Chapter one
Introduction and literature review

1.1Introduction:

Haemostasis in normal pregnancy involves a complex network of interactions with positive and negative feedback loops, integrating blood vessels; platelets, coagulation factors, coagulation inhibitors and Fibrinolysis and has evolved to maintain the integrity of the vasculature. Normal pregnancy is associated with substantial changes in the tissue factor pathway and in the wider haemostatic system (Buseri *et al.,* 2008).

These changes serve to protect the mother from the hazard of bleeding imposed by placentation and delivery, but they also carry the risk of an exaggerated response, localized or generalized, to coagulant stimuli. The coagulation cascade is in an activated state in pregnancy. Activation includes increased concentrations of all clotting factors, except factors XI, XIII, with increased levels of High molecular weight fibrinogen complexes.Changes in the haemostatic mechanism also involve decreased levels of anticoagulant proteins such as protein C and Protein S as well as enhanced thrombin generation (Hanes *et al.,* 1999)

Pregnancy is accompanied by changes in the expression of coagulation and fibrinolytic proteins that favour a balance towards clot formation. Increases in the activation of the coagulation cascade contribute to a reduction in hemorrhage risk that could otherwise be detrimental to fetal and maternal health. Alterations in both the extrinsic and intrinsic pathways of coagulation have been well-characterized and show that prothrombin, coagulation factors VII, X, XII and VIII are increased during pregnancy the precursor of fibrin, has been shown to increase during pregnancy contributing to a procoagulant environment (Stirling *et al.*, 1984).

1.2 Literature review

1.2.1 Overview of coagulation

Coagulation is a complex network of interaction involving vessels and factors the ability to form and to remove aclot is truly asystem dependant on many synergistic forces, haemostasis dependes on asystem of checkes and balances between thrombosis and haemorrhage that includes both procoagulantes and anticoagulants. This scale needs to be kept in balance. Thrombosis is an activation of the haemostatic system at an inappropriate time in vessels. Thrombi formed in this fashion are pathological and beyond the normal hemostatic mechanism If physiological anticoagulants are decreased in the circulation there will be clot If procoagulants or clotting factors are decreased this scale will tip toward bleeding, hemorrhage or excessive bleeding may be due to blood vessel disease, platelets abnormalities and acquired or congenital abnormalities . (Bouma and Meijers, 2004)

Hemostasis is comprised of the vascular system, platelet and series of enzymatic reactions of the of coagulation factors. Under physiological conditions, fluidity is maintained by the anticoagulants, profibrinolytic, and antiplateletes properties of the normal endothelium. Coagulation is divided into tow major systems, the primary and secondary systems of hemostasis. (Lozier, 2004)

The primary systems comprise platelets function and vasoconstriction. The secondary system involves coagulation proteins and aseries of enzymatic reactions once the coagulation protein become involved fibrin is formed and this reinforces platelets plug formation until healing is complete the product of the coagulation cascade is the conversion of soluble fibrinogen into insoluble fibrin clot. This is accomplished by the action of powerful coagulant thrombin. Thrombin is formed by aprecursor circulating protein and prothrombin. Dissolution of the platelets plug is achieved by the fibrinolytic process. (Üstün *et al.*, 2005).

1.2.2 Components of the primary haemostatic system:

1.2.2.1 Blood vessel:

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 of this layer themselves that differentiate arties from veins. The intima is the innermost layer and the surface is cover with a single layer of the endothelium (ECs). Which rest on a basement of collagen fibres and some elastin. The media or middle layer contains mianly circularl arranged smooth muscle cells and collagenous fibrils and is divided form the adventitia by the internal elastic lamina. The muscle cells contract and relax whereas the elastin allows vessels to stretch and recoil. The adventitia or outermost layer is composed of collagen fibres and fibroblasts that protect the blood vessel and ancher it to surrounding structures. (Diebold *et al.,* 2008).

Endothelial cells are not passive blood vessel wall linings. They are active participants in global hemostasis. Endothelial cells are particularly important in the prevention of coagulation. Endothelial cells express several molecules on their surface membranes that are important in regulation of coagulation. Examples are heparin sulfate and thrombomobulin. Which activate anticoagulant systems (antithrombin and the protein C protein S system respectively). Endothelial cells produce a variety of metabolic products that are critical in the prevention of thrombosis, including tissue plsminogen activator (t-PA). The primary initiator of the fibrinolytic system is tissue factor pathway inhibitor (TFPI). Which inhibits coagulation via the prostacyclin, potent vasodilator and platelet antagonist. Endothelial cells also produce nitric oxide (NO) which originally called the endothelial – derived relaxing factor (EDRF) which is a potent vasodilator and platelet antagonist. (Schaefer *et al.*, 2006) .

1.2.2.2 Platelets:

Platelets, or thrombocytes , are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes one of the largest cells in the body . The megakaryocyte arises by process of differentiation from the heamopoietic stem cell. The megakaryocyte matures by endomitotic synchronous replication (i.e.DNA replication in the absence of nuclear or cytoplasmic division). Enlarging the cytoplasmic volume

as the number of nuclear lobes is increase. Thrombopoietin is the major regulater of platelet production and is constitutively produced by the liver and kidneys. Thrombopoietin increases the number and rat of maturation of megakaryocytes . .Platelets are extremely small and discoid 3.0 x 0.5 um in diameter. With a mean volume of 7-11 fl. (Bouma, 2004)

Adhesion to collagen is facilitated by glycoprotein la (GPla). Glycoproteins Ib (defective in thrombasthenia) are important in the attachment of platelet to von Willebrand factor (VWF) and hence to vascular subendothelium where metabolic interactions occur. The binding site for GP IIb, IIIa is also the receptor for fibrinogen which is impotan in platelet - platelet aggregation. The plasma membrane invaginates into the platelet interior to form an open membrane (canalicular) system which provides a large reactive surface to which the plasma coagulation proteins may be selectively absorbed. The membrane phospholipids (previously known as platelet factor 3) are of particular importance in the conversion of coagulation factor X to Xa and prothrombin (factorII) to thrombin (factorIIa). (Diebold *et al.,* 2008)

The platelet contains three types of granules: dense, alpha and lysosomes. The main function of platelets is the formation of mechanical plug during the normal haemostatic response to vascular injury. In the absence of platelets spontaneous leakage of blood through small vessels may occure. **(** Lang *et al.*, 2009)

1.2.3 Coagulation Cascade

The blood clotting system or coagulation pathway, like the complement system, is a proteolytic cascade. Each enzyme of the pathway is present in the plasma as a zymogen, in other words in an inactive form, which on activation undergoes proteolytic cleavage to release the active factor from the precursor molecule. The coagulation pathway functions are a series of positive and negative feedback loops which control the activation process. The ultimate goal of the pathway is produce thrombin, which can then convert soluble fibrinogen into fibrin to, which forms a clot. (O'Riordan, *et al*., 2003).

1.2.4 Coagulation Factors

The operations of the enzyme cascade require local concentration of circulating coagulation factors at the site of injury .coagulation factors can be classified into:

Table (1.1) classification of coagulation factors (Dawood *et al.*, 2012).

Name	Function
1-2-4-1 plasma protein	Proteins in blood plasma
1-2-4-2 factor I, fibrinogen	A protein present in blood plasma; converts to fibrin when blood clots
1-2-4-3factor II, prothrombin	A protein in blood plasma that is the inactive precursor of thrombin
1-2-4-4 factor III, thrombokinase, thromboplastin	An enzyme liberated from blood platelets that converts prothrombin into thrombin as blood starts to clot
1-2-4-5 calcium ion, factor IV	Ion of calcium; a factor in the clotting of blood
1-2-4-6 accelerator factor, factor V, proaccelerin, prothrombin accelerator	A coagulation factor
1-2-4-7 cothromboplastin, factor VII, proconvertin, stable factor	A coagulation factor formed in the kidney under the influence of vitamin K
1-2-4-8 antihaemophilic factor, antihaemophilic globulin, antihemophilic factor, antihemophilic globulin, factor VIII, Hemofil	A coagulation factor (trade name Hemofil) whose absence is associated with hemophilia A
1-2-4-9 Christmas factor, factor IX	Coagulation factor whose absence is associated with hemophilia B
1-2-4-10 factor X, prothrombinase	Coagulation factor that is converted to an enzyme that converts prothrombin to thrombin in a reaction that depends on calcium ions and other coagulation factors
1-2-4-11 factor XI	Coagulation factor whose deficiency results in a hemorrhagic tendency
1-2-4-12 factor XII, Hageman factor	coagulation factor whose deficiency results in prolongation of clotting time of venous blood
1-2-4-13 factor XIII, fibrinase	In the clotting of blood thrombin catalyzes factor XIII into its active form (fibrinase)

1.2.5 Intrinsic pathway

It is activated when blood comes into contact with sub-endothelial connective tissues or with negatively charged surface that are exposed as a result of tissue damage. Quantitatively it is the more important of the two pathways, but is slower to cleave fibrin than the extrinsic pathway. The Hageman factor (factor XII), factor XI, prekallikrein, and high molecular weight kininogen (HMWK) are involved in this pathway of activation .This pathway provides a further of the interrelationship between the various enzyme cascade systems in plasma. The first step is the binding of Hageman factor to a sub-endothelial surface exposed by an injury. A complex of prekallikrein and HMWK also interacts with the exposed surface in close proximity to the bound factor XII, which becomes activated. During activation, the single chain protein of the native Hageman factor is cleaved into two chains (50 and 28 kDa), that remain linked by a disulphide bond. The light chain (28kDa) contains the active site and the molecule is referred to as activated Hageman factor (factor XIIa). There is evidence that the Hageman factor can autoactivate, thus the pathway is self-amplifying once triggered (compare with the alternative pathway of complement). (Buseri *et al*., 2008).

Activated Hageman factor in turn activates prekallikrein. The *kallikrein* produced can then also cleave factor XII, and a further amplification mechanism is triggered. The activated factor XII remains in close contact with the activating surface, such that it can activate factor XI, the next step in the intrinsic pathway which, to proceed efficiently, requires Ca^{2+} . Also involved at this stage is HMWK, which binds to factor XI and facilitates the activation process. Activated factors XIa, XIIa, and kallikrein are all serine proteases, like many of the enzymes of the complement system.

Eventually the intrinsic pathway activates factor X, a process that can also be brought about by the extrinsic pathway. Factor X is the first molecule of the common pathway and is activated by a complex of molecules containing activated factor IX, factor VIII, calcium, and phospholipid which is provided by the platelet surface, where this reaction usually takes place. The precise role of factor VIII in this reaction is not clearly understood. Its presence in the complex is obviously essential, as evidenced by the serious consequences of factor VIII deficiency

experienced by haemophiliacs. Factor VIII is modified by thrombin, a reaction that results in greatly enhanced factor VIII activity, promoting the activation of factor X.(Bogdanich and Koli, 2003).

1.2.6 Extrinsic pathway

It is an alternative route for the activation of the clothing cascade. It provides a very rapid response to tissue injury, generating activated factor X almost instantaneously, compared to the seconds or even minutes required for the intrinsic pathway to activate factor X. The main function of the extrinsic pathway is to augment the activity of the intrinsic pathway. (Thornton and Douglas, 2010).

There are two components unique to the extrinsic pathway, tissue factor or factor III, and factor VII. Tissue factor is present in most human cells bound to the cell membrane. The activation process for tissue factor is not entirely clear. Once activated, tissue factor binds rapidly to factor VII which is then activated to form a complex of tissue factor, activated factor VII, calcium, and a phospholipid, and this complex then rapidly activates factor X.

The intrinsic and extrinsic systems converge at factor X to a single common pathway which is ultimately responsible for the production of thrombin (factor IIa). (Kenet *et al.*, 1999).

1.2.7 Clot formation:

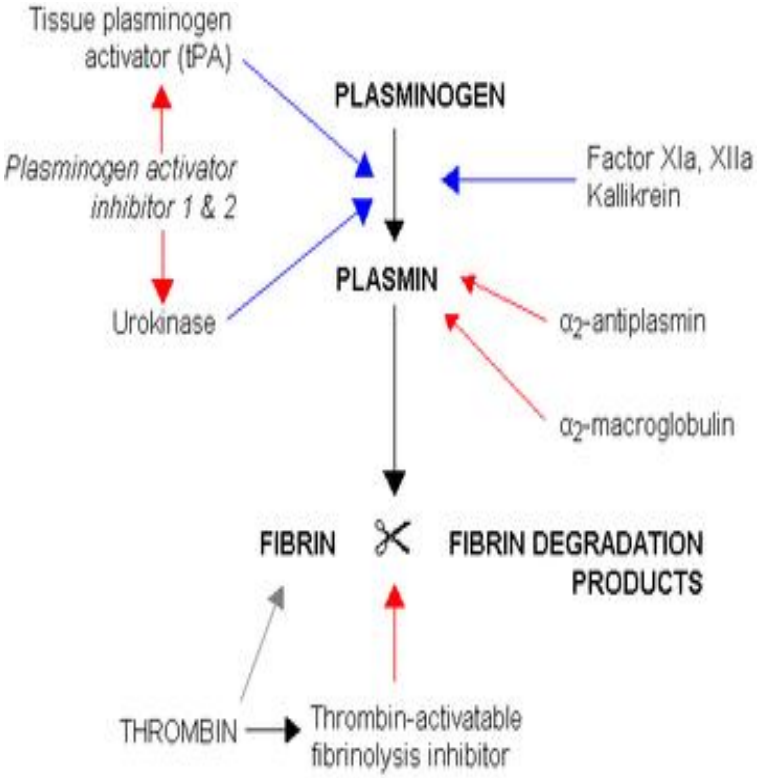
The end result of the clotting pathway is the production of *thrombin* for the conversion of fibrinogen to fibrin. Fibrinogen is a dimer soluble in plasma. Exposure of fibrinogen to thrombin results in rapid proteolysis of fibrinogen and the release of fibrinopeptide A. The loss of small peptide A is not sufficient to render the resulting fibrin molecule insoluble, a proces that is required for clot formation, but it tends to form complexes with adjacent fibrin and fibrinogen molecules. A second peptide, fibrinopeptide *B*, is then cleaved by thrombin, and the fibrin monomers formed by this second proteolytic cleavage polymerize spontaneously to form an insoluble gel. The polymerized *fibrin*, held together by noncovalent and electrostatic forces, is stabilized by the transamidating enzyme factor XIIIa, produced by the action of thrombin on factor XIII. These insoluble fibrin aggregates (clots), together with aggregated

platelets (thrombi), block the damaged blood vessel and prevent further bleeding (Lang *et al*., 2009).

1.2.8 Fibrinolysis

It is a process that prevents blood clots from growing and becoming problematic. Primary or secondary fibrinolysis, this process has two types: primary fibrinolysis and secondary fibrinolysis. The primary type is a normal body process, whereas secondary fibrinolysis is the breakdown of clots due to a medicine, a medical disorder, or some other cause (Pabinger, 2009).

In fibrinolysis, a fibrin clot, the product of coagulation, is broken down "Molecular mechanisms of fibrinolysis· Its main enzyme plasmin cuts the fibrin mesh at various places, leading to the production of circulating fragments that are cleared by other proteases or by the kidney and liver.



Fig(1.1) Fibrinolysis (Dugdale**,** David *et al* , 2008)

Fibrinolysis (simplified). Blue arrows denote stimulation, and red arrows inhibition. Plasmin is produced in an inactive form plasminogen, in the liver. Although

plasminogen cannot cleave fibrin, it still has an affinity for it, and is incorporated into the clot when it is formed; Tissue plasminogen activator and urokinase are the agents that convert plasminogen to the active Plasmin, thus allowing fibrinolysis to occur. t-PA is released into the blood very slowly by the damaged endothelium of the blood vessels, such that, after several days (when the bleeding has stopped), the clot is broken down. This occurs because plasminogen became entrapped within the clot when it formed; as it is slowly activated, it breaks down the fibrin mesh. t-PA and urokinase are themselves inhibited by plasminogen activator inhibitor-1 and plasminogen activator inhibitor-2 (PAI-1 and PAI-2). In contrast, plasmin further stimulates plasmin generation by producing more active forms of both tissue plasminogen activator (tPA) and urokinase. Alpha 2-antiplasmin and alpha 2-macroglobulin inactivate plasmin. Plasmin activity is also reduced by thrombin-activatable fibrinolysis inhibitor (TAFI), which modifies fibrin to make it more resistant to the tPA-mediated plasminogen, when plasmin breaks down fibrin, a number of soluble parts are produced. These are called fibrin degradation products (FDPs). FDPs compete with thrombin, and thus slow down clot formation by preventing the conversion of fibrinogen to fibrin. This effect can be seen in the thrombin clotting time (TCT) test, which is prolonged in a person that has active fibrinolysis (Kumar *et al.*, 2005).

Fibrin degradation products, the D-dimer, can be measured using antibody-antigen technology, and confirms that fibrinolysis has occurred. It is therefore used to indicate deep-vein thrombosis, pulmonary embolism, and efficacy of treatment in acute myocardial infarction. Alternatively, a more rapid detection of fibrinolytic activity, especially hyperfibrinolysis, is possible with thromboelastometry (TEM) in whole blood, even in patients on heparin. In this assay, increased fibrinolysis is assessed by comparing the TEM profile in the absence or presence of the fibrinolysis inhibitor aprotinin. Clinically, the TEM is useful for near real-time measurement of activated fibrinolysis for at-risk patients, such as that experiencing significant blood loss during surgery (Thornton and Douglas, 2010).

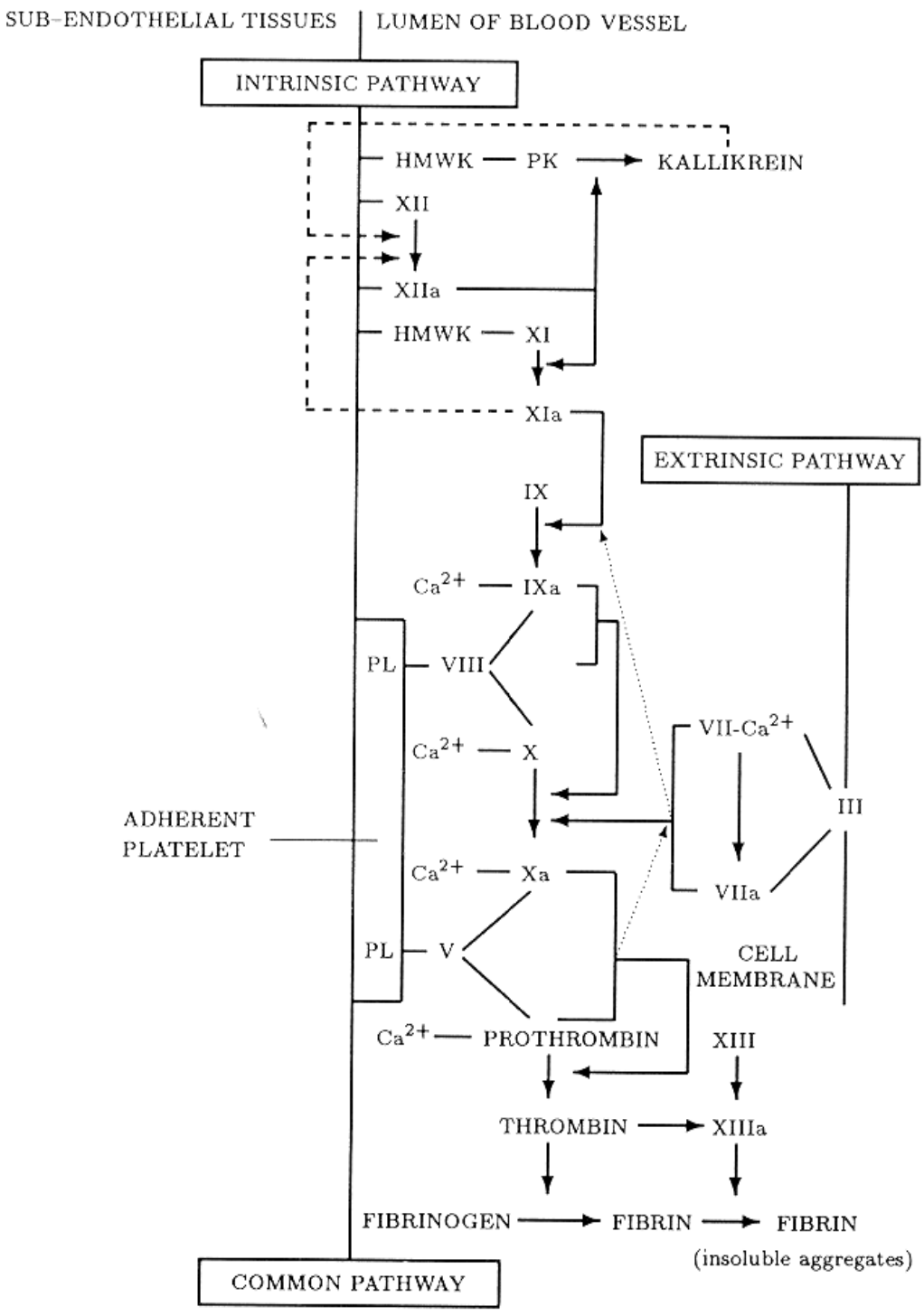


Fig (1-2) Coagluation Cascade (Muszbek *et al.*, 2008).

1.2.9 Coagulation inhibitors

Clot formation is the final result of interaction among multiple plasma proteins. after activation, it results in the conversion of fibrinogen to fibrin and cross-linking of fibrin by activated factor XIII, which stabilizes the formed clot. Deficiency or functional abnormality of the factors involved in these reactions causes bleeding disorders. Natural inhibitors of clotting factors include antithrombin III, protein S, and protein C. When activated, these proteins inactivate specific clotting factors, providing a regulatory mechanism that serves to control the coagulation response and limit the extension of the clot. Physiologic or natural inhibitors should not be confused with acquired inhibitors of coagulation factors, Inhibitors to coagulation factors, also known as circulating anticoagulants, are antibodies that neutralize specific clotting proteins, thereby interfering with their normal function. Antibodies may be directed against isolated clotting factors, as is the case with factor VIII or IX inhibitors. On the other hand, the antiphospholipid antibodies are known to develop against multiple coagulation proteins.Acquired inhibitors of coagulation are antibodies that either inhibit the activity or increase the clearance of a clotting factor.Some disorders may be associated with antibodies to a variety of clotting factors. In systemic lupus erythematosus, for example, antiphospholipid antibodies and antibodies directed against factors II, VIII, IX, XI, XII, and XIII Antiphospholipid antibodies represent a special problem; these antibodies prolong certain clotting assays, but result in thrombosis rather than bleeding. The pathogenesis, diagnosis, and treatment of conditions associated with antiphospholipid antibodies A common clinical manifestation in affected patients is a hemorrhagic diathesis. Acquired coagulation inhibitors that lead to bleeding will be reviewed here. The natural history and management of these inhibitors are quite different from inhibition due to alloantibodies that occur in patients with various inherited bleeding disorders (eg, congenital deficiencies of factors VIII, IX, or XI) treated with factor replacement (Gilliam *et al.*, 2011).

1.2.9.1 Factor VIII inhibitors

The most common autoantibodies that affect clotting factor activity and lead to a bleeding disorder are directed against, and interfere with, the activity of factor VIII, a condition also called acquired hemophilia Although there are no large series

describing the immunoglobulin class of the factor VIII autoantibodies, most have been IgG antibodies that do not bind complement. The reasons for the production of factor VIII autoantibodies in a particular individual are not clear, but may involve the presence of certain gene polymorphisms (eg, HLA, CTLA4) and/or autoreactive CD4+ T lymphocytes (Levy, 2005).

1.2.9.2 Feedback inhibition

Tissue factor pathway inhibitor (TFPI) is a multivalent, Kunitz-type plasma proteinase inhibitor that regulates tissue factor-induced coagulation. TFPI directly inhibits activated factor X and, in a factor Xa-dependent fashion, produces feedback inhibition of the factor VIIa/tissue factor catalytic complex. The properties of this rediscovered inhibitor appear, at least in part, to explain the clinical requirement for both the extrinsic and intrinsic pathways of the cascade and waterfall theories of blood clotting and have led to a reformulation of the coagulation mechanism. In the revised hypothesis, factor VIIa/tissue factor is responsible for the initiation of coagulation, but owing to TFPI-mediated inhibition, sustained hemostasis requires the persistent and amplified procoagulant action of intrinsic factors VIII, IX, and XI (Jenkins *et al*., 2012).

1.2.10 Fibrinogen

Fibrinogen was first isolated from horse plasma by Hammarsten in 1876, although an inactive precursor to fibrin was proposed to exist as early as 1859 by Deni de Commercy. Fibrinogen can undergo a remarkable transformation from soluble monomers (fibrogen) to an insoluble polymer gel (polymerised fibrin) , Fibrinogen is a plasma glycoprotein synthesised in the liver that is essential for haemostasis (stopping blood loss from damaged tissues), wound healing, fibrinolysis, inflammation, angiogenesis, cellular and matrix interactions, and neoplasia. These processes involve the conversion of fibrinogen to fibrin, and often the interaction of fibrinogen to various proteins and cells. People usually carry about 2.5g fibrinogen/L of blood, however, concentrations of fibrinogen can increase by as much as 200-400% during times of physiological stress (primarily due to the actions of macrophage-derived interleukin-6). Fibrinogen is a large, complex glycoprotein composed of three pairs of polypeptides: two A, two B, These polypeptides are linked together by 29

disulphide bonds, some of which are depicted in Figure 2 below. The polypeptides are oriented so all six N-terminal ends meet to form the central E domain. Two regions of coiled coil alpha helices stretch out on either side of the E domain, each consisting of one A, one B and one polypeptide. Each coiled coil region ends in a globular D domain consisting of the C-terminal ends of B and, as well as part of A. The C-terminal end of A then protrudes from each D domain as a long strand; these A protuberances can interact with each other and with the E domain during fibrin clot cross-linking. Both the E and D domains contain important binding sites for the conversion of fibrinogen to fibrin, for fibrin assembly and cross-linking, and for platelet aggregation. Bound calcium ions are important to help maintain the structure of fibrinogen. The N-terminal ends of both the A and B polypeptides are cleaved by thrombin in order to turn soluble fibrinogen into gel-forming fibrin. Once cleaved from fibrinogen, the N-terminal ends are known as fibrinopeptide A (from A polypeptide) and fibrinopeptide B (from B polypeptide) (Antonarakis, 1995).

1.2.11 Factor VIII

(FVIII) is an essential blood-clotting protein, also known as anti-hemophilic factor (AHF). In humans, factor VIII is encoded by the *F8* gene Defects in this gene results in hemophilia A, a recessive X-linked coagulation disorder. Factor VIII is produced in liver sinusoidal cells and endothelial cells outside of the liver throughout the body. This protein circulates in the bloodstream in an inactive form, bound to another molecule called von Willebrand factor, until an injury that damages blood vessels occurs (Jenkins *et al.*, 2012).

In response to injury, coagulation factor VIII is activated and separates from von Willebrand factor. The active protein (sometimes written as coagulation factor VIIIa) interacts with another coagulation factor called factor IX. This interaction sets off a chain of additional chemical reactions that form a blood clot. "NIH: F8 - coagulation factor VIII". National Institutes of Health Factor VIII participates in blood coagulation; it is a cofactor for factor IXa which, in the presence of Ca^{+2} and phospholipids forms a complex that converts factor X to the activated form Xa. The factor VIII gene produces two alternatively spliced transcripts. Transcript variant 1 encodes a large glycoprotein, isoform a, which circulates in plasma and associates with von Willebrand factor in a noncovalent complex. This protein undergoes

multiple cleavage events. Transcript variant 2 encodes a putative small protein, isoform b, which consists primarily of the phospholipid binding domain of factor VIIIc. This binding domain is essential for coagulant activity. Entrez Gene: FVIII coagulation factor VIII, procoagulant component (hemophilia A)" People with high levels of factor VIII are at increased risk for deep vein thrombosis and pulmonary embolism. The gene for factor VIII is located on the X chromosome (Xq28). The gene for factor VIII presents an interesting primary structure, as another gene is embedded in one of its introns (Levinson *et al.*, 1990).

FVIII is a glycoprotein procofactor. Although the primary site of release in humans is ambiguous, it is synthesized and released into the bloodstream by the vascular, glomerular, and tubular endothelium, and the sinusoidal cells of the liver. Hemophilia A has been corrected by liver transplantation. Transplanting hepatocytes was ineffective, but liver endothelial cells were effective in the blood, it mainly circulates in a stable noncovalent complex with von Willebrand factor. Upon activation by thrombin, (factor IIa), it dissociates from the complex to interact with factor IXa in the coagulation cascade. It is a cofactor to factor IXa in the activation of factor X, which, in turn, with its cofactor factor Va, activates more thrombin. Thrombin cleaves fibrinogen into fibrin which polymerizes and crosslinks (using factor XIII) into a blood clot. No longer protected by vWF, activated FVIII is proteolytically inactivated in the process (most prominently by activated protein C and factor IXa) and quickly cleared from the blood stream.Factor VIII is not affected by liver disease. In fact, levels usually are elevated in such instances (Rubin and Leopold, 1998).

FVIII concentrated from donated blood plasma (Aafact or Alphanate, Monoclate-P®), or alternatively recombinant FVIII can be given to hemophiliacs to restore hemostasis.The transfer of a plasma byproduct into the blood stream of a patient with hemophilia often led to the transmission of diseases such as hepatitis B and C and HIV before purification methods were improved. Antibody formation to factor VIII can also be a major concern for patients receiving therapy against bleeding; the incidence of these inhibitors is dependent of various factors, including the factor VIII product itself (Lozier, 2004).

1.2.12 Factor VII

Blood-coagulation factor VIIa, activated blood coagulation factor VII, formerly known as [proconvertin] is one of the proteins that causes blood to clot in the coagulation cascade. It is an enzyme of the serine protease class. A recombinant form of human factor VIIa (NovoSeven, eptacog alfa (activated) has U.S. Food and Drug Administration approval for uncontrolled bleeding in hemophilia patients. It is sometimes used unlicensed in severe uncontrollable bleeding, although there have been safety concerns. A Biosimilar form of recombinant activated factor VII (AryoSeven) is manufacturing by AryoGen Biopharma and since 2012 is available in the market. The main role of factor VII (FVII) is to initiate the process of coagulation in conjunction with tissue factor (TF/factor III). Tissue factor is found on the outside of blood vessels - normally not exposed to the bloodstream. Upon vessel injury, tissue factor is exposed to the blood and circulating factor VII. Once bound to TF, FVII is activated to FVIIa by different proteases, among which are thrombin (factor IIa), factor Xa, IXa, XIIa, and the FVIIa-TF complex itself. The most important substrate for FVIIa-TF is Factor X. The action of the factor is impeded by tissue factor pathway inhibitor (TFPI), which is released almost immediately after initiation of coagulation. Factor VII is vitamin K dependent; it is produced in the liver. Use of warfarin or similar anticoagulants decreases hepatic synthesis of FVII. The gene for factor VII is located on chromosome 13 (13q34). Deficiency is rare (congenital proconvertin deficiency) and inherits recessively. Factor VII deficiency presents as a hemophilia-like bleeding disorder. It is treated with recombinant factor VIIa (NovoSeven or AryoSeven). Recombinant factor VIIa is used for people with hemophilia (with Factor VIII or IX deficiency) who have developed inhibitors against replacement coagulation factor. It has also been used in the setting of uncontrollable hemorrhage,but its role in this setting is controversial with insufficient evidence to support its use outside of clinical trials. The first report of its use in hemorrhage was in an Israeli soldier with uncontrollable bleeding in 1999. [1] Risks of its use include an increase in arterial thrombosis; Factor VII has been shown to interact with Tissue factor (Carlsson *et al*, 2003).

1.2.13 Physiological Changes in Pregnancy

Female undergo several anatomical changes during pregnancy, including cardiovascular, hematologic, metabolic, renal, and respiratory changes that provide adequate nutrition and gas exchange for the developing fetus.Progesterone and estrogen levels rise continually through pregnancy together with blood sugar, breathing rate, and cardiac output.The body's posture changes during pregnancy to accommodate the growing fetus and the mother will experience weight gain. Breasts grow and change in preparation for lactation once the infant is born. Once lactation begins, the e's breasts swell significantly and can feel achy, lumpy, and heavy (engorgement). This is relieved by nursing the infant Plasma and blood volume increase over the course of the pregnancy leading to changes in heart rate and blood pressure. The e may also have a higher risk of blood clots, especially in the weeks following labor (McCrory *et al.*, 2010).

human chorionic gonadotropin A peptide hormone, produced during pregnancy, that prevents the breakdown of the corpus luteum and maintains progesterone production.human placental lactogen Human placental lactogen (HPL), also called human chorionic somatomammotropin (HCS), is a polypeptide placental hormone. Its structure and function is similar to that of human growth hormone. It modifies the metabolic state of the mother during pregnancy to facilitate the energy supply of the fetus (Whitcome *et al.*, 2007).

Changes in gait, especially during the last trimester, limit a e's mobility. This limited mobility acted as a selection factor on the timing of birth in humans. Compared to the other great apes, a full-term human infant is actually premature. The human e pelvis has been modified as much as possible to both maintain mobility and to allow the passage of a large-brained infant. If the human infant were born at the same developmental stage as a chimp infant, pregnancy would last about 18 months. The pelvic changes necessary for this would mean a e would be unable to walk. Natural selection has resulted in a balance of e mobility and infant survivability. Maternal physiological changes in pregnancy are the normal adaptations that a female undergoes during pregnancy to better accommodate the embryo or fetus, and include cardiovascular, hematologic, metabolic, renal, and respiratory changes. The e body must change its physiological and homeostatic mechanisms in pregnancy to ensure

proper fetal development. Increases in blood sugar, breathing, and cardiac output are all required (Campolongo and Marianne, 2007).

Pregnant female also experience adjustments in their endocrine system. Levels of progesterone and estrogens rise continuously throughout pregnancy, suppressing the hypothalamic axis and, subsequently, the menstrual cycle. Estrogen produced by the placenta is associated with fetal well being. also experience increased human chorionic gonadotropin (β-hCG), which is produced by the placenta, maintaining progesterone production by the corpus luteum. The increased progesterone production primarily functions to relax smooth muscle. Prolactin levels increase due to maternal pituitary gland enlargement, mediating a change in the structure of the mammary gland from ductal to lobular-alveolar. Parathyroid hormone increases, leading to increased calcium uptake in the gut and reabsorption by the kidney. Adrenal hormones such as cortisol and aldosterone also increase. Human placental lactogen (hPL) is produced by the placenta, stimulating lipolysis and fatty acid metabolism by the female and conserving blood glucose for use by the fetus. It can also decrease maternal tissue sensitivity to insulin, resulting in gestational diabetes (Wood *et al*, 2008).

The body's posture changes as the pregnancy progresses. The pelvis tilts and the back arches to help keep balance. Poor posture occurs naturally from the stretching of the female's abdominal muscles as the fetus grows. These muscles are less able to contract and keep the lower back in proper alignment. The pregnant female has a different gait: the step lengthens as the pregnancy progresses, due to weight gain and changes in posture. In addition, the increased body weight of pregnancy, fluid retention, and weight gain lowers the arches of the foot, further adding to the foot's length and width. The influences of increased hormones such as estrogen and relaxin initiate the remodeling of soft tissues, cartilage, and ligaments. Certain skeletal joints (e.g. the pubic symphysis and sacroiliac) widen or have increased laxity (Kaushansky *et al*., 2010).

One of the most noticeable alterations in pregnancy is the gain in weight. The enlarging uterus, the growing fetus, the placenta and liquor amnii, the acquisition of fat and water retention, all contribute to weight gain. The weight gain varies and can be anywhere from five pounds (2.3 kg) to over 100 pounds (45 kg). In the U.S., the

doctor-recommended weight gain range is 25 pounds (11 kg) to 35 pounds (16 kg), less if the female is overweight; more (up to 40 pounds (18 kg) if the female is underweight. A female's breasts grow during pregnancy, usually one to two cup sizes, but possibly larger. A female who wore a C cup bra prior to her pregnancy may need to buy an F cup or larger bra while nursing. A female's torso also grows and her bra band size may increase one or two sizes. Once the baby is born (about 50 to 73 hours after birth), the mother will experience her breasts filling with milk, at which point changes in the breast happen very quickly. Once lactation begins, the female's breasts swell significantly and can feel achy, lumpy, and heavy (engorgement). Her breasts may increase again in size; individual breast size can vary daily or for longer periods depending on how much the infant nurses from each breast (Robert *et al*., 2004).

Plasma and blood volume slowly increase by 40-50% over the course of the pregnancy (due to increased aldosterone) to accommodate the changes, resulting in an increase in heart rate (15 beats/min more than usual), stroke volume, and cardiac output. Cardiac output increases by about 50%, primarily during the first trimester. The systemic vascular resistance also drops due to the smooth muscle relaxation and overall vasodilation caused by elevated progesterone, leading to a fall in blood pressure. Diastolic blood pressure consequently decreases between 12–26 weeks, and increases again to pre-pregnancy levels by 36 weeks. Edema, or swelling, of the feet is common during pregnancy, partly because the enlarging uterus compresses veins and lymphatic drainage from the legs. Apregnant e will also become hypercoagulable, leading to increased risk for developing blood clots and embolisms due to increased liver production of coagulation factors. Female are at highest risk for developing clots (thrombi) during the weeks following labor. Clots usually develop in the left leg or the left iliac venous system because the left iliac vein is crossed by the right iliac artery. The increased flow in the right iliac artery after birth compresses the left iliac vein leading to an increased risk for thrombosis (clotting) which is exacerbated by lack of ambulation (walking) following delivery. Both underlying thrombophilia and cesarean section can further increase these risks (Dunning *et al.*, 2003).

During pregnancy, both protein metabolism and carbohydrate metabolism are affected. One kilogram of extra protein is deposited, with half going to the fetus and placenta, and another half going to uterine contractile proteins, breast glandular tissue,

plasma protein, and haemoglobin. An increased requirement for nutrients is given by fetal growth and fat deposition. Changes are caused by steroid hormones, lactogen, and cortisol. Maternal insulin resistance can lead to gestational diabetes. Increased liver metabolism is also seen, with increased gluconeogenesis to increase maternal glucose levels. Nutritionally, pregnant female require a caloric increase of 300 kcal/day and an increase in protein to 70 or 75 g/day.] There is also an increased folate requirement from 0.4 to 0.8 mg/day (important in preventing neural tube defects). On average, a weight gain of 20 to 30 lb (9.1 to 14 kg) is experienced The fetus inside a pregnant e may be viewed as an unusually successful allograft, since it genetically differs from the eIn the same way, many cases of spontaneous abortion may be described in the same way as maternal transplant rejection (McCrory *et al.*, 2010).

Neuromechanical adaptation to pregnancy refers to the change in gait, postural parameters, as well as sensory, due to the numerous anatomical, physiological, and hormonal changes female experience during pregnancy. Such changes increase their risk for musculoskeletal disorders and fall injuries. Musculoskeletal disorders include lower-back pain, leg cramps, and hip pain. Pregnant female fall at a similar rate (27%) to female over age of 70 years (28%). Most of the falls (64%) occur during the second trimester. Additionally, two-thirds of falls are associated with walking on slippery floors, rushing, or carrying an object The body's posture changes as the pregnancy progresses. The pelvis tilts and the back arches to help keep balance. Poor posture occurs naturally from the stretching of the e's abdominal muscles as the fetus grows. These muscles are less able to contract and keep the lower back in proper alignment. The pregnant e has a different pattern of gait. The step lengthens as the pregnancy progresses, due to weight gain and changes in posture. On average, a e 's foot can grow by a half size or more during pregnancy. In addition, the increased body weight of pregnancy, fluid retention, and weight gain lowers the arches of the foot, further adding to the foot's length and width. The influences of increased hormones such as estrogen andrelaxin initiate the remodeling of soft tissues, cartilage and ligaments. Certain skeletal joints such as the pubic symphysis and sacroiliac widen or have increased laxity (Jenkins *et al.*, 2012).

1.2.14 Hypercoagulability In Pregnancy

Hypercoagulability in pregnancy is the propensity of pregnant female to develop thrombosis (blood clots). Pregnancy itself is a factor of hypercoagulability (pregnancy-induced hypercoagulability), as a physiologically adaptive mechanism to prevent *post partum* bleeding However, when combined with an additional underlying hypercoagulable states, the risk of thrombosis or embolism may become substantial (Paolo, 2008).

Pregnancy-induced hypercoagulability is probably a physiologically adaptive mechanism to prevent *post partum* hemorrhage. Pregnancy changes the plasma levels of many clotting factors, such as fibrinogen, which can rise up to three times its normal value. Thrombin levels increase Protein S, an anticoagulant, decreases. However, the other major anticoagulants, protein C and antithrombin III, remain constant. Fibrinolysis is impaired by an increase in plasminogen activator inhibitor-1 (PAI-1 or PAI) and plasminogen activator inhibitor-2 (PAI-2), the latter synthesized from the placenta Venous stasis may occur at the end of the first trimester, due to enhanced compliance of the vessel walls by a hormonal effect . Also, pregnancy can cause hypercoagulability by other factors, e.g. the prolonged bed rest that often occurs *post partum* that occurs in case of delivery by forceps, vacuum extractor or Caesarean section. Pregnancy after the age of 35 augments the risk of VTE, as does multigravidity of more than four pregnancies. Several pregnancy complications, such as pre-eclampsia, cause substantial hypercoagulability (McCrory *et al*., 2010).

General causes of hypercoagulability, about as common in pregnancy as in the general population, include both acquired ones such as antiphospholipid antibodies, and congenital ones, including factor V Leiden, prothrombin mutation, protein C and S deficiencies, and antithrombin III deficiency. Hypercoagulability in pregnancy, particularly due to inheritable thrombophilia, can lead to placental vascular thrombosis. This can in turn lead to complications like early-onset hypertensive disorders of pregnancy, pre-eclampsia and small for gestational age infants (SGA). Among other causes of hypercoagulability, Antiphospholipid syndrome has been associated with adverse pregnancy outcomes including recurrent miscarriageDeep vein thrombosis has an incidence of one in 1,000 to 2,000 pregnancies in the United

States, and is the second most common cause of maternal death in developed countries after bleeding. (Uppsala Academic Hospital, 2008).

During pregnancy, there is increase in concentration of clotting factors II, V, VII, VIII, IX, X and XII. Plasma fibrinogen level is significantly increased. Plasma fibrinolytic activity is suppressed during pregnancy and labour. It returns to normal within 1 hour of delivery of the placenta. This is due to liberation of plasminogen inhibitor from the placenta. Because of the hypercoagulable state in pregnancy, presence of any provocative factor can easily upset the normal balance injury, the delicate haemostatic mechanism is triggered, which leads to coagulation failure (Dawood et al., 2012).

1.2. 15 Previous studies.

Fibrinogen is protein that plays akey role in blood clotting' fibrinogen is sticky fibrous coagulant in the blood that appears to significantly increase the risk of experiencing one of the leading causes of death band disability-stroke (Dugdale and David, 2008).

Analysis of the large-scale eurostaoke showed that fibrinogen is powerful predictor of stroke : including fatal and non fatal stroke , first time strokes and hemorrhagic and ischemic strokes Dividing the population into four groups {quartiles} based on their fibrinogen levels researchers estimated that the risk of stroke increased by nearly 50% for each ascending quartile individuals whose fibrinogen levels were in the highest quartile were almost seven time more likely to suffer aheamorrhagic stroke , and mor than twice as likely to die from astroke (Simpson *et al*., 2012).

Their were study in high molecular weight of fibrinogen during and after normal pregnant found that pregnancy has recently been described as generalized intravascular inflammatory response to the concept us total fibrinogen concentration increase during pregnancy the percentage high molecular weight fibrinogen of the concentration total fibrinogen is known to increase during acute phase conditions like inflammation therefore ,we investigated whether the percentage high molecular weight fibrinogen increase during pregnancy. The study done in Gerontology and Gynaecology University medical center Utrecht.

Other study done in annals of hematology to investigate the relationship between coagulation activities and the fibrinolytic system during normal pregnancy , they measured the plasma concentration of coagulation factors , antithrombin III, D-dimer and plasminogen activator inhibitor type 1 in 436 apparently healthy pregnant , and non pregnant females , there were no significant changes in anti thrombin III and factor XI concentration during pregnancy . However factor VII, VIII, IX, XII activities increased gradually as pregnancy progressed , reached maximum values in the third trimester (Bezemer *et al*., 2008).

Their was study undertaken in Safdarjang Hospital, New Delhi to determine the coagulation profile of pregnant female compared with control non pregnant female. (Osmanagaoglu *et al*.,2005).

In the Hamamatsu University School of Medicine, Hamamatsu City, Shizuoka, Japan conducted a study to establish a new coagulation index Haemostasis in normal and abnormal pregnancy. (O' Riordan and Higgins, 2003) .

1.3 Rationale:

The determination level of plasma fibrinogen, activated partial thromboplastin time, prothrombin time in pregnant female is very important in diagnosis of disease and follow up of pregnancy (Paolo, 2008).

Fibrinogen is clotting protein, when its levels increase it may lead to thrombosis , this study carried out to asses PT , APTT and Fibrinogen level during pregnancy and monitoring homeostatic status of pregnant female (Bengt, 2008).

Pregnancy itself is a factor of hypercoagulability (pregnancy- induced hypercoagulability]. And Deep vein thrombosis the second most common cause of maternal death in countries after bleeding.This study was conducted to determine the frequency of hypercoagubility in pregnancy.

1.4 objectives

1.4.1 General objectives:

To measure some haemostatic parameter among Sudanese pregnant females in Khartoum state.

1.4.2 Specific objective:

1- To measure PT , APTT and fibrinogen level among Sudanese Pregnant female and compare with control group .

2- To determine the relationship between PT, APTT and Fibrinogen level according to gestational age.

3- To measure PT, APTTand Fibrinogen level according to the age group .

Chapter tow

Materials and methods

2.1 Study design:

This is an analytical case control study was conducted in Khartoum state to measure some coagulation parameter in patient attending Turkish Teaching Hospital during the period from April to July 2013.

2.2 Data collection:

Data was collected using design questionnaire including age, gestational age, medical history and other information.

2.3 Sample size:

Hundred healthy pregnant females as case group and fifty healthy females serve as controls group was included in the study. Both cases and controls were randomly selected**.**

2.4 Inclusion criteria:

Hundred healthy pregnant females in first, second and third trimester not complicated with any disease and no anticoagulant treatment. No history of significant gynecological problems, healthy females with no history of significant medical illness, who agreed to participate for the study voluntarily were included into study.

2.5 Exclusion criteria:

Pregnant female suffer from cardiovascular, renal, liver , endocrinal disorders, bleeding disorders or anticoagulant therapy, and pregnant female suffer from any condition affected the results were excluded from the study.

2.6 Sample collection:

Two ml of blood sample was collected in sodium citrate tubes from the antecubital fossa. The ratio of blood to citrate 9:1 they were centrifuged for 15minute at 3000xg to isolate platelets poor plasma and examined immediately by coagulometer.

2.7 Data analysis:

The data were analyzed using Statistical Package for Social Science SPSS computer program version 15.1.

2.8 Ethical consideration:

All information obtained from participants was kept as highly security data. Verbal consent was taken from all participants. The participants were provided with information about the study and any risk that may arise especillay when the collection technique was applied.

2-9 Material:

1. Pipette.
2. Cup Array for Sample Cups (Controls/Calibrators)
3. Reagent Block (removable)
4. Washer / Cleaner Position
5. Rinsing Station
6. Measuring / Cuvette Ring Rotor
7. STAT Positions
8. Sample Positions
9. USB Stick Interface (undern eath) + handles to carry analyzer
10. Touch sensitive color display
11. Safety Shield Handling

2.10 Methods:

2.10.1 Principle of coagulometer:

The automatic coagulometer [clot] is an instrument for the determination of the main parameter used in the plasma coagulation method.

The coagulometer has an optical measurement system which detects sudden variation in optical density when clot is formed.

The chronometer and the stirring system are activated by sudden change in optical density. This permits the initiation of the time measurement when the sample is added to the reagent and stop the measurement time at the moment that the clot is formed. The continues mixing is guarantees a perfect homogenization and make the measurement possible of low concentration of fibrinogen by grouping the fibrin filament in the center of the optical pass.

2.10.2 Procedure of coagulometer:

First of all the Cuvette was placed corresponding to the determination that were done on the thermostat. A magnetic stirrer was installed in every Cuvette and waited for the instrument to reach 37 ^{0}C after that into the Cuvette the sample or reagent volume required were introduced.

PT= [200ml of reagent].

APTT= [100ml of reagent+ 100ml of plasma].

Fibrinogen= [200ml of diluted plasma].

When thermo station time was finished the Cuvette was placed on reading well the chronometer was remained inactive for some second and when it was showed 000,0 at this moment the reagent of plasma was added with disposable tip pipette.

The liquid was left to get down with one below and all the reaction was started at the same time.

100ml of starter was added:

PT= plasma

APTT=cacl2

Fibrinogen= thrombin reagent.

When the reagent and plasma were in contact and optical density variation were produced that automatically activate the digital chronometer and the magnetic mixer. When the clot was start to formed and optical density was produced and stopped the chronometer and mixer the clotting time appears on the display.

2.10.3 Prothrombin time:

2.10.3.1Principle:

The PT test measures the clotting time of recalcified plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the 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 the fibrinogen concentration of the plasma (Buseri *et al.,* 2008).

2.10.3.2 Procedure:

- The PT reagent was incubated at (37 ^{0}C) for at least 10 minutes.
- Then 25µl of sample was pipette into a test cuvette, incubated at (37 C) for 1-2 minutes.
- Add 50 µl of PT was added plus reagent (37^{o}C) and simultaneously start test .The clotting time was recorded in seconds.

Table (2.1) Prothrombin time (plasma) normal range: (Abbassi *et al*, 2009)

Units	Nonpregnant Adult	First Trimester	Second Trimester	Third Trimester
seconds	12.7 - 15.4	9.7 - 13.5	9.5 -13.4	9.6 - 12.9

2.10.4 Activated partial thromboplastin time:

2.10.4.1 Principle:

The test measures the clotting time of plasma after the activation of contact factors and the addition of phospholipids and $CaCl_2$ but without added tissue thromboplastin. And so indicate the overall efficiency of the intrinsic pathway. To standardize the activation of contact factor, the plasma is first preincubated for a set period with a contact activator such as kaolin, silica or ellagic acid. During this phase of the test, factor XIIa is produced, which cleaves factor XI to factor XIa, but coagulation does not proceed beyond this in the absence of calcium. After recalcification, factor XIa activate factor ix and coagulation follows. Astandardized phospholipid is provided to allow the test to be performed on platelets poor plasma. The test depends not only on the contact factors and on factors VIII and IX but also on the reactions with factors X, V, prothrombin and fibrinogen. It is also sensitive to the presence of circulating anticoagulants [inhibitors] and heparin.

2.10.4.2 Procedure

- Pipette 100 µl from sample or control plasma into a test cuvette.
- Incubated at 37°C for 1 to 2 minute.
- Then add 100 ml of APTT reagent to the cuvette containing the plasma we maintained the suspension of the reagent by magnetic stirring.
- Incubate the mixture at 37c for 3 minutes then rapidly add 100 ml of the pre-incubated calcium chloride (0.02) and start the timer finally the clotting time was recorded in second.

Table (2.2): Activated partial thromboplastin time normal range: (Abbassi *et al.*, 2009)

Units	Nonpregnant Adult	First Trimester	Second Trimester	Third Trimester
Seconds	**26.3 - 39.4**	**24.3 - 38.9**	**24.2 - 38.1**	**24.7 - 35.0**

2.10.5 Measurement of fibrinogen concentration:

2.10.5.1Principle:

Diluted plasma is clotted with strong thrombin solution the plasma must be diluted to give a low level of any inhibitors (FDPs and heparin) .A strong thrombin solution must be used so that the clotting time over a wide range is independent of the thrombin concentration.[clauss a rapid physiological coagulation method in determination of fibrinogen acta haematologica{basal] 1957"17"237 } reagents calibration plasma with known level of fibrinogen calibrated against an international reference standard ppp frome patient and control thrombin solution freshly reconstituted to 100NIH u per ml in 9g/l Nacl owrens " veronal buffer

2.10.5.2 Procedure

- Dilute PPP 1:10 prepared from specimen with owren"s buffer solution.
- Add 200ml of 1: 10 dilution of plasma sample to be tested to test tube at 37°C.
- Incubate at 37c° for one minute and to the test tube.
- Add 100ml of thrombin reagent and started the stopwatch and stopped it after the appearance of the fibrin web then we repeated steps 1-5 for a duplicate test then we calculated the mean clotting time for the plasma specimen.

Table (2.3) Plasma Fibrinogen normal range: (Abbassi *et al*., 2009)

Units	Nonpregnant Adult	First Trimester	Second Trimester	Third Trimester
mg/dL	**233 - 496**	**244 - 510**	**291 -538**	**373 -619**
g/L	**2.3 - 5**	**2.4- 5.1**	**2.9- 5.4**	**3.7 - 6.2**

Chapter Three
Results

3.1 Results

Hundred healthy pregnant females when selected as case group and fifty females serve as control group were conducted in the study to measure the PT, APTT and fibronogin.

The results showed that the case groups has mean prothrombin time of 15.7 ± 1.4 sec where as the control group showed mean PT of 13.3 ± .94sec P < 0.000(by the independent sample test) which is significant. The case group has mean APTT of 31.8 ± 3.8 sec where as the control group showed mean APTT of 29.8 ± 4.11sec with P.value < 0.006.

The case group has a mean of fibrinogen level 467 ± 227mg/dl where as the control group showed mean of fibrinogen level of 292 ± 70.6 mg/dl , P < 0.000.table (3.2).

When comparing the PT, APTT and Fibrinogen level with the age of study group, the study found that the mean of PT in age group between 15 – 25 years old was 15.5± 1.6sec and the P > 0.478. The mean of PTT was 31.9± 3.3sec and the p > 0.973. The mean of Fibrinogen was 458.6±224.3mg/dl and the P > 0.955 that show there is no significant. Table (3.3).

When comparing the mean (PT, APTT and Fibrinogen) in the three trimesters of gestational age, the result found that the mean of PT in the first trimester was 15.3±.9sec in second trimester was 15.9±1.3sec and in third trimester was 15.7±1.6sec with P. value > 0.597.

The mean of PTT in first trimester was 27.4±3.7sec, in second trimester was 31.3±3.9sec and in third trimester 32.5±3.3sec with P.value > 0.009. The mean of Fibrinogen in first trimester was 502±229.9mg/dl, in second trimester was 468±213.4mg/dl, and in third trimester 471±244.0mg/dl the P > 0.953. That shows there in significant increase the PTT.table (3.4).

Table (3.1): age distribution for case and control:

Age group / years	Case No.	Control No.
15 - 25	32	15
26 – 35	63	25
> 35	5	10
Total	100	50

Table (3.2): Comparison of mean (PT, PTT and Fibrinogen) between case and control

Test		N	Mean	Std. Deviation	P value
PT/seconds	Case	100	15.8	1.4	0.000
	Control	50	13.4	0.9	
APTT/seconds	Case	100	31.8	3.8	0.006
	Control	50	29.9	4.1	
Fibrinogen mg/dl	Case	100	467.8	227.5	0.000
	Control	50	292.1	70.6	

Table(3.3): Correlation between the PT, APTT and Fibrinogen according to the age group of study population.

Dependant Variable	Age Group/years	No.	STD	Mean	p. Value
PT/ Seconds	15-25	32	1.6	15.5	0.478
	26-35	63	1.3	15.9	
	>35	5	0.7	16.2	
	Total	100	1.4	15.8	
PTT/ Seconds	15-25	32	3.3	31.9	0.973
	26-35	63	4.4	31.7	
	>35	5	3.8	31.6	
	Total	100	224.3	31.8	
Fibrinogen / mg/dl	15-25	32	234.8	458.6	0.955
	26-35	63	187.5	473.2	
	>35	5	227.5	459.8	
	Total	100	890.6	1.58	

Table (3.4) Comparison of mean PT, APTT and Fibrinogen according to gastaional age of study population.

Dependant Variable	Mean of First trimester	STD of First trimester	Mean of Second trimester	STD of Second trimester	Mean of Third trimester	STD of Third trimester	P. Value
PT/ Seconds	15.3	0.9	15.9	1.3	15.7	1.6	0.597
APTT/Seconds	27.4	3.7	31.3	4.0	32.5	3.3	0.009
Fibrinogen/ mg/dl	502	230.0	468	213.4	471	244.1	0.953

Chapter four
Discussion, conclusion and Recommendation

4.1 Discussion

The study showed that the mean of prothrombin time of the study group was significally prolonged than that of control group, and activated partial thromboplastin time (APTT) significantly prolonged in all pregnant females of the study group compared to control group, this agreed with **(**McCrory, 2010), who stated that there was prolongation in PT ,APTT in case group . Normal pregnancy accompanied by changes in coagulation and fibrinolytic system – which include some of coagulation factors (I, II, VII, VIII, IX and XII).

Fibrinogen level also increases in study group compared to control with statistical significance (P value > 0.000). During normal pregnancy there is generalized intravascular inflammatory response to concept as total fibrinogen concentration increase during pregnancy. Also their was increased in fibrinogen level in first trimester, This study disagreed with that done in Obstetric and Gynecology University center Utrecht in 2005,who found increases in fibrinogen from first to third trimester.

The activated partial thromboplastin (APTT) significantly prolonged in third trimester of gestational age comparing with the first and second trimester (P value > 0.000). Prothrombin time showed slightly increase in second trimester and fibrinogen level increase in first trimester. However factor VII, VIII, IX, XII activities increased gradually as pregnancy progressed , reached maximum values in the third trimester.

This result disgreed with study of (Rubin, 1998), who stated that in the third trimester, their was shortening of prothrombin time and activated partial thromboplastin time, and agreed with study conducted in medical collage cuttack in 2007. Which show increase in coagulation profile in all three trimester of gestation (Thornton, 2010).

4.2 Conclusion

This study concluded the following**:**

- Normal pregnancy state results in the significant increase of most coagulation parameter.
- Coagulation, anti-coagulation, fibrinolytic and anti-fibrinolytic activities are enhanced and balanced at a higher level during pregnancy.
- PT, APTT and Fibronogen level of pregnant females when compared to control group showed that there was prolongation with APTT.
- According to gestational age there was prolongation in APTT in third trimester and no significant with PT and Fibronogen level.
- The finding of PT in age group more than 35 years showed prolongation.

4.3 Recommendation

1- Recommended for further work up to involve large number of pregnant female in different states of Sudan to get over view.

2- Regular check up during pregnancy is very important so as to detect all abnormalities raised during pregnancy. Fibrinogen level is one of the factors affecting coagulation process, so it must be measured as routin investigation during pregnancy.

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Appendices

Appendix I

Questionaire

Sudan University of science and Technology

College of Graduate Studies

رسالة ماجستير بعنوان:-

قياس بعض معدلات تخثر الدم عند النساء الحوامل

Questionaire

- Number
- Age
- Gender
- Gestational age
- Medical condition eg: (cardio vascular disease, renal disease and liver disease)
- Other disease
- Treatment
- Investigation : PT , PTT , Fibrinogen level.

Appendix II

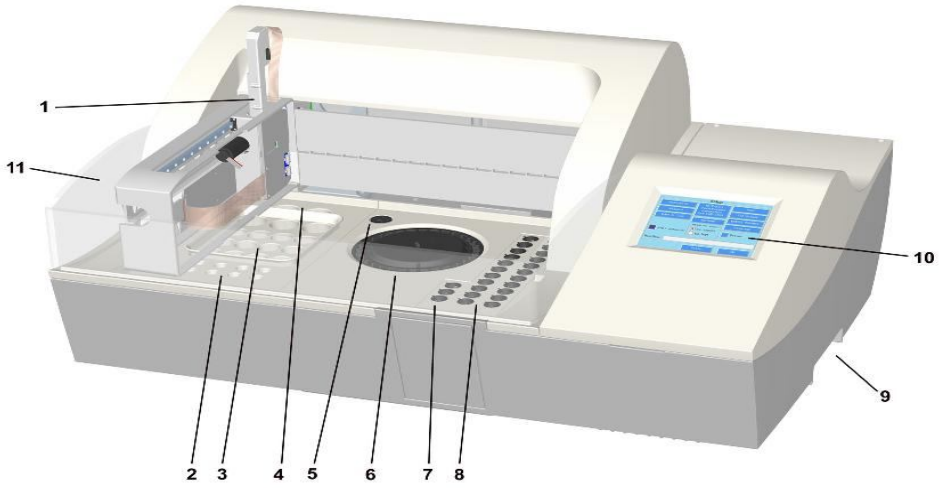

Fig (2.1) Coagulometer (kim, *et al*., 2006)