Chapter One

Introduction and literature review

1.1. Introduction

Haemostasis

The maintenance of circulatory hemostasis is achieved through the process of balancing bleeding (hemorrhage) and clotting (thrombosis). The arresting of bleeding, depends on several components. The four major components are the vascular system, platelets (thrombocytes), blood coagulation factors, fibrinolysis and ultimate tissue repair. Three other, less important, components are the complement and kinin systems as well as serine protease inhibitors. 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 platelet, and

Coagulation proteins

4. Development of a blood clot around the injury
5. Fibrinolytic removal of excess hemostatic material to reestablish vascular integrity (Ciesla,2007)

. Quality Assurance and Quality control:

It covers the part of quality assurance which primarily concerns the control of errors in the performance of tests and verification of test results. Quality control should cover the aspects of every procedure within the laboratory; it must be practical, achievable and affordable. Laboratory QC is designed to detect, reduce and correct deficiencies in a laboratory's internal analytical process prior to release of patients results and improve the quality.

1.2. Literature review

1.2.1 Haemostasis

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1.2.1.1. Blood vessel spasm

Arteries and Veins

Arteries are the distributing vessels that leave the heart, and veins are the collecting vessels that return to the heart. Arteries have the thickest walls of the vascular system. Although variations in the size and type of vessel exist, the tissue in a vessel wall is divided into three coats or tunics. These coats are the tunica intima, tunica media, and tunica adventitia. The tunica intima forms the smooth glistening surface of endothelium that lines the lumen (inner tubular cavity) of all blood and lymphatic vessels and the heart. The simple squamous

epithelium that lines these vessels is referred to as endothelium. The tunica intima consists of a single layer of endothelial cells thickened by a sub endothelial connective tissue layer containing elastic fiber .The tunica media thickest coat, is composed of smooth muscle and elastic fibers. The tunica adventitia consists of fibrous connective tissue that contains autonomic nerve endings and the vasavasorum, small networks of blood vessels that supply nutrients to the tissues. Veins are larger and have a more irregular lumen than arteries. In comparison with arteries veins are relatively thin walled with a weaker middle coat. Elastin fibers are usually found only in larger veins, and there are fewer nerves distributed to the veins than to the arteries (Ciesla,2007).

The Role of Vasoconstriction in Hemostasis

Vascular injury to a large or medium-size artery or vein requires rapid surgical intervention to prevent exsanguinations. When a smaller vessel, such as an arteriole, venule, or capillary, is injured, contraction occurs to control bleeding. This contraction of the blood vessel wall is called **vasoconstriction**. Vasoconstriction is a short-lived reflex reaction of the smooth muscle in the vessel wall produced by the sympathetic branches of the autonomic nervous system. Thin narrowing, orstenosis, of the lumen of the blood vessel decreases the flow of blood in the injured vessel and surrounding vascular bed and may be sufficient to close severed capillaries (Turgeon , 2010).

The Role of the Endothelium

The endothelium contains connective tissues such as collagen and elastin. This connective tissue matrix regulates the permeability of the inner vessel wall and provides the principal stimulus to thrombosis following injury to a blood vessel. The endothelium is highly active metabolically and is

Involved in the clotting process by producing or storing clotting components. It is also rich with plasminogen activator, which activates plasminogen, which

ensures rapid lyses of fibrin clots. Additionally, the endothelium elaborates prostacyclin, which is synthesized bythe endothelium from prostaglandin precursors and strongly inhibits platelet aggregation and adhesion minimal interactions leading to platelet activation or clot formation occur between the circulating blood and intact endothelial surfaces. However, disrupted endothelial cells release thromboplastic substances that can initiate coagulation. Collagen, in particular, initiates contact activation of factor XII, thereby initiating blood coagulation (Turgeon , 2010).

1.2.1.2. Formation of platelet plug

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 megakaryocyte is megakaryoplast which arise by a process of differentiation from haemopoietic stem cell. Approximately each megakaryocyte giving 1000_5000 platelet. The time interval from differentiation of stem cell to production of platelet average approximately 10 days. Thrombopoietin is the major regular of platelet production and is constitutively produced by the liver and kidneys. He normal platelet count is approximately range 150_450 * 10^9 /l, and the normal lifespan 7_10 days (Ciesla,2007).

Platelet function:

The main function of platelet is the formation of mechanical plugs during the normal haemostatic response to vascular injury. The immobilization of platelet at the site of vascular injury requires specific platelet vessel wall (adhesion) and platelet- platelet (aggregation).

Platelet adhesion

Following blood vessel injury, platelets adhere to the exposed sub endothelial matrix protein via specific adhesive glycoprotein (GP). Under condition of high shear, e.g. arterioles, the exposed sub endothelial matrix is initially coated with VWF multi\mares. The platelet then make contact with VWF via GPIb_XI-V complex on platelet. This initiates platelet rolling in the direction of blood flow over the exposed VWF with activation of GPIIb/IIIa receptors. firm adhesion is established by interaction of other glycoprotein including activated GPIIb/IIIa with VWF and GPVI with collagen and component of sub endothelial matrix. Collagen binds initially to GPIa/IIa, cross-links many of these integral molecules, and in this way activates platelet. The events that follow are shape change adhesion , activation of GPIIb/IIIa and granule secretion (Turgeon ,2010).

Platelet aggregation:

Platelet aggregation in vivo is a much more complex and dynamic process than previously thought. Over the last decade, it has become clear that platelet aggregation represents a multistep adhesion process involving distinct receptors and adhesive ligands, with the contribution of individual receptor-ligand interactions to the aggregation process dependent on the prevailing blood flow conditions. It is now believed that three distinct mechanisms can initiate platelet aggregation. A variety of agents are capable of producing in vitro platelet. It characterized by cross-linking of platelets through active GPIIb/III are captor with fibrinogen bridges. A resting platelet has about 50-80000 GPIIb/IIIa receptor (Turgeon , 2010).

1.2.1.3. Coagulation factors:

Bleeding from small blood vessels may be stopped by vasoconstriction and the formation of a platelet plug, bu tthe formation of a clot (thrombus) usually occurs as part of the normal process of hemostasis. The soluble blood coagulation factors are critical components in the formation of at hrombus hepatic cells are the principal site of the synthesis of coagulation factors. However, other cells, such as endothelial cells, also play an important role in the normal process of hemostasis and thrombosis. Classically, the coagulation factors have been described as reacting in a cascading sequence (Ciesla, 2007).

1.2.1.3.1. Common Characteristics of Coagulation Factors

Proteins that are clotting factors have four characteristics in common. These characteristics are as follows:

1. A deficiency of the factor generally produces a bleeding tendency disorder with the exception of factor XII,prekallikrein (Fletcher factor), and high molecular weight kininogen (HMWK; Fitzgerald factor).
2. The physical and chemical characteristics of the factor are known.
3. The synthesis of the factor is independent of other proteins.
4. The factor can be assayed in the laboratory.

To develop an understanding of the theory of coagulation and the underlying principles of related laboratory procedures, it is helpful to compare the characteristics of various coagulation factors. Three groups of factors exist: the fibrinogen group, the prothrombin group, and the contact group. The fibrinogen group consists of factors I, V, VIII, and XIII. These factors are consumed during the process of coagulation Factors V and VIII are known to decrease during blood storage in vitro. These factors are known to increase during pregnancy, in the presence of conditions of inflammation and subsequent to the use of oral contraceptive drugs. The **prothrombin group** consists of factors II, VII, IX, and X. All these factors are dependent on vitamin K during their synthesis. Vitamin

K is available to the body through dietary sources and intestinal bacterial production. This group is

Inhibited by warfarin. This group is considered to be stable and remains well preserved in stored plasma. The **contact group** consists of factors XI, XII, prekallikrein(Fletcher factor), and HMWK (Fitzgerald factor). These factors are involved in the intrinsic coagulation pathway (Ciesla,2007).

1.2.1.3.2. Characteristics of Individual Factors

Each of the individual coagulation factors has some unique characteristics. These characteristics include the following:

Factor I (Fibrinogen)

Fibrinogen is a large, stable globulin protein (molecular weight, 341,000). It is the precursor of fibrin, which forms the resulting clot. When fibrinogen is exposed to thrombin, two peptides split from the fibrinogen molecule, leaving a fibrin monomer. These monomers aggregate together to form the final polymerized fibrin clot.

Factor II (Prothrombin)

Prothrombin is a stable protein (molecular weight, 63,000).In the presence of ionized calcium, prothrombin is converted to thrombin by the enzymatic action of thromboplastinfrom both extrinsic and intrinsic sources. Prothrombin has a half-life of almost 3 days with 70% consumption during clotting.

Factor IIa (Thrombin)

Thrombin (molecular weight, 40,000) is the activated form of prothrombin, which is normally found as an inert precursor in the circulation. This proteolytic enzyme, which interacts with fibrinogen, is also a potent platelet-aggregating substance. A large quantity of thrombin is consumed during the process of

converting fibrinogen to fibrin. A unit of thrombin will coagulate 1mL of a standard fibrinogen solution in 15 seconds at 28°C.

Tissue Thromboplastin (Formerly Factor III)

Tissue thromboplastin is the term given to any nonplasma substance containing lipoprotein complex from tissues. These tissues can be from the brain, lung, vascular endothelium, liver, placenta, or kidneys; these tissue types are capable of converting prothrombin to thrombin.

Ionized Calcium (Formerly Factor IV)

The term *ionized calcium* has replaced the term factor IV. Ionized calcium is necessary for the activation of thromboplastinand for the conversion of prothrombin to thrombin. Ionized calcium is the physiologically active form of calcium in the human body, and only small amounts are needed for blood coagulation. A calcium deficiency would not be expressed as a coagulation dysfunction, except in cases of massive transfusion.

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 the clotting process and is essential to the later stages of thromboplastin formation.

Factor VII (Proconvertin)

Factor VII, a beta-globulin, is not an essential component of the intrinsic thromboplastin-generating mechanism. It is not destroyed or consumed in clotting and is found in both plasma and serum, even in serum left at room temperature for up to 3 days. The action of factor VII is the activation of tissue thromboplastin and the acceleration of the production of thrombin from prothrombin. This factor is reduced by vitamin K antagonists.

Factor VIII (Antihemophilic Factor)

This factor, an acute-phase reactant, is consumed during the clotting process and is not found in serum. Factor VIII is extremely labile, with a 50% loss within 12 hours at 4°C invitro and a similar 50% loss in vivo within 8 to 12 hours after transfusion. In addition, factor VIII can be falsely decreased in the presence of lupus anticoagulant (LA).Factor VIII can be subdivided into various functional components .The total molecule, consisting of both a high–molecular-weight fraction and a low–molecular-weight fraction, is described by the nomenclature VIII/vWF. Factor VIII/vWF

consists of two major moieties. The high– molecular-weight moiety consists of the vWF, VIIIR:RCo, and VIIIR:Ag components. The low–molecular-weight moiety consists of theVIII:C and VIIIC:Ag components. Factor VIII:C has procoagulant activity as measured byclotting assay techniques. Factor VIII/vWF multimers formionic bonds with factor VIII:C and transport VIII:C in thecirculation. Factor VIIIC:Ag is a procoagulant antigen as measured by immunological techniques using antibodies for factor VIII:C.Factor VIIIR:Ag is a related factor VIII antigen that has be enidentified using immunological techniques employing heterologous antibodies to VIII/vWF.

Factor VIIIR:RCo demonstrates ristocetin cofactor activity, which is required for the aggregation of human platelets induced by the antibiotic ristocetin factor VIII/vWF is factor VIII-vWF. Endothelial cells are known to synthesize and secrete VIII/vWF multimers.

Factor IX (Plasma Thromboplastin Component)

Factor IX is a stable protein factor that is neither consumed during clotting nor destroyed by aging at 4°C for 2 weeks. Itis an essential component of the intrinsic thromboplastin generating system, where it influences the amount rather than the rate of thromboplastin formation.

Factor X (Stuart Factor)

This alpha-globulin is a relatively stable factor that is not consumed during clotting. Together with factor V, factor X in the presence of calcium ions forms the final common pathway through which the products of both the extrinsic and intrinsic thromboplastin-generating systems merge to form the ultimate thromboplastin that converts prothrombin to thrombin. The activity of factor X appears to be related to factor VII.

Factor XI (Plasma Thromboplastin Antecedent)

Factor XI, a beta-globulin, can be found in serum because It is only partially consumed during the clotting process. This factor is essential to the intrinsic thromboplastin-generating mechanism.

Factor XII (Hageman Factor)

Factor XII is a stable factor that is not consumed during the coagulation process. Adsorption of factor XII and kininogen (with bound prekallikrein and factor XI) to negatively charged surfaces such as glass or sub endothelium (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 a feedback mechanism, kallikrein (activated Fletcher factor) cleaves partially activated factor XIIa molecules adsorbed onto the sub endothelium to produce a more kinetically effective form of XIIa.

Factor XIII (Fibrin-Stabilizing Factor)

Fibrin-stabilizing factor in the presence of ionized calcium produces a stabilized fibrin clot.

Fine fibrin clots factor XIII calcium ions stable fibrin clot(Turgeon , 2010).

1.2.1.3.3. Coagulation Pathways

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 the two pathways and subsequently catalyzes the conversion of prothrombin to thrombin (Hoffbrand *et al.*,2006).

The Extrinsic Coagulation Pathway

The extrinsic pathway (Fig. 23.9) is initiated by the entry of tissue thromboplastin into the circulating blood. Tissue thromboplastin is derived from phospholipoproteins and organelle membranes from disrupted tissue cells. These membrane lipoproteins, termed **tissue factors**, are normally extrinsic to the circulation. Platelet phospholipids are not necessary for activation of the extrinsic pathway because tissue factor supplies its own phospholipids. Factor VII binds to these phospholipids in the tissue cell membranes and is activated to factor VIIa, a potent enzyme capable of activating factor X to Xa in the presence of ionized calcium. The activity of the tissue factor–factor VII complex seems to be largely dependent on the concentration of tissue thromboplastin. The proteolytic cleavage of factor VIIa by factor Xa results in inactivation of factor VIIa. Factor VII participates *only* in the extrinsic pathway. Membranes that enter the circulation also provide a surface for the attachment and activation of factors II and V. The final step is the conversion of fibrinogen to fibrin by thrombin (Hoffbrand *et al.*,2006).

The Intrinsic Coagulation Pathway

The intrinsic pathway (Fig. 23.10) involves the contact activation factors prekallikrein, HMWK, factor XII, and factorXI. These factors interact on a surface to activate factor IXtoIXa. Factor IX are acts with factor VIII, PF 3, 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. Strong negatively charged solids that can participate in the

activation of factor XII include glass and kaolin in vitro as well as elastin, collagen, platelet surfaces, kallikrein, plasmin, and high–molecular-weight kininogen in vivo. Collagen exposed by blood vessel injury greatly influences the rateofreaction Factor XIIa interacts in a feedback loop to convert prekallikreinto additional kallikrein. This reaction is facilitated by the action of HMWK. In the absence of prekallikrein, factor XIIa is generated more slowly. Ionized calcium plays an important role in the activation of certain coagulation factors in the intrinsic pathway. Calcium is not required for the activation of factor XII, prekallikrein,

or factor XI but is necessary for the activation of factor IX (Hoffbrand *et al.*,2006).

Final 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 the platelet surface is formed near platelet-bound factor II molecules. In turn, the platelet-bound Xa/Va complex cleaves factor II into thrombin, factor IIa. The stage is accelerated by factor V and ionized calcium (Hoffbrand *et al.*,2006).

Fibrin Formation

Clotting is the visible result of the conversion of plasma fibrinogen into a stable fibrin clot. Thrombin plays a major role in converting factor XIII to XIIIa and in converting fibrinogen to fibrin. Fibrin formation occurs in three phases: proteolysis, polymerization, and stabilization. Initially, thrombin, a protease enzyme, cleaves fibrinogen, which results in a fibrin monomer, fibrinopeptide A,

and fibrinopeptideB fragments. In the second step, the fibrin monomers spontaneously polymerize end-to-end due to hydrogen bonding.

Finally, the fibrin monomers are linked covalently by factor XIIIa into fibrin polymers. These polymers form a mesh network, and the final fibrin solution is converted to a gel when

More than 25% of the fibrinogen is converted to fibrin. Factor XIII is converted to the active form, factor XIIIa, in two steps. In the first step, thrombin cleaves a peptide from each of the two alpha chains of factor XIII with formation of an inactive intermediate form of factor XIII. In the second step, calcium ions cause factor XIII to dissociate, forming factor XIIIa.Fibrinogen is normally present in the plasma as a soluble molecule. Subsequent to the action of thrombin, fibrinogen is transformed into fibrin, an insoluble gel. This conversion of fibrinogen to a cross-linked gel occurs in several stages factor XIIIa introduces peptide bonds within the polymerized fibrin network. This cross-linking makes the fibrin more elastic and less susceptible to lyses by fibrinolytic agents. Fibrin forms a loose covering over the injured area, reinforces the platelet plug, and closes off the wound. After a short period, the clot begins to retract and becomes smaller and denser. This retraction process is thought to be caused by the action of platelets trapped along with erythrocytes and leukocytes in the clot. As the fibrin filaments gather around the aggregated platelets, the platelets send out cytoplasm processes that attach to the fibrin and pull the fibers closer together (Ciesla,2007).

1.2.1.4. Coagulation Inhibitor

The coagulation inhibition activity displayed by thrombin is the binding of AT-III to inhibit serine proteases and binding to thrombomodulin to activate protein C. In addition, the other activity in this category is the promotion of endothelial cell release of t-PA (O'Shaughnessy *et al.*,2005).

1.2.1.5. Fibrinolysis

Fibrin clots are temporary structures that seal off a damaged area until healing can take place. Fibrinolysis is the physiological process that removes insoluble fibrin deposits by enzymatic digestion of the stabilized fibrin polymers. As healing occurs, the clots themselves are dissolved by plasmin. Plasmin digests fibrin and fibrinogen by hydrolysis to produce progressively smaller fragments. This slow-acting process gradually dissolves away the clot as tissue repair is taking place, with the particulate matter being phagocytized by the mononuclear phagocytic system. Inactive **plasminogen** circulates in the plasma until an injury occurs. The activators of plasminogen consist of endogenous and exogenous groups . Plasminogen activation to plasmin is the result of the activity of a number of proteolytic enzymes. These enzymes, the kinases, are referred to as the **plasminogen activators**. Plasminogen activators are found in various sites, such as the vascular endothelium or lysosomal granules, and biological fluids. At least two forms of tissue activators have been described: those that seem related to urokinase, a urinary activator of plasminogen and those unrelated to urokinase. The activators unrelated to urokinase include thrombin, bacterial products such as streptokinase from beta-hemolytic streptococci, and staphylokinase. Plasma activators of plasminogen include plasma kallikrein, activated plasma thromboplastin antecedents(factor XI), and activated Hageman factor (factorXIIa).It is estimated that 1.5 million Americans have a heart attack Each year. Most are caused by clots that cut off blood flow to the heart muscle. Tissue-type plasminogen activator (t-PA) is present in minute quantities in the vascular endothelium (Hoffbrand *et al.*,2006).

When t-PA encounters a blood clot, t-PA transforms plasminogen to plasmin, and plasmin then degrades the clot's fibrin network. As a result of biotechnology (recombinant DNA), asynthetic tissue type plasminogen has been developed and

is used clinically to treat post myocardial infarction and pulmonary emboli. t-PA is considered by many to be more specific and twice as effective as streptokinase in dissolving clots and has caused fewer side effects. Through its lysis of fibrin or fibrinogen, plasmin is responsible for forming degradation or fibrin split products consisting of intermediate fragments X and Y, and fragments D and E. These fragments exert an anti-thrombin effect, Inhibit the hemostasis system through interference with fibrin monomer polymerization, and interfere with platelet aggregation (Hoffbrand *et al.*,2006).

1.2.1.5. Protease Inhibitors

Because the fibrinolytic system is activated when the coagulation cascade is activated, extra fibrin is degraded and eliminated along with some coagulation factors. Enzymes such as plasmin and kallikrein still circulate until they are eliminated by various mechanisms: liver hepatocytes, mononuclear
phagocytic cells, or serine protease inhibitors present in the plasma. Serine protease inhibitors attach to various enzymes and inactivate them. Serine protease inhibitors include

1. a-Anti plasmin
2. a-Antitrypsin
3. a-Macroglobulin
4. AT-III
5. C1 esterase inhibitor

(O'Shaughnessy *et al.*,2005).

1.2.2. BLEEDING DISORDERS RELATED TO BLOOD CLOTTING

Vascular response and platelet plug formation are responsible for the initial phases of hemostasis. Subsequent to these activities, the clotting factors are initiated to form the fibrin clot. Fibrin formation can occur if the activity of various factors is at least 30% to 40% of normal. Bleeding and defective fibrin clot formations are frequently related to a coagulation factor. Disorders of the blood coagulation factors can be grouped into three categories:

1. Defective production
2. Excessive destruction
3. Pathological inhibition

(Hoffbrand *et al.*,2006).

1.2.2.1. Defective Production

Vitamin K Deficiency

A condition of defective production may be related to a deficiency of vitamin K. The synthesis of vitamin K and dependent factors can be disrupted because of disease or drug therapy (e.g., cephalosporin antibiotics). Vitamin K deficiencies are also encountered in neonates, mala absorption syndrome, biliary obstruction, and patients taking oral anticoagulants. Vitamin K depletion develops within two weeks if both intake and endogenous production are eliminated. Factors II, VII, IX, and X are vitamin K dependent. Factor VII has the shortest half-life and usually declines in the early stages of vitamin K depletion. A mild deficiency of vitamin K may present as an asymptomatic prolongation of a patient's PT assay (Beak.2009).

Severe Liver Disease

Because the liver is the primary site of synthesis of coagulation factor, severe liver disease can cause defective production of coagulation factors. Severe liver disease may produce decreased plasma levels of fibrinogen, although low levels

of fibrinogen rarely produce hemorrhage. In patients with liver disease, thePT is noticeably prolonged, whereas the aPTTs are variable (Beak.2009).

Hereditary Clotting Defects

Classic hemophilia (hemophilia A) and von Willebrand disease are examples of hereditary disorders that represent functionally inactive factor VIII (Hoffbrand *et al.*,2006).

1.2.2.2. Hemophilia

Etiology

Hemophilia has been used as a paradigm for understanding the molecular pathological processes that underlie hereditary disease. The cloning of factor VIII facilitated the identification of mutations that lead to hemophilia A, an inherited deficiency of factor VIII coagulant activity that causes severe hemorrhage. Two types of mutations dominate the defects identified so far: gene deletions and point mutations. Gene deletions are associated with severe hemophilia A in which no factor VIII circulates in the blood. To date, approximately50 deletion mutations in the gene for factor VIII have been characterized at the molecular level, and 34 independent deletion mutations in the factor IX gene have been found to be the cause of hemophilia B. Point mutations, in which a single base in DNA is mutated to another base, represent a second type of mutation that causes hemophilia (Ciesla, 2007).

Epidemiology:

Individuals with hereditary clotting defects may be either genetically homozygous or heterozygous carriers of the trait. The level of factor activity ranges from 0% to 25% in persons homozygous for the trait and from 15% to 100% in persons heterozygous for the trait. Defects of this origin may result from the decreased production of a clotting factor, factor VIII, or the production of functionally inactive molecules of the clotting factor. Hemophilia A, a sex-

linked homozygous disorder expressed in males, occurs in 1 in 10,000 males (Ciesla, 2007).

Path physiology:

Classic hemophiliacs have an intact high–molecular-weight moiety and a deficient low–molecular-weight procoagulantportion. This disorder of procoagulant synthesis expresses itself by decreased factor VIII clotting activity in laboratory assay and a normal bleeding time. Conversely, severe von Willebrand disease has both a decreased high–molecular-weight portion and a decreased low–molecular-weight portion. Plasma levels of factor VIII can be temporarily corrected and the bleeding tendency reversed in most patients following infusion of factor VIII in appropriate blood products. One would expect that correction of the hemostatic defect would place a patient at the same risk of thromboembolism as an unaffected individual, but thromboembolic events in patients with hemophilia A are distinctly un-common. Itself by decreased factor VIII clotting activity in laboratory assay and a normal bleeding time. Conversely, severe von Willebrand disease has both a decreased high–molecular-weight portion and a decreased low–molecular-weight portion. Plasma levels of factor VIII can be temporarily corrected and the bleeding tendency reversed in most patient's following infusion of factor VIII in appropriate blood products. One would expect that correction of the hemostatic defect would place a patient at the same risk of thromboembolism as an unaffected individual, but thromboembolic events in patients with hemophilia A are distinctly uncommon Willebrand disease may complicate other diseases such as lymphoproliferative and autoimmune disorders, and proteolytic degradation of vWF complicates myeloproliferative disorders. Variant forms of von Willebrand disease can be identified by their patterns of genetic transmission and the vWF abnormalities in the plasma and the cellular compartment. Distinguishing

between various subtypes of vonWillebrand disease is important in determining appropriate therapy (Ciesla , 2007).

1.2.2.3. Other Hereditary Deficiencies

A deficiency of factor IX is known as **hemophilia B** or **Christm disease**. This form of hemophilia is non–sex linked and occurs at a rate of 1/50,000 in the general population, with a defective molecule being the usual cause. It is clinically indistinguishable from hemophilia A and must be differentiated by laboratory testing. A deficiency of factor XI is referred to as **hemophilia C**. This genetic defect is an autosomal recessive trait that occurs almost exclusively in people of Jewish descent. It is usually a mild disorder characterized by easy bruising, epistaxis, and hemorrhage in conjunction with trauma. The laboratory results in this defect, as well as those of other hemophilias and von Willebrand disease (Hoffbrand *et al.*,2006).

Hereditary fibrinogen deficiency

may exist as absent or decreased levels of fibrinogen, a fibrinogenemia, or hypofibrinogenemia, respectively. Production of dysfunction al molecules produces dysfibrinogenemia. Afibrinogenemia associated with a severe bleeding tendency but is less common than hypofibrinogenemia. Patients with hypo fibrinogenemiaare usually asymptomatic except in situations of surgery or trauma. Patients with dysfibrinogenemia may be asymptomatic or experience a mild bleeding tendency if heterozygous for the defect, or they may have a severe bleeding tendency if homozygous for the defect. Hereditary deficiencies of the other coagulation factors are relatively rare. Examples of rare defects include **factor XII deficiency**, in which no clinical bleeding tendencies are apparent, and **factor XIII deficiency**, which is associated with spontaneous abortion and poor wound healing (Hoffbrand *et al.*,2006).

1.2.2.4. Disorders of Destruction and Consumption

Enhanced fibrin deposits can result in thrombosis and damage to organs owing to impeded blood flow and ischemia. The fibrinolytic system serves as a protective mechanism against excessive fibrin deposits by lysing both fibrin and fibrinogen. Blood coagulation factors can be destroyed in vivo by Enzymatic degradation or by pathological activation of coagulation with excessive utilization of the clotting factors. Enzymatic destruction can result from bites by certain species of snakes whose venom contains an enzyme that degrades fibrinogen to a defective fibrin monomer. In vivo activation of coagulation by tissue thromboplastin–like materials can produce excessive utilization of clotting factors. Conditions associated with this consumption of coagulation factors include obstetrical complications, trauma, burns, prostatic and pelvic surgery, shock, advanced malignancy, septicemia, and intravascular hemolysis (Beck.2009).

General Features of Fibrinolysis

Primary and secondary fibrinolysis are recognized as extreme complications of a variety of intravascular and extra vascular disorders and may have life-threatening consequences. Primary fibrinolysis is associated with conditions in which gross activation of the fibrinolytic mechanism with subsequent fibrinogen and coagulation factor consumption occurs. The important characteristic of primary fibrinolysisis that no evidence of fibrin deposition occurs. Primary fibrinolysis occurs when large amounts of plasminogen activator enter the circulatory system as a result of trauma, surgery, or malignancies. Although the same clinical conditions may also induce secondary fibrinolysis or DIC, the distinction between the two is essentially in the demonstration of fibrin formation.

In secondary fibrinolysis, excessive clotting and fibrinolytic activity occur. Increased amounts of fibrin split (degradation products (FSPs) and fibrin monomers are detectable because of the action of thrombin on the fibrinogen molecule. This fibrinolytic process is only caused by excessive clotting; therefore, it is a secondary condition. Distinguishing between primary and secondary fibrinolysis (Beck.2009).

1.2.2.5. Disseminated Intravascular Coagulation

Etiology

DIC is actually a complication or intermediary phase many diseases and does not constitute a disorder in itself. It is also known as consumptive coagulopathy or defibrination syndrome. Triggering events that may predispose patients to DIC include alterations in the endothelium, direct activation of fibrinogen, release of thromboplastin like substances, and erythrocyte or platelet destruction. Extra vascular trauma, advanced malignancy, leukemia, and retained fetal syndrome are examples of clinical situations in which tissue thromboplastin can activate coagulation. Infections, most commonly Gram-negative microorganisms, can trigger DIC by producing endotoxins that expose collagen. Stasis, shock, or tissue necrosis can have the same effect. Snakebites may introduce substances that initiate coagulation by direct activation of fibrinogen to form fibrin. Red blood cell or platelet injury may contribute to the consumptive coagulopathy by releasing phospholipids that accelerate coagulation. Red cell injury may be a result of intravascular hemolysis caused by malaria, incompatible transfusion Other causes can include liver disease, lymphoproliferative disorders, and renal disease. In addition, DIC can also be triggered by trauma including shock, hypothermia, and extensive tissue damage, such as in myocardial infarction and eclampsia. It has been associated with multiple surgical,

Obstetrical, and medical disorders. Coma and convulsions can result (Ciesla,2007).

Pathophysiology

The overall DIC process involves coagulation factors, platelets, vascular endothelial cells, fibrinolysis, and plasma inhibitors. This major breakdown of the hemostatic mechanism occurs when the procoagulant factors outweigh the anticoagulant mechanisms. Initiation of DIC can be caused by a number of factors. If vascular endothelial damage results in the exposure of collagen and basement membrane, collagen can activate factor XII. Factor XII has multiple roles in the direct or indirect activation of coagulation including

1. Initiation of the intrinsic clotting cascade resulting in thrombin formation
2. Participation as a cofactor for the conversion of prekallikre into kallikrein
3. Initiation of fibrinolysis

Regardless of the initiating event, DIC is characterized by excess thrombin formation, conversion of fibrinogen to fibrin, and platelet consumption and deposition. Secondary fibrinolysis occurs as a result of fibrin deposition and can decrease plasma coagulation factors, leading to a hemorrhagic diseases.

Thrombin is central to the mechanism of consumptive coagulopathy. The action of thrombin on the coagulation systems includes :

1. Proteolytic cleavage of fibrinogen to fibrin monomer, releasing fibrinopeptides A and B (fibrin monomer may form soluble complexes with fibrinogen or form fibrin thrombi that entrap platelets during thrombus formation)
2. Activation of factor XIII, which stabilizes fibrin by crosslinking

3. Stimulation of platelets, resulting in decreased circulating platelets. These stimulated platelets undergo shape change, adhesion, aggregation, and secretion. The contents of the dense alpha-granules are released, leading to an acquired storage pool deficiency. If, during perhaps a 3-hour span, platelet counts and fibrinogen levels decrease significantly in a critically ill patient.

4. Activation of factors V and VIII; however, thrombin activation results in unstable end products that have decreased factor V and VIII activity

5. Activation of protein C, which degrades factors V and VIII The deposition of fibrin thrombi in the vasculature, primarily in the microvasculature, initiates fibrinolysis. This secondary fibrinolysis is responsible for the hemorrhagic complication of DIC. When the fibrinolytic system is activated, plasminogen is converted to plasmin. Alpha-2 anti-plasmin is the fibrinolytic inhibitor uniquely designed to cope with plasmin. The moreplasmin generated, the more alpha-2antiplasmin the patient consumes. This produces a vicious cycle in which increased activation leads to decreased inhibitors; this, in turn, allows more increased activation to continue. This is known as a positive feedback loop and leads to a situation incompatible with life. Damaged tissue, especially renal cells, releases plasminogen activators that convert plasminogen to plasmin. Plasmin is a proteolytic enzyme that destroys fibrin, fibrinogen, and clotting factors V and VIII. Circulating plasmin may lead to systemic fibrinolysis, causing increased hemorrhagic events. In the microcirculation, plasmin's action is primarily directed against fibrin. In the circulation, the breakdown of fibrin results in FSPs, labeled X, Y, D, and E, which inhibit thrombin and normal platelet, functions. As fibrinogen is degraded by plasmin, FSPs form. Degradation occurs whether the plasmin comes from DIC or primary fibrinogenolysis. FSPs compete with regular fibrinogen molecules for thrombin molecules. This competitive binding makes the thrombin unavailable for the conversion of fibrinogen to fibrin. In this

situation, patients with high FDP/FSP levels have a circulating anticoagulant behaving like heparin. If the FSP level is high, the thrombin clotting time is significantly prolonged and fibrinogen quantitation is low. The second effect is on platelets. These split products coat the platelet surface, blocking the receptor site needed for further platelet activation.

When pathological fibrinolysis occurs, not only are factors destroyed, but, through the destruction of fibrinogen, a profound anti-clotting effect inhibits secondary hemostasis and platelets. If the fibrinolytic system is activated, it will contribute to the consumption of many coagulation factors. Plasmin, the primary proteolytic enzyme of fibrinolysis, directly attacks and destroys them. This becomes another form of consumptive coagulopathy originating from an entirely differen toccur with the same end result. When systemic clotting activation begins, the body usually attempts to stop it (Hoffbrand *et al.*,2006).

Clinical Signs and Symptoms

The DIC phenomenon has varied clinical and laboratory manifestations (Table 24.15) owing to the many physiological abnormalities associated with the syndrome. DIC maybe acute or sub-acute (chronic). Chronic DIC is more common than acute DIC but is often more difficult to diagnose.

Chronic DIC can convert to acute consumption if the balance of procoagulant-anticoagulant is lost.

Either form may initially be seen with varying degrees of thrombosis and hemorrhage, but bleeding is usually the major symptom, particularly in acute cases. Both hemorrhagic and thrombotic complications may accompany DIC, often being manifested in the same patient. Thrombosis may predominate in chronic or low-grade DIC. Thrombotic complications can include deep venous thrombosis. Acute DIC is severe and often life threatening. Its onsets rapid, and both fibrinogen and platelets may be depleted. Patients with chronic DIC may

have mild manifestations of the disorder or be recognizable only by laboratory data. Hemorrhagic complications are also seen but are generally milder than in acute DIC. Clinical manifestations of DIC include petechiae, purpura, hemorrhagic bullae, surgical wound bleeding, traumatic wound bleeding, venipuncture site bleeding, arterial line oozing, and subcutaneous hematomas. TTP is a condition that is similar to DIC. In addition, pediatric respiratory distress syndrome (PRDS), adult respiratory distress syndrome (ARDS), HUS, preeclampsia or frank eclampsia (Ciesla, 2007).

Laboratory Findings

Although the quantitative measurement of FSPs cannot distinguish between primary and secondary fibrinolysis, such measurement plays the major role in diagnosing and monitoring these conditions. Laboratory diagnosis of DIC requires the availability of tests that are rapid and simple to perform. There is no single test that confirms the diagnosis, but rather a combination of tests. Because DIC is a dynamic process, values from tests performed a single time, whether normal or abnormal, cannot be used as diagnostic indicators.

Sequential testing is necessary to provide an accurate\diagnosis and effectively manage therapy. The most important consideration in the treatment of DIC is the resolution of the underlying disease or triggering event (Dacie and Lewis, ,2011).

Tests for Fibrinolysis and DIC

Because the manifestations of fibrinolysis and DIC are extremely variable, diagnosis depends on laboratory testing. Coagulation assays such as the platelet count, fibrinogen levels, FSP test, factor V assay, ethanol gelation test, and thrombin time–reptilase test can all be useful. Prekallikrein andAT-III have also been suggested to be of prognostic value. The key feature is an elevation of circulating fibrinogen-FSPs. Typical results in DIC include prolonged aPTT, PT,

and thrombin time and an increased level of D-dimers. Fibrinogen levels and the total platelet count may vary, although thrombocytopenia and a decrease in fibrinogen are common. The platelet count decreases earlier than fibrinogen in endotoxin-induced DIC. The reverse is true when tissue factor release is responsible, such as in obstetrical accidents or trauma. Excessive fibrinolysis with the release of FSPs occurs secondary to intravascular fibrin formation. Although the presence of FSPs is characteristic, the finding is not specific for DIC and cannot be used as the sole criterion for diagnosis (Dacie and Lewis, 2011).

1.2.2.6. THE HYPERCOAGULABLE STATE

Systemic inflammation has long been recognized as being associated with hyper-coagulability. It commonly occurs in patients with DIC in severe sepsis. Recently, the molecular basis of the influence of inflammation has been recognized. Most of the hyper coagulable effects of inflammation are mediated by inflammatory cytokines, including IL-1, IL-6and tumor necrosis factor (TNF).

The processes of coagulation, thrombosis, and inflammation do not occur in isolation. There is interaction between these systems. Thrombosis and coagulation can act as triggers for inflammation, and severe or systemic inflammatory responses can trigger coagulation. A laboratory assay,

High-sensitivity C-reactive protein (hsCRP), may herald an impending acute thrombotic event (Turgeon, 2010).

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 state**.

Thrombosis is promoted by vascular damage, by retarded blood flow, and by alterations in the blood that increase the likelihood of clotting. A variety of high-

and low-incidence disorders are associated with thrombosis . A number of factors may contribute to hyper coagulation (Turgeon, 2010).

1.2.2.6.1. Primary States of Hypercoagulability

Hyper coagulable states include various inherited and acquired clinical disorders characterized by an increased risk for thromboembolism. Primary hyper coagulable states include relatively rare inherited conditions that lead to disordered endothelial cell thromboregulation. These conditions include decreased thrombomodulin dependent activation of AP. The major inherited inhibitor disease states include ATIII 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, 2010).

1.2.2.6.2. Secondary States of Hypercoagulability

Secondary hyper coagulation states may be seen in a number of heterogeneous disorders. In many of these conditions, endothelial activation by cytokines leads to the loss of normal vessel-wall anticoagulant surface functions, with conversion to a pro inflammatory 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 increase in procoagulant functions, an inhibition of fibrinolysis, and a down regulation of the three major physiologic anticoagulant systems of protein C, AT-III, and tissue factor inhibitor (Turgeon, 2010).

Laboratory Assessment

A panel of assays is required to assess hyper coagulability. Functional screening tests include the following:

- PT
- Activate and partial thromboplastin time (aPTT)
- Lupus anticoagulant (LA) screening
- Factor VIII and fibrinogen (factor I) assays
- APC assay
- Protein C and protein S assays
- D-dimer screening test

In addition, acute-phase reactants (e.g., C-reactive protein [CRP]) may be assayed.

Traditionally, the APC resistance assay identifies patient insensitivity to APC. The assay is based on the aPTT assay with and without reagent APC. The aPTT in the presence of APC (CaCl2/APC) is divided by the unaltered (CaCl2) aPTT to yield a unit less ratio. A ratio of greater than 2 (a longer clotting time) generally indicates an unaffected condition. A ratio of less than 2 (a shorter clotting time) indicates a potential factor V (Leiden) mutation and resistance to APC.

Factor V–deficient plasma may be added to the test system to correct for any existing factor deficiencies. The APC resistance assay may be affected by other conditions (e.g., LA, elevated factor VIII and fibrinogen levels, oral contraceptive use, or pregnancy).Another method of testing for the mutation is by a dilute Russell's viper venom time (DRVVT) based test. The DRWT method avoids limitations inherent in the aPTT –based method, which requires a normal baseline aPTT and may

be affected by high concentrations of factor VIII, LA, and anticoagulant therapy. The DRWT also eliminates the technical requirement of pre diluted patient samples with factor V deficient plasma (Turgeon , 2010).

Genetic Testing

Single-nucleotide polymorphisms (SNPs) are major contributors to genetic variation, comprising approximately 80%of all known polymorphisms. Their density in the humangenome is estimated to be on average 1 per 1,000 base pairs. APC-resistant patients may be confirmed for factor V (Leiden) mutation by DNA PCR amplification of a segment of the potentially affected gene. General population screening is not recommended. At this point, the recommendations for testing focus primarily on individuals younger than age50 who have already had an idiopathic thrombotic event.

The three most common assays ordered to investigate a genetic predisposition to thrombosis are

1. Factor V (Leiden)
2. Prothrombin mutation
3. Methy lenetetrahydrofolate reductase enzyme (MTHFR)

Prothrombin (factor II) mutation leads to an increased risk of cerebral vascular thrombosis. MTHFR deficiency leads to hyper homocysteinemia that may injure the vascular endothelium. It may play a role in venous thromboembolism. These three assays can be performed simultaneously by analyzing genomic DNA in peripheral blood mononuclear cells using polymerase chain reaction (PCR) (O'Shaughnessy *et al.*,2005).

1.2.2.7. Circulating Anticoagulants

Acquired inhibitors of clotting proteins, also known as circulating anticoagulants, inactivate or inhibit the usual procoagulant activity of

coagulation factors. Inhibitors are frequently characterized as specific, those directed against a coagulation factor, or nonspecific, those directed against a complex of factors, such as the LA. The majority of these inhibitors exhibit biochemical properties, suggesting that they are immunoglobulin. Inhibitors may arise following transfusion of blood products or inpatients with no previous hemostatic disorders. Acquired inhibitors can be a significant cause of hemorrhage. Specific inhibitors against factors II, V, VII, VIII, IX, XII,XIII, and vWF have been detected in patients with individual factor deficiencies. However, some inhibitors of factors

II, V, VII, IX, XII, and vWF have been observed in patients having no deficiencies of coagulation factors. Patients with acquired specific inhibitors may exhibit hemorrhagic episodes, whereas nonspecific inhibitors are not generally associated with bleeding tendencies (O'Shaughnessy *et al.*,2005).

Etiology

The incidence of circulating anticoagulants has been bench marked at 0.75% of the general population, but certain patient populations have a higher incidence of inhibitor development. Inhibitors, found in both serum and plasma, are not inactivated by heating at 56°C for 30 minutes and remain stable when stored at –20°C. Inhibitors are more stable than clotting factors and more tolerant of changes in pH and temperature. Inhibitors may remain in the circulation for months and in some instances have been found inpatients years after development (O'Shaughnessy *et al.*,2005).

1.2.2.8. Specific Inhibitors

Antiphospholipid Antibodies (Lupus Anticoagulant and Anticardiolipin Antibodies)

The **lupus anticoagulant (LA)** occurs in approximately 30% to40% of patients with SLE. LA is the most common coagulation inhibitor found in SLE patients,

although these patients may have other acquired inhibitors as well. LA occurs in the presence of disease states other than SLE, such as acquired immunodeficiency syndrome (AIDS) and malignancy, and in procainamide, hydralazine, or chlorpromazine therapy. Although LA exhibits an anticoagulant effect, it is rarely associated with bleeding.LA, an IgM, IgG, or IgA immunoglobulin, interferes with phospholipid-dependent coagulation reactions in laboratory assays but does not inhibit the activity of any specific coagulation factor. LA is an inhibitor that prolongs phospholipid dependent clotting tests in vitro. LA is the most common cause of prolonged aPTT. In 1995 the Subcommittee on Lupus Anticoagulant Standardization Committee published criteria for the diagnosis of LA. This guideline recommends at least two screening tests based on different assay principles. In addition, a mixing study for the verification of the presenceof a coagulation inhibitor and a confirmation test for the documentation of phospholipids dependency should also be performed. All assays should be performed on citrate anticoagulated specimens that are platelet poor and free of underlying defects.

In comparison, anti cardiolipin antibodies (ACAs), IgM,IgG, or IgA immunoglobulin, bind to the phospholipids cardiolipin in the presence of beta 2-GP 1-cardiolipin complex.

It may be detected in healthy patients and in those with a variety of conditions (e.g., SLE).

LA and ACA are risk factors for thrombosis but the mechanism of action is unclear. **(**Frenette PS .1996).

1.2.2.8.1. Antiphospholipid Syndrome

The APS is defined by the persistent presence of anti phospholipidantibodies. APS is a prothrombotic disorder with various manifestations in patients with a

history of recurrent venous or arterial thromboembolism or a history of miscarriages. APS is an important cause of acquired thrombophilia.

APS can occur alone or in association with other autoimmune conditions, particularly SLE. The core clinical manifestation is thrombosis. In women, it can be associated with recurrent fetal loss. Fetal morbidity and mortality maybe due to factors such as placental thrombosis and placental inflammation due to complement activation. Antiphospholipid antibodies include

- LA
- Anti cardiolipin antibodies
- Anti-b2-glycoprotien-1 antibodies. In the laboratory, elevated levels of antibody are required to establish a diagnosis. The predominant antigenic targets in APS areb 2-GP I and prothrombin. Complement activation is suspected because increased complement activation products have been found in APS patients who have suffered from a cerebral ischemic event. Dysregulated platelet activation may contribute to thrombotic manifestations. Elevated levels of platelet-derived thromboxane metabolic breakdown products have been demonstrated in the urine of APS patients. **(**Frenette PS .1996).

1.2.2.8.2. Factor VIII Inhibitor

Factor VIII inhibitors are the most common specific factor inhibitors. Inhibitors of factor VIII develop in 10% to 15%of patients with factor VIII deficiency (hemophilia A), and the majority occur in patients with severe hemophilia (those having less than 1% factor VIII activity). Inhibitors have developed in patients exposed to factor VIII after as few as10-exposure days but may develop after several hundred days. Approximately 65% of patients with hemophilia who develop inhibitors do so before the age of 20. Non hemophiliac women have been reported to develop factor VIII inhibitors during the postpartum period, most frequently after the birth of their first child. Patients with underlying

immunological disorders such as RA, SLE, drug allergies, ulcerative colitis, and bronchial asthma also have an increased tendency to develop factor VIII inhibitors. Many patients have been Inhibitors against vWF occur in patients with vonWillebrand disease, underlying diseases such as malignancy or SLE, and in previously healthy persons. A familial tendency for the development of vWF inhibitors has been noted. (Turgeon, 2010).

1.2.2.8.2. Factor IX Inhibitor

Inhibitors are found in approximately 2% to 3% of factor IX–deficient (hemophilia B) patients, but the incidence of inhibitors in severe hemophilia B may be as high as 12%.Although these inhibitors are predominantly a result oftransfusion of blood products, spontaneous inhibitor formation has been reported. (Turgeon, 2010).

1.2.2.8.3. Factor V Inhibitor

Factor V inhibitors are rare and are not generally associated with hereditary factor V deficiency. Some patients have had exposure to streptomycin but no causal relationship has been established. (Turgeon, 2010).

1.2.2.8.4. Fibrinogen, Fibrin, and Factor XIII Inhibitors

Inhibitors of fibrinogen, fibrin, and factor XIII have been reported. These inhibitors have occurred following plasma transfusions or appeared spontaneously. Some patients have a common denominator of taking isoniazid, an antituberculosis drug. (Turgeon, 2010).

1.2.2.8.5.Factor II, VII, IX, and X Inhibitors

Factor II, VII, IX, and X inhibitors are rare. The causes for factor inhibitor development are varied and include congenital deficiencies, immune disorders, and amyloidosis. (Turgeon, 2010).

1.2.2.8.6. Factor XI and XII Inhibitors

Inhibitors of factors XI and XII have been reported infrequently in patients with SLE, Walden ström macroglobulinemia, and other disorders, as well as with chlorpromazine administration. (Turgeon, 2010).

Clinical Presentation

The LA is the most commonly acquired and has an interesting presentation. In the absence of other hemostatic abnormalities, the LA is rarely associated with bleeding tendencies, even with surgical procedures. Bleeding episodes in these patients are usually the result of thrombocytopenia or another anomaly. Paradoxically, patients with LA are at increased risk for arterial and venous thromboembolism. Venous thrombosis involving the leg veins, with associated pulmonary emboli, is the most frequent complication. Spontaneous abortion and intrauterine deaths are also increased in patients with LA. The presence of a specific factor inhibitor can be suspected in patients with no history of bleeding episodes who experience hemorrhage from various sites or in hemophiliac patients not responsive to their usual dosage of blood product infusion. Bleeding episodes in hemophiliac patients with inhibitors do not appear to be any more frequent or severe than in patients without inhibitors. When hemorrhagic events do occur, treatment of a patient with inhibitor is difficult

No hemophiliac patients with acquired inhibitors of factor VIII can have major bleeding requiring transfusion. Patients with inhibitors to vWF, factor XI, and factor XII do not generally exhibit a hemorrhagic tendency. However, therapy

for these patients can be complicated by the presence of the inhibitor. Patients with acquired factor IX inhibitors have clinical courses similar to hemophilia patients with inhibitors. Factor V inhibitors may cause clinical bleeding, although the degree of hemorrhage varies considerably. Inhibitors of factors XIII, II, VII, IX, and X; fibrin; or fibrinogen can result in serious hemorrhagic events.

Laboratory Findings

Prolonged PT or aPTT are classic laboratory findings. Incubation of patient's plasma with normal plasma at37°C (mixing study) and determination of aPTT and PT may detect the presence of an inhibitor. The mixing study will be prolonged in the presence of an inhibitor. Inhibitors are more time and temperature stable than their specific clotting factors. To quantities the levels of inhibitors, the Bethesda assay is most commonly used in the United States. One Bethesda unit is defined as the amount of antibody that will neutralize 50% of the inhibitor activity in a mixture of equal parts of normal plasma and antibody containing plasma that has been incubated

for 2 hours at 37°C.Detection of anti-phospholipid antibody is based on prolongation of phospholipid-dependent coagulation assays Antiphospholipid antibody is considered one of the most common causes of a prolonged aPTT. Assays include the Russell's viper venom time, kaolin clotting time, platelet neutralization procedure, and tissue thromboplastin inhibition test (Dacie and Lewis , 2011).

1.2.2.9. Impaired Fibrinolysis

Impaired fibrinolytic mechanisms have been noted to be both genetic and acquired in their origin. Impairment of fibrinolysis may predispose an individual to thrombosis. Patients with type II hyper lipoproteinemia caused by familial

hypercholesterolemia demonstrate impairment of fibrinolysis. A high incidence of recurrent thrombosis has been noted inpatients with hereditary deficiencies of protein C or AT-III. Protein S deficiency also joins the group of other plasma protein deficiencies associated with inherited thrombophilia. Deficiencies of inhibitors to factors VIII and V have also been correlated with recurrent thrombosis. Francis CW, Marder VJ (1986).

1.2.2.9.1. Protein C Deficiencies

Protein C activity has been demonstrated to be related to the commonly occurring thrombotic episodes in patients with an inherited deficiency of protein C and protein S. However, the hyper coagulable state in patients with proteinuria is not caused by decreased levels of protein C. Elevated protein

C levels may represent a protective mechanism to the hypercoagulable state in patients with proteinuria becausethe anticoagulant activities of AT-III and protein C. Deficiencies of protein C and protein S can be acquired or congenital. Acquired deficiencies occur in DIC, severe liver disease, vitamin K deficiency, and oral anticoagulation therapy. Congenital deficiencies are transmitted in an autosomal dominant fashion. Thrombotic complications usually involve the venous system, although more recently protein S has been associated with arterial as well. Several types of protein C defects have been reported. Type I protein C deficiency is characterized by low antigenic and functional levels of the protein. In those with type II deficiency, the antigenic level of protein C is normal, but the function of the molecule is impaired. Two sub types of the type II defect have been described: classic type IIa, in which both chromogenic and clotting functional assays are abnormal, and type IIb, in which only the clotting functional method is abnormal. Protein C deficiencies should, accordingly, be screened by using a protein C functional assay (clot based or chromogenic),

because this will detect both types I and II. Once a low level of protein C activity is determined,

An immunological assay should be performed to distinguish type I from type II protein C deficiency.(Esmon. CT .1990).

1.2.2.9.2. Activated Protein C Resistance

APC resistance, a new discovery, has been added to the list of causes of thrombotic disease. APC resistance may be caused by an inherited deficiency of an anticoagulant factor that functions as a cofactor to APC. APC resistance appears to be inherited as an autosomal dominant trait, suggesting that a single gene is involved. It is possible that patients with severe APC resistance are homozygous for the genetic defect, whereas an APC response closer to the normal range indicates heterozygosity. The genetically determined defect in anticoagulation characterized by resistance to APC is highly prevalent in patients with venous thrombosis. This defect appears to be at least 10 times more common in such patients than any of the other known inherited deficiencies of anticoagulant Proteins.(Esmon. CT .1990).

The anticoagulant cofactor that corrects inherited APC resistance is identical to un activated factor V. APC-resistant plasma contains normal levels of factor V procoagulant, which suggests that APC resistance may be caused by a selective defect in an anticoagulant function of factor anticoagulation characterized by resistance to APC is highly prevalent in patients with venous thrombosis. This defect appears to be at least 10 times more common in such patients than any of the other known inherited deficiencies of anticoagulant proteins. The anticoagulant cofactor that corrects inherited APC resistance is identical to inactivated factor V. APC-resistant plasma contains normal levels of factor V

procoagulant, which suggests that APC resistance may be caused by a selective defect in an anticoagulant function.

1.2.2.9.3. Protein S Deficiency

Familial studies indicate that patients with a deficiency of protein S have an increased incidence of thrombosis. Early descriptions indicate that protein S deficiency is much more common than either protein C or AT-III deficiency. The congenital deficiency of protein S is associated with an increased risk of recurrent juvenile venous and arterial thrombo embolism. The association of a thrombotic diathesis with acquired protein S deficiency is less clear cut.(deFouw NJ, *et al.*, 1986).

1.2.2.9.4. Congenital Protein S Deficiency

Diagnosis of protein S deficiency differs significantly from that of vitamin K–dependent plasma proteins owing to protein S binding with C4b-BP and repartitioning between free (functional) and bound (nonfunctional) forms. The classification of congenital protein S is based on the comparison of functional and antigenic (free and total) as well as C4b-BP levels. Currently, three types of congenital deficiencies have been identified: type I, low

functional and antigenic protein S levels; type II, low functional protein S levels with a normal antigenic repartition(molecule dysfunctional); and type III, low functional protein S levels corresponding to a decrease in free antigenic protein S along with a normal C4b-BP. However, a decrease in free/functional protein S caused by increased synthesis of C4b-BP can occur transiently during acute phase reactions. (Fair DS, *et al* (1986).

A protein S functional assay should be used to screen for all types of protein S deficiencies. Antigenic levels of both free and total forms of protein, as well as C4b-BP, will then be determined to differentiate types I, II, and III.(Fair DS, *et al* (1986).

1.2.2.9.5. Anti-thrombin III Deficiency

Hereditary defects of AT-III may be caused by quantitative or qualitative defects. Quantitative deficiency of AT-III is transmitted as an autosomal dominant disorder. Type I(quantitative) deficiencies represent the majority of cases Familial studies reveal that severe thromboembolic problems usually begin to be manifested in late adolescence or early adulthood. Manifestations of AT-III deficiency are rare in infancy. Women with the deficiency have a much higher incidence of thrombosis because pregnancy, delivery, and oral contraceptives are causative factors. Defects of a qualitative nature (type II deficiency) are often characterized by decreased heparin cofactor activity. This functional manifestation of defective AT-III is not associated with a reduction in molecular concentration. More than half of patients with type II deficiency develop recurrent deep venous thrombosis. (Turgeon, 2010).

1.2.2.9.6. Decreased AT-III Levels: Congenital

The relative incidence of congenital AT-III deficiency is between 1:2,000 and 1:5,000. AT-III deficiency is inherited as an autosomal dominant disorder. Homozygotes have not been reported in AT-III deficiency. Patients manifest signs and symptoms of between 10 and 30 years of age, their first thrombotic event. An initial event is spontaneous in approximately half of patients. Women frequently experience manifestations during pregnancy or because of oral

contraceptive use. Decreased levels of AT-III usually correlate with the severity of venous thrombosis. Arterial thrombosis is a less common finding in AT-III deficiency. (Turgeon, 2010).

1.2.2.9.7. Decreased AT-III Levels: Acquired

Acquired AT-III deficiency can be caused by decreased synthesis, increased consumption, or other disorders; it can also be drug induced. The associated disorders are decreased synthesis: arteriosclerosis, cardiovascular disease chronic hepatitis, cirrhosis, type II diabetes mellitus Increased consumption: DIC, homocystinuria, nephroticsyndrome, postoperative, postpartum, protein-losingenteropathy, pulmonary embolism, stroke, thrombophlebitis Drug induced: fibrinolysin, heparin, L-asparaginase, oral contraceptives other disorders: burns, malignancies. (Turgeon, 2010).

1.2.3.Laboratory quality control

is designed to detect, reduce, and correct deficiencies in a laboratory's internal analytical process prior to the release of patient results, in order to improve the quality of the results reported by the laboratory. Quality control is a measure of precision, or how well the measurement system reproduces the same result over time and under varying operating conditions. Laboratory quality control material is usually run at the beginning of each shift, after an instrument is serviced, when reagent lots are changed, after calibration, and whenever patient results seem inappropriate. Quality control material should approximate the same matrix as patient specimens, taking into account properties such as viscosity, turbidity, composition, and color. It should be simple to use, with minimal vial to vial variability, because variability could be misinterpreted as systematic error in the method or instrument. It should be stable for long periods of time, and available

in large enough quantities for a single batch to last at least one year. Liquid controls are more convenient than lyophilized controls because they do not have to be reconstituted minimizing pipetting error.(Tietz.1987)

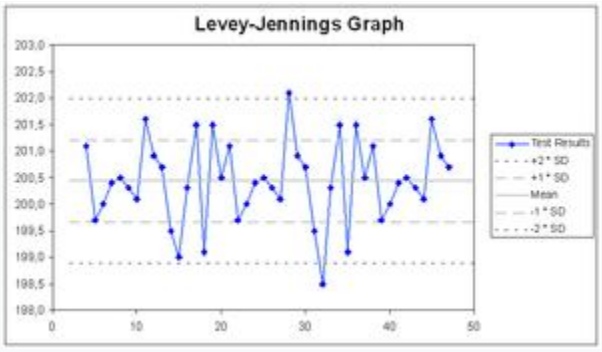
1.2.3.1.Interpretation

Interpretation of quality control data involves both graphical and statistical methods. Quality control data is most easily visualized using a Levey-Jennings chart. The dates of analyses are plotted along the X-axis and control values are plotted on the Y-axis. The mean and one, two, and three standard deviation limits are also marked on the Y-axis. Inspecting the pattern of plotted points provides a simple way to detect increased random error and shifts or trends in calibration.(Grant and Lavenworth.1988).

1.2.3.2.The control chart

The control charts: a statistical approach to the study of. manufacturing process variation for the purpose of improving the economic effectiveness of the process. These methods are based on continuous monitoring of process variation. The control chart, also known as the 'Shewhart chart' or 'process-behavior chart' is a statistical tool intended to assess the nature of variation in a process and to facilitate forecasting and management. A control chart is a more specific kind of a run chart. The control chart is one of the seven basic tools of quality control which include the histogram, pare to chart, check sheet, control chart, cause and effect diagram, flowchart and scatter diagram. Control charts prevents unnecessary process adjustments; provides information about process capability; provides diagnostic information and it is a proven technique for improving productivity

Levey–Jennings chart



An example of a Levey–Jennings chart with upper and lower limits of one and two times the standard deviation.

Levey-Jennings chart is a graph that quality control data is plotted on to give a visual indication whether a laboratory test is working well. The distance from the mean is measured in standard deviations (SD). It is named after S. Levey and E. R. Jennings who in 1950 suggested the use of Shewhart's individuals control chart in the clinical laboratory. On the x-axis the date and time, or more usually the number of the control run, are plotted. A mark is made indicating how far away the actual result was from the mean (which is the expected value for the control). Lines run across the graph at the mean, as well as one, two and three standard deviations to either side of the mean. This makes it easy to see how far off the result was (Westgard *.et al.*,1981).

Rules, such as the Westgard rules can be applied to see whether the results from the samples when the control was done can be released, or if they need to be rerun. The formulation of Westgard rules were based on statistical methods. Westgard rules are commonly used to analyse data in Shewhart control charts. Westgard rules are used to define specific performance limits for a particular assay and can be used to detect both random and systematic errors. Westgard rules are programmed in to automated analyzers to determine when an analytical

run should be rejected. These rules need to be applied carefully so that true errors are detected while false rejections are minimized. The rules applied to high volume chemistry and hematology instruments should produce low false rejection rates (Westgard.*et al.*,1986).

1.2.3.3. Quality Assurance and Quality control:

It covers the part of quality assurance which primarily concerns the control of errors in the performance of tests and verification of test results. Quality control should cover the aspects of every procedure within the laboratory, it must be practical, achievable and affordable. Laboratory QC is designed to detect, reduce and correct deficiencies in a laboratory's internal analytical process prior to release of patients results and improve the quality of results reported by the laboratory. Quality control system divided into two main types, and these are

Internal quality control

External quality control (assessment)

Tietz, N.W. (1987).

1.2.3.4.Terms Used in Clinical Quality Control

In the clinical hematology laboratory, several terms are used to describe different aspects of Quality Assessment:

1. Accuracy describes how close a test result is to the true value. This term implies freedom from error. Reference samples and standards with known values are needed to check accuracy.

2. Calibration is the comparison of an instrument measure mentor reading to a known physical constant.

3. Control (Known) represents a specimen that is similar in composition to the patient's whole blood or plasma. The value of a control specimen is known. A

control specimen must be carried through the entire test procedure and treated in exactly the same way as any unknown specimen it must be affected by all the variables that affect the unknown specimen. Control specimens are tested daily or in conjunction with the unknown patient specimen. Controls are the best measurements of precision and may represent normal or abnormal test values.

4. Precision describes how close the test results are to one another when repeated analyses of the same materials are performed. Precision refers to the reproducibility of test results. It is important to make a distinction between precision and accuracy. The term accuracy implies freedom from error; the term precision implies freedom from variation

5. Standards are highly purified substances of a known composition .A standard may differ from a control in its overall composition and in the way it is handled in the test. Standards are the best way to measure accuracy. Standards are used to establish reference points in the construction of graphs (e.g., manual hemoglobin curve) or to calculate a test result. Tietz, N.W. (1987).

1.2.3.5.systemic variations

It is a degree of deviation or shifting away from the mean or true target value. Also systemic variation can be thought of as reflection of the accuracy of a diagnostic test

Aging reagents (Expired reagents), Aging calibrators, Instrument components, Optical changes, Wear and tear of instrument, reagent lot variability, and Calibration differences

2-random variations

Random variation could be equated to the reproducibility or precision of an assay it could be due to:

Fluctuation in electrical and or optical sensor, variation in pipetting and dispensing system, reagent stability, temperature fluctuation, and evaporation of specimens and reagents. Tietz, N.W. (1987).

1.2.3.6.Coagulation analyzer

neamly blood coagulation analyzer, is to the blood clots and stop bleeding for laboratory medical apparatus and instruments. Stop bleeding and thrombosis molecular markers detection indicators and clinical various diseases are closely linked, such as atherosclerosis, cardiovascular disease, diabetes, arteriovenous thrombosis, thromboangiitis obliterans, pulmonary embolism, pregnancy-induced hypertension syndrome, diffuse intravascular coagulation, hemolysis urine poison syndrome, chronic obstructive pneumonia, etc. The theory of traditional Chinese medicine for activating blood circulation and eliminating stasis about treatment and research on the work also involves bleeding and blood clots. Use blood clots apparatus to blood clots and stop bleeding for laboratory become necessary. And blood analyzer, blood clots instrument is divided into two types of automatic and semi-automatic

At present the which can develop thrombosis/hemostatic ingredients detection method mainly has solidified method, the substrate show color method, immune method, latex aggregation method, etc. In the table can be noticed, in thrombosis/hemostatic inspection of the most commonly used prothrombin time (PT), activate part blood coagulation analyzerlive enzyme (APTT), time fibrinogen (FIB), thrombin time (TT), endogenous clotting factor, exogenous clotting factors, high molecular weight heparin, low molecular weight heparin, protein C, protein S, etc can all be method for measuring the solidification. So right now, semi-automatic blood clots apparatus basically solidification is

measured to give priority to, and in full automatic blood coagulation analyzer must also have solidified measured. PERLONG MEDICAL.(2012).

In the law of the solidification and can be divided into optical method and the method of magnetic beads of two categories. Due to the optical method is almost covers all kinds of test method, in order to reduce the cost of manufacturing equipment, automatic blood clots in optical method is in the majority. But also have a few senior automatic blood clots instrument measured by method of solidification without the interference of sample double magnetic circuit magnetic beads method, and the other is measured by optical method, and at the same time for the testing.

Optical method blood coagulation analyzer is according to the plasma solidification process to determine the turbidity change blood clotting. According to different optical instrument measuring principle, can divide again scattering method and transmission than muddy than turbidity method two kinds. Scattering turbidity method is better than for inspection according to the sample during solidification in scattering light changes to determine the end of the test. In this method detection channel monochrome light source and light detectors with a 90 O right Angle .PERLONG MEDICAL.(2012).

when in the sample to join blood coagulation activation agent, with samples of fibrin clots forming process, and the scattering of light intensity sample gradually increase. When the sample after completely solidification, the scattering intensity of no longer changes, usually is the starting point of the solidification as 0%, solidification end as 100%, 50% as the coagulation time. Light detectors receiving the optical change, turn it into electrical signals, amplified to be transmitted to monitor on processing, trace solidification curve. Transmission than turbidity method, is based on data for the samples during

solidification in absorbency change to determine the end of the solidification, and scattering turbidity method is different than the method of the light path the same color as a straight line method than arrangement: from the light source the light processed into parallel light, through for the samples to cell into electrical signals after irradiation, amplified monitoring processing. When added to the sample of blood after activation agent, the start of the spectrophotometery is very weak, with the reaction tube fibrin clot formation, specimens spectrophotometry is also increasing, when clots after fully formed, absorbency tend to be constant. Blood clots instrument can be described absorbency and set curve of a point of the corresponding time for the coagulation time. PERLONG MEDICAL.(2012).

1.2.4.Previous studies:

1.2.4.1 Pervious study in Bangkok

The average values by automated method were 35.13 seconds for aPTT. The average values by semi-automated method were 34.50 seconds for aPTT. It revealed that there was no significant between aPTT values determined by both methods

The aPTT assay results from automated and semi-automated methods are not significant different. Recommendation for using of semi-automated method to perform clotting assay tests in small setting is set.(Viroj Wiwanitkit, MD. Chulalongkom University, Bangkok).

1.2.4.2. Previous study in India

Large numbers of activated partial thromboplastin time (aPTT) reagents are sold in the market. The phospholipid content and its source, nature and the amount of activators are highly varied in different aPTT reagents. The present study was

undertaken to evaluate which of the four aPTT reagents commonly used is suitable as an all-purpose reagent for a modest haemostasis laboratory. Four aPTT reagents (reagent A, Platelin LS; reagent B, Silimat; reagent C, Actin FSL reagent D, CK Prest) were tested against 75 different plasmas obtained from normal patients as well as from patients with different haemostatic problems. All the tests were conducted by one of us (S.S.) in duplicates. Different aPTT reagents missed different proportions of mild factor VIII and factor IX deficiency (36.4, 18.2, 4.6 and 13.6% for reagents A, B, C and D, respectively) and showed abnormal results with normal plasmas (i.e. more than 5 s prolongation) (29.2, 25, 8.3 and 12.5% for reagents A, B, C and D, respectively). All the reagents faithfully picked up moderate and severe factor VIII and factor IX deficiency. There was no difference among the four aPTT reagents regarding their ability to prolong aPTT to therapeutic dosage of heparin or in their ability to give comparable factor VIII or factor IX levels in one-stage aPTT-based assays. There were differences in aPTT reagents in their ability to pick up mild deficiency of coagulation factor VIII and factor IX. Some reagents showed abnormal aPTT results in mild cases of factor VIII and factor IX deficiency without producing a large number of falsely prolonged aPTT with normal plasma. (shetty et al 2003).

1.2.4.3.Previous study:

Previous study in America

They have evaluated the performance of a centrifugal analyzer, specifically designed for hemostasis tests, for methods based on clotting time as measured by light scattering, or based on splitting of chromogenic substrates. The results were compared with those obtained with a semi automated coagulometer (clotting tests) or with a manually operated photometer (chromogenic tests). In general,

the results with the centrifugal analyzer were at least as precise as those of the comparative methods, with greater output and ease of operation. Disadvantages of the instrument are its partial incompatibility with some commercial reagents (V Chantarangkul et al 1987).

The values observed in this study were different from previous reports. This could be due to differences in methodology or other factors such as the manner of blood coagulation, the type of container, the type of anticoagulant, specimen transport and storage conditions, incubation time and temperature, assay reagents, and the method of end point detection as reported by (Tseng *et al*. ,2001) and(Mischke. 2002).

Given these variations, it was advised that each laboratory must establish their own references ranges as suggested by(Lopez *et al*.,2005). In this backdrop the present laboratory.

1.2.5.Rationale:

The rationale for conducting this research in patients is to advanced knowledge about the quality system in different medical laboratories to produce accurate and reliable result of these tests (PT and PTT) in the follow up of patient with coagulation disorder.

1.2.6. Objectives

1.2.6.1.General objective

To determine the PT and PTT by using the same sample in tow equipment, that applied different quality control samples in sprites laboratory.

1.2.6.2. Specific objectives

To compare between the result that obtained from different laboratory

To determine the PT and PTT result in laboratory number (1) in Khartoum teaching hospital. By using in (STAGO) number (1)) pool plasma quality control srea.

To determine the PT and PTT result in laboratory number (2) in Khartoum advance hospital. By using in (STAGO) number (2) commercial quality control sera.

Chapter Two

Material and methods

2.1. Study design:

This study is cross sectional study in period from august to October 2015 to compare between Prothrombin Time (PT) and Activated Partial Thromboplastin Time (PTT) that obtained from the same sample by using same device in different laboratory, were performed using control pooled plasma reagent in lab (1)and commercial control in lab(2).All assay tests were determined by same methods- using semi automated analyzer (STAGO)

2.2. Study population:

Study carried out in 100 individual as study group without control group in Khartoum stat.

2.3. Inclusion criteria:

Any patient come to Khartoum hospital with request form included coagulation test and all age group were included

2.4. Exclusion criteria:

Presence of hemolysis or lipidema in sample.

2.5. Ethical consideration:

An informed consent from individual to be study was taken after being informed with all detailed objective of the study.

2.6.Data collection:

Data was collected from random Sudanese patient have coagulation profile in his request form.

2.7. Sample collection:

3 ml venous blood were collected from individual under study and dispensed in Tri sodium citrate for coagulation tests then separated to Platelet Poor Plasma (PPP) after centrifugation.

2.8. Procedure of coagulation tests (PT and APPT)

The coagulation tests (PT and APTT) were performed using semiautomatic device (STAGO).

2.8.1. Prothrombin time (PT)

Test principle:

The principle of the test consists of the use of calcium thromboplastin to measure the clotting time of patient's plasma. The test measure, as a whole. The activity of extrinsic coagulation factors: factor 11 (prothrombin), factor V (proaccelerin), factorV11 (proconvertin), and factor X (Stuart factor).

Reagents and equipments:

Thromboplastin with calcium reagent

System control (control plasma, commercial and pool plasma, normal and pathological levels) .

Parameters programming:

From the menu select test parameters by pressing the (3) key and confirm with (Enter)

Select PT by pressing (1) and confirm with (Enter)

Method:

1. Was centrifugation of blood sample at 2000 r for 15 min at 4c to prepared Platelet Poor Plasma (PPP).
2. Was delivered 0.1 ml of plasma into a clean cuvite in the device which contain the magnetic bills.

3. Was added 0.1 ml of thrompoblastin (PT) reagent.
4. Than was reared the result and statistical analysis was done.

2.8.2. Activated partial thromboplastin time (APTT)

Test principle:

The test involved the re calcification of plasma in the presence of a standardized amount of platelet substitute and a specific activator. This procedure minimizes test variables by standardized the contact activation and optimizes the concentration of platelet like phospholipids. The APTT explores the intrinsic coagulation pathway (factors X11, X1, 1X.V111, X.V.11.1).

Reagents and equipments:

$CaCl_2$ 0.025 M

System control (control plasma, commercial and pool plasma, normal and pathological levels) .

Parameters programming:

From the menu select test parameters by pressing the (3) key and confirm with (Enter)

Select APTT by pressing (2) and confirm with (Enter)

Method:

5. Was centrifugation of blood sample at 2000 r for 15 min at 4c to prepared Platelet Poor Plasma (PPP).
6. Was delivered 0.1 ml of plasma into a clean cuvite in the device which contain the magnetic bills.
7. Was added 0.1 ml of PTT reagent.
8. After 2 minute.
9. Was add 0.1 ml of Calcium chloride Ca+2
10. Than was reared the result and statistical analysis was done.

2.8.3. Data analysis:

Data were entered into computer and analyzed by T-test of the SPSS v 17.

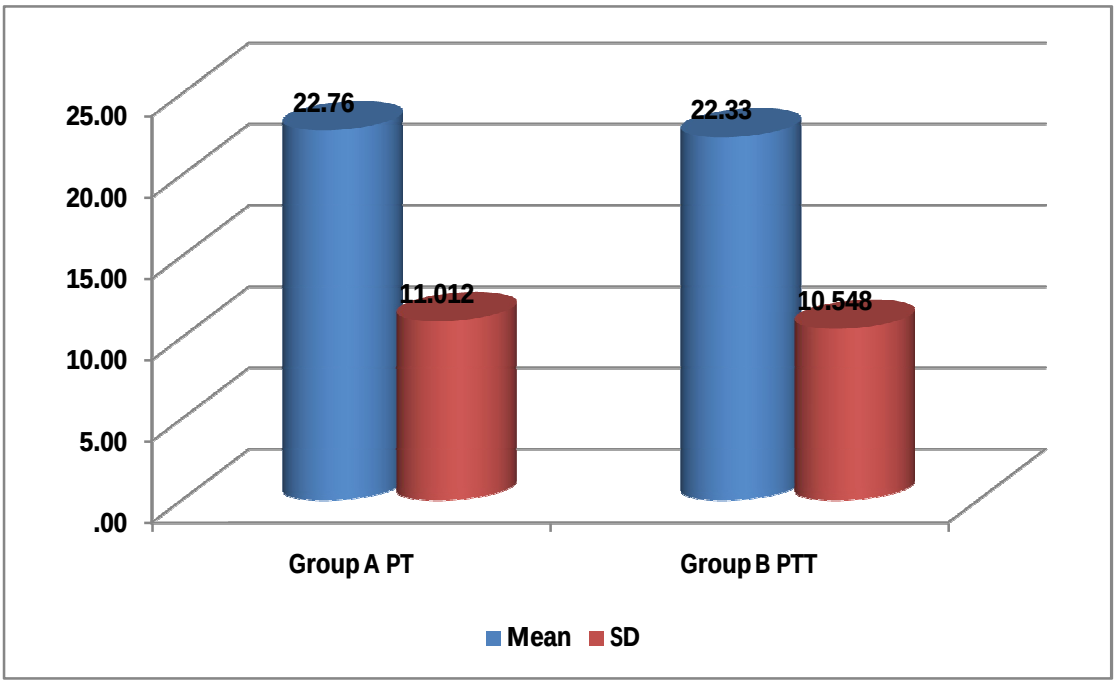
Chapter Three

RESULT

100 blood sample was taken from patient as study group without control group in Khartoum locality (Regardless to Age and Sex) and the result below

- There was no significant difference in means of PT between the two different laboratories
- There was no significant difference in means of APTT between the two different laboratories.
- **Table 1 :**

	Mean	SD	P-value
Group A PT	22.76	11.012	.783
Group B PTT	22.33	10.548	


25.00
20.00
15.00
10.00
5.00
.00
22.76
11.012
22.33
10.548
Group A PT
Group B PTT
Mean
SD

Chapter Four

Discussion, Conclusion, and Recommendation

4.1 Discussion

The result of this study showed that there no significant difference in mean of PT Between different hospitals (P = 0.783).

Also study showed that there no significant difference in mean of PT Between different hospitals (P = 0.498).

In pervious study in Bangkok The aPTT assay results from automated and semi-automated methods are not significant different. Recommendation for using of semi-automated method to perfom clotting assaytests in small setting is set.(Viroj Wiwanitkit, MD. Chulalongkom University, Bangkok).

N other previous study in America the performance of a centrifugal analyzer, specifically designed for hemostasis tests, for methods based on clotting time as measured by light scattering, or based on splitting of chromogenic substrates. The results were compared with those obtained with a semiautomated coagulometer (clotting tests) or with a manually operated photometer (chromogenic tests). In general, the results with the centrifugal analyzer were at least as precise as those of the comparative methods (V Chantarangkul *et al.*, 1987).

They are many factors that affect the PT and PTT result such as Calibration, and controls of devices and reagents, Time, and Temperature.

4.2 Conclusions

The result of this study showed that there no significant difference in mean of PT Between different hospitals (P = 0.783).

Also study showed that there no significant difference in mean of PT Between different hospitals (P = 0.498).

4.3. Recommendations

Follow up of patient with any hemostatic disorders.

Semi-automated device. Should be close control and calibrate before use.

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Appendix (1)

Figure(1-1)STAGO