Sudan University of Science & Technology

College of Graduate Studies

Estimation of Antithrombin III Level in Cord Blood of New Born from Gestational Diabetic Sudanese Women

قياس مستوى مضاد الثرومبين٣ في دم الحبل السري لدى الاطفال حديثي الولاده من نساء سودانيات مصابات بسكرى الحمل

A dissertation submitted in partial fulfillment of the requirements for M.Sc. degree in Medical Laboratory Science (Hematology and Immunohematology)

Submitted by

Sara Elkhatim Mohamed Usif
(BSc. MLs, SUST, 2016)

Supervisor

Dr. Hiba BadrEldin Khalil, Ph.D

Associate Professor of Haematology and Stem Cell Technology

Alneelain University

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بسم الله الرحمن الرحيم

قال تعالى: {أَلَمْ تَرَ أَنَّ اللَّهَ أَنْزَلَ مِنَ السَّماءِ ماءً فَأَخْرَجْنا بِهِ ثَمَراتٍ مُخْتَلِفًا أَلْوانُها وَمِنَ الْجِبالِ جُدَدٌ بِيضٌ وَحُمْرٌ مُخْتَلِفٌ أَلْوانُها وَغَرابِيبُ سُودٌ (٢٧) وَمِنَ النَّاسِ وَالدَّوَابِّ وَالْأَنْعامِ مُخْتَلِفٌ أَلْوانُهُ كَذلِكَ إِنَّما يَخْشَى اللَّهَ مِنْ عِبادِهِ الْعُلَماءُ إِنَّ اللَّهَ عَزِيزٌ غَفُورٌ (٢٨)}

صدق الله العظيم

سورة فاطر الآيات ٢٧-٢٨

Dedication

Every challenging work needs self-efforts as well as guidance of elders especially those who were very close to our hearts…

My humble effort I dedicate to my sweet and loving father and mother, whose affection, love, encouragement and prays of day and night make me able to get such success and honor…

And I dedicate my research to my lovely friends for supporting me…

Acknowledgement

First of all, I’m grateful to the Almighty ALLAH for giving me strength and ability to complete this research.

I would like to express my sincere gratitude to my supervisor Dr. Hiba BadrEldin Khalil For her support and guidance...

I would like to thank my friends and colleagues for their help…

My thanks also extended to all people who help me in the sample collection, estimation and analysis...

Abstract

Introduction; Hyperglycemic state in women affected by gestational diabetes mellitus leads to change in structure and functions in endothelial cells which promote the hypercoagulable status and increase the consumption of antithrombin III in the cord blood of the newborn. **Materials and Methods;** An analytical cross sectional study conducted at Sudan University of Science and Technology in Khartoum from March 2018 to January 2019; 30 newborn's cord blood samples collected after delivery from women clinically diagnosed by gestational diabetes mellitus and 30 newborn's cord blood samples collected after delivery from women with normal pregnancy as control group recruited in this study. Five ml Citrated Cord blood samples (3.8 % Tri-sodium Citrate) were collected from Saad-Aboulela University Hospital, Alsaaha Specialized Hospital and Al-Qma Specialized Hospital. Antithrombin III level was measured by using automated Mindray, and then Data analyzed by SPSS version 20, and expressed as means ± SD, tables and figures **Results;** The mean and standard deviation of antithrombin III level in newborn's cord blood from women with gestational diabetes was 19.43 ± 6.62 mg / dl , while the mean ± standard deviation of antithrombin III level in newborn's cord blood from women with normal pregnancy was 24.43 ± 6.28 mg / dl. A significant decreased of antithrombin III level in cord blood from women with gestational diabetes compared with antithrombin III level in cord blood from women with normal pregnancy (P. value = 0.005). In addition, there was a significant decreased of antithrombin III level in cord blood from pregnant women with gestational diabetic and fasting blood glucose level > 100 mg/dl in compare with antithrombin III level in cord blood from pregnant women with gestational diabetic and fasting blood glucose < 100 mg/dl. In contrast, there was no correlation between antithrombin III level and both gestational weeks and weight of newborn. **Conclusions**; There was significant decreased in level of antithrombin III in newborn's cord blood from women with gestational diabetes

more than level of antithrombin III in newborn’s cord blood from women with normal pregnancy. Additionally, antithrombin III level in newborn’s cord blood of pregnant women gestational diabetes affected by blood glucose level but, it did not affect by both gestational weeks and weight of baby.

مستخلص البحث

مقدمه: حاله زياده السكر في الدم عند النساء المصابات بسكر الحمل تؤدي الى تغيير في شكل ووظائف الخلايا البطانيه والتي تحفز حالة زياده التجلط وزياده استهلاك معدل مضاد الثرومبين ٣ في دم الحبل السري لدى الاطفال حديثي الولاده. **طرق البحث:** هذه دراسة تحليليه مقطعيه أجريت في جامعه السودان للعلوم والتكنولوجيا في منطقه الخرطوم, في الفتره من شهر مارس ٢٠١٨ الى يناير للعام ٢٠١٩, ثلاثون عينه دم من الحبل السري لاطفال حديثي الولاده جمعت من نساء تم تشخيصهم اكلينيكيا بسكر الحمل باستخدام قياس سكر الدم عند الصيام كحاله دراسه و ثلاثين عينه دم من الحبل السري لاطفال حديثي الولاده جمعت من نساء ذوات حمل طبيعي كحاله قياس تطوعن لهذه الدراسه , جمعت عينات الدم من الحبل السري للاطفال حديثي الولاده من مستشفى سعد ابو العلا الجامعي و مستشفى الساحه التخصصي ومستشفى القمه التخصصي بكمية 5 ماي ليتر في (TSC) المضاده للتخثر وذلك لقياس معدل مضاد الثرومبين باستخدام جهاز (Mindary), وتم تحليل النتائج بواسطة برنامج الحزم الاحصائية للعلوم الاجتماعية اصداره٢٠ وتم توضيحها في شكل متوسطات و جداول و رسومات توضيحه. **النتائج:** متوسط النتائج ± الانحراف المعياري لمعدل مضاد الثرومبين٣ للنساء المصابات بسكر الحمل ١٩.٤٣ ±٦٢,٦ ملجرام / ديسيليتر, ومتوسط النتائج ± الانحراف المعياري لمعدل مضاد الثرومبين٣ للنساء ذوات الحمل طبيعي ٢٤.٤٣ ±٢٨,٦ ملجرام / ديسيليتر, وأيضا وجد نقصان واضح لمعدل مضاد الثرومبين٣ في دم الحبل السري لحديثي الولاده عند النساء المصابات بسكر الحمل عند مقارنته بمعدل مضاد الثرومبين٣ في دم الحبل السري لحديثي الولاده عند النساء ذوات الحمل الطبيعي (القيمة المعنوية اصغر من٠,٠٥). بالاضافه الى انه وجد انخفاض ملحوظ في معدل مضاد الثرومبين٣ في دم الحبل السري عند النساء المصابات بسكر الحمل وكان معدل سكر الدم عند الصيام اعلى من 100 مليغرام / ديسيلتر مقارنته بمعدل مضاد الثرومبين٣ في دم الحبل السري عند النساء المصابات بسكر الحمل وكان معدل سكر الدم عند الصيام اقل من 100 مليغرام / ديسيلتر, في المقابل لايوجد علاقه بين معدل مضاد الثرومبين ٣ وبين الاسبوع الذي تم فيه الانجاب ووزن الطفل. **الخلاصه:** في كل من مجموعتي عينات دم الحبل السري للاطفال حديثي الولاده, وجد انخفاض في

معدل مضاد الثرومبين٣, ولكن يوجد انخفاض ملحوظ في معدل مضاد الثرومبين ٣ في دم الحبل السري عند مجموعه النساء المصابات بسكر الحمل اكثر من معدل مضاد الثرومبين ٣ في دم الحبل السري عند مجموعه النساء ذوات الحمل الطبيعي, بالاضافه الى ان معدل مضاد الثرومبين ٣ في دم الحبل السري عند مجموعه النساء المصابات بسكر الحمل يتاثر بمعدل سكر الدم ولكنه لا يتاثر بالاسبوع الذي تم فيه الانجاب ووزن الطفل حديث الولاده.

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Abbreviations

Term	Abbreviations
ADP	Adenosine Diphosphate
AT III	Antithrombin III
BMI	Body Matrix Index
DM	Diabetes Mellitus
EPO	Erythropoietin
FBG	Fasting Blood Glucose
GDM	Gestational Diabetes Mellitus
GP	Glycoprotein
Hb	Hemoglobin
HMWK	High Molecular Weight Kininogen
NRBC	Nucleated Red Blood Cell
P. value	Probability value
Plt	Platelet
PPP	Platelet Poor Plasma
SD	Mean ± Standard deviation
SOP	Standard Operational Procedure
TAT	Thrombin Anti-Thrombin complex
TFPI	Tissue Factor Pathway Inhibitor
Tpa	Tissue Plasminogen Activator
TPO	Thrombopoeitin
TSC	Tri Sodium Citrate
TXA2	Thromboxan A2
VTE	Venous Thromboemoblism
vWF	Von Willebrand Factor

Chapter One

1. Introduction

1.1. Gestational Diabetes Mellitus (GDM)

Gestational diabetes mellitus is the most common metabolic condition during pregnancy which defined as glucose intolerance first diagnosed during pregnancy (Ryckman *et al*, 2015). Up to 22% of all pregnancies affected by GDM, and this prevalence may be higher under new diagnostic criteria, this is a growing concern as women with GDM are at increased risk of developing diabetes post-pregnancy, in addition to hypertension, hyperlipidemia and coronary heart disease, the offspring of women with GDM are also more likely to develop diabetes and metabolic syndrome in childhood or adulthood, more immediate consequences of GDM include fetal macrosomia, preeclampsia and cesarean delivery, although older maternal age and ethnicity are risk factors for GDM, maternal obesity is the strongest known risk factor (Ryckman *et al*, 2015).

1.1.1. Pathophysiology

During pregnancy, the maternal tissues become insensitive to insulin, it occurs due to the placental lactogen hormone and other hormones, such as progesterone, cortisol and growth hormone, when the pancreas is unable to offer an appropriate response of insulin to compensate normal insulin resistance, which leads to maternal hyperglycemia, and this stimulates the fetal hyperinsulinemia, insulin secretion increases at the beginning of pregnancy. Whereas, the sensitivity to insulin remains unchanged, at around 20 weeks of pregnancy, insulin sensitivity reduces progressively and it is even lower in the third trimester. However, after birth, the GDM disappears almost immediately and the earlier pregnant women treated in order to implement diagnosis and thus, minimize complications caused by the disease. The risk factors are: obesity, increased maternal age, previous occurrence of GDM, family history of diabetes, polycystic ovary syndrome,

persistent glycosuria, pregnancy-induced hypertension, history of recurrent miscarriage, unexplained fetal death history, macrosomia (Bortolon *et al*, 2016).

1.1.2. 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, and shoulder dystocia (Reece, 2010).

Macrosomia is the most common fetal complication, with a reported incidence of 15 % – 45 %, followed by hyperbilirubinemia in 10 % – 13 % of neonates. Hypoglycemia can occur in 3 % – 5 % of infants because 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 *et al*, 2014).

Maternal diabetes mellitus increases the risk of fetal morbidity and mortality and exposes the neonate to metabolic abnormalities and to the state of relative fetal hypoxia, the most important cytokine regulator of mammalian erythropoiesis is erythropoietin (EPO), unlike hormones, which regulated by ambient levels of target products; EPO is regulated by tissue hypoxia and vasoconstriction. Fetal hypoxia induces EPO secretion and increased erythropoiesis causes elevated nucleated red blood cells (NRBC) and many studies have documented an association between fetal hypoxia and elevated umbilical cord plasma EPO levels and NRBC counts (Madazli *et al*, 2008).

1.1.3. Effect of GDM on Hemostasis

Impact of diabetes mellitus on the coagulation system and endothelial functions has known for many years, hemostatic factors and activities 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 (Lemkes, *et al*, 2010). Pregnancy creates a hypercoagulable state as a physiological and adaptive mechanism to ensure the hemostatic balance by preventing excessive maternal blood loss at delivery. However, this physiological mechanism may convert into a pathologic process in a pregnancy complicated by GDM and / or eclampsia, preeclampsia, 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 (Gorar *et al*, 2016).

1.1.4. Risk Factors

Screening of GDM is important because it aims to identify women who are at high risks to develop the disease, in order to reduce or avoid risks to maternal and fetal health; it carried out by identifying the risk factors. The parameters that determine the greatest risk to disease are : previous GDM, family history of diabetes mellitus, overweight or obesity, increased maternal age. Given these factors, the woman is required to fasting blood glucose, which should held in early pregnancy (Bortolon *et al*, 2016).

1.1.5. Management of GDM

Women with gestational diabetes can be divided into two functional classes using fasting glucose levels. Pharmacological methods are usually recommended if diet

modification does not consistently maintain the fasting plasma glucose levels < 95 mg / dl or the 2-hour postprandial plasma glucose < 120 mg/dl, whether pharmacological treatment should be used in women with lesser degrees of fasting hyperglycemia 105 mg / dl or less before dietary intervention unclear. There have been no controlled trials to identify ideal glucose targets for fetal risk prevention, the Fifth International Workshop Conference recommended that fasting capillary glucose levels must be kept ≤ 95 mg / dl (Boyd, 2010).

1.2. Cord Blood

The umbilical cord is the linkage between the fetus and mother, it initially presents as connecting stalk at the caudal end of embryo before folding, later shifted to ventral surface of embryo to umbilical ring after folding of the embryo, it is lined by fetal membranes and the main constituents are extra embryonic mesoderm containing the extra embryonic coelem, the length of the fully developed umbilical cord is 50 cm but the length varies from 20 - 120 cm and the diameter is 1 - 2 cm (Rohinidevi *et al*, 2016).

The rudimentary umbilical cord is formed during the 4^{th} to 8^{th} weeks of gestation (calculated from the first day of the last menstrual cycle) by the expanding amnion enveloping tissue from the body stalk, the omphalomesenteric duct and the umbilical coelom, blood flow is established within the umbilical cord by the end of the 5^{th} week of gestation (Spurway *et al*, 2012).

It inserts into the chorionic plate of the placenta and composed of two arteries and a single umbilical vein, these vessels are developmentally derived from the allantoic vessels, at the junction of umbilical cord and placenta, the umbilical arteries branch radially to form chorionic arteries. The chorionic arteries further branch before they enter into the villi. In the villi, they form an extensive arteriocapillary venous system, bringing the fetal blood extremely close to the maternal blood; but no intermingling of fetal and maternal blood occurs.

This is defined as the placental barrier and allows selective movement of endogenous and exogenous compounds across the placenta. This protects the developing foetus from the harmful effects of toxins, chemicals, cytokines, and microorganisms. (Habibollah, 2010).

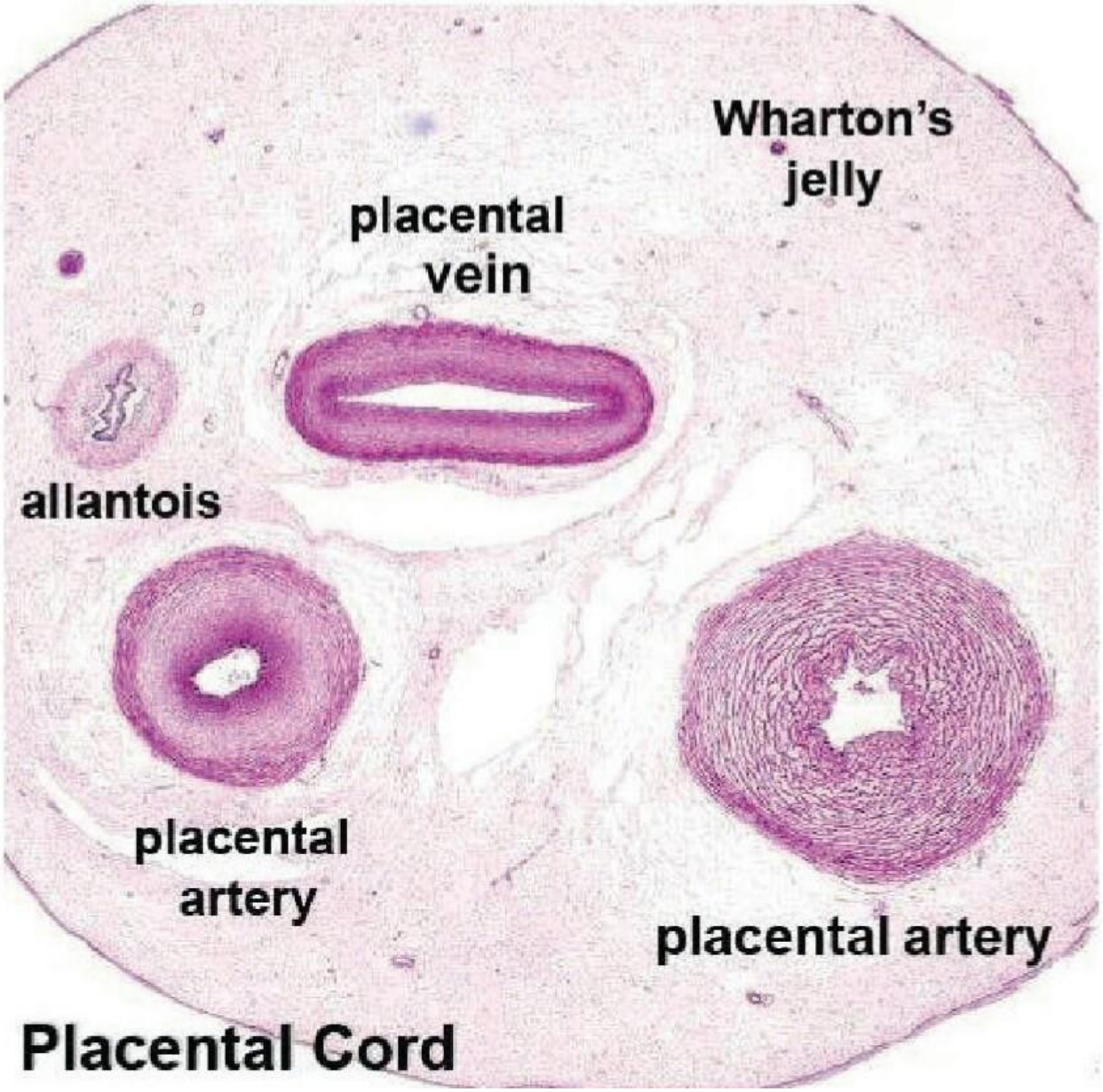

Figure A: A cross-sectional image of a postpartum umbilical cord showing the major structures (Spurway *et al*, 2012).

1.3. Impaired Placental Development in GDM

The placenta acts as a natural selective barrier between maternal and fetal blood circulations and is capable of controlling nutrient and gas exchange. Moreover, human placenta is responsible for important endocrine function and local maternal immune tolerance and due to its location, this organ may be exposed to adverse intrauterine conditions and act as a target for maternal and/or fetal metabolic alterations associated with pregnancy pathologies and according to the current diagnostic standards, GDM may be diagnosed at any time in pregnancy, if diabetes develops early in pregnancy it affects mainly the structure of the placenta. Whereas, later disturbances in glucose metabolism are more likely to affect its function, in the second half of pregnancy, placental villi undergo extensive angiogenesis and vascularization. In hyperglycemic environment, both of them may remain uncompleted. Placental development disorders such as villous immaturity and alteration in villous branching are suggested to be an adaptation to particular intrauterine conditions, mainly related to early onset of diabetes (Jarmuzek *et al*, 2015).

1.4. Hemostasis

The maintenance of circulatory hemostasis is achieved through the process of balancing between bleeding (hemorrhage) and clotting (thrombosis). Hemostasis, the arresting of bleeding, depends on several components. The four major components are the vascular system, platelets (thrombocytes), blood coagulation factors, and fibrinolysis and ultimate tissue repair. Three other, less important, components are the complement and kinin systems as well as serine protease inhibitors (Turgeon, 2012).

1.4.1. 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 and many structures (Palta *et al*, 2014).

1.4.1.1 The Vessels Wall

Endothelial cells line the surface of the entire circulatory tree, totaling (1 - 6) $\times 10^{13}$ cells. The endothelium normally presents an antithrombotic surface but rapidly becomes prothrombotic when stimulated, which promotes coagulation, inhibits fibrinolysis, and activates platelets. In many cases, Endothelium-derived vasodilators are also platelet inhibitors (e.g., nitric oxide) and, conversely, endothelium-derived vasoconstrictors (e.g., endothelin) can be platelet activators, which are promoting blood fluidity and hemostasis (Fauce *et al*, 2010).

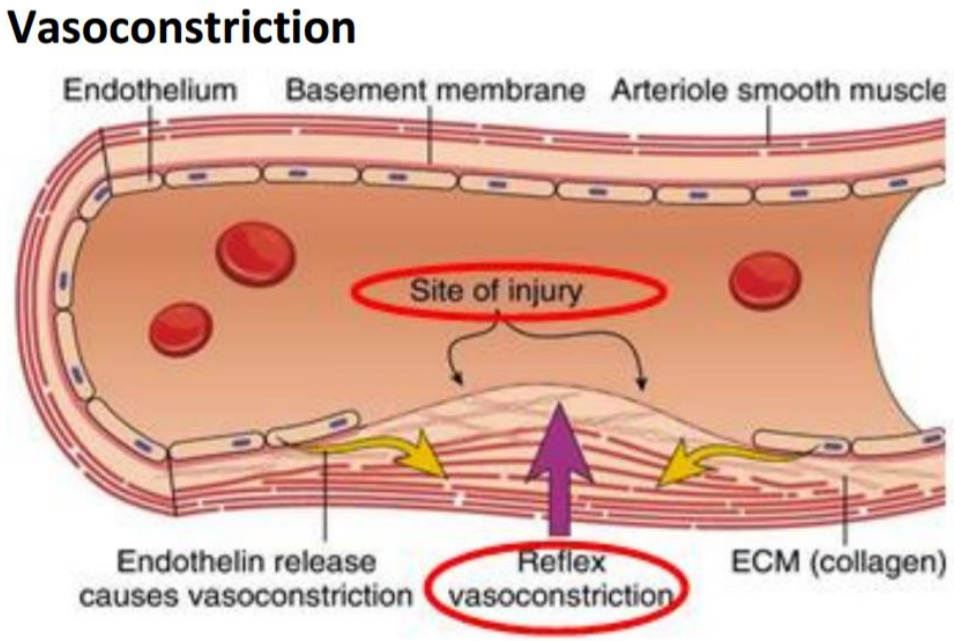

Figure B: Vasoconstriction phase, Primary hemostasis is characterized by vasoconstriction, which is the initial phase for stopping the blood flow (Kumar *et al*, 2009).

1.4.1.2. Platelets

Are small anuclear cell fragments that bud off from megakaryocytes, specialized large polyploid blood cells that originate in the bone marrow, platelets are present at 150 to 400 million per milliliter of blood and circulate for about ten days. In a healthy blood vessel, and under normal blood flow, platelets do not adhere to surfaces or aggregate with each other (Andrew, 2011).

Thrombopoietin (TPO) is the major regulator of platelet production and is constitutively produced by the liver and kidneys. This increases the number and rate of maturation of megakaryocytes via c-MPL receptor. The main function of platelets is the formation of mechanical plugs during the normal hemostatic response to vascular injury via many process, platelet adhesion, aggregation and activation (Hoffbrand and Moss, 2016).

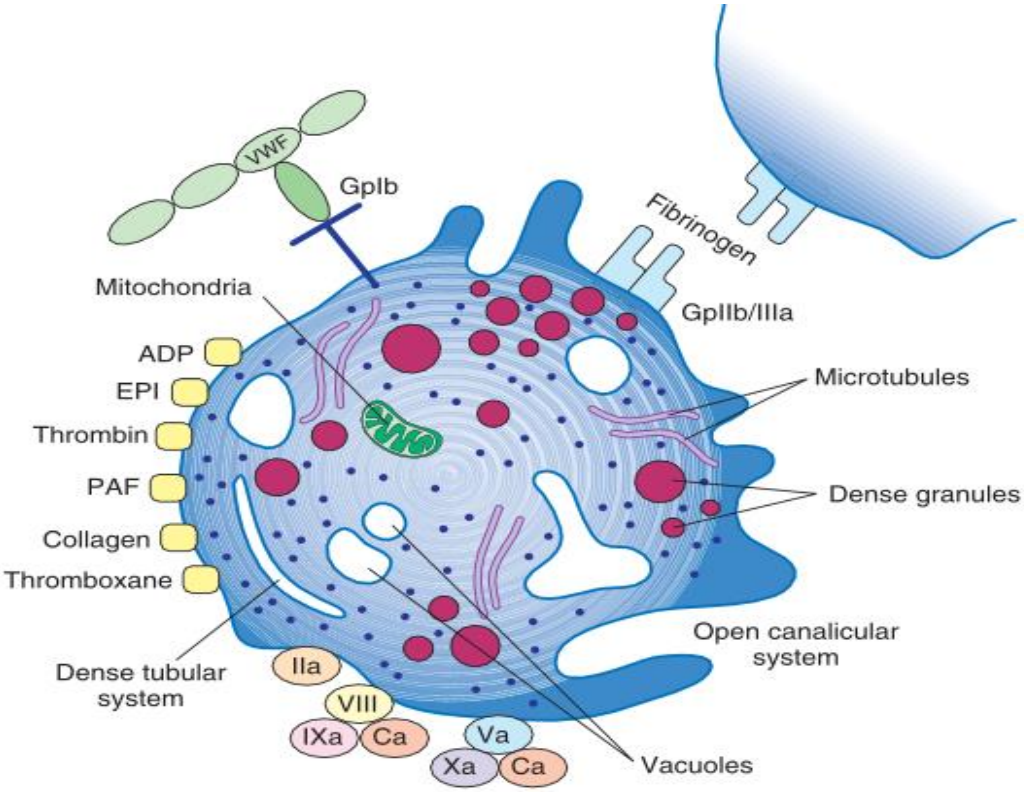

Figure C: Schematic diagram of platelet morphology (Ciesla, 2007).

1.4.1.2.1. Platelet Adhesion

Mechanism generally supported by the particular interactions between the membrane receptors and absorbed plasma proteins. The platelet membrane receptors are enriched with glycoprotein (GP) receptors embedded in the phospholipid bilayer, including tyrosine kinase receptors, integrin, leucine rich receptors; GP coupled transmembrane receptors, selectin and immunoglobulin domain receptors. These are the important proteins involved to facilitate hemostatic function by mediating the interactions within cell-platelet and platelet substratesand the initial event that occurs upon hemostasis is the rolling and adherence of the platelets to the exposed subendothelium. The adhesion mediated by von Willebrand Factor (vWF) that binds to GPIb-IX in the platelet membrane, vWF is a blood GP that serves as an adhesive protein, which could bind to other proteins, especially factor VIII at the wound sites (Rumbaut, and Thiagarajan, 2010).

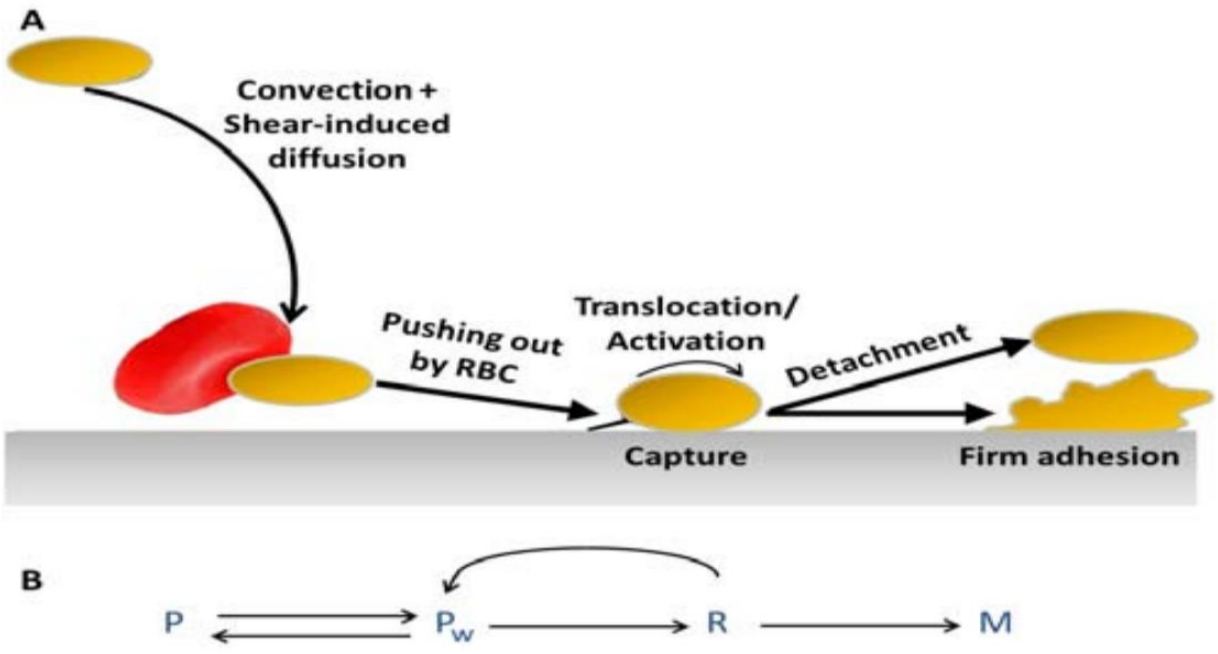

Figure D: platelet adhesion stages (Tafti, 2017).

1.4.1.2.2. Platelet Activation

Platelet cells can be activated upon biomaterial surface stimulation. Adhered platelets undergo degranulation and release cytoplasmic granules that contain serotonin, platelet activating factors and adenosine diphosphate (ADP) which is an important physiological agonist stored in the dense bodies of platelets that play an essential function in normal hemostasis and thrombosis (Gupta, 2014). Platelets are activated to change shapes into a pseudopodal form upon the adhesion to the injured area, which will activate the collagen receptors on their surface membrane, named GPIIbIIIa, to undergo release reactions. The GPIIbIIIa complex, organized through calcium-dependent association of GPIIb and GPIIIa that is a necessary step in platelet aggregation and endothelial adherence, at the same time, platelets tend to synthesize and discharge thromboxane A2 (TXA2), aiding in vasoconstriction and platelet aggregation. In addition, GPIIbIIIa integrin and P-selectin move from the α-granule membrane to the platelet membrane to support platelet aggregation (Gupta, 2014).

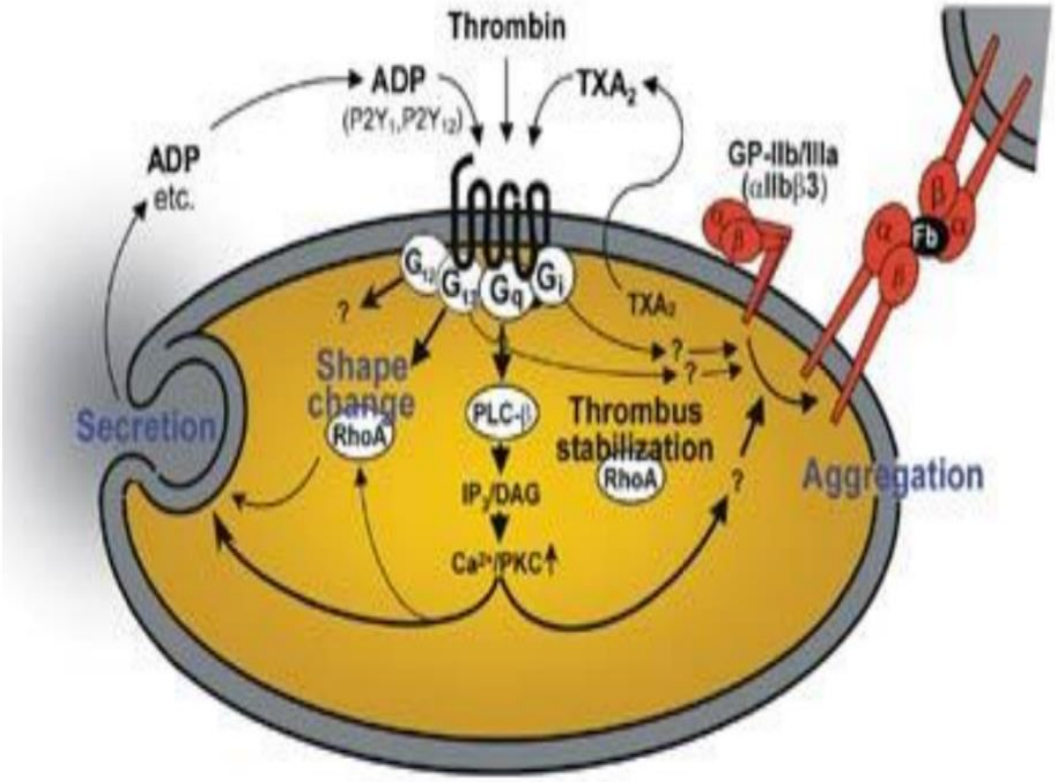

Figure E: Platelet activation process. The schematic diagram portrays the internal organelles with prominent crucial storage contents (Moers *et al*, 2004).

1.4.1.2.3. Platelet Aggregation

Begins once platelets become activated, triggering the GpIIbIIIa receptor (50 - 100 / platelets), which attach to vWF or Fibrin. Each activated platelet extends pseudopods, clumping and becoming aggregated, these activations are further heightened by the generation of thrombin via the hemostasis mechanism, and the aggregation promotes a primary platelet plug, ADP receptor interconnects with a family of ADP receptors (P2Y1 and P2Y12), P2Y1 receptors assist in stimulating the initial platelet shape changes and platelet aggregation, at the same time, P2Y12 is an important mediator for blood clotting. It increases significantly, responding to ADP to complete the aggregation process. Eventually, the formed platelet plug ought to be stabilized by the formation of fibrin (Kumar *et al*, 2009).

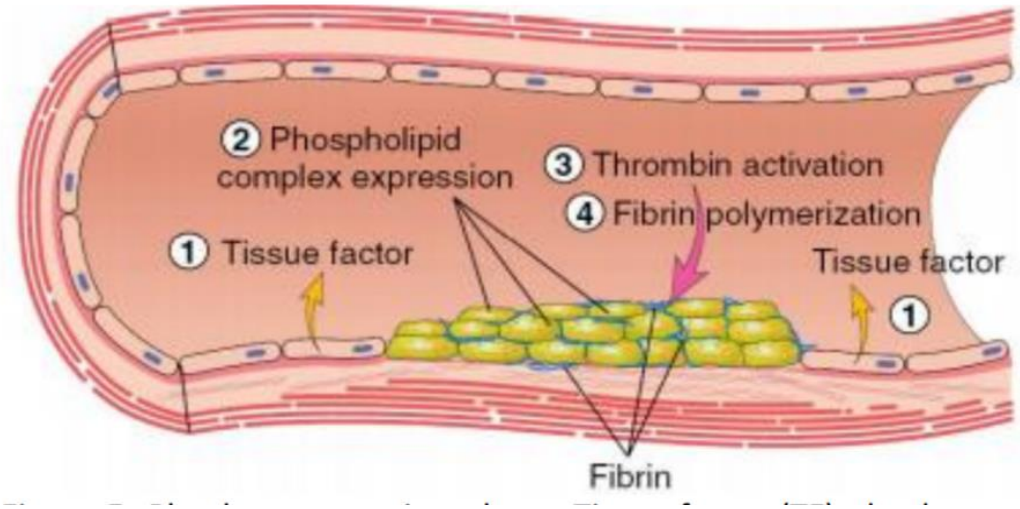

Figure F: Platelet aggregation phase (Kumar *et al*, 2009).

1.4.2. Secondary Hemostasis

It consists of the cascade of coagulation 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).

in intact and healthy blood vessels this cascade is not activated and several anticoagulant mechanisms prevent its activation. These include the presence of thrombomodulin and heparan sulfate proteoglycans on vascular endothelium. Thrombomodulin is a cofactor for thrombin that converts it from a procoagulant to an anticoagulant by stimulating activation of the anticoagulant serine protease protein C, Heparan sulfateproteoglycans stimulate the activation of the serine protease inhibitor (or serpin) antithrombin, which inactivates thrombin and factor Xa (Furie, 2009).

1.4.2. Coagulation System

Plasma transports at least 16 procoagulants, also called coagulation factors or clotting factors. Nearly all are glycoproteins synthesized in the liver, although a few are made by monocytes, endothelial cells, and megakaryocytes, eight are enzymes that circulate in an inactive form called zymogens, others are cofactors that bind and stabilize their respective enzymes. Then, the procoagulants become activated and produce a localized thrombus. Additional plasma glycoproteins are controls that regulate the coagulation process (Bernadette *et al*, 2012).

Table 1.1: Coagulation Factors (Hoffbrand and Moss, 2016)

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
-	Fibrin stabilizing factor	Transglutaminase
-	Prekallikrein (Fletcher factor)	Serine protease
-	HMWK (Fitzgerald factor)	Cofactor

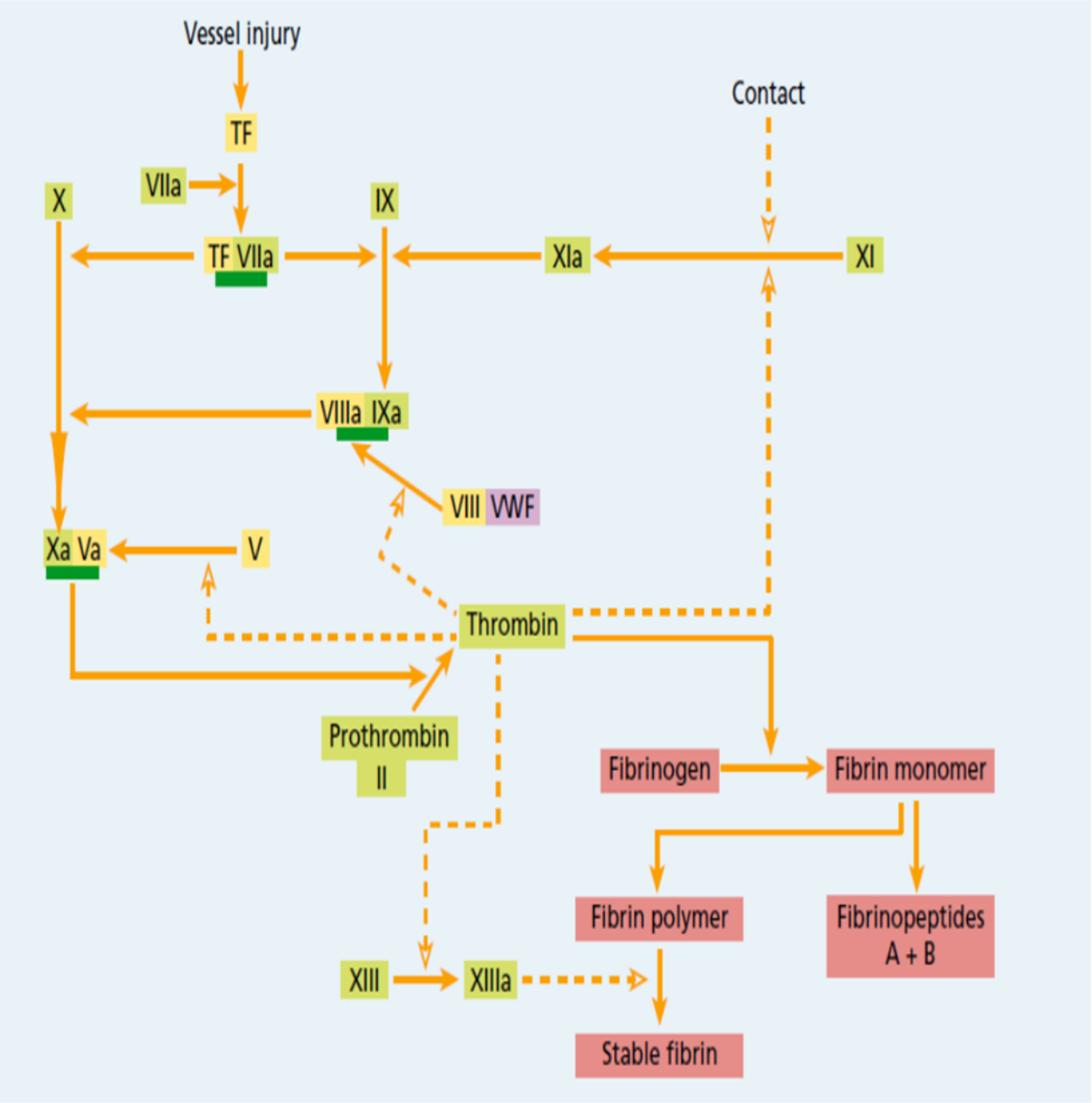

Figure G: The pathway of blood coagulation cascade. (Hoffbrand and Moss, 2016).

1.4.3. Fibrinolysis

Is a normal hemostatic response to vascular injury, Plasminogen, a B-globulin proenzyme in blood and tissue fluid, is converted to the serine protease plasmin by activators either from the vessel wall (intrinsic activation) or from the tissues (extrinsic activation). The most important route follows the release of tissue plasminogen activator (tPA) from endothelial cells. tPA is a serine protease that binds to fibrin. This enhances its capacity to convert thrombus bound plasminogen into plasmin. This fibrin dependence of tPA action strongly localizes plasmin generation by tPA to the fibrin clot. Release of tPA Plasmin generation at the site of injury limits the extent of the evolving thrombus. The split products of fibrinolysis are also competitive inhibitors of thrombin and fibrin polymerization (Hoffbrand and Moss, 2016).

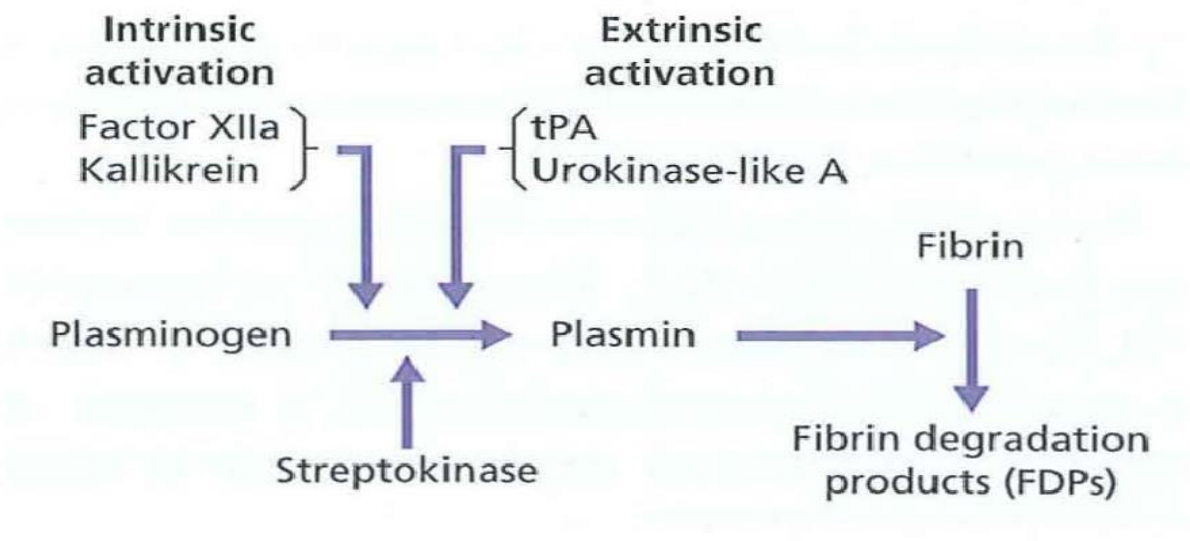

Figure H: the fibrinolytic system (tPA) (Hoff brand and Moss. 2016)

1.4.4. Coagulation Regulatory Mechanisms

Serine proteases and cofactors in the coagulation system are regulated by inhibitors, cofactors, and feedback loops to maintain a complex and delicate balance between thrombosis and abnormal bleeding. The principal regulators are TFPI, AT, and the protein C pathway. These function as natural anticoagulants, because they inhibit the action of procoagulants and prevent excessive clot formation or thrombosis (Bernadette *et al*, 2012).

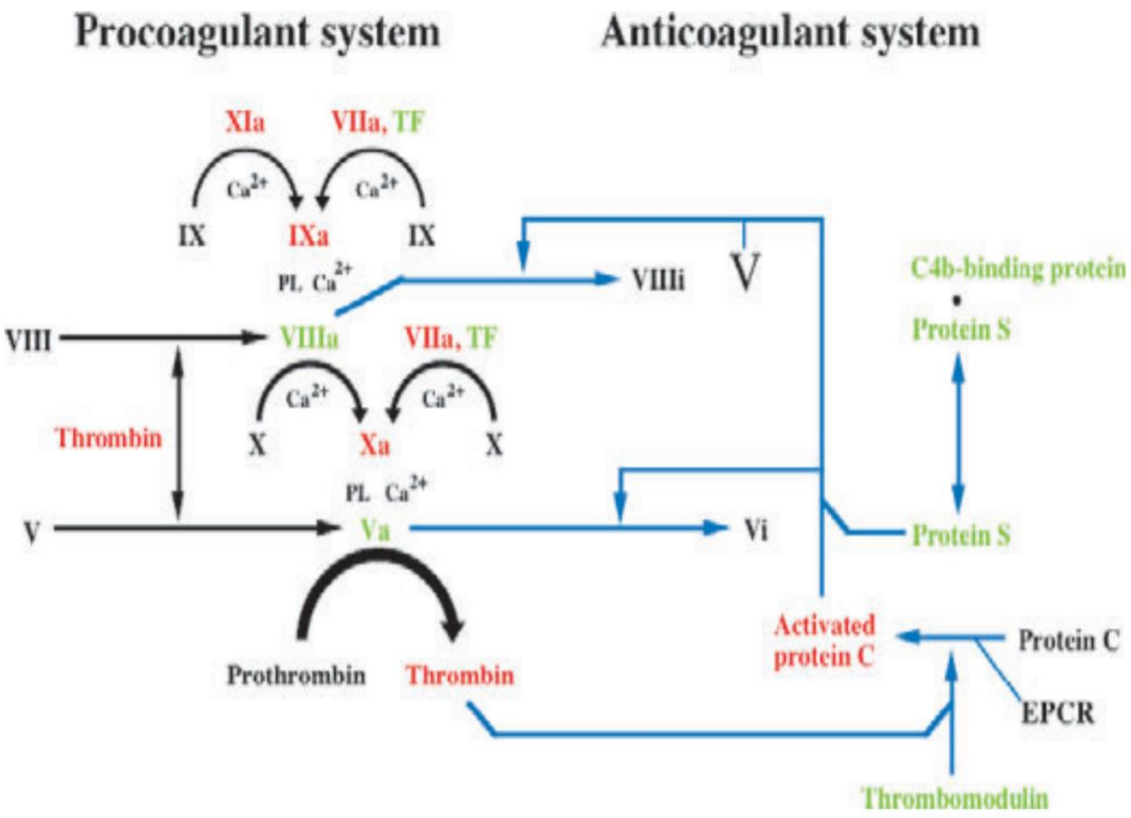

Figure I: Coagulation Regulatory Mechanisms (Dahlback, 2005).

1.5. Antithrombin (AT)

AT is a natural anticoagulant that inhibits thrombin (factor IIa), factor Xa, and other serine proteases in the coagulation cascade. As a serine protease inhibitor, its activity accelerated more than 1000 -fold by heparin binding. In the absence of AT, heparin has little effect on anticoagulation (Refaei *et al*, 2017).

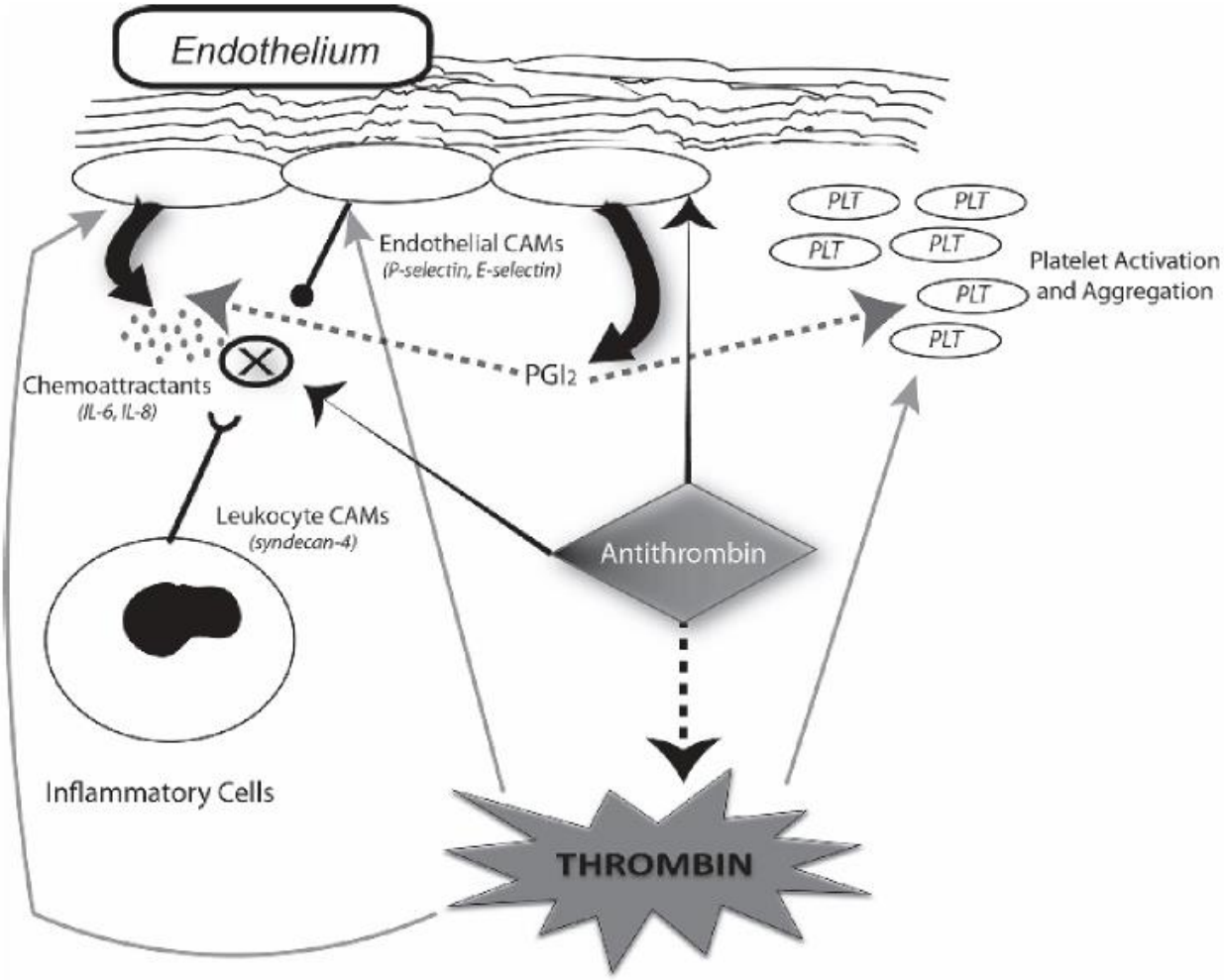

Figure J: antithrombin action (Jerrold *et al,* 2016).

1.5.1. Genetic and Physiology of AT III

It's encoded by the gene SerpinC1**;** SerpinC1 is the short name for serpin peptidase inhibitor, clade C (antithrombin), member 1. This gene encodes 464 amino acids and is located on chromosome 1q23-25.1. It is composed of seven exons that span 13.4 kb of genomic DNA. SerpinC1 provides instructions for the production of antithrombin III (Zeyuan *et al*, 2017).

Antithrombin III is an alpha-2 globulin with a molecular weight of approximately 65,000 Daltons and a half-life of 96 hours in the circulation. It is a glycoprotein containing approximately 15% carbohydrate. Antithrombin III appears to be synthesized in the liver, although recent work suggests that endothelial cells may be a source of antithrombin III as well. antithrombin III forms an inactive complex by binding irreversibly in a 1:1 ratio with an activated enzyme (thrombin, Xa, etc). Therefore, the functional activity of antithrombin III is determined by the amount of thrombin inhibited by a plasma or serum sample (McGann and Triplett, 2018).

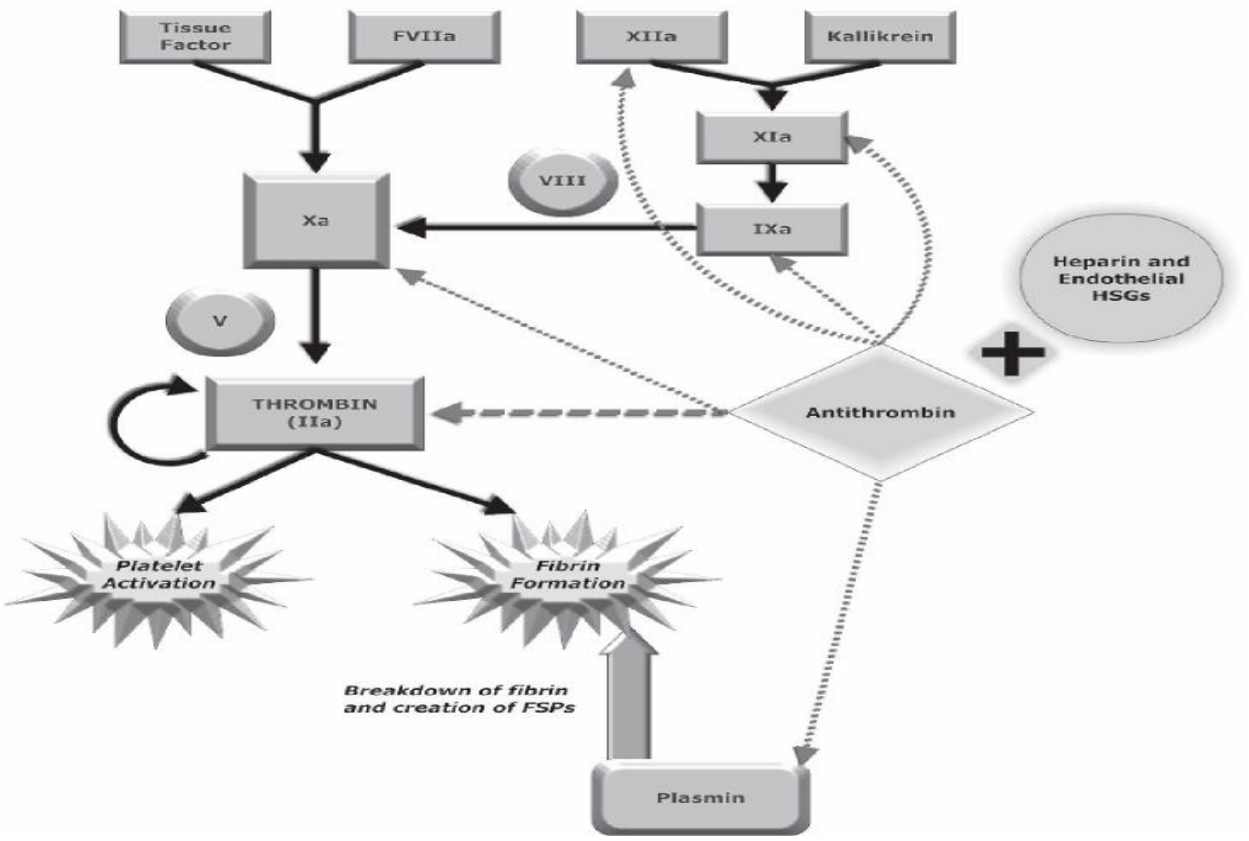

Figure K: antithrombin effect (Jerrold *et al,* 2016).

Antithrombin is also used in the therapeutic treatment of acute thrombotic episodes and prophylaxis during surgery and pregnancy in patients with AT shortage (Mallu *et al,* 2016).

Antithrombin is a natural anticoagulant which plays a potentially important role in whether women develop thromboembolism during pregnancy. In pregnancy, the rate of venous Thromboembolism (VTE) is correspondingly higher. Historic series have reported rates as high as 18 to 70% (James *et al,* 2014).

In the general population, pregnant women have a 5-fold higher risk for VTE compared to non-pregnant women. The risk is higher in the postpartum period and may extend for up to 12 weeks. Women with hereditary antithrombin deiciency who are also pregnant are at further risk of VTE during pregnancy, although estimates are highly variable (Refaei *et al,* 2017).

1.5.2. AT and Pregnancy

Several studies suggest that diabetes is associated with a hypercoagulable state. The determination of thrombin-antithrombin complex (TAT) is a useful clinical marker of coagulation activation. TAT concentration was significantly higher during normal pregnancy and labor. Slightly high TAT levels in GD women suggest that diabetes itself may induce activation of the coagulative system. This agrees with the increase in TAT levels observed in nonpregnant diabetic patients. The inhibitors of coagulation antithrombin III play a major role in maintaining the hemostatic balance, alterations in this inhibitor that lead to a decrease in its activity was found in GD women. These decreases were most evident in the third trimester, during the active phase of labor and after delivery and coincide with the metabolic imbalance (Bellar *et al*, 1998).

1.6. Previous studies

- Study performed by Hassan Bahakim *et al*, 1989, in blood samples collected randomly from 300 Saudi mothers and their babies at the time of delivery, all mothers were healthy and had normal full-term (37 to 42 weeks) pregnancy, labor, and uneventful delivery at the Riyadh maternity hospital, the coagulation parameters in maternal and cord blood at delivery showed that the plasma levels of antithrombin III was significantly lower in cord plasma than in maternal and control plasma.
- Also, study did by Schneider *et al* in 1997, therefor they used the blood samples from 59 newborns and their mothers to obtain a number of different components of the coagulation and fibrinolytic system including AT III, their result approved that the infant showed clearly significantly lower level of antithrombin III.

1.7. Rationale

Pregnancy is a state in which intense change in maternal hemostasis occur. During normal pregnancy, coagulation processes predominate over fibrinolytic activity, and the previous studies have reported hematological abnormalities in diabetes that affect the function, morphology, and metabolism of blood cells and the coagulation system and that result in a state of thrombophilia (Bronisz *et al*, 2008). Diabetic patients are prone to develop vascular complications. Increased procoagulatory factors and reduced fibrinolytic potential are considered as thrombogenic risk factors and are involved in thromboembolic complications in diabetic patients (Bronisz *et al*, 2008).

Umbilical cord blood evaluation is emerging as a potential means to aid in the diagnosis of neonatal pathology (Voller and *et al*, 2014) , instead of that available source of blood wasting it can be useful for predication of early thrombotic event, moreover, not many studies are available on hemostatic change in GDM. So, by doing so, the findings of this study could play an important role in decreasing morbidity and mortality of Infants of diabetic mothers. Furthermore, this study did not perform in Sudan so it could be used as a reference for related studies.

1.8. Objectives

General Objective

- To estimation antithrombin III level in cord blood of newborn from gestational diabetic Sudanese women.

Specific Objectives

- To estimate the level of antithrombin III in cord blood of newborn from normal pregnant women and gestational diabetic women using specific antibody and immunological complex.
- To compare between levels of antithrombin III in cord blood of newborn from normal pregnant women and gestational diabetic women.
- To correlate between levels of antithrombin III in cord blood of newborn within gestational weeks of delivery, weight of newborn, age of mother, glucose level of mother and family history of DM.

Chapter Two

2. Materials and Methods

2.1 Study design:

This an analytical cross sectional study design.

2.2 Study area:

The study performed in Khartoum state in Sudan.

2.3 Study duration:

The study conducted in period from March 2018 to January 2019.

2.4 Study population

Sixty cord blood samples collected from volunteer women with normal pregnancy and gestational diabetes.

2.5 Inclusion Criteria

- Sudanese volunteer Healthy pregnant women.
- Sudanese volunteer women with gestational diabetes.

2.6 Exclusion Criteria

- Newborn with heart disease, renal or hepatic dysfunction and inflammatory diseases.
- Premature newborn.
- Mother presents with coagulation diseases under treatment such as anticoagulant therapy.

2.7 Data collection

Data collected using informed consent and designed written questionnaire to obtain information about demographical and clinical data that helped in either including or excluding certain subjects.

2.8 Sample Collection

Immediately after caesarian delivery of the baby under observation of specialist, the cord clamped, before the placenta separated, cord blood dispensed in 3.8 % Tri-sodium citrate container (1ml of TSC : 9 ml of blood), blood mixed gently for avoidance of clotting and hemolysis.

2.9 Data Quality Assurance

- Samples checked whether they were in the acceptable criteria like; hemolysis, clotting, volume, time of collection, the correct labeling with safety procedure and specimen handling procedure, and manufacturer procedures and standard operational procedures (SOPs) were strictly followed.
- Samples were separated to form platelet poor plasma by using centrifuge for 15 minute and insure there was no hemolysis or clotting then they freezed at the allowed temperature (- 20°C) and analyzed at allowed time.
- The performance of Automated Mendiry checked by running the control and insured all data and sample results were correct.

2.10 antithrombin III Estimation

Cord blood in TSC container separated by using centrifuge for 15-minute lead to formation of platelet poor plasma (PPP).

After addition of antisera to antithrombin III, which present in a PPP lead to formation of specific Antibody an Immunological complex. The increase of turbidity after the addition of antiserum is directly proportional to antithrombin III concentration in the sample (appendix).

2.11 Quality Control

- For internal quality control it was recommended to use the cormay immuno-control III (Cat. No 4-291) with each batch of samples, for the calibration of automatic analyzers systems the cormay immuno-mulyical (Cat. No 4-287)

is recommended. As a 0 calibrator 0.9 % NaCl should be used. The calibration curve should be prepared every 4 weeks, with change of reagent lot number or as required e.g. quality control findings outside the specified range (Accent - 200 AT III, appendix).

- It is prefers to use syringe with TSC anticoagulant during the collection of cord blood to avoid the rapid clotting of sample.
- Avoid excessive mixing of the blood with the anticoagulant since the Hb affect in the result of antithrombin III.

2.12 Ethical Considerations

Ethical committee of research in Sudan University of Science and Technology, Faculty of Medical Laboratory Science approved the study. The purpose and objectives of the study was explained to each one of participants, who they had right to draw at any time without any deprivation, assured them that the data collected remained confidential and it was not allowed for any person to identify it. The questionnaire filled in their rest time, and participant had right to benefit from the researcher knowledge and skills.

2.13 Data analysis

Statistical Package for the Social Sciences (SPSS) version 20 software program used for statistical analysis of results, which expressed as means ± SD, tables and figures.

Chapter Three

3. Results

3.1 Demographic Data of Pregnant Women

Sixty cord blood samples of newborns from Sudanese volunteers with gestational diabetic pregnant women and matched healthy pregnant women as control enrolled in this study, 73.3 % of gestational diabetic pregnant women aged < 35 years whereas 26.7 % of them were > 35 Years. Also 53.3 % of them had history of gestational diabetes mellitus whereas 46.7% were not. On the other hand, 37 % of them had family history of GDM whereas 63 % were not. Whereas, 83 % healthy pregnant women < 35 years whereas 17 % of them > 35 Years (Table 3.1.1 represents the results).

Table 3.1.1 Frequency of Clinical Data among Study Groups

Variables	Case		Control	
	Frequency	**Percentage**	**Frequency**	**Percentage**
Age of mothers				
< 35 Years	22	73.3	25	83
> 35 Years	8	26.7	5	17
History of GDM				
Yes	16	53.3	-	-
No	14	46.7	-	-
Family History of DM				
No	19	63.0	-	-
Yes	11	37.0	-	-
Total	**30**	**100**	**30**	**100**

3.2 Means of Age of mothers, FBS of mothers, Gestational Weeks, Weights of newborns and BMI of newborns

Mean ± SD of age of mothers with gestational diabetes 31.03 ± 3.55 years. While, mean ± SD of age of mothers with normal pregnancy 29.10 ± 5.66 years (P. value = 0.112). Mean ± SD of FBG of mothers with gestational diabetes 110.53±19.15 mg / dl. While, mean ± SD of FBG of mothers with normal pregnancy 85.57±8.98 mg / dl (P. value = 0.002). Mean ± SD of gestational weeks of delivery in mothers with gestational diabetes 37.70 ± 0.65 weeks. When, mean ± SD of gestational weeks of delivery in mothers with normal pregnancy 37.77 ± 0.68 weeks (P. value = 0.699). On the other hand mean ± SD of BMI of the newborns from mothers with gestational diabetes 28.22 ± 3.67 and mean ± SD BMI of the newborns from mothers with normal pregnancy 24.67 ± 4.05 (P. value = 0.001). Mean ± SD of weight of newborns from mothers with gestational diabetes 3.47 ± 0.47 Kg, while mean ± SD of weight of the newborns from mothers with normal pregnancy3.07 ± 0.51Kg (P. value = 0.003). (Table 3.2.1 represents the results)

Table 3.2.1: Means of Age of mothers, FBS of mothers, Gestational Weeks, Weights of newborns and BMI of newborns

Variables	Case (Mean ± SD)	Control (Mean ± SD)	P. value
Age of mothers	31.03 ± 3.55 years	29.10 ± 5.66 years	0.112
FBG	110.53±19.15 mg / dl	85.57±8.98 mg / dl	0.002
Gestational weeks	37.70 ± 0.65 weeks	37.77 ± 0.68 weeks	0.699
BMI	28.22 ± 3.67	24.67 ± 4.05	0.001
Weight of baby	3.47 ± 0.47 Kg	3.07 ± 0.51Kg	0.003

3.3 Antithrombin III Level in cord blood of newborns from mothers with Gestational Diabetic Women and Normal Pregnant Women

The means ± SD of antithrombin III level in cord blood of newborns from mothers with Gestational Diabetes and normal pregnancy are 19.43 ± 6.62 mg / dl and 24.43 ± 6.28 mg / dl respectively. In addition, there is a significant decrease of antithrombin III level in cord blood of newborns from both gestational diabetic and normal pregnant women. While, there is a significant decrease of antithrombin III level in cord b blood of newborns from mothers with Gestational Diabetes in compare with antithrombin III level in cord b blood of newborns from mothers with normal pregnancy (P. value = 0.005) (Table 3.3.1 represents the results).

Table 3.3.1 antithrombin III Level in cord b blood of newborn from mother with Gestational Diabetic Women (Case) and Normal Pregnant Women(Control)

Parameter	Case (Mean ± SD)	Control (Mean ± SD)	P. value
antithrombin III (mg/dl)	19.43 ± 6.62	24.43 ± 6.28	0.005

3.4 Mean ± SD of antithrombin III level in cord blood of newborn from mother with GDM and Compare between FBG < 100 mg / dl and > 100 mg / dl

There was a significant decrease of antithrombin III level in cord blood of newborns from women with FBG > 100 mg/dl in compare with pregnant women with FBG < 100 mg / dl (P. value = 0.042) (Table 3.4.1 represents the results)

Table 3.4.1 Mean ± SD of antithrombin III level in cord blood of newborn from mother with GDM and Compare between FBG < 100 mg / dl and > 100 mg / dl

Parameter	FBG < 100 mg/dl (Mean ± SD)	FBG > 100 mg/dl (Mean ± SD)	P. value
antithrombin III (mg/dl)	22.89 ± 5.18	17.95 ± 6.72	0.042

3.5 Mean ± SD of antithrombin III level in Cord Blood of Newborns from Women with GDM in Compare between the Age < 35 Years and > 35 Years

There was a significant decrease of antithrombin III level cord blood of newborns from pregnant women with age more than 35 years in compare with age less than 35 years (P. value = 0.036) (Table 3.5.1 represents the results)

Table 3.5.1: Mean ± SD of antithrombin III level in Cord Blood of Newborns from Women with GDM in Compare between the Age < 35 Years and > 35 Years

Parameters	< 35 Years (Mean ± SD)	> 35 Years (Mean ± SD)	P. value
antithrombin III (mg/dl)	20.00 ± 7.05	17.88 ± 5.33	0.036

3.6 Mean ± SD of antithrombin III level in Cord Blood of Newborns from Pregnant Women with GDM in Compare between the Family History of Diabetes

There was a significant decrease in antithrombin III level in cord blood of newborns from pregnant women who have family history of diabetes mellitus with pregnant women who have not family history diabetes mellitus, (P. value = 0.555) (Table 3.6.1 represents the results).

Table 3.6.1: Mean ± SD of antithrombin III level in Cord Blood of Newborns from Pregnant Women with GDM in Compare between the Family History of Diabetes

Parameters	Yes (Mean ± SD)	No (Mean ± SD)	P. value
antithrombin III (mg / dl)	18.75 ± 6.71	20.21 ± 6.68	0.555

3.7 Correlation between Gestational Weeks, Weight of Baby and antithrombin III level

There was no correlation between antithrombin III level in a cord blood of newborns with both gestational weeks and Weight of baby and (P. value = 0.781 and 0.391, respectively). (fiqure L,M represent the results)

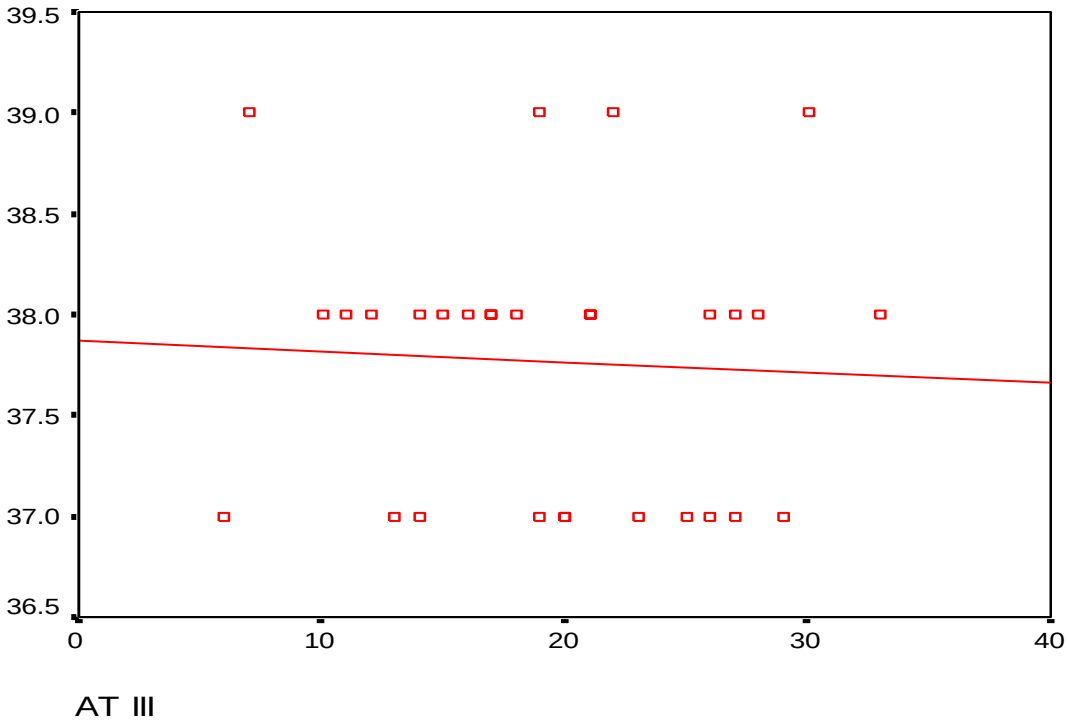

Figure L: Correlation between Gestational Weeks and antithrombin III level

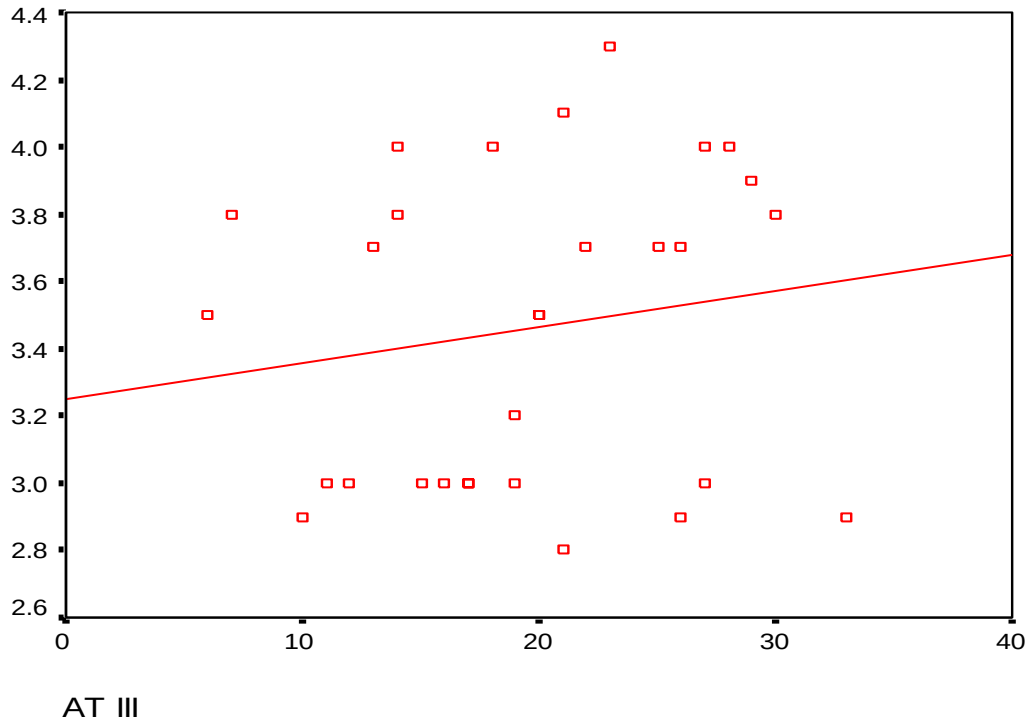

Figure M: Correlation between weight of newborn and antithrombin III level

Chapter Four

4. Discussion, Conclusions and Recommendations

4.1 Discussion

This study was conducted in cord blood of Sudanese pregnant women with gestational diabetes mellitus which is a systemic disease that affects both the mother and fetus. In fact recent study reported that, these patients are more likely to develop Type 2 DM, at risk of the developing in the following 10 years increases by 20 - 30% (Sak *et al*, 2012), the present study revealed that, there was a significant decrease of antithrombin III in cord blood of both normal pregnancy and gestational diabetes and also antithrombin III level significantly decreased in pregnant women as compared to control group (P. value = 0.005).

Althought the hemostatic factors influenced by the hyperglycemic state in GDM by causing of endothelial cells injuries that there is increase in prothrombotic state due to increase in activation of platelets and prothrombotic coagulation. The determination of TAT is a useful clinical marker of coagulation activation which is significantly higher lead to consumption of more antithrombin III concentration.

The present finding agreed with previous study in evaluation of antithrombin III in cord blood which performed by **Bahakim** *et al*, in 1989, the blood samples collected randomly from 300 Saudi mothers and their babies at the time of delivery, all mothers were healthy and had normal full-term (37 to 42 weeks) pregnancy, labor, and uneventful delivery at the Riyadh Maternity Hospital, their result show that the plasma levels of AT III was significantly lower in cord plasma than in maternal and control plasma. In addition, study have been done by **Schneider** *et al* in 1997 , therefor they used the blood samples from 59 newborns and their mothers to obtain a number of different components of the coagulation and fibrinolytic system including antithrombin III, and their result approved that the infant showed clearly significantly lower level of antithrombin III.

4.2 Conclusions

- There was decrease in the level of antithrombin III in cord blood of newborn from normal pregnant women and GD women.
- There was significant decrease in levels of antithrombin III in cord blood of newborn observed in GD women compared with normal pregnant women.
- There was significant decrease of antithrombin III level in cord blood of newborn from pregnant women with FBG more than 100 mg / dl in compare with antithrombin III level in cord blood of newborn from pregnant women with FBG less than 100 mg / dl. In addition, there was significant decrease of antithrombin III level in cord blood of newborn from women with family history of DM more than women with no family history of DM and there was significant decrease of antithrombin III in cord blood of newborn from pregnant women with age more than 35 years in compare with antithrombin III in cord blood of newborn from pregnant women with age less than 35 years.
- There was no correlation between antithrombin III level in a cord blood of GDM pregnant women with both gestational weeks delivery and weight of newborn.

4.3 Recommendations

- Increased sample size.
- More researches should perform for monitoring the complete coagulation profile.
- Analyze the sample as soon as possible or insure the suitable temperature for storage.

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Appendix (1)

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Department of Hematology

Estimation of AntithrombinIII level in cord blood on gestational diabetes

قياس معدل مضاد الثرومبين٣ في دم الحبل السري في الاطفال حديثي الولاده عند النساء السودانيات المصابات بسكر الحمل

-Name: …….. -ID code ……

-Age: ……

-History of diabetes? .....

-History of thrombosis? .....

-Family history of thrombosis ……...

-Number of previous pregnancy? .....

-Weight of the infant? ..... -Time of delivery …….

-Fasting blood glucose level ……..

-Medical condition ……

-Medication of mother……..

-Antithrombin level ……..

-informed consent:

أنا المذكوره أعلاه أوافق على سحب عينات دم مني لغرض البحث العلمي ولي الحق بالأنسحاب متى ما شئت ولي الحق في الحصول على النتائج الخاصة بي وأن لا يتم أستخدام هذه العينات لأغراض التجارب الأنسانيه اوالجيوانيه.

Signature: …….