.Chapter One
Introduction and literature review

1.1 Introduction

In chemistry, analcohol is anorganic compound in which the hydroxyl functional group (-OH) is bound to acarbon atom. In particular, this carbon atom center should be saturated, having single bonds to three atoms (IUPAC). An important class of alcohols is the simple acyclic alcohols, the general formula for which is $C_nH_{2n+1}OH$. Of these ethanol (C_2H_5OH) is alcohol found in alcoholic beverages; in common speech the word alcohol refers to ethanol. Other alcohols are usually described with aclarifying adjective, as in isopropyl alcohol or wood alcohol (methyl alcohol, or methanol). The suffix –OH appears in the IUPAC chemical name of substrates where the hydroxyl group is functional group which the highest priority; in substrates where ahigher priority group is present the prefix hydroxyl- will appear in IUPAC name. In everyday the "alcohol" without classification usually refers to ethanol, or abeverages based on ethanol (as in term "alcohol abuse"). Alcoholic beverages have been consumed by humans since prehistoric times for avariety of hygienic, dietary, medicinal, religious, and recreational reasons. Primary alcohols ($R\text{-}CO_2\text{-}OH$) can be oxidized either to aldehydes or to carboxylic acid, while oxidation of secondary alcohol ($R_1R_2CH\text{-}OH$) normally terminates at the ketones stage(Fowkes et al., 2012). Ethanol is obtained by fermentation using glucose produced from sugar from hydrolysis of starch, in the presence of yeast and temperatures of less than 37^0C to produce ethanol. For instance, such aprocess might proceed by the conversion of sucrose by the enzyme invertase into glucose and fructose, then the conversion of glucose by the enzyme zymase into ethanol (and carbon dioxide). Several of the benign bacteria in the intestine use fermentation as form of anaerobic metabolism. This metabolic reaction produces ethanol as awaste product, just like aerobic respiration produces

carbon dioxide and water. Thus, human bodies contain some quantity of alcohol endogenously produced by bacteria(Geertinger et al.,1982). Alcoholic beverages are divided into three general classes: bears, wines, and spirit (or distilled beverage). They are legally consumed in most countries, and over 100 countries have laws regulating their production, sales, and consumption(Hall, 1995) Alcoholism represents one of the most serious worldwide socioeconomic and health problems. An alcoholic is a person who consumes an amount of alcohol capable of producing pathological changes(Criteria committee, 1972) The amount of alcohol capable of producing disease depends on a variety of factors. Including genetic predisposition (Bailey *et al*., 1976), malnutrition (Mendenhall, 1984), and concomitant viral infections of the liver (Oduola *et al*,.2005).

Locally, there are two types of alcohol marisa and aragi which are prepared by fermentation of seed and date respectively.

The normal haemostatic response to vasculor damage depends on a closely linked interation between the blood vesselwall, circulating platelets and blood coagulation factors. An efficient and rapid mechanism for stopping bleeding from sites of blood vessel injury is clearly essntial for survival. The haemostatic system thus represents a delicate balance between procoagulant and anticogulant mechanisms allied to a process for fibrinolysis(Hoffbrand *et al*.,2011). The maintenance of circulatory hemostasis is achieved through the process of balancing bleeding (hemorrhage) and clotting (thrombosis). Functionally, several processes are involved in hemostasis following injury to a small blood vessel:

1-Blood vessel spasm.

2-Formation of a platelet plug.

3-Contact among damaged blood vessel, blood platlet, and coagulation proteins.

4-Development a blood clot around the injury.

5-Fibrinolytic removal of excess hemostatic material to reestablish vascular injury(Turgeon, 2012). The haemostatic mechanisms have several important functions:

(a)To maintain blood in a fluid state while it remains circulating within vascular system.

(b)To arrest bleeding at the site of injury or blood loss by formation of a haemostatic plug.

(c)To ensure the eventual removal of the plug when healing is complete (Dacie *et al*., 2006).

Hemostasis is a balanced process that halts bleeding after blood vessels have been traumatized, a five major components involved in maintaining hemostasis: vascular integrity, platelet function, fibrin formation(coagulation), control (inhibitory) systems, and lysis (breakdown) of fibrin clots (fibrin lysis) (Rodak, 1995).

1.2 Literature review

1.2.1 Components of normal hemostasis:

The hemostatic system consists of blood vessels, platelets, and the plasma coagulation system including the fibrinolytic factors and their inhibitors. When blood vessel is injured, three mechanisms operate locally at the site of injury to control bleeding. These are vessel wall contraction, platelet adhesion and aggregation (platelets plug formation), and plasmatic coagulation to form afibrin clot. All three mechanisms are essential for normal hemostasis. Abnormal bleeding usually results from defects in one or more of three mechanisms. For better understanding of the pathogenesis of pathological bleeding, it is customary to divide hemostasis into two stages (i.e., primary and secondary hemostasis). Primary hemostasis is term used for the instantaneous plug formation upon injury of the vessel wall, which is achieved by vasoconstriction, platelet adhesion, and aggregation. The fibrin formation is not required for hemostasis at this stage.

Primary hemostasis is, however, only temporarily effective. Hemorrhage may start again unless the secondary hemostasis reinforces the platelet plug by formation of astable fibrin clot. Finally, mechanisms within the fibrinolytic system lead to dissolution of the fibrin clot and to arestoration of normal blood flow(Hoffbrad *et al.*, 2005). The maintenance of circulating hemostasis is achieved through the process of balancing bleeding (hemorrhage) and clotting (thrombosis). Hemostasis, the arresting of bleeding, depends on several components. The four major components are vascular system, platelets (thrombocytes), blood coagulation factors, and fibrinolysis and ultimate tissue repair(Turgeon,2012).

1.2.1.1 The vascular system:

The vascular system prevents bleeding through vessel contraction, diversion of blood flow from damaged vessels, initiation of contact activation of platelets with aggregation, and contact activation of the coagulation system (Harmening, 1997).

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 intima is the innermost layer and the surface is convered with asingle layer of the endothelium, which rest on abasement membrane of subendothelial microfibrils that are composed of collagen fibers and some elastin. The media contains mainly circularly arranged smooth muscle cells and collagenous fibrils, and is divided from the adventitia by the elastic lamina. The muscle cells contract and relax, whereas the elastin allows vessels to stretch and recoil. The adventitia is composed of collagen fibers and fibroblasts that protect the blood vessel and anchor it to surrounding structures(Hoffbrand *et al* .,2005).

1.2.1.2 Platelets :

Platelets are anuclear cells released from megakaryocytes in the bone marrow. Their lifespan in the peripheral blood is approximately 9 days. The average platelet count in peripheral blood ranges from 150.000 to 400.000 per microliters. The exterior coat of platelets is comprised of several glycoproteins, including integrins and leucine-rich glycoproteins. They mediate platelet adhesion and aggregation as receptors for agonists such as adenosine diphosphate (ADP), archidonic acid, and other molecules. Electron microscopic examination shows the presence of many cytoplasmatic bodies such as alpha-granules and dense bodies. Alpha-granules are

storage site of beta-thromboglobin , platelet factor (PF)4 , platelet – derived growth factor (PDGF) , vWF , fibrinogen , factor v , PAI – 1 , and thrombospondin . Dense bodies contain ADP, ATP, calcium, and serotonin. Platelets also contain actin filaments and acircumferential band of microtubules, which are involved in maintaining the shape of platelets. The open canicular system has its role in the exchange of substances from the plasma to the platelets and vice versa. Whereas normal platelets circulate in the blood and do not adhere to normal vasculature, activation of platelet causes a number of changes resulting in promotion of hemostasis by two major mechanisms. These mechanisms are formation of the hemostatic plug at the site of injury (primary hemostasis) and provision of phospholipids as a procoagulant surface for plasmatic coagulation (Reinhold *et al*., 2007). Platelets are extremely small and discoid with a mean volume of 7-11 fl. The glycoproteins of the surface coat are particularly important in 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). Glycoprotein Ib (defective in Bernard-Soulier syndrome) and IIb/IIIa (defective in Ganzmans thrombasthenia) are important in the attachment of platelets to von Willebrand factor and hence to vascular subendothelium. The binding site for IIb/IIIa is also the receptor for fibrinogen which is important in platelet-platelet aggregation. The membrane phospholipids are of particular importance in the conversion of factor X to Xa and prothrombin to thrombin (Turgeon,2012). Alcohol has anti-aggregatory effect on platelet suggesting the beneficial effect of alcohol in preventing coronary heart diseasebut when consumed moderately. It was also found that alcohol decreases both platelet aggregation and the circulating fibrinogen level (Renaud *et al*.,1992) Haut and Cowan, in 1974, were among the first to report the effect of alcohol on platelet activity. They demonstrated that alcohol added to human platelets in vitro , injested

by subjects , or infused intravenously into men markedly decreased platelet aggregation in response to thrombin , collagen , epinephrine , and adenosine- 5 – diphosphate (ADP) (Haut *et al*.,1974) . Subsequent epidemiologic and clinical studies confirmed these results and showed that an increase in ethanol intake leads to a decrease in platelet aggregation in response to ADP and collagen (Pikaar *et al*.,1987).

1.2.1.3 Coagulation :

Coagulation is a complex network of interactions involving vessels, platelets, and factors. The ability to form and to remove a clot is a truly a system dependent on synergistic forces. Coagulation is divided into two major systems; the primary and secondary systems of hemostasis. The primary system comprises platelet function and vasoconstriction. The secondary system involves coagulation proteins and series of enzymatic reactions. Once the coagulation proteins become involved, fibrin is formed, and this reinforces platelet plug formation until healing is complete. The product coagulation cascade is conversion of soluble fibrinogen into an insoluble fibrin clot. This is accomplished by the action of a powerful coagulant, thrombin. Thrombin is formed by a precursor circulating protein, prothrombin. Dissolution of the platelet plug is achieved by the fibrinolytic process (Ciesla ,2007). Blood coagulation is a series of steps in which plasma zymogens of serine proteases are transformed in active enzymes. These enzymes act to convert their procofactor substances to cofactors, which assemble these proteases on cell surfaces. This assembly increases the local concentration of the reactions. The final event is the formation of thrombin, which converts a soluble protein, fibrinogen, into an insoluble polymer, fibrin, that forms the clot(Corriveau *et al*., 1988).

1.2.1.3.1 Coagulation Factors:

Most of coagulation factors are synthezised in the liver and secreted into the plasma (Corriveau *et al.*,1988). Hepatic cells are the principal site of the synthesis of coagulation factors. However, other cells such as endothelial cells(Harmening, 1997).

1.2.1.3.1.1 Factor I ,Fibrinogen:

Substance for thrombin and precursor of fibrin, it is a large globin protein (340.000 D) produced by the liver. Its function is to be converted into an insoluble protein and then back to soluble components. When exposed to thrombin, two peptides split from fibrinogen molecule, leaving a fibrin monomer to form a polymerized clot (Ciesla , 2007).

1.2.1.3.1.2 Factor II (Prothrombin):

Prothrombin is a stable protein (630.000 D). In the presence of ionized calcium, prothrombin is converted to thrombin by the enzymatic action of thromboplastin form both extrinsic and intrinsic sources. Prothrombin has half life of a most 3 days with 70 per cent consumption during clotting(Turgeon, 2012).

1.2.1.3.1.3 Factor III:

Originally assigned to tissue thromboplastin, factor III is now referred to as tissue factor. A 45.000 – D transmembrane lipoprotein, tissue factor is a cofactor found in all tissues but the highest concentrations are found in the brain, liver, lung, and placenta. Normally, no tissue factor is found in plasma. It is released from traumatized tissue and carried into the extravascular spaces by escaping blood,

where it serves as a cofactor for activation of factor VII in extrinsic coagulation because extrinsic activation of coagulation is depended upon tissue factor(Rodak,1995).

1.2.1.3.1.4 Factor IV:

This name was originally given to calcium; however, the chemical designation for ionized calcium is used instead of the Roman numeral IV. Calcium has molecular weight of 40.000 – D. Ionized calcium is the mediator of platelet activation and binds vitamin k – dependent factors to phospholipid surface (factor III and platelet factor 3 (PF3)). Without calcium ions, vitamin k – dependent factors will not anchor to thrombogenic phospholipids and clotting would be prevented. Chelating agents such as citrate, oxalate, and EDTA are used as anticoagulants to remove calcium from the blood to prevent clotting(Rodak, 1995).

1.2.1.3.1.5 Factor V (Proaccelerin):

Factor V is an extremely labile globulin protein. It deteriorates rapidly, having a half life of 16 hours. Factor V is consumed in clotting process and is essential to later stages of thromboplastin formation(Turgeon, 2012).

1.2.1.3.1.6 Factor VII (Proconvertin):

Plasma FVII binds to TF, for example after vessel trauma or plaque rupture, to form a complex that initiates coagulation by directly activating FX and to lesser extent FIX. The half life of FVII zymogen in plasma is 3 hours and, exceptionally, the half life of the FVIIa enzyme is 2.5 hours, probably because there is no plasma inhibitor capable of effectively neutralizing free FVIIa (Hoffbrad *et al*., 2005).

1.2.1.3.1.7 Factor VIII – von willebrand Factor complex (VIII : VWF):

This factor consists of one factor VIII portion bound to one multimeric von Willebrand factor polymer. The factor VIII portion is controlled by genes on X chromosome and has molecular weight 260.000 D. Von Willebrand protein is autosomal and controlled by chromosome 12. von Willebrand factor is produced by megakaryocytes and endothelial cells. Factor VIII is produced in several tissues; however, the major production site is hepatic. Factor VIII and vWF are bind together tightly in plasma to form a variable molecular weight complex that weights over 1 million Daltons. The complexing of the two proteins leads stability to the VIII protein. Factor / vWF complex plays two pivotal roles in hemostasis. Initially, platelets adhere to collagen bound vWF by their glycoprotein Ib (GP Ib) receptors after trauma. Subsequently, more vWF in the blood binds to the adherent platelets resulting in platelet aggregates that stop bleeding (Rodak, 1995).

1.2.1.3.1.8 Factor IX (Plasma Thromboplasin Component):

Factor IX is a stable protein that is consumed during clotting or destroyed by ageing at 40 c for 2 weeks. It is an essential component of the intrinsic thrombopastin – generating system, where it influences the amount rather than the rate of thromboplastin formation (Turgeon, 2012).

1.2.1.3.1.9 Factor X (Sturat Factor):

Factor X (molecular weight 58.000 D) is a vitamin k – dependent factor glycoprotein that participates in the middle phase of blood coagulation. Like manyn other blood coagulation proteins, it is synthesized in the liver and secreted into plasma as precursor to a serine protease (Corriveau *et al*., 1987). Factor X can be activated into a serine protease by both factors VIIa and IXa. Factor IXa activation of X requires calcium and factor VIII as a cofactor. Factor VIIa

activation requires calcium and tissue factor as a cofactor. Factor Xa, along with calcium, phospholipid, and modified factor V, form a complex known as prothrombinase (prothrombin activator) complex (Rodak, 1995).

1.2.1.3.1.10 Factor XI (Plasma Thromboplatin Antecedent):

The protein circulates as a homodimer. Activation of FXI is by a single cleavage performed by thrombin; FXIa then activates FIX directly in free solution. FXI activation by contact protein FXIIa was originally thought to be a relevant step in "intrinsic " or contact – activated coagulation , but it is now considered that the feedback activation of FXI by trace thrombin provides a physiological relevant route for generation of increased amounts of FXIa to assemble FX – ase during the amplification of initial TF stimulus (Hoffbrand *et al*., 2005).

1.2.1.3.1.11 Factor XII (Hageman Factor):

Factor XII is a stable factor that is not consumed during coagulation process. Adsorption of factor XII and kininogen (with bound prekallikrein and factor XI) to negatively charge d surfaces such as glass or subendothelium (collagen) exposed by blood vessel injury initiates the intrinsic coagulation pathway. Surface absorption alters and partially activates factor XII to factor XIIa by exposing an active enzyme (protease) site. Because of feedback mechanism, kallikrein (activated Fletcher factor) cleaves partially activated factor XIIa molecules adsorbed onto the subendothelium to produce a more kinetically effective form of XIIa (Turgeon, 2012).

1.2.1.3.1.12 Factor XIII (Fibrin – stabilizing Factor):

Factor XIII is the proenzyme for plasma transglutaminase. In the presence of fibrin, thrombin converts factor XIII to enzyme called factor XIIIa. Because

thrombin and fibrin are generated in the final stage of coagulation process, factor XIII activation is one of the last events in the blood coagulation casecade. Factor XIII circulates in blood association with fibrinogen. It is a heterotetrmer composed of two a subunits and two b subunits. The A and B dimmers are held together by noncovalent bonds. The A subunit contains the catalytic site with transglutaminase activity, whereas B subunit appears to protect or stabilize the A subunit (Corriveau *et al.*, 1988).

1.2.1.3.1.13 Fletcher Factor:

Fletcher factor is also known as prekallikrein (PK). It is a fast gamma globulin with serine protease activity when activated by factor XIIa. Like factor XI, prekallikkrein is attached to HMWK, which acts as a cofactor for its activation by factor XIIa. The active enzyme form, kallikrein, activates more factor XII in a positive feedback loop. Kallikrein activates plasminogen to plasmin initiating the fibrin lysis system and it also hydrolyzes low – molecular weight bradykinins from HMWK to initiate the kinin system. The kinins act as vasodilators and smooth muscle relaxants to reestablish blood flow after serotonin and thromboxane A2 from platelets have cause vessel constriction (Rodak, 1995).

1.2.1.3.1.14 Fitzgerald Factor:

The Fitzgerald factor, also known as HMWK, is glycoprotein produced by the liver. HMWK complexes with factor XI and prekallikrein and is a member of the contact group. It acts as a cofactor to accelerate the activation of factor XI by factor XIIa and acts as a substrate for kallikrein in the production of kinins (Rodak, 1995).

1.2.1.3.2 Coagulation Inhibitors:

When thrombin and other activated clotting factors escape the clot site, they are neutralized by inhibitor proteins. Activated clotting factors attach to the inhibitors because the inhibitors have peptide bonds similar to those of their normal substrate. The active enzyme cannot, however, hydrolyze the inhibitor and is irreversibly complexed with it. Active factor – inhibitor complexes are removed by phagocytosis in the spleen, liver, and other organs (Rodak, 1995).

1.2.1.3.2.1 Blood Flow and Hepatic Degradation of Clotting Factor:

Normal blood flow dilutes the activated clotting factors below the level required to propagate the cascade. Hepatoytes in the liver digest and destroy the activated clotting factors, washed away from the site of clot formation. (William ,2002)

1.2.1.3.2.2 Antithrombin:

Antithrombin (AT; previously called antithrombin III) is the major inhibitor of factor IXa, Xa, and thrombin. Although enough AT – III is present to neutralize three times the total amount of thrombin that could form in the blood, a decrease to 40 per cent to 50 per cent predispose to thrombotic disorders. The fact that congenital AT – III deficiency is associated with a strikingly increased risk of venous thromoboembolism indicates that inhibitors play major regulatory role and that delicate balance exists between the procoagulant and anticoagulant forces (Corriveau *et al.,* 1988).

1.2.1.3.2.3 Protein C and Protein S:

These are inhibitors of coagulation cofactors V and VIII. Thrombin binds to an endothelial cell surface receptor, thrombomodulin. The resulting complex activates the vitamin k – dependent serine protease protein C which is able to destroy

activated factor V and VIII, thus preventing further thrombin generation. The action of protein C enhanced by another vitamin k – dependent protein, S, which binds protein C to the platelet surface (Hoffbrand *et al*.,2011).

1.2.1.3.2.4 Heparin Cofactor II:

This serpin is present in plasma at high concentration. It appears to be a specific inhibitor of thrombin and to have little or no anti – Fxa activity. The role of thrombin neutralization by HCII is increased approximately 1000 – fond by heparin (Hoffbrand *et al.,* 2005).

1.2.2 Coagulation Pathway :

Initiation of clotting begins with either the extrinsic or the intrinsic pathway. Factor X activation is the point of convergence. Factor X can be activated by either of two pathways and subsequently catalyzes the conversion of prothrombin to thrombin (Turgeon, 2012). Although it has been traditional (and useful for in vitro laboratory testing) to divide the coagulation system into intrinsic and extrinsic pathways , such a division does not occur in vivo because tissue factor / factor VIIa complex is a potent activator of factor IX as well as factor X (Corriveau *et al*., 1988). The process of blood coagulation is initiated by the exposure of cells expressing TF to flowing blood. Thrombin generation is propagated by a series of positive feedback loops, leading to fibrin deposition. This process is controlled by a series of negative feedback steps; the initiation complex is inhibited by the formation of the quaternary complex TF – FVIIa – TFPI and the active proteases FIXa, FXa and thrombin are inactivated by the serpin AT (Hoffbrand *et al.*, 2005).

1.2.2.1 The Extrinsic Pathway:

The extrinsic pathway is initiated by entry of tissue thromboplastin into circulating blood. Tissue thromboplastin is derived from phospholipoproteins and organelle membranes from disrupted tissue cells. These membrane lipoproteins, termed tissue factor, are normally extrinsic to the circulation. Platelet phospholipids are not necessary for activation of extrinsic pathway because tissue factor supplies its own phospholipids. Factor VII binds to phospholipids in the tissue cell membrances and is activated to factor VIIa, a portent enzyme capable of activating factor X to Xa in presence of ionized calcium. The activity of tissue factor – factor VII complex seem to be largely dependent on the concentration of tissue thromboplastin. Membrances that enter the circulation also provide a surface for attachment and activation of factor X and V. The final step is the conversion of fibrinogen to fibrin by thrombin (Turgeon, 2012). Alcohol consumption has complex associated with lower levels of plasma viscosity, factor VII, and(Pikaar *et al.*,1987)

1.2.2.2 The intrinsic Pathway:

Parallel with the extrinsic system is the intrinsic system, which could be defined as coagulation initiated by components entirely contained within the vascular system. This pathway results in activation of IX by a novel dimeric serine protease; factor XIa, providing a pathway independent of factor VII for blood coagulation. However, an important difference exists between these two pathways in the clotting cascade. Whereas the activation of factor IX by XIa requires only the presence of ionized calcium, the activation of factor IX by VIIa requires calcium and the protein cofactor, tissue factor, embedded in a cell membrane. The role of the contact system proteins in initiation of the intrinsic pathway of coagulation in

hemostasis is questionable, because only a deficiency of factor XI is associated with a hemorrahagic tendency. The zymogen factor XII is the first protein in the series of tightly regulated reactions and binds to negatively charged surfaces such as kaolin, dextran, sulfate, and sulfatides. Negative feedback regulation is characteristic of coagulation system. Similarly, thrombin activates factors V and VIII (Corriveau *et al*., 1998). These factors interact on the surface to activate factor 1X to 1Xa. Factor 1Xa reacts with factor VIII, PL3, and calcium to activate factor X to Xa. In the presence of factor V, factor Xa activates prothrombin (factor II) to thrombin, which in turn converts fibrinogen to fibrin (Turgeon, 2012). Alcohol consumption has complex associated with lower levels of fibrinogen, plasma viscosity, factor VII, and VWF (Pikaar *et al*.,1987).

1.2.2.3 The Common Pathway:

Once factor X is activated to Xa, the extrinsic and intrinsic pathways enter a common pathway. Factor II, prothrombin, is activated to thrombin (factor IIa) which normally circulates in the blood as an inactive factor. Following the activation of factor Xa, it remains platelet bound and activates factor V. The complex of factors Xa and Va on platelet surface in formed near platelet – bound factor II molecules. In turn, the platelet – bound factor Xa/Va complex cleaves factor II into thrombin. The stage is accelerated by factor V and ionized calcium (Turgeon, 2012).

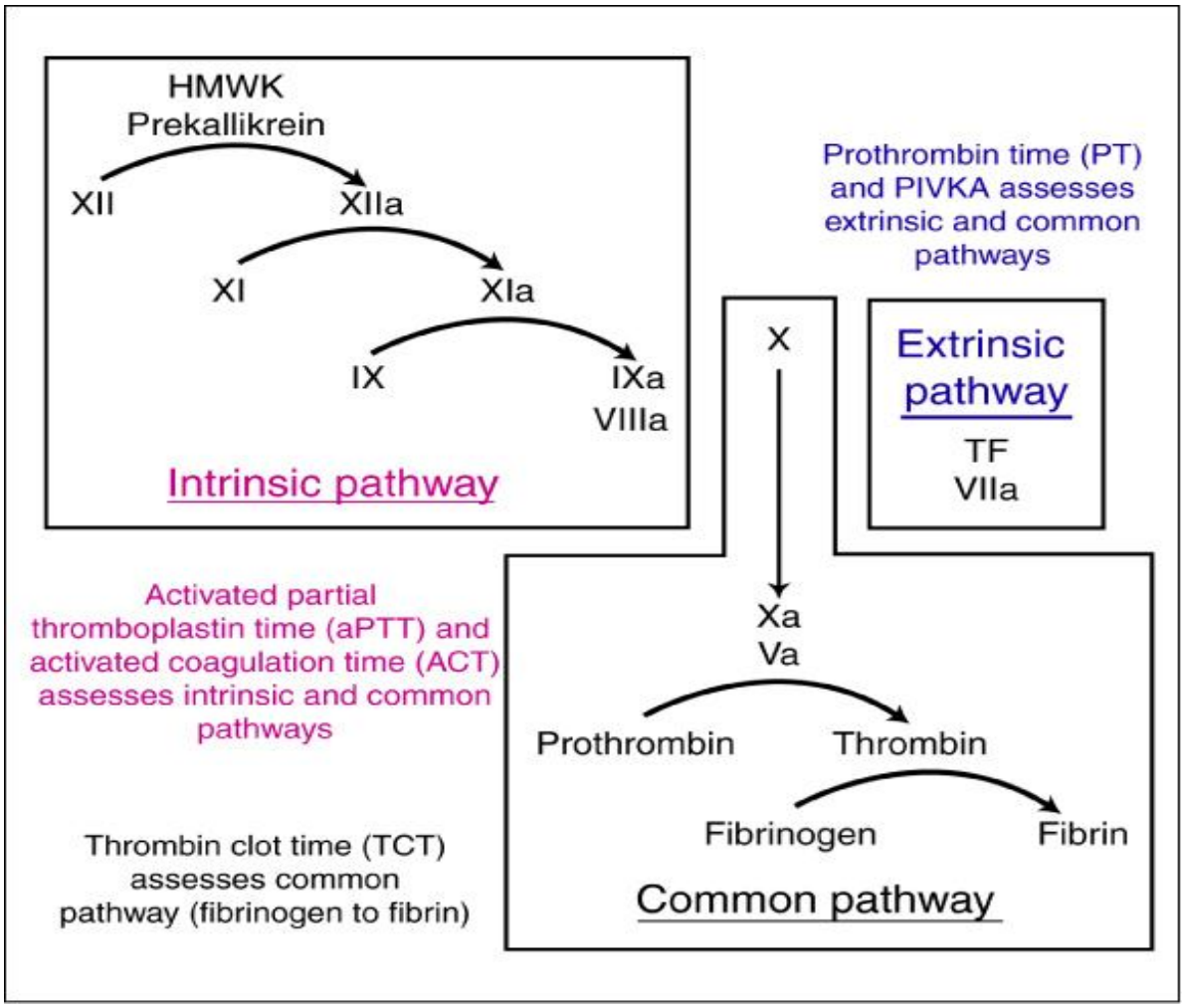

Figure 1.1 Classical coagulation cascade:

1.2.2.4 Fibrinolysis:

Once a clot has served its useful purpose, it becomes a waste product that must be discarded. The fibrin mesh is too large to be phagocytized. The fibrin mesh is systematically degraded by plasmin, the enzyme form of zymogen plasminogen . This is called fibrin lysis system. Blood plasminogen activators are produced when factor XIIa and kallikrein are produced by contact activation. Plasminogen is a complex molecule produced in the liver. It has five loops in its structure that are heavily populated with carbohydrates attached to protein backbone. The major activation of plasminogen to plasmin is mediated by tissue plasminogen activator

(tPA) released from nearby endothelial cells. Interestingly, tPA is initially released when the vessel wall is damaged, however, both activated platelets and injured endothelial cells release plasminogen activator inhibitor 1 (PAI – 1) to neutralize tPA. tPA is strongly attached to the site where plasminogen is attached to crosslinked fibrin. Therefore, activation of plasminogen to plasmin by tPA is localized and generally clot specific. tPA hydrolyzes the serine protease enzyme plasmin from plasminogen. Plasmin degrades the fibrin mesh into the most basic units. First, large degradation products that contain three E domains and three crosslinked D domains are carved away from the clot. Normal amounts of E – and D- dimer degradation products from fibrinolysis are released into the blood, from which they are removed by liver and spleen macrophages. Degradation of fibrinogen, which yields D and E fragments, is not a normally process. Plasmin degrades native fibrinogen into individual D and E domain fragments. These particles are not detectable by plasma D-dimer testing. They can be measured by tests that measure total degradation products such as the serum latex FDP tests or monoclonal tests that measure E fragments. Both types of degradation products, when elevated, interfere with normal coagulation and may produce a bleeding diathesis. Plasmin has the ability, when not controlled by inhibitors, to degrade factors VIII and V as well, which adds to the bleeding syndrome (Rodak, 1995).

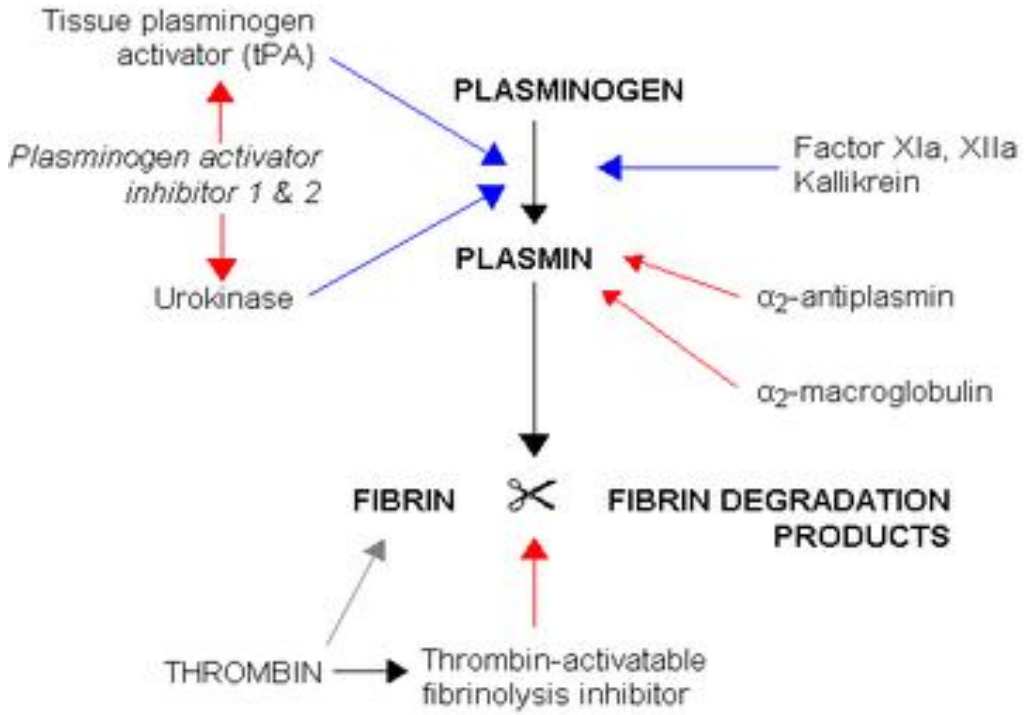

Figure 1.2 Fibrinolysis

1.2.3 Bleeding Disorders:

The pattern of bleeding is relatively predictable depending on the etiology. Vascular and platelet disorders tend to be associated with bleeding from mucous membranes and into skin whereas in coagulation disorders the bleeding is often into joins and soft tissue (Hoffbrand *et al.*, 2011).

1.2.3.1 Vascular Bleeding Disorders:

The vascular disorders are a heterogeneous group of conditions characterized by an easy bruising and spontaneous bleeding from small vessels. The underlying abnormality is either in the vessels themselves or in perivascular connective tissues. Most cases of bleeding caused by vascular defects alone are not severe (Hoffbrand *et al.*, 2011). Abnormal bleeding involving the loss red blood cells from microcirculation expresses itself as the condition of purpura, which is characterized by hemorrhages in the skin, mucous membrane, and internal organs.

Purpura may be produced by a variety of vascular abnormalities. These abnormalities include the following:

1. Purpura associated with direct endothelial cell damage. The overall action of endothelins is increase blood pressure and vascular tone. Endothelial damage may result from physical or chemical injury to the tissue caused by microbial agents such as in rickettsial disease.
2. Purpura associated with an inherited disease of connective tissue. Alterations of the vascular supportive framework can occur in disorders such as diabetes.
3. Purpura associated with decreased mechanical strength of the microcirculation. Decreased strength can be seen in conditions such as scurvy and amyloidosis.
4. Purpura associated with mechanical disruption of small venules. This condition can be observed around the ankles with prolonged standing and may be caused by presence of abnormal proteins in macroglobulinemias or hyperviscosity disorders.
5. Purpura associated with microthrombi (small clot). This type of disorder is associated with abnormal intravascular coagulation conditions.
6. Purpura associated vascular malignancy. Purpura of this origin is observed in Kaposi sarcoma and vascular tumors (Hoffbrand *et al.*, 2011).

1.2.3.2 Platelet Disorders:

Clinical manifestations of bleeding disorders can be divided into two broad and rather poorly defined groups (1) superficial bleeding (such as petechiae, epistaxis, or gingival bleeding), which is usually associated with platelet defect or vascular disorder, and (2) deep bleeding (such as hemtomas, or hemarthrosis), which is associated with plasma clotting factor deficiencies (Rodak, 1995). The normal

range of circulating platelets is 150.000 to 450.000. When the quantity of platelets decreases to levels below this range, a condition of thrombocytopenia exists. If the quantity of platelets increases, thrombocytosis is the result. Disorders of platelets can be classified as quantitative (thrombocytopenia or thrombocytosis) or qualitative (thrombocytopathy) (Turgeon, 2012).

1.2.3.2.1 Thrombocytopenia:

A correlation exists between severe thrombocytopenia and spontaneous clinical symptoms usually include the presence of petechiae or purpura. Petechiae appear as small, purplish hemorrhagic spots on the skin or mucous membranes; purpura is characterized by extensive areas of red or dark – purple discoloration. Thrombocytopenia can result from a wide variety of conditions, such as after the use of extracorporeal circulation in cardiac bypass surgery or in alcoholic liver disease. Thrombocytopenia in itself rarely poses a threat to affected patients, but disorders associated with it – which include deep venous thrombosis, disseminated intravascular coagulation (DIC), pulmonary embolism, cerebral thrombosis, myocardial infractions, and ischemic injury to the legs or arms. Most thrombocytopenic condition can be classified into major categories. These categories are:

1. Disorders of production
2. Disorders of destruction, including decreased megakaryocytopoiesis and ineffective platelet production, and disorders of utilization.
3. Disorders of platelet distribution and dilution

(Turgeon, 2012).

1.2.3.2.2 Thrombocytosis:

Thrombocytosis is defined as an abnormally high platelet count (more than 450.000/cumm) in whole blood. In most cases thrombocytosis is secondary to inflammation or trauma and is termed reactive thrombocytosis, in which case the platelet count is elevated for a limited period and rarely exceeds 800.000/cumm. A marked and persistent elevation in platelet count may accompany a myeloproliferative disorder. The platelet count may exceed 1000.000 /cumm when it is a primary symptom of a myeloprolifrative disorder such as polycythemia vera, chronic myelocytic leukemia, or myelofibrosis with myeloid metaplasia (Rodak, 1995).

1.2.3.2.3 Qualitative Platelet Disorders:

Qualitative platelet disorders are suggested by a prolonged bleeding time (abnormal platelet function screen) or clinical evidence of bleeding in setting of normal platelet count and coagulation studies. They are most commonly acquired, but can be inherited. Anew platelet function test, PFA – 100, has a 96 per cent sensitivity for detecting von willebrand disease and three separate categories of platelet dysfunctions can be identified based on etiology (Acquired, Drug – induced, and Hereditary). These include the more common acquired causes and the less frequent hereditary causes (Mamen *et al*., 1998).

1.2.3.2.3.1 Acquired:

Acquired platelet function defects can be caused by a blood plasma inhibitory substance. Examples of disorders or diseases that may exhibit this dysfunction include infused dextran, uremia, liver disease, and pernicious anemia (Turgeon,2012).

1.2.3.2.3.2 Hereditary:

Hereditary platelet dysfunctions are caused by an inherited platelet defect that is either structural or biochemical. Examples of adhesion disorders include Bernard – Soulier syndrome, a collagen receptor defect, Glanzmann thrombasthenia, and storage granule abnormalities (Turgeon,2012).

1.2.3.2.3.2.1 Thrombasthenia (Glanzmann`s disease):

This autosomal recessive disorder leads to failure of primary platelet aggregation because of deficiency of the membrane glycoproteins IIb/IIIa which together forms the vWF and fibrinogen receptor. It usually presents in the neonatal period (Hoffbrand *et al.*,2011).

1.2.3.2.3.2.2 Bernard- Soulier Syndrome:

Bernard – Soulier syndrome is a moderate bleeding disorder associated with prolonged bleeding time, enlarged platelets, and thrombocytopenia. The primary platelet defects in this syndrome (1) an inability of platelets to participate in vWF – dependent adhesion to endothelium exposed by vascular damage and (2) no response to microfibrils (Rodak, 1995).

1.2.3.2.3.2.3 Storage Pool diseases:

In the rare grey platelet syndrome, the platelets are large than normal and there is a virtual absence of a granules with deficiency of their proteins (Hoffbrand *et al* .,2011).

1.2.3.3 Coagulation Disorders:

The diverse defects of plasma clotting factors can be divided into major groups; hereditary and acquired forms. The defects in the clotting factors can be produced by one of the following:

- Decreased synthesis of the factors.

- Production of abnormal molecules that interfere with the coagulation cascade.

- Loss or consumption of the coagulation factors.

- Inactivation of these factors by inhibitors or antibodies. (Thorup *et al*., 1987)

1.2.3.3.1 Hereditary Coagulation Disorders:

Hereditary coagulation disorders are the result of a deficiency or functional abnormality of a single factor. They most often result in a lifelong bleeding diathesis that occurs in a consistent genetic pattern. However, some hereditary coagulation disorders may be "silent" whereas others lead to thrombosis. The most common hereditary disorders of coagulation are hemophilia A, von Willebrand`s disease and hemophilia B. These three disorders together constitute more than 90 per cent of the hereditary coagulation disorders, hemophilia A and von Willebrand`s disease being the most common (Rodak, 1995).

1.2.3.3.1.1 Hemophilia A:

This disorder is the most common hereditary coagulation disorder and one of the oldest diseases known to humankind. It occurs in all geographic areas and in ethnic groups with approximately equal frequencies. The genetic abnormality gives rise to

deficiency or aberration of low – molecular – weight subunit of factor V111, designated VIII:C (Rodak, 1995).

Hemophilia A is the most common of the hereditary clotting factor deficiencies. The prevalence is of 30 – 100 per million of population. The inheritance is a sex-linked but up to one-third of patients have no family history and result from recent mutation (Hoffbrand et al., 2011).

The major of patients are male, but hemophilia can occur very rare in females. The severity and frequency of bleeding in X-linked factor VIII deficiency are inversely correlated with residual factor VIII level. The main load- or strain- bearing joints – ankles, knees and elbows – are most affected but any joint can be the site of bleeding. If untreated, this intracapsular bleeding causes severe swelling, pain, stiffness and inflammation, which gradually resolves over days or weeks. It is not clear why bleeding in hemophilia shows a predilection joints, but it has been suggested recently that synthesis of tissue factor pathway inhibitor (TFPI) in synovial tissue may be at least part of the explanation. Muscle bleeding can be seen in any anatomical site, but it most often presents in the large load-bearing groups of the thigh, calf, posterior abdominal wall and buttocks. Local pressure effects often cause entrapment neuropathy, particularly of the femoral nerve, with iliopsoas bleeding (Hoffbrand *et al*.,2005).

1.2.3.3.1.2 Hemophilia B:

Hemophilia B is an X-linked deficiency of FIX and behaves clinically like hemophilia A. FIX is responsible for the activation of FIX in the presence of activated FVIII, calcium, and phospholipid. FIX is synthesized in the liver and is a vitamin k-dependent protease that is similar to prothrombin, FVII, FX, and protein C (Hoffbrand *et al*.,2005).

The inheritance and clinical features of factor IX deficiency (Christmas disease, hemophilia B) are identical to those of hemophilia A. The incidence is one-fifth than hemophilia A (Hoffbrand et al.,2011). Factor IX is a vitamin k-dependent factor. It is synthesized in the liver and is present in serum. Although the bleeding abnormality is usually less severe than in factor VIII deficiency, it can be clinically indistinguishable from it. Hemophilia B is identical to the more common hemophilia A in the terms of bleeding symptoms, varying degrees of severity, and complications. As in hemophilia A, the severity of factor IX deficiency tends to be the same for affected members of a family (Rodak,1995).

1.2.3.3.1.3 von Willebrand Disease:

von Willebrand disease is the most common hereditary coagulation abnormality described in humans, although it can be acquired as result other medical conditions. It arises from a qualitative or quantitative deficiency of von Willebrand factor (vWF), a multimeric protein that is required for platelet adhesion. There are three forms of vWD: inherited, acquired, and pseudo or platelet type. There are three types of hereditary vWD: vWD type I, vWD type II, and type III. vWD is named after Erik Adolf Von Willebrand, a Finnish pediatrician who first described the disease in 1926. Most cases are hereditary, but acquired forms of vWD have been described. The International Society on Thrombosis and Hemostasis (ISTH) classification depends on the definition of qualitative and quantitative defect (Sadler ,1994). The prevalence of vWD is about 1 in 100 individuals. However, the majority of these people do not have symptoms. The prevalence of clinically significant cases is 1 per 10.000. Because most forms are rather mild, they are detected more often in women, whose bleeding tendency shows during menstruation. It may be more severe or apparent in people with blood type O (Rodak,1995).

1.2.3.4 Hypercoagulability State:

Systematic inflammation has long been recognized as being associated with hypercoagulability. It common occurs in patients with DIC in severe sepsis. Most of the hypercoagulable effects of inflammation are mediated by inflammatory cytokines, including IL-1, IL-6, and tumor necrosis factor (TNF) and interaction between these systems. Thrombosis and coagulation can act as triggers for inflammation, and severe systemic inflammatory responses can trigger coagulation. Thrombi may form because coagulation is enhanced or because protective devices such as fibrinolysis are impaired. An increase in the likelihood of blood to clot is referred to as the hypercoagulable. Thrombosis is promoted by vascular damage, by retarded blood flow, and by alteration in the blood that increase the likelihood of clotting. A number of factors may contribute to hypercoagulaion (Turgeon,2012).

1.2.3.4.1 Primary States of Hypercoagulability:

Hypercoagulable states include various inherited and acquired clinical disorders characterized by n increased risk for thromboembolism. Primary hypercoagulable states include relatively rare inherited conditions that lead to disordered endothelial cell thromboregulation. The major inherited inhibitor disease states include AT-III deficiency, protein C deficiency, and protein S deficiency. These conditions should be considered in patients who have recurrent, familial, or juvenile deep venous thrombosis or occlusion in an unusual location such as a mesenteric, brachial, or cerebral vessel(Turgeon,2012).

1.2.3.4.2 Secondary States of Hypercoagulability:

Secondary hypercoagulation states may be seen in a number of heterogeneous disorders. In many of these conditions, endothelial ctivation by cytokines leads to

the loss of normal vessel-wall anticoagulant surface functions, with conversion to a proinflammatory thrombogenic phenotype. Important clinical syndromes associated with substantial thromboembolic events include the APS, heparin-induced thrombopathy, myeloproliferative syndromes, and cancer. Hypercoagulability can be associated with systemic inflammation due primarily to an incease in procoagulant functions, an inhibition of fibrinolysis, and a downregulation of the three major physiologic anticoagulant systems of protein C, AT-III, and tissue factor inhibitor(Corriveau *et al*.,1988).

1.2.3.5 Laboratory Screening Tests:

Despite the leads derived from the clinical examination, diagnosing the type of hemostatic disorder ultimately depends on laboratory testing. Platelet plug formation reflects primary hemostatic events and is evaluated through the bleeding time and the platelet count. Abnormalities of coagulation are then screened for by measuring the fibrinogen concentration, thrombin time (TT), prothrombin time (PT), and partial thromboplastin time (PTT). Once screening test results have indicated the type of disorder, additional studies and factor assays are used to establish a specific diagnosis (Thompson *et al*.,1992).

1.2.3.5.1 Prothrombin Time:

The PT procedure evaluates the generation of thrombin and the formation of fibrin via extrinsic and common pathway. Thromboplastin reagent is used for this assay. Thromboplastin can be prepared by various methods; tissue extraction of rabbit brain or lung, tissue culture, and molecular methods. Thromboplastin reagent is a mixture of tissue factor, phospholipid, and calcium ions and is used to initiate clotting measure as the PT. Thromboplastin forms complexes with and activates factor VII. This provides surfaces for attachment and activation of factors X, V,

and II. Thromboplstin, derived from tissues that supply phospholipoprotein, and calcium are added to the blood plasma. The time required for fibrin clot to form is measured. Reference ranges are from 10 to 13 seconds. Prolonged results can indicate a deficiency of one or more factors in the extrinsic pathway; factors VII, X, V, and II or I (Turgeon,2012).

1.2.3.5.2 Partial Thromboplastin Time:

The PTT procedure measures the time required to generate thrombin and fibrin polymers via the intrinsic and common pathways. In the PTT assay, calcium ions and phospholipids that substitute for platelet phospholipids are added to blood plasma. In vitro, the activation factor XII to XIIa, prekallakrein, and factor XI to Xia occurs on the negatively charged glass surface. The generation of fibrin is the end point. The PTT assay reflects the activity of prekallikrein, HMWK, and factors XII, XI, IX, VIII, X, V, II, and I. PTT may be prolonged because of a factor decrease, such as fibrinogen (factor I), or the presence of circulating anticoagulants. The reference range for PTT is less than 35 seconds (depending on the activator used) (Turgeon,2012).

1.2.3.5.3 Thrombin Time:

The thrombin time test determines the rate of thrombin-induced cleavage of fibrinogen to fibrin monomers and the subsequent polymerization of hydrogen-bonded fibrin polymers to form an insoluble fibrin clot. The normal value is less than 20 seconds. Prolonged results will be seen if the fibrinogen concentration is less than 100 mg/dl. Abnormal results will also be encountered in the presence of thrombin inhibitors or substances that interfere with fibrin formation, high concentration of immunoglobulins that interfere with fibrin monomer polymerization such as in cases of multiple myeloma(Turgeon,2012).

1.2.3.5.4 Test of Fibrinolsis:

Increased levels of circulating plasminogen activator may be detected by demonstrating shortened euglobulin clot lysis times. A number of immunological methods are available to detect fibrinogen or fibrin degradation products (including D-dimers) in serum. In patients with enhanced fibrinolysis, low levels of circulating plasminogen may be detected(Hoffbrand *et al*,.2011).

1.2.3.5.5 Platelet Count:

A platelet count is test to measure how many platelets you have in your blood. Platelets are parts of the blood that help the blood clot. They are smaller than red or white blood cells. The number of platelets in your blood can be affected by many diseases. Platelets may be counted to monitor or diagnose diseases, or to look for the cause of excess bleeding or clotting. There are two methods for counting platelets; manual (using counting chamber) and automation. The normal number of platelet in the blood is 150.000 – 400.000 /cumm. A lower – than normal platelet count is called thrombocytopenia whereas, a higher – number of platelets (thrombocytosis) refers to when your body is making too many platelets (Abrams ,2011).

Previous study:

A study conducted in Nigeria with prospective case-control as study design included 200 adults ($18 years) age range (25–65 years) and mean age (45.25 ± 11.50 years). There were 120 alcohol dependent (subjects), and 80 age, gender-matched nondrinkers (controls).The coagulation parameters, PT and APTT, were investigated among alcoholics and nondrinkers. The mean values of PT (20.7 sec) and APTT(48.1 sec) were significantly higher among alcoholics compared to the nondrinkers (P = 0.04 and P = 0.02 respectively).(T.oduola *et al,* .2005)

A study was carried out among the inhabitant of Ile-Ife, who gave the consent of participation. One hundred subjects that volunteered known as alcoholics were recruited the result shows that there is Statistically significant differences in all the parameters estimated which are PLT mean (228.0 $x10^9$c/l) (p= 0.3), PT mean(16.39 sec) (p= 0.02) and PTT mean (40.21 sec) (p= 0.06) (Oke *et al,*.2013).

Objectives:

General objective.

To evaluate PT, PTT, and Platelets count among alcohol consumers.

Specific Objectives:

To compare the results of these tests between study group and control group.

To study the effect of alcohol on hemostatic parameters**.**

To compare age and duration of alcohol consumers.

Rationale:

Alcohol consumption has complex associated with hemostatic that has great impact on both individuals and society. Recent studies indicate that individuals that taken alcohol may have ability to stimulate hemostatic disorders. This research was conducted to evaluate some of hemostatic parameters in Sudanese those drinks alcohol so as to detect changes which may be associated alcohol consumption. Knowledge of these changes will be valuable so as to avoid complications associated with alcohol consumption. So mortlity and morbidity rate to be reduce.

Chapter Tow
Materials and Methods

2.1 Study design:

This is case control analytical study conducted in Khartoum during the period from May to August 2014. to evaluate PT, PTT, and platelets count in alcohol consumers.

2.2 Study Population:

Fifty individuals from Khartoum who drinks alcohol and Fifty non alcoholic as control group.

2.3 Inclusion criteria:

Individuals who drinks alcohol in Khartoum .

2.4 Exclusion criteria:

Presence of other coagulation disorders not associated with alcohol abuse.

2.5 Ethical consideration:

A consent of the selected individuals to study was taken after being informed with all detailed and objectives of the study.

2.6 Data collection:

Data were collected using self – administered questionnaire which was designed to obtain information.

2.7 Sampling:

Non – probability sampling method was used (individual who accepted to participate in the study)

2.8 Data analysis:

The data were analyzed using the SPSS computer program version 11.5.

2.9 Blood Sample:

5.7 ml of blood was obtained by clean venepuncture from each subjects. 2.7 ml was dispended into bottle containing 0.25 ml tri sodium citrate (3.8%) and 3 ml dispended into dipotassium ethylenediamine tetraacetic acid (EDTA)

2.10 Methodology:

2.10.1 Prothrombin Time:

2.10.1.1 Principle:

The PT test measures the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiecy of extrinsic clotting system. Although originally thought to measure prothrombin, the test is now known to depend also on reactions with factors V, VII, and X and on fibrinogen concentration of the plasma (Dacie *et al,*. 2006).

2.10.1.2 Reagents:

1- **Thromboplastin:**

Thromboplastins were originally tissue extracts obtained from different species and different organs containing tissue factor and phospholipid. Because of the

potential hazard of viral and other infections from handling human brain, it should not longer be used as a source of thromboplastin. They are manufactured using recombinant human tissue factor produced in Escherichia Coli. (Dacie *et al*,. 2006)

2- **$Cacl_2$;**

0.025 mol/l

2.10.2 Activated Partial Thromboplastin Time:

2.10.2.1 Principle:

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

2.10.2.2 Reagents:

1- **Kaolin:**

5g/l (laboratory grade) in barbitone buffered saline, PH 7.4. Add a few glass beads to aid resuspension. Other insoluble surface active substances such as silica,

Celite, or ellagic acid can also be used.(Dacie *et al*,. 2006).

2- **Phosphplipids:**

Cephalin as phospholipids substitution.

3- **$Cacl_2$:**

0.025 mol/l.

2.10.3 Platelet Count:

2.10.3.1 Principle of automated analyzer system:

The counting of cellular elements in the blood sample was be done with the impendencemetery technique. This technique is based on the modification of the impedance of calibrated aperture soaking in an electrolyte and going through a constant course delivered by two electrodes located on both sites of the aperture. A vacuum applied on the site of the aperture allow the cell passage. They oppose their physical volume to course passage. A voltage impulse is registered at the electrodes terminal. The highest of this impulse is proportional to the cell volume.

The innovative optical detection system is covered by two patents pending. This technology called: OCHF (optimal cytometer hydrofocus free) is based on an unique and innovative concept of an active sample flow and passive sample flow is introduced in the flow cell under pressure and the sheath is only dedicated to maintain it. For each cell throwing the optical detection area, to pulses are generated. When for the axis loss light (ALL) measurement and the forward side scatter (FSC) measurement. The result of those two axis of measurement is high dilution matrix that unable to identify the WBC population, the five parts different is obtained by the optical matrix analysis after action of the lytic reagent (banding pattern). The reagent destroys RBCs and their stromas, composes the oxyhaemoglobin chromogen and product the white blood cell membrane to keep it in closed native state. Platelet analysis is made by impedancemetery in RBCs counting champer at the same time with the red blood cell s, four parameters are Obtained, platelet, MPV. PDW, and P-LCR (William ,2002).

Chapter Three
Results

Demographic characteristics of study population:

The study was conducted to measure of PT, PTT, and Plts count in Khartom.

Fifty of alcohol consumers were participate in this study, all of them are males ,range between 20 and 70 years, and fifty (non alcohol consumers) selected as control.

Table 3.1 shows Age group among alcohol consumer.

Age group	Frequency	Percent
20-30	11	22.0
31-40	23	46.0
41-50	7	14.0
51-60	6	12.0
61-70	2	4.0
71-80	1	2.0
Total	50	100.0

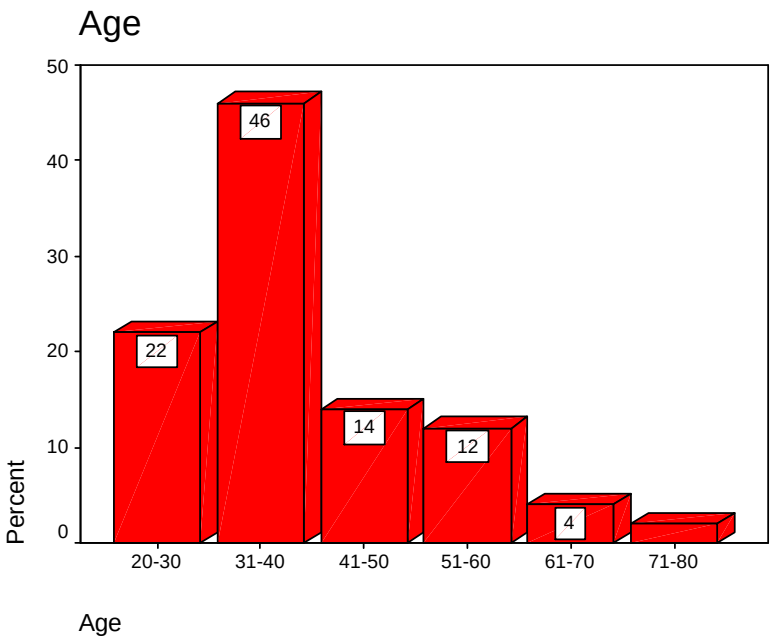

Figure No(1) show age group of study pupulation.

Table 3.2 shows duration of alcoholics.

Duration	Frequency	Percent
1-10	32	64
11-20	15	30
21-30	2	4
31-40	1	2
Total	50	100

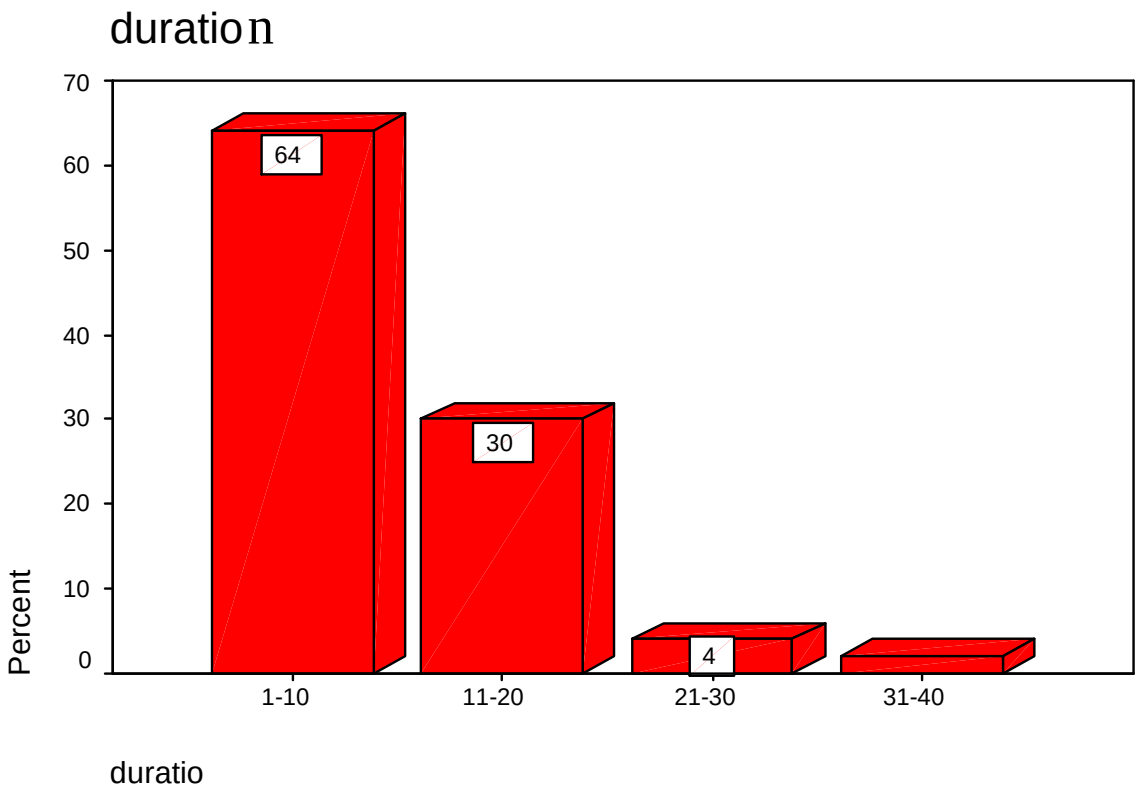

Figure No(2) shows duration of alcoholics.

Table 3.3 shows education level of alcoholics.

EDUCATION	Frequency	Percent
Primery	31	62.0
Secondery	15	30.0
Unversity	4	8.0
Total	50	100.0

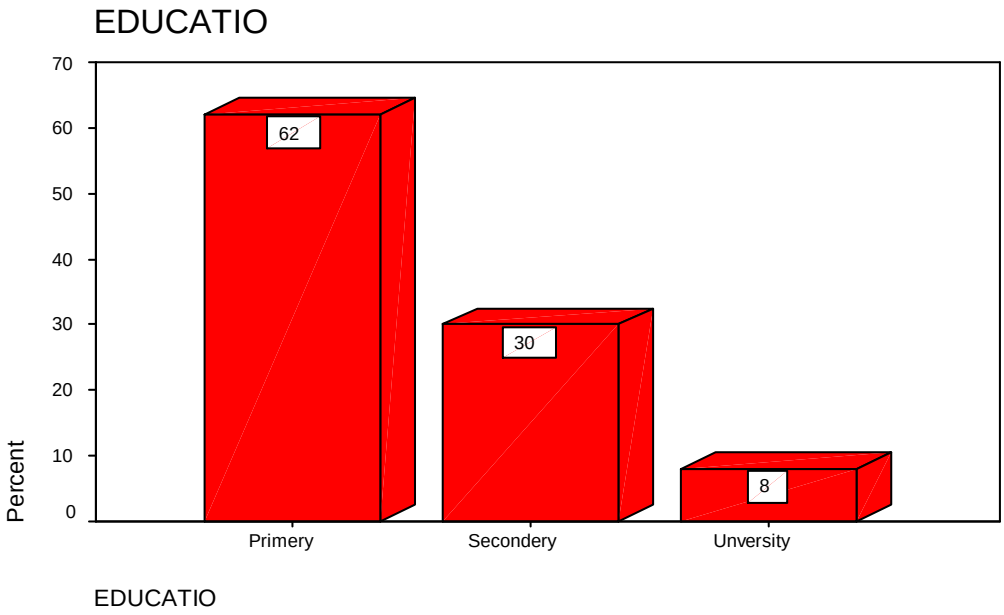

Figure No(3) shows education level of alcoholics.

Table 3.4 shows Age of control group.

Age	Frequency	Percent
20-30	8	16.0
31-40	20	40.0
41-50	12	24.0
51-60	6	12.0
61-70	4	8.0
Total	50	100.0

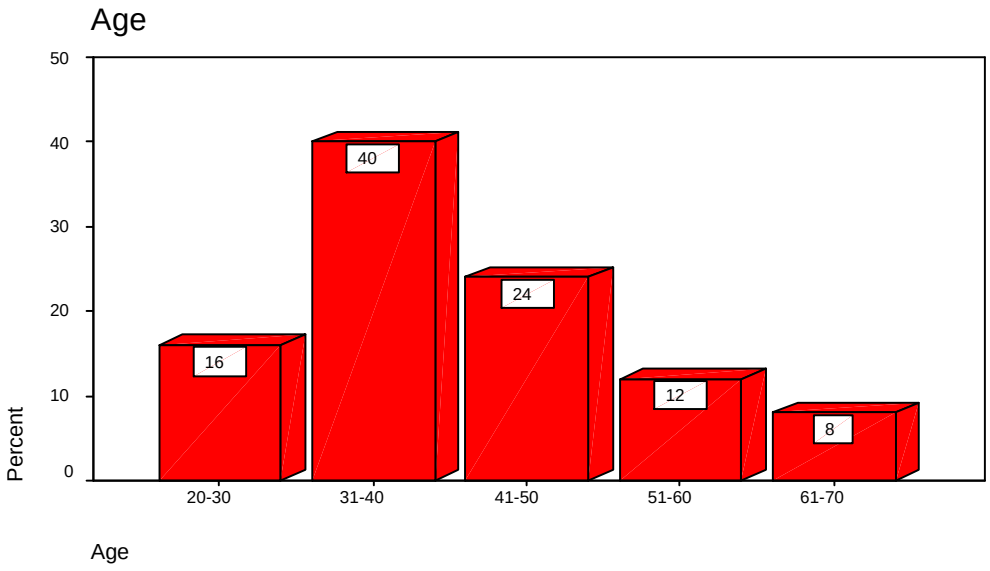

Figure No(4) shows Age of control group.

Table 3.5 shows education level of control group .

EDUCATIO	Frequency	Percent
Primary	3	6.0
Secondary	8	16.0
University	39	78.0
Total	50	100.0

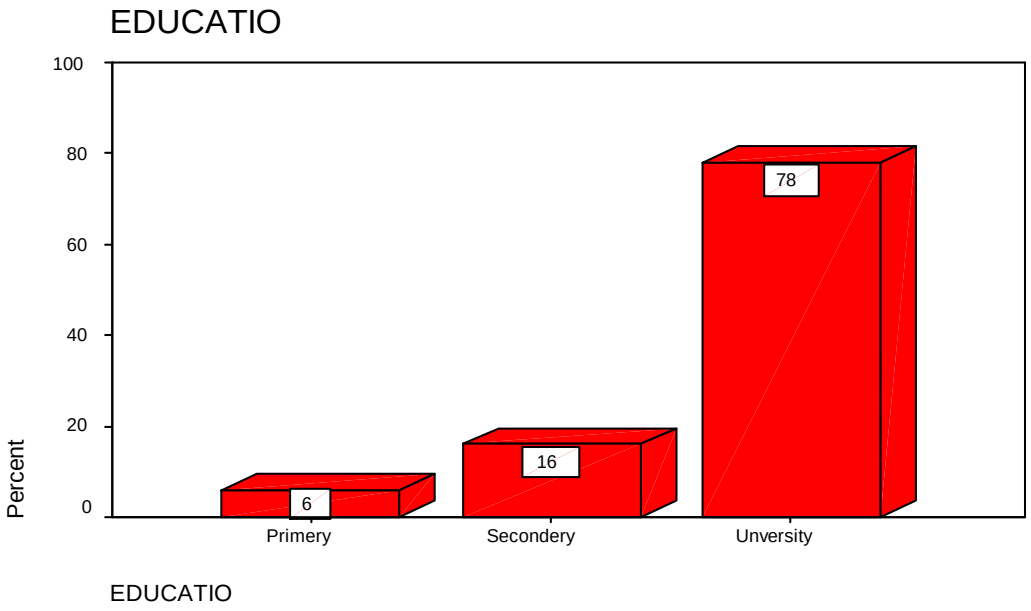

Figure No (5) shows education level of control group .

Table 3.6 shows significant increase of the mean PT result in cases (16.6) when compare with control (13.6) with (p.value 0.00).

parameter	sample	N	mean	P.valvue
PT/seconds	Case	50	16.6 ± 5.2	0.01
	control	50	13.6 ± 5.2	

Table 3.7 shows insignificant difference in the mean of PTT result between case (32.7) and control 31.1) with (p.value 0.22).

parameter	sample	N	mean	P.valvue
PTT/seconds	Case	50	32.7 ± 7.2	0.22
	control	50	31.1 ± 5.3	

Table 3.8 shows insignificant difference in the mean of platelet result between case (272) and control (263) with (p.value 0.44).

parameter	sample	N	mean	P.valvue
Platelet/cumm	Case	50	272 ± 60.7	0.44
	control	50	263 ± 59.0	

Chapter Four

Discussion, Conclusion and Recommendations

Discussion

This is case control study was conducted in Khartoum during the period from May to August 2014 to evaluate the Prothrombin time, Activated Partial Thromboplastin time, and Platelet count among hundred (100) subjects fifty (50) as cases (alcohol consumer) and fifty (50) controls (non alcoholics). with age ranged between 20 to 70 years.

The findings of this study showed that the mean of PT result of alcoholics was (16.6 seconds) which significantly increased than that of control group (13.6) with (p.value 0.01) so there is significant difference in PT. This result is agree with the study of T.oduola *et al* which conducted in Nigeria at 2005 with significant increased (p.value 0.04). And also agree with the study of Oke et al which conducted in IIe- Ife at 2013 (p.value 0.03). The mean of PTT result of alcoholics was (32.7 seconds) which slightly increased than that of control group (31.1 seconds) but without statistical significance (p.value 0.22). This result agree with the study of Oke et al which conducted in IIe- Ife at 2013 with (p.value 0.06) and diagree with the study of T.oduola et al which conducted in Nigeria at 2005 with significant increased (p.value 0.02) .The mean of Platelet count result of study group was (272/cumm) which slightly increased than that of control group (263/cumm) but without statistical significance (p. value 0.44). This result is agree with study of Oke *et al* which conducted in IIe- Ife at 2013 with (p.value 0.3) .

Conclusion:

This study concluded that the consumption of alcohol significantly increase the PT result in compare with control.

On the other hand, no significant changes occurred in PTT and platelet count.

The majority of alcohol consumers are primary education.

Recommendation:

- Another study should be conducted to control the confound factors (Nutrition, drinks other than alcohol,etc) .
- Another research should be conducted to study platelet function .
- Education should be available for all community so as to rise awareness so alcohol drinking will be avoided.

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Appendix–1

Sudan University of Science and Technology

College of Graduate Studies

Department of Hematology

Measurement of Some Hematological Parameters among

Alcohol abuse persons - Khartoum State

Serial NO [ ]

الاسم:.....

الموضوع:_ جمع عينات لأجراء البحث التكميلي (سحب 5.7 مل دم).

Sex	male	female

Age	0	1	2	
	<20	21-40	>41	

Types of alcohol	0	1
	local	Exported

Duration	0	1	2	3
	<1year	2-5year	6-10 year	>10year

Education	0	1	2	3
	primary	secondary	university	H.degre

أمضاء الباحث ..... امضاء المتبرع .....

Appendix–2

بسم الله الرحمن الرحيم

جامعه السودان للعلوم والتكنولوجيا

كليه الدراسات العليا برنامج ماجستير - مختبرات طبية

قسم أمراض الدم ومبحث المناعة

براءة أخلاقية

الاسم:.....

سوف يتم اخذ عينه من الدم (5.7 مل) من الوريد بواسطة حقنه وذلك بعد مسح منطقه اخذ العينة بواسطة المطهر كل الأدوات المستخدمة لأخذ العينة معقمه ومتبع فيها كل وسائل السلامة المعملية.

الإمضاء:.....

التاريخ:.....