بسم الله الرحمن الرحيم

Sudan University of Science & Technology

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Estimation of Fibrinogen Level in Umbilical Cord Blood among Gestational Diabetes Mothers

قياس مستوى الفيبرينوجين في دم الحبل السري لدى الأمهات اللاتي لديهن سكر الحمل

A dissertation submitted in partial fulfillment of the requirements for the degree of M.Sc. in Medical Laboratory Science,

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الآية

قال تعالى في محكم تنزيله :

وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ"

صدق الله العظيم

سورة التوبة الآية رقم

(١٠٥)

Dedication

To my mother, who gives me care and love

To my father, who is the source of help fullness

To my brothers & sisters

To my colleagues & friends

I dedicate this work

Acknowledgments

All great thanks are firstly to Allah who guided me to the strait way in my life.

Then, I would like to express my gratitude and thanks to my supervisor Dr. Hiba BadrEldin Khalil for her guidance, helpful suggestions, solving problems and her precious advices as well as continuous assistance through the whole process of the research.

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List of Abbreviations

Abbreviation	Full text
AA	Arachidonic Acid
ACOG	American College of Obstetricians and Gynecologists
ADA	American Diabetes Association
ADP	Adenosine Diphosphate
Ang	Angiotensin
AT	Antithrombin
ATP	Adenosine triphosphate
BMI	Body Mass Index
CaCl2	Calcium Chloride
DIC	Disseminated Intravascular Coagulation
DM	Diabetes Mellitus
ECs	Endothelial Cells
EDRF	Endothelial-Derived Relaxing Factor
FBG	Fasting Blood Glucose
FDPs	Fibrin Degradation Products
GDM	Gestational Diabetes Mellitus
GP	Glycoproteins
HAPO	Hyperglycemia and Adverse Pregnancy Outcomes
HT	Hypertention
IADPSG	International Association of Diabetes and Pregnancy Study Group
IGT	Impaired Glucose Tolerance
INR	International Normalized Ratio

IR	Insulin Resistance
ISI	International Sensitivity Index
LGA	Large for Gestational Age
NO	Nitric Oxide
NDDG	National Diabetes Data Group
NIH	National Institutes of Health
NPH	Neutral Protamine Hagedorn
OGTT	Oral Glucose Tolerance Test
PDGF	Platelet Derived Growth Factor
PED	Pregestational Diabetes
PK	Prekallikrein
PPP	Platelets Poor Plasma
PTT	Partial Thromboplastin Time
PT	Prothrombin Time
RAAS	Renin Angiotensin Aldosterone
RT	Reptilase Time
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TFPI	Tissue Factor Pathway Inhibitor
TF	Tissue Factor
TNF	Tumor Necrosis Factor
T-PA	Tissue Plasminogen Activator
TT	Thrombin Time
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organization

Abstract

Background: Gestational diabetes mellitus (GDM) is the most common medical complication of pregnancy. It is associated with maternal and neonatal adverse outcomes. Fibrinogen level has been the intensive study in latest years. In addition, there is an association mentioned in previous records between fibrinogen level and newborn metabolic diseases, liver disorders and some cancers.

Objectives: To evaluate and estimate fibrinogen level in newborn cord blood of gestational diabetes mellitus mothers from February to August 2018, and to compare with fibrinogen level of newborn cord blood of healthy pregnancy. Moreover, to correlate fibrinogen levels according to the gestational weeks and weight of newborns.

Materials and Methods: A case control study was conducted on 60 newborns from 30 mothers with GDM and 30 healthy mothers after ethical consent and hospital approval. Citrate cord blood samples were performed for fibrinogen estimation.

Results: There were statistically significant increase of fibrinogen level (*P.value*=0.026) among newborns with GDM mothers (257.66±32.61mg/dl) compared with control group (236.77±37.93mg/dl). And there was significant increase in fibrinogen level in newborns with mothers FBG<100 mg/dl (249.44 mg/dl) compared with mothers FBG>100mg/dl (231.33 mg /dl) (*P. value*=0.031). No correlation between mentioned level and gestational weeks (*P. value*=0.414) and also the weight of newborns (*P. value*=0.401).

Conclusions: There were significant increase of fibrinogen level among newborns with GDM mothers and no correlation between mentioned level and gestational weeks and also the weight of newborns.

Increased fibrinogen level may contribute to the development of thrombotic risk.

ملخص البحث

الخلفية: يعتبر سكر الحمل من الأمراض الخطيرة على صحة المرأة الحامل ورضيعها. كما ان التركيز الفيبرينوجين في الدم يساعد في تنبؤ تكوين الجلطة. بالإضافة إلى ذلك هناك علاقة بين الفيبرينوجين وبين حالات الأمراض الإستقلابية ،وأمراض الكبد وبعض أنواع السرطان.

الأهداف: تحديد مستوى الفيبرينوجين في دم الحبل السري للمصابات بسكر الحمل من الفترة فبراير الى أغسطس ٢٠١٨، ومقارنتها مع مستوى الفيبرينوجين في دم الحبل السري للحمل الطبيعي.وإيجاد علاقة مترابطة بين مستوى الفيبرينوجين و أسابيع الحمل و وزن حديث الولادة.

طرق البحث: أجريت هذه الدراسة على 60 مولود منهم 30 من أمهات لديهن سكر الحمل و30 من أمهات سليمات. كما تم تحليل الفيبرينوجين من عينة دم الحبل السري بعد الحصول على البلازما الخالية من الصفائح الدموية ومن ثم اجراء تحليل احصائي للمقارنة بين النتائج العينات. تم ترتيب البيانات وإدخالها وتحليلها باستخدام برنامج SPSSالإصدار20 و باستخدام اختبارT المستقل ومعامل الإرتباط و اعتبار قيمة P بقيمة <0.05 على أنها ذات دلالة إحصائية.

النتائج: كان هناك فرق معنوي واضح في مستوى الفيبرينوجين (*P. value 0.*026)، بين حديثي الولادة من الأمهات المصابات بسكر الحمل وكان المتوسط (257.66±32.61mg/dl) مع عينات السليمة للمقارنة حيث كان المتوسط (236.77±37.93mg/dl).كمان ان هناك فرق معنوي في مستوى الفيبرينوجين بين سكرالصائم اقل من 100mg/dlوسكري الصائم اكثر من 100mg/dl (*P. value 0.*031). أظهرت النتائج عدم وجود فروقات معنوية بين مستوى الفيبرينوجين ووزن حديث الولادة و أسبوع الولادة.

مستخلص الدراسة: هناك زيادة ذات دلالة معنوية في مستوى الفيبرينوجين لدى حديثي الولادة من الأمهات المصابات بسكر الحمل عن مستوى الفيبرينوجين في الامهات السليمات ، وليس هناك ارتباط بين مستوى الفيبرينوجين ووزن حديث الولادة و أسابيع الحمل. كما يمكن اعتبار تقدير مستوى الفيبرينوجين كطريقة للتنبؤ بتكون الجلطة.

Chapter One

Introduction and Literature Review

1. Introduction

1.1Gestational Diabetes Mellitus (GDM)

Gestational diabetes mellitus (GDM) is defined as glucose intolerance of various degrees that is first detected during pregnancy. GDM is detected through the screening of pregnant women for clinical risk factors and, among at risk women, testing for abnormal glucose tolerance that is usually, but not invariably, mild and asymptomatic. GDM appears to result from the same broad spectrum of physiological and genetic abnormalities that characterize diabetes outside of pregnancy. GDM is a form of hyperglycemia. In general, hyperglycemia results from an insulin supply that is inadequate to meet tissue demands for normal blood glucose regulation (Thomas and Anny,2005).

1.1.1 Classification

A uniform classification of diabetic in pregnancies is still needed for both epidemiological and clinical purposes. Both the World Health Organization (WHO) and National Diabetes Data Group (NDDG) of National Institutes of Health (NIH) have endorsed a classification based on the etiology. WHO classification differ only by recognizing impaired glucose tolerance (IGT) before pregnancy. This is simple but of no prognostic value. Classification of maternal diabetes in pregnancy:
Pregestational diabetes; preexisting type1 or type2 or secondary, and gestational diabetes; diagnosis is made post gestationally, normal glucose tolerance.

Currently, the term diabetes in pregnancy has been suggested to include all cases of hyperglycemia observed during pregnancy comprising GDM and PED. The latter include pre-gestational type 2 diabetes mellitus

(T2DM) and type 1 diabetes mellitus (T1DM), and GDM is defined as any degree of hyperglycemia that is recognized for the first time during pregnancy. This definition of GDM should be understood as to include cases of undiagnosed T2DM "overt diabetes" identified early in pregnancy and true GDM which develops later in pregnancy (Chamberlain *et al*. ,2013).

1.1.2 Epidemiology

The prevalence of GDM has been progressively increasing and it reflects the background prevalence of obesity and T2DM in general population. Higher rates of GDM were found to raise in parallel with higher rates of T2DM. This may be related to the common risk factors including obesity, physical inactivity, ethnic background and urbanization. In Northern Californian pregnant women without pre-existing diabetes, reported that the prevalence of GDM was low among non Hispanic white women and African Americans, and high in Asians and Filipinas. Interestingly higher rates of GDM were demonstrated among those with lowest BMI (Asians and Filipinas) and lower rates were found in those with highest BMI (non-Hispanic white women and African Americans) (Hedderson *et al*., 2012).

1.1.3 Etiology

Pregnancy represents a complex metabolic and physiological condition that can be considered as a status of biological tolerance test which has the ability to detect insulin resistance earlier. Insulin resistance (IR) in pregnancy could be the result of maternal obesity with varying degree of adipocytokine production, or increased production of diabetogenic placental hormones. In addition to insulin resistance, pancreatic β-cell dysfunction might also play a role in the pathophysiology of GDM (Abdel Hameed and John,2017).

1.1.4 Pathophysiology

During normal pregnancy, a progressive IR develops beginning around mid-pregnancy, and progresses during the third trimester. Hormones and adipokines secreted from the placenta, including tumor necrosis factor (TNF)-α, human placental lactogen, and human placental growth hormone are possible causes of IR in pregnancy. In addition, increased estrogen, progesterone, and cortisol during pregnancy contribute to a disruption of the glucose insulin balance. To compensate for the peripheral IR during pregnancy, insulin secretion increases from a woman's pancreas. The development of GDM occurs when a woman's pancreas does not secrete enough insulin to keep up with the metabolic stress of the IR. In addition, increased maternal adipose deposition, decreased exercise, and increased caloric intake contribute to this state of relative glucose intolerance (Eman,2015).

1.1.5 Risk Factors for GDM

Several risk factors are associated with the development of GDM. The most common risk factors are; obesity, BMI >30 kg/m^2 older maternal age, past history of GDM, strong family history of diabetes, member of an ethnic group with a high prevalence of T2DM, polycystic ovary syndrome, and persistent glucosuria. A history of macrosomia (birth weight ≥4000 g), history of recurrent abortions, and history of unexplained stillbirths, and history of essential hypertension, or pregnancy-related hypertension are other risk factors for GDM (Abdel Hameed and John,2017).

1.1.6 Maternal Complications

Potential maternal complications associated with GDM include gestational hypertension, preeclampsia, and non-elective cesarean delivery. The Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study was an international, multicenter study designed to assess the risks

of adverse outcomes associated with maternal glucose concentrations (HAPO ,2008). Regarding long-term complications, up to 50% of women with GDM will develop type 2 diabetes later in life. On average, this occurs 22–28 years after pregnancy (England *et al*., 2009 and O'Sullivan, 1982). Ethnicity and obesity (BMI > 30 kg/m^2) may play a role in the risk and timing of the subsequent diagnosis of diabetes. For example, as many as 60% of Latino women with GDM may develop diabetes within 5 years (Kjos *et al*.,2000). Other long-term complications include increased risk of developing metabolic syndrome and cardiovascular disease, respectively (Gunderson *et al*., 2009 and Shah *et al*. ,2008).

1.1.7 Fetal Complications

Neonates of women with GDM are at increased risk of macrosomia, which is defined as a birth weight over 4000 g, as well as neonatal hypoglycemia, hyperbilirubinemia, birth trauma, respiratory distress syndrome RDS, and shoulder dystocia (Reece, 2010). The over transfusion from placenta of GDM lead to hyper viscosity which may contribute to induce thrombosis. Macrosomia is the most common fetal complication, with a reported incidence of 15%–45%, followed by hyperbilirubinemia in 10%–13% of neonates (Esakoff et al., 2009).Hypoglycemia can occur in 3%–5% of infants as a result of increased fetal insulin production in response to maternal hyperglycemia, which can increase the risk of seizures. Shoulder dystocia is a rare, but serious complication that can lead to brachial plexus injury. Long-term complications of infants born to mothers with GDM include increased risk of impaired glucose tolerance, type 2 diabetes, hypertension, obesity, and dyslipidemia (Mitanchez, 2014).

1.1.8 Screening and Diagnosis for GDM

Screening and diagnosis of GDM may consist of either a one or a two-step approach (Figure1.1). The one-step approach was initially recommended by the ADA in 2011 for use in all pregnant women without preexisting diabetes. It involves a 75-g OGTT at 24–28 weeks' gestation. This was based on recommendations from the International Association of Diabetes and Pregnancy Study Group (IADPSG). The IADPSG recommendations were based primarily on the results of the HAPO study. The two-step approach has been recommended by ACOG and an NIH consensus development program (ACOG ,2013).

The IADPSG and ADA recognize that using the one-step approach would likely increase the number of women with a GDM diagnosis because only one abnormal value is needed for diagnosis. Although this may lead to increased health care costs, the ADA believes that the benefits outweigh these disadvantages. Data are unavailable from randomized controlled trials regarding outcomes for these additional women whose GDM would be diagnosed by the one-step method (Karen *et al*., 2017).

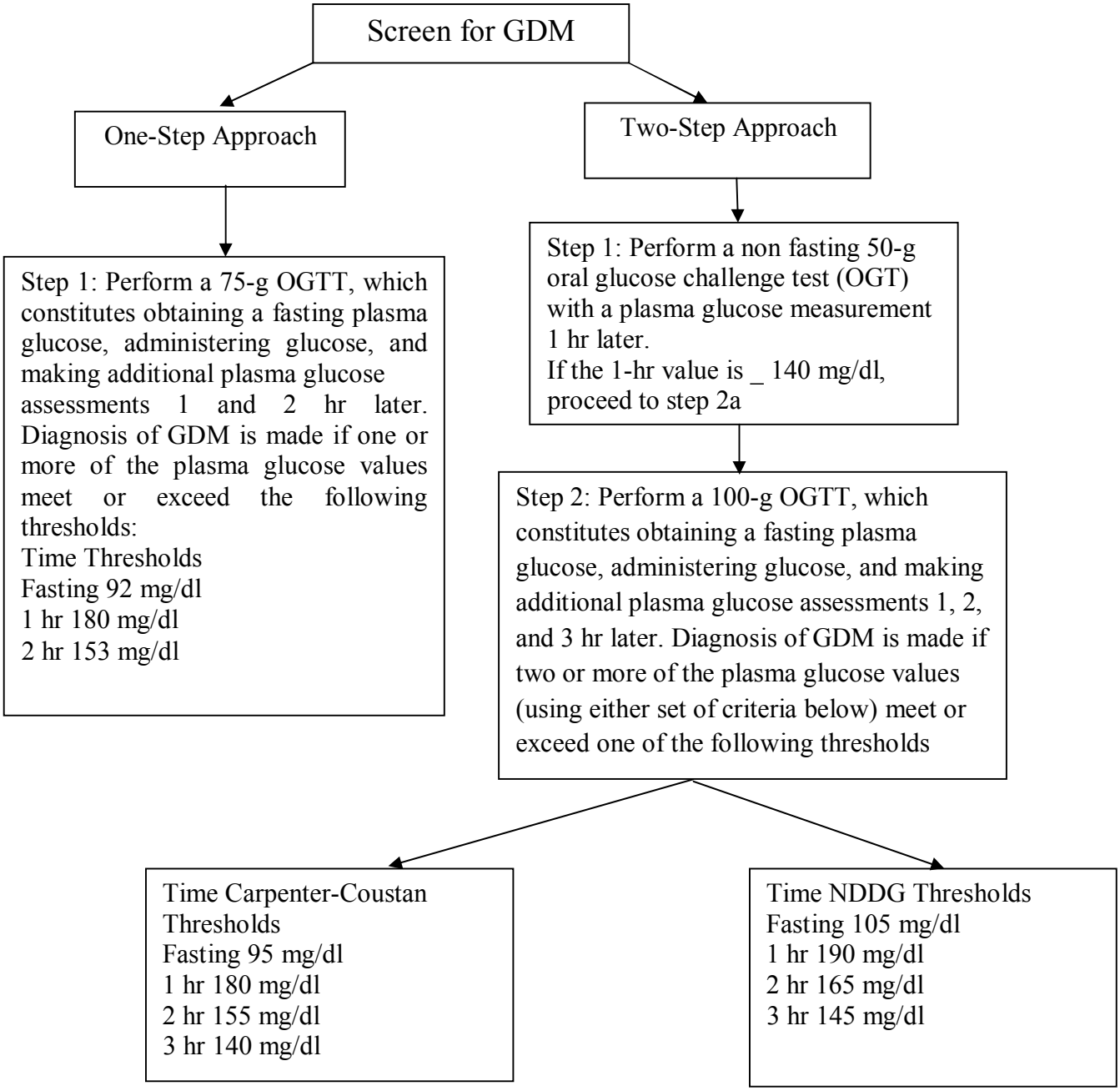


Figure1.1 Screening and diagnostic criteria for gestational diabetes mellitus. Adopted by Karen et al., 2017.

1.1.9 Management of GDM

The cornerstone of GDM management is glycemic control. The initial treatment for GDM is lifestyle interventions, which include medical nutrition therapy and daily exercise. Patients are required to check their glucose level frequently at home to assure that the glycemic targets are achieved. If the glycemic goals are not accomplished with these measurements, medical therapy should be initiated (Eman,2015).

1.1.9.1 Blood Glucose Monitoring

Women are instructed to carry out self monitoring of blood glucose 4 times a day, fasting glucose (upon awakening), and one or 2 hour post-meals (after the first bite of a meal). In GDM, monitoring of blood glucose after meals is preferred over pre-meal testing as the risk of macrosomia increases with increased maternal glucose levels post-meals. This was illustrated in a randomized clinical trial, which compared preprandial glucose monitoring to one hour post-prandial testing, and found macrosomia, cesarean deliveries, and neonatal hypoglycemia were significantly less frequent in women who monitor their glucose post-meals. However, it is not known whether a one hour, or 2-hour post-prandial testing is the ideal goal for the prevention of fetal risks. Therefore, patients can monitor their glucose levels at one or 2 hours post-meal, whatever is convenient, or at the estimated peak blood glucose is most likely to occur post prandial, for example, choosing the time at which glucose was elevated during OGTT (Eman,2015).

1.1.9.2 Pharmacological Interventions (Insulin Therapy)

If the medical nutrition therapy and exercise fail to achieve glycemic goals for a woman with GDM, insulin therapy should be initiated. The type and timing of insulin should be chosen based on the specific blood

glucose elevation. If the fasting glucose is greater than 90-95 mg/dl (whole blood capillary) then basal insulin, long-acting insulin analog, or Neutral Protamine Hagedorn (NPH); 4 units for example, should be started before bedtime. If fasting glucose level is too high, then basal insulin dose can be calculated according to the patient's weight, 0.2 units/kg/day. In cases where glucose level is elevated following a meal, rapid-acting insulin, or regular insulin should be prescribed before that specific meal, beginning with 2-4 units, or a dose of one unit per 10-15 g of carbohydrates. If both fasting and PP glucose levels are elevated, a 4-injections-per-day regimen "basal and meal time insulin regimen" should be prescribed. Basal and meal time insulin regimen is preferred over twice dose regimen because it is more likely achieves, maintains target blood glucose, and allows more flexibility. One could start by 2-4 units of rapid-acting insulin, or regular insulin before each meal, and 2-4 units of basal insulin before bed time. Another approach to determine the insulin doses is based on a woman's body weight and gestational week. In the first trimester, the total daily insulin requirement is 0.7 units/kg/day, in the second trimester it is 0.8 units/kg/day, and in the third trimester it is 0.9-1.0 units/kg/day. In a morbidly obese woman, the initial doses of insulin may need to be increased to 1.5-2.0 units/kg to overcome the combined IR of pregnancy and obesity. Subsequently, the calculated total daily dose of insulin should be divided into 2 halves; one half given as basal insulin at bed time, and the other half divided between 3 meals, and given as rapid-acting, or regular insulin before meals (Eman,2015).

1.2 Umbilical Cord Blood

Umbilical cord blood or "cord blood" is the blood left in the umbilical cord and placenta after a baby is born. This blood is usually thrown away. It can be saved and stored in a cord blood bank for use in the future. Cord

blood contains stem cells. Stem cells are special cells that can be used to treat certain diseases in children and adults. These diseases include some cancers (leukemia and lymphoma), blood disorders (sickle cell and thalassemia major), and other life-threatening diseases. When stem cells are used to treat a disease, it is called a stem cell transplant. Cord blood is collected after the baby is born and the umbilical cord has been clamped and cut. The umbilical vessels were identified and blood was drawn by needle puncture (18-gauge needle on a 10ml syringe) and transferred to an appropriate blood bottle (3ml tube) containing sodium citrate and massaging the umbilical cord during collection allowed for better blood flow. There are no risks to the baby or mother. At times, however, it may not be possible to collect the cord blood. This may happen if the delivery becomes complicated. If this happens, the mother's health care provider may not advise cord blood collection for medical reasons (Baer *et al*., 2013).

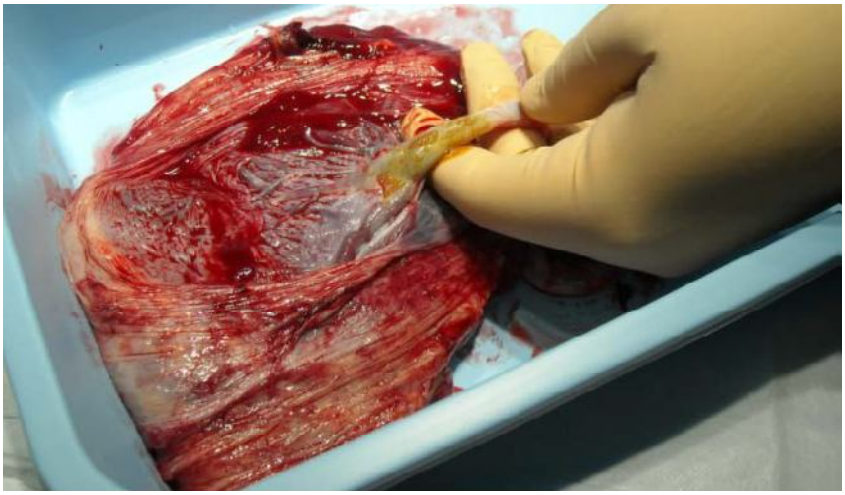


Figure1.2 Typical size and appearance of placenta/ umbilical cord obtained. Adopted by Baer *et al*., 2015.

1.2.1 Coagulation Parameters in Umbilical Cord Blood

Normal values coagulation parameters in the newborn adopted by Lippi, *et al* .,2007

- Platelets count 150-400,000 cell/cumm
- Prothrombin time (sec) 11-15
- Partial thromboplastin time (sec) 30-40
- Fibrinogen (mg/dl) 175-350
- Fibrin split products (mcg/ml) <10
- Thrombin time (sec)15-20

1.3 Hemostasis

Hemostasis defined as arrest of bleeding, comes from Greek, haeme meaning blood and stasis meaning to stop (Thornton and Douglas, 2010).The normal hemostatic response to vascular damage depends on closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors. An efficient and rapid mechanism for stopping bleeding from sites of blood vessel injury is clearly essential for survival. Nevertheless, such a response needs to be tightly controlled to prevent extensive clots developing and to break down such clots once damage is repaired. The hemostatic system thus represents a delicate balance between procoagulant and anticoagulant mechanisms allied to a process for fibrinolysis. The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels (Hoffbrand *et al*.,2011)

1.3.1 Primary Hemostasis

Primary hemostasis results from complex interactions between platelets, vessel wall and adhesive proteins leading to the formation of initial platelet plug. The formation of the platelet plug involves a series of steps (Palta *et al*., 2014).

1.3.1.1 Platelet Adhesion

During an injury, subendothelial collagen from the extracellular matrix beneath the endothelial cells is exposed on the epithelium as the normal epithelial cells are damaged and removed, which releases von willebrand factor (VWF). VWF causes the platelets to change form with adhesive filaments (extensions) that adhere to the subendothelial collagen on the endothelial wall (Varga *et al*.,2008).VWF acts as a bridge between endothelial collagen and platelet surface receptors Gp-Ib and promotes platelet adhesion. The platelet glycoprotein complex I (GP-Ib) is the principal receptor for VWF (Ruggeri, 2007).

1.3.1.2 Platelet Secretion

After adhesion, degranulation from both types of granules takes place with the release of various factors. Release of calcium, which binds to the phospholipids that appear secondary to the platelet activation and provides a surface for assembly of various coagulation factors. Also, release of adenosine diphosphate (ADP), which helps additional platelets to adhere to the injury site. Prostaglandins and Phospholipids, which also maintain vasoconstriction and help to activate further clotting chemicals. Thromboxane is an arachidonic acid derivative (similar to prostaglandins) that activates other platelets and maintains vasoconstriction. Serotonin is a short-lived inflammatory mediator with a vasoconstrictive effect that contributes to vascular changes associated with inflammation during an injury. PDGF and VEGF are involved in angiogenesis, the growth of new blood vessels and cell cycle proliferation (division) following injury. The coagulation factors include factor V and VIII, which are involved in the coagulation cascade that converts fibrinogen into fibrin mesh after platelet plug formation (Jackson ,2007).

1.3.1.3 Platelet Aggregation

Thromboxane A2 produced by activated platelets provide stimulus for further platelet aggregation. TxA2 along with ADP enlarge this platelet aggregate leading to the formation of the platelet plug, which seals off vascular injury temporarily. ADP binding also causes a conformational change in GpIIb/IIIa receptors presents on the platelet surface causing deposition of fibrinogen. Thrombin generation also catalyses the conversion of this fibrinogen to fibrin which adds to the stability of the platelet plug and is now known as secondary hemostasis (Andrews RK and Berndt MC, 2004). Prostacyclin inhibits platelet aggregation (platelet anti aggregating effect) and the balance between TxA2 and prostacyclin leads to localized platelet aggregation thus preventing extension of the clot thereby maintaining the vessel lumen patency (Palta *et al*., 2014).

1.3.2 Secondary Hemostasis

Secondary hemostasis refers to the coagulation cascade, which produces a fibrin mesh to strengthen the platelet plug. The coagulation factors circulate as inactive enzyme precursors, which, upon activation, take part in the series of reactions that make up the coagulation cascade. The coagulation factors are generally serine proteases that culminates in cleavage of soluble fibrinogen by thrombin, Thrombin cleavage generates insoluble fibrin that forms a cross linked fibrin mesh at the site of an injury. Fibrin generation occurs simultaneously to platelet aggregation (Furie,2009). It has been traditionally classified into intrinsic and extrinsic pathways, both of which converge on factor X activation (Figure 1.4).

Table 1.1 Coagulation factors and relative substances

Number and/or name	Function
I (fibrinogen)	Forms clot (fibrin)
II (Prothrombin)	Its active form (IIa) activates I, V, VII, VIII, XI, XIII, protein C, platelets
III Tissue factor	Co-factor of VIIa (formerly known as factor III)
IV Calcium	Required for coagulation factors to bind to phospholipid (formerly known as factor IV)
V (Proaccelerin) labile factor	Co-factor of X with which it forms the prothrombinase complex
VI	Unassigned – old name of Factor Va
VII (stable factor) proconvertin	Activates IX, X
VIII (Anti hemophilic factor A)	Co-factor of IX with which it forms the tenase complex
IX (Anti hemophilic factor B or Christmas factor)	Activates X: forms tenase complex with factor VIII
X (Stuart-Prower factor)	Activates II: forms prothrombinase complex with factor V
XI (plasma thromboplastin antecedent)	Activates IX
XII (Hageman factor)	Activates factor XI, VII and prekallikrein
XIII (fibrin-stabilizing factor)	Crosslink fibrin
Von Willebrand Factor	Binds to VIII, mediates platelet adhesion
Prekallikrein (Fletcher factor)	Activates XII and Prekallikrein; cleaves HMWK
High-molecular-weight kininogen(HMWK) (Fitzgerald factor)	Supports reciprocal activation of XII, XI, and Prekallikrein

Fibronectin	Mediates cell adhesion
Anti thrombin III	Inhibits IIa, Xa, and other proteases;
Heparin cofactor II	Inhibits IIa, cofactor for heparin and dermatan sulfate ("minor anti thrombin")
Protein C	Inactivates Va and VIIIa
Protein S	Cofactor for activated protein C (APC, inactive when bound to C4b-binding protein)
Protein Z	Mediates thrombin adhesion to phospholipids and stimulates degradation of factor X by ZPI
Protein Z-related protease inhibitor (ZPI)	Degrades factors X (in presence of protein Z) and XI (independently)
Plasminogen	Converts to plasmin, lyses fibrin and other proteins
Alpha 2-antiplasmin	Inhibits plasmin
Tissue plasminogen activator (tPA)	Activates plasminogen
Urokinase	Activates plasminogen
Plasminogen activator inhibitor-1 (PAI1)	Inactivates tPA & urokinase (endothelial PAI)
Plasminogen activator inhibitor-2 (PAI2)	Inactivates tPA & urokinase (placental PAI)
Cancer procoagulant	Pathological factor X activator linked to thrombosis in cancer

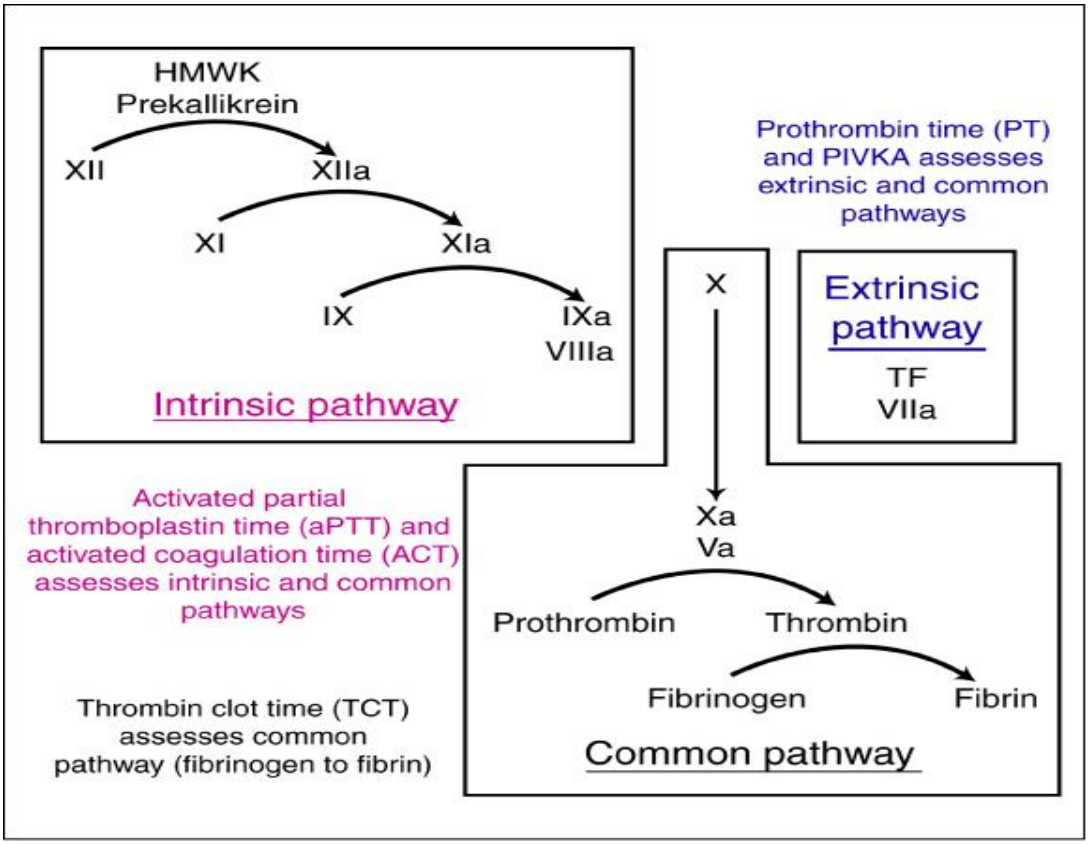


Figure 1.3 Classical coagulation cascade adopted by Mann et al., 2003.

1.3.2.1 Extrinsic Pathway

The quicker responding and more direct extrinsic pathway (also known as the tissue factor pathway) begins when damage occurs to the surrounding tissues, such as in a traumatic injury. Upon contact with blood plasma, the damaged extravascular cells, which are extrinsic to the bloodstream, release factor III (thromboplastin). Sequentially, Ca^{2+}then factor VII (proconvertin), which is activated by factor III, are added, forming an enzyme complex. This enzyme complex leads to activation of factor X (Stuart–Prower factor), which activates the common pathway. The events in the extrinsic pathway are completed in a matter of seconds (Owens and Mackman , 2010).

1.3.2.2 Intrinsic Pathway

It is a parallel pathway for thrombin activation by factor XII. It begins with factor XII, HMW kininogen, prekallekerin and factor XI (contact family) which results in activation of factor XI. Activated factor XI further activates factor IX, which then acts with its cofactor (factor VIII) to form tenase complex on a phospholipid surface to activate factor X (Hall, 2010).

1.3.2.3 Common Pathway

Activated factor X along with its cofactor (factor V), tissue phospholipids, platelet phospholipids and calcium forms the prothrombinase complex which converts prothrombin to thrombin. This thrombin further cleaves circulating fibrinogen to insoluble fibrin and activates factor XIII, which covalently cross links fibrin polymers incorporated in the platelet plug. This creates a fibrin network which stabilises the clot and forms a definitive secondary haemostatic plug (Kumar *et al*., 2010).

1.3.3 Plasma Anticoagulants

Several circulating plasma anticoagulants play a role in limiting the coagulation process to the region of injury and restoring a normal, clot-free condition of blood. For instance, a cluster of proteins collectively referred to as the protein C system inactivates clotting factors involved in the intrinsic pathway. TFPI (tissue factor pathway inhibitor) inhibits the conversion of the inactive factor VII to the active form in the extrinsic pathway. Antithrombin inactivates factor X and opposes the conversion of prothrombin (factor II) to thrombin in the common pathway. And as noted earlier, basophils release heparin, a short-acting anticoagulant that

also opposes prothrombin. Heparin is also found on the surfaces of cells lining the blood vessels. A pharmaceutical form of heparin is often administered therapeutically, for example, in surgical patients at risk for blood clots (Quinsey *et al*.,2004).

1.3.4 Fibrinolytic System

The role of the fibrinolytic system is to dissolve blood clots during the process of wound healing and to prevent blood clots in healthy blood vessels. The fibrinolytic system is composed primarily of three serine proteases that are present as zymogens (i.e., proenzymes) in the blood. Plasmin cleaves and breaks down fibrin. Plasmin is generated from the zymogen plasminogen by the proteases tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). TPA and plasminogen come together on the surface of a fibrin clot, to which they both bind. TPA then activates plasminogen, which subsequently cleaves fibrin. UPA activates plasminogen in the presence of the u-PA receptor, which is found on various cell types (Lijnen *et al*., 2000). All three of these serine proteases are down-regulated by serpins that are present in blood. Alpha-2-antiplasmin inhibits plasmin, and plasminogen activator inhibitors 1 and 2 inhibit tPA and uPA (Rau *et al*., 2007).

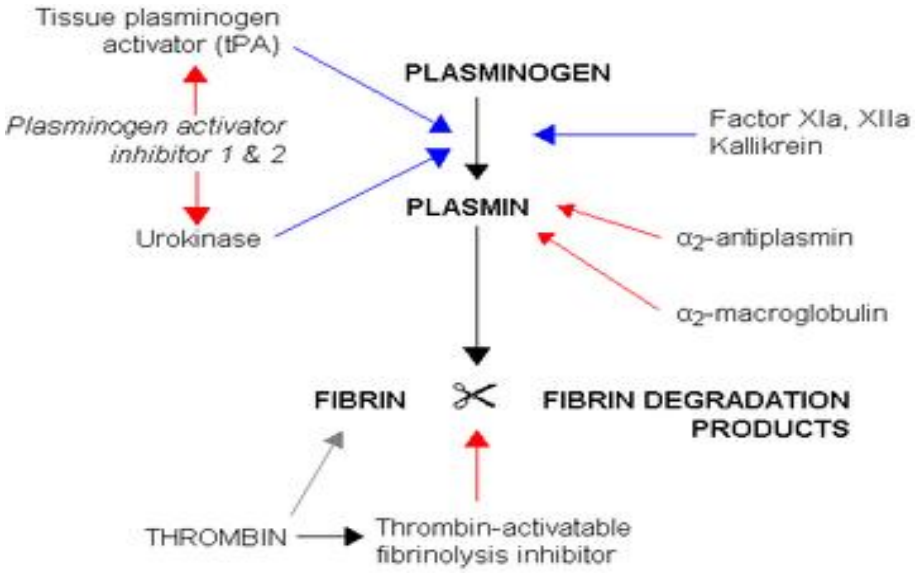


Figure 1.4 Fibrinolysis adopted by Rau et al., 2007.

1.4 Fibrinogen

Fibrinogen is a soluble plasma glycoprotein protein produced in the liver by hepatocytes. It is a large molecule(340,000 D), made up of two identical halves, each half composed of three protein chains (α alpha, β beta, and γ gamma)It is substrate for thrombin and precursor of fibrin.

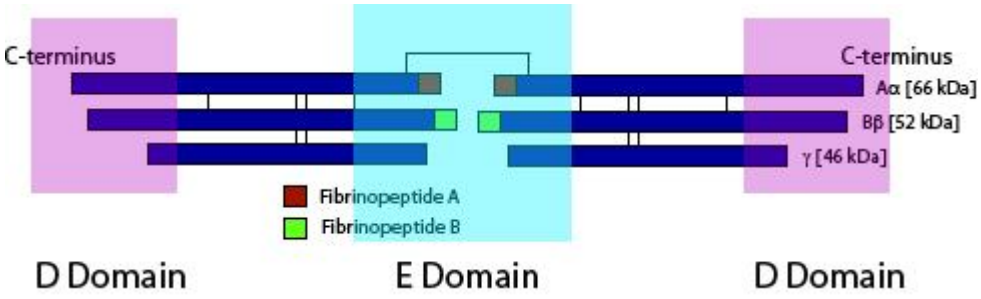


Figure1.5The structure of Fibrinogen adopted by Hanss et al. ,2013.

Thrombin cleaves fibrinogen with the release of fibrinopeptides A and B, producing fibrin monomer which then polymerizes and is stabilized by the action of factor XIII, Fibrin is then cross linked by factor XIII to form a clot. In its natural form, fibrinogen can form bridges between platelets, by binding to their GpIIb/IIIa surface membrane proteins. Thrombin is the essential enzyme product of the blood coagulation enzymatic cascade.

Thrombin is a "trypsin-like" serine protease protein that in humans is encoded by the F2 gene (Asselta *et al*.,2006).

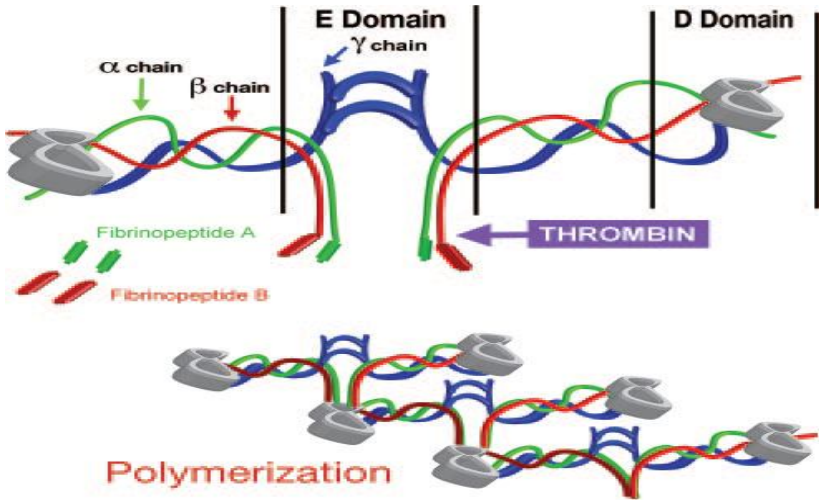


Figure1.6 The Thrombin cleavage of fibrinogen and polymerization of fibrin monomers to Fibrin, adopted by Jerrold H et al.,2012.

1.4.1 Fibrinogen Disorders

Several disorders in the quantity and/or quality of fibrinogen cause pathological bleeding, pathological blood clotting, and/or the deposition of fibrinogen in the liver, kidneys, and other tissues (Casini *et al*.,2016)**.**

1.4.1.1 Congenital Afibrinogenemia

It is a rare and generally autosomal recessive inherited disorder in which blood does not clot due to a lack of fibrinogen**.** This severe disorder is usually caused by mutations in both the maternal and paternal copies of either the FGA, FGB, or FBG gene (Casini *et al*.,2016)**.**

1.4.1.2 Congenital Hypofibrinogenemia

It is a rare inherited disorder in which blood may not clot normally due to reduced levels of fibrinogen **.**The disorder reflects a disruptive mutation in only one of the two parental FGA, FGB, or FBG genes (Casini *et al* .,2016).

1.4.1.3 Congenital Dysfibrinogenemia

It is a rare autosomal dominant inherited disorder in which plasma fibrinogen is composed of a dysfunctional fibrinogen made by a mutated FGA, FGB, or FBG gene inherited from one parent plus a normal fibrinogen made by a normal gene inherited from the other parent (Casini *et al*.,2016*)*.

1.4.1.4 Acquired Dysfibrinogenemia

Acquired dysfibrinogenemia is a rare disorder in which circulating fibrinogen is composed at least in part of a dysfunctional fibrinogen due to various acquired diseases. One of the disorder is severe liver disease including hepatoma, chronic active hepatitis, cirrhosis, and jaundice due to biliary tract obstruction. The diseased liver synthesizes a fibrinogen which has a normally functional amino acid sequence but is incorrectly glycosylated The incorrectly glycosalated fibrinogen is dysfunctional and may cause pathological episodes of bleeding and/or blood clotting. Other causes are plasma cell dyscrasias and autoimmune disorders in which a circulating abnormal immunoglobulin or other protein interferes with fibrinogen function, and rare cases of cancer and medication toxicities (Besser *et al*.,2016).

1.4.1.5 Acquired Hypofibrinogenemia

Acquired hypofibrinogenemia is a deficiency in circulating fibrinogen due to excessive consumption that may occur as a result of trauma, certain phases of disseminated intravascular coagulation, and sepsis. It may also occur as a result of hemodilution as a result of blood losses and/or transfusions with packed red blood cells or other fibrinogen-poor whole blood replacements (Fries *et al*.,2009).

1.4.1.6 Hyperfibrinogenemia

Levels of functionally normal fibrinogen increase in pregnancy. They may also increase in various forms of cancer, particularly gastric, lung, prostate, and ovarian cancers. In these cases, the hyperfibrinogenemia may contribute to the development of pathological thrombosis (Repetto *et al*.,2007).

1.4.2 Measurement of Fibrinogen

Fibrinogen levels are a useful as part of the investigation of a bleeding tendency or an unexplained prolongation of the APTT or PT. Elevated levels may correlate with increased risk of thrombosis in epidemiological studies(Mackie *et al*.,2003).Numerous methods of determining fibrinogen concentration have been devised including clotting, immunological, physical, and nephelometric techniques, and all tend to give slightly different results, presumably partly because of the heterogeneous nature of plasma fibrinogen (Carroll *et al*.,2008).

1.2.4 Cord Blood Coagulation Factors Relationship

The hemostasis of healthy newborn differs from those of normal adult but remains well balanced without bleeding or thrombosis. However, this equilibrium is unstable, and the neonate is exposed to acquired or inherited hemostasis disorders that necessitate to be early diagnosed in order to be appropriately treated. The newborn is at risk for vitamin K deficiency with bleeding due to poor transport of vitamin K across the placenta and low levels of coagulation factors (Gruel,2010).

Coagulation factors do not cross the placental barrier but are synthesized independently by the conceptus. At birth, activities of the vitamin K dependent factors II, VII, IX, and X and the concentrations of the contact factors XI and XII are reduced to about 50% of normal adult values. The levels of the factors V, VIII, XIII, and fibrinogen are similar to adult

values. Plasma concentrations of the naturally occurring anticoagulant proteins (antithrombin, protein C, and protein S) are significantly lower at birth than during the adult years. The diagnosis of some inherited coagulation deficiencies can be difficult in the newborn due to physiologically low levels of coagulation factors (Pichler *et al*,2008)**.**

However, there were studied in coagulation factors, which measure fibrinogen level in newborn cord blood between GDM and normal pregnancy. This physiological mechanism may convert into a pathologic process in a pregnancy complicated by GDM. Since the coagulation cascade and the fibrinolytic system involve various coagulation factors interacting through complex pathways, it becomes difficult to reveal and even understand the underlying mechanisms of the hemostatic changes occurring in the glucose metabolism. Considering the impact of GDM on the coagulation system, the dynamics involved at a pathophysiological level and the exact mechanism remain still unclear (Lemkes *et al*., 2010).

1.5 Previous Studies

Neary, *et al* (2015) conducted a study in Rotunda Hospital, Dublin, Ireland, One hundred and sixteen infants were recruited. Cord and peripheral blood of neonates < 30 weeks gestational age (GA) was drawn at birth, on days 1 and 3 and fortnightly until 30 weeks corrected gestational age. The following parameters were studied - Prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and coagulation factor levels. There were prolongation of PT, APTT, and in significant increase in plasma fibrinogen between the two groups was observed (*P.value* >0.05).

Christensen *et al* (2014) a study of coagulation tests at 175 preterm deliveries from the umbilical vein near the placenta. Fibrinogen, prothrombin time, activated partial thromboplastin time, D-dimer,

platelet (PLT) count, and mean PLT volume were measured. There were no abnormal coagulation value.

Datonye, *et al* (2007) at Nigerian Newborn, the study involving 60 subjects consisting of 30 newborn neonates and their respective mothers. Hematocrit, erythrocyte sedimentation rate, hemoglobin concentration, red blood cell count, white blood cell count, whole blood relative viscosity, relative plasma viscosity, fibrinogen concentration, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular hemoglobin concentration were determined. There was significantly lower value of fibrinogen found in umbilical cord blood compared to maternal blood ($P.value < 0.05$).

Lippi G, *et al* (2007) a study the postnatal development of the human coagulation system in newborn infants and to develop appropriate reference ranges for prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen. The mean Fibrinogen level was within the adult reference range in newborns at birth.

In addition, a study done by Hacer, *et al*(2000) at Çukurova University, Adana, Turkey, the recruited newborn infants with cord bleeding. There were prolongation of thrombin time, partial thromboplastin time, prothrombin time and decreased fibrinogen level. In conlusion, a report genetic disorders congenital hypofibrinogenemia and should be remembered in newborn infants with cord bleeding.

1.6 Rationale

Contact of diabetes mellitus (DM) on the coagulation system and endothelial functions is known for many years. Hemostatic factors and activities are influenced both by the hyperglycemic state in DM and hypoglycemia induced by hypoglycemic agents. There is an increased prothrombotic state due to increased activation of platelets and prothrombotic coagulation factors coupled with a decrease in fibrinolysis. Pregnancy is a hypercoaguble state in itself. However, this physiological mechanism may convert into a pathologic process in a pregnancy complicated by GDM. Thrombosis have a tendency to occur in neonatal of diabetic mothers (Edstrom and Christensen,2000). So, there is evidence that the risk of thrombosis is increased. The determination of the level of plasma fibrinogen in the umbilical cord not have any effect in newborn and no need for venipuncture in diagnosis of diseases and follow up of newborns. Because of the importance of fibrinogen in the process of clotting, and in the maintenance of the fluid state of blood in vessels. The newborns with low level of fibrinogen, platelet count and prolong in PT and APTT can occur due to development of DIC. Also, hepatic dysfunction due to several causes can lead to low level of fibrinogen. Also, in the cases of increase fibrinogen may contribute to the development of thrombosis. Studies of the effect of gestational diabetes in cord blood in Sudan are not yet conducted so this study take place as starter for coming studies to verify the relation between the gestational diabetes and the thrombosis.

1.7 Objectives

1.7.1 General objective

To estimate fibrinogen level in newborn cord blood samples among mothers with gestational diabetes mellitus.

1.7.2 Specific objectives

- To estimate fibrinogen level in newborn cord blood of mothers with gestational diabetes mellitus and newborn cord blood of healthy mothers.

- To compare the fibrinogen level in newborn cord blood of gestational diabetes mellitus mothers and newborn cord blood of healthy mothers.

- To compare the cord blood fibrinogen level according to different FBG levels among gestational diabetes mellitus mothers and their gestational weeks and weight of newborns.

Chapter Two

2. Materials and Methods

2.1 Materials

A case control study conducted at the faculty of Medical laboratory science, Sudan University of Science and Technology from February to August 2018. Sixty (60) Newborns from 30 mothers with GDM and 30 healthy mothers were enrolled in this study after ethical consent and hospital approval. Citrate cord blood samples were performed for fibrinogen estimation. The practical work and newborns selection were performed at Saad Aboulela University Hospital and, Al-Qma Specialized Hospital and, Alsaaha Specialized Hospital, Khartoum, Sudan.

2.1.1 Inclusion criteria

- Gestational diabetes mellitus pregnant women.
- Healthy pregnant women (as control).

2.1.2 Exclusion criteria

- Presence of coagulation disorders.
- Intra-uterine fetal death (IUFD).
- New born with heart disease, renal or hepatic dysfunction and inflammatory diseases.
- Premature newborn.

2.1.3 Ethical consideration

Ethical committee of research in the Faculty of Medical Laboratory Science was approved the study. The purpose and objectives of the study was explained to each one of participants, the participant has right to voluntary informed consent, has right to withdraw at any time without any deprivation, assured them that the data collected will remain

confidential and it's not allowed for any person to identify it. The questionnaire was filled in their rest time, and participant has right to benefit from the researcher knowledge and skills. Samples were coded and confidentiality of patient data was maintained throughout the study by locking hard copies and password protecting electronic files.

2.1.4 Study Variables

2.1.4.1 Dependent Variable

- Fibrinogen level.

2.1.4.2 Independent Variables

- Infants of Diabetic Mothers.
- Gestational Age.
- Age of Women.
- FBG.
- BMI.

2.2 Methods

2.2.1 Sample Collection

Volunteering pregnant women after getting an informed consent and before delivery were clinically examined by the physician whether she is gestational diabetes mellitus or normal.

Two and half ml of new born umbilical cord blood was collected in plastic container containing 3.2% ml tri sodium citrate as anticoagulant, and then the blood is centrifuged, after thoroughly mixing, for 15 minutes at 3000 rpm to obtain platelets poor plasma (PPP).

2.2.2 Fibrinogen Estimation

2.2.2.1 Principle

Thrombin converts soluble fibrinogen into insoluble fibrin, which when cross-linked becomes the fibrin clot as the last step in the coagulation cascade. Fibrinogen test is based on the clauss method of quantifying

plasma fibrinogen. The clauss method measures the rate of fibrinogen fibrin conversion in the presence of excess thrombin and has been shown to be rapid, sensitive and precise. When diluted plasma is clotted with excess thrombin, the fibrinogen level is inversely proportional to the clotting time. A calibration curve is prepared from a fibrinogen reference and plotted on log-log paper. This calibration curve is used to determine the fibrinogen concentration in the test.

2.2.2.2 Reagents

- Thrombin reagent, which is a lyophilized preparation from bovine source 50 NIH units per vial.
- 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).
- Imidazol buffer solution with stabilizer.

2.2.2.3 Procedure for Fibrinogen Calibration Curve Preparation

The citrated platelet poor plasma of control pool plasma was used and the following dilutions in buffer were made: Dilution: 1:5 1:10.

In plastic plane tube 90ul of buffer + 10ul of control plasma was added to obtain a 1:5 dilution. Then, In each of a second and a third plastic plain tubes 50 ul of buffer was added. In the second tube 50 ul from the first tube was added to 50 ul buffer to obtain a dilution of 1:10.

Alternatively, a calibration curve which was provided with each kit. The same calibration curve could be used when using the same lot of reagent and performing a daily quality control.

2.2.2.4 Test Procedure for Sample

The PPP samples were diluted 1:10 with Imidazol buffer solution. Then the diluted samples were assayed for fibrinogen assay using the automated coagulometer and the clotting time of each sample was blotted

on the log-log paper and the corresponding concentration was gotten from the curve.

Reference values : 200 - 400 mg/dl .

2.2.3 Data Analysis

The collected data proceed for analysis using SPSS version 20 computerized program by independent T test and correlation test and the data presented in form of tables and figures.

Chapter Three

3.Results

3.1 Demographic Data of GDM Women

A total of 60 Newborns from 30 mothers with GDM with gestational diabetic pregnant women were enrolled in this study; 73% of them have age less than 35 years whereas 27% of them were more than 35 Years. Also, 53% of them had history of diabetes mellitus whereas 47% were not. On other hand 27% of them had family history of DM whereas 40% they not and, 23% were having hypertension, and 10% were having both diabetes mellitus and hypertension (Table 3.1)**.**

Table(3.1) Age and Clinical Data Frequencies of GDM Women.

Variables	Frequency	Percentage (%)
Age		
<35 Years	22	73
>35 Years	8	27
History of DM		
Yes	16	53
No	14	47
Family history of DM		
No	12	40
HT	7	23
DM	8	27
HT+DM	3	10
Total	**30**	**100**

3.2 Demographic Data of Healthy Women

Thirty healthy mothers as control were enrolled in this study; 83% of them have age less than 35 years whereas 17% of them were more than 35 Years. On other hand 20% of them had family history of DM whereas

43% they not and, 20% were having hypertension, and 17% were having both diabetes mellitus and hypertension (Table 3.2)**.**

Moreover, significant increased in BMI, FBG level and newborn weight among GDM mother. While, insignificant increased in age of mothers among GDM mother and Gestational weeks among control mother.

Table(3.2) Age and Clinical Data Frequencies of Healthy Women

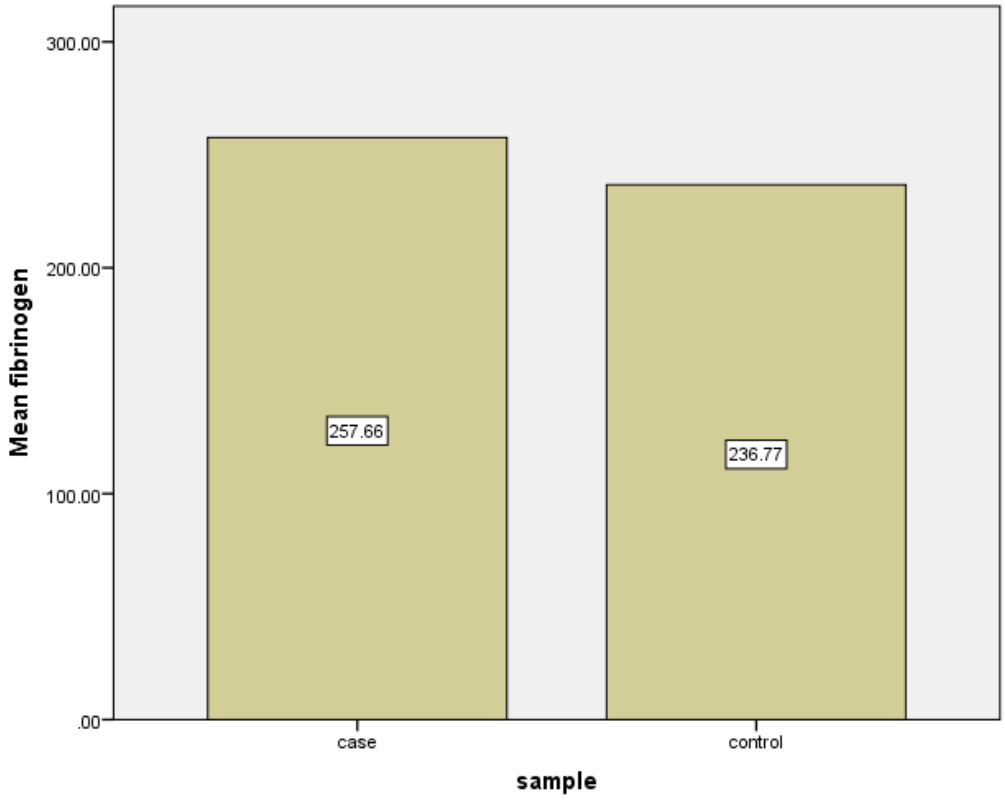
Variables	**Frequency**	**Percentage (%)**
Age		
<35 Years	25	83
>35 Years	5	17
History of DM		
Yes	0	0
No	30	100
Family history of DM		
No	13	43
HT	6	20
DM	6	20
HT+DM	5	17
Total	**30**	**100**

Table (3.3) Mean of Age, Weights, FBS,BMI and Gestational Weeks

Variables	Case (Mean±SD)	Control (Mean±SD)	*P-value*
Age of Mothers	31.03±3.55	29.10±5.66	0.112
Weight of Newborns	3.47±0.47	3.07±0.51	0.003
Gestational Weeks	37.70±0.65	37.77±0.68	0.699
BMI	28.22±3.67	24.67±4.05	0.001
FBG	110.53±19.15	85.57±8.98	0.002

3.3 Fibrinogen Level

The mean of fibrinogen level in newborn cord blood and healthy controls was significant increased in fibrinogen level among newborn from mothers with GDM compared to control group (*P. value* <0.05) (Figure3.1).



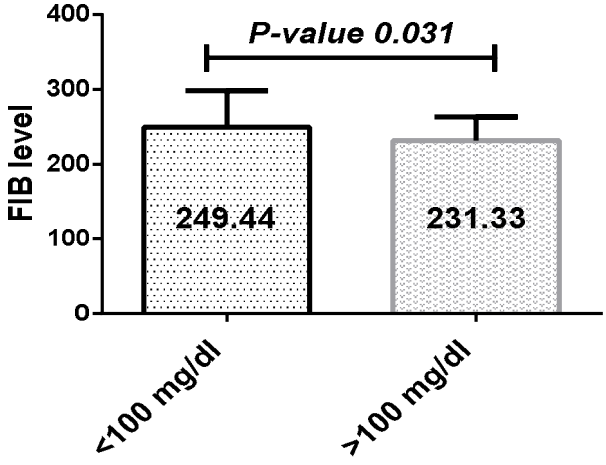
Figure(3.1) mean of fibrinogen level among cases (GDM) and controls

3.4 FBG of Gestational Diabetes Mellitus and Fibrinogen Level

The mean of fibrinogen level in newborn group FBG less than 100mg/dl was significant increased than newborn group FBG more than 100mg/dl.

Table 3.3 : Mean of Fibrinogen Level among Cases according to FBG level

Parameters	<100 mg/dl (Mean±SD)	>100 mg/dl (Mean±SD)	*P-value*
Fibrinogen level (mg/dl)	249.44±49.15	231.33±31.88	0.031



Figure(3.2) mean of fibrinogen level among GDM according to FBG level

3.4 Correlation between Gestational Weeks, Weight of Newborns and Fibrinogen Level in GMD

In newborns of mothers with GDM there were no correlation in the fibrinogen level with gestational weeks, and weight of newborns.

Table (3.4) Correlation between gestational week , weight of newborns and fibrinogen level in GMD

Variable		FIB (mg/dl)
Gestational weeks	R-value	0.155
	P-value	0.414
Weight of newborns	R-value	0.159
	P-value	0.401

Chapter Four

4.Discussion, Conclusions and Recommendations

4.1 Discussion

Gestational diabetes mellitus (GDM) is the most common medical complication of pregnancy. It is associated with maternal and neonatal adverse outcomes. GDM constitutes a greater contact on diabetes epidemic as it carries a major risk of developing T2DM to the mother and fetus later in life. In addition, Gestational diabetes mellitus (GDM) has also been linked with cardiometabolic risk factors such as lipid abnormalities, hypertensive disorders and hyperinsulinemia. These might result in later development of cardiovascular disease and metabolic syndrome(Abdel Hameed and John,2017).This study, aimed to estimate fibrinogen level in newborns cord blood of gestational diabetes mellitus mothers, the two groups were comparable as regard to fibrinogen level. In the current study, there is a significant increase in fibrinogen level in newborns cord blood of GDM mothers (257.66±32.61mg/dl) to newborns healthy mothers (236.77±37.93mg/dl) with *P.value*=0.026. This observation is disagreed Neary, *et al* 2014 who concluded that insignificant increase in fibrinogen level(*P. value*>0.05). Moreover, a study done in 2007 by Lippi G, *et al* supported this result, and concluded that coagulation system in newborn infants was within the adult reference range in newborns at birth. In addition, Christensen *et al,* 2014 and concluded there were no abnormal coagulation value.

Moreover, the present study was significance increase in fibrinogen level in newborns of mothers with GDM who has FBG <100 (249.44mg/dl) ,while fibrinogen level in newborns of mothers with GDM who has FBG >100 (231.33mg/dl) is decrease (*P. value*< 0.05). This finding supported by Neary, *et al* 2014 who concluded that

insignificant increase in fibrinogen level in newborn group FBG less than 100mg/dl.

In this study there was no correlation between newborns cord blood fibrinogen level in GDM mothers and gestational weeks, also there was no correlation between newborns cord blood fibrinogen level in GDM mothers and weight of newborns.

These findings of this study could play an important role in the health impact of newborn of diabetic mothers. Furthermore, this study could be used as a reference or a benchmark study for related studies.

4.2 Conclusions

- There was significant increase in fibrinogen level in newborns cord blood among gestational diabetes mellitus mothers compare to newborns healthy mothers.
- There was a significant reverse association between FBG level of GDM and Fibrinogen level.
- The results of this work concluded that there is insignificantly difference in newborns cord blood fibrinogen level in gestational weeks and weight of newborns.

4.3 Recommendations

- Sample size should be increase.
- Regular follow up during GDM to avoid the sudden crisis which may occur.
- The role of changes in the parameters in the hemostatic system during diabetic pregnancy and the possible clinical relevance concerning the risk for thrombosis call for further studies.
- Comparison between venous newborn and newborn cord blood.
- Cord blood is not a waste, and it has many benefits beside the clinical application.
- Follow up after management macrosomia.

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بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Medical Laboratory Science

Questionnaire

Estimation of Fibrinogen Level in Umbilical Cord Blood among Gestational Diabetes Mothers

Name: …… ID code……

Age:……

BMI…….....

Gestational Diabetes onset?.....

Weeks of gestation?.....

History of DM?.....

Family history of DM?.....

Weeks of gestation on delivery time? .....

Weight of newborn? .....

Maternal Glucose Level: …….....

Health problem of newborn?.....

Apgar score: …….

Newborn Investigation:

Fibrinogen level.....mg/dl

Informed Consent:

انا المذكورة اعلاه اوافق على المشاركة في البحث الذكور اعلاه لغرض البحث العلمي فقط بمنح عينة دم ولي كل الحق بالانسحاب من الدراسة متى ما شئت ولي الحق ايضا في الحصول عى النتائج الخاصة بي و أن لا يتم استخدام هذه العينات لأغراض التجارب الانسانية أو الحيوانية.

الامضاء.....

FIBRONOGEN

PRODUCT CODE: COAG116A/COAG116B

COAG116A	COAG116B
1x10ml/1x2ml/1x1ml	1x20ml/2x2ml/1x1ml
STORE AT 2-8°C	
INSTRUCTIONS FOR USE	
FOR IN-VITRO DIAGNOSTIC USE ONLY	

IVD For In Vitro Diagnostics Use Only
LOT Lot Number
REF Catalogue Number
Storage Temperature
Expiry Date (Year / Month)
Warning, Read Enclosed Documents
Instructions For Use
Manufactured By

FIBRINOGEN Liquid stable

HAEMOSTASIS

Intended Use:
Fortress Diagnostics Fibrinogen reagent is an *in vitro* diagnostic assay intended for quantitative determination of fibrinogen in plasma.

Summary:
Thrombin converts soluble fibrinogen into insoluble fibrin, which when cross-linked becomes the fibrin clot as the last step in the coagulation cascade. Fibrinogen is an acute-phase reactant protein in that the concentration rises sharply in response to many different physiological stimuli such as tissue inflammation or injury. High fibrinogen levels are associated with atherosclerotic cardiovascular disease and with the occurrence of myocardial infarction and stroke. Other conditions in which fibrinogen is elevated are cancers of the stomach, breast, or kidney, and inflammatory disorders like rheumatoid arthritis. Reduced fibrinogen levels are prevalent in liver disease, prostate cancer, lung disease, bone marrow lesions, malnourishment, and disseminated intravascular coagulation. Other conditions of deficient fibrinogen are congenital afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemia.

Principle:
Fortress Fibrinogen test kit is based on the Clauss method of quantifying plasma fibrinogen. The Clauss method measures the rate of fibrinogen fibrin conversion in the presence of excess thrombin and has been shown to be rapid, sensitive and precise. When diluted plasma is clotted with excess thrombin, the fibrinogen level is inversely proportional to the clotting time. A calibration curve is prepared from a fibrinogen reference and plotted on log-log paper. This calibration curve is used to determine the fibrinogen concentration in the test sample.

Reagents:
A) 1x10ml OR 1x20ml of Imidazole buffer – Contents are ready to use and stable upto expiry when stored at 2-8°C.

B) 1x2ml OR 2x2ml of Thrombin Reagent – The Thrombin reagent is liquid stable and has to be stored at 2-8°C. Once opened the reagent is stable for a period of 1 month when stored tightly capped at 2-8°C. DO NOT FREEZE. Mix the reagent before use.

C) 1x1ml of Human reference Plasma – Reconstitute using 1ml of distilled water. Stable for 8 hours when stored at 2-8°C.

Precautions:
Do not ingest. Avoid contact with skin, eyes or clothing. Fibrinogen Normal control is a potentially biohazardous material. Source materials from which this product was manufactured were found negative for HBsAg and for antibodies against HCV, HIV-1 and HIV-2 using approved methods; however, no test method can offer complete assurance that infectious agents are absent. As with all materials of human origin, this product should be handled as a potentially infectious material.

Specimen Collection and Preparation:

Mix 9 parts of freshly collected whole blood with 1 part of 3.8% sodium citrate. Centrifuge for 10 minutes at 1000 g.

Samples thus collected have to be used within 2 hours of the collection period.

Test plasma should be prepared from citrated whole blood without heparin, EDTA or oxalate.

For description of collection of blood using vacuum tubes, refer manuals of the vacuum tube manufacturers.

Procedure:
Material Provided
COAG 116A
1. Fortress Diagnostics THROMBIN reagent, 1 x 2 mL
2. Fortress Diagnostics Human reference plasma 1 x 1 mL
3. Fortress Diagnostics Imidazole Buffer, 1 x 10 mL

COAG 116B
1. Fortress Diagnostics THROMBIN reagent, 2 x 2 mL
2. Fortress Diagnostics Human reference plasma 1 x 1 mL
3. Fortress Diagnostics Imidazole Buffer, 1 x 20 mL

This procedure pertains to manual or semi-automated coagulation systems. Refer to your instrument manual for more detailed instrument specific instructions.

1. Prepare 1/3.5,1/5, 1/10, 1/15, 1/35 dilutions of Fortress Human reference plasma with Fortress Imidazole buffer using the following table:

	Normal Plasma	Imidazole Buffer	Total Volume
1/3.5	100 µL	250 µL	350 µL
1/5	50 µL	200 µL	250 µL
1/10	50 µL	450 µL	500 µL
1/15	50 µL	700 µL	750 µL
1/35	50 µL	1700 µL	1750 µL

2. Dilute Quality controls and Patient plasmas 1:10 using the Fortress Imidazole Buffer.
3. All the above dilutions have to be pre-warmed at 37°Cfor 3 minutes. Use 0.2 ml of each dilution for testing purposes.
4. Add 0.1ml of the Fortress Thrombin reagent to the pre-warmed dilutions and record the clotting time in seconds. (DO NOT PREWARM THE FORTRESS THROMBIN REAGENT AFTER ENSURING THAT THE REAGENT IS AT ROOM TEMPERATURE).
5. Create a standard curve, plotting the average clotting time against fibrinogen concentration on a log-log graph. Use all the Calibrator values for plotting the curve. Draw a best fit straight line and if necessary omit the non linear points. The final curve to be reliable has to have at least 3 consecutive points. Plot the concentration (mg/dl) on x-axis and clotting time (sec) on the y-axis. Use the assigned fibrinogen value on the Human reference Plasma to determine fibrinogen values for the dilutions. The following table has an example where in the Human reference plasma has a value of 230 mg/dl.

Dilution Factor	Factor	Conc. mg/dl
1/3.5	2.9	667
1/5	2	460
1/10	1	**230**
1/15	0.67	154.1
1/35	0.29	66.7

Quality Control:
Use Fortress Plasma Controls L1 and L2 to determine the reliability of the standard curve.

Limitations:
If the clotting time of the test plasmas fall outside the limits of the graph drawn, prepare a dilution of 1:5 or 1:20 dilutions as needed. If the sample is diluted 1:5, divide the result by 2 and if the dilution is 1:20 multiply the result by 2 to get the final result.

Expected Values:
The normal range for fibrinogen levels in human plasma is considered to be 200-400 mg/dl. Each laboratory should establish its own mean normal and normal range because of variances among different laboratories.

Performance Characteristics:
Precision: Within-run precision was assessed using **Fortress Diagnostics** Fibrinogen Normal and Low Control plasmas on both optical and a mechanical instruments. The results are shown in the following table:

Warranty:
This product is warranted to perform in accordance with its labeling and literature. **Fortress Diagnostics Ltd.** disclaims any implied warranty of merchantability or fitness for any other purpose. Purchaser must calibrate and determine the suitability of **Fortress Diagnostics** products for their specific applications. In no event will **Fortress Diagnostics Ltd.** be liable for any consequential damages arising out of aforesaid express warranty.

Reference:

Kessler C. Hemorrhagic disorders: Coagulation factor deficiencies. In: Goldman L, Ausiello D, eds. *Cecil Medicine*. 23rd ed. Philadelphia, Pa: Saunders Elsevier; 2007:chap 180.