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Determination of Prothrombin Time, Activated Partial Thromboplastin Time and level of D- Dimer among Sudanese Patients with Diabetes Mellitus

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عند المرضى السودانيين المصابين بمرض السكري

A thesis submitted in partial fulfillment for the requirement of degree of M.SC. In haematology.

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October 2018

الآية الكريمة

قال تعالي:

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ لاَ يُكَلِّفُ اللّهُ نَفْساً إِلاَّ وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لاَ تُؤَاخِذْنَا إِن نَّسِينَا أَوْ أَخْطَأْنَا رَبَّنَا وَلاَ تَحْمِلْ عَلَيْنَا إِصْراً كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِن قَبْلِنَا رَبَّنَا وَلاَ تُحَمِّلْنَا مَا لاَ طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا أَنتَ مَوْلاَنَا فَانصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ(286)﴾

صدق الله العظيم

سورة البقرة الاية (286)

Dedication:

To the one who brightens our days "Mother".

To the one who makes the world a better place "Father".

To my husband and my beautiful daughter.

To My beautiful & wonderful friends you are my best thing.

To my wonderful supervisor;

Prof. Babiker Ahmed Mohamed who was with me when need.

I dedicate this work.

Acknowledgment:

-First of all I would like to thank God for giving me the health and strength which without it I could not be able to complete this research.

-Gentle and deeply grateful to my supervisor Prof. Babiker Ahmed Mohamed who encouraged me and providing me the expertise to accomplish this work.

-My gratitude to hospital members for their cooperation and kindness.

- Finally, thanks extend to all people whom the blood sample has been collected from. To all them very thanks with regards**.**

Abstract

This is a case control study carried out in Khartoum state in Yastabshiron hospital, in the period from (October 2017 _ October 2018) to evaluate some coagulation parameters in Sudanese patient with Diabetes mellitus in Khartoum state , 40 diagnosed Diabetic patients were selected 17 (42.5%) male and 23(57.5%) female and 40 healthy individuals were selected as control group, 3 ml of venous blood was withdrawn from each patient, placed in tri sodium citrate container , then centrifuge to get platelet poor plasma (PPP) to measure PT,INR, APTT and D-dimer , The result was analyzed by SPSS version 15 , and express as means, The mean of Prothrombin time, INR, and Activated partial thromboplastin time and D- dimer in patients group were(18.19 sec), (1.36), (37.73 sec) and (992.91 ng /ml) respectively , The means of PT, INR, APTT and D-dimer in control group ,(13.85 sec),(1.03), (32.55 sec) and (16.27 ng / ml) respectively.

The result showed that there was significant differences in mean of PT, INR, APTT, and D-Dimer between patients group and control group (P.value < 0.05), There was no significant differences in mean of PT, INR, APTT, and D-Dimer between both group of sex (P. value > 0.05), according to the duration of disease there was no significant differences in PT, INR, APTT, and D-Dimer (P. value > 0.05).

The results of this study reveal that measurement of PT, INR, APTT, and D-dimer should be use in identifying risk group of Diabetes Mellitus who are likely to develop thrombotic events.

الخلاصة

هذه دراسة تحليلية حالة وحالة ضابطة في ولاية الخرطوم في مستشفى يستبشرون في الفترة من اكتوبر 2017 الي يوليو2018 لقياس بعض معاملات تخثر الدم في مرضي السكري السودانيين في ولاية الخرطوم، تم اختيار اربعين شخص مشخصين كمرضى سكري منهم سبعة عشر من الذكور وثلاثة وعشرون من الاناث كما تم أخذ اربعين عينة من الاصحاء كمجموعة ضبط، تم اخذ 3 مليلتر من الدم الوريدي من كل شخص وتم وضعه في وعاء يحتوي على مانع التجلط ثلاثي سترات الصوديوم واستخلص المصل الدموي لقياس زمن البروثرومبين ،النسبة الطبيعية العالمية، زمن الثرمبوبلاستين الجزئي المنشط و دي دايمر وتم تحليل النتائج بواسطة برنامج الحزم الاحصائية للعلوم الاجتماعية اصدار ١٥، تم حساب المتوسط وكان كالاتي : متوسط زمن الثرومبين، متوسط النسبة الطبيعية العالمية، متوسط زمن الثرمبوبلاستين الجزئي المنشط ومتوسط دي دايمر في مجموعة المرضى (19،18ثانية)، (36،1)، (73،37 ثانية) و (91،992 نانوجرام/مليلتر) علي التوالي، بينما كان متوسط زمن البرثرومبين، متوسط النسبة الطبيعية العالمية، زمن البروثرومبين الجزئي المنشط و دي دايمر (85،13ثانية)، (03،1)، (55،32 ثانية) و(27،16 نانوجرام/مليلتر) على التوالي.

اظهرت النتائج وجود فروقات ذات دلالة احصائية في متوسط زمن البروثرومبين، النسبة الطبيعية العالمية، زمن الثرومبوبلاستين الجزئي المنشط و دي دايمر (القيمة المعنوية اصغر من ٠،٠٥) بين مجموعة المرضي ومجموعة الضبط، كما اظهرت عدم وجود فروقات ذات دلالة احصائية في متوسط زمن البروثرومبين، النسبة الطبيعية العالمية، زمن الثرومبوبلاستين الجزئي المنشط و دي دايمر بين الجنسين (القيمة المعنوية اكبر من ٠،٠٥) وبالنسبة لمدة المرض عند المصابين بمرض السكري لا توجود فروقات ذات دلالة احصائية في متوسط زمن البروثرومبين، النسبة الطبيعية العالمية، زمن الثرومبوبلاستين الجزئي المنشط و دي دايمر (القيمة المعنوية اكبر من ٠،٠٥).

اشارت نتيجة الدراسة الي ان قياس مستويات زمن البروثرومبين، النسبة الطبيعية العالمية، زمن الثرومبوبلاستين الجزئي المنشط و دي دايمر يمكن ان يكون مفيد في تحديد مجموعة مرضى السكري الذين يحتمل تعرضهم لمخاطر التجلط.

List of Content

List of Tables

Abbreviations

ADP: Adenosine diphosphate.

ATP: Adenosine Triphosphate.

AA: Arachidonic acid.

AMP: Adenosine Monophosphate**.**

ADA: American Diabetes Association.

AGEs: Advanced glycation end products.

APTT: Activated Prothrombin Time.

β TG: β – Thromboglobulin.

BSA: Bovine serum albumin.

CSF: Colony Stimulating Factor.

CLEC-2: C-type lectin-like receptor 2.

CD62P: Cluster of Differentiation 62P.

CaCl2: Calcium Chloride.

DVT: Deep Venous Thrombosis.

DM: Diabetes mellitus.

DKA: Diabetic ketoacidosis.

ECs: Endothelial Cells.

ECM: Endothelial Cell Matrix.

F2R: Factor II Receptors.

FEU: Fibrinogen equivalent units.

Gp1b: Glycoprotein 1b.

G-CSF: Granulocyte Colony Stimulating Factor.

GP: Glycoproteins.

GPCR: G Protein-Coupled Receptors.

GPIIb: Glycoprotein IIb**.**

GPIIIa: Glycoprotein IIIa.

GLP-1: Glucagon-like peptide 1.

GADA: Glutamic acid decarboxylase.

GFR: Glomerular filtration rate.

HMW: High-molecularweight kininogen.

HLA: Human Leukocyte Antigen.

HDL: High-density lipoprotein.

IL-3: Interleukin-3.

IL-6: Interleukin-6.

ITAM: Immunoreceptor Tyrosine-based Activation Motif .

IDDM: Insulin-dependent Diabetes mellitus.

ICA: Islet cell antibodies.

IAA: Insulin autoantibodies.

IA-2A: Islet antigen-2A.

INR: International normalized Ratio.

LDL: Low-density lipoprotein.

Meg-CSF: Megakaryocyte Colony Stimulating Factor.

MI: Myocardial Infarction.

NIDDM: Non-insulin-dependent Diabetes mellitus.

PC: Protein C.

PAIs: Plasminogen Activator Inhibitors.

PAI-1: Plasminogen Activator Inhibitor type 1.

PCF: Platelet Contractile force.

PECAM-1: Platelet-endothelial cell adhesion molecule-1.

PGI2: Prostaglandin I2.

PF4: Platelet factor 4.

PTGS1: Prostaglandin (PG)-endoperoxide synthase 1.

PE: Pulmonary Embolism.

PT: Prothrombin Time.

PPP: Platelet poor plasma.

PBS: Phosphate buffered saline.

TFPI: Tissue Factor Pathway Inhibitor.

TBXA2: Thromboxane A2.

TBXA2R : TBXA2 receptor .

t-PA: tissue-type plasminogen activator .

TSC: Tri Sodium Citrate.

u-PA : urokinase-type plasminogen activator.

u-PAR: urokinase-type plasminogen activator Receptor.

vWF: von Willebrand Factor.

VTE: Venous thromboembolism.

ZnT8: Zinc transporter 8.

Chapter One

Introduction and Literature Review

1.1 Introduction:

Diabetes mellitus is characterized by a high risk of atherothrombotic events. What is more, venous thrombosis has also been found to occur more frequently in this diseased group. This prothrombotic condition in diabetes is underpinned by laboratory findings of elevated coagulation factors and impaired fibrinolysis. Hyperglycemia plays an important role in the development of these hemostatic abnormalities, as is illustrated by the association with glycemic control and the improvement upon treatment of hyperglycemia. Interestingly, stress induced hyperglycemia, which is often transient, has also been associated with poor outcome in thrombotic disease. Similar laboratory findings suggest a common effect of acute vs. chronic hyperglycemia on the coagulation system. Many mechanisms have been proposed to explain this prothrombotic shift in hyperglycemia, such as a direct effect on gene transcription of coagulation factors caused by hyperglycemia-induced oxidative stress, loss of the endothelial glycocalyx layer, which harbours coagulation factors, and direct glycation of coagulation factors, altering their activity. (Lemkes, B. A et al, 2010).

80% of patients with diabetes mellitus die a thrombotic death. 75% of these deaths are due to cardiovascular complications and remainder due to cerebrovascular events and peripheral vascular complications. (Madan ,R . et al, 2010).

Vascular endothelium, the primary defense against thrombosis, is abnormal in diabetes. Endothelial abnormalities undoubtedly play a role in the enhanced activation of platelets and clotting factors seen in

diabetes. Coagulation activation markers, such as prothrombin activation fragment 1+2 and thrombin–anti-thrombin complexes, are elevated in diabetes. The plasma levels of many clotting factors including fibrinogen, factor VII, factor VIII, factor XI, factor XII, kallikrein, and von Willebrand factor are elevated in diabetes. Conversely, the level of the anticoagulant protein C (PC) is decreased. The fibrinolytic system, the primary means of removing clots, is relatively inhibited in diabetes due to abnormal clot structures that are more resistant to degradation and an increase in plasminogen activator inhibitor type 1 (PAI-1). Increased circulating platelet aggregates, increased platelet aggregation in response to platelet agonists, increased platelet contractile force (PCF), and the presence of higher plasma levels of platelet release products, such as β-thromboglobulin, platelet factor 4, and thromboxane B_2, demonstrate platelet hyperactivity in diabetes.(Carr, M.E, 2001).

1.2 Literature Review:

1.2.1Normal hemostasis:

Hemostasis is the physiologic mechanism that stems bleeding after injury to the vasculature. Normal hemostasis depends on both cellular components and soluble plasma proteins. (DeSancho, M.T and Rand,J.H, 2003).

The hemostatic system consists of blood vessels, platelets, and the plasma coagulation system including the fibrinolytic factors and their inhibitors. When a blood vessel is injured, three mechanisms operate locally at the site of injury to control bleeding: (1) vessel wall contraction, (2) platelet adhesion and aggregation (platelet plug formation), and (3) plasmatic coagulation to form a fibrin clot. All three mechanisms are essential for normal hemostasis. Abnormal bleeding usually results from defects in one or more of these three mechanisms. For a better understanding of the pathogenesis of pathological bleeding, it is customary to divide hemostasis into two stages (i.e., primary and secondary hemostasis). Primary hemostasis is the term used for the instantaneous plug formation upon injury of the vessel wall, which is achieved by vasoconstriction, platelet adhesion, and aggregation. The fibrin formation is not required for hemostasis at this stage. Primary hemostasis is, however, only temporarily effective. Hemorrhage may start again unless the secondary hemostasis reinforces the platelet plug by formation of a stable fibrin clot. Finally, mechanisms within the fibrinolytic system lead to a dissolution of the fibrin clot and to a restoration of normal blood flow. (Munker ,R. etal, 2007).

1.2.2 Blood vessel structure:

The basic structure of blood vessels can be broken down into three layers: the intima, the media and the adventitia. It is the materials that make up these layers and the size of these layers themselves that differentiate arteries from veins, and indeed one artery or one vein from another artery or vein. The intima is the innermost layer and the surface is covered with a single layer of ECs (the endothelium), which rest on a basement membrane of subendothelial microfibrils that are composed of collagen fibres and some elastin. The media or middle layer contains mainly circularly arranged smooth muscle cells and collagenous fibrils, and is divided from the adventitia by the external elastic lamina. The muscle cells contract and relax, whereas the elastin allows vessels to stretch and recoil. The adventitia or outermost layer is composed of collagen fibres and fibroblasts that protect the blood vessel and anchor it to surrounding structures. (Hoffbrand, A.V .et al, 2005).

1.2.2.1 Endothelial Structure:

In contrast to circulating blood cells and vascular smooth muscle cells, but similar to epithelial cells, the endothelium exhibits polarity manifested by the asymmetric distribution of cell-surface glycoproteins and by the unidirectional secretion of some extracellular matrix proteins and chemical mediators.

Four types of intercellular junctions have been described between adjacent endothelial cells: tight junctions, gap junctions, adherens junctions, and syndesmos.

Their distribution varies along the vascular tree with tight junctions being more frequent in the larger arteries, correlating with a more stringent requirement for permeability control. The molecular structure of endothelial tight junctions is similar to that of epithelium, consisting of a

network of fibrils with the critical component being a transmembrane protein, occludin. The distribution of gap junctions tends to follow that of tight junctions. Connexin-40, connexin-37, and, in arterioles, connexin-43 are gap junction proteins that have been detected in endothelial cells. Communication between adjacent endothelial cells and between endothelial cells and pericytes or smooth muscle cells is mediated by gap junctions. Adherens junctions are formed by transmembrane glycoproteins called cadherins, which make the link between cell-to-cell contacts and the cytoskeleton. Several different types of cadherins are expressed in endothelial cells. Recently, an endothelial-specific cadherinVE-cadherin/cadherin-5was identified and found to be expressed on virtually all types of endothelium. Similar to other cadherins, VE cadherin forms homotypic contacts with VE-cadherin on adjacent cells. Within the cell VE-cadherin complexes with catenins which, through other proteins, contact the actin cytoskeleton. Homotypic engagement of VE-cadherin has been reported to be responsible for the density-dependent inhibition of endothelial cell growth. The structure of a fourth type of junction, the syndesmos, is not well-elucidated. Other membrane proteins that are located at interendothelial junctions include platelet-endothelial cell adhesion molecule-1 (PECAM-1), which may be important in directing the formation of junctions, and the integrins (particularly 2 1 and 5 1). (Hoffman, R. etal, 2000).

1.2.2.2 Function of blood vessels:

After an injury, the damaged vessels initiate hemostasis. The first response of vessels to injury is constriction or narrowing of the lumen of the arterioles to minimize the flow of blood into the wound area and the escape of blood from the wound site. Vasoconstriction also brings the hemostatic components of the blood (the platelets and the plasma proteins) closer to the vessel wall, facilitating their interactions.

Vasoconstriction occurs immediately and lasts a short time. The mechanism of vasoconstriction is complex. It is caused in part by neurogenic factors and in part by several regulatory substances that interact with receptors on the surface of cells of the blood vessel wall. The regulating substances include serotonin and thromboxane A_2 (both products of platelet activation) and endothelin-1 (which is produced by damaged endothelial cells). These substances can aid in prolonging vasoconstriction.2 In contrast, healthy intact endothelial cells synthesize and secrete a prostaglandin PGI_2, also called prostacyclin. PGI_2 counteracts constriction by causing vasodilation of the arterioles.3 Vasodilation increases blood flow into the injured area to bring fresh supplies of plasma-blood clotting substances and causes redness of the skin at the wound site. Also after injury, endothelial cells of the venules contract, producing gaps between them and allowing plasma leakage into the tissues causing swelling or edema (increased vascular permeability). The increased blood flow into the area (vasodilation) and the increased vascular permeability are components of the inflammatory response, a normal physiologic response to injury. (McKenzie, S.B, 2014).

1.2.2.3 Function of endothelium:

Endothelial cells are central regulators of hemostasis; the balance between the anti- and prothrombotic activities of endothelium determines whether thrombus formation, propagation, or dissolution occurs. Normal endothelial cells express a variety of anticoagulant factors that inhibit platelet aggregation and coagulation and promote fibrinolysis; after injury or activation however, this balance shifts, and endothelial cells acquire numerous procoagulant activities.

Antithrombotic activities: (1) Intact endothelium prevents platelets (and plasma coagulation factors) from engaging the highly thrombogenic subendothelial ECM. Nonactivated platelets do not adhere to normal

endothelium; even with activated platelets, prostacyclin (i.e., prostaglandin I2 [PGI2]) and nitric oxide produced by endothelium impede their adhesion. Endothelial cells also produce adenosine diphosphatase, which degrades adenosine diphosphate (ADP) and further inhibits platelet aggregation. (2) Inhibitory Effects on Coagulation Factors; these actions are mediated by factors expressed on endothelial surfaces, particularly heparin-like molecules, thrombomodulin, and tissue factor pathway inhibitor. The heparin-like molecules act indirectly: They are cofactors that greatly enhance the inactivation of thrombin (and other coagulation factors) by the plasma protein antithrombin III. Thrombomodulin also acts indirectly: It binds to thrombin, thereby modifying the substrate specificity of thrombin, so that instead of cleaving fibrinogen, it instead cleaves and activates protein C, an anticoagulant. Activated protein C inhibits clotting by cleaving and inactivating two procoagulants, factor Va and factor VIIIa; it requires a cofactor, protein S, which is also synthesized by endothelial cells. Finally, tissue factor pathway inhibitor (TFPI) directly inhibits tissue factor–factor VIIa complex and factor Xa. (3) Fibrinolysis: Endothelial cells synthesize tissue-type plasminogen activator, a protease that cleaves plasminogen to plasmin; plasmin, in turn, cleaves fibrin to degrade thrombi. (Kumar, V. et al, 2003).

Prothrombotic activities : (1) Activation of Platelets; Endothelial injury brings platelets into contact with the subendothelial ECM, which includes among its constituents von Willebrand factor *(vWF)*, a large multimeric protein that is synthesized by EC. vWF is held fast to the ECM through interactions with collagen and also binds tightly to Gp1b, a glycoprotein found on the surface of platelets. These interactions allow vWF to act as a sort of molecular glue that binds platelets tightly to denuded vessel walls. (2) Activation of Clotting Factors; endothelial cells produce tissue factor,

the major in vivo activator of coagulation, and down regulate the expression of thrombomodulin. Activated endothelial cells also bind coagulation factors IXa and Xa, which augments the catalytic activities of these factors. (3) Antifibrinolytic Effects. Activated endothelial cells secrete plasminogen activator inhibitors (PAIs), which limit fibrinolysis and thereby favor thrombosis. (Kumar, V. et al, 2003).

1.2.3 Platelets:

Platelets are anucleate circulating blood particles. They circulate around the body in an inactive state until they come into contact with areas of endothelial damage or activation of the coagulation cascade. Here they adhere to the endothelial defect, change shape, release their granule contents, and stick together to form aggregates. Physiologically these processes help to limit blood loss; however, inappropriate or excessive platelet activation results in an acute obstruction of blood flow, as occurs, for example, in an acute myocardial infarction. However, activated platelets also express and release molecules that stimulate a localized inflammatory response through the activation of leukocytes and endothelial cells, and it is now clear that platelet function is not merely limited to the prevention of blood loss. Indeed, platelets have been implicated in many pathological processes including host defense, inflammatory arthritis, adult respiratory distress syndrome, and tumor growth and metastasis. (Quinn, M and Fitzgerald, D , 2005).

1.2.3.1 Platelets Production:

Platelets, also called thrombocytes, are cytoplasmic fragments released from a parent cell known as a megakaryocyte. Megakaryocyte are large cells (80-150µ in diameter), which are found predominantly in the bone marrow and to a smaller degree in the spleen and lungs. Similar to erythrocyte and leukocyte , megakaryocyte develop from a pluripotential stem cell that has been influenced by colony stimulating factor (CSF)

produced by macrophages , fibroblast , T lymphocyte , and stimulated endothelial cells. Additional influences, such as interleukin-3 and -6 (IL-3 and IL-6), which appear to be instrumental in differentiation of stem cell into platelet producing Megakaryoblasts, and megakaryocyte CSF (Meg-CSF) and granulocyte CSF (G-CSF) synergistically stimulate production of progenitor cells. Meg-CSF is thought to be generated by bone marrow cells in response to megakaryocytic mass. as the number of megakaryocyte decrease, the amount of Meg-CSF increases. Thrombopoietin is generated predominantly by the kidney, and to a lesser amount, by the liver and spleen, in response to a demand for platelets. It stimulates megakaryocyte progenitor cell to mature and release platelets, although its chemical nature is still not completely known. The spleen is the part of the regulatory system for platelet production wherein approximately 30% of peripheral blood platelets are sequestered .sudden depletion of platelets, resulting from consumption in clotting or immune and nonimmune destruction, may rapidly empty the splenic pool. In response, Thrombopoietin causes maturation of the Megakaryoblasts to produce a marrow response equal to the loss of platelets. Because the action of Thrombopoietin is similar to that of erythropoietin, any Increases in Thrombopoietin will speed up the maturation of megakaryocyte. This accelerated maturation results in less platelet production per cell. If the consumption or destruction of platelets continues, the platelet count will fall to a level incapable of maintaining normal vascular and hemostatic integrity and a condition called acute thrombocytopenia. (Bernadette, F, 1997).

Megakaryocytes do not undergo complete cellular division but undergo a process called endomitosis or endoreduplication creating a cell with a multilobed nucleus. Each megakaryocyte produces about 2000 platelets.

Platelet development occurs in the following sequence:

- Megakaryoblasts are the most immature cell (10 to 15 μm) with a high nuclear to cytoplasmic ratio and two to six nucleoli.
- Promegakaryocyte is a large cell of 80 μm with dense alpha and lysosomal granules.
- Basophilic megakaryocyte shows evidence of cytoplasmic fragments containing membranes, cytotubules, and several glycoprotein receptors.
- The megakaryocyte is composed of cytoplasmic fragments that are released by a process called the budding of platelets. (Ciesla, B**,** 2007).

1.2.3.2 Platelet Structure:

Platelets have several unique features that enable them to efficiently perform their primary function, namely the rapid formation of a vascular plug following vessel injury. Platelets are extremely small and discoid in shape, with dimensions of approximately 3.0 μm by 0.5 μm, and a mean volume of 7–11fL. This shape and small size enables the platelets to be pushed to the edge of the vessel, placing them next to the endothelial cells and in the right place to respond to vascular damage. They are present at a high level in the human circulation, usually between 150 and $400x10^9$ platelets/L. This level of expression appears to represent a considerable degree of redundancy, as individuals with platelet counts as low as $10x10^9$ platelets/L tend to exhibit only occasional, major spontaneous bleeds, although they are at considerable risk of bleeding during major trauma. (Hoffbrand, A.V, et al, 2005).

Electron microscopy reveals a fuzzy coat (glycocalix) on the platelet surface composed of membrane glycoproteins (GP), glycolipids, mucopolysaccharides and plasma proteins. The plasma membrane is a bilayer of phospholipids in which cholesterol, glycolipids and glycoproteins are embedded. Platelets have an elaborate channel system, the open canalicular system, which is composed of invaginations of the plasma membrane. In addition, they have a dense tubular system, a closed

- channel network derived from the smooth endoplasmic reticulum; it is the major site of platelet thromboxane synthesis. The discoid shape of resting platelets is maintained by a cytoskeleton consisting of a spectrin membrane skeleton, a microtubule coil, and an actin scaffold. Platelets contain several organelles: mitochondria and glycogen stores, lysosomes, dense granules and alpha granules. The dense (δ) granules contain calcium, ATP, ADP, magnesium and serotonin. The alpha (α) granules contain numerous proteins, including β - thromboglobulin (β TG) and platelet factor 4 (PF4), which are considered platelet - specific, several higher molecular mass coagulation factors, (e.g., fibrinogen, factor V, high molecular weight kininogen, factor XIII), von Willebrand factor (vWF), growth factors (e.g., platelet - derived growth factor, vascular endothelial growth factor), protease inhibitors (e.g., plasminogen activator inhibitor - 1, C1 inhibitor, amyloid - β - protein precursor), thrombospondin, P - selectin. albumin and IgG. The lysosomes contain acid hydrolases and other enzymes. (Schmaier, A.H and Lazarus, H.M, 2012).

1.2.3.3 Platelet Function:

The main ftU1ction of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury. In the absence of platelets, spontaneous leakage of blood through small vessels may occur. The immobilization of platelets at the sites of vascular injury requires specific platelet-vessel wall (adhesion) and platelet-platelet (aggregation) interactions. (Hoffbrand, A.V, et al 2006).

1.2.4 Coagulation Factors:

The fibrin clot is the end product of a multiplicity of complex reactions of plasma proteins called coagulation or clotting factors. Most of the clotting factors are zymogens of serine proteases and are converted to active enzymes during the process of blood coagulation. The six serine

proteases are the activated forms of the clotting factors II, VII, IX, X, XI, and XII. The letter "a" accompanying a Roman numeral (e.g., factor Xa) indicates that the factor is in its activated form. Factors V and VIII are not enzymes but co-factors, which, after activation, modify the speed of the coagulation reaction. The reactions of the coagulation factors take place on the surface of phospholipids. Following platelet activation, certain phospholipids (i.e., phosphatidyl ethanolamine, phosphatidyl serine, and phophatidyl choline) that were not present on the surface of the resting platelet become exposed on the platelet surface. These newly exposed phospholipids provide the appropriate phospholipid surface upon which reactions of the coagulation factors take place.

We have grouped the coagulation factors as (1) the vitamin K–dependent zymogens (prothrombin; factors VII; IX, and X; and protein C); (2) the soluble cofactors [protein S, factor V, factor VIII, and von Willebrand factor (vWf)]; (3) factor XI and the other "contact" factors; (4) cell-associated cofactors (tissue factor and thrombomodulin); (5) fibrinogen; (6) factor XIII; and (7) the plasma coagulation protease inhibitors. (Roberts, H.R, et al, 2000).

1.2.5 Hemostatic Mechanisms:

Coagulation (clotting) is traditionally divided into two systems: primary hemostasis and secondary hemostasis. (Kern, W, 2002).

1.2.5.1 Primary hemostasis:

Platelet adhesion: In a healthy blood vessel, and under normal blood flow, platelets do not adhere to surfaces or aggregate with each other. However, in the event of injury platelets are exposed to subendothelial matrix, and adhesion and activation of platelets begins. Multiple receptors on the surface of platelets are involved in these adhesive interactions, and these receptors are targeted by multiple adhesive proteins. The key for all

of these receptors is that the adhesive interaction only takes place in the event of an injury to the blood vessel. This restriction is maintained in several different ways. Receptor GPIb-IX-V binds to immobilized von Willebrand factor (VWF) specifically through an interaction between GPIbα and the A1 domain of VWF. VWF is a large multimeric protein secreted from endothelial cells and megakaryocytes that is always present in the soluble state in the plasma as well as in the immobilized state in subendothelial matrix. However, soluble VWF in the circulation does not bind with high affinity to GPIba; The high affinity interaction may be dependent upon high sheer stress exerted by flowing blood on immobilized VWF, whether that VWF is immobilized on subendothelial matrix or other activated platelets. Receptor GPVI is constitutively active but its ligand is collagen, which is present in the subendothelial matrix and thus is only exposed to the blood in the event of injury. GPVI and GPIb-IX-V are critical for adhesion of platelets to subendothelial matrix at the site of injury and for their subsequent activation. (Gale, A. J, 2011)

Platelet activation: is stimulated by bound platelet secretion products and local prothrombotic factors such as tissue factor. Multiple pathways can lead to platelet activation. There are two principle activating pathways in platelets. GP Ib-IX-V, GP VI, or C-type lectin-like receptor 2 (CLEC-2) are all membrane glycoproteins exclusively expressed in platelets and megakaryocytes and have closely related signal transduction pathways. GP VI is thought to be the major signaling receptor involved in platelet activation on exposed collagen. Following GP VI interactions with collagen, platelets initiate strong activation and release the content of α- and dense granules. Recently, CLEC-2 has been identified as mediating the potent platelet activation response to rhodocytin, a platelet-activating snake venom. Platelet activation by GP VI and CLECL-2 is through

receptors containing the immunoreceptor tyrosine-based activation motif (ITAM) sequence. There is a growing evidence that platelets also function independently of full aggregation to regulate vascular permeability and development. Many of these novel platelet function depend on ITAM signaling rather than via activation through G protein-coupled receptors (GPCR). (Yun, S.H, et al, 2016).

Platelet secretion (Release reaction): The presentation of the procoagulant surface results in the co localization of different coagulation factors on the surface of the activated platelet, which triggers a series of zymogen conversions, resulting in the release of active thrombin from prothrombin (Coughlin, S.R, 2005). Adenosine diphosphate (ADP), adenosine triphosphate, and serotonin are released from the dense platelet granule. Activated phospholipase A2 enzymes release arachidonic acid (AA) by the cleaving of fatty acids, especially phosphatidylcholine and phosphatidylethanolamine, at their sn-2 position. AA is a precursor for thromboxane A2 (TBXA2) synthesis. In the first step in platelets, prostaglandin (PG)-endoperoxide synthase 1 (PTGS1; also known as cyclooxygenase 1) catalyzes the transformation of AA into cyclic endoperoxide PG G2 and H2. In platelets, PGG2 and PGH2 are then mainly converted by TBXA synthase into TBXA2. (Stassen, J.M, et al, 2004).

Platelet aggregation: The processes described above result in the local accumulation of molecules such as thrombin, TBXA2, and ADP, which are important for the further recruitment of platelets and the amplification of activation signals as described above. The secreted agonists activate their respective G protein-coupled receptors: coagulation factor II (thrombin) receptors (F2R also known as protease-activated receptor 1; F2RL3 also known as protease-activated receptor 4), TBXA2 receptor

(TBXA2R), and ADP receptors (P2RY1 and P2RY12). The P2RY12 receptor couples to Gi, and when activated by ADP, inhibits adenylate cyclase. This interaction counteracts the stimulation of cyclic AMP formation by endothelial-derived PGs, which alleviates the inhibitory effect of cyclic AMP on inositol 1,4,5-trisphosphate-mediated calcium release. P2RY12 has a major role in arterial thrombosis and pharmacologic targeting of this receptor, which is an important strategy in the treatment of cardiovascular diseases. Thienopyridines (ticlopidine, clopidogrel, prasugrel), a class of oral anti-platelet agents, permanently inhibit P2RY12 signaling by irreversibly binding the receptor and blocking ADP-induced platelet activation and aggregation.

F2R, TBXA2R, and P2RY1 couple to Gq-phospholipase C–inositol 1,4,5-trisphosphate–Ca2+ pathway, inducing shape change and platelet aggregation. In addition, receptor signaling by $G_{12/13}$ (F2R; TBXA2R) contributes to morphologic changes through the activation of kinases. Platelet adhesion, cytoskeletal reorganization, secretion, and amplification loops are all different steps toward the formation of a platelet plug. These cascades finally result in the activation of the fibrinogen receptor (GPIIb/GPIIIa) expressed on platelet cells. This activation results in the exposure of the binding sites for fibrinogen, which are not available in inactive platelets. The binding of fibrinogen results in the linkage of the activated platelets through fibrinogen bridges, thereby mediating aggregation. (Sangkuhl, K, et al, 2011).

1.2.5.2 Secondary Hemostasis:

Secondary hemostasis 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 crosslinked

fibrin mesh at the site of an injury. Fibrin generation occurs simultaneously to platelet aggregation (Furie, B , 2009).

Coagulation cascade has been traditionally classified into intrinsic and extrinsic pathways, both of which converge on factor X activation. (Palta, S ,et al.2014).

The extrinsic pathway is initiated by the entry of tissue thromboplastin into the circulating blood. Tissue thromboplastin is derived from phospholipoproteins and organelle membranes from disrupted tissue cells. These membrane lipoproteins, termed **tissue factors**, are normally extrinsic to the circulation. Platelet phospholipids are not necessary for activation of the extrinsic pathway because tissue factor supplies its own phospholipids. Factor VII binds to these phospholipids in the tissue cell membranes and is activated to factor VIIa, a potent enzyme capable of activating factor X to Xa in the presence of ionized calcium. The activity of the tissue factor–factor VII complex seems to be largely dependent on the concentration of tissue thromboplastin. The proteolytic cleavage of factor VIIa by factor Xa results in inactivation of factor VIIa. Factor VII participates *only* in the extrinsic pathway. Membranes that enter the circulation also provide a surface for the attachment and activation of factors II and V. The final step is the conversion of fibrinogen to fibrin by thrombin. (Turgeon, M.L, 2012).

The early part of the intrinsic pathway is called contact phase. This phase is carried out by factor XII (contact factor), prekallikrein, and high-molecularweight (HMW) kininogen. In vitro contact phase is initiated by the binding of factor XII to negatively charged surfaces, such as glass or kaolin. This leads to the formation of the enzyme factors XIIa and kallikrein, and the release of bradykinin from HMW kininogen. Factor XIIa then activates factor XI. The resulting factor XIa converts factor IX

to factor IXa, a reaction that requires the presence of calcium. Factor IXa then forms a complex with its co-factor protein factor VIIIa on a negatively charged membrane surface. This enzymatic complex, also referred to as tenase complex, converts factor X to factor Xa.

After both the extrinsic and intrinsic pathways have resulted in the formation of factor Xa, the ensuing reactions of the coagulation pathway are the same and are referred to as the common pathway. Based on the discovery that the factor VIIa-TF complex also activates factor IX to factor IXa, which appears to be the favored reaction, a new revised concept of coagulation was suggested in which factor VIIa-TF complex is thought to be the main initiator of coagulation, whereas the intrinsic pathway is considered necessary to sustain the coagulation response. (Munker, R, etal. 2007).

Once factor X is activated to Xa, the extrinsic and intrinsic pathways enter a common pathway. Factor II, prothrombin, is activated to thrombin (factor IIa), which normally circulates in the blood as an inactive factor. Following the activation of factor Xa, it remains platelet bound and activates factor V. The complex of factors Xa and Va on the platelet surface is formed near platelet-bound factor II molecules. In turn, the platelet-bound Xa/Va complex cleaves factor II into thrombin, factor IIa. The stage is accelerated by factor V and ionized calcium.

Clotting is the visible result of the conversion of plasma fibrinogen into a stable fibrin clot. Thrombin plays a major role in converting factor XIII to XIIIa and in converting fibrinogen to fibrin. Fibrin formation occurs in three phases: proteolysis, polymerization, and stabilization.

Initially, thrombin, a protease enzyme, cleaves fibrinogen, which results in a fibrin monomer, fibrinopeptide A, and fibrinopeptide B fragments. In the second step, the fibrin monomers spontaneously polymerize end-to-end due to hydrogen bonding. Finally, the fibrin monomers are linked

covalently by factor XIIIa into fibrin polymers. These polymers form a meshy network, and the final fibrin solution is converted to a gel when more than 25% of the fibrinogen is converted to fibrin. (Turgeon, M.L .2012).

1.2.5.3 Fibrinolysis:

The fibrinolytic system in mammalian blood plays an important role in the dissolution of blood clots and in the maintenance of a patent vascular system. The fibrinolytic system comprises an inactive proenzyme, plasminogen, that can be converted to the active enzyme, plasmin, that degrades fibrin into soluble fibrin degradation products. Two immunologically distinct physiologic plasminogen activators have been identified in blood: tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). Inhibition of the fibrinolytic system may occur either at the level of the plasminogen activators, by specific plasminogen activator inhibitors (PAI-1 and PAI-2), or at the level of plasmin, mainly by 2 -antiplasmin.

Tissue-type plasminogen activator mediated plasminogen activation is primarily involved in the dissolution of fibrin in the circulation, whereas u-PA binds to a specific cellular receptor (u-PAR) resulting in enhanced activation of cell-bound plasminogen. The main role of u-PA is the induction of pericellular proteolysis during tissue remodeling and repair, macrophage function, ovulation, embryo implantation, and tumor invasion. (Hoffman, R, et al. 2000).

1.2.6 Thrombosis:

The term thrombosis refers to the formation, from constituents of the blood, of a mass within the venous or arterial vasculature of a living animal. Hemostatic thromboses, namely, self-limited and localized thromboses that prevent excessive blood loss, represent the body's natural and desired response to acute vascular injury. Pathologic thromboses such

as deep venous thrombosis (DVT), pulmonary embolism (PE), coronary arterial thrombosis leading to myocardial infarction (MI), and cerebrovascular thrombotic occlusion represent the body's undesired response to acute and chronic perturbations of the vasculature, blood, or both. Thrombosis of the veins and arteries, together with complicating embolic phenomena, is perhaps the most important cause of sickness and death in the developed countries of the world at the present time.

In the mid-nineteenth century (1854), German pathologist Rudolph Virchow postulated that vascular obstruction was precipitated by, and thrombosis resulted from, three interrelated factors: (a) "decreased blood flow" (stasis of blood flow), (b) "inflammation of or near the blood vessels" (vascular endothelial injury), and (c) "intrinsic alterations in the nature of the blood itself" (hypercoagulability). (Greer, J.P, etal. 2009).

Arterial thrombosis usually occurs after the erosion or rupture of an atherosclerotic plaque and, through platelet-mediated thrombi, can cause ischaemic injuries especially in tissues with a terminal vascular bed. Indeed, cardiac ischaemia and stroke are the most severe clinical manifestations of atherothrombosis. Ischaemia can arise slowly from the progression of atherosclerotic disease (stable angina, claudication) or acutely in the case of vascular (atherosclerotic plaque rupture) or intracardiac (atrial fibrillation, mechanical valve prostheses) thromboembolisation. Venous thromboembolism (VTE) is the most common vascular disease after acute myocardial infarction and stroke. It is represented by two main clinical events: deep venous thrombosis (DVT) and pulmonary embolism (PE), which often constitute an unique clinical picture in which PE follows DVT. Although VTE is a common disease, the underlying pathogenic mechanisms are only partially known, particularly in comparison to those of atherothrombosis.

Venous thrombi are mainly constituted by fibrin and red blood cells, and less by platelets. In contrast, platelets are essential for primary haemostasis, repair of damaged endothelium and play a pivotal role in the development of atherosclerosis. Inflammation, lipids and the immune system, through a complex interplay, are also important determinants of arterial and, albeit to a lesser extent, of venous thrombosis. (Previtali, E, etal. 2011).

We now know that abnormally high levels of some coagulation factors and defects in the natural anticoagulants contribute to thrombotic risk. Among these, factor V Leiden, which renders factor Va resistant to activated protein C, is the most prevalent with approximately 5% of the Caucasian population having this genetic alteration. These genetically controlled variants in coagulation factors work in concert with other risk factors, such as oral contraceptive use, to dramatically increase thrombotic risk. While these abnormalities in the blood coagulation proteins are associated with thrombotic disease propensity, they are less frequent contributors to thrombosis than age or cancer. Cancer increases thrombotic risk by producing tissue factor to initiate coagulation, by shedding procoagulant lipid microparticles or by impairing blood flow. Age is the strongest risk factor for thrombosis. Among possible reasons are fragility of the vessels potentially contributing to stasis, increased coagulation factor levels, impaired function of the venous valves, decreases in the efficacy of natural anticoagulants associated with the vessel wall, increased risk of immobilization and increased risk of severe infection.(Esmon, C. T. 2010).

1.2.7 Bleeding:

The main bleeding disorders are genetically inherited. Hemophilia results from defects in secondary hemostasis. Hemophilia A is due to deficiency

of factor VIII, and hemophilia B is due to deficiency of factor IX. Activated factor VIII and activated factor IX together form the intrinsic factor Xase complex on activated membrane surfaces that is critical in the positive feedback loop of blood coagulation (Dahlback, B. 2000). Therefore, deficiency in either of these proteins causes a very similar bleeding phenotype characterized by excess bruising, spontaneous bleeding into joints, muscles, internal organs and the brain. Factor VIII and factor IX are both X-linked genes. Thus hemophilia is primarily expressed in males, with hemophilia A present in about 1 in 5000 males and hemophilia B present in about 1 in 20,000 males. However, multiple mutations in either factor VIII or factor IX have been identified, and not all of them cause complete loss of protein or protein function. In fact, almost half of hemophilia A sufferers have *de novo* mutations that were not inherited from their parents. Depending on the mutation, hemophilia can be severe (<1% function), moderate (1–5%) or mild (5–20%) (Mannucci, P.M and Tuddenham, E.G. 2001)**.**

Von Willebrand disease (VWD) is a bleeding disorder caused by deficiency or defect in von Willebrand factor (VWF). VWF is involved in platelet aggregation and is also a carrier for factor VIII. Thus deficiency of VWF causes defects in platelet aggregation but also causes a deficiency of factor VIII. VWD can result from multiple different mutations in VWF. These different mutations cause various different forms of VWD. These have been grouped into three overall categories. Type 1 is a partial quantitative defect, while type 3 results from a complete absence of VWF. In type 2 VWD, a normal amount of VWF is present but it has functional defects. Type 2 VWD is broken down into several subcategories. VWF is an autosomal gene, so the disease is present equally in men and women. The severity of the different types of

VWD varies, and various therapies are available and preferred for different forms of the disease. (Gale, A. J. 2011).

There are several genetic platelet disorders that derive from defects in platelet receptors. Bernard-Soulier syndrome is due to a deficiency of GPIb-IX-V. As a consequence, these individuals are defective in platelet aggregation because their platelets do not bind VWF. Bernard-Soulier syndrome is also characterized by abnormally large platelets and thrombocytopenia. Glanzmann thrombasthenia is a deficiency of αIIbβ3. Since αIIbβ3 is also critical for platelet aggregation; and since it binds to fibrinogen, collagen, VWF, fibronectin and vitronectin; these individuals are also defective in platelet aggregation. (Gale, A. J. 2011).

1.2.8 Diabetes Mellitus:

Diabetes mellitus (DM) is a common disease in which the blood sugar (glucose) is abnormally elevated. Normally, the body obtains glucose from food, and additional glucose is made in the liver. The pancreas produces insulin, which enables glucose to enter cells and serve as fuel for the body. In patients with diabetes, glucose accumulates in the blood instead of being properly transported into cells. Excess blood sugar is a serious problem that may damage the blood vessels, heart, kidneys, and other organs. (Hightower, C. 2016).

1.2.8.1 Pancreas:

The pancreas, named for the Greek words *pan* (all) and *kreas* (flesh), is a 12-15–cm long J-shaped (like a hockey stick), soft, lobulated, retroperitoneal organ. It lies transversely, although a bit obliquely, on the posterior abdominal wall behind the stomach, across the lumbar (L1-2) spine. (Kapoor, V.K. 2015).

Gross Anatomy: In adult humans, the pancreas weighs about 80 g. The pancreas is a retroperitoneal organ and does not have a capsule. The

second and third portions of the duodenum curve around the head of the pancreas. The spleen is adjacent to the pancreatic tail. The regions of the pancreas are the head, body, tail and uncinate process. The distal end of the common bile duct passes through the head of the pancreas and joins the pancreatic duct entering the duodenum.

Because of its posterior position, the pancreas is usually protected from trauma. However, it is just anterior to the vertebral column, and severe blunt trauma to the upper abdomen as might occur from a steering wheel in an auto accident can "crush" the pancreas against the vertebral column and cause severe injury. (Pandol, S.J. 2010).

The pancreas is typically divided into five parts;

- Head: This is the widest part of the pancreas. It lies within the C-shaped curve created by the duodenum, and is connected to it by connective tissue.
- Uncinate process: This is a projection arising from the lower part of the head and extending medially to lie beneath the body of the pancreas. It lies posterior to the superior mesenteric vessels.
- Neck: Located between the head and the body of the pancreas. It overlies the superior mesenteric vessels which form a groove in its posterior aspect.
- Body: The body is centrally located, crossing the midline of the human body to lie behind the stomach and to the left of the superior mesenteric vessels.
- Tail: The left end of the pancreas that lies within close proximity to the hilum of the spleen. It is contained within the splenorenal ligament with the splenic vessels. This is the only part of the pancreas that is intraperitoneal. (Thompson, L. 2017).

The arterial blood supply to the pancreas is from two major arteries supplying the abdominal organs, the celiac and superior mesenteric arteries. Because of the dual blood supply, ischemia to the pancreas from vascular obstruction is uncommon. Venous drainage of the pancreas is via the splenic vein and the superior mesenteric vein draining into the portal vein. The splenic vein runs along the body of the pancreas.

The pancreas is innervated by both the parasympathetic and sympathetic nervous systems. The efferent parasympathetic system is contained within the branches of the vagus nerve that originates in the dorsal vagal complex (tenth cranial nerve nucleus) of the brain. The terminal branches of the vagus synapse with intrapancreatic ganglia. The postganglionic fibers innervate both exocrine and endocrine structures that are described in the next section. The sympathetic innervation originates in the lateral grey matter of the thoracic and lumbar spinal cord. The bodies of the postganglionic sympathetic neurons are located in the hepatic and celiac plexuses. The postganglionic fibers innervate blood vessels of the pancreas. (Pandol, S.J. 2010).

1.2.8.2 Classification of diabetes mellitus:

If any characteristic can define the new intentions for DM classification, it is the intention to consolidate etiological views concerning DM. The old and confusing terms of insulin-dependent (IDDM) or non-insulin-dependent (NIDDM) which were proposed by WHO in1980 and 1985 have disappeared and the terms of new classification system identifies four types of diabetes mellitus: type 1, type 2, “other specific types” and gestational diabetes. (Baynes, H.W .2015).

Type 1 diabetes is an autoimmune disease in which the β-cells of the pancreas do not produce sufficient insulin, a hormone which helps use

blood sugar (glucose) for energy. The cells become starved of energy and there will be excess of glucose in the blood. This is then followed by life threatening conditions of hypoglycemia, low blood sugar, and hyperglycemia, high blood sugar. When hypoglycemia develops, cells do not get enough glucose and patients suffer of confusion, loss of consciousness, and coma. Even death can results when the brain is deprived of glucose for too long.

Hyperglycemia and prolonged absence of insulin may lead to ketoacidosis, which is accumulation of ketones in the blood when the body uses fat for energy instead of glucose. This is because fatty acids cannot be converted into glucose at steady state. Ketones make the blood acidic and slow down all body functions. This also leads to a coma and eventually death**.** (Siddiqui, A.A, et al .2013).

Type 2 diabetes mellitus is a complex endocrine and metabolic disorder. The interaction between several genetic and environmental factors results in a heterogeneous and progressive disorder with variable degrees of insulin resistance and pancreatic β-cell dysfunction. Overweight and obesity are major contributors to the development of insulin resistance and impaired glucose tolerance. When β cells have not longer able to secrete sufficient insulin to overcome insulin resistance, impaired glucose tolerance progresses to type-2 diabetes. Abnormalities in other hormones such as reduced secretion of the incretin glucagon-like peptide 1 (GLP-1), hyperglucagonaemia, and raised concentrations of other counter-regulatory hormones also contribute to insulin resistance, reduced insulin secretion, and hyper glycaemia in type 2 diabetes. Overweight and obesity contribute to insulin resistance through several pathways, including an imbalance in the concentrations of hormones (eg, increased leptin, reduced adiponectin, and increased glucagon), increased

concentrations of cytokines (eg, tumour necrosis factor α, interleukin 6), suppressors of cytokine signalling (eg, suppressor of cytokine signalling), other inflammatory signals, and possibly retinol-binding protein 4.1, Concurrent alterations in β-cell function often include a period of compensatory hyperinsulinaemia with abnormal secretory dynamics. When insulin secretion is no longer sufficient to overcome insulin resistance, glucose intolerance progresses to type 2 diabetes. The decline in β-cell function seems to involve chronic hyperglycaemia (glucotoxicity), chronic exposure to non-esterifies fatty acids (lipotoxicity), oxidative stress, inflammation, and amyloid formation Patients with type 2 diabetes usually have pancreatic α-cell dysfunction that results in increased (or nonsuppressed) glucagon secretion in the presence of hyperglycaemia and probably reduced prandial GLP- 1 secretion. **.** (Siddiqui, A.A, et al .2013).

Gestational diabetes mellitus is an operational classification (rather than a pathophysiologic condition) identifying women who develop diabetes mellitus during gestation. (Women with diabetes mellitus before pregnancy are said to have “pregestational diabetes” and are not included in this group.) Women who develop type 1 diabetes mellitus during pregnancy and women with undiagnosed asymptomatic type 2 diabetes mellitus that is discovered during pregnancy are classified with gestational diabetes mellitus. However, most women classified with gestational diabetes mellitus have normal glucose homeostasis during the first half of the pregnancy and develop a relative insulin deficiency during the last half of the pregnancy, leading to hyperglycemia. The hyperglycemia resolves in most women after delivery but places them at increased risk of developing type 2 diabetes mellitus later in life. (Mayfield, J .1998).

Types of diabetes mellitus of various known etiologies are grouped together to form the classification called “Other Specific Types”. This group includes persons with genetic defects of beta-cell function (this type of diabetes was formerly called MODY or maturity-onset diabetes in youth) or with defects of insulin action; persons with diseases of the exocrine pancreas, such as pancreatitis or cystic fibrosis; persons with dysfunction associated with other endocrinopathies (e.g. acromegaly); and persons with pancreatic dysfunction caused by drugs, chemicals or infections and they comprise less than 10% of DM cases. (Baynes, H.W. 2015).

1.2.8.3 Pathogenesis of Type 1 Diabetes Mellitus:

The risk of developing diabetes is strongly influenced by genes affecting immune function, particularly the HLA system, but other factors are involved. Prospective studies in human populations reveal that circulating autoantibodies directed against the islets typically appear in the first 5 years of life, and may be present for many years (sometimes 20 years or more) before the disease develops.

Humoral immunity: The first evidence of circulating antibodies directed against the islets came from the demonstration of islet cell antibodies (ICA) by indirect immunofluorescence in 1974. ICA staining is a composite of antibodies directed against a variety of islet-associated molecular entities, and was superseded by specific assays as these entities came to be identified. Insulin autoantibodies (IAA) were reported in 1984, followed by antibodies directed against glutamic acid decarboxylase (GADA), islet antigen-2 (IA-2A) and the zinc transporter ZnT8. All these autoantigens are related to the beta cell secretory apparatus. Islet autoantibodies can typically be detected within the first few years of life, but may appear later; the appearance of multiple

antibody species indicates established islet autoimmunity and strongly predicts the subsequent onset of diabetes.

Cellular immunity: Type 1 diabetes appears to develop as the consequence of an imbalance between pathogenic and regulatory T lymphocytes. $CD4^+$ T cells and $CD8^+$ T cells appear to mediate beta cell destruction, and diabetes can be induced in animal models by transfer of these cells. $CD8^+$ T lymphocytes are capable of destroying beta cells by release of granules containing granzyme or perforin, or via Fas-ligand interactions. Circulating T lymphocytes directed against a wide range of islet antigens have been identified, but do not as yet offer the degree of specificity or disease prediction provided by measurement of islet autoantibodies. (Gale, E.A.M .2014).

1.2.8.4 Pathogenesis of Type 2 Diabetes Mellitus:

Understanding the pathogenesis of type 2 diabetes is complicated by several factors. Patients present with a combination of varying degrees of insulin resistance and relative insulin deficiency, and it is likely that both contribute to type 2 diabetes. Furthermore, each of the clinical features can arise through genetic or environmental influences, making it difficult to determine the exact cause in an individual patient. Moreover, hyperglycemia itself can impair pancreatic beta-cell function and exacerbate insulin resistance, leading to a vicious cycle of hyperglycemia causing a worsening metabolic state. Type 2 diabetes is often accompanied by other conditions, including hypertension, high serum low-density lipoprotein (LDL) cholesterol concentrations, and low serum high-density lipoprotein (HDL) cholesterol concentrations that, like type 2 diabetes, increase cardiovascular risk. This constellation of clinical conditions is referred to as the metabolic syndrome. Hyperinsulinemia occurring in response to insulin resistance may play an important role in

the genesis of these abnormalities. Increased free fatty acid levels, inflammatory cytokines from fat, and oxidative factors have all been implicated in the pathogenesis of metabolic syndrome, type 2 diabetes, and their cardiovascular complications. (McCulloch, D.K and Robertson, R.P . 2016).

1.2.8.5 Clinical Feature of Diabetes mellitus:

Thirst: arises as a consequence of dehydration resulting from loss of fluid, salt and other electrolytes in the urine. The acute thirst of type 1 diabetes may be almost unquenchable. Some attempt to slake their thirst with sugar-containing fluids such as Coca-Cola, thus creating a spiral of hyperglycaemia, dehydration and increased craving for fluids.

Polyuria: develops when the rate at which glucose enters the proximal tubules of the kidney exceeds the capacity of the tubules to pump glucose back into the circulation. This is achieved by an active transport system which (in most people) can extract almost all glucose below a concentration of ~10 mmol/l (180 mg/dl). Above this point, known as the renal threshold for glucose, glucose spills over into the urine. This exerts an osmotic effect, causing loss of water, salt and other electrolytes from the body, and resulting in dehydration and thirst.

Weight loss: is a consequence of calories lost as glucose in the urine, amounting to hundreds of grams of glucose per day in severely uncontrolled diabetes. This is aggravated by insulin deficiency, which accelerates glucose production by the liver while promoting breakdown of fat and protein; the glucose loss and metabolic inefficiency of uncontrolled diabetes thus produces a state of accelerated catabolism.

Tiredness and lack of energy: are common symptoms, but not at all specific for diabetes.

Changing glucose levels can produce osmotic changes in the lens of the eye, causing changes in visual accommodation resulting in visual blurring, and many freshly diagnosed patients have a new pair of glasses which (unfortunately) will no longer be right for them once the glucose abnormality has been corrected! Another common presentation is with pruritus vulvae (genital itching) in women or balanitis (inflammation of the prepuce) in men. This is due to *Candida albicans*, a fungal infection which grows more readily in the presence of glucose. (Diapedia Collective, 2014).

1.2.8.6 Complication of Diabetes Mellitus:

Diabetic ketoacidosis (DKA): is a life-threatening condition associated with increased fat metabolism and production of harmful acids (ketones). Patients diagnosed with DKA develop very high blood sugar levels, abdominal pain, fruity-smelling breath, dehydration, severe weakness, lethargy, and coma.

Damage to nerves (neuropathy): in the arms and legs causes decreased sensation, numbness, and tingling. Neuropathy and poor blood circulation in the feet can cause non-healing wounds and infections that may necessitate lower extremity amputations. (Hightower, C . 2016).

Eye: Hyperglycaemia has direct effects on the hydration state of the ocular lens, which explains why some patients complain of a blurry vision when acutely hyperglycaemic. And chronic hyperglycaemia can lead or contribute to cataract which gradually impairs vision. However, the true damage to the eye, diabetic retinopathy, starts with small retinal changes and only results in visual problems at the advanced proliferative stage when vitreous bleeding followed by retinal detachment can result in permanent visual loss.

Kidney: The first sign of diabetic nephropathy is micro-albuminuria, the presence of small quantities of albumin in the urine (30-300mg/24 hrs). At this stage of incipient nephropathy, kidney function as expressed by the glomerular filtration rate (GFR) is generally preserved. Moreover, the progression of albuminuria can be slowed or indeed reversed by appropriate treatment. Once macro-albuminuria (albumin excretion of over 300mg/24 hrs) develops, i.e. overt nephropathy, it is usually harder to stop the progression of the disease and a decline in kidney function (GFR) may ensue which can eventually necessitate renal replacement therapy (dialysis or kidney transplantation). (Holleman, F . 2014).

Macrovascular Complications of Diabetes: The central pathological mechanism in macrovascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls throughout the body. Atherosclerosis is thought to result from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system.

In addition to atheroma formation, there is strong evidence of increased platelet adhesion and hypercoagulability in type 2 diabetes. Impaired nitric oxide generation and increased free radical formation in platelets, as well as altered calcium regulation, may promote platelet aggregation. Elevated levels of plasminogen activator inhibitor type 1 may also impair fibrinolysis in patients with diabetes. The combination of increased coagulability and impaired fibrinolysis likely further increases the risk of vascular occlusion and cardiovascular events in type 2 diabetes. (Fowler, M.J .2008).

1.2.8.7 Diagnosis of Diabetes Mellitus:

The diagnosis of diabetes is based on one of three methods of blood glucose measurement. Diabetes can be diagnosed if the patient has a

fasting blood glucose level of 126 mg per dL (7.0 mmol per L) or greater on two separate occasions. The limitations of this test include the need for an eight-hour fast before the blood draw, a 12 to 15 percent day-to-day variance in fasting blood glucose values, and a slightly lower sensitivity for predicting microvascular complications.

Diabetes can also be diagnosed with a random blood glucose level of 200 mg per dL (11.1 mmol per L) or greater if classic symptoms of diabetes (e.g., polyuria, polydipsia, weight loss, blurred vision, fatigue) are present.

The oral glucose tolerance test is considered a first-line diagnostic test. Limitations include poor reproducibility and patient compliance because an eight-hour fast is needed before the 75-g glucose load, which is followed two hours later by a blood draw. The criterion for diabetes is a serum blood glucose level of greater than 199 mg per dL (11.0 mmol per L). **(**Patel, P and Macerollo, A . 2010).

A1C is a widely used marker of chronic glycemia, reflecting average blood glucose levels over a 2- to 3-month period of time. The test plays a critical role in the management of the patient with diabetes, since it correlates well with both microvascular and, to a lesser extent, macrovascular complications and is widely used as the standard biomarker for the adequacy of glycemic management. Prior Expert Committees have not recommended use of the A1C for diagnosis of diabetes, in part due to lack of standardization of the assay. However, A1C assays are now highly standardized so that their results can be uniformly applied both temporally and across populations. In their recent report, an International Expert Committee, after an extensive review of both established and emerging epidemiological evidence, recommended the use of the A1C test to diagnose diabetes, with a threshold of ≥6.5%, and ADA affirms this decision. (American Diabetes Association . 2010).

1.2.8.8 Treatment:

- Controlling blood sugar (glucose) levels is the major goal of diabetes treatment, in order to prevent complications of the disease.
- Type 1 diabetes is managed with insulin as well as dietary changes and exercise.
- Type 2 diabetes may be managed with non-insulin medications, insulin, weight reduction, or dietary changes.
- The choice of medications for type 2 diabetes is individualized, taking into account:
    - the effectiveness and side effect profile of each medication,
    - the patient's underlying health status,
    - any medication compliance issues, and
    - cost to the patient or health-care system.
- Medications for type 2 diabetes can work in different ways to reduce blood glucose levels. They may:
    - increase insulin sensitivity,
    - increase glucose excretion,
    - decrease absorption of carbohydrates from the digestive tract, or
    - work through other mechanisms.
- Medications for type 2 diabetes are often used in combination. (Ferry, R . 2016).

1.2.8.9 Diabetes as Hypercoagulable state:

Diabetes-related mortality is associated with thrombotic events, especially cardiovascular. In general, patients with diabetes present symptoms of hypercoagulability and hypofibrinolysis.

Diabetics have abnormalities of the endothelium, platelets, clotting factors, natural anticoagulants and the fibrinolytic system; all these changes are directly and/or indirectly caused by hyperglycemia. Thus, analytes such as von Willebrand factor, factor VIII, fibrinogen and D-dimer are markers that should be interpreted differently in diabetic patients. Laboratory evidence of hemostatic abnormalities in diabetic patients supports clinical observations that diabetes is a state of hypercoagulability and hypofibrinolysis. (Soares, A.L, etal . 2010).

Hyperglycemia directly contributes to endothelial injury through irreversible glycation of collagen and other subendothelial structural proteins of the vessel, forming advanced glycation end products (AGEs). AGEs accumulate in the subendothelium over time influenced by increases in blood sugar levels and are directly related to atherosclerosis and renal failure. AGEs cause changes in structure and biophysical properties of the basement membrane causing changes in permeability and vasodilation of blood vessels. (Tooke , J.E .1995).

Hyperglycemia increases the propensity of platelets to aggregate and degranulate. *In vivo* studies have shown evidence of increased platelet activation in patients with metabolic syndrome and T2DM due to increased levels of beta-thromboglobulin and platelet factor 4 in the plasma; these markers are only stored in platelet granules. An increase of P-selectin (CD62P) on the platelet surface of diabetic individuals has also been described. This is a platelet activation marker directly related to the formation of thrombi.

Metabolic syndrome subjects and T2DM patients have increased Factor VII (FVII) levels. This increase in FVII is related to the dyslipidemia present in both conditions. There is a positive correlation between FVII

and triglycerides, with one hypothesis being that part of FVII circulates in plasma bound to very low-density lipoprotein particles rich in triglycerides, thereby prolonging the plasma half-life of FVII.

The factor VIII/vWF complex is also increased in individuals with insulin resistance and T2DM. This increase may be related to the presence of endothelial dysfunction and/or an inflammatory process, since vWF is an acute phase protein that is stored in the form of multimers in endothelial cells. Fibrinogen, another acute phase protein, is increased in diabetic patients.

Hyperglycemia acts on the fibrinolytic system by stimulating PAI-1 production. This condition favors the permanence of the fibrin clot, and consequently the development of thrombi. In many studies, diabetic patients had increased levels of D-Dimer. However, under normal conditions, when there is a hypercoagulable state, there is consequently a state of hyperfibrinolysis. As hypercoagulability and hypofibrinolysis states are present in diabetic patients, the expression of markers such as Di-Dimer may be underestimated. . (Soares, A.L, et al . 2010).

1.3 Rationale:

Diabetes Mellitus is Associated with many hemostatic abnormalities, and considered as a hypercoagulable state**,** it‘s characterized by a high risk of atherothrombotic events, and venous thrombosis has also been found to occur more frequently in this patient group.

This prothrombotic condition in diabetes is underpinned by laboratory findings of elevated coagulation factors and impaired fibrinolysis. Hyperglycemia plays an important role in the development of these hemostatic abnormalities, as is illustrated by the association with glycemic control and the improvement upon treatment of hyperglycemia.

In order to assess these hemostatic abnormalities, in this study we demonstrate PT, APTT, and D Dimer in diabetic patients to help in early diagnosis and management of hemostatic abnormalities.

1.4 Objectives:

1.4.1 General objectives:

To determine Prothrombin Time (PT), Activated Prothrombin Time (APTT), and D dimer in patients with diabetes mellitus in Sudan.

1.4.2 Specific objectives:

1. To measure Prothrombin Time (PT) in patients with diabetes mellitus.
2. To measure Activated Prothrombin Time (APTT) in patients with diabetes mellitus.
3. To measure D dimer in patients with diabetes mellitus.
4. To compare between coagulation parameters and sex among group of DM.
5. To determine the effect of duration of DM on coagulation parameters in study group.

Chapter two

Material and method

2.1 Study design:

This is Descriptive analytical case control study conducted from October 2017 _October 2018. Aimed to measure prothrombin time, activated partial thromboplastin time, and D dimer in Diabetes Mellitus patients (case) and non Diabetes Mellitus individuals (control).

Sample size formula: $n = \frac{z^2\, pq}{d^2}$

Where: z is the z score

d is the margin of error

p is population proportion

n $= \frac{1.96 \times 1.96 \times 0.5 \times 0.5}{0.05 \times 0.05} = 384.16 \approx 385$

The sample size is 385samples and according to my budget I select 40 samples.

2.2 Study area and population:

This study was conducted in Ystabshiron Hospital, sample size of 40 venous blood samples was collected from diagnosed Diabetic patients and 40 samples were collected from healthy individuals as control.

2.3 Sampling:

Individual whom diagnosed as Diabetic were selected and data collected using self –administrated per-coded questionnaire which was specifically designed to obtain informative data.

2.4Sample:

The venous puncture site was cleaned by 70% alcohol, and with a sterile

disposable syringe, 3ml of blood was collected and transfer to Tri Sodium Citrate (TSC) buffered blood containers and gently mixed.

The sample was centrifuged at 1300 rpm for 15min to obtain platelet poor plasma (PPP) .the PPP used for prothrombin time (PT), activated partial thromboplastin time (APTT) and D- dimer.

2.5 Inclusion criteria:

- Diagnosed Diabetes Mellitus patients.
- Non Diabetes Mellitus individual as control group for comparing.

2.6 Exclusion criteria:

- Thrombotic patients.
- Hypertension patients.
- Pregnant women.
- Smokers.

2.7 Data analysis:

- The collected data proceed for analysis using SPSS version 11.5 computerized program and the data presented in form of tables.

2.8 methods:

2.8.1 Prothrombin time PT by Stago Hemostasis Analyzer:

2.8.1.1 Principle of Stago Hemostasis Analyzer:

STAGO STart Max Coagulation Analyzer is an efficient semi-automated bench top system, integrated with Stagos patented electro-mechanical clot detection method (Viscosity-based Detection System).

2.8.1.2 Principle of PT:

The PT was performed by automated testing measure the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) with calcium chloride (cacl2) which indicates over all the efficiency of the extrinsic clotting system.

2.8.1.3 Reagent and materials:

1. Pooled normal plasma control.

2. Prothrombin time kits.

Kit contents: PT reagent contains an extract of rabbit brain with buffer, stabilizer and calcium chloride.

3. Coagulation analyzer

4. Small cuvettes

5. Magnetic.

6. Pipette tips.

7. Calibrated pipettes.

2.8.1.4 Assay procedure:

-Cuvettes were placed in incubation area for pre warming at 37c for at least 3 minutes.

- A magnetic was dispensed to each Cuvette, in the incubation area 100ul of ppp of patient or control was dispensed in each Cuvette.

- After warming the Cuvette transferred to test column area, 100ul of the reagent (Ca+ /thromboplastin) was dispensed into Cuvette in the test column area.

- The time was started immediately, Then after clot produced the instrument automatically stopped the timer and the result of PT appear at the display of the instrument per seconds.

2.8.1.5 Normal value:

11-17 seconds (depend on PT reagent).

2.8.2 Activated Partial Thromboplastin time (APTT) by Stago Hemostasis Analyzer:

2.8.2.1 Principle of APTT:

The APTT was performed by automated testing in the batch or state mode. In the APTT an aliquot of undiluted platelet poor plasma was incubated at 37c with a particulate factor XII activator(i.e. ,silica, celite, kaolin ,ellagic acid ,etc).A reagent containing phospholipid (partial thromboplastin) was added, followed by CaCl2 .the time required for clot formation after the addition of CaCl2 it measure over all the activity of intrinsic pathway.

2.8.2.2 Reagent and Materials:

-Pooled normal plasma control.

-Activated partial thromboplastin time kits.

Kit contents: APTT reagent contains kaolin cephalin with phospholipid, buffer and preservatives. CaCl2 (.025M) contain sodium azide.

-Coagulation analyzer.

-Small cuvettes.

-Magnetic.

-Pipette tips.

-Calibrated pipettes.

2.8.2.3 Assay procedure:

- Cuvettes were placed in incubation area for pre warming at 37c for at least 3 minutes.

- A magnetic was dispensed to each cuvette in the incubation area, 100ul of PPP of patient or control was dispensed in each cuvette.

- 100 of phospholipid reagent was added to each cuvette .

- After incubation for 3 minutes the cuvette transformed to test column area, then 100ul of calcium reagent was added to each cuvette in the column area.

- The timer was started immediately by pressing the pipette key, then after clot produce the instrument automatically stopped the timer and the result of APTT appear at the display of the instrument per seconds.

2.8.2.4 Normal value:

27 - 42 seconds (depend on APTT reagent).

2.8.3 D dimer using ichroma™ Reader:

2.8.3.1Principle of ichroma™ D-Dimer:

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

The working range of ichroma™ D-Dimer test is 50 – 10,000 ng/ml.

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

2.8.3.2Components and Reagents:

Ichroma™ D-Dimer consists of Cartridge, an ID Chip, and Detection Buffer tubes.

The test cartridge contains a test strip, the membrane which has antibodies against D-Dimer and streptavidin have been immobilized at the test line and the control line respectively.

- Each test cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed test cartridges are packed in a box which also contains an ID chip.

- The detection buffer pre-dispensed in a tube contains fluorochrome-labeled anti-D-Dimer antibodies, fluorescent labeled biotin-BSA, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.

- 25 detection buffer tubes are packed in a separate pouch and further packed in a Styrofoam box with ice packs for the purpose of shipment.

2.8.3.3 Test procedure:

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

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

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

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

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

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

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

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

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

2.8.3.4Interpretation of the result:

- Ichroma™ Reader calculates the test result automatically and displays D-Dimer concentration of the test sample as ng/mL.

- Working range of ichroma™ D-Dimer is 50-10,000 ng/mL.

- Reference value of ichroma™ D-Dimer is 500 ng/ml. (FEU: Fibrinogen equival units).

Chapter three

Results

A Case control study 40 sample collected from Diabetes Mellitus patients and 40 samples collected as control from healthy (non Diabetes Mellitus individuals) include frequency of sex was 17 male (42.5%) and 23 female(57.5%) ,frequency of age group <40 years 12 (30%), 40-60 years 16(40%) and > 60years 12(30%), and frequency of DM duration 1-15 years 29 (72.5%), 16-30 years 11 (27.5%) .

Characteristic		Frequency	Percent%
Sample	Case	40	50
	Control	40	50
Sex	Male	17	42.5
	Female	23	57.5
Age	20-40 years	12	30
	40-60 years	16	40
	>60 years	12	30
DM duration	1-15 years	29	72.5
	16-30 years	11	27.5

Table (3.1) Demographic characteristic of study participants:

Table (3.2) show significant increase in mean of PT, INR, APTT, and D-dimer when compared with control (p. value < 0.05).

Table (3.2): Mean and standard deviation of coagulation parameters among study population:

Coagulation parameters	Sample N=40	Mean±SD	P.value
PT	Case	18.19±7.7	0.001
	Control	13.85±1.9	
INR	Case	1.36±0.6	0.003
	Control	1.03±0.1	
APTT	Case	37.73±15.2	0.042
	Control	32.55±3.3	
D-dimer	Case	992.91±1488.7	0.00
	Control	16.27±49.8	

Table (3.3) show means of PT, INR. APTT and D dimer among sex group of DM, there is no significant differences between means of males and females (P. value > 0.05).

Table (3.3): Correlation between coagulation profile and sex among study population:

Coagulation Parameters	Sex	Number	Mean±SD	P.value
PT	Male	17	20.56±11.1	0.154
	Female	23	16.43±2.7	
INR	Male	17	1.56±0.9	0.142
	Female	23	1.21±0.2	
APTT	Male	17	38.44±7.8	0.803
	Female	23	37.20±19.2	
D-dimer	Male	17	815.85±1290.3	0.525
	Female	23	1123.77±1635.9	

Table (3.4) show means of PT, INR. APTT and D dimer among DM duration groups, there is no effect of duration of DM on coagulation parameters (PT, INR, APTT, and D-dimer) (P-value > 0.05).

Table (3.4) Correlation between coagulation profile and duration of DM among study population:

Coagulation parameters	Duration groups	Number	Mean±SD	P-value
Pt	1-15 years	29	18.59±8.9	0.597
	16-30 years	11	17.12±2.3	
INR	1-15 years	29	1.39±0.7	0.654
	16-30 years	11	1.29±0.1	
APTT	1-15 years	29	35.05±7.5	0.249
	16-30 years	11	44.78±26.0	
D-dimer	1-15 years	29	969.61±1180.9	0.875
	16-30 years	11	1054.32±2175.6	

Chapter four

Discussion, Conclusion and Recommendations

4.1Discussion:

Diabetes is a major public health problem that is approachin*g* epidemic proportions globally. Worldwide, the prevalence of chronic, noncommunicable diseases is increasing at an alarming rate. About 18 million people die every year from cardiovascular disease, for which diabetes and hypertension are major predisposing factors. Today, more than 1.7 billion adults worldwide are overweight, and 312 million of them are obese. In addition, at least 155 million children worldwide are overweight or obese. A diabetes epidemic is underway. According to an estimate of International Diabetes Federation comparative prevalence of Diabetes during 2007 is 8.0 % and likely to increase to 7.3% by 2025. Number of people with diabetes is 246 million (with 46% of all those affected in the 40–59 age group) and likely to increase to 380 m by 2025. Almost 80% of the total adult diabetics are in developing countries. The regions with the highest rates are the Eastern Mediterranean and Middle East, where 9.2 % of the adult population is affected, and North America (8.4%). The highest numbers, however, are found in the Western Pacific, where some 67 million people have Diabetes, followed by Europe with 53 million. India leads the global top ten in terms of the highest number of people with diabetes with a current figure of 40.9 million, followed by China with 39.8 million. Behind them come USA; Russia; Germany; Japan; Pakistan; Brazil; Mexico and Egypt. (Tabish, S. A. (2007).

The prevalence of diabetes is highest among adults, and in Khartoum is 19.2% (Khartoum state risk factor survey 2005-2006) and 14.5% (Sudan House Hold survey 2010). Some community based studies revealed a high prevalence of diabetes of 24.3% in Argo Town in Dongola (2003) and 24.6% in Alkabashi Area 2006. (Almogtarebin University).

Diabetes mellitus (DM), a growing health problem itself, is accompanied by an increased risk of cardiovascular and thrombotic complications. The imbalance between coagulation and fibrinolysis processes observed in patients with diabetes may be defined as diabetic thrombophilia. Several mechanisms are involved in the hypercoagulability state in diabetics, including endothelial cell damage, altered platelet structure and function, increased microparticle formation, different structure of fibrin clots, disturbances in the activity of coagulation factors, fluctuations in the concentrations of fibrinolysis activators and inhibitors, and qualitative changes of proteins due to glycation and oxidation processes. These all are the reasons why DM is the most common cause of acquired thrombophilia. Moreover, diabetes changes the efficacy of certain medications. Results of various trials seem to suggest that thrombolytic drugs are less effective in patients suffering from this disease. The impact of DM on the effectiveness of treatment with acetylsalicylic acid (ASA) remains unclear. Awareness of thrombotic complications in diabetic patients may enable earlier diagnosis and proper therapy. (Kwiatkowski, J. etal. 2018).

The research of (Lodigiani, C. etal. 2009) pointed out an interesting aspect of the linking between factor V Leiden gene variant , diabetes a atherothrombosis and other vascular complication.

The research of (Sdogou, T. etal. 2013) report a case of 5.5 year old boy who was admitted to the pediatric department with Diabetic Ketoacidosis

(DKA) due to newly diagnosed type 1 diabetes, after DKA management, the child developed symptoms of iliofemoral deep vein thrombosis, A family history of protein S deficiency was revealed.

Vitamin B12 deficiency may be induced by long-term use of metformin, which may in turn lead to Hyperhomocysteinemia. Thus hyperhomocysteinemia may increase the risk of vascular thrombosis in diabetic patients, when metformin is used and a homozygous methylenetetrahydrofolate reductase C677T mutation is present. (Shen, M.C. etal. 2007).

This is case control study carried out in Khartoum state in Yastabshiron hospital, in the period from (October 2017 _ October 2018) to evaluate some coagulation parameters in Sudanese patient with Diabetes Mellitus in Khartoum state, 40 diagnosed Diabetes Mellitus patients were selected 17 (42.5%) male and 23(57.5%) female and 40 healthy individuals were selected as control group.

The results showed that there was significant differences in mean of PT, INR, APTT, and D-Dimer between patients group and control group (P.value < 0.05), and there was no significant differences in mean of PT, INR, APTT, and D-Dimer between both group of sex (P. value > 0.05), according to the duration of disease there was no significant differences in PT, INR, APTT, and D-Dimer (P. value > 0.05).

These results agree with study of (O Alao. etal. 2009) which observed that PT of diabetic subjects (15.7+\- 2.1) was significantly prolonged compared to that of non diabetic controls (14.9+\- 2.3) and APTT in diabetic subjects was significantly prolonged than that of controls ($p<0.05$),

These results also agree with study of (Pan, L. etal. 2018) which said that diabetes mellitus exhibited significantly different APTT and D-Dimer

levels compared with controls. Also agree with result of study of (Junbin, h. etal. 1998) in which the diabetic subjects displayed higher levels of D-Dimer.

The result of this study reveals that there was disturbance in coagulability in diabetic patients and this responsible from development of microvascular complications of diabetes mellitus, this is agree with study of (Madan, R. Etal, 2010) who have concluded that hypercoagulable state as indicated by decreased fibrinolysis and increased coagulability is responsible as one of the factors for development of microvascular complications of diabetes mellitus.

4.2 Conclusion:

Prothrombin time, Activated partial thromboplastin time, and D-dimer were increase, so patients with diabetes mellitus at risk to develop microvascular complications and thrombosis.

4.3 Recommendations:

- Prothrombin time, Activated partial thromboplastin time, and D-dimer should be considered for patients with diabetes mellitus to fallow up and management to avoid risk of thrombosis.
- Anti –coagulant therapy recommended for diabetics once Prothrombin time, Activated partial thromboplastin time, and D-dimer are increased.

Chapter five

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Sudan University of Science and Technology

College of Medical Laboratory Science

Department of Hematology

Questionnaire

Name: …… Age: ……

Gender: Male ( ) Female ( ).

Duration of disease: ……

Symptoms:

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History:

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Investigations:

Prothrombin Time: ……

Activated Prothrombin Time: ……

D- Dimer: ……..

Signature: ……**Date**……...