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Evaluation of Haemostatic Changes Among Non Complicated pregnancy in Shendi Locality - Nile River State - Sudan

A thesis Submitted in Fulfillment For the Requirement the M.Sc. Degree in (Hematology)

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الآية

قال تعالى:

(اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ (2) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ (3) الَّذِي عَلَّمَ بِالْقَلَمِ (4))

سورة العلق : الايات 1- 4

Dedication

To my wonderful parents who strongly supported me all throughout.

To my beloved brothers.

To all those whom I always love, care and respect.

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First of all I would like thank Allah, for giving me health strength and patience to complete this work. I would like to formally thank:

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Abstract:

Background: Normal pregnancy is associated with haemostatic changes which have been linked to a complex physiological adaptation but these changes return to that of non-pregnant state at about 4 weeks of post-delivery. The study aimed to assess the PT,PTT, Fibrinogen and D-Dimer level among pregnant women .

Methodology: This was a cross-sectional descriptive study, has been conducted at Mek Nemir hospital and Shendi Teaching Hospital from August 2015 to August 2017, Following informed consent 145 pregnant women in different trimesters and different ages were enrolled in this study, their aged ranged from (17- 40) years with mean average of SPSS version ---was used to calculate the mean , STD, frequency and independent *t* test , the p values were considered to be significant < (0.05)

Results: The mean age of pregnant women was (24.4) years. Pregnant women with Haemostasis change in different trimesters 60% were in third trimester. In the study population the values of *PT* (14.3) second during pregnancy and control (12.5) second showed significant differences (P>0.00) but within normal range, values of *APPT* (34.1) second during pregnancy and control (32.4) second showed no significant differences (P=0.30), fibrinogen levels during pregnancy (548.1)mg/dl and control (269.6) mg/dl showed significant differences (P>0.00), and D-Dimer levels during pregnancy (609.7)ng/ml and control (285.5) ng/ml showed significant differences (P>0.00) without normal range.

Conclusion: The study concluded that changed values of PT, APTT, fibrinogen levels and D-Dimer levels during pregnancy, It is recommended that fibrinogen level and D-Dimer level be monitored during pregnancy to minimize thrombotic effect.

المستخلص :

خلفية : يرتبط الحمل الطبيعي مع التغيرات في الارقاء الدموي والتي ترتبط بالتكليفات الفسيولوجية المعقدة ولكن هذه التغييرات تعود للطبيعي بعد أربعة أسابيع بعد الولادة , وقد أجريت هذه الدراسة تقييم اختبارات التخثر أثناء الحمل في مدينة شتدي.

الهدف : هدفت هذه الدراسة إلى تقييم تأثير الحمل على أنواع مختلفة من اختبار التخثر. وقد أجريت في مستشفى الملك نمر ومستشفى شتدي التعليمي بين أغسطس 2015 إلى أغسطس 2017. والتي شملت 145 امرأة حامل في مختلف الأعمار والشهور.

الطريقة : تم رصد 145 امرأة حامل ، تتراوح أعمارهم بين (40-17) سنة وتختلف شهور الحمل للارتقاء الدموي بين أغسطس 2015 وأغسطس 2017 في مستشفى الملك نمر ومستشفى شتدي التعليمي. تم تحليل البيانات عن طريق برنامج SPSS لحساب المتوسط ، وتحليل عينات الدم التي تم جمعها باستخدام تقنيات المختبر القياسية.

النتائج : كان متوسط عمر النساء الحوامل (24.4 ± 6.1) سنة. وكانت النسبة الاكبر في تغيرات الارقاء الدموي في اجزاء الحمل الثلاث للجزء الثالث بنسبة 60 % . أظهرت نتائج الدراسة وجود فورقات معنوية بين النساء الحوامل والغير حوامل في وقت البروثرومبين **(P>0.00)** وكانت داخل المعدل الطبيعي. وفي قيمة قيم وقت الثرومبوبلاستين الجزئي لا توجد فروق ذات دلالة إحصائية (P = 0.30) . ام ا مستويات الفيبرينوجين ومستويات دي-ديمر أظهرت فروق معنوية **(P>0.00)** دون المعدل الطبيعي .

الاستنتاج : خلصت هذه الدراسة الى ان هناك زيادة في وقت البروثرومبين وقت الثرومبوبلاستين الجزئي و مستويات الفيبرينوجين ومستويات دي-ديمر اثناء فترة الحمل الطبيعي، ووجد ايضا من المستحسن رصد تغيرات مستويات الفيبرينوجين و دي-دايمر اثناء فترة الحمل للحد من اخطار الجلطات .

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Abbreviations:

Term	Full term
APS	Antiphospholipid syndrome
APTT	Activated partial thromboplastin time.
AT	anti-thrombin
CSF	Cerebrospinal fluid.
DDAVP	Desmopressin
DIC	Disseminated intravascular coagulation.
ELISA	Enzyme-linked immunosorbent assay.
ELT	Euglobulin clot lysis time.
ET	Essential thrombocythemia
F1+2	Prothrombin fragment 1 + 2
FDPs	Fibrin degradation products.
FNP	fresh normal pooled plasma
HbH	Hemoglobin H disease
HELLP	hemolysis, elevated liver enzyme levels, and low platelet levels.
HUS	Hemolytic uremic syndrome
INR	International Normalized Ratio.
ISI	International Sensitivity Index
ITP	Immune thrombocytopenia purpur
LMP	last menstrual period

Term	Full term
LMoWH	low molecular weight heparin
MCV	mean red cell volume
MMA	methyimalonic acid
MNPT	Mean normal prothrombin time
MPD	Myeloproliferative disorders
PAI	plasminogen activator inhibitor
PPH	Postpartum bleeding or postpartum hemorrhage
PT	prothrombin time.
R	Ratio
RBCs	Red Blood Cells
SLE	Systemic lupus erythematosis
TAFI	Thrombin-activatable fibrinolysis inhibitor.
TAT	thrombin-antithrombin III complex
TDI	total dose infusion
TEG	Thromboelastography
t-PA	type plasminogen activator.
TTP	Thrombotic thrombocytopenic purpura
uPA	urokinase-type plasminogen activator.
VAD	vascular access device
VTE	venous thromboembolism.
VWF	Von Willebrand factor
WHO	World Health Organization.
XDP	fibrin derivatives in plasma containing D-Dimer.

Chapter one

Introduction

Rationale

Objectives

1.1. Introduction:

The coagulation involves a series of enzymatic reactions leading to the conversion of soluble plasma fibrinogen to fibrin clot . Roman numeral are used for most of the factor, but I and II are referred to as fibrinogen and prothrombin respectively ; III, IV and VI are redundant. The active forms are denoted by (a). [1]

The generation of thrombin is depended on three enzyme complexes, each consisting of protease, cofactor, phospholipids and calcium. They are extrinsic Xase (VIIa, TF, PL, Ca^{2+})and intrinsic Xase (IXa, VIIIa, PL, Ca^{2+}) generation FXa, prothrnombinase complex (Xa, Va, PL, Ca^{2+}) generation thrombin. [2]

The coagulation disorder is deficiencies of each of the coagulation factor. May be hereditary or acquired. The hereditary include hemophilia A that is most common of the hereditary clotting factor deficiencies. The inheritance is sex-linked, factor IX deficiency (hemophilia B, Christmas disease) the inheritance is X chromosome, von Willebrand disease is reduced level or abnormal function of VWF. [2]

 Coagulation acquired include vitamin K deficiency is obtained green vegetables and bacteria synthesis in the gut, liver disease, hemorrhagic disease of the newborn and disseminated intravascular coagulation . [2]

 The prothrombin time is functional determination of the extrinsic (tissue factor) pathway of coagulation and is extremely sensitive to the vitamin-K dependent clotting factors (factors II, VII, and X). Tissue factor (factor III) is a transmembrane protein that is widely expressed on cells of non-vascular origin, which activates factor VII during the initiation of the extrinsic coagulation pathway. A cascade mechanism results in fibrin production and clot formation. [3]

The partial thromboplastin time is originally performed to screen the function of coagulation system, mainly for the intrinsic pathway factors: factor XII, XI, IX and VIII. Various APTT reagents for different purposes are now available, including for screening of factor deficiencies and lupus anticoagulant. [4]

Pregnancy changes the plasma levels of many clotting factors, such as fibrinogen, which can rise up to three times its normal value. Venous stasis may occur at the end of the first trimester, due to enhanced compliance of the vessel walls by a hormonal effect. Hypercoagulability states as an pre-existing condition in pregnancy 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. [5]

During normal pregnancy, the concentration of many of the clotting factors rise, thereby increasing the potential to generate fibrin. There is aso evidence of increased thrombin activity during normal pregnancy which sharply increased during placental separation. Antithrombin III, the main inhibitor of thrombin and activated factor X, shows no compensatory rise during pregnancy bu increase during the puerperium. [6]

1.2. Rationale:

Normal pregnancy is accompanied by changes in the coagulation and fibrinolytic systems. These include increases in a number of clotting factors and inhibition of fibrinolysis. These changes may be important for reducing intrapartum blood loss, but they determine an increased risk of thromboembolism during pregnancy, this study was can ducted to determine the effect of pregnancy on *PT*, *APTT*, Fibrinogen level and D-Dimer among pregnant women in Shendi locality.

1.3. Objectives of the study*:*

1.3.1. General objectives:

To determine the effect of pregnancy on coagulation screening test, fibrinogen and D-Dimer.

1.3.2. Specific objectives:

1. To determine the effect of pregnancy on prothrombin time.
2. To determine the effect of pregnancy on partial thromboplastin time.
3. To determine the effect of pregnancy on fibrinogen level.
4. To determine the effect of pregnancy on D-Dimer.
5. To compare prothrombin time, partial thromboplastin time, fibrinogen and D-Dimer between different stage of pregnancy.

Chapter 2

Literature Review

2.1. pregnancy :

Pregnancy, also known as gravidity or gestation, is the time during which one or more offspring develops inside a woman. A multiple pregnancy involves more than one offspring, such as with twins. Pregnancy can occur by sexual intercourse or assisted reproductive technology. It usually lasts around 40 weeks from the last menstrual period (LMP) and ends in childbirth. This is just over nine lunar months, where each month is about (29½) days. When measured from conception it is about (38) weeks. An embryo is the developing offspring during the first eight weeks following conception, after which, the term fetus is used until birth. Symptom of early pregnancy may include a missed periods, tender breasts, nausea and vomiting, hunger, and frequent urination. Pregnancy may be confirmed with a pregnancy test.[7]

Normal pregnancy is associated with major changes in many aspects of haemostasis all contributing to maintain placental function during pregnancy and to prevent excessive bleeding in delivery. Most changes in blood coagulation and fibrinolysis create a state of hypercoagulability. This phenomenon protects the woman from haemorrhage during delivery but predisposes her to thromboembolism both during pregnancy and in puerperium. The changes in the coagulation system in normal pregnancy are consistent with a continuing low-grade process of intravascular coagulation. [8]

Coagulation system During pregnancy the concentrations of coagulation factors VII, VIII, IX, X, XII and von Willebrand factor rise significantly, accompanied by a relevant increase in the concentration of plasma fibrinogen. Plasma fibrinogen often increases to over (600) mg/dL in late pregnancy. Factor VII may increase as much as tenfold in pregnancy. The von Willebrand factor and factor VIII are elevated in late pregnancy, when coagulation activity is about twice that in the non-pregnant state.1 The increase in factor IX concentrations during pregnancy is reported by several authors to be small, as is the decrease in factor XI. After an initial increase, factor XIII falls gradually, reaching (50%) of the normal no

pregnant value at term. Factors II and V do not change significantly in pregnancy.
[8]

Table (2 - 1): Main changes in haemostasis factors during pregnancy.

Platelet count	↓
II, V	=
Fibrinogen, VII, VIII, von Willebrand factor, IX, X, XII	↑
XI	= / ↓
XIII	↑ / ↓
Antithrombin	=
Protein C	= / ↑
Protein S	↓
Heparin cofactor II	↑
F1+2,TAT,d-dimer	↑
t-PA	↓
ELT,PAI, TAFI	↑

↑ : increased

↓ : decreased

= : not changes

2.1.1.Hematology of pregnancy:

Pregnancy place extreme stresses on the hematological system and an understanding of the physiological changes that result is obligatory in order to interpret any need for therapeutic intervention. [1]

Pregnancy leads to a hypercoaguable state with consequent increased risks of thromboembolism and *DIC*. These is an increased in plasma factors VII, VIII, X and fibrinogen and fibrinolysis is suppressed. These changes last for up to (2 months) into the puerperal period and the increased of thrombosis during this period is increased. There is an association between thrombophilic conditions in the mother and with recurrent fetal loss. This is presumed to result from placental thrombosis and infarction. [1]

Warfarin has no role in management. It crosses the placenta and in addition is associated with embryopathy, especially between (6 and 12) weeks' gestation. Heparin does not cross the placenta but a significant side-effect of prolonged use is maternal osteoporosis. Low molecular weight heparin is now the treatment of choice because it can be given once daily and is less likely to cause osteoporosis. [1]

Normal pregnancy involves many changes in maternal physiology including alterations in hematologic parameters. These changes include expansion in maternal blood and plasma volume. The increase in plasma volume is relatively larger than the increase in red cell mass resulting in a decrease in hemoglobin concentration. An increase in the levels of some plasma proteins alters the balance of coagulation and fibrinolysis. Worldwide, the predominant cause of anemia in pregnancy is iron deficiency. Fetal requirements for iron are met despite maternal deficiency, but maternal iron deficiency has a number of adverse consequences including an increased frequency of preterm delivery and low-birth-weight infants. Bleeding disorders in pregnancy are a common reason for hematologic consultation and evoke concern for both the mother and child. Life-threatening bleeding caused by disseminated intravascular coagulation is seen with some complications unique to pregnancy, including placental abruption, retained dead

fetus, and amniotic fluid embolism. Von Willebrand disease is the commonest inherited bleeding disorder, but because of increases in factor VIII level and von Willebrand factor (VWF) during pregnancy, excessive bleeding at delivery is rarely a problem. Factor levels fall rapidly postpartum, and serious hemorrhage can occur during this period. Carriers of hemophilia A and B should be monitored during pregnancy to determine if factor levels will be adequate for delivery at term. Caution should be exercised at delivery and during the first few days of life with offspring of hemophilia carriers until hemophilia testing is completed and the infant's status is known. Acquired hemophilia as a result of factor VIII autoantibodies is rare, but can occur during pregnancy or the puerperium. Thrombocytopenia is not uncommon in pregnancy, and its causes include several conditions that are unique to pregnancy, such as preeclampsia. Idiopathic thrombocytopenic purpura (ITP) is common, it is often exacerbated in pregnancy, and is managed conservatively if possible; close followup of newborns of mothers with *ITP* is essential. *HELLP* syndrome and *TTP, HUS* are also seen in pregnancy and the puerperium. *HELLP* syndrome is managed with delivery if possible, whereas *TTP* requires plasma exchange. Inherited and acquired prothrombotic conditions can be exacerbated by pregnancy and can result in adverse reproductive outcomes as well as venous thromboembolism. The strongest evidence for an association between a thrombophilia and recurrent fetal loss exists for antiphospholipid antibody syndrome; however, evidence is mounting for a connection between inherited thrombophilias and the severity of some complications of pregnancy. These thrombophilias increase the risk of venous thromboembolism in pregnancy and the puerperium. Treatment of hematologic malignancies in pregnancy can present a difficult dilemma both in terms of staging studies and management. In many cases of Hodgkin lymphoma, treatment can be delayed safely until after delivery. [9]

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Blood volume changes in pregnancy both red cell mass and plasma volume increase during pregnancy. Since the increase in red cell mass is around (25%), well below the plasma volume increase of (40%) (up to (55%) in multiple pregnancies) the hemoglobin level falls, particularly between weeks (24 and 32) of pregnancy. This decrease in hemoglobin level occurs even when iron and folate stores are adequate, but is accentuated by deficiencies. The consequent decrease in blood viscosity facilitates placental perfusion. Women who do not exhibit a fall in haemoglobin during a pregnancy have a high incidence of complications such as preeclampsia and stillbirth, according to the Swedish Medical Birth Register 1. Although (110g/l) is commonly accepted as the lower limit of normal hemoglobin in pregnancy there are many patients in whom the hemoglobin falls as low as (95g/l) without any deleterious effects or evidence of iron or folate deficiency. [10]

Folate status at conception and in pregnancy it is now widely accepted that folate deficiency at conception is strongly correlated with neural tube defects. It is strongly recommended that all women take folate supplementation for (3 months) prior to conception and throughout pregnancy. Women with a family history of neural tube defect, with diabetes or on anti-epileptiform drugs constitute a high risk group and require a daily (5 mg) folate dose. For standard risk women the recommended folate supplement is (0.5mg) daily. It should be noted that the standard Fefol tablet only contains(0.3mg) of folate, well short of the recommended pre-conception intake. In addition, since intolerance to the iron in Fefol may hinder compliance, it makes sense to prescribe folate alone initially, switching to a combined iron/folate preparation only if the serum ferritin is reduced. [10]

Folate and Vitamin B12 metabolism in pregnancy a slight increase in mean red cell volume (MCV) may occur in normal pregnancy. However, an increase in *MCV* to over (105fl) (given a normal range of 80-100fl) warrants assessment of red cell and serum folate and serum Vitamin B12 levels. Neutrophil hyper segmentation is not a reliable indicator of megaloblastic anemia in pregnancy due to the tendency for a left shift to occur. Decreased folate intake due to such causes as vomiting in pregnancy and malabsorption, combined with increased requirements at pregnancy, make folate deficiency a much more common cause of megaloblastic anemia than VitaminB12 deficiency. A fall in the inactive transcobalamin I during pregnancy results in a decrease in Vitamin B12 levels which does not accurately reflect either the active transcobalamin II or body stores, which on average last for (2 years). In fact the B12 levels may fall to (100pmol/l) or even below without any functional disturbance of B12 metabolism. When the patient has a macrocytic anaemia, a balanced diet, normal folate levels but reduced Vitamin B12, it is worth checking serum homocysteine and urine methyimalonic acid (MMA) levels and anti-parietal cell and anti-intrinsic factor antibodies before giving empiric parenteral Vitamin B12. If genuine Vitamin B12 deficiency is confirmed investigation can then be undertaken at leisure once breast feeding has been completed. [10]

Iron deficiency in pregnancy Iron deficiency accounts for (75%) of anaemias of pregnancy. The markedly raised red cell mass, together with the requirements of the placenta and foetus necessitate a considerable increase in recommended daily iron intake of around (30mg) daily, compared with (15mg) daily in the non pregnant state. A serum ferritin estimation at the initial antenatal visit will help to determine whether iron supplementation will be needed and whether this should be taken as Fefol or equivalent or whether iron containing multivitamin preparations will suffice. In the second half of pregnancy symptomatic iron deficiency, developing in the setting of intolerance to oral iron can be effectively treated with intravenous total dose infusion (TDI). These infusions are safe and effective but

need to be given by experienced staff adhering to a strict protocol. Additional important causes of a microcytic blood film (MCV < 8Ofl) are Thalassemias / haemoglobinopathies. As thalassemia trait may be masked by iron deficiency, it is important to check not only Hb electrophoresis and *HbH* bodies but also serum ferritin. This also avoids the unnecessary administration of iron supplementation to patients whose primary problem is not iron deficiency but thalassemia. Checking the partner is important once a diagnosis of thalassemia trait/haemoglobinopathy is confirmed so that genetic advice can be given promptly if appropriate. [10]

Platelets in pregnancy in up to (10%) of pregnancies mild thrombocytopenia supervenes after week (20). The platelet count rarely fails below (75/nl) and pregnancy proceeds uneventfully. There is typically a history of similar mild thrombocytopenia with previous pregnancies but normal platelet count between pregnancies. For this reason the term gestational thrombocytopenia is usually applied. The baby's platelet count at birth is normal. Platelet antibodies are rarely found, but it remains likely that the transient thrombocytopenia is of autoimmune a etiology. An autoimmune mechanism also accounts for most cases of severe isolated thrombocytopenia in pregnancy. *ITP* which accounts for (5-10%) of thrombocytopenia in pregnancy is important as the severity of the thrombocytopenia often posing major problems for pregnancy, delivery and for the baby. Systemic lupus erythematosis (SLE), the antiphospholipid syndrome (APS) and pre-eclampsia and its *HELLP* syndrome variant also need to be excluded. Platelet-specific antibodies are found in over (50%) of patients with immune thrombocytopenia. However the presence or absence of antibodies does not correlate with severity. Bone marrow examination is usually noncontributory. Typically, the platelet count falls , sometimes profoundly, as pregnancy progresses. In severe acute *ITP* high dose corticosteroid therapy and intravenous immunoglobulin may be required. In the most extreme cases splenectomy may be unavoidable, but can only be performed with acceptable safety to mother and foetus during the second trimester. In the milder acute *ITP* and in chronic *ITP* it

may be possible to delay treatment until the last few weeks of pregnancy providing there is no active bleeding and the platelet count remains above (25/nl). In the last few weeks of pregnancy however, treatment aimed at increasing the platelet count to (75-100/nl) is needed since this enables delivery and epidural anesthesia to be performed safely. In maternal *ITP*, particularly in splenectomized patients there is a significant risk of severe neonatal thrombocytopenia. This holds true even when the maternal count is normal or near normal at the time of delivery. In *ITP*, the mode of delivery should be determined by obstetric indications. When the thrombocytopenia is associated with pregnancy-specific causes such as pre-eclampsia, *HELLP* syndrome and acute fatty liver it is important to perform a coagulation profile including D-dimers to exclude *DIC*. *TTP* and *HUS* is an important diagnosis since the disease complex is life threatening but responds rapidly to early intensive plasma exchange therapy. The red cell appearances on the peripheral blood film in *TTP* are often characteristic, with variable numbers of red cell fragments. *MPD* and other haematological malignancies occur occasionally in pregnancy. *ET* is the most common of these. Despite the potential hazards of thrombosis and haemorrhage it is usually possible to avoid exposure to myelosuppressive agents during pregnancy. [10]

Thrombosis and homeostasis in pregnancy the incidence of *VTE* increases three-fold in late pregnancy and ten-fold in the immediate post partum period. This risk reflects a variety of pregnancy induced changes in the haemostatic mechanism including an increase in fibrinogen and Factor VIII, the development of acquired resistance to activated protein C, a reduction in the antithrombotic factor protein S, and reduced fibrinolytic activity. In pregnancy the ESR reflects the hyper fibrinogenaemia and loses its usefulness as a marker for systemic disease. Inherited thrombophilias, whether due to genetic mutations such as factor V Leiden and prothrombin, gene mutation or deficiencies of protein C, S and *AT*, increase the risk, not only of *VTE* in pregnancy and the post-partum period but also of recurrent fetal loss. However, the increase in risk is not considered sufficient to warrant

funding of thrombophilia screening for recurrent fetal loss under the Medical Benefits Schedule. Anti-cardiolipin antibodies and lupus anticoagulant, particularly when present at high titer, markedly increase the risk of fetal loss and maternal *VTE*, and may warrant the use of prophylactic *LMWH* during pregnancy and the puerperium. Patients with homozygous or multiple thrombophilic defects are also at greatly increased risk, and likewise merit *LMWH* prophylaxis. If therapeutic anticoagulation is required during pregnancy *LMWH* is the drug of choice for most conditions. In view of the risk of bleeding complicating epidural an aesthesia it is mandatory to obtain expert advice concerning the timetabling of heparin dosage particularly *LMWH*. *VWF* disease is the most common inherited bleeding disorder, mild (type 1) disease accounting for (90%) of cases. In pregnancy, extending through to the immediate post partum period, there is usually a temporary normalization of the disease, measurable as a return of Factor Vlll complex levels and platelet function studies to normal. A check early in the third trimester revealing that these parameters are all within the normal range clears the way for epidural an aesthesia to be performed safely if required. However, a history of *PPH* in a previous pregnancy may override these considerations and mandate the prophylactic use of specific therapy such as *DDAVP* (Minirin) or Factor Vlll concentrate, depending on the severity of the von Will brand's disease. [10]

2.1.2. Coagulation changes in pregnancy:

Pregnancy is a state of hypercoagulation, which is likely an adaptive mechanism to reduce the risk of hemorrhage during and after the delivery process. Unfortunately as a result of the hypercoagulable state, thromboembolism is one of the leading causes of death associated with pregnancy, particularly in developed nations, with clinically significant venous thromboembolism occurring in 1 of every 1000 to 2000 pregnancies. The hypercoagulability of blood during pregnancy has been confirmed with Thromboelastography (TEG) and is thought mainly due to the increased production of factor VII and fibrinogen. Although many of the coagulation factors are increased during pregnancy, none are quite to the extent of factor VII and fibrinogen. The risk of developing a venous thromboembolism increases about 100-fold when a parturient is admitted to a hospital and also seems to be worse during the third trimester. This is thought to be due to changing hormonal levels, in particular increased estrogen as pregnancy progresses. It is important to note that the classic Virchow's Triad favors thrombus formation in pregnancy with increased venous stasis, increased coagulability factors, and increased endothelial damage. Risk of venous thromboembolism in pregnancy increases with the following additional risk factors: obesity, smoking, multiple gestations, advanced maternal age, increased parity, cesarean section, as well as the presence of a concomitant thrombophila. Unfractionated heparin and low molecular weight heparin are the modalities of choice for anticoagulation during pregnancy.[11]

There is some evidence to suggest that the body creates a level of homeostasis by also increasing fibrinolysis to a certain extent. During pregnancy, we see increases in plasma activity of plasminogen alpha2-antiplasmin and elevated concentrations of D-dimer, which suggests increased fibrinolytic activity and the restriction of fibrin formation. This would explain that despite all the factors favoring a hypercoagulable state in pregnancy, venous thromboembolism remains a relatively rare event.[11]

2.2. Haemostasis:

Haemostasis is a complex interaction between the vessel and components of blood, and has the function of preventing excessive blood loss after vascular damage while maintaining a viable circulation by preventing thromboembolic conditions. [12]

A normal hemostatic mechanism is a very important aspect of human survival. Small, repeated traumas of everyday life continuously produce minor injuries to the vessels and, thus, the danger of spontaneous hemorrhage. "Extrinsic" trauma may, by severing vessels, cause bleeding which must be promptly controlled. Both functions require a perfect integration of a number of elementary mechanisms. The vascular wall must present normal resistance and contractility, the platelets and many factors which take part in the coagulation process must be normal in number or concentration, and in activity. Other mechanisms, such as fibrinolysis (which may play a role in limiting the undue extension of the fibrin clot within the vessel) and all the agents which bring about "organization" of the clot, recanalization of the vessel, etc. must also be normal. The very complexity of these mechanisms, then, creates many situations.

This short description of an hypothesis of the hemostatic process indicates the advisability of individual analysis of the various mechanisms:

(a) the vascular mechanism;

(b) the platelet factor;

(c) the blood coagulation mechanism;

(d) the autocatalytic mechanisms;

(e) the anticoagulant factors; finally

(f) the fibrinolytic mechanism. It should not be forgotten, however, that these various mechanisms are very closely integrated, as is clearly revealed by the study of the pathogenesis of bleeding in various types of hemorrhagic tendency. The single abnormality of one of the hemostatic mechanisms is not necessarily followed by bleeding, if all others are normal. Thus bleeding is moderate and may

occur only following serious trauma or operative procedures in pseudohemophilia, congenital deficiency of prothrombin, labile factor, stable factor and fibrinogen, severe as the defect of the hemostatic mechanism involved might be. In many, better defined hemorrhagic syndromes, there is multiple involvement of hemostatic mechanisms. Thus, in some cases of Werlhof's disease, particularly of the acute variety, thrombocytopenia is accompanied by pronounced involvement of the vascular wall (polyarteritis). [12]

2.2.1. Coagulation:

The involves a series of enzymatic reaction leading to the conversion of soluble plasma fibrinogen fibrin clot. The coagulation factors (factor I, II, V, VII, IX, X, XI, XII and XIII) are primarily synthesized in the liver and the synthesis of four of these (factor II, VII, IX and X) is vitamin K dependent. The site of synthesis of factor VIII is uncertain. [13]

Coagulation proceeds through a series of sequential enzymatic reactions which can be initiated by a variety of stimuli and terminate with the conversion of soluble fibrinogen into insoluble fibrin. Most coagulation factors occur in trace concentration (1/10,000 the concentration of albumin), yet, for coagulation to occur, each must interact with its specific substrate. These interaction also require a specific tertiary alignment of the coagulation proteins. The coagulation process could not occur if the protein-protein interaction occurred at random unless they were present in much higher concentration. [1]

Blood coagulation may be produced by either activation of the intrinsic pathway which is relatively slow, or by activation of extrinsic pathway which is a faster process. While these two pathways are inter-related in vivo, it is convenient to consider them separately. [13]

2.2.1.1. Intrinsic pathway:

The intrinsic pathway is initiated by the activation of factor XII when this factor is exposed to a non-endothelial surface. It activates factor XI in the presence of high molecular weight kininogen. High molecular weight kininogen is not modified by this interaction, and it is likely that it facilitates the binding of factor XI to a surface. Conformationally changed factor XII, (XIIA) slowly activates prekallikrein to kallikrein (fletcher factor). Kallikrein participates in a number of processes including inflammation and kinin formation. Kallikrein cleaves factor XII enzymatically to produce factor XIIa that slowly activates factor XI. The physiological importance of factor XII, prekallikrein and high molecular weight kininogen is not clearly understood, since deficiencies of each are not associated with bleeding.[14]

In the presence of calcium, factor XI activates factor IX. Factor IXa binds to a surface (platelet phospholipid) and activates factor X, a reaction requiring factor VIII. Factor Xa activates prothrombin and this activation occurs on a phospholipid surface and requires calcium and factor V. [14]

2.2.1.2. Extrinsic pathway:

The main role of the tissue factor pathway (extrinsic pathway) is to generate a "thrombin burst", a process by which thrombin, the most important constituent of the coagulation cascade in terms of its feedback activation roles, is released very rapidly. FVIIa circulates in a higher amount than any other activated coagulation factor. The process includes the following steps:

- Following damage to the blood vessel, FVII leaves the circulation and comes into contact with tissue factor (TF) expressed on tissue-factor-bearing cells (stromal fibroblasts and leukocytes), forming an activated complex (TF-FVIIa).

- TF-FVIIa activates FIX and FX.

- FVII is itself activated by thrombin, FXIa, FXII and FXa.

- The activation of FX (to form FXa) by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI).

- FXa and its co-factor FVa form the prothrombinase complex, which activates prothrombin to thrombin.

- Thrombin then activates other components of the coagulation cascade, including FV and FVIII (which activates FXI, which, in turn, activates FIX), and activates and releases FVIII from being bound to vWF.

- FVIIIa is the co-factor of FIXa, and together they form the "tenase" complex, which activates FX; and so the cycle continues. ("Tenase" is a contraction of "ten" and the suffix "-ase" used for enzymes.) [14]
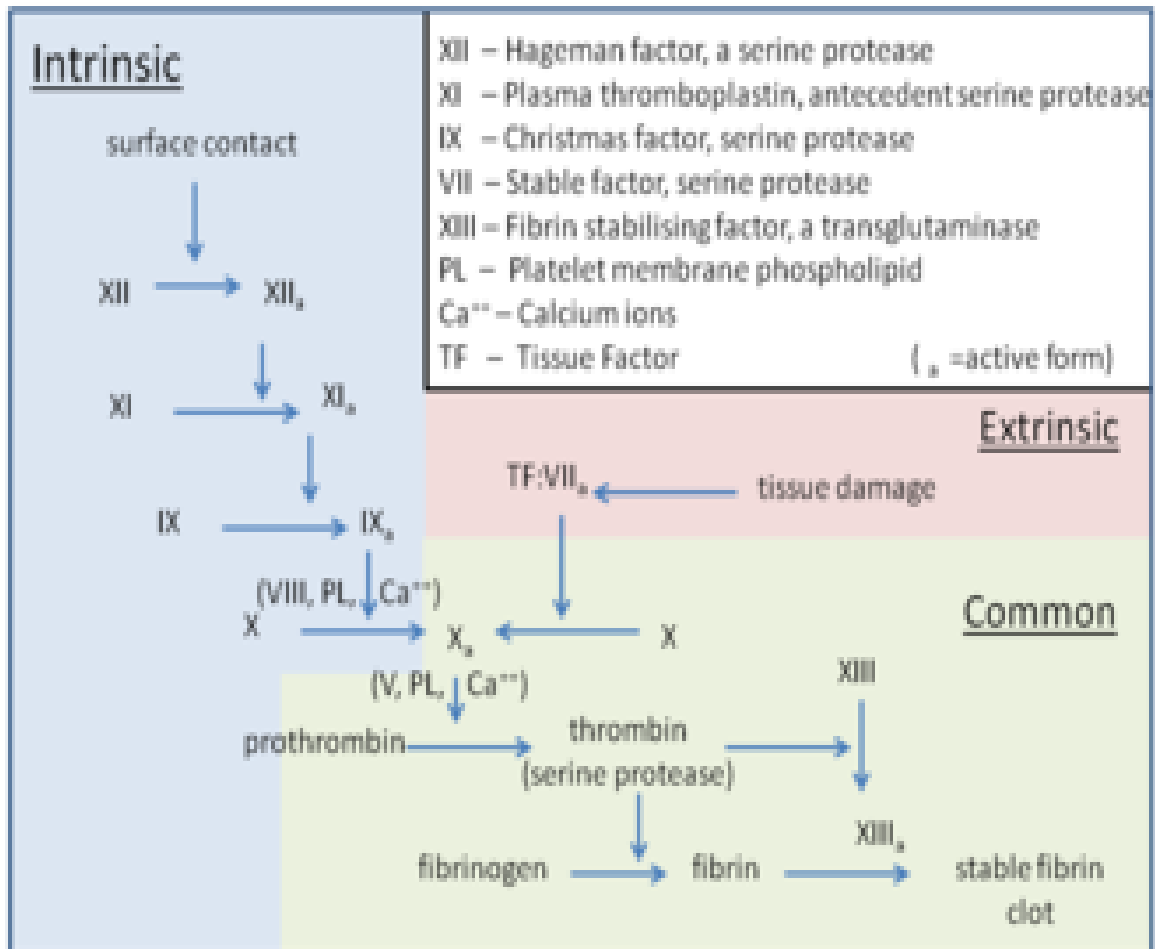
2.2.1.3. Final common pathway:

The division of coagulation in two pathways is mainly artificial, it originates from laboratory tests in which clotting times were measured after the clotting was initiated by glass (intrinsic pathway) or by thromboplastin (a mix of tissue factor and phospholipids). In fact thrombin is present from the very beginning, already when platelets are making the plug. *Thrombin* has a large array of functions, not only the conversion of fibrinogen to fibrin, the building block of a hemostatic plug. In addition, it is the most important platelet activator and on top of that it activates Factors VIII and V and their inhibitor protein C (in the presence of thrombomodulin), and it activates Factor XIII, which forms covalent bonds that crosslink the fibrin polymers that form from activated monomers.[14]

Following activation by the contact factor or tissue factor pathways, the coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX to form the tenase complex, until it is down-regulated by the anticoagulant t pathways.[14]

Fig (2 - 1): The classical blood coagulation pathway :

The three pathways that makeup the classical blood coagulation pathway

Intrinsic

surface contact

XII → XII$_a$

XI → XI$_a$

IX → IX$_a$

(VIII, PL, Ca^{++})
X → X$_a$ ← X

(V, PL, Ca^{++})

prothrombin → thrombin (serine protease)

fibrinogen → fibrin → stable fibrin clot

TF:VII$_a$ ← tissue damage

Extrinsic

Common

XIII

XIII$_a$

XII – Hageman factor, a serine protease
XI – Plasma thromboplastin, antecedent serine protease
IX – Christmas factor, serine protease
VII – Stable factor, serine protease
XIII – Fibrin stabilising factor, a transglutaminase
PL – Platelet membrane phospholipid
Ca^{++} – Calcium ions
TF – Tissue Factor   ($_a$ =active form)

2.2.1.4. Coagulation factors:

The coagulation factor are either enzyme precursors or cofactors. All the enzyme, except factor VIII, are serine proteases. [15]

Table (2 - 2): The coagulation factors.

Factor number	Descriptive name	Active form
I	Fibrinogen	Fibrin subunit
II	Prothrombin	Serine protease
III	Tissue factor	Receptor/cofactor*
V	Labile factor	Cofactor
VII	Proconvertin	Serine protease
VIII	Antihaemophilic factor	Cofactor
IX	Christmas factor	Serine protease
X	Stuart–Prower factor	Serine protease
XI	Plasma thromboplastin antecedent	Serine protease
XII	Hageman (contact) factor	Serine protease
XIII HMWK, high molecular weight kininogen.	Fibrin-stabilizing factor Prekallikrein (Fletcher factor) HMWK (Fitzgerald factor)	Transglutaminase Serine protease Cofactor*
* Active without proteolytic modification		

2.2.2. Coagulation disorders :

The coagulation disorder is deficiencies of each of the coagulation factor. May be hereditary or acquired. [16]

2.2.2.1. Inherited disorders :

Inherited deficiencies of each of the coagulation factors have been described. These deficiencies can either be quantitative due to impaired synthesis of the coagulation factor, or qualitative due to the synthesis of a biologically inactive molecule. There is a rough correlation between the severity of bleeding in patients with an inherited coagulation abnormality and the biological amount of the deficient coagulation factor. [14]

2.2.2.2. Acquired disorders :

The acquired coagulation disorders are more frequently encountered in clinical practice than the inherited disorders and, unlike inherited disorders, are almost invariably associated with multiple coagulation factor deficiencies. Acquired coagulation disorders can be caused by impaired synthesis of coagulation factors, circulating inhibitors to coagulation factor (or reaction) or by increased consumption of coagulation factors. [14]

2.2.2.2.1. Impaired synthesis:

Defective or decreased synthesis of coagulation factors occurs in vitamin K deficiency and in liver disease. Vitamin K deficiency can occur in states of impaired fat absorption and in patients with a poor diet. The physiological function of vitamin K is to catalyze the carboxylation of the coagulation factor protein after its synthesis. [14]

The haemostatic defect in liver disease is multifactorial, and can include decreased synthesis of clotting factor, decreased production of the natural inhibitors, decreased clearance of activated coagulation factors and excessive fibrinolysis. [14]

2.2.2.2.2. Circulating inhibitors:

A number of inhibitors interfere with coagulation. Heparin catalyses the antithrombin III-dependent inactivation of thrombin and factor Xa, IXa, XIIa and Xia. Heparin is used therapeutically in the treatment of venous thrombosis.

Non-specific inhibitors interfere with a general coagulation sequence rather than a specific factor. The fibrin split products that are produced by plasmin digestion of fibrin and fibrinogen inhibit the conversion of fibrinogen to fibrin, by interfering with the normal polymerization of fibrin and competing with systemic lupus erythematosus is termed the lupus inhibitor. [14]

2.2.2.2.3. Reduced survival of coagulation factor:

The consumption of many coagulation factor occurs in a syndrome termed disseminated intravascular coagulation (DIC). *DIC* can be considered to result from coagulation activation which overwhelms the normal control mechanisms.[14]

2.2.3. Coagulation tests:

Fig (2- 2): The Coagulation Cascade **:**
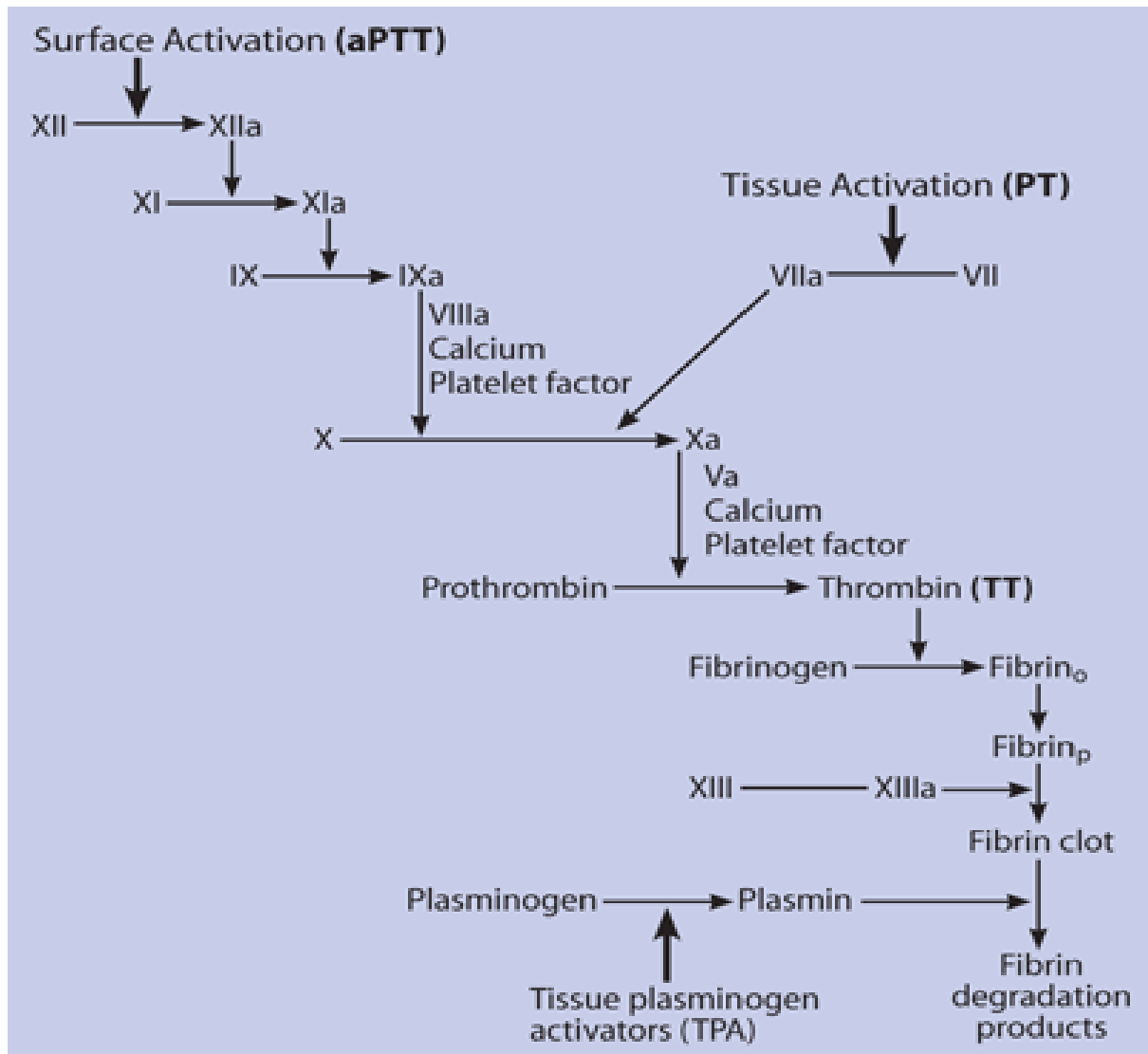
2.2.3.1.1. Prothrombin time (PT) :

The *PT* test measures coagulation factors of the extrinsic pathway. It is carried out by adding thromboplastin (containing tissue factor), phospholipid and an excess of calcium to anticoagulated plasma and measuring the clotting time. *PT* is the most commonly used coagulation test in routine laboratories. The *PT* test is used for assessment in pre-operative detection of bleeding tendencies in risk groups, the monitoring of anticoagulation therapy; used for prevention and treatment of venous thromboembolism. [15]

 The *PT* is performed on platelet poor plasma prepared from blood collected into citrate anticoagulant. In the *PT* an aliquot of plasma is incubated at ($37^{o}C$) with a reagent containing a phospholipid-protein extract of tissue (thromboplastin). [15]

Many physiologic factors can influence the *PT* as well. The *PT* is prolonged in cord blood and newborns due to relatively low levels of vitamin K-dependent clotting factors, which may not increase to the normal adult range until several weeks after birth. Lipemia, hyperbilirubinemia, and hemolysis interfere with the detection of clot formation by photo-optical methods and cause falsely elevated values. Heparin at therapeutic doses usually does not interfere with the *PT*, but *PT* prolongation can result in patients receiving higher doses of Heparin. [16]

2.2.3.1.2. International Normalized Ratio (INR) :

The *INR* was introduced by the World Health Organization (WHO) in the early (1980)'s as a means of standardizing *PT* results. Since there is a linear relationship between the logarithms of the *PT* ratios obtained with different extracts of human and rabbit brain, a calibration system was developed to relate any *PT* ratio to a *WHO* standard.

 INR units were originally not recommended for screening and follow-up of patients with liver diseases. However, kovacs and associates(1994) have since demonstrated that *INR* can also be used to monitor patients with severe form of a liver disease.[16]

2.2.3.2. Activated partial thromboplastin time(APTT) :

The *APTT* is functional determination of the intrinsic pathway of coagulation (factors XII, XI, IX, VIII, V, II, I, prekallikrein, high molecular weight kininogen). This pathway is intitated by the interaction of Factor XII with a negatively charged surface. A cascade mechanism results in fibrin production and clot formation. The *APTT* is utilized to detect congenital and acquired abnormalities of the intrinsic coagulation pathway and to monitor patients receiving heparin.[17]

No specific patient preparation is required. However, since lipemia may interfere with photo-electric measurements of clot formation, specimens should not be obtained after a meal. In patients receiving intermittent heparin injections, peripheral blood for *APTT* analysis should be obtained one hour before the next dose of heparin is scheduled. The specimen should not be drawn from an arm with a heparinized catheter or heparin lock. [17]

The *APTT* is increased above the upper limit of normal with hereditary or acquired intrinsic factor deficiencies < (40%) (factor VIII:C, Factor IX, Factor XI, Factor XII, vWf), lupus anticoagulants, or specific inhibitors of the intrinsic coagulation factors. Other causes of anelevated *APTT* include liver disease, *DIC*, heparin or anticoagulant therapy, or improper specimen collection (i.e., traumatic phlebetomy or hemolyzed specimen). Normal Values and Critical Limits(24 – 37) seconds .[17]

2.3. The Fibrinolytic systems:

The responsible for the degradation and removal of fibrin clots, thereby restoring eventually obstructed circulation. This system is also involved in tissue repair, macrophage function, ovulation and malignant transformation. Plasminogen plays a central role in fibrinolysis. It is an inactive proenzyme, which upon activation to plasmin may degrade fibrin. Activation of plasminogen is catalysed by tissue plasminogen activator (*tPA*) or urokinase-type plasminogen activator(*uPA*). Plasmin bound to the fibrin surface is relatively protected from inactivation by antiplasmin. Both *tPA* and *uPA* are serine proteases, which exist as single-chain or two-chain molecules.[18]

Fig (2 - 3): fibrinolysis (simplified). Blue arrows denote stimulation, and red arrows inhibition:
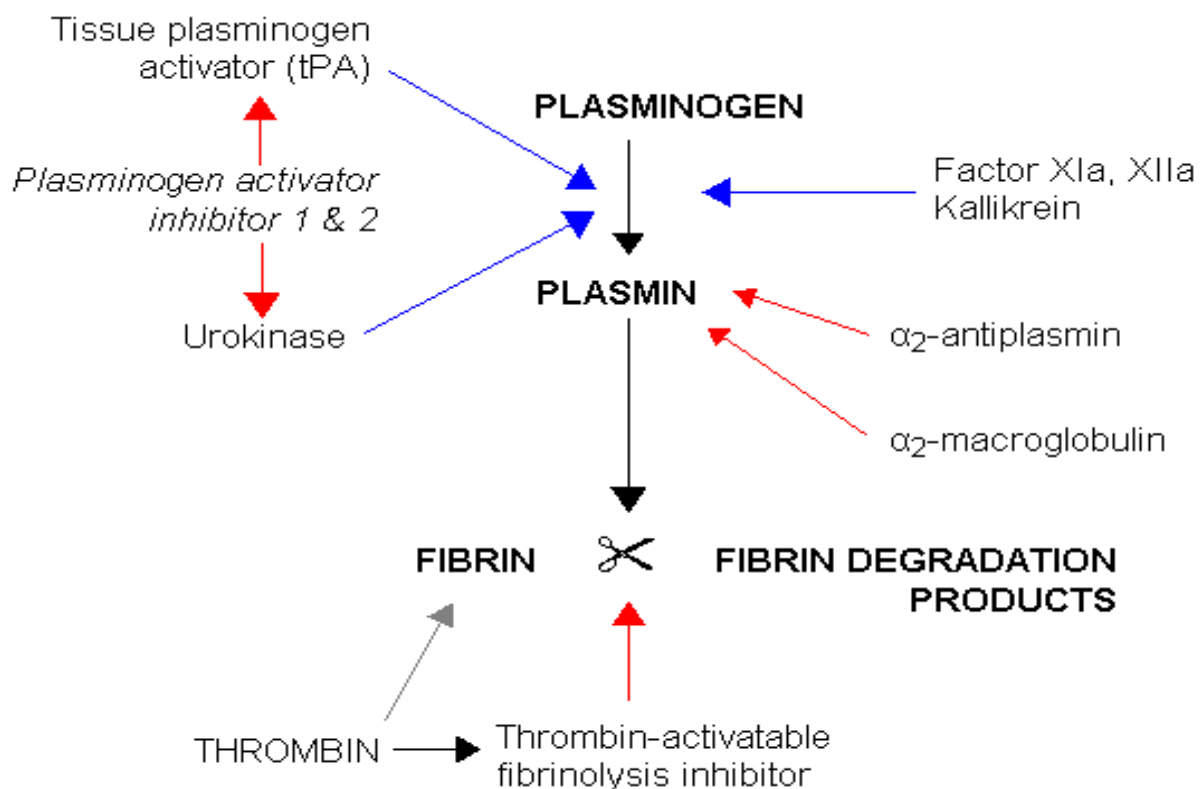
2.3.1. Fibrinogen:

is a plasma protein critical to hemostasis and clot formation. The blood plasma concentration of fibrinogen ranges between (1.5 and 4.0 g/L) but it can be higher, particularly in certain conditions such as pregnancy. Structurally, human fibrinogen comprises outer D domains, which are both linked by a central E domain. Each D domain is made up of (3) polypeptide chains (α, β, γ), which together form a coiled-coil configuration. These domains are linked at the *N*-terminus to the central E domain via a series of disulfide bonds. Thrombin cleavage occurs at specific amino-acid sequences present on the _ and _ polypeptide chains, removing the *N*-terminal peptides (fibrinopeptides) and exposing the polymerization sites. Fibrin polymerization then occurs via noncovalent interaction of the exposed polypeptide chain with complementary binding sites present on the D domain of a neighboring molecule. Furthermore, recent preliminary data have suggested that fibrinogen may be heme associated and could play a role in carbon monoxide sensing. Studies from our laboratory and others have demonstrated the importance of thrombin generation and hemostatic activation for clot formation.(6–11) Functionally, fibrinogen molecules act during both cellular and fluid phases of coagulation. In the cellular phase, it facilitates the aggregation of platelets via binding of glycoprotein IIb/IIIa receptors on platelet surfaces. In the fluid phase, it is cleaved by thrombin to produce fibrin monomers, which polymerize to form the basis of the clot , Fibrinogen also plays other important roles, functioning in vivo as an acute phase reactant, helping modulate inflammatory cellular reactions and also increasing in plasma concentration after injury. When acute hemorrhage occurs, the resulting blood loss and consumption of procoagulants combine to reduce the circulating concentration of multiple clotting factors. Derangement in common measures of coagulation (prothrombin time and activated partial thromboplastin time) can develop in cases of acute trauma, before administration of fluid therapy. [18]

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2.3.2. Fibrinogen and hemostasis :

Fibrinogen plays several key roles in the maintenance of hemostasis. Its cleavage by thrombin and subsequent polymerization to form fibrin strands provides the structural network required for effective clot formation. During cases of acute blood loss, attempts to maintain circulating volume and tissue perfusion often involve the infusion of crystalloids, colloids, and red blood cells. Intravascular volume resuscitation, although vital, frequently results in dilution of the remaining clotting factors and onset of dilutional coagulopathy. In such cases, fibrinogen is the first coagulation factor to decrease to critically low levels. There currently is a lack of awareness among physicians regarding the significance of fibrinogen during acute bleeding and, at many centers, fibrinogen is not monitored routinely during treatment. We reviewed current studies that demonstrate the importance of considering fibrinogen replacement during the treatment of acquired bleeding across clinical settings. If depleted, the supplementation of fibrinogen is key for the rescue and maintenance of haemostatic function; however, the threshold at which such intervention should be triggered is currently poorly defined. Although traditionally performed via administration of fresh frozen plasma or cryoprecipitate, the use of lyophilized fibrinogen (concentrate) is becoming more prevalent in some countries. Recent reports relating to the efficacy of fibrinogen concentrate suggest that it is a viable alternative to traditional haemostatic approaches, which should be considered. The prospective study of fibrinogen supplementation in acquired bleeding is needed to accurately assess the range of clinical settings in which this management strategy is appropriate, the most effective method of supplementation and a comprehensive safety profile of fibrinogen concentrate used for such an approach. [18]

2.3.3. Acute blood loss and massive transfusion coagulopathy :

In cases of acute blood loss, restoring circulatory volume is a primary objective often addressed with volume expanders such as crystalloids, colloids, or a combination of both. The ideal volume expander has been the subject of significant debate; however, the administration of any volume expander will result in the reduction of platelets and plasma clotting factor concentrations. In such cases, the commonly observed change is dilutional thrombocytopenia, but continuing blood loss can lead to a more complex coagulopathy. Neither concentrates of *RBCs* or platelets contain enough plasma to supplement the depleted factors sufficiently to maintain haemostatic balance , continued consumption of clotting factors coupled with their dilution with volume expanders can lead to the development of dilutional coagulopathy. The critical role of fibrinogen deficiency and fibrinolysis in cases of major bleeding is increasingly described. The preoperative measurement of plasma fibrinogen concentration was found to be predictive of postoperative bleeding volume and transfusion requirements in a prospective observation of coronary bypass grafting surgical patients (n 170). In another example, a multivariate analysis of postpartum hoemorrhage (n 128) reported that fibrinogen concentration was the only haemostatic marker consistently associated with the occurrence of severe postpartum hemorrhage. It was concluded that the early measurement of fibrinogen was able to detect reductions in plasma fibrinogen concentration, allowing the risk of severe bleeding to be predicted. As such, monitoring of this kind is recommended during the management of obstetric-related bleeding events. A greater understanding of the predictive value of plasma fibrinogen concentrations has led to the potential for laboratory-guided, prophylactic supplementation of coagulation factors in cases of elective procedures. Thus, in events when hemorrhage is likely, the onset of coagulopathy can be delayed and the extent of bleeding reduced. A recent prospective randomized controlled pilot study (n 20) investigating prophylactic fibrinogen supplementation before coronary artery bypass grafting showed that postoperative

bleeding was reduced by (32%) in patients receiving (2 g) fibrinogen concentrate preoperatively in comparison with the control group (565 150 vs 830 268 mL; P 0.010), without any evidence of hypercoagulability. Recognizing the emerging evidence, which highlights the importance of maintaining adequate plasma fibrinogen concentrations, European guidelines now include the administration of fibrinogen concentrate among their recommendations for the treatment of trauma-related, life threatening hemorrhage; however, it should be noted that this recommendation is based upon the lowest level of evidence available to the guideline authors.[19]

2.3.4. Fibrinogen deficiency :

Factor I (or fibrinogen) deficiency is a very rare inherited disorder with complications that vary with the severity of the disorder. It is not well known, even among health professionals. People affected by this disorder and those close to them have very little written information about it. This booklet therefore seeks to provide information for people trying to cope with this health problem. It explains the causes of the disorder and currently available treatments.There are three types of deficiency: [19]

2.3.4.1. Afibrinogenemia (absence of fibrinogen) :

In this type of factor I deficiency, there is a complete absence of fibrinogen. The fibrinogen level is < (0.2 g/l) of plasma. About(5) people out of(10) million are affected by it. of the three types, this one causes the most serious bleeding. [19]

2.3.4.2. Hypofibrogenemia:(lower than normal level):

In this anomaly, fibrinogen is present, but at a lower level than normal, between (0.2 g/l and 0.8 g/l). This anomaly is less frequent than Afibrinogenemia. Bleeding problems may be mild, moderate or severe. [19]

2.3.4.3. Dysfibrinogenemia: (malfunctioning) :

In dysfibrinogenemia, the fibrinogen level is normal, which means between (2 and 4 g/l), but the fibrinogen does not function properly. About (1) person in (1) million is affected by this condition. More than (100) different types of dysfibrinogenemia have been reported. Those affected rarely suffer from hemorrhaging problems. They may even present the opposite condition: thrombosis (blood coagulates in the blood stream). [19]

2.4. D-Dimer:

The D-dimer assay is specific for fibrin derivatives. In this assay, the presence of cross-linked D-dimer domain is diagnostic for lysis of a fibrin clot, and confirm that thrombin was formed and Factor XIII was activated with reactive fibrinolysis. Since fibrinogen derivatives do not contain the cross-linked D-dimer domain, they are not recognized by the D-dimer assay, even when present in high concentration. In other words, fibrin derivatives in plasma containing D-Dimer *XDP* are specific markers for fibrinolysis, as opposed to fibrinogenolysis. D-Dimers are detected by immunoassays using monoclonal antibodies specific for the cross-linked D-dimer domain in fibrinogen. Commercially available assays include latex agglutination, immunoturbidimetry, and *ELISA*. No specific patient preparation is required for the measurement of D-Dimers. [20]

XDPs are cross-linked fibrin degradation products which arise directly from fibrin. Thus, the measurement of *XDPs*, unlike total *FDPs*, is a specific measure of fibrinolysis. Elevated D- dimers are seen in *DIC*, pulmonary embolism, arterial and venous thrombosis, septicemia, cirrhosis, carcinoma, sickle cell crisis, and following operative procedures. Both *FDPs* and *XDPs* are present during late pregnancy and for approximately (48) hours post-surgery.

During fibrinolytic therapy the *FDP* test is positive, while the D-dimer test is negative in the absence of thrombolysis. Disseminated intravascular coagulation (*DIC*, consumption coagulopathy) is one of the most common and clinically important acquired disorders of hemostasis. In *DIC*, intravascular activation of the coagulation system results in the widespread deposition of fibrin microthrombi in the microcirculation, the consumption of platelets and clotting factors, and activation of the fibrinolytic system. At the same time that thrombin converts fibrinogen to fibrin, it also activated Factor XIII to form a plasma transglutaminase, Factor XIIIa, which stabilizes fibrin by cross-linking the gamma chains of fibrinogen in the region of the D-domain. Plasmin digests fibrin and fibrinogen to produce fibrin(ogen) degradation (*FDP*) (or split, *FSP*) products (X,Y,D, and E), which are removed from the circulation by the reticuloendothelial system. [20]

DIC is not a specific disease, but a sequalae of many pathologic conditions, including acute intravascular hemolysis, hemolytic transfusion reactions, shock, hyperthermia, extensive tissue damage, malignancies, obstetric complications, hyperthermia, snake bites, etc. Prompt diagnosis and therapy of *DIC* is essential, since the associated hemorrhage, small vessel thrombosis, and occasional large vessel thrombosis can lead to the impairment of blood flow, ischemia, end-organ damage, and death. The clinical signs and symptoms in *DIC* are variable and non-specific, and include fever, hypotension, acidosis, proteinuria, hypoxia, petechiae and purpura, subcutaneous hematomas, bleeding (surgical wound, traumatic wound, venipuncture), and arterial line oozing. [20]

 The analysis of plasma D-dimers has been reported to be of diagnostic value in patients with suspected complications of pregnancy such as pre-eclampsia and the *HELLP* syndrome, to monitor anticoagulant and thrombolytic therapy, and to correlate with disease severity in rheumatoid arthritis. *CSF* D-dimers have been reported positive in patients with subarachnoid hemorrhage, but not in normal patients or those with traumatic lumbar puncture. [20]

2.5. contraceptive hormones:

Oral contraception with conventional estrogen / progestogen preparations produces raised levels of clotting factors and increased platelet aggregation. Although these changes do not involve as broad a spectrum of clotting factors as thrombosis and the third trimester of pregnancy, they do not appear a desirable side effect and may be responsible for the increased risk of thrombosis. Oral contraception with progestogen alone does not appear to cause similar clotting and platelet changes. [21]

2.6. Previous studies :

An increase in the concentration of plasma fibrinogen and factors VII, VIII, and X in association with pregnancy has been reported (Pechet and Alexander, 1961; Kasper et al. 1964; Talbert and Langdell, 1964), and the findings in this

study have shown that a considerable deviation from the normal occurs in the components of the fibrinolytic enzyme system. The results reported here of clearly increased levels of plasminogen during pregnancy are in agreement with the findings of Mitchell and Cope (1965) and Nilsson and Kullander (1967). [22]

Pregnancy was associated with some physiological ,biochemical and anatomical changes , This study was carried out to determine plasma fibrinogen level in pregnant women .samples were collected from women in their first ,second ,third trimester as well as from non pregnant women as control group totaling sixty samples all range between (15-45) years. All were collected from Giad hospital during period (January to – March 2010). .fifteen plasma sample were collected from pregnant women in the first trimester .fifteen plasma sample were collected from pregnant women in second trimester .Fifteen plasma sample were collected from pregnant women in third trimester This result showed that there was a significant increase in the levels of plasma fibrinogen in pregnant women when compared with non pregnant women (first trimester mean=3.1600g/l, second trimester) mean = (4.2000 g/l) (, third trimester mean =5.4867 g/l) comer with that

of control (mean value =2.493g/l) there was correlation between number of pregnancies before and the level of fibrinogen. [23]

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. [24]

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. A study of more than (200,000) women came to the result that admission to inpatient care during pregnancy was associated with an 18-fold increase in the risk of venous thromboembolism (*VTE*) during the stay, and a (6) -fold increase in risk in the four weeks after discharge, compared with pregnant women who did not require hospitalization. The study included women admitted to hospital for one or more days for reasons other than delivery or *VTE*. [24]

Pregnancy after the age of (35) augments the risk of *VTE*, as does multigravidity of more than four pregnancies. And in itself causes approximately a five-fold increased risk of deep venous thrombosis. Several pregnancy complications, such as pre-eclampsia, cause substantial hypercoagulability. [24]

Hypercoagulability states as a pre-existing condition in pregnancy include both acquired ones, such as antiphospholipid antibodies, and congenital ones, including factor V Leiden, prothrombin mutation, proteins C and S deficiencies, and antithrombin III deficiency. [24]

Before delivery the fibrinogen concentration is about (50%). above the non-pregnant value. Bearing in mind the increase in circulating blood volume, there is about double the amount of fibrinogen available to the pregnant woman at delivery compared to the non-pregnant state. After delivery there is a secondary rise in fibrinogen followed by a return to normal. [25]

Chapter 3

Materials & Methodology

Material and Methodology

3.1. Study design:

This is a case central, Hospital based study, conducted in Shendi town, and aimed to evaluate the effect of pregnancy coagulation test .

3.2. Study area:

Shendi locality, River Nile State, Sudan. Shendi is a town in northern of Sudan on the east bank of the River Nile (150) km northeast of Khartoum (16°41'N 33°25'E). The area is inhabited by the Ga'aleen Tribe.

3.3. Study duration:

 The study conducted in the period from August 2015 to August 2017.

3.4. Study population:

Pregnant women a total (145) as case study and non pregnant women a total (50) as control group.

3.4.1. Inclusion criteria:

Pregnant females in different ages and trimesters .

3.4.2. Exclusion criteria:

Any Pregnant females suffering from haematological diseases, liver diseases and under treatment were excluded.

3.5. Sample size:

Sample was calculated from free online web site http://www.calculator.net by using confidence level 95% and confidence interval 8%, One hundred ninety one.

Sample size = Z^2 x (p) x (p-1) / C^2

Z = Z value – confidence level 95% (1.96),

C = Confidence interval (.08 = ± 8).

 P = standard division (0.5).

The calculated sample size based on this formula was (145).

3.6. Scientific & Ethical considerations:

The study proposal was reviewed and ethically approved by the scientific and the pregnant women agree to participate in this study.

3.7. Statstical analysis:

3.8. Data collection:

Data was collected by using questionnaire.

3.9. **Materials:**

- BioMed-Liquiplastin for P.T determination: (ISI – 1.05). For quantitative determination of Thromboplastin activity in plasma.

- BioMed-Liquicellin-Efor APTT determination:. For quantitative determination of Partial the Biomed- LIQUICELIN-E activity in plasma.

- BioMed-Fibrinogen : Reagent for quantitative estimation of fibrinogen. For quantitative determination of Fibrinogen in plasma.

- Ichroma™ D-Dimer : Reader is a fluorescence immunoassay that quantifies the total D-Dimer concentration in plasma. The test is used as an aid in the post therapeutic evaluation of thromboembolic disease patients.

- Disposable Syringe: Disposable syringe EL-WALIEF, 3mL 22Gx1/4.

- Vacuum Citrate Tube: The tube is (Glass,3.2 % Sodium Citrate additive ,13x75mm,1.8ml/2.7ml) Contains trisodium citrate 3.2% 0.9mL anticoagulant Add to 1.8mL for freshly blood.

- Tourniquet

- Water Bath

- centrifuge

- Stop Watch

- Immunoassay-Analysis-System *i-CHROMA™*,

Methods:

3.9.Specimen collection:

Two and half ml of whole blood with (1:9) ratio of tri sodium citrate were collected from all participants, then centrifuged to take plasma to perform *PT*, *APTT*, fibrinogen and D-Dimer using manual method, automated coagulometer and Immunoassay-Analysis-System i-CHROMA™

.3.10.0 Principle for *PT* Test:

Tissue Thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulation mechanism. reagent is added to normal citrated plasma, the clotting mechanism is initiated, forming a solid gel clot within a specified period of time. The time required for clot formation would be prolonged if there is acquired or congenital deficiency of factors/ factor activity in the extrinsic pathway of the coagulation mechanism or reduction in the activity of Vitamin K dependent clotting factors during oral anticoagulant therapy.

3.10.1 Procedure for *PT* Test

Patients samples were tested in parallel fresh plasma and suitable controls and centrifuged immediately for (15 minutes) at *RCF* (1500-2000 g) and the plasma was separated into a clean test tube, the reagent vial was token to room temperature (20 – 37°C) then the contents was mixed, enough reagent was aspirated for immediate testing requirements in a thoroughly clean and dry test glass tube , then the reagent was re warmed to (37°C) . In (12 x 75 mm) tube, (0.1ml) of plasma was added and placed in a water bath for (3–5) minutes at (37°C), then (0.2 ml) of reagent was added (prewarmed at 37°C for at least 10 minutes) and simultaneously start stop watch, the tube was shake gently to mix contents. Gently we tilted the tube back and forth and stop the stopwatch as soon as the first fibrin strand is visible and the gel / clot formation begins record the time in seconds. The same steps were repeated for four time on the same sample. The find the average of the duplicate test values. This is the Prothrombin time.

3.10.2 Calculation:

The results reported directly in terms of the mean of the double determination of *PT* of the test plasma in seconds.

$$\text{Or as a ratio 'R': } R = \frac{\text{Mean of the patient plasma PT in seconds}}{\text{MNPT for the reagent}}$$

Or as International Normalized Ratio, *INR = (R)ISI* , where *ISI* = International Sensitivity Index of the reagent.

It is recommended by the *WHO* that *MNPT* should be established for each lot of PT reagents by each laboratory, since *PT* result are dependent on the combination of reagent lot, instrument and technique followed at each laboratory.

Usually plasma from at least (20) normal healthy individuals should be used to establish the *MNPT*. The average of such *PT* results in seconds = *MNPT*.

Normal values using are between (10-15) seconds. For photo optical instruments, it is

INR between (2.0 - 3.5).

3.11.0. Principle for *APTT* Test:

Cephaloplastin activates the coagulation factors of the intrinsic pathway of the coagulation mechanism in the presence of calcium ions.

APTT is prolonged by a deficiency of one or more of these clotting factors of the intrinsic pathway and in the presence of coagulation inhibitors like heparin.

3.11.1.Method for *APTT* Test:

Patients samples were tested in parallel fresh plasma and suitable controls and centrifuged immediately for (5 minutes) at *RCF* (1500-2000 g) and the plasma was separated into a clean test tube. the Calcium Chloride Reagent vial was token to room temperature $(20 – 37^{o}C)$ for at least (10 minutes), added (100μ) of test or control plasma into a test cuvette. The plasma was Incubated at (37°C) for (1 to 2 minutes), taken (100μl) of the *APTT* reagent, mixed with the plasma with Maintain the suspension of the APTT reagent by magnetic stirring or mixing by inversion immediately prior to use and Incubated at (37°C) for(3 minutes), then (100μl) of preincubated Calcium Chloride solution Added and simultaneously start the timer

and then record the clotting time in seconds.

3.11.2.Calculation:

(a) The results may be reported directly in terms of the mean of the double determination of *APTT* of the test plasma.

$$R = \frac{\text{APTT of patient plasma (in seconds)}}{\text{APTT of } FNP \text{ (in seconds)}}$$

(b) Or as a ratio 'R':

Normal values using reagent are between (28-40) seconds at (3 minutes) activation time.

3.12.0. Principle for Fibrinogen Levels Test:

The addition of thrombin coagulates fresh citrated plasma. The coagulation time is proportional to the fibrinogen concentration. This allows the estimation of plasma fibrinogen by functional clotting assay.

3.12.1. Method for Fibrinogen Levels Test :

Patients samples were tested in parallel fresh plasma and suitable controls and centrifuged immediately for (5 minutes) at *RCF* (1500-2000 g) and the plasma was separated into a clean test tube. the was Prepared (1:10) dilution of plasma specimen with Owren's buffer solution, in (10 x 75 mm) test tube was added (0.2 ml) of (1:10) dilution of plasma sample to be tested and Incubated at (37°C) for one minute. To the test tube was added (0.1 ml) of fibrinogen thrombin reagent (prewarmed at 37°C for one minutes) and start the stopwatch simultaneously, then Stoped the stopwatch at the first appearance of the fibrin web, as the gel clot began to form and then record the clotting time in seconds. The steps (1-5) was repeated for a duplicate test. The normal expected value: (150 – 400 mg/dl)

3.13.0.Principle for D-Dimer Levels Test:

The test uses the sandwich immunodetection method, such that the detection antibody in buffer binds to D-Dimer in the plasma sample and antigen-antibody complexes are captured by antibodies that have been immobilized on the test strip as sample mixture migrates through nitrocelluose matrix. The more D-Dimer antigen in the plasma, the more antigen-antibody complexes are accumulated on test strip. Signal intensity of fluorescence on detection antibody reflects amount of antigen captured and is processed by ichroma™ Reader to show D-Dimer concentration in sample

3.13.1.Method for D-Dimer Levels Test:

Patients samples were tested in parallel fresh plasma and suitable controls and centrifuged immediately for (5 minutes) at *RCF* (1500-2000 g) and the plasma was separated into a clean test tube and was added (10 µL) of plasma sample by a transfer pipette to a tube containing the detection buffer. After closed the lid of the detection buffer tube, was mixed the sample thoroughly by shaking it about (10) times The sample mixture must be used immediately, was taked (75 µL) of a sample mixture and added in the test cartridge and leave the test cartridge at room temperature for (12 minutes). For scanning, was insert it into the test cartridge holder of the ichroma™ Reader. And ensured proper orientation of the test cartridge before pushing it all the way inside the test cartridge holder. An arrow has been marked on the test cartridge especially for this purpose, then was Pressed 'Select' button on the ichroma™ Reader to start the scanning process. Was started scanning the sample-loaded test cartridge immediately. The test result was read on the display screen of the ichroma™ Reader.

Reference value of D-Dimer is up to (500 ng/ml).

Chapter 4

Results

4.0. Results:

Mean of *PT* among pregnant women was (14.3) sec and the mean of control group was (12.9) sec. The P. value was (0.000) significant. The mean of *PT* in pregnant women in first trimester was (12.5) sec, second trimester (13.8) sec and third trimester (13.6) sec, with was statistically significant difference as the P. value was (0.001).

Mean of *APTT* among pregnant women was (34.1) sec and the mean of control group was (32.4) sec. The P. value was equal (0.03) significant . More than 50% were in their 2d trimester. The mean of pregnant women in first trimester was (32.4) sec, second trimester (34.1) sec and third trimester (33.4) sec, with was statistically significant difference as the P. value was (0.000).

Mean of fibrinogen among pregnant women was (548.1) mg/dl and the mean of control group was (296.6) mg/dl . The P. value was (0.000) significant. More than 50% were in their 3d trimester. The mean of pregnant women in first trimester was (257.3) mg/dl, second trimester (478.0) mg/dl and third trimester (788.0) mg/dl, with was statistically significant difference as the P. value was (0.000).

Mean of D-Dimer among pregnant women was (609.7) ng/ml and the mean of control group was (285.5) ng/ml. The P. value was (0.000) significant. More than 50% were in their 3d trimester. The mean of D-Dimer level pregnant women in first trimester was (208.3) ng/ml, second trimester (472.7) ng/ml and   third trimester (928.6) ng/ml, with was statistically significant difference   as the P. value was (0.000).

Table (4 – 1) : Distribution of the pregnant women according to age.

Age group	Frequency	Percentage
Less than 24	30	20.7%
25 – 34	110	75.9%
35 – 44	4	2.8%
More than 44	1	0.6%
Total	**145**	**100%**

Age (mean ± SD) = (24.4 ± 6.1)

Table (4 – 2): Distribution of the pregnant women according to trimester.

Trimester	Frequency	Percentage
First	20	13.8%
Second	90	62.1%
Third	35	24.1%
Total	**145**	**100%**

Table (4 – 3): The mean of *PT* in pregnant women and control women:

Group	Mean	std. Deviation	P. value
pregnant women	14.3 sec	1.9	
control women	12.9 sec	0.9	.000

Table (4 – 5): The mean of compare *PT* in first, second and third trimester :

Group	Mean	std. Deviation	P. value
first trimester	12.5 sec	1.4	
second trimester	13.8 sec	1.2	.001
third trimester	13.6 sec	1.5	

Table (4 – 6) : The mean of *APTT* in pregnant sample and control sample:

Group	Mean	std. Deviation	P. value
pregnant sample	34.1 sec	4.8998	
control sample	32.4 sec	2.9347	.030

Table (4 –7): The mean of compare *APTT* in first, second and third trimester :

Group	Mean	std. Deviation	P. value
first trimester	32.4 sec	3.5	
second trimester	34.1 sec	2.9	.000
third trimester	33.4 sec	2.9	

Table (4 – 8): The mean of fibrinogen level in pregnant women sample and control sample:

Group	Mean	std. Deviation	P. value
pregnant sample	548.1 mg/dl	219.0	
control sample	296.6 mg/dl	59.6	.000

Table (4–9): The mean of compare fibrinogen level in first, second and third trimester :

Group	Mean	std. Deviation	P. value
first trimester	257.3 mg/dl	90.0	
second trimester	478.0 mg/dl	108.9	.000
third trimester	788.0 mg/dl	155.6	

Table (4 – 10): The mean of D-Dimer level in pregnant women sample and control sample:

Group	Mean	std. Deviation	P. value
pregnant sample	609.7 ng/ml	554.7	
control sample	285.5 ng/ml	129.7	.000

Table (4 – 11): The mean of compare D-Dimer level in first, second and third trimester :

Group	Mean	std. Deviation	P. value
first trimester	208.3 ng/ml	116.0	
second trimester	472.7 ng/ml	144.9	.000
third trimester	928.6 ng/ml	945.3	

Chapter 5

Discussion

Conclusion

Recommendations

5.1. Discussion:

Normal pregnancy is associated with major changes in many aspects of haemostasis all contributing to maintain placental function during pregnancy and to prevent excessive bleeding in delivery. Most changes in blood coagulation and fibrinolysis create a state of hypercoagulability. [24]

The current study reveal that the mean of *PT* during pregnancy was (14.3 ± 1.9) sec compared to the non pregnant women (12.9 ± 0.9) sec. The result showed slightly increase of *PT* in the study group as the p. value was (0.000), but still within the normal range (10 -15) seconds. *PT* values fluctuated during the periods of pregnancy as slight increase during the first and the second trimester but decresed in the last trimester , these observations are in line with (Momodu and Ajay) [27] , also our findings are supported by (Hellgren M)[28] who proposed that during pregnancy there were increase in endogenous thrombin generation. However, variability in *PT* results from different researchers have been traced to the differing sensitivities of the thromboplastin reagents used, concentration of citrate anticoagulant, Study population and method of analysis. [27]

Regarding *APTT*, the result was(34.1 ± 4.9) sec in pregnant women compared to the control women (32.4 ± 2.9) sec. there was statistically significant difference as the p. value was (0.03), but the result still within the normal range (28 -40) seconds, the same result have been expressed by various authors (Momodu and Ajay) [27]. Also most authors reveal the same meaning of slightly increase in *APTT* in pregnancy, but there were different reading within this scope of finding by the researchers, this may be due to different sensitivities of *APTT* reagents, poor end-point detection by analyst and poor storage of plasma or delay in running the samples as the heat-labile factors may be affected. However, different *APTT* results have been associated with sensitivities of *APTT* reagents used, study population and methods of analysis. [27]

the present study illustrated that there was a positive correlation between the PT and APTT in trimester , as PT and PTT were slightly increase in the second and third trimester compared to the first trimester , these findings were consistent with some published work [28–29]

The fibrinogen levels was evaluated in pregnant women (548.1 ± 219.0) mg/dl compared to non pregnant women (296.6±59.6) mg/dl. p. value was (0.000), our result were supported by (Journal of Dental and Medical Sciences) who concluded (even in normal pregnancies, there is an elevated level of fibrinogen concentration but in severe pre eclamptic women showed interestingly more elevation in their levels. [33] , also the same observational was reported by (Ibrahim, Leila Abdallah Ibrahim) as they concluded that (there was a significant increase in the levels of plasma fibrinogen in pregnant women when compared with non pregnant women. This highly elevated fibrinogen concentration was markedly seen in the third trimester. [23]

In this study, pregnant women have higher D-dimer concentrations (609.0 ± 554.7) ng/ml than age matched non pregnant women (285.5 ± 129.7) ng/ml,
 this result was in agreement with (Kline JA[1], Williams GW, Hernandez-Nino J) they found that pregnancy increased the D-dimer concentration in a stepwise fashion from preconception to the third trimester. [32]

5.2. Conclusion:

- PT was slightly increased in pregnant women compare to non women but still within normal range.

- APPT was slightly increased in pregnant women compare to non women but still within normal range.

- D-Dimer and fibrinogen levels were significantly increased in pregnant women compare to non pregnant women , with highly significant increased level in the third trimester.

5.3. Recommendations

On the base of the obtained result we recommended that ;-

- Conducting furthers studies regarding the effect of pregnancy on coagulation factors and Fibrinolytic systems
- Regular follow up of pregnant women for coagulation factors and Fibrinolytic systems
- Community awareness of the importance of the study.

Chapter 6

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Appendices:

Appendix 1:   Questionnaire

Republic of Sudan

Ministry of Higher Education and Scientific Research

Shendi University

Faculty of Graduate Studies and Scientific Research

In Vitro Screening prothrombin time , partial thromboplastin time, Fibrinogen level and D-Dimer level among Pregnant Woman.

Name:……………………………………………………………………………

City:……………………………………………………………………………

Village:……………………………………………………………………………

Sample number:

- **Age:**

  a- <15 – 24 (……)   c- 25 – 34 (……).   d- 35 – 44 (……....).   e- 45 and more (……).

- **Occupations:**

  a- House wife (……)   b- Other (……)

- **Pregnancy trimester:**

First trimester (……) Second trimester (……)Third trimester (…….).

- **did you use contraceptives ?**

  a-Yes (……)   b- No (……)

- **History of haemostatic change in previous pregnancy:**

  a-Yes (……)   b- No (……)

- **Other disease:**

  a-Diabetes (…….)   b- Hypertension (……)   c- Other (……)   d- No
  (……)

Appendix 2:

The clot form :

Mindray
C2000-1coagulation analyzer

Water bath

Appendix 3:

BioMed-Liquiplastin for P.Tdetermination

BIOMED
DIAGNOSTICS

Prothrombin Time (ISI - 1.5 / 1.05)

REF:	PT201240	(6×8 ml)	(ISI - 1.5)
	PT200150	(6×5 ml)	(ISI - 1.05)

INTENDED FOR USE:
For quantitative determination of Thromboplastin activity in plasma

PRINCIPLE:
Tissue Thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulation mechanism. When Biomed-LIQUIPLASTIN reagent is added to normal citrated plasma, the clotting mechanism is initiated, forming a solid gel clot within a specified period of time. The time required for clot formation would be prolonged if there is acquired or congenital deficiency of factors/ factor activity in the extrinsic pathway of the coagulation mechanism or reduction in the activity of Vitamin K dependent clotting factors during oral anticoagulant therapy

SPECIMEN COLLECTION:
The arrest of bleeding depends upon primary platelet plug formed along with the formation of stable fibrin clot. Formation of this clot involves the sequential interaction of series of plasma proteins in a highly ordered and complex manner and also the interaction of these complexes with blood platelets and materials released from the tissues.

Tissue Thromboplastin, in the presence of calcium, is an activator, which initiates the extrinsic pathway of coagulation factors VII, X, V, Prothrombin and Fibrinogen.

During oral anticoagulant therapy most of the Vitamin K dependent factors such as II, VII, IX, X, Protein S are depressed, as also during the deficiencies of clotting factor activity which may be hereditary or acquired. Prothrombin Time determination is the preferred method for presurgical screening, as a liver function test, determination of congenital deficiency of factors II, V, VII and X and for monitoring of patients on oral anticoagulant therapy

REAGENT COMPOSITIONS :
LIQUIPLASTIN is a novel, highly sensitive, low opacity, ready to use liquid Calcified liquiplastin Reagent, which is derived from rabbit brain.

Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.

PACKAGE: Collection and storage.
- Store the reagent at 2-8°C. DO NOT FREEZE.

- The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. The uncontaminated reagent is stable at 2-8°C up to the expiration date.

PRECAUTIONS & WARNING :
Avoid pipetting with mouth.
The preparation, according to current regulation, is classified as not dangerous.
The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation.
Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes.
The samples must be handle as potentially infected from HIV or Hepatitis.

REAGENT PREPARATION & STABILITY :
- Use freshly collected blood taken into 0.11 mol/L trisodium citrate in the ratio 9 parts blood to 1 part anticoagulant.

- Centrifuge immediately for 5 minutes at RCF 1500-2000 g and separate plasma into a clean test tube.
- Plasma should be tested within 3 hours (keep refrigerated).

REQUIRED MATERIALS NOT PROVIDED:
General Laboratory Equipment and instrumentations

PROCEDURE:
Manual method
- Patients samples should be tested in parallel with pooled fresh normal plasma (FNP) and suitable controls.

1. Bring the reagent vial to room temperature (20-30°C). Mix the contents of the vial to homogenise the suspension completely.

2. Aspirate from the reagent vial enough reagents for immediate testing requirements in a thoroughly clean and dry test tube. (Plastic test tubes are preferred).

3. Prewarm the reagent and bring to 37°C before use in test procedure (5-10 minutes may be required depending on the reagent volume to attain 37°C before testing).

4. Recap the reagent vial and replace immediately to 2-8°C.

5. To a 12 x 75 mm tube add 0.1 ml of plasma (PPP) and place the tube in a waterbath for 3 to 5 minutes at 37°C.

6. To the tube forcibly add 0.2 ml of BioMed- LIQUIPLASTIN reagent (prewarmed at 37°C for at least 10 minutes) and simultaneously start a stopwatch. Shake the tube gently to mix contents.

7. Gently tilt the tube back and forth and stop the stopwatch as soon as the first fibrin strand is visible and the gel / clot formation begins record the time in seconds.

8. Repeat steps from 4 to 6 for a duplicate test on the same sample.
9. Find the average of the duplicate test values. This is the Prothrombin time (PT).

CALCULATION:
Manual Method

The results may be reported directly in terms of the mean of the double determination of PT of the test plasma in seconds.

Or as a ratio 'R':
$$R = \frac{\text{Mean of the patient plasma PT in seconds}}{\text{MNPT for the reagent}}$$

Or as International Normalized Ratio (INR), INR = (R)ISI , where ISI = International Sensitivity Index of the reagent.

It is recommended by the WHO that MNPT should be established for each lot of PT reagents by each laboratory, since PT result are dependent on the combination of reagent lot, instrument and technique followed at each laboratory.

Usually plasma from at least 20 normal healthy individuals should be used to establish the MNPT. The average of such PT results in seconds = MNPT.

EXPECTED VALUE:

Normal values using BioMed-LIQUIPLASTIN are between 10-15 seconds. Between manual and Turbo densitometric instrument results a variation of 1-2 seconds may be expected. For photo optical instruments, it is recommended that each laboratory must establish its own MNPT for each lot of BioMed- LIQUIPLASTIN. Oral Anticoagulant Therapeutic range: INR = 2.0 - 3.5

WASTE DISPOSAL:

The disposal of the product must be in accordance with local regulation concerning waste disposal.

REFERENCES:

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PERFORMANCE:

1. It is recommended that controls (PLASMATROL H-I/II) with known factor activity should be run

    simultaneously with each test series to validate test run.

2. Incorrect mixture of blood and tri-sodium citrate, insufficient prewarming of plasma and reagent,

    contaminated reagents, glassware etc. are potential source of errors.

3. Oxalated plasma may induce prolonged clotting times.

4. Since the PT test functions correctly only at 37± 0.5°C, temperature of all equipment must be calibrated

    daily.

5. Clotting time of patients on anticoagulant and also the time lag between the specimen collected and the last

    dose.

6. Turbid, icteric, lipemic,or grossly hemolysed samples may generate erroneous PT results.

7. Glasswares and cuvettes used in the test must be scrupulously clean and free from even traces of acids

    /alkalies or detergents.

8. Plasma samples held at 4-8°C may undergo 'cold activation' leading to marked shortening of the PT.

9. The PT may be shortend during acute inflammatory conditions, which are accompanied by increase in

    Fibrinogen levels and also by agents such as antihistamines, butabarbital, Phenobarbital, caffeine, oral

    contraceptives and vitamin K. The PT may be prolonged by corticosteroids, EDTA, asparaginase, clofibrate,

    erythromycin, ethanol, tetracycline, aspirin and anticoagulants such as heparin and warfarin.

10. It is important that each laboratory express the results in terms of INR for patients on oral anticoagulant

    therapy for the clinician to adjust the dosage based on INR.

11. Since the test uses platelet poor plasma, each laboratory must calibrate the necessary force and time required

    during centrifugation to yield the PPP. Contamination of plasma with excess platelets could falsely elevated

    levels of some of the factors.

12. Homogenisation of LIQUIPLASTIN reagent suspension before use is important to achieve accurate and
    consistent results.

LIMITATIONS :

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.

2. BioMed-LIQUIPLASTIN reagent is not from human source hence contamination due to HbsAg and HIV is practically excluded.

3. It is very important that scrupulously clean and dry micropipette tips be used to aspirate / dispense the reagent.

4. Avoid exposure of the BioMed-LIQUIPLASTIN reagent to elevated temperatures, contamination and undue stress due to high and low temperature exposure cycles. Immediately replace reagent cap after use and store at recommended temperatures only.

5. On prolonged storage at 2-8°C the BioMed-LIQUIPLASTIN suspension has a tendency to settle down. Homogenize the reagent by resuspending before use.

🛈	Consult Instructions for Use
⚠	Caution, Consult accompanying Documents
IVD	In Vitro Diagnostic Medical Device
🌡	Temperature Limitation
🏭	Manufacturer
EC REP	Authorized Representative in the European Community
REF	Catalogue Number
LOT	Batch Code
⧗	Use by

EGY- CHEM for lab technology
Badr City, Industrial Area Piece 170
250 Fadan In East of Elrubaki, EGYPT
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CE

EC REP

MDSS GmbH
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30175 Hannover,
Germany

BioMed-Liquicellin-E for APTT determination

Activated Partial Thromboplastin Time

REF: PTT202180   (6x3 ml)

INTENDED FOR USE:

For quantitative determination of Partial the Biomed- LIQUICELLIN-E activity in plasma

PRINCIPLE:

Cephaloplastin activates the coagulation factors of the intrinsic pathway of the coagulation mechanism in the presence of calcium ions.

APTT is prolonged by a deficiency of one or more of these clotting factors of the intrinsic pathway and in the presence of coagulation inhibitors like heparin.

SPECIMEN COLLECTION:

The arrest of bleeding depends upon primary platelet plug formed along with the formation of stable fibrin clot. Formation of this clot involves the sequential interaction of series of plasma proteins in a highly ordered and complex manner and also the interaction of these complexes with blood platelets and materials released from the tissues.

Activated Partial Biomed- LIQUICELIN-E Time is prolonged by a deficiency of coagulation factors of the intrinsic pathway of the human coagulation mechanism such as factor XII, XI, VIII, X, V, II and Fibrinogen. Determination of APTT helps in estimating abnormality in most of the clotting factors of the intrinsic pathway including congenital deficiency of factor VIII, IX, XI and XII and is also a sensitive procedure for generating heparin response curves for monitoring heparin therapy

REAGENT COMPOSITIONS :

Biomed-LIQUICELIN-E is liquid ready to use activated cephaloplastin reagent for the determination of Activated Partial Thromboplastin Time. It is a phospholipids preparation derived from rabbit brain with ellagic acid as an activator.

Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.

PACKAGE: Collection and storage.

- Store the reagent at 2-8°C. DO NOT FREEZE.

- The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. The reagent is stable at 2-8°C up to the expiration date.

PRECAUTIONS & WARNING :

Avoid pipeting with mouth.

The preparation, according to current regulation, is classified as not dangerous.

The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation.

Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes. The samples must be handle as potentially infected from HIV or Hepatitis.

REAGENT PREPARATION & STABILITY :

- Use freshly collected blood taken into 0.11 mol/L trisodium citrate in the ratio 9 parts blood to 1 part anticoagulant.

- Centrifuge immediately for 5 minutes at RCF 1500-2000 g and separate plasma into a clean test tube.
- Plasma should be tested within 3 hours (keep refrigerated).

REQUIRED MATERIALS NOT PROVIDED:

(a) 12 X 75 mm glass test tubes.

(b) 0.1 ml.

(c) Stop watch.

(d) Water bath or heating block at 37°C.

(e) Fresh Normal Pooled Plasma.

(f) CaCl2 (0.02 mol/l).

PROCEDURE:

Manual method

1. Pre-incubate the Calcium Chloride Reagent to 37°C for at least 10 minutes. Pipette 100µl of test or control plasma into a test cuvette.

2. Incubate the plasma at 37°C for 1 to 2 minutes.

3. Pipette 100µl of the APTT reagent, into reagent cuvette containing the plasma. Maintain the suspension of the APTT reagent by magnetic stirring or mixing by inversion immediately prior to use.

4. Incubate at 37°C for 3 minutes.

5. Add 100µl preincubated Calcium Chloride solution and simultaneously start the timer.

6. Record the clotting time in seconds.

Calibration Curve Method (For determination of heparin concentration):

1. Dilute heparin (as used for treatment) with physiological saline to a concentration of 10 U/ml.

2. Mix 0.2 ml of 10 U/ml diluted heparin with 1.8 ml of FNP to give a heparin standard of 1U/ml concentration.

3. Dilute the heparin standard as prepared above (1U/ml) with FNP as follow

Test tube no.	1	2	3	4	5	6	7
Heparin standard (1U/ml) in ml	0.5	0.4	0.3	0.2	0.1	0.1	–
FNP in ml	–	0.1	0.2	0.3	0.4	0.9	0.5
Heparin concentration (U/ml)	1.0	0.8	0.6	0.4	0.2	0.1	0.0

4. Pipette 0.1 ml each of the seven heparin dilutions into clean test tubes.

5. Add 0.1 ml LIQUICELIN-E reagent to each test tube.

6. Mix well and incubate each test tube at 37°C for exactly 3 minutes before testing.

7. Forcibly add 0.1 ml calcium chloride (prewarmed at 37°C) to each test tube, one by one and simultaneously start the stopwatch.

8. Gently tilt the tube back and forth and stop the stopwatch as the first fibrin strand is visible and the gel/clot formation begins. Record the time in seconds.

9. Repeat steps 4-8 for each dilution for duplicate test, and find the average of the duplicate test values.

10. Plot the mean of the double determination in seconds, against each heparin concentration using BioMed-LIQUICELIN-E graph paper.

11. Clotting times (APTT) of test specimens can be interpolated against the heparin concentration to determine the heparin concentration of the sample in U/ml.

CALCULATION:

Manual Method

(a) The results may be reported directly in terms of the mean of the double determination of APTT of the test plasma.

(b) Or as a ratio 'R':
$$R = \frac{\text{APTT of patient plasma (in seconds)}}{\text{APTT of FNP (in seconds)}}$$

Calibration Curve Method

Heparin concentration in the test sample can be directly obtained from the Biomed- LIQUICELIN-E calibration curve by interpolating the test plasma clotting time against the heparin concentration in U/ml.

EXPECTED VALUE:

Normal values using LIQUICELIN-E reagent are between 28-40 seconds at 3 minutes activation time. Between manual and Turbo densitometric instrument results a variation of 1-2 seconds may be expected. For photo optical instruments, it is recommended that each laboratory must establish its own normal range.

WASTE DISPOSAL:

The disposal of the product must be in accordance with local regulation concerning waste disposal.

REFERENCES:

1. Biggs R. and R.G. McFarlane: Human Blood Coagulation and its Disorders, Blackwell Scientific Publications, Oxford,1962.
2. Hoffmann, J.J.M.L. and Neulendijk P.N.: Thrombos. Haemosta. (Stuttgart) 39, 640 (1978).
3. CRC, Handbook Series in Clinical Laboratory, Science, Section 1: Haematology, Volume III, 1980. CRC Press, Inc. Boca Raton, Florida

PERFORMANCE:

1. Due to inter and intra laboratory variations users must establish their own normal population range as well as normal and abnormal range.
2. It is recommended that controls with known factor activity should be run simultaneously with each test series routinely.
3. Incorrect mixture of blood and tri-sodium citrate, insufficient prewarming of plasma and reagent, contaminated reagents, glassware etc. are potential source of errors.
4. Incorrect dilution of heparin is also a potential source of error.
5. Oxalated plasma may include prolonged clotting times.
6. Clotting time of patients on anticoagulant therapy depends upon the time lag between the specimen collected and the last dose.
7. Abnormalities of coagulation factor VII, factor XIII and platelets are not detected by this test procedure.
8. For automated equipment it is strongly recommended that the equipment manufacturer methodology be strictly adhered to.
9. In heparin monitoring time of collection of blood sample is important since the in-vitro half-life of heparin is approximately 1.5hours. When it is administered intravenously it has an immediate anti coagulant effect but its efficacy decreases rapidly with time.
10. Platelet factor IV, a heparin-neutralizing factor can be released due to platelet aggregation or damage. In order to prevent this phenomenon in-vitro the specimen should be collected with a minimum of trauma.
11. Decrease in APTT time is observed in males under estrogen therapy and oral contraceptive administration in females

LIMITATIONS :

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.

2. LIQUICELIN-E reagent is not from human source hence contamination due to HBsAg and HIV is practically excluded.

3. Reagent contains 0.01% Thimerosal as preservative.

4. It is very important that clean and dry micropipette tips be used to dispense the reagent.
5. Avoid exposure of the reagent to elevated temperatures, contamination. Immediately replace cap after use and store at recommended temperatures only.

🛈	Consult Instructions for Use
⚠	Caution, Consult accompanying Documents
IVD	In Vitro Diagnostic Medical Device
🌡	Temperature Limitation
🏭	Manufacturer
EC REP	Authorized Representative in the European Community
REF	Catalogue Number
LOT	Batch Code
⧖	Use by

EGY- CHEM for lab technology
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CE

EC REP

MDSS GmbH
Schiffgraben 41
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BioMed-Fibrinogen

BIOMED DIAGNOSTICS

Reagent for Quantitative Estimation Of Fibrinogen

REF: FIB203020   (20 test)

INTENDED FOR USE:

For quantitative determination of Fibrinogen in plasma

PRINCIPLE:

The addition of thrombin coagulates fresh citrated plasma. The coagulation time is proportional to the fibrinogen concentration. This allows the estimation of plasma fibrinogen by functional clotting assay.

SPECIMEN COLLECTION:

At present there are known to be at least eleven factors in circulating blood, are required for normal haemostasis. Deficiency in any of these, Factors I,II,V,VII,VIII,IX,X,XI and XIII, results in a notable hemorrhagic condition, and the severity of the bleeding is proportional to the degree of deficiency. In order to treat the hemorrhagic condition, it is important to identify and quantify the deficient factor.

Fibrinogen (Factor) is a high molecular weight glycoprotein synthesized in the liver, which plays an important role in haemostasis. For normal haemostasis to occur in response to injury or tissue damage, A sufficient concentration of fibrinogen must be present in plasma. Fibrinogen is converted into fibrin by the action of thrombin and is a key component of clot formation.

FIBRINOGEN kit contains lyophilised thrombin and fibrinogen calibrator to determine the quantitative reactivity of fibrinogen calibrator to determine the quantitative reactivity of fibrinogen. Since the reagent system contains heparin-neutralizing substances, heparin levels upto 0.4 IU/ml does not interfere with test results. When used as a front line test with PT, APTT, platelet count and thrombin time, fibrinogen assay helps in investigating acute haemostatic failure.

REAGENT COMPOSITIONS :

FIBRINOGEN kit contains:

1. Thrombin reagent, which is alyophilized preparation from bovine source ~ 50 NIH units per vial.

2. Fibrinogen calibrator, which is a lyophilized preparation of human plasma equivalent to stated amount of fibrinogen on a mg basis (refer FIBRINOGEN graph paper supplied with each kit for the value of each lot).

3. Owren's buffer, ready to use (pH 7.35).

PACKAGE: Collection and storage.

1. Store the unopened reagent vials at 2-8°C. DO NOT FREEZE.

2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial labels.

3. Once reconstituted the FIBRINOGEN thrombin reagent is stable for 6 days when stored at 2-8°C and for 4 hours at room temperature (20-25°C), provided it is not contaminated. Extreme care has to be taken to maintain aseptic precautions while reconstituting, retrieving and handling reagents to prevent contamination. The reagent vial must be replaced to 2-8°C immediately upon retrieving the reagent for the day's work.

4.The reconstituted FIBRINOGEN calibrator is stable for 6 hours at 2-8°C and for 2 hours at room temperature (20-25°C).

PRECAUTIONS & WARNING :

Avoid pipetting with mouth.
The preparation, according to current regulation, is classified as not dangerous.

The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation.
Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes.
The samples must be handle as potentially infected from HIV or Hepatitis

REAGENT PREPARATION & STABILITY :

No special preparation of the patient is required prior to sample collection by approved techniques. Withdraw blood without undue venous stasis and without frothing into a plastic syringe fitted with a short needle of 19 to 20 SWG. The venapuncture must be a "clean" one and, if there is any difficulty, take a new syringe and needle and try another vein. Transfer the blood into tubes, after detaching the needle from the syringe.

Mix nine parts of freshly collected blood with one part of sodium citrate (0.109mol/l, 3.2%). Centrifuge immediately for fifteen minutes at 3000rpm (approximately2000 g) and transfer the plasma into a clean test tube. Plasma must be tested within 3 hours of collection.

REQUIRED MATERIALS NOT PROVIDED:

General Laboratory Equipment and instrumentations.

PROCEDURE:

Bring all the reagent and sample to room temperature before testing.

A) Procedure for fibrinogen Calibration Curve Preparation

• The FIBRINOGEN thrombin reagent vial must be reconstituted exactly with one ml of distilled water; wait for 5 minutes, do not shake but gently swirl the vial till the solution attains homogenity. Further keep the vial aside for 10 minutes to attain equilibrium. Once reconstituted it is ready to use for the fibrinogen test.
• The FIBRINOGEN calibrator vial must be reconstituted exactly with one ml of distilled water; wait for 5 minutes, do not shake, gently swirl the vial till the solution attains homogenity. Further keep the vial aside for 10 minutes to attain equilibrium. This is the fibrinogen calibrator stock solution

• Dilute fibrinogen calibrator stock solution with Owren's buffer as follows:

Test tube no.	I	II	III
Owren's buffer	NIL	0.8 ml	0.9 ml
Fibrinogen calibrator	0.2 ml	0.2 ml	0.1 ml
Dilution(Calibrator)	NIL	1 : 5	1 : 10

1. Pipette 0.2 ml of each fibrinogen calibrator dilution into clean test tubes and prewarm for 3 minutes at 37°C.

2. Add 0.1 ml of reconstituted thrombin reagent (prewarmed at 37°C for one minute) and simultaneously start stopwatch.

3. Stop the stopwatch at the first appearance of the fibrin web, as the gel clot begins to form and record the time in seconds.

4. Repeat steps 1-3 for a duplicate test on each calibrator dilution.

5. Plot average of the duplicate test values on FIBRINOGEN graph paper.

6. Connect the points, which should produce a straight line.

7. The calibration curve may be extended beyond the lowest and highest point.

8. The calibration curve is valid only for the same lot of FIBRINOGEN thrombin reagent.

B) Test Procedure for sample

1. Prepare 1:10 dilution of plasma specimen with Owren's buffer solution.

2. To a 10 x 75 mm test tube at 37°C add 0.2 ml of 1:10 dilution of plasma sample to be tested.

3. Incubate at 37°C for one minute.

4. To the test tube add 0.1 ml of FIBRINOGEN thrombin reagent (prewarmed at 37°C for one minute) and start the stopwatch simultaneously.

5. Stop the stopwatch at the first appearance of the fibrin web, as the gel clot begins to form and record the time in seconds.

6. Repeat steps 1-5 for a duplicate test.

7. If at the sample dilution of 1:10 the observed clotting time is usually between 8-25 seconds, the fibrinogen content is normal (Fibrinogen content between 150 and 400 mg/dl). Assay results can be read off directly from the graph paper provided with the FIBRONIGEN kit for the fibrinogen concentration.

8. If the fibrinogen content is high the clotting time will be less than 8 seconds. In such cases repeat the test at 1:20 dilution of the sample or 1:30 dilution of the sample. The results read off the graph will be multiplied by a factor 2 or 3 for the respective dilution.

9. Conversely, if fibrinogen content is low, the clotting time will be over 25 seconds. Repeat the assay at 1:5 dilution, or if necessary at 1:2 dilution. In this case the results read off the graph will be divided by a factor of 2 or 5 for the respective dilution.

EXPECTED VALUE:

150 mg/dl - 400 mg/dl

WASTE DISPOSAL:

The disposal of the product must be in accordance with local regulation concerning waste disposal.

QUALITY CONTROL:

A known normal control should be run in parallel with each batch of tests. This control may be plasma coagulation control (PLASMATROL) or freshly drawn normal plasma.

REFERENCES:

1. Significant levels of heparin and elevated levels of fibrinogen degradation products (FDP) in the patient plasma can cause falsely low fibrinogen results.

2. Insufficient prewarming of plasma and reagent or contaminated glassware may cause erroneous results.

3. EDTA should not be used as an anticoagulant.

4. Use reagents of the same lot for performing the test.
5. Do not interchange reagents from different lots.

PERFORMANCE:

1. Biggs R. and R.G. McFarlane: Human Blood Coagulation and its disorders, Blackwell Scientific Publications, Oxford 1962.
2. Quick A.J.: Haemorrhagic diseases and thrombosis, 2nd Edition, Philadelphia, Lea and Febiger 1966.
3. CRC handbook Series in Clinical Laboratory Science, Section 1: Haematology, Volume III,1980.
4. Dacie J.V. and Lewis S.M.: Practical Haematology, 8th Edition, Churchill Livingstone Publications, 1995.
5. Provan D. et al. Oxford Handbook of Clinical Haematology,8th Edition, 1998.

LIMITATIONS :

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.

2. The individual reagents contain 0.1% sodium azide as preservative.

3. FIBRINOGEN thrombin reagent is not from a human source hence contamination due to HBsAg, HIV and HCV is practically excluded.

4. Fibrinogen calibrator provided in the fibrinogen kit is from a human source, which was tested and found to be non-reactive for HBsAg, HCV and HIV. However no known test methods can assure that infectious agents are absent. Handle all human products as potentially infectious.

5. It is very important that absolutely clean and dry micropipettes be used to aspirate and dispense the reagent.

6. Avoid exposure of the reagent to elevated temperatures, direct light and contamination. Immediately replace the cap after use and store at recommended temperature.

📖	Consult Instructions for Use
⚠	Caution, Consult accompanying Documents
IVD	In Vitro Diagnostic Medical Device
🌡	Temperature Limitation
🏭	Manufacturer
EC REP	Authorized Representative in the European Community
REF	Catalogue Number
LOT	Batch Code
⏳	Use by

ichroma™ D-Dimer

INTENDED USE

ichroma™ D-Dimer along with the ichroma™ Reader is a fluorescence immunoassay that quantifies the total D-Dimer concentration in plasma. The test is used as an aid in the post therapeutic evaluation of thromboembolic disease patients.

INTRODUCTION

D-dimer, a degradation product of cross-linked fibrin formed during activation of the coagulation system, is commonly used to exclude thromboembolic disease in outpatients suspected of having deep venous thrombosis (DVT) and pulmonaryembolism (PE).[1] DVT and PE is relatively common and can cause sudden, fatal embolic events in the pulmonary arteries and other regions. [2-3]

Measurement of the D-Dimer level in plasma has been used as a screening strategy for subclinical DVT. A systematic review reported that a normal range of a highly sensitive D-dimer level accurately ruled out DVT in patients classified as having a low or moderate clinical probability of DVT. The DVT is a high-risk factor for the stroke because of advanced age, hemiplegia, and coagulation disorders, and DVT can cause paradoxical embolic stroke via a right-toleft shunt. Thus, it is important to monitor the level of D-Dimer the incidence and characteristics of DVT in acute stroke patients.[4-7] The Plasma D-dimer level has proven to be useful for DVT screening in chronic stroke patients undergoing rehabilitation.[8-10] National and international scientific organizations have suggested the use of these markers when implementing new diagnostic strategies in patients with coronary syndrome. Since D-Dimer is well known to be an important prognostic indicator of heart diseases, its most definitive role is on monitoring post-treatment clinical status and the post therapeutic evaluation of patients.

ichroma™ D-Dimer Test measures quantitative D-Dimer concentration in human plasma.

PRINCIPLE

The test uses the sandwich immunodetection method, such that the detection antibody in buffer binds to D-Dimer in the plasma sample and antigen-antibody complexes are captured by antibodies that have been immobilized on the test strip as sample mixture migrates through nitrocellulose matrix. The more D-Dimer antigen in the plasma, the more antigen-antibody complexes are accumulated on test strip. Signal intensity of fluorescence on detection antibody reflects amount of antigen captured and is processed by ichroma™ Reader to show D-Dimer concentration in the specimen. The working range of ichroma™ D-Dimer test is 50 – 10,000 ng/mL.

* Reference Value: 500 ng/mL. (FEU: Fibrinogen equivalent units)

COMPONENTS AND REAGENTS

ichroma™ D-Dimer consists of Cartridge, an ID Chip, and Detection Buffers.

- The test cartridge contains a test strip; on the membrane of which, antibodies against D-Dimer and streptavidin have been immobilized at the test line and the control line respectively.
- Each test cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed test cartridges are packed in a box which also contains an ID chip.
- The detection buffer pre-dispensed in a tube contains fluorochrome-labeled anti-D-Dimer antibodies, fluorescent-labeled biotin-BSA, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is dispensed in each detection buffer tube. 25 detection buffer tubes are packed in a separate pouch which is further packed in a Styrofoam box provided with ice packs for the purpose of shipment.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use only.
- Carefully follow the instructions and procedures described in this insert.
- Lot numbers of all the test components (test cartridge, ID chip and detection buffer) must match with each other.
- Do not interchange the test components from different lots or use the test components beyond the expiration date.
- Test performed by using any test component with mismatching lot number or that beyond the expiration date may yield misleading test result(s).
- The test cartridge should remain sealed in its original pouch until use. Do not use the test cartridge that is damaged or already opened.
- Allow a minimum of 30 minutes for the test cartridge to attain room temperature, which has been stored in a refrigerator.
- The detection buffer should attain room temperature prior to performing the test.
- ichroma™ D-Diemr as well as the ichroma™ Reader should be used away from vibration and /or magnetic field. During normal usage, ichroma™ Reader may produce minor vibrations which should be regarded as normal.
- A detection buffer tube should be used for processing one sample only. Similarly a test cartridge should be used for testing one processed sample only. Both the detection buffer

tube as well as the test cartridge should be discarded after single use.

- Used detection buffer tubes, pipette tips, and test cartridges should be handled carefully and disposed of by an appropriate method in accordance with relevant local regulations.

- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.

STORAGE AND STABILITY

- The test cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4 - 30°C.

- The detection buffer dispensed in a tube is stable for 20 months if stored at 2 - 8°C.

- After the test cartridge pouch is opened, the test should be performed immediately.

LIMITATIONS OF THE TEST SYSTEM

ichroma™ D-Dimer provides accurate and reliable results subject to the following constraints:

- Use ichroma™ D-Dimer should be used only in conjunction with ichroma™ Reader.

- The test should always be performed on freshly collected sample(s).

- The test sample must be at room temperature prior to testing. If the test samples are to be shipped for the purpose of this test, appropriate precautions must be exercised.

- Effectiveness of the test is highly dependent on storage of test components and test samples at prescribed optimal conditions.

- The test may yield false positive result(s) due to cross-reactions of some components of serum with the capture/detector antibodies and/or non-specific adhesion of certain components having similar epitopes to bind with these antibodies.

- The test may also yield false negative results; the most common factor being non-responsiveness of the antigen to the antibodies due to its epitopes being masked by some unknown components such that the antigen cannot be detected or captured by the antibodies. False negative results may also be obtained due to instability or degradation of the D-Dimer antigen with time and/or temperature making it unrecognizable by the antibodies.

- Other factors interfering with the test and causing erroneous results include technical/procedural errors, degradation of the test components/reagents as well as presence of interfering substances in the test samples.

- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

SAMPLE COLLECTION AND PREPARATION

The test can be performed with plasma.

- It is recommended to test the sample within 24 hours after collection.

- The serum and plasma should be prepared by centrifugation within 3 hours after the collection of whole blood.

- Take precautions on the handling and storage of sample blood because it's analyzed the concentration of D-Dimer is sensitive to anticoagulant and storage conditions

- Preparing the Plasma specimen: Collect the blood in a tube treated with **sodium citrate**.

- Be careful not to have blood sample hemolyzed in the course of handling and centrifugal process

- Do not keep the sample in a freezer, which could affect the test value of D-Dimer

- It is recommended to avoid using severely **hemolyzed and hyperlipidemia** specimens whenever possible. If the specimen appears to be severely hemolyzed, another specimen should be obtained and tested.

MATERIALS PROVIDED

REF CFPC-25

Components of ichroma™ D-Dimer

- **Test Cartridge Box:**
  - Sealed Test Cartridges   25
  - ID Chip   1
  - Package Insert   1
- **Box containing Detection Buffer Tube**
  - Detection Buffer Tubes   25

MATERIALS REQUIRED BUT NOT PROVIDED

Following items can be purchased separately from ichroma™ D-Dimer. Please contact our sales division for more information.

- ichroma™ Reader REF FR203
- Thermal Printer

TEST SETUP

1. Check the contents of ichroma™ D-Dimer: Sealed Test Cartridge, ID Chip, and Detection Buffer Tube.

2. Ensure that the lot number of the test cartridge matches with that of the ID chip as well as the detection buffer tube.

3. Keep the sealed test cartridge (if stored in refrigerator) and the detection buffer tube at room temperature for at least 30

minutes just prior to the test. Place the test cartridge on a clean, dust-free and flat surface.

4. Turn on the power supply of the ichroma™ Reader.

5. Insert the ID Chip into the ID chip port of the ichroma™ Reader.

6. Press the 'Select' button on the ichroma™ Reader. (Please refer to the 'ichroma™ Reader Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

1. Transfer 10 μL of serum/plasma/control sample using a transfer pipette to a tube containing the detection buffer.

2. Close the lid of the detection buffer tube and mix the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately.).

3. Pipette out 75 μL of a sample mixture and dispense it into the sample well on the test cartridge.

4. Leave the sample-loaded test cartridge at room temperature for 12 minutes.

5. For scanning, insert it into the test cartridge holder of the ichroma™ Reader. Ensure proper orientation of the test cartridge before pushing it all the way inside the test cartridge holder. An arrow has been marked on the test cartridge especially for this purpose.

6. Press 'Select' button on the ichroma™ Reader to start the scanning process.

7. ichroma™ Reader will start scanning the sample-loaded test cartridge immediately.

8. Read the test result on the display screen of the ichroma™ Reader.

INTERPRETATION OF TEST RESULT

- ichroma™ Reader calculates the test result automatically and displays D-Dimer concentration of the test sample as ng/mL.
- Working range of ichroma™ D-Dimer is 50-10,000 ng/mL.
- Reference value of ichroma™ D-Dimer is 500 ng/mL. (FEU: Fibrinogen equival units)

Quality Control

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Before testing a clinical sample using a new test lot, control reagents should be tested to confirm the test procedure, and to verify whether the test produces the expected results.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control reagents are not provided with ichroma™ D-Dimer. For more information regarding obtaining the control reagents,

contact Boditech Med Inc.'s Technical Services for assistance.

- ichroma™ D-Dimer test has a built-in internal control that satisfies the routine quality control requirements. This internal control test is performed automatically each time a clinical sample is tested. An invalid result from the internal control leads to display an error message on the ichroma™ Reader indicating that the test should be repeated.

PERFORMANCE CHARACTERISTIC

1. Specificity

Other bio-molecules, such as Hb, CEA, AFP, ALP, CRP, Troponin I, CK-MB, Myoglobin, Albumin and specially hyperlipid were added to test specimen with much higher level than their physiological level in normal blood. There was no significant interference with the D-Dimer measurement, nor was their any significant assay cross-reactivity with those bio-molecules tested.

2. Imprecision:
For studying intra-assay imprecision, 10 replicates of each of the eight concentrations of spiked plasma samples with Bio-Rad D-Dimer control were tested. For studying inter-assay imprecision, 10 replicates of each of the eight concentrations of spiked plasma samples with Bio-Rad D-Dimer control were tested by four different persons

Intra-and inter-assay precision

Conc. [ng/ml]	Intra Assay			Inter Assay		
	Mean	SD	CV	Mean	SD	CV
100	100.82	6.36	6.31	94.38	7.27	7.70
1000	1021.49	53.03	5.19	1080.20	63.23	6.14
5000	5060.42	209.29	4.14	4986.93	274.43	5.50
10000	9553.36	407.76	4.27	9514.11	502.71	5.28

3. Comparability

Total D-Dimer concentrations of 100 plasma samples were quantified independently with ichroma™ D-Dimer device and bioMerieux VIDAS automatic analyzer according to established standard test procedure. Test result was compared and their compatibility was investigated with linear regression and correlation of coefficient (R). Linear regression and correlation of coefficient were Y=0.955X + 47.13 and R=0.965, respectively.

y=0.96x+7.13
R=0.965
N=100

Relative VIDAS D-Dimer [ng/ml]
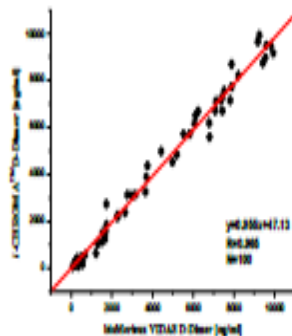
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