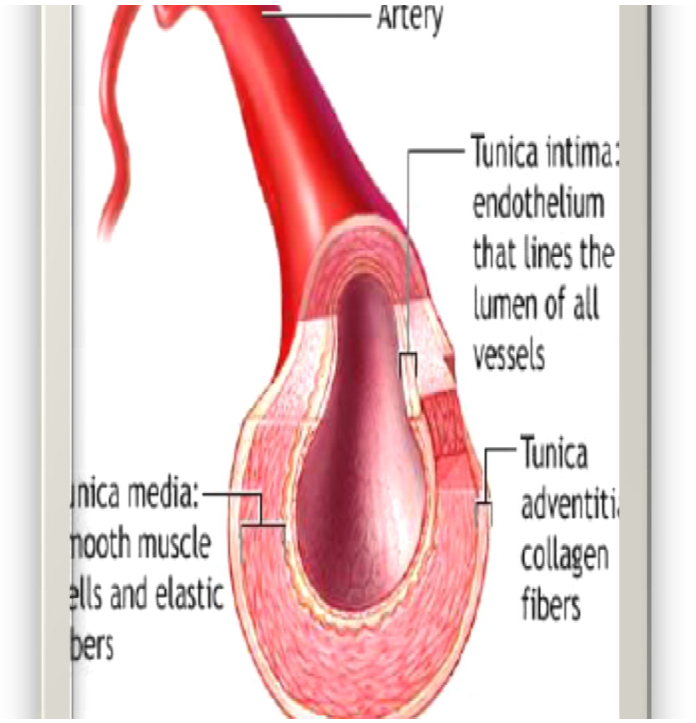

Figure (1.1) blood vessel structure(Marlies, 2006)**:**

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, 2010)

The megakaryocyte matures by endomitotic synchronous replication (i.e. 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, 2010)

Thrombopoietin is the major regulator of platelet production and is constitutively produced by the liver and kidneys. The normal platelet count is approximate250 x 10^9/L (range 150-400 x 10^9/L) and the normal platelet lifespan is 7-10 days. Up to one-third of the marrow output of platelets may be trapped in spleen but this rises to90% in cases of massive splenomegaly. (Hoffbrand, 2010)

1.2.1.1.2.2 Platelet structure and function:

Platelets are extremely small and discoid, 3.0 x 0.5 um 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 la (Gpla). 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 Ilb /IIIa is also the receptor for fibrinogen which is important in platelet-platelet aggregation.(Hoffbrand, 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, 2010).

The platelet contains three types of storage granules: dense, alpha and lysosomes. The more frequent specific a 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 canalicularsystem.Platelets are also rich in signaling and cytoskeletal proteins which support the rapid switch from quiescent to activation that follows vessel damage.(Hoffbrand, 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 fall 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, 2010)

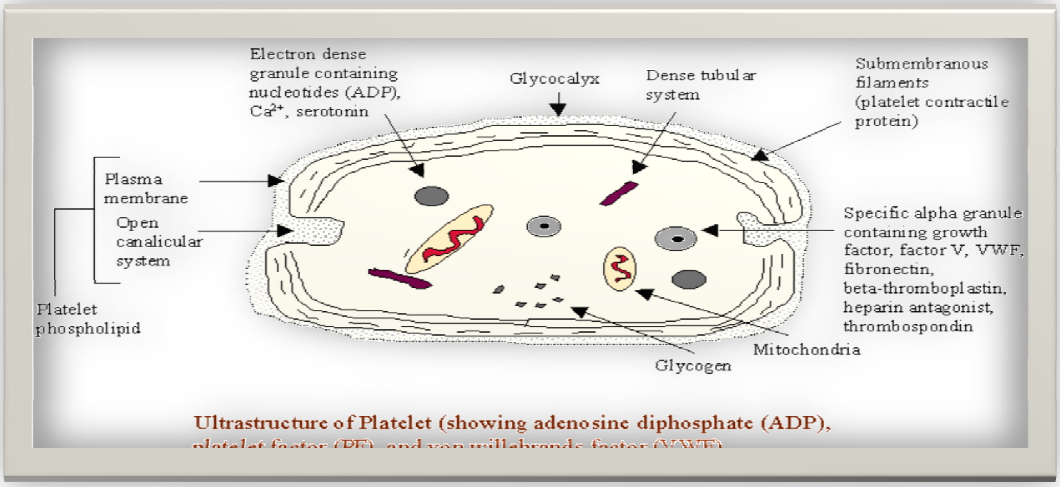

Figure (1.2)platelet structure(Hoffbrand, 2010)**:**

1.2.1.1.3Mechanism of primary hemostasis:

1.2.1.1.3.1Vasoconstriction:

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 A2 liberated from platelets, and the fibrinopeptides liberated during fibrin formation, also have vasoconstrictive activity.(Hoffbrand, 2010)

1.2.1.1.3.2Platelet 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 thromboxaneA2. Released ADP causes platelets to swell and aggregate.(Hoffbrand, 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, 2010)

1.2.1.1.4Causes of primary hemostasis deficiencies:

Causes of primary hemostasis deficiencies may include: Thrombocytopenia, Inherited disorders of platelet function (Bernard-Soulier syndrome, Glanzmann'sthrombasthenia and storage pool deficiency), vonWillebrand's disease. Also medications: aspirin, ticlopidine, etc.

And defects in the blood vessel wallwhich include:Hereditary hemorrhagic telangiectasia(HHT; Osler Weber-Rendu syndrome), Scurvy: Scurvy (vitamin C deficiency), Vasculitis, Amyloidosis and Senile purpura .(Kern, 2002)

1.2.1.2 Secondaryhemostasis:

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)

1.2.1.2.1Coagulation 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 The coagulation factors. (Hoffbrand,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 (Fletcher factor) HMWK (Fitzgerald factor)	Transglutaminas Serine protease Cofactor*

HMWK, high molecular weight kininogen.

*Active without proteolytic modification.

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 Concepts 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.We now know that there is really only one pathway; the intrinsic pathway is largely a laboratory artifact. However, because we still use the same two main tests to investigate the status of the coagulation cascade, you must understand 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 transmembraneprotein 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 offtwo 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.

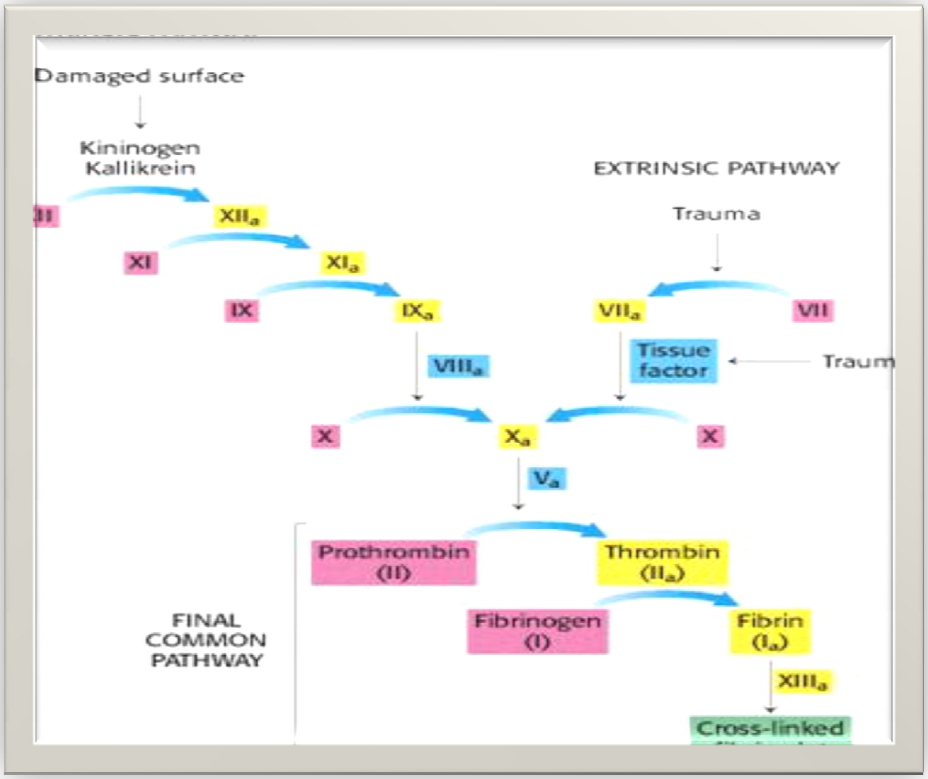

Figure (1.3) Coagulation Cascade(Miracles and Killers, 2009)**:**

1.2.1.2.3Natural 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 (i.e. not X-linked) 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 t-PA. There are also plasmin inhibitors, the most important of which is _2-antiplasmin, 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 fibrinolyis to the clot.(Kern, 2002)

There is also a circulating inhibitor of plasmin, _2antiplasmin,which inactivates any plasmin that is free in circulation. Plasmin bound to fibrin is protected from inhibition by _2-antiplasmin. (Kern, 2002)

1.2.1.3.2 Inhibition of Plasminogen Activation:

Just as there are inhibitors to plasmin, there are also inhibitors of plasmin 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 inthrombocytopenia but is normal in vascular causes of abnormal bleeding. The test involves the application of pressureto 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, 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 20×10^9/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.(Monica, 2000)

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. Prewarmed PT reagent suspended in calcium chloride (CaCl2) is added to the test plasma, which is anticoagulated with citrate. The CaCl2 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 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 = (patient PT _ 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. CaCl2 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)

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)

1.2.1.5.4Second line investigation:

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

1.2.2 Liver Disease:

The liver plays a major role in hemostasis, as most of the coagulation factors, anticoagulant proteins and components of the fibrinolytic system are synthesized by hepatic parenchymal cells. Additionally, the reticuloendothelial system of the liver helps to regulate coagulation and fibrinolysis by clearing this coagulation

factors from the circulation. Finally, because the liver is a highly vascularizedorgan with vital venous systems draining through the parenchyma, liver diseases
can affect abdominal blood flow and predispose patients to significant bleeding problem (Quick, 1973).

The etiology of impaired hemostasis resulting from abnormal liver function is often multifactorial. (Peltz, 1991) And may include impaired coagulation factor synthesis, synthesis of dysfunctional coagulation factors, increased consumption of coagulation factors, altered clearance of activated coagulation factors and quantitative and qualitative platelet disorders. (Mammen, 1994). Various hemostatic abnormalities can occur in patients with liver diseases such as chronic liver disease (CLD), liver cirrhosis (LC), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatocellular carcinoma (HCC) and obstructive jaundice (Czaja*et al*., 2002). The severity of these abnormalities is dependent on the degree of hepatic dysfunction. (Mammen, 1994). Liver disease can cause both gross abnormalities in coagulation factors. (Kramer *et al*., 2002) Commonly, the vitamin K-dependent factors decreased first, starting with factor VII and protein C owing to their short half-life of six hours, followed by reductions in factor II and X levels. Factor V level decreased in both acute and chronic liver disease. (Lee *et al*., 2004). Factor IX level is modestly reduced until advanced stages of liver disease occurs. In contrast, von Willebrand Factor (vWF) which synthesized by the endothelial cells and factor VIII levels may be normal even in the presence of advanced liver disease because there is an increased production of factor VIII by the sinusoidal endothelial cells when the liver is damaged, combined with decreased clearance of the vWF/VIII complex. Fibrinogen levels are rarely decreased and may even be elevated because of abnormal non-functional fibrinogen (dysfibrinogenaemia) related to defective polymerization. A decrease in fibrinogen levels may indicate

the presence of disseminated intravascular coagulation (DIC) or progression to fulminant hepatitis with hepatic failure. (Pernambuco*et al*.,1993).

In a study of patients with significant liver injury and associated coagulopathy, factors II, V, VII and X were reduced to a similar degree and were significantly lower than factors IX and XI. (Pernambuco*et al*,. 1993).

In patients with liver cirrhosis, most coagulation factors and inhibitors of the coagulation and fibrinolytic systems are markedly reduced because of impaired protein synthesis, except for factor VIII and fibrinogen levels, which may be normal or increased. Possible explanations for the increased factor VIII levels are the increased hepatic biosynthesis of vWF and decreased expression of low-density lipoprotein receptor related protein, both of which modulate the level of factor VIII in plasma, rather than increase factor VIII synthesis. Because fibrinogen is an acutephasereactant, its synthesis tends to bepreserved in patients with stablecirrhosis. (Sanjo*et al*., 2003).

Patients with advanced hepatic failure may present with the entire spectrum of coagulation factor deficiencies. (Heathcoat, 2000). The levels of coagulation activity markers in patients with chronic liver disease were significantly different in comparison to those in healthy (Sheikh Sajjadieh*et al*., 2008). Liver cirrhosis causes significant morbidity and mortality, (Albornoz*et al*., 1999). However early diagnosis prevents complications and carries good prognosis. Estimation of fibrinogen level may be helpful in preventing bleeding tendencies. (Mackie *et al*., 2003).Many of these hepatic functions may be assessed by laboratory procedures to gain insight into the integrity of the liver. (Zimmerman, 1999)

1.3 Rational:

Liver disease are considered as major causes of health problem in Sudanese patients and might cause many complications of high morbidity and mortality including bleeding and thrombosis because the liver disease has vital role in synthesis of coagulation proteins and hemostatic regulatory agent hence liver disease generally lead to gross change in normal coagulation mechanism.

1.4 Objectives:

1.4.1 General objective:

To measure Hemostatic profile in liver disease among patients in Khartoum state.

1.4.2 Specific objective:

-To determine PT, APTT activity and calculate INR in liver disease patients attending Omdurman teaching hospital and compered them with control group.

-To compare between PT, APTT and INR with age, gender and duration of the disease among study population.

-To compare result of PT, APTT and INR between different type of liver disease.

Chapter Two

2-Material and methods

2.1 Study Design:

This is a case control study was conducted in Omdurman teaching hospital during the period from April to June 2014 to measureProthrombin time(PT) Partial Thromboplastin Time(APTT)and The INR in patients with liver disease in Khartoum state.

2.2 Study Population:

Liver disease patients attending Omdurman Teaching Hospital, 50 patients who were diagnosed as liver disease patients as case groupand 50 apparently healthy individuals as control group.

2.3 Inclusion criteria:

All liver disease patients attending Omdurman teaching hospital, who are not on anticoagulant treatment or with no other medical condition affecting in results were included in the study.

2.4 Exclusion criteria:

Patients with no liver disease, who on anticoagulant treatment, presence of other coagulation disorders were excluded from the study.

2.5 Data collection:

Data were collected using self-administered pre-coded questionnaire which was specifically designed to obtain information including age, sex, duration of the disease and other information.

2.6 Sample collection:

Five ml of venous blood were collected in plastic container containing Tri sodium citrate as anticoagulant in ratio 1:9,and then the blood is centrifuged after thoroughly mixing for 15 minutes at 3000rpm to obtain platelet poor plasma (PPP).

2.7 Ethical consideration:

The consent of the selected individuals to the study was taken after being informed with all detailed objectives of the study and its health benefit future.

2.8 Data analysis:

The data were analyzed by independent t test,one way a nova test and correlation using the SPSS computer programmed version 11.5. The data were presented in tables and figures.

2.9 Methods:

2.9.1 Prothrombin Time:

2.9.1.1 Principle:

The PT test measure the clotting time of the plasma in the present of optimal concentration of tissue extract (Thromboplastin)and indicate the overall efficiency of the extrinsic pathway.Although originally thought to measure prothrombin,the test is now known to depend also on reaction with factor V,Vll and X and of the fibrinogen concentration of the plasma (Lewis *et al*., 2006).

2.9.1.2Reagent:

Thromboplastin were originally tissue extracts obtained from different species and different organs containing tissue factor and phospholipid.The majority of animal thromboplastin now in use is extracts of rabbit brain or lung.

Cacl2:0.025mol/L.

2.9.2Activated partial thromboplastin time:

2.9.2.1 Principle:

The test measure the clotting time of plasma after the activation of contact factors but without added tissue thromboplastin and so indicator the overall efficiency of intrinsic pathway. The test depends not only on the contact factors and on factor VIII and IX but also on the reaction with factor X, V, prothrombin, and fibrinogen. It is also sensitive to the presence of circulating anticoagulants (inhibitors) and heparin (Lewis et al., 2006).

2.9.2.2Reagents:

Kaolin 5g/l (laboratory grade)in barbitone buffer saline,PH 7.4. A few glass beads were added to aid resuspension. The suspension is stable at room temperature. Other insoluble surface active substance such as silica,celite,or ellagic acid can also be used.

Cephalin as phospholipids substitution.

CaCl:0.025mol/l

2.9.3 The coagulometer:

The automatic coagulometer (clot) is an instrument for the determination of the main parameters used in the plasma coagulation methods:

2.9.3.1Theory and principle:

The coagulometer has an optical measurement system which detects asudden variation in optical density when aclot is formed. The chronometer and the stirring system are activated by sudden change of the optical density.This permitsthe 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 aperfect homogenization and make the measurement possible of low concentration of fibrinogen by grouping the fibrin filament in the center of the optical pass.

2.9.3.2Procedure:

-First of all,the cuvette was placed and a magnetic stirrer was inserted in every cuvette and waited for instrument to reach 37˚c.

-After that into the cuvette the reagent or sample volumes required were introduced.

-PT 200µl of reagent

-APTT 100µl of reagent+100µl of plasma then incubated for 3-5min.

-When the incubation time is finished,the cuvettewas placed on the reading well the chronometer was remained inactive for some second and then it was showed 000:0. At this moment 100µl of the starter was added:

-PT100µl of plasma was added

-APTT 100µl of CaCl was added

-When the reagent and the plasma were in contact an O.D variation wasproduced,that automatically activated the digital chronometer and the magnetic mixer, when the clot was start to formed,an O.D variation was produced and stopped the chronometer and the mixer. The clotting time appeared on the display.

Normal range:

PT: 11 -16 sec

APTT:30-40 sec (Lewis *et al*., 2006).

Q.C:

MAINTENANCE MANUAL															
LABORATORY						**INSTRUMENT: *CLOT***								**Nº:**	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DAILY CONTROL															
Read a control.															
MONTHLY CONTROL															
Read a high control.															
Read a low control.															
ANNUAL CONTROL															
Clean the optical group.															
Read a high control.															
Read a low control.															
Operator															
TAS															
NOTES															

Chapter three

3. Results

This is study was conducted in Omdurman teaching hospital, blood sample were collected from fifty patients with liver disease as case group and other fifty samples were collected from apparently healthy individuals as control group. The data analyzed statistically using computer program and the results presented in tables and figure as follow:

Figure (3.1) shows the frequency of case and control among study group. Fifty samples from liver disease patients as case group and fifty samples from healthy apparently individual as control group.

Figure (3.2) shows the distribution of the study population according to gender, thirty male and twenty female in case and control group.

Figure (3.3) shows the mean of age in case group (43years) and control group (35years) of study population.

The mean values of PTin case group (16sec±4.6) and control group (12.4sec±0.7), APTTin case group (39sec±7.5) in control group (31sec±1.7) and INR in case (1.4±0.4) in control group (1.0±0.7). Table (3.1).

The frequency and percentage of different type of liver disease. ShowsHBV were 26 (52%), obstructive jaundice were 6 (12%), liver cirrhosis were 7 (14%), hepatocellular carcinoma were 2(4%), chronic Liver disease were 5(10%), hepatic encephalopathy were 2(4%), HAV were 2(4%), as in Table (3.2)

Comparison of mean of PT,APTT and INR in case and control group in Table (3.3) shows the mean ofPT, APTT and INR (16.0sec±4.6),(39.9sec±7.5) and (1.41±0.4) respectively in case group and (12.4sec±0.7) (31.3sec±1.7) and (1.08±0.07) respectivelyin control group (P value .000).

According to age groups, the mean ofPT (13.6sec±2.3) in age group<35 years, and (15.1sec±5.0) inAge group>35years(P.value0.090). The mean of APTTwas (33.6sec±4.9) in age group<35 years and (38.5sec±8.3)inAge group>35years

(P.value0.064). The mean of INR (1.1±0.2) in age group<35, and (1.3±0.4)inAge group>35years (P.value 0.094).(Table 3.4).

Table (3.5) shows the mean of PT (13.8sec±2.1) in male group and (14.8± 5.3) in female group (P.value 0.167).The mean APTT(35.7±7.2) in male group, and (35.5± 6.5)in female group (P.value 0.871).The mean of INR (1.20±0.2) in male group, and (1.31± 0.9)in female group (P.value 0.156).

Table (3.6) shows the mean values of PT, APTTand INR among case group according to different type of liver disease. In Hepatitis B Virusthe mean of PT (15.4sec±3.0), APTT (38.2sec±6.6) and INR (1.3±0.27).Obstructive jaundice the mean of PT (13.7sec±1.6) APTT (35.8sec±2.7) INR (1.2±0.2).Liver Cirrhosis the mean of PT (15.7sec± 2.2) APTT (45.2sec±7.9) INR (1.3±0.2).Hepatocellular Carcinomathe mean of PT (16.8sec±1.6) APTT (51sec±2.8) INR (1.3±0.2).Chronic liver Disease the mean of PT (14.4sec±1.4) APTT (39.3sec±8.3) INR (1.2±0.1).Hepatic Encephalopathy the mean of PT (33.1±9.8) APTT (51.9sec±9.8) INR (3.0±0.9).Hepatitis A Virusthe mean of PT (18.9sec±1.9) APTT (35.5sec±4.5) INR (1.6±0.1).

Table (3.7) shows a significant difference in PT, APTT and INR results according to different liver disease in which there was increase in PT, APTT and INR results in H.E when compared with (HBV,Obstructive jaundice,Liver Cirrhosis,Hepatocellular Carcinoma, Chronic liver Diseaseand HAV) also there was increase in APTT inHepatocellular Carcinomawhen compared with (Obstructive jaundice,Chronic liver Disease and HAV) and Liver Cirrhosiswhen compared with (HBV,Obstructive jaundice) (P.value .000). One way ANOVA test is used for comparisons.

Figure (3.4) Scatter plot shows insignificant weak negative correlation between duration of liver disease and PT/sec (r 0.024 P.value0.773).

Figure (3.5) Scatter plot shows insignificant weak positive correlation between duration of liver disease and APTT/sec (r 0.151 P.value0.295).

Figure (3.6) Scatter plot shows insignificant weak negative correlation between duration of liver disease and INR (r0.04 P.value0.761).

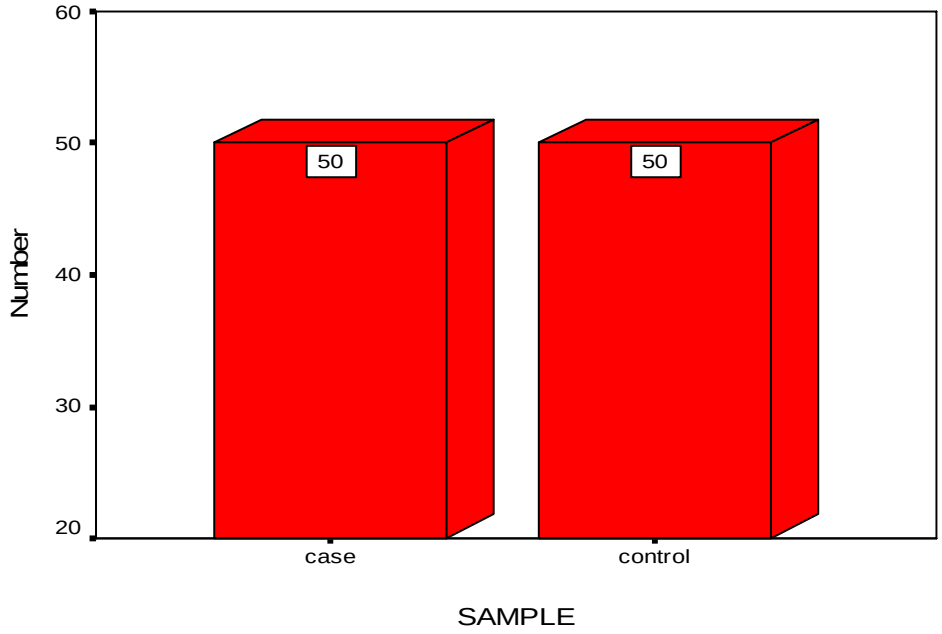

Figure (3.1) Frequency of case and control among study group:

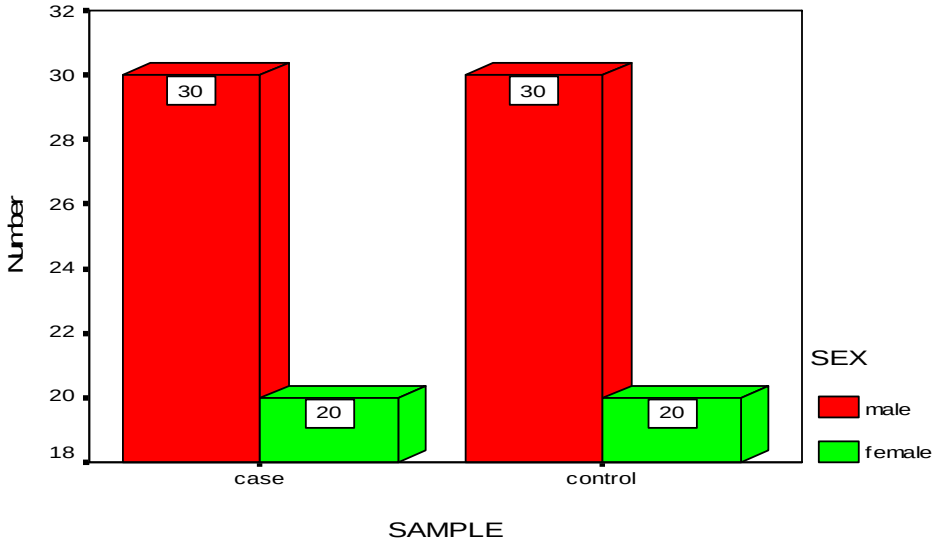

Figure (3.2) Distribution of study population according to gender:

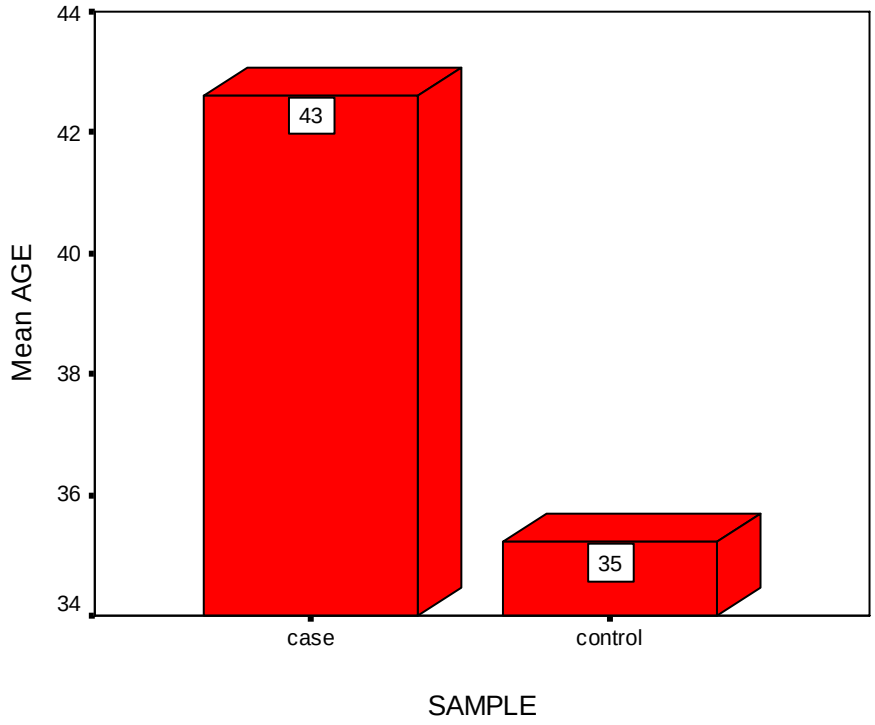

Figure (3.3) the Mean of age among case and control group:

Table (3.1)The mean values of PT, APTT and INR in case and control group:

Demographic data	Sample	Mean	SD	Minimum	Maximum
PT/sec	Control	12.4	0.7	10.8	14.8
	Case	16	4.6	11.9	40.1
PTT/sec	Control	31	1.7	27	35
	Case	39	7.5	28.6	62.0
INR	Control	1.0	.07	0.93	1.3
	Case	1.4	0.4	1.03	3.7

Table (3.2) Frequency and percentage of case group according to different type of liver disease:

Parameters	Frequency	Percentage
Hepatitis B Virus	26	52
Obstructive jaundice	6	12
Liver Cirrhosis	7	14
Hepatocellular Carcinoma	2	4
Chronic liver Disease	5	10
Hepatic Encephalopathy	2	4
Hepatitis A Virus	2	4
	50	100

Table (3.3) Comparison of mean of PT/sec, PTT/sec, INR in case and control group:

Parameter	Sample	NO	Mean	SD	P.value
PT/sec	Case	50	16.0	4.6	.000
	Control	50	12.4	0.7	
PTT/sec	Case	50	39.9	7.5	.000
	Control	50	31.3	1.7	
INR	Case	50	1.41	0.4	.000
	Control	50	1.08	0.07	

Table(3.4) Comparison of meanPT/sec, PTT/sec, INR in case group according to age group:

Parameter	Age/years	Frequency	Mean	SD	P.value
PT/sec	<35	59	13.6	2.3	0.090
	>35	41	15.1	5.0	
PTT/sec	<35	59	33.6	4.9	0.064
	>35	41	38.5	8.3	
INR	<35	59	1.1	0.2	0.094
	>35	41	1.3	0.4	

Table(3.5) Comparison of meanPT/sec,PTT/sec, INR in male and female group:

Parameter	Sample	NO	Mean	SD	P.value
PT/sec	Male	60	13.8	2.1	0.167
	Female	40	14.8	5.3	
PTT/sec	Male	60	35.7	7.2	0.871
	Female	40	35.5	6.5	
INR	Male	60	1.20	0.2	0.156

Table (3.6) The Mean values of PT/sec, APTT/sec and INR among case group according to different types of liver disease:

Disease	Parameter	mean	SD	Minimum	Maximum
Hepatitis B Virus	PT/sec	15.4	3.0	11.9	26.5
	PTT/sec	38.2	6.6	28.6	52.3
	INR	1.35	0.27	1.03	2.38
Obstructive jaundice	PT/sec	13.7	1.6	12.3	18
	PTT/sec	35.8	2.7	32	38
	INR	1.25	0.21	1.08	1.64
Liver Cirrhosis	PT/sec	15.7	2.2	12.0	18.6
	PTT/sec	45.2	7.9	36.4	62
	INR	1.37	0.2	1.04	1.64
Hepatocellular Carcinoma	PT/sec	16.8	1.6	15.6	18
	PTT/sec	51	2.8	49	53
	INR	1.38	0.02	1.36	1.40
Chronic liver Disease	PT/sec	14.4	1.4	13	16.8
	PTT/sec	39.3	8.3	30	52
	INR	1.26	0.1	1.13	1.49
Hepatic Encephalopathy	PT/sec	33.1	9.8	26.2	40.1
	PTT/sec	51.9	2.0	50.5	53.4
	INR	3.0	0.9	2.38	3.70
Hepatitis A Virus	PT/sec	18.9	1.9	17.5	20.3
	PTT/sec	35.5	4.5	32.3	38.8
	INR	1.69	0.1	1.56	1.82

Table (3.7)Comparisonof mean PT/sec,PTT/sec and INR resultsaccording to different type of liver disease:

Parameter	Liver Disease	Comparison with other Disease	Mean Different	P.value
PT/sec	Hepatic Encephalopathy	HBV	17.6*	.000
		OJ	19.4*	.000
		LC	17.4*	.000
		HCC	16.3*	.000
		CLD	18.7*	.000
		HAV	14.2*	.000
APTT/sec	Hepatic Encephalopathy	HBV	13.7*	.006
		OJ	16.1*	.004
		CLD	12.6*	.025
		HAV	16.4*	.016
	Hepatocellular Carcinoma	HBV	12.7*	.011
		OJ	15.1*	.007
		CLD	11.7*	.038
		HAV	15.1*	.022
	Liver Cirrhosis	HBV	7.0*	.015
		OJ	9.4*	.013
INR	Hepatic Encephalopathy	HBV	1.6*	.000
		OJ	1.7*	.000
		LC	1.6*	.000
		HCC	1.6*	.000
		CLD	1.7*	.000
		HAV	1.3*	.000

(*)The mean difference is significant at the .05 level

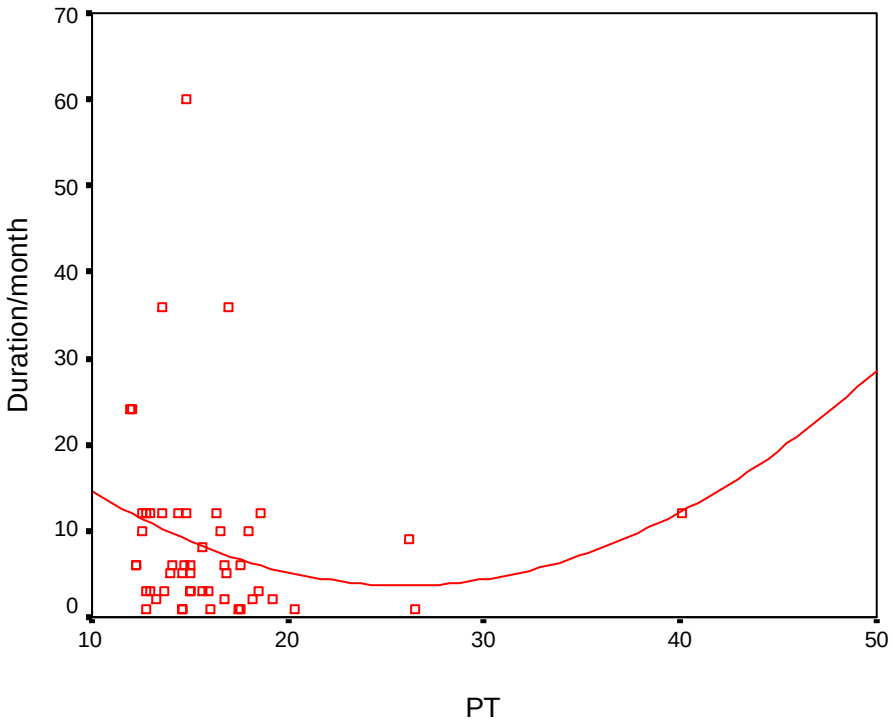

Figure (3.4) Correlation between the duration of the disease and PT/sec among case group:

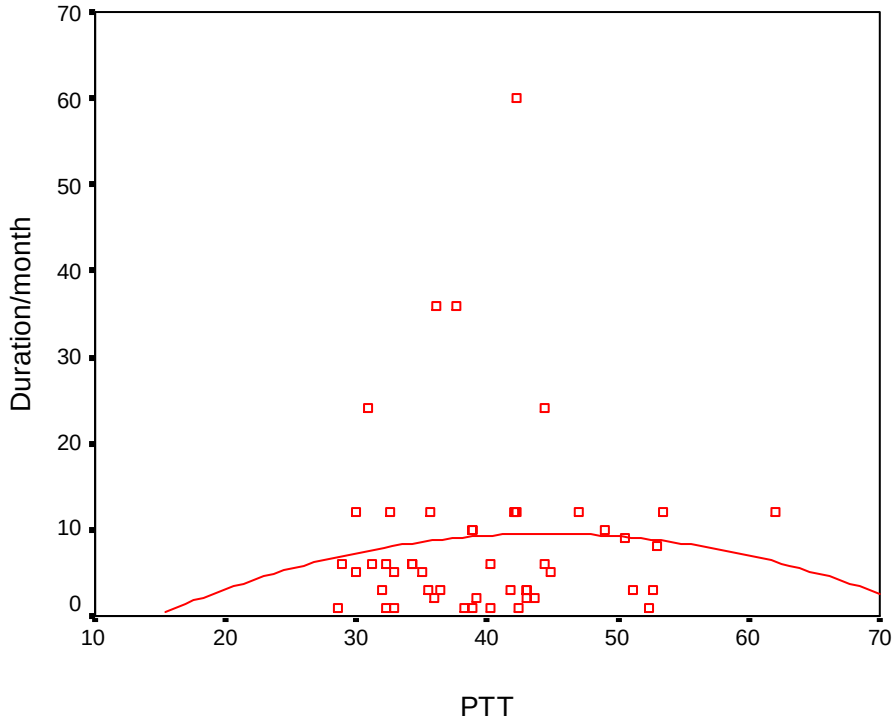

Figure (3.5) Correlation between the duration of the disease and APTT/sec among case group:

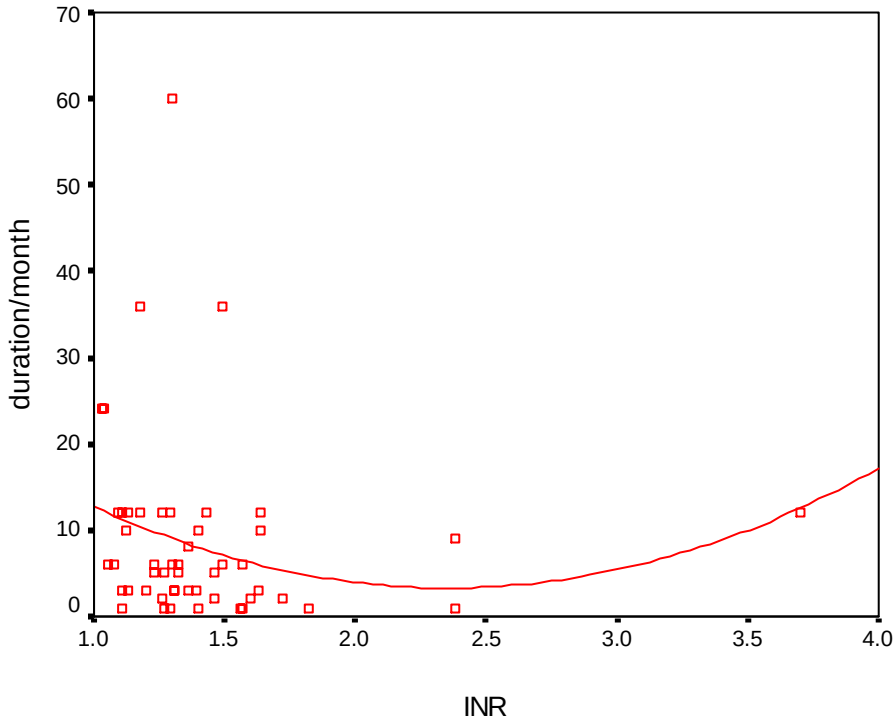

Figure (3.6) Correlation between the duration of the disease and INR among case group:

Chapter Four

Discussion, Conclusion andRecommendation

4.1 Discussion

This is Case control study conducted in Omdurman Teaching Hospital during the period from April to June 2014 to measureProthrombintime(PT) Partial Thromboplastin Time(APTT)and The INR of Sudan's patients with liver disease. Fifty citrated venous blood were collected from liver diseases patients according to inclusion criteria and consider as case and other fifty samples were collected from apparently healthy individuals as control group. The study revealed the following:

The mean of PT/sec, APTT and INRof study group significantly increased when compared with control group (P. value .000).The finding was consistent with a study in Sudanof (Mahmoud Mohamed Elgari, 2013) which revealed that there was a significant increase in PT (P. value 0.00), and APTT (P.value 0.00).

The present study revealed that there was a significant difference in PT/sec, APTT/sec and INR results according to different liver diseases in which there was an increase in PT/sec, APTT/sec and INR results inHepatic Encephalopathywhen compared to other type of liver disease, also there was increase in APTT/sec inHepatocellular Carcinoma andLiver Cirrhosis when compared with other liver disease, the finding is consisted with study of (Shah shaila, TruptiJansari, 2014), in which there study concluded that the coagulation abnormalities were significantly associated with the extend of the liver disease.

The study explained that there was no any effect of gender and age on PT,APTT and INR results among the study group, which consistent with thestudy in Sudan of (Mahmoud Mohamed Elgari, 2013).This revealed that there were no significant change in coagulation parameters related to age and gender.

According to duration there was no any significant difference in mean level of PT,APTT and INR results among the study group.

4.2 Conclusion:

After completion of the study the results concluded that:

- There was significantly increasing in PT/sec, APTT/sec and INR results in patients with liver diseases in compare with control.
- The study revealed that a significant difference in PT/sec, APTT/sec and INR results according to different liver disease.
- There were no significant changes in coagulation parameters related to age and gender.
- According to duration of the disease there was no any significant difference in mean level of PT/sec, APTT/sec and INR results among the study group.

4.3 Recommendations:

After completion of this study,the following were recommended:

1. Sample size should be increased.
2. Other clear cut tests are requested as indicator for precise final diagnosis.
3. Coagulation tests should be a routine tests and it could be done regularly for patients with bleedingtendency.

REFERENCES:

Albornoz L.;Bandi JC.andOtaso JC. (1999). Prolonged bleeding time in experimental cirrhosis: role of nitric oxide. *J Hepatol* 30(3):456-460.

Czaja A, Frcese D.(2002) Diagnosis and treatment of autoimmune hepatitis Hepatology; 36:479.

Heathcote E.(2000) Management of primary biliary cirrhosis. The American Association for the Study of Liver Diseases practice guidelines. Hepatology; 31:1005-13.

HoffbrandP.A.H.Moss, (2010):Essential hematology,6th edition, 315, 318-322,328.

Hoffbrand AV, Catovsky D, Edward G.D(2005): Post graduate hematology, Fifth edition. Blackwell UK, 787-788, 793-798.

Lee WM.(2004). Acute Liver Failure Study Group: lowering the risks of hepatic failure. Hepatology; 40:6-9.

Lewis S.M, Bain B.J and Bates I. (2006) Daice and Lewis Practical Hematology.9th edn.

Mackie IJ, Kitchen S, Machin SJ,(2003) Guidelines on fibrinogen assays. British Journal of Haematology; 121:396-404

Mammen EF (1994) Coagulation defects in liver disease. Med Clin North Am 78(3): 545–554.

Marliesledfard-Kraemer, (2006), Hemostasis principle, 11thedition.5:8:14.

Michael andNathanson**,**(2006), Hepatology, USA, 44:1039-1046.

Miracles and Deadly Killers(2009), Cancer and clotting, *Journal of Advancing Cancer Education*.

Monica(2000)cheesbrough:District laboratory practice in tropical countries,low price edition,united kingdom,.

Peltz S (1991) Sever thrombocytopenia secondary to alcohol use Postgraduate 89(6): 75–76, 85.

Pernambuco JR, Langley PG, Hughes RD et al. (1993) Activation of the fibrinolytic system in patients with fulminant liver failure.Hepatology 18(6): 1350–1356.

Quick AJ: Quick on "Quick agglutination venostasis" bleeding time technique. Journal of Laboratory and Clinical Medicine 1973; 26:1812.

SaateaArif, A Sattar Khan, and Arif Raze Khan, (1999) University of Peshawar and Police Services Hospital, Peshawar, Pakistan.

Sanjo A, SatoiJ, Ohnishi A et al. (2003) Role of elevated platelet associated immunoglobulin G and hypersplenism in thrombocytopenia of chronic liver diseases. J GastroenterolHepatol 18(6):638–644.

Sheikh Sajjadieh, Mohammad Reza(2008) Department of Clinical Laboratory Diagnosis, National Medical Academy for Post-graduate Education, 9 Dorogozhytska ST, Kiev, Ukraine

Sir john et al.,(1995)Practicalhematology, eighth edition, New York USA,(310)

Tripodi A, Salerno F,ChantarangkulV, Clerici M, Cazzaniga M, Marigrazia C, et al., (2005) Evidence of normal thrombin generation in cirrhosis. abnormal conventional coagulation tests.HEPATOLOGY;41:553-558.

WillamKernw. (2002):PDQ Hematology,1st edition.USA., 384-398.

Zimmerman H(1999)hepatotoxicology: The adverse effects of drugs and other chemicals on the liver, 2nd ed. Pihiiadeiphia: JB Lippincott,.

Appendix1:

Sudan University of science and technology

College of Graduate studies

Questionnaire

Name: ……

Age: ……

Gender: ……

History of the Diseaes: ……

Duration of the disease: ……

Drugs:……
……

Others:……
……

Result:

PT:……

APTT:……

INR:……

Appendix2:

Clot

ابراءذمه

اناطالبهماجستير:

رزانحسنمحمدمدثر

المنتسبهالىجامعهالسودانللعلومو التكنولوجيا
.احتاجمنكمعيناتمنالدمالوريدىلاجراءاختباراتالسيولهعندالمرضىالذينيعانونمنامراضالكبدو التناحتاجهالاتمامبحثنا لتكميلى

واللهالموفق

التوقيع:

.....