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Estimation of Platelets Count, Platelets Indices, Coagulation Profile and D-dimer among Sudanese Patients with Type 2 Diabetes Mellitus in Khartoum State 2022

قياس تعداد الصفائح الدموية، مؤشرات أحجام الصفائح الدموية، معاملات التخثر وثنائيات الدال عند المرضى السودانيين المصابين بداء السكر في ولاية الخرطوم 2022

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الأية

بسم الله الرحمن الرحيم

قال الله تعالى: (دَعْوَىٰهُمْ فِيهَا سُبْحَٰنَكَ ٱللَّهُمَّ وَتَحِيَّتُهُمْ فِيهَا سَلَٰمٌۚ وَءَاخِرُ دَعْوَىٰهُمْ أَنِ ٱلْحَمْدُ لِلَّهِ رَبِّ ٱلْعَٰلَمِينَ)

صدق الله العظيم

سورة يونس (10)

Acknowledgment

Firstly, all the thank goodness to ***Allah*** *who guided me to the strait way in all my life and give me the time, health, patience, and strength, for accommodating to complete this work in one piece, secondly thanks so much to my supervisor* ***Abdallah Musa Abdallah*** *this research couldn't be done without him, to* ***everybody*** *who helped me to complete this project, to* ***anyone*** *who participates with me in this work.*

Giving this humble research may help humanity.

Abstract

Diabetic patients are suffering from complication that with long term could lead to death; one of the important complications is hematological changes. This is an analytical case control study that aimed to evaluate platelet count, platelet indices, coagulation profile and D-dimer in type 2 diabetic patients. This study is conducted on 50 diabetic patients and 50 controls in Khartoum state in period between August to October 2022.

One hundred (100) venous blood samples were collected in EDTA anticoagulant and Sodium citrate anticoagulant tubes, blood samples were analyzed by Mission HA-360 3-Diff Automatic hematology analyzer, COARTON IM and BIOTIME instruments. Data obtained were analyzed statistically by SPSS version 16 using independent T test and One-way ANOVA test.

The results showed that platelet large cell ratio (P-LCR) was significantly increased in diabetic patients compared with control (18.69±6.90)and (14.90±4.42) (*p* value=0.03), Plateletcrit (PCT) also showed differences in case (0.28±0.08) in compare with control (0.26±0.06) with (*p* value = 0.04). And D-dimer also revealed significant change when compared with control group (0.50±0.05 vs 0.01±0.06) with *p* value=0.00. But there were no significant differences in platelet count, MPV, PDW, P-LCC, PT, INR and APTT when compared the diabetics to non-diabetics, with *p* values (0.22, 0.72, 0.43, 0.94, 0.46, 0.06 and 0.29) respectively.

Its concluded from the study that the variation in P-LCR, PCT and D-dimer between diabetic patients and non-diabetic indicate there was variation in platelet size, platelet volume and the ability to formation the thrombus that mean they are large and activated platelet which can cause thrombosis.

المستخلص

يعاني مرضى داء السكر من النوع الثاني من مضاعفات والتي بمرور الزمن قد تؤدي الى الوفاة، أهم هذه المضاعفات هي التغيرات الدموية .هذه دراسة تحليلية بين المرضى والفئة الضابطة بهدف تقدير بعض المعاملات في مرضى السكر النوع الثاني. أقيمت الدراسة على 50 من مرضى السكر و50 غير مصابين بالسكر في مدينة الخرطوم في الفترة بين اغسطس واكتوبر لعام 2022.

سحبت 100عينة دم وريدية في وعاء يحتوي على مانع التخثر إيثيلين ثنائي الأمين رباعي حمض الخليك وثلاثي سترات الصوديوم لتحليل الدم الكامل في جهاز التحليل الآلي وجهاز كوارتون وبايوتايم. البيانات المكتسبة تم تحليلها إحصائيا باستخدام برنامج الحزم الإحصائية للعلوم المجتمعية النسخة 16 باستعمال الاختبار الفرضي المستقل واختبار الطريقة الواحدة انوفا.

أظهرت النتائج وجود زيادة معنوية في مرضى السكر مقارنة بالأصحاء في نسبة الصفائح الدموية الكبيرة (18.69±6.90) و(14.90±4.42) (القيمة الإحصائية=30.0)، وأيضا حجم الصفائح الدموية المكدسة ظهر اختلاف عند مقارنة الفئة المريضة مع الفئة الضابطة (0.28±0.08) و (0.26±0.06) (القيمة الإحصائية=0.04) وثنائيات الدال ايضا كشفت عن اختلاف عندما تمت مقارنتها مع الفئة الضابطة (0.50±0.05) و(0.01±0.06) (القيمة الإحصائية=0.00) لكن لا يوجد اختلاف في عدد الصفائح الدموية, متوسط حجم الصفائح الدموية, نطاق توزيع الصفائح الدموية ,تركيز الصفائح الدموية الكبيرة, زمن البروثرومبين, معدل التطبيع العالمي والزمن الجزئي للثرومبوبلاستين المفعل عندما تمت مقارنة المصابين بالسكر مع غير المصابين مع وجود هذه القيم الإحصائية (0.22, 0.72, 0.43 , 0.94 ,0.46, 0.06 و 0.29) على التوالي.

استنتجت من الدراسة أن الفرق في نسبة الصفائح الدموية الكبيرة، حجم الصفائح الدموية المكدسة وثنائيات الدال بين مرضى السكر وغير المصابين بالسكر يدل على وجود اختلاف في حجم وكمية الصفائح الدموية والقدرة على تكوين الجلطة مما يدل على وجود صفائح دموية كبيرة ونشطة قد تؤدي إلى جلطة.

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Abbreviations	**Full text**
ADAMTS	A distingrin and metalloproteinase with a thrombospondin type 1 motif
APTT	Activated partial thromboplastin time
APC	Activated Protein C
APCR	Activated protein C resistant
ADP	Adenosine di-phosphate
AMP	Adenosine mono-phosphate
ATP	Adenosine tri-phosphate
AT	Antithrombin
AIDS	Aquired immune deficiency syndrome
Arg	Arginine
BSS	Bernard soulier syndrome
BM	Bone marrow
Ca^{+2}	Calcium ions
CB/P-PT	Capillary blood/ plasma- prothrombin time
CB/B-PLT	Capillary blood/ whole blood- platelet
CHS	Chediak hegashi syndrome
CD	Cluster of differentiation
CFU-MK	Colony forming unit megakaryocytic
CMV	Cytomegalovirus

DNA	Deoxyriboneuclic acid
DM	Diabetes mellitus
DIC	Disseminated intravascular coagulation
ELISA	Enzyme Linked Immune Sorbent Assay
EDTA	Ethylene diamine tetra acetic acid
F	Factor
FBG	Fasting blood glucose
Fl	Femtoliter
FDPs	Fibrin Degradation Products
Fc	Fragment crystallization
GTT	Glucose tolerant test
GLA	Glutamic acid
Gln	Glycin
GP	Glycoprotein
GPS	Grey platelet syndrome
HB/HGB	Hemoglobin
HBA1c	Hemoglobin A1c
HUS	Hemolytic uremic syndrome
HIT	Heparin Induced Thrombocytopenia
HPS	Hermanskpudlak syndrome
HDL	High density lipoprotein
HMWK	High molecular weight kininogen
hs-CRP	High sensitive C-Reactive Protein

HIV	Human immundefficient virus
HLA	Human leukocyte antigen
HPA	Human platelet antigen
ITP	Idiopathic thrombocytopenic purpura
Ig	Immunoglobulin
IFG	Impaired fasting glucose
IP3	Inositol tri-phosphate
IDDM	Insulin dependent diabetes mellitus
IL	Interleukin
INR	International Normalization Ratio
ISI	International Sensitivity Index
KD	Kilo Dalton
LADA	Latent autoimmune diabetic in adult
LED	Light emiting diode
L	Liter
LAC	Lupus anticoagulant
LYST	Lysosomal trafficking regulator gene
Mg^{+2}	Magnesium
MPV	Mean platelets volume
Mb	Meta base
um	Micro meter
NIDDM	Non-insulin dependent diabetes mellitus
OGTT	Oral glucose tolerant test

P-APT	Plasma-Activated partial thromboplastin
PLG	Plasminogen
PAIs	Plasminogen activator inhibitors
PLT	Platelet
PDGF	Platelet derived growth factor
PDW	Platelet distribution width
PF	Platelet factor
PI	Platelet Indices
P-LCC	Platelet large cell concentration
P-LCR	Platelet large cell ratio
PPP	Platelet poor plasma
PCT	Plateletcrit
PG	Prostaglandine
PC	Protein C
PS	Protein S
PZ	Protein Z
PT	Prothrombine time
RBG	Random blood glucose
RBC	Red blood cell
SD	Standard deviation
SPSS	Statistic package for social science
SDF-1a	Stromal cell derived factor 1 alpha
SCCS	Surface connected canalicular system

SCOCS	Surface connected open canalicular system
SLE	Systemic lupus erythromatosus
TAFI	Thrombin activatable fibrinolytic inhibitor
TAR	Thrombocytopenia-absent radius
TM	Thrombomodulin
TPO	Thrombopoitein
TTP	Thrombotic thrombocytopenic purpura
TXA	Thromboxane A
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
t-PA	Tissue plasminogen activator
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
VTE	Venous Thromboemobolism
vWF	von Willebrand factor
WBC	White blood cell
WAS	Wiskottaldrich syndrome

Chapter One

Introduction

CHAPTER I

Introduction

1.1. Introduction:

Diabetes mellitus is actually a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Classified into two broad categories: type 1, insulin dependent diabetes mellitus (IDDM); and type 2, non-insulin dependent diabetes mellitus (NIDDM) (Bishop *et al*., 2013).

Type 2 diabetes mellitus is characterized by hyperglycemia as a result of an individual's resistance to insulin with an insulin secretary defect. This resistance result in a relative, not an absolute, insulin deficiency. Type 2 constitutes the majority of the diabetes cases. Most patients in this type are obese or have an increased percentage of body fat distribution in the abdominal region. This type of diabetes often goes undiagnosed for many years and is associated with a strong genetic predisposition (Bishop *et al*., 2013; Dasgupta and Wahed, 2014).

The hemostatic system thus represents a delicate balance between pro-coagulant and anticoagulant mechanisms allied to a process for fibrinolysis. Physiologic hemostasis is an amazing and complex process that keeps blood fluid in the circulation and then when an injury occurs produce a clot to stop the bleeding, keeps the clot confined to the site of injury, and finally dissolves the clot and the wound heals (Hoffbrand *et al*., 2006; Rodak *et al*., 2012).

The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolytic elements and blood vessels (Hoffbrand *et al*., 2006).

Platelets are a-nucleated discoid shaped blood cells derived from megakaryocyte and have glycoprotein attached to the outer surface that serves receptors (Wahed and Dasgupta, 2015).

Platelets normally move freely through the lumen of blood vessels as components of the circulatory system. Maintenance of normal vascular integrity involves nourishment of the endothelium by some platelet constituents or the actual incorporation of platelets into the vessel wall. This process requires less than 10% of the platelets normally in the circulating blood. For hemostasis to occur, platelets not only must be present in normal quantities but also must function properly. When endothelium damaged series event occur including adhesion to the injured vessel, shape change, aggregation, and secretion (Turgeon, 2012).

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 (Munker *et al*., 2007).

D-dimer is a soluble fibrin degradation product that results from ordered breakdown of thrombi by the fibrinolytic system. Numerous studies have shown that D-dimer serves as a valuable marker of activation of coagulation and fibrinolysis. Consequently, D-dimer has been extensively investigated for the diagnosis of venous thromboembolism (VTE) and is used routinely for this indication. In addition, D-dimer has been evaluated for determining the optimal duration of anticoagulation in VTE patients, for diagnosing and monitoring disseminated intravascular coagulation, and as an aid in the identification of medical patients at high risk for VTE. Thus, quantification of D-dimer levels serves an important role in guiding therapy (Weitz *et al*., 2017).

Diabetes (both type-1 and type-2) affects millions of individuals worldwide. A major cause of death for individuals with diabetes is cardiovascular diseases, in part since both types of diabetes lead to physiological changes that affect haemostasis. Those changes include altered concentrations of coagulatory proteins, hyper-activation of platelets, changes in metal ion homeostasis, alterations in lipid metabolism (leading to lipotoxicity in the heart and atherosclerosis), the presence of pro-coagulatory microparticles and endothelial dysfunction. In this review, we explore the different mechanisms by which diabetes leads to an increased risk of developing coagulatory disorders and how this differs between type-1 and type-2 diabetes (Scobczak *et al*., 2019).

In patients with diabetes, metabolic disorders disturb the physiological balance of coagulation and fibrinolysis, leading to a prothrombotic state characterized by platelet hypersensitivity, coagulation disorders and hypofibrinolysis (Li *et al*., 2021). The increased platelet activity is emphasized to play a role in the development of vascular complications of this metabolic disorder. Platelet volume, a marker of the platelet function and activation, is measured as mean platelet volume (MPV) by hematology analyzers. Diabetic patients have an increased risk of developing micro and macro-vascular disease, and platelets may be involved as a causative agent with respect to altered platelet morphology and function (Kodiatte, 2012).

Diabetes augments the capability of coagulation. Increased level of plasminogen activator inhibitor-1 leads to impaired fibrinolysis in Type 2 diabetes. Increased expression of tissue factor and coagulation factors along with decrease in endogenous anticoagulants such as protein C, antithrombin 3 is seen in diabetes. Thus, there is an increased tendency for coagulation combined with impaired fibrinolysis in patients with Type 2 diabetes (Sherin *et al*., 2020).

D-dimer levels had risen in diabetic patients. In normal conditions, when there is 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 D-D-dimer may be under estimated. Moreover, the more rigid fibrin from glycated fibrinogen may also contribute to relatively low values as this fibrin is more difficult to break down (Elbadri *et al*., 2020).

1.2. Rationale:

The number of people with type 2 diabetes mellitus (DM) is increasing in every country, with 80% of people with DM living in low and middle-income countries. DM caused 4.6 million deaths in 2011 (Olokoba *et al.*, 2012). 80% of diabetic mortality is due to cardiovascular disorder these due to hypercoagulability state during the disease (Riaz, 2009). Prevalence of diabetes rise from 9.3% in 2010 to 10.6% in four states in Sudan (Gadarif, River Nile, Gazera and Northern Sudan) (Balla *et al.*, 2014).

Patients with DM have a high risk of developing atherothrombotic events. This leads to initiation and progression of microvascular and macrovascular complications (Sherin *et al*., 2020). There are few studies about evaluation of platelets indices, coagulation profile and D-dimer among diabetic patients was commonly performed. There for this study is to determine if hemostatic system was activated in diabetes, by measuring platelets indices, coagulation profile and D-dimer in the diabetics compared to the non-diabetics.

1.3. Objectives:

1.3.1. General objective:

To estimate platelet count, platelets indices, coagulation profile and D-dimer among type 2 diabetic patients in Khartoum state.

1.3.2. Specific objectives:

To measure platelet, count and indices in type 2 diabetic patients and non-diabetic individuals by hematological analyzer.

To estimate prothrombin time and activated partial thromboplastin time among diabetes patients and non-diabetes individuals by COARTON IM device and calculate international normalization ratio.

To measure D-dimer among diabetes patients and non-diabetes people by BIOTIME device.

To compare between them among case and control groups and correlate it according to duration of disease.

Chapter Two

Literature review

CHAPTER II

Literature review

2.1. Diabetes mellitus:

Diabetes mellitus (DM) is a syndrome of chronic hyperglycemia due to relative insulin deficiency, resistance or both (Kumar and Clark, 2012).

The term is derived from the Greek words dia (=through), bainein (=to go) and diabetes literally means pass through. The disease causes loss of weight as if the body mass is passed through the urine. The Greek word, mellitus, means sweet, as it is known to early workers, that the urine of the patient contains sugar (Vasudevan *et al*., 2011).

2.1.1. Classification of diabetes mellitus:

Clinically, patients with diabetes mellitus may be classified as having primary or secondary diabetes mellitus (Gad Allah, 2009).

2.1.1.1. Primary diabetes mellitus:

This may be caused by a factor in the blood which antagonize or inhibits the action of insulin, or by production of abnormal form of insulin, or by inability of the pancreas to produce sufficient insulin from the earliest stage of the disease. It is divided into type 1 and type 2 (Gad Allah, 2009).

2.1.1.1.1. Type 1 diabetes mellitus:

Formerly known as insulin dependent DM (IDDM), type I, or juvenile-onset diabetes. Approximately 5% to 10% of all individuals with diabetes mellitus are in this category (Burtis *et al*., 2008).

Type 1 diabetes is a disease of insulin deficiency, reaching a peak incidence around the time of puberty, but can present at any age, a ‘slow burning’ variant with slower

progression to insulin deficiency occurs in later life and is sometimes called latent autoimmune diabetes in adults (LADA). LADA may be difficult to distinguish from type 2 diabetes. Clinical clues are: leaner, rapid progression to insulin therapy following an initial response to other therapies, and the presence of circulating islet auto-antibodies (kumar and Clark, 2012).

Type 1 diabetes is due to autoimmune destruction of pancreatic beta cells by T lymphocytes. Markers of immune destruction of beta cells in these patients include islet cell auto-antibodies as well as auto-antibodies to insulin and glutamic acid decarboxylase (Dasgupta and Wahed, 2014).

Type 1 diabetes is an auto immune disease with the greatest contribution from the human leukocyte antigen (HLA) region (Kumar and Clark, 2012).

2.1.1.1.2. Type 2 Diabetes Mellitus:

Formerly type 2 known as non-insulin-dependent DM (NIDDM), type 2 diabetes constitutes approximately 90% of all cases of diabetes. Patients have minimal symptoms, are not prone to ketosis and are not dependent on insulin to prevent ketonuria. Insulin concentration may be within the reference interval, decreased or increased, and most people with this form of diabetes have impaired insulin action (Burtis *et al.*, 2008).

Type 2 diabetes is associated with central obesity which increases the risk of type 2 diabetes 80-100 fold, hypertension, hyper-triglyceridaemia, decreased high density lipoproteins (HDL) cholesterol, disturbed hemostatic variables and modest increases in a number of pro-inflammatory markers (Kumar and Clark, 2012).

2.1.1.1.2.1. Pathophysiology of type 2 diabetes:

There is no evidence of an autoimmune etiology, rather polymorphism in genes associated with beta-cell function and insulin secretion confer the greatest genetic risk. Type 2 diabetes is characterized by decrease response of peripheral tissue to

insulin and beta-cell dysfunction (inadequate insulin secretion in the setting of hyperglycemia) (Mitchell *et al*., 2012).

2.1.1.2. Secondary diabetes Mellitus:

This occurs as a consequence of other diseases, either pancreatic or endocrine. With pancreatic diabetes, the secretion of insulin is reduced due to pancreatitis, hemochromatosis or resection of the pancreas. In diabetes secondary to other endocrine disorders, ineffective insulin caused by abnormal secretion of hormones with diabetogenic activity. Several drugs adversely affect glucose tolerance and a number of genetic disorders (Gad Allah, 2009).

2.1.2. Clinical Presentation of Diabetes Mellitus:

When the blood glucose level exceeds the renal threshold, glucose is excreted in urine (glocosuria)**.** Due to osmotic effect, more water accompanies the glucose (polyuria). To compensate for this loss of water, thirst center is activated, and more water is taken (polydypsia). To compensate the loss of glucose and protein, patient will take more food (polyphagia). The loss and ineffective utilization of glucose leads to break down of fat and protein, this would lead to loss of weight. Important differential diagnosis for weight loss is diabetes mellitus, tuberculosis, hyperthyroidism, cancer and acquired immune deficiency syndrome (AIDS). Often the presenting complaint of the patient may be chronic recurrent infections such as boils, abscesses, etc. Any person with recurrent infections should be investigated for diabetes. When glucose level in extracellular fluid is increased, bacteria get good nutrition for multiplication. At the same time, macrophage function of the host is inefficient due to lack of efficient utilization of glucose (Vasudevan *et al*., 2011).

2.1.3. Diabetic complications:

Diabetic complications may be classified based on whether the manifestation is physical such as: lipo-hypertrophy or metabolic as: hyper and hypoglycemia. However, diabetic complication can be classified into microvascular complications

(neuropathy, nephropathy and retinopathy) concern in type 1 and type 2 patients, and macrovascular complications (cardiovascular, cerebrovascular and peripheral vascular disease) which are common in type2 patients (Dunning, 2003; Ojo, 2016).

2.1.3.1. Diabetic nephropathy:

It is a syndrome characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions and loss of glomerular filtration rate in diabetic. It is a significant cause of chronic kidney disease and end-stage renal failure globally (Lim, 2014).

2.1.3.2. Diabetic retinopathy:

Is characterized by a spectrum of lesions within the retina and is the leading cause of blindness among adult (Hirral *et al.*, 2011).

These include changes in vascular permeability, capillary micro aneurysms, capillary degeneration and excessive new vascularization. The neural retina is also dysfunctional with death of some cells, which alters retinal electrophysiology and results in an inability to discriminate between colors (Frank, 2004).

2.1.3.3. Diabetic neuropathy:

It is a syndrome which encompasses both the somatic and autonomic divisions of the peripheral nervous system. There is a growing appreciation that damage to the spinal cord (Selvarajah *et al.*, 2006).

And the higher central nervous system can also occur (Wesseles *et al.*, 2006).

And that neuropathy is a major factor in the impaired wound healing, erectile dysfunction and cardiovascular dysfunction seen in diabetes (Obrosova, 2009).

2.1.3.4. Cardiovascular diseases:

Cardiovascular disorders in diabetes include premature atherosclerosis, manifest as myocardial infarction and stroke as well as impaired cardiac function, predominantly diastolic dysfunction (Okon *et al.*, 2005).

Cardiovascular disease account for more than half of the mortality seen in the diabetic population (Laing *et al.*, 2003).

2.1.3.5. Cerebrovascular diseases:

They are the most severe complication especially in patients with type 2 DM. Cerebrovascular diseases include the ischemic stroke and hemorrhagic stroke, both of which happen in patients with microvascular or macrovascular diseases (Zhou *et al.*, 2014).

2.1.3.6. Peripheral vascular disease:

It is the atherosclerosis of lower extremity arteries and also associated with atherothrombosis of other vascular beds, including the cardiovascular and cerebrovascular system. The presence of diabetes mellitus greatly increases the risk of peripheral vascular disease as well as accelerates its course, making these patients more susceptible to ischemic events (Thiruvopati *et al.*, 2015).

2.1.4. Laboratory diagnosis of diabetes mellitus:

Criteria for the Diagnosis of Diabetes Mellitus:

Four methods of diagnosis are suggested:

- Hemoglobin A1c (HbA1c) ≥ 6.5%.
- A fasting blood glucose (FBG) ≥ 126 mg/dl.
- An oral glucose tolerance test (OGTT) with a 2-hour post-load (75 g glucose load) level ≥ 200 mg/dl.
- Symptoms of diabetes plus a random blood glucose level (RBG) ≥ 200 mg/dl.

Each of which should be confirmed on a subsequent day by any one of the first three methods. Any of the first three methods are considered appropriate for the diagnosis of diabetes (Bishop *et al*., 2013).

2.1.4.1. Random blood glucose (RBG):

A random blood glucose test can be used to diagnose diabetes. A blood glucose level of 200 mg/dl in two different occasions indicates diabetes (Gad Allah, 2009).

2.1.4.2. Fasting blood glucose (FBG):

The fasting blood glucose test is the preferred way to diagnose diabetes. It is easy to perform and convenient. After the patient has fasted overnight (at least 8 hours), a single sample of blood is drawn and sent to the laboratory for analysis. This can also be done accurately in doctor own office using a glucose meter (Harris, 1993; ADA, 2007).

Fasting blood glucose exceeding 126 mg/dl on more than one occasion are diagnostic of diabetes mellitus (Burtis *et al.*, 2008).

Impaired fasting glucose (IFG) an intermediate stage, in which the fasting glucose is increased above normal limits but not the level of diabetes (Bishop *et al*., 2013).

2.1.4.3. Oral Glucose Tolerance Test (OGTT):

The OGTT is more sensitive than fasting glucose early in the course of type 2 diabetes, resulting in a lack of equivalence between the fasting and 2-hour glucose values. Although more sensitive than FBG determination, GTT is affected by large numbers of factors that results in a poor reproducibility. In addition, approximately 20% of OGTTs fall into the non-diagnostic categories (e.g., only one blood sample exhibits increased glucose concentration). Unless results are grossly abnormal initially, the OGTT should be performed on two separate occasions before the results are considered abnormal (Burtis *et al*., 2008).

Preparing for a glucose tolerance test: continue to eat a normal diet in the days leading up to the test. Consult with your doctor about any medications you're currently taking. Some medications, such as corticosteroids, beta-blockers, diuretics, and antidepressants, can interfere with the results. Abstain from food for at least eight hours before the scheduled test. You may drink water, but avoid other beverages, including coffee and caffeinated tea, as these can interfere with the results. Avoid going to the bathroom just before the procedure because you may need to provide a urine sample. Bring something to read or an activity to keep you

busy while you wait. Once you are in the laboratory, the technician will take a blood sample (fasting sample). Then you will be given a syrup containing 75 mg of glucose or dextrose. After 2 hours, another sample is drawn (two hours' sample) (Pletcher, 2016).

2.1.4.4. Glycated hemoglobin:

Glycated hemoglobin produced when glucose (a reducing sugar) reacts with the amino group of hemoglobin (a protein). The glucose molecule attaches non-enzymatically to the hemoglobin molecule to form ketoamine. The rate of formation is directly proportional to the plasma glucose concentrations. Because the average red blood cell lives approximately 120 days, the glycosylated hemoglobin level at any one time reflects the average blood glucose level over previous 2 to 3 months. HbA1c, the most commonly detected glycosylated hemoglobin, is a glucose molecule attached to one or both N-terminal valines of the beta-polypeptide chains of normal adult hemoglobin. HbA1c is more reliable method of monitoring long-term diabetes control than random plasma glucose. Normal values range from 4% to 6%. Two factors determine the glycosylated hemoglobin levels: the average glucose concentration and the blood cell life span. If the red blood cell life span is decreased because of another disease state such as hemoglobinopathies, the hemoglobin level will be lower (Bishop *et al*., 2013).

2.1.5. Treatment:

2.1.5.1. Treatment of type I:

Individuals with type 1 diabetes must rely on exogenous insulin injected subcutaneously to control the hyperglycemia and ketoacidosis. Two therapeutic regimens are currently in use standard and intensive insulin treatment. Insulin may also be delivered by a pump, which allows continuous subcutaneous infusion of insulin 24 hours a day at present levels and the ability to program doses (a bolus) of insulin as needed at meal times (Harvey and Ferrier, 2011).

2.1.5.2. Treatment of type 2:

The goal in treating type 2 diabetes is to maintain blood glucose concentrations within normal limits, and to prevent the development of long-term complications. Weight reduction, exercise, and medical nutrition therapy (dietary modifications) often correct the hyperglycemia of newly diagnosed type 2 diabetes. Hypoglycemic agents or insulin therapy may be required to achieve satisfactory plasma glucose level (Harvey and Ferrier, 2011).

2.2. Haemastasis:

Haemostasis is one of a number of protective processes that have evolved in order to maintain a stable physiology. Systemic anticoagulant and clot-dissolving components have also evolved to prevent extension of the procoagulant response beyond the vicinity of vascular injury resulting in unwanted thrombus formation. 'Haemostasis' refers more widely to the process whereby blood coagulation is initiated and terminated in a tightly regulated fashion, together with the removal (or fibrinolysis) of the clot as part of vascular remodeling. The resultant haemostatic system is thus a complex mosaic of activating or inhibitory pathways that integrates its five major components (blood vessels, platelets, coagulation factors, coagulation inhibitors and fibrinolytic elements). In the most simplistic terms, blood coagulation occurs when the enzyme thrombin is generated and proteolyses soluble plasma fibrinogen, forming the insoluble fibrin polymer, or clot (Hoffbrand *et al*., 2006).

2.2.1. Blood Vasculature:

2.2.1.1. The Role of Vasoconstriction in Hemostasis

Vascular injury to a large or medium-size artery or vein requires rapid surgical intervention to prevent exsanguination. When a smaller vessel, such as an arteriole, venule, or capillary, is injured, contraction occurs to control bleeding. This contraction of the blood vessel wall is called vasoconstriction. Vasoconstriction is a short-lived reflex reaction of the smooth muscle in the vessel wall produced by the

sympathetic branches of the autonomic nervous system. This narrowing, or stenosis, of the lumen of the blood vessel decreases the flow of blood in the injured vessel and surrounding vascular bed and may be sufficient to close severed capillaries (Turgeon, 2012).

2.2.1.2. Endothelium:

The endothelium functions in a multitude of physiological processes, including intracellular transport, the regulation of vasomotor tone and maintenance of blood flow. Endothelial cells possess surface receptors for a variety of physiological substances, for example thrombin and angiotensin II, which may influence vascular tone directly or indirectly through various haemostasis-related events. Once activated, endothelial cells express a variety of intracellular adhesion molecules, some of which are released into the plasma. These include vascular cell adhesion molecule (VCAM), E-selectin, P-selectin and von Willebrand factor (vWF), which modulates leucocyte and platelet adhesion, inflammation, phagocytosis and vascular permeability (Hoffbrand *et al*., 2006).

2.2.2. Platelets:

Platelets were described by Addison in 1841 as “extremely minute … granules” in clotting blood. In 1882 Bizzozero recognized the platelet as a cell structure different from red blood cells and white blood cells. It was not until 1970, however, that scientists recognized the relationship of platelets to hemostasis and thrombosis as significant and extensive. 9 every cubic millimeter of healthy blood contains 250 million platelets, resulting in approximately 1 trillion platelets in the blood of an average woman. Each platelet makes 14,000 trips through the bloodstream during its life span of 7-10 days. The same elements were identified by microscopic examination of blood smears by Osler and Schaefer and by Hayem in the late nineteenth century (Greer *et al*., 2014; Ciesla, 2012).

2.2.2.1. Platelets production:

It is a process by which platelet produce known as megakaryopoiesis or thrombopoiesis is a process of development of megakaryocytes and platelet within the bone marrow (BM) (Porwit *et al*., 2011).

The primary regulator of platelet production is thrombopoietin (TPO) it is protein produced by the liver with molecular weight of about 335kDa, it is increased in thrombocyopenias and thrombocythemia. IL-6 and IL-11 also play role in generation of megakaryocyte colony-forming unit (CFU-MK). CFU-MK is a diploid cell population, in which DNA synthesis and nuclear division (karyoinesis) is followed by cell division (cytokinesis). CFU-MK undergoes further maturation to megakaryoblasts (Bain *et al*., 2011).

2.2.2.1.1. Megakaryoblast:

Proliferative cells with single, large and oval nucleus, not generally recognizable in normal BM. Have nucleoli, some degree of nuclear lobulation and azurophilic cytoplasmic granules with 2-6 um in diameter (Bain *et al*., 2010; Kawthalkar, 2013).

2.2.2.1.2. Promegakaryocyte:

Have strongly basophilic cytoplasm and very high nucleocytoplasmic ratio. It is larger than megakaryoblast and have 15-30 um in diameter, may contain azurophilic granules and have lobulated shape nucleus (Bain *et al*., 2010; Kawthalkar, 2013).

2.2.2.1.3. Granular megakaryocyte:

Their cytoplasm is less basophilic and contains some azurophilic granules. Have a diameter 40-60 um and large multi-lobed nucleus (Bain *et al*., 2010; Kawthalkar, 2013).

2.2.2.1.4. Mature megakaryocyte:

Are mature cells capable of production of platelet, produce about 1000-5000 platelets, their cytoplasm is weakly basophilic and contain abundant azurophilic granules, have pyknotic nucleus (Bain *et al*., 2010; Kawthalkar, 2013).

2.2.2.1.5. Platelet:

Are small fragments of megakaryocyte cytoplasm with an average volume of 7-8fl. Have a diameter of 2-3 um. They have an irregular outline, stain light blue and contain a number of small azurophilic granules. The normal range for the platelet count in peripheral blood is about 150-450× 10^9/L, the life span of normal platelet is 7-10 days (Porwit *et al*., 2011).

2.2.2.2. Platelets structure:

Platelets divided into three regions; peripheral zone, Sol-gel zone, and organelle zone (Hoffbrand *et al*., 2006).

2.2.2.2.1. Peripheral zone:

The major structural elements of the platelet peripheral zone are the cell surface and channels of the surface-connected open canalicular system (SCOCS). The peripheral zone is made up of the three structural domains: the exterior coat, the unit membrane, and the sub-membrane. The exterior coat is glycocalyx covering the outer surface of platelets. Many different glycoproteins have been defined by on the exterior coat, including glycoproteins (GP);

GPIaIIa: allow platelet adhesion to collagen.

GPIIbIIIa: allow platelet to aggregate.

GPIb–IX: initial adhesion of platelet to vWF and thrombin.

GPV: allow platelet adhesion.

GPIV: platelet adhesion to collagen.

GPVI: activation of collagen (Hoffbrand *et al*., 2006).

2.2.2.2.2. The sol–gel zone:

It is the matrix of the platelet cytoplasm. It contains several fiber systems in various states of polymerization that support the discoid shape of unaltered platelets and provide a contractile system involved in shape change, pseudopodia extension, internal contraction, and secretion. Elements of the contractile system appear to be

major components, since they constitute approximately 30–50% of the total platelet protein. Masses as well as discrete particles of glycogen are distributed in the sol–gel matrix (Hoffbrand *et al*., 2006).

2.2.2.2.3. The organelle zone:

It consists of granules, electron-dense peroxisomes, lysosomes, glycosomes and mitochondria randomly dispersed in the cytoplasm. It serves in metabolic processes and for the storage of enzymes, non-metabolic adenine nucleotides, serotonin, a variety of protein constituents, and calcium destined for secretion (Gresele *et al*., 2002).

Platelets contain four main types of storage granules; dense granules, α-granules, lysosomes and peroxisomes, and several mitochondria. There are between five and nine dense granules in platelets, which contain high levels of ADP (increase adhesive of platelet), ATP, polyphosphates, 5-hydroxtryptamine (serotonin) promote vasoconstrction and Ca2+. There are approximately 80 α-granules per platelet and these contain a rich diversity of proteins and membrane receptors that support hemostasis, vascular repair, inflammation and host defense. Major components of α-granules include clotting factors such as fibrinogen, vWF, factor FV, protein sand tissue factor pathway inihibtor (TFPI), the chemokines stromal cell derived factor-1 alpha (SDF-1α), platelet factor 4 (PF4) and β thromboglobulin. The growth factors platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) A and C. Platelet α-granules also express key transmembrane proteins including integrin αIIbβ3, P-selectin (CD62) and CD40L that are only expressed on activated cells. Platelets are enriched in signalling and cytoskeletal proteins that enable them to undergo dramatic changes in morphology and which enable the aggregate to withstand the high shear forces of the vasculature. Platelets have a network of intracellular membranes known as the dense tubular system that release intracellular Ca2+ in response to the second messenger inositol 1,4,5-

trisphosphate (IP3). They also have a network of invaginations of the surface membrane, known as the surface-connected canalicular system (SCCS), which increase the surface area of the plasma membrane during platelet spreading. The SCCS also gives rise to membrane tethers that play a vital role in supporting adhesion (Hoffbrand *et al*., 2016).

In addition to containing substantial quantities of the contractile proteins, including actomyosin (thrombosthenin), myosin, and filamin, the cytoplasm of the platelet contains glycogen and enzymes of the glycolytic and hexose pathways. Energy for metabolic activities and cellular contraction is derived from aerobic metabolism in the mitochondria and anaerobic glycolysis utilizing glycogen stores. The platelet is a very high-energy cell with a metabolic rate 10 times that of an erythrocyte. Based on energy availability and endogenous constituents, the platelet is effectively equipped to fulfill the role of protecting the body against vascular trauma (Turgeon, 2012).

2.2.2.3. Platelets antigens:

Several platelet surface proteins important antigens in platelet-specific autoimmunity and they have been termed human platelet antigens (HPA). In most cases, two different alleles exist, termed α or β alleles (e.g. HPA-la). Platelets also express ABO and human leukocyte antigen (HLA) class I but not class II antigens (Hoffbrand *et al*., 2006).

2.2.2.4. Platelets function:

Platelets play an important role in the formation of a primary plug and the coagulation cascade (Ciesla, 2007).

Activation of platelet refers to adhesion, aggregation and release of reaction of platelet which occurs after platelet stimulation (i.e. after vascular damage) (Kawthalkar, 2013).

2.2.2.4.1. Adhesion:

This means binding of platelet to non-endothelial surface, particularly sub-endothelial which uncovered following vascular injury. Von Willebrand factor (vWF) mediates adhesion of platelet to sub-endothelium via glycoprotein Ib (GPIb) on the surface of platelet. Congenital absence of glycoprotein receptor GPIb (Bernard-Soulier syndrome) or von Willebrand in plasma (von Willebrands disease) cause defective platelet adhesion and bleeding disorder. Platelet normally circulates as round to oval disc-like structures. With activation, platelets undergo shape change, i.e. they become more spherical and form pseudopodia. This shape change is due to reorganization of microtubules and contraction of actomyosin of microfilaments (Kawthalkar, 2013).

2.2.2.4.2. Release reaction:

Immediately after adhesion and shape change, process of release reaction or secretion begins. In this process, contents of platelet organelles and released to the exterior. ADP released from dense granules promotes platelet aggregation. Platelet factor 4 released from alpha granules neutralizes the anticoagulant activity of heparin while platelet-derived growth factor stimulates proliferation of vascular smooth muscle cells and skin fibroblasts and play a role in wound healing. Activated platelets also synthesise and secrete thromboxane A2 (TxA2). Platelet agonist such as ADP, epinephrine, and low-dose thrombin bind to their specific receptors on platelet surface, and activate phospholipase enzymes, which release arachidonic acid from membrane phospholipids. Arachidonic acid is converted to cyclic endoperoxidesby the enzyme cycloxygenase. These are then converted to thromboxane A2 by thromboxane synthetase. Thromboxane A2 has a very short half-life and is degraded into thromboxane B2 which is biologically inactive. TxA2 also induces aggregation of other platelet and local vasoconstriction (Kawthalkar, 2013).

2.2.2.4.3. Platelets aggregation:

This may be defined as binding of platelet to each other. ADP released from platelet or from damaged cells binds to specific receptors on platelet surface. This is cause inhibition of adenylcyclase and reduction in the level of cyclic AMP in platelet. A configurational change in the membrane occurs so that receptors for fibrinogen (GPIIb/IIIa) become exposed on the surface. Binding of fibrinogen molecules to GPIIb/IIIa receptors on adjacent platelet causes platelet aggregation. The activated platelet release ADP and TxA2 leading to the formation of a platelet plug. Also thrombin causes platelet aggregation (Kawthalkar, 2013).

2.2.2.4.4. Platelet functions testing:

Platelet function tests are primarily used to aid in the diagnosis of patients presenting with bleeding problems. A variety of tests can be used to diagnose an underlying cause of the bleeding problem. Normal platelet function is highly dependent on extracellular Ca2+ and Mg2+ concentrations and so the choice of anticoagulant is important. Most current testing is still performed on citrated blood within a few hours of sampling. Global tests of platelet function are often initially used as screening tests during the laboratory investigation of individuals with suspected haemostatic defects. Since global tests of platelet function do not enable specific diagnosis of platelet disorders, they are normally performed as the first part of a two-step strategy that requires further testing with more specialized assays of platelet function to confirm or refute any clinical diagnosis. The most commonly proposed rationale for testing global platelet function as a first-line investigation is exclusion of a platelet function disorder so that further specialized testing can be avoided. For this reason, global platelet function tests are usually initially performed at the same time as global assays of coagulation pathway function (prothrombin time and activated partial thromboplastin time), vWF screening tests and measurement of platelet number with a full blood counter. The most widely performed test for initial

global screening platelet function disorders are currently the template bleeding time. The bleeding time was the first *in vivo* test of platelet function and is performed by timing the arrest of bleeding from standard-sized cuts made in the skin of the forearm. Automated cell counting of whole blood in the modern full blood count investigation is an essential screening test in patients with abnormal bleeding. Modern blood counters can rapidly detect abnormalities in platelet number, platelet size distribution and mean platelet volume (MPV) and provide a reliable method for screening samples from patients. If any abnormalities in platelet count, MPV or distribution are flagged by the instrument, then a blood smear should be examined to confirm defects in platelet number, size and granule content. Whole-blood morphology may also assist in the diagnosis of platelet disorders by indicating abnormalities such as red cell schistocytes in TTP. Depending on the results of clinical and laboratory screening of patients, a series of diagnostic platelet function tests are usually performed. Modern aggregometers are now multichannel, fully computerized, easy to use compact instruments and some can also simultaneously measure ATP secretion levels by luminescence. Measurement of the storage and release of the dense granular nucleotides can also be performed by a variety of alternative methods for confirming either storage and/or release defects, Flow cytometry provides an exquisite, sensitive and powerful tool for studying and diagnosing various platelet defects. Flowcytometric analysis of platelets is performed in fresh whole blood or in platelet-rich plasma, and the technique can be used with very small fluid volumes, even in thrombocytopenia. This technique is used to determine the copy density of platelet membrane glycoproteins and receptors, and is therefore useful for confirming the absence of various glycoproteins or receptors in disease (Hoffbrand *et al.,* 2016).

2.2.2.5. Platelets disorders:

Disorders of platelets can be classified as quantitative (thrombocytopenia or thrombocytosis) or qualitative (thrombocytopathy) (Turgeon, 2012).

2.2.2.5.1. Quantitative platelets disorders:

The normal range of circulating platelets is 150×10^9/L to 450×10^9/L. When the quantity of platelets decreases to levels below this range, a condition of thrombocytopenia exists. If the quantity of platelets increases, thrombocytosis is the result (Turgeon, 2012).

2.2.2.5.1.1. Thrombocytopenia:

This may arise through decreased production or accelerated peripheral destruction and/or hypersplenism. The former may result from extensive marrow infiltration or chemotherapy or radiotherapy (Hoffbrand *et al*., 2016).

Is characterized by a decrease in the number of circulating platelets (i.e., <100,000/mm3). Clinical evidence of thrombocytopenia includes an increased number of petechiae, hemorrhages, prolonged bleeding time, and impaired clot retraction (Ciulla and Lehman, 2010).

Thrombocytopenia in itself rarely poses a threat to affected patients, but disorders associated with it which includes deep venous thrombosis, disseminated intravascular coagulation (DIC), pulmonary embolism, cerebral thrombosis, myocardial infarction, and ischemic injury to the legs or arms can produce severe morbidity and mortality (Turgeon, 2012).

2.2.2.5.1.1.1. Disorders of platelets production:

Generalized bone marrow failure or selective megakaryocyte depression results in decreased platelet formation. Congenital diseases associated with reduced platelet production include hereditary thrombocytopenias, macro-thrombocytopenia with neutrophilic inclusions, Wiskott Aldrich syndrome (X-linked recessive), and Bernard_Soulier disease (Wahed and Dasgupta, 2015).

Decreased production of platelets may be caused by hypoproliferation of the megakaryocytic cell line or ineffective thrombopoiesis caused by acquired conditions or hereditary factors. A hypoproliferative state frequently affects other normal cell lines of the bone marrow and platelets. Thrombocytopenia owing to hypoproliferation can result from acquired damage to hematopoietic cells of the bone marrow caused by factors such as irradiation, drugs (e.g., chloramphenicol and chemotherapeutic agents), chemicals (e.g., insecticides), and alcohol. Infiltration of the bone marrow by malignant cells in the conditions of metastatic cancer, leukemia, and Hodgkin disease can produce hypoproliferative state. Hypoproliferation may also result from nonmalignant conditions, such as infections, lupus erythematosus, granulomatous disease such as sarcoidosis, and idiopathic causes. Ineffective thrombopoiesis may result in decreased platelet production. Thrombocytopenias of this type may be the manifestation of a nutritional disorder, such as a deficiency of vitamin B12 or folic acid. In these megaloblastic anemias caused by deficiencies of vitamin B12 or folic acid, the defect in thymidine and DNA synthesis affects megakaryocytes and causes decreased or ineffective thrombopoiesis. Another disorder related to ineffective thrombopoiesis is iron deficiency anemia, which usually results in a decrease in megakaryocyte size and the suppression of megakaryocyte endoproliferation and size. Hereditary thrombocytopenias include Fanconi syndrome, constitutional aplastic anemia and its variants, ameiosis thrombocytopenia (TAR syndrome), X linked megakaryocytic thrombocytopenia, Wiskott-Aldrich syndrome, May-Hegglin anomaly, and hereditary macrothrombocytopenia (e.g., Alport syndrome) (Turgeon, 2012).

2.2.2.5.1.1.2. Increase in destruction of platelets:

2.2.2.5.1.1.2.1. Thrombotic Thrombocytopenic Purpura (TTP):

It is caused by excessive deposition of platelet aggregates in renal and cerebral vessels. Physiologically, TTP is believed to be caused by vascular wall dysfunction,

which disrupts the inert basement membrane. An increase in a platelet-aggregating factor, a possible deficiency of platelet-aggregating factor inhibitor, a decrease in prostacyclin (PGI2), an increase in PGI2 degradation, or an absence of a plasminogen activator this cause damage of endothelial cells of vascular wall.TTP is three times more prevalent in women than men at an average age of 35 years. The majority of patients are seen with micro-angiopathichemolytic anemia, thrombocytopenia, neurologic symptoms (e.g., headaches, seizures), fever, and renal disease. Lab diagnosis includes a prothrombin time (PT) that is normal in 88% of patients, an activated partial thromboplastin time (APTT) that is normal in 94% of patients, and a fibrinogen level that is normal in 79% of patients. Fibrin-degradation products (FDP) are normal in 53% of patients, weakly positive in 23%, and positive in 24% (Hoffbrand *et al.,* 2016).

2.2.2.5.1.1.2.2. Hemolytic uremic syndrome:

It is a clinical syndrome with presentation and manifestations similar to TTP. Unlike TTP, which has a peak age incidence in the third decade, HUS has a peak incidence between 6 months and 4 years of age. Unlike TTP, HUS is characterized by:

- Association with *Escherichia coli* O 157:H7 in 80% of cases.
- Renal failure.
- Small vWF multimers predominate.
- Normal level of ADAMTS13 activity (Turgeon.2012).

2.2.2.5.1.1.2.3. Vasculitis:

As seen with systemic lupus erythematosus (SLE) (Hoffbrand *et al.,* 2016).

2.2.2.5.1.1.2.4. Idiopathic (immunologic) thrombocytopenia purpura (ITP):

It is an autoimmune disorder. Common laboratory findings include an increase in mean platelet volume (MPV), decreased platelet count, increased bone marrow platelet production, increased marrow megakaryocyte, a normal bleeding time, and platelet-associated IgG (Hoffbrand *et al.,*2016).

2.2.2.5.1.1.2.4.1. Acute ITP:

It occurs in children 2 to 6 years of age. There is a sudden onset of thrombocytopenia, which often follows viral infections, such as rubella, chickenpox, cytomegalovirus (CMV), and toxoplasmosis. Usually lasts for 2 to 6 weeks with a spontaneous remission in 80% of patients. Platelet count is usually <20,000/mm3 in patients with acute ITP. It is caused by viral attachment and antigenic alteration of platelet membrane proteins that result in the formation of platelet auto-antibodies, which are most often IgG. IgG coated platelets are removed by macrophages in the spleen. Because acute ITP is usually self-limited, corticosteroids are the treatment of choice when therapy is instituted. Steroids suppress macrophage phagocytic activity, decrease Fc-receptor function, and decrease antibody platelet binding. Splenectomy is rarely needed, and platelet transfusion is ineffective (Hoffbrand *et al.,* 2016).

2.2.2.5.1.1.2.4.2. Chronic ITP:

It occurs in adults 20 to 40 years of age. It is found in women three times more than in men and has a slow, asymptomatic onset of thrombocytopenia. Platelet count usually ranges from 30,000 to 80,000/mm3. It is often associated with SLE. Splenectomy is the most common treatment because it decreases the number of macrophage with Fc receptors. Immunosuppressive chemotherapy with vincristine or vinblastine is used in severely affected patients (Hoffbrand *et al.,* 2016).

2.2.2.5.1.1.5. Post-transfusion purpura:

It occurs in 1% to 2% of persons who receive blood transfusions. Production of antiplatelet antibodies by the recipient of platelet transfusions results in the destruction of platelets (Hoffbrand *et al.,* 2016).

2.2.2.5.1.1.6. Isoimmune neonatal purpura:

It is caused by maternal viremia (e.g., CMV or rubella) or maternal drug ingestion (Hoffbrand*et al.,* 2016).

2.2.2.5.1.1.7. Drug-induced antibody:

Formation is most commonly seen with the use of quinidine and heparin. The drugs function as haptens, combining with a serum protein and causing an antibody response. The drug-antibody complex attaches to platelets, which results in agglutination, complement fixation, and destruction by macrophages (Hoffbrand *et al.,* 2016).

2.2.2.5.1.1.8. Heparin-induced thrombocytopenia (HIT):

It is observed in more than 10% of patients who undergo heparin therapy. Risk from thrombosis without heparin therapy is greater than the risk of bleeding from heparin-induced thrombocytopenia. The mechanism of thrombocytopenia is due to a direct platelet-aggregating effect, as well as immune destruction by anti-platelet antibodies. A normal aggregation pattern in platelet aggregometer studies is found, except that adding heparin as a stimulant will increase aggregation instead of blunting the aggregation reaction (Hoffbrand *et al.,* 2016).

2.2.2.5.1.1.9. Thrombocytopenia associated with human immunodeficiency virus (HIV) infection:

It is immune origin, severe but rarely hemorrhagic. Characteristics similar to classic ITP include: Abundant megakaryocytes**,** occasional giant platelets and absence of splenomegaly. And differ from ITP in: it has greater levels of bound antibody and involvement of immune complexes (Hoffbrand *et al.,* 2016).

2.2.2.5.1.1.10. Combined consumption of both platelet and coagulation factors:

It is seen with: Toxicity due to snake venoms, Tissue injury, obstetric complications (e.g., aborted fetus, toxemia of pregnancy), neoplasms (e.g., promyelocytic leukemia, carcinoma), bacterial and viral infections and disseminated intravascular coagulation (Hoffbrand *et al.,* 2016).

2.2.2.5.1.1.3. Disorders of platelets distribution:

A platelet distribution disorder can result from a pooling of platelets in the spleen, which is frequent if splenomegaly is present. This type of thrombocytopenia develops when more than a double or triple increase in platelet production is required to maintain the normal quantity of circulating platelets. Disorders that may produce splenomegaly with resultant splenic pooling or delayed intrasplenic transit include alcoholic or posthepatic cirrhosis with portal hypertension, lymphomas and leukemias, and lipid disorders such as Gaucher disease (Turgeon, 2012).

2.2.2.5.1.1.4. Falsely low platelet count:

2.2.2.5.1.1.4.1. Platelet satellitosis:

Platelets can adhere to neutrophils when exposed to EDTA. Redraw in sodium citrate to correct; multiply obtained platelet count by 1.1 to correct for dilution factor in sodium citrate tube (Ciullla and Lehman, 2010).

2.2.2.5.1.1.4.2. EDTA-dependent platelet agglutinins:

Platelets can adhere to each other when exposed to EDTA. Correction of the problem is the same as for platelet satellitosis (Ciullla and Lehman, 2010).

2.2.2.5.1.1.4. Thrombocytopenia in pregnancy:

Pregnant women generally have lower platelet counts than non-pregnant women. Gestational thrombocytopenia is caused by a combination of hemodilution and increased platelet activation and clearance. A decrease of approximately 10% in the platelet count is typical toward the end of the third trimester of pregnancy (Turgeon, 2012).

2.2.2.5.1.2. Thrombocytosis:

Is characterized by an increase in circulating platelet counts>450,000/mm3. A raised platelet count is frequently seen as a reactive phenomenon in patients with malignancy, but is usually less than 1000×10^9/L and is rarely of clinical significance.

Iron deficiency may also cause a rise in platelets (Hoffbrand *et al.,* 2016; Ciulla and Lehman, 2010).

2.2.2.5.1.2.1. Essential thrombocytosis:

It is characterized by an increased number of platelet; platelet count can be >1000 X 109/L. As a result of a primary bone marrow disorder it is caused by a clonal proliferation that affects all hemopoietic cells. Associated with either hemorrhagic or thrombotic complications, often patients with a thrombocytosis will have increased bleeding tendencies because of possible accompanying functional abnormalities. Uncontrolled, malignant proliferation of platelets, not in response to thrombopoietin; can be caused by essential thrombocythemia, polycythemia vera, and chronic myelocyticleukemia (Hubbard, 2010; Ciulla and Lehman, 2010).

2.2.2.5.1.2.2. Secondary thrombocytosis:

It is a secondary response most commonly associated with the following disorders:

- Iron-deficiency anemia associated with chronic blood loss. Iron regulates thrombopoiesis by inhibiting thrombopoietin; deficiency causes increased thrombopoietin and stimulates thrombopoiesis.
- Chronic inflammatory disease may be associated with high platelet counts e.g. tuberculosis, cirrhosis.
- Splenectomy-associated thrombocytosis.
- Rebound thrombocytosis, which may occur after a platelet depletion through a massive blood loss.
- Exercise, prematurity, and response to drugs.
- Other conditions: Cytotoxic drug withdrawal, post-operative state from tissue damage, and splenoctomy (Hubbard, 2010; Ciulla and Lehman, 2010).

2.2.2.5.2. Qualitative platelets disorders:

If platelets are normal in number but fail to perform effectively, a platelet dysfunction exists. In addition to both an individual and family medical history, laboratory tests are critical in determining a platelet dysfunctional diagnosis. Laboratory tests of platelet function include bleeding time, clot retraction, platelet aggregation, platelet adhesiveness, and anti-platelet antibody assay. Divided into acquired and hereditary (Turgeon, 2012).

2.2.2.5.2.1. Acquired qualitative platelets disorders:

Acquired platelet function defects can be caused by a blood plasma inhibitory substance. Examples of disorders or diseases that may exhibit this dysfunction include infused dextran, uremia, liver disease, and pernicious anemia. Laboratory testing reveals the presence of fibrinolytic degradation or split products (Turgeon, 2012).

2.2.2.5.2.1.1. Myeloproliferative syndromes:

Acquired platelet dysfunction is commonly seen in the myeloproliferative syndromes (Turgeon, 2012).

2.2.2.5.2.1.2. Uremia:

Uremia is commonly accompanied by bleeding caused by platelet dysfunction. It is proposed that circulating guanidine succinic acid or hydroxyl phenolic acid interferes with platelet function (Turgeon, 2012).

2.2.2.5.2.1.3. Paraprotein disorders:

Paraprotein disorders including malignant or benign paraprotein, such as multiple myeloma, Waldenström's macroglobulinemia, or other monoclonal gammopathies, harbor platelet dysfunction. Dysfunction results from the paraprotein coating the platelet membranes but does not depend on the type of paraprotein present (Turgeon, 2012).

2.2.2.5.2.1.4. Drug induced platelets dysfunction:

Many drugs can induce platelet function defects, resulting in hemorrhage. A typical example of this dysfunction is the ingestion of aspirin (Turgeon, 2012).

2.2.2.5.2.2. Herediatory qualitative platelets abnormalities:

2.2.2.5.2.2.1. Surface membrane defects:

2.2.2.5.2.2.1.1. Glanzmanthrombasthenia:

This disease is a homozygous autosomal recessive disorder in which one of two genes coding for either the membrane receptor glycoprotein IIb (GPIIb) or for GPIIIa is affected. Both genes are found on chromosome 17. Males as well as females are affected. Both receptors function in the aggregation process to anchor platelets to exposed collagen in the sub-endothelial tissue of damaged blood vessel walls commonly present in infants and in early childhood; Bruising, Nose bleeds, bleeding from the gums started by trauma or simple sneezing and menorrhagia. Normal platelet counts and morphology, significantly prolonged bleeding time, platelet aggregometer profile shows failure to aggregate with all agents (i.e., ADP, adrenaline, thrombin, collagen), except von Willebrand factor (Hubbard, 2010).

2.2.2.5.2.2.1.2. Bernard-Soulier syndrome (BSS):

It is directly due to an inherited quantitative or qualitative defect in the platelet glycoprotein (GP) Ib/IX complex consisting of four platelet surface glycoproteins (Ib α, Ib β, IX, and V). The GP Ib α gene is located on the short arm of chromosome 17, the GPIb β gene is on the long arm of chromosome 22, and the GP IX and GPV genes are located on the long arm of chromosome 3. The Ib molecule carries both the high- and moderate-affinity receptor sites for thrombin. This receptor complex mediates adhesion of platelets to the blood vessel wall at sites of injury by binding vWF and facilitates the ability of thrombin to activate platelets. BSS is extremely rare, occurring <1 per 1 million. Can present early in life and most commonly include frequent bouts of epistaxis, gingival and cutaneous bleeding, and

hemorrhage associated with trauma. Platelet counts may range from very low (i.e., <30,000/mm3) to slightly low or low normal. Bleeding time results range from slightly prolonged (8–10 minutes) to significantly prolonged (>20 minutes), commonly results in a prolonged Ivy bleeding time, examination of a blood smear and the mean platelet volume (MPV) for giant platelets and thrombocytopenia. Platelet aggregation studies will show a reduced aggregation with ristocetin and a normal response to other agonists (Hubbard, 2010).

2.2.2.5.2.2.1.3. Abnormalities in the granular fraction of the platelets:

2.2.2.5.2.2.1.3.1. Defects in dense granules:

Some platelet functional abnormalities are due to abnormalities in the granular fraction of the platelet. Congenital deficiencies in dense granules, which contain ADP, ATP, serotonin, and calcium, show diminished platelet aggregation in the second wave of aggregation (Hubbard, 2010).

2.2.2.5.2.2.1.3.1.1. Hermansky-Pudlak syndrome (HPS):

Due to decreased numbers of platelet dense granules due to an autosomal recessive trait caused by mutations in the HPS1 gene on chromosome10q23. The gene controls functions involved in the production and control of melanosomes, platelet dense bodies, and lysosomes resulting in a defectin platelet ADP release and a disruption in the ADP/ATP ratio granular content. Common symptoms consist of a triad phenotype of albinism, prolonged bleeding time, and the accumulation of ceroid pigment in lysosomal organelles (Hubbard, 2010).

2.2.2.5.2.2.1.3.1.2. Chediak-Higashi syndrome (CHS):

CHS is a generalized autosomal recessive genetic disorder with recurrent infections in combination with ocular, neurological**,** and skin manifestations. Onset in early childhood and death often occurs before the age of 7. It caused by mutations in the lysosomal trafficking regulator gene (LYST) found on chromosome 1, results in abnormal membrane fluidity, uncontrolled granule membrane effusion and

formation of giant cytoplasmic granules; and a lack of distinguishable dense granules in leukocytes, melanocytes and platelets (Hubbard, 2010).

2.2.2.5.2.2.1.3.1.3. Wiskott-Aldrich syndrome (WAS):

This disorder is a rare x-linked immunodeficiency disorder highlighted by thrombocytopenia, eczema, recurrent infections, and a predisposition for secondary leukemia or lymphoma (Hubbard, 2010).

2.2.2.5.2.2.1.3.2. α-Granule deficiencies:

Are rare platelet functional abnormalities in which both aggregation and release properties are diminished (Hubbard, 2010).

2.2.2.5.2.2.1.3.2.1. Storage pool disease:

Also known as Grey platelet syndrome (GPS) is a congenital platelet disease which associates thrombocytopenia and aggregation abnormalities. Inheritance is an autosomal dominant. The disorder is typically mild and patients commonly present with mildepistaxis, easy bruising, and long-lasting hemorrhages after accidental cuts. It is characterized by a marked decrease or absence of platelet α-granules and specific α-granule proteins. Morphologically, platelets appear large and contain few granules, giving them a grey appearance with Wright-Giemsa stained blood smears (Hubbard, 2010).

2.2.2.6. Platelets indices:

Platelet indices are group of platelet parameters determined together with automated complete blood counter, are biomarkers of platelet activation. They allow extensive clinical investigations focusing on the diagnostic and prognostic values in a variety of settings without bringing extra cost (Budak *et al*, 2016; Bashir *et al*, 2017).

Platelet indices (PI), markers of platelet activation, are parameters obtained daily as a part of an automatic blood count. PI are related to platelet's morphology and proliferation kinetics. Most commonly assessed PI include the mean platelet volume (MPV), platelet distribution width (PDW), platelet-large cell ratio (P-LCR) and the

plateletcrit (PCT). Blood-based platelet parameters, due to their fairly easy accessibility and inexpensive methods of measurement, seem to be on the rise as potential novel biomarkers of numerous, both acute and chronic, diseases. However, despite numerous attempts to determine the parameters' clinical correlations, their direct association to clinics in terms of both diagnosis and prognosis, is yet to be substantially verified (Jindal *et al*., 2011).

2.2.2.6.1. Mean platelets volume:

The mean platelets volume (MPV) is the calculated measurement of the average size of platelet found in blood. The normal range is given as 7.5 – 10.4 fl it is measure of thrombocyte volume, is determind directly by analyzing the platelets distribution curve. When platelets production is decreased, young platelets become bigger and more active and MPV level is increased. Low MPV is associated with low grade inflammation (Rheumatoid artharitis), MPV is increased in chronic disorders (Budak *et al*, 2016).

Elevated MPV levels have been identified as an independent risk factor for myocardial infarction in patients with coronary heart disease and for death or recurrent vascular event after myocardial infarction. Moreover, increased platelets size has been reported in patients with vascular risk factor such as diabetes, and smoking. Previous studies have demonstrated higher levels of MPV in patients with acute ischemic stroke than in control subject. In contrast, data regarding the association between MPV and stroke severity or stroke outcome have been controversial. The Mean platelet volume can be an indication of platelet turnover because younger platelets tend to be larger. A spectrum of platelet size is seen in patient with rapid turnover. MPV is recently taken as a determinant for platelet function, as it is positively associated with platelets reactivity function (Elbadri *et al*., 2020).

2.2.2.6.2. Platelets distribution width:

The platelets distribution width (PDW) is a measure of the uniformity of platelet size in a blood specimen. This is a measure of platelet anisocytosis. A normal PDW is less than 20%. The causes of increased PDW are not known but are probably related to dysfunctional megakaryocytic development. High PDW may indicate peripheral immune destruction of platelets. The PDW has been found to be of some use in distinguishing essential thrombocythaemia (PDW increased) from reactive thrombocytosis (PDW normal) (Bain *et al*., 2011; Turgeon, 2012).

PDW seems to be proportionally related to MPV in healthy individuals, however, under non physiological conditions, such as, threatened preterm labor, they show significant dissonance – a rise in PDW and decrease in MPV. Also found that higher level of PDW in patients with vaso occlusive crisis in the course of sickle cell disease can be contributed to megakarycote hyperplasia (Jindal *et al*., 2011).

2.2.2.6.3. Platelet larger cell ratio:

Platelet larger cell ratio (P_LCR) is an indicator of circulating larger platelets (more than 12fl) which is presented as percentage, the normal range is15-35%, use to monitor platelet activity (Budak *et al*, 2016).

2.2.2.6.4. Platelet large cell concentration:

It is the actual concentration of that large platelet in the total platelet count presented as count by 10^9cell/L (Bashir, 2017).

2.2.2.6.5. Plateletcrit:

Plateletcrit (PCT) is the volume occupied by platelets in blood as percentage and calculated according to the formula PCT = PLT count × MPV÷10000. The normal range is 0.22-0.24%. Higher with acute colitis and cholecystitis (Budak *et al*, 2016). Plateletcrit (PCT) measures total platelet mass as a percentage of volume occupied in the blood. It seems to play an effective screening role in detecting platelet quantitative abnormalities. The PCT is nonlinearly correlated to the platelet count

and indicates comparable clinical implication. PCT potential as a novel biomarker of active Crohn's disease in patients with low high sensitivity C-reactive protein (hs-CRP) (Jindal *et al*., 2011).

2.2.3. Coagulation:

MacFarlane and Davie and Ratnoff provided the first integrated descriptions of the coagulation system. They proposed a "cascade" or "waterfall" sequence of events in which the reactions occur in a defined series leading to prothrombin activation and fibrin clot formation. Each reaction shares a similar mechanism in which an inactive zymogen is converted to an active enzyme (Greer, *et al*., 2014).

2.2.3.1. Coagulation Factors:

2.2.3.1.1. FI (Fibrinogen):

The fibrinogen gene cluster is located on chromosome Polymerization of fibrinogen occurs when thrombin cleaves two short, negatively charged fibrinopeptides A and B from the N-termini of the α- and β-chains, respectively. This reveals new N-terminal sequences in the fragment E region (called knobs) that fit into holes in the fragment D regions (Hoffbrand *et al.,* 2016).

2.2.3.1.2. F II (Prothrombin):

The mRNA encodes a preproleader sequence similar to that found in other Vitamin-K-dependent proteins, followed by a GLA domain, two kringle domains, an activation peptide and a serine protease domain. FXa complexed with FVa activates prothrombin zymogen to thrombin on a phospholipid surface (prothrombinase) on cleavage of two peptide bonds. Fully cleaved thrombin is termed α-thrombin, and is rapidly released from its site of production to participate in numerous haemostatic functions free in solution: acting as a procoagulant against many substrates including fibrinogen, FV, FVIII and FXI; acting in complex with TM as an anticoagulant against PC; and acting to activate cellular transmembrane protease-activated

receptors. Thrombin also activates thrombin activatable fibrinolytic inhibitor (TAFI), in complex with TM, inhibiting fibrionlysis (Hoffbrand *et al.,* 2016).

2.2.3.1.3. F III (Tissue factor):

The formation of the FVIIa–TF complex is regarded as the sole initiator of coagulation in both normal and pathological coagulation. The extracellular region binds tightly to FVIIa to form a highly active procoagulant complex. TF is also expressed by monocytes and endothelium after activation by inflammatory cytokines or by endotoxin, as occurs in sepsis, and on cancerous tissues (Hoffbrand *et al.,* 2016).

2.2.3.1.4. Ionized Calcium (Formerly Factor IV):

The term ionized calcium has replaced the term factor IV. Ionized calcium is necessary for the activation of thromboplastin and for the conversion of prothrombin to thrombin. Ionized calcium is the physiologically active form of calcium in the human body, and only small amounts are needed for blood coagulation. A calcium deficiency would not be expressed as a coagulation dysfunction, except in cases of massive transfusion (Turgeon, 2012).

2.2.3.1.5. Factor V (Proaccelerin):

The structure of FV, like that of FVIII, can be represented as A1–A2–B–A3– C1–C2. In contrast with FVIII the B domain is required for full procoagulant function. FV also differs from FVIII in that it lacks the three short acidic interdomain peptides (a1, a2 and a3) implicated in FVIII function. FV is the cofactor for the activation of prothrombin by FXa. It has no cofactor activity until proteolysed by thrombin or FXa, FVa is inactivated by APC The initial cleavage is at Arg506 this is the site of the mutation in FV Leiden (FV Arg506Gln) that is resistant to APC, leading to the most common form of familial thrombophilia (Hoffbrand *et al.,* 2016).

2.2.3.1.6. Factor VII (Proconvertin):

Plasma FVII binds to TF, for example after vessel trauma to form a complex that initiates coagulation by directly activating FX and to a lesser extent FIX. The FVII gene (*F7*) lies adjacent to the factor X gene (*F10*), In common with the other serine proteases of the coagulation network (FIX, FX, prothrombin, PC), as well as PS and PZ, FVII has an N-terminal domain that contains a number (9– 12) of glutamic acid residues that are post-translationally modified by the addition of a carboxyl group to the γ-carbon by a Vitamin-K-dependent carboxylase. This γ-carboxyglutamic acid (GLA) domain confers affinity to negatively charged phospholipidmembranes, such as those of activated platelets or endothelial cells, promoting the assembly of functional multiprotein complexes on these surfaces. Proteins containing GLA modules are commonly referred to as 'vitamin K-dependent factors' as this vitamin is a cofactor in the carboxylation reaction. Blocking this post-translational modification with coumarin derivatives such as warfarin is one of the main treatments for the long-term prevention of thromboembolic events. The half-life of FVII zymogen inplasma is 3 hours and, remarkably, thehalf-life of the FVIIa enzyme is 2.5 hours, probably because there is no plasma inhibitor capable of effectively neutralizing free FVIIa. Recombinant FVIIa is in clinical use as a treatment for haemophiliacs with inhibitors and, for a wider range of bleeding problems and general surgical intervention (Hoffbrand *et al.,* 2016).

2.2.3.1.7. Factor VIII (Antihemophilic Factor):

The FVIII gene (*F8*) located on X chromosome (Xq28) FVIII is the essential cofactor for activation of FX by FIXa in the tenase complex. It has no function until proteolysed to FVIIIa by thrombin or FXa. FVIIIa is directly inactivated by APC however functional activity of FVIIIa also decays rapidly by dissociation of the A2 subunit from FVIIIa (Hoffbrand *et al.,* 2016).

2.2.3.1.8. Factor IX (Plasma Thromboplastin Component):

The FIX gene (*F9*) is located at Xq26, about 15.2 Mb from the FVIII gene (*F8*); thus deficiencies of both factors are X-linked disorders. Deficiency of FIX results in clinical haemophilia B, as the main function of FIX is to participate in the tenase complex (FIXa–FVIIIa). Plasma half-life of FIX is 18 hours (Hoffbrand *et al.,* 2016).

2.2.3.1.9. Factor X (Stuart Factor):

The gene and protein structures of FX closely resemble those of FVII. The half-life of FX in plasma is 36 hours. FX is activated by either FIXa–FVIIIa or TF–FVIIa on phospholipid surfaces in the presence of Ca2+ ions. FXa forms a phospholipid bound complex with FVa, which efficiently activates prothrombin (prothrombinase complex) (Hoffbrand *et al.,* 2016).

2.2.3.1.10. Factor XI (Plasma Thromboplastin Factor):

FXI is a zymogenof a serineprotease The protein circulates as a homodimer, with the two monomer subunits linked via a series of interactions It is clear that dimerization is required for full function of this factor. There is increasing evidence that FXI has other roles in haemostasis, such as regulation of fibrinolysis by the thrombinactivatable fibrinolysis inhibitor (TAFI). The fact that bleeding symptoms in FXI deficiency correlate poorly with FXI procoagulant level suggest that these other functions may be important in determining the phenotype in this mild bleeding disorder (Hoffbrand *et al.,* 2016).

2.2.3.1.11. Factor XII (Hageman Factor):

Factor XII is a stable factor that is not consumed during the coagulation process. Adsorption of factor XII and kininogen (with bound prekallikrein and factor XI) to negatively charged surfaces such as glass or subendothelium (collagen) exposed by blood vessel injury initiates the intrinsic coagulation pathway. Surface absorption alters and partially activates factor XII to factor XIIa by exposing an active enzyme (protease) site. Because of a feedback mechanism, kallikrein (activated Fletcher

factor) cleaves partially activated factor XIIa molecules adsorbed onto the subendothelium to produce a more kinetically effective form of XIIa (Turgeon, 2012).

2.2.3.1.12. Factor XIII (Fibrin-Stabilizing Factor):

FXIII circulates as a tetramer of two A-chains and two B-chains. The B-chains function as carriers for the A-chains which, after activation by thrombin, function as a transglutaminase to crosslink fibrin and other proteins in the clot, resulting in a stable structure. FXIIIa contains a free sulfydryl group at the active site. Platelets also contain FXIIIA-chain dimers, which are fully functional after thrombin activation (Hoffbrand *et al.,* 2016).

2.2.3.2. Coagulation Inhibitor:

The coagulation inhibition activity displayed by thrombin is the binding of AT-III to inhibit serine proteases and binding to thrombomodulin to activate protein C. In addition, the other activity in this category is the promotion of endothelial cell release of t-PA. The naturally occurring inhibitors include AT-III, alpha-2 macroglobulin inhibitor, and alpha-1 antitrypsin (Turgeon, 2012).

2.2.3.3. Coagulation Pathways:

Initiation of clotting begins with either the extrinsic or the intrinsic pathway. Factor X activation is the point of convergence. Factor X can be activated by either of the two pathways

and subsequently catalyzes the conversion of prothrombin to thrombin (Turgeon, 2012).

2.2.3.3.1. The Extrinsic Coagulation Pathway:

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, 2012).

2.2.3.3.2. The Intrinsic Coagulation Pathway:

The intrinsic pathway involves the contact activation factors prekallikrein, HMWK, factor XII, and factor XI. These factors interact on a surface to activate factor IX to IXa. Factor IXa reacts with factor VIII, PF 3, and calcium to activate factor X to Xa. In the presence of factor V, factor Xa activates prothrombin (factor II) to thrombin, which in turn converts fibrinogen to fibrin. Strong negatively charged solids that can participate in the activation of factor XII include glass and kaolin in vitro as well as elastin, collagen, platelet surfaces, kallikrein, plasmin, and high–molecular-weight kininogen in vivo. Collagen exposed by blood vessel injury greatly infl uences the rate of reaction. Factor XIIa interacts in a feedback loop to convert prekallikrein to additional kallikrein. This reaction is facilitated by the action of HMWK. In the absence of prekallikrein, factor XIIa is generated more slowly. Ionized calcium plays an important role in the activation of certain coagulation factors in the intrinsic pathway. Calcium is not required for the activation of factor XII, prekallikrein, or factor XI but is necessary for the activation of factor IX by factor Xia (Turgeon, 2012).

2.2.3.3.3. Final Common Pathway:

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 (Turgeon, 2012).

2.2.3.3.4. Fibrin Formation:

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. Factor XIII is converted to the active form, factor XIIIa, in two steps. In the first step, thrombin cleaves a peptide from each of the two alpha chains of factor XIII with formation of an inactive intermediate form of factor XIII. In the second step, calcium ions cause factor XIII to dissociate, forming factor XIIIa. Fibrinogen is normally present in the plasma as a soluble molecule. Subsequent to the action of thrombin, fibrinogen is transformed into fibrin, an insoluble gel. This conversion of fibrinogen to a cross-linked gel occurs in several stages (Turgeon, 2012).

2.2.3.4. Fibrinolysis:

Once damaged endothelium is repaired the fibrin thrombus must be removed to restore normal blood flow. Thrombus removal is facilitated by a fibrin-splitting serine protease, plasmin. Release of tissue plasminogen activator (t-PA) from endothelial cells leads to conversion of the proenzyme plasminogen into plasmin. t-PA is most active when bound to fibrin, thus maximising its action at the site of the thrombus. Plasmin has the capacity to digest fibrin in addition to fibrinogen and a number of other proteins. Digestion of a crosslinked thrombus by plasmin leads to the formation of 'degradation products' which themselves act as anticoagulants. Fibrinolysis is under strict control; circulating plasmin is inactivated by the protease inhibitor α2-antiplasmin (Howard and Hamilton., 2013).

Inactive plasminogen circulates in the plasma until an injury occurs. The activators of plasminogen consist of endogenous and exogenous groups. Plasminogen activation to plasmin is the result of the activity of a number of proteolytic enzymes. These enzymes, the kinases, are referred to as the plasminogen activators. Plasminogen activators are found in various sites, such as the vascular endothelium or lysosomal granules, and biological fluids. At least two forms of tissue activators have been described: those that seem related to urokinase, a urinary activator of plasminogen, and those unrelated to urokinase. The activators unrelated to urokinase include thrombin, bacterial products such as streptokinase from beta-hemolytic streptococci, and staphylokinase. Plasma activators of plasminogen include plasma kallikrein, activated plasma thromboplastin antecedents (factor XI), and activated Hageman factor (factor XIIa). Tissue-type plasminogen activator (t-PA) is present in minute quantities in the vascular endothelium. When t-PA encounters a blood clot, t-PA transforms plasminogen to plasmin, and plasmin then degrades the clot's fibrin network. As a result of biotechnology (recombinant DNA), a synthetic tissue-type plasminogen has been developed and is used clinically to treat postmyocardial

infarction and pulmonary emboli. t-PA is considered by many to be more specific and twice as effective as streptokinase in dissolving clots and has caused fewer side effects. Through its lysis of fibrin or fibrinogen, plasmin is responsible for forming degradation or fibrin split products consisting of intermediate fragments X and Y, and fragments D and E. These fragments exert an antithrombin effect, inhibit the hemostasis system through interference with fibrin monomer polymerization, and interfere with platelet aggregation. Small amounts of plasmin become trapped in the clot. The specificity of plasmin ensures that clot dissolution occurs without widespread proteolysis of other proteins. Plasmin also activates the complement system, liberates kinins from kininogen, and can hydrolyze coagulation factors V, VIII, and XII. Further clot formation is impeded by antiplasmins and naturally occurring inhibitors, some of which prevent the activation of plasminogen. Plasmin is not normally found in plasma because it is neutralized by an excess of inhibitors (Turgeon, 2012).

2.2.3.4.1. Inhibitors of fibrinolysis:

The plasmin-generating potential of plasma is sufficient to completely degrade all the fibrinogen in the body in a very short period of time. It is prevented from doing so by the PLG activator inhibitors or PAIs, most of which belong to the serpin family, and by a number of circulating inhibitors of plasmin itself (the antiplasmins) (Hoffbrand *et al.,* 2016).

2.2.3.5. Coagulation Profile:

Coagulation and hemostasis involve interactions between tissue and coagulation factors as well as blood and endothelial cells, finally resulting in formation of fibrin clots stopping bleeding. During this process, the fibrinolytic system decomposes generated clots to prohibit widespread thrombus formation and vascular occlusion (Li *et al*., 2021).

At the time of injury to the endothelium, the integrity of the high-pressure circulatory system is maintained through the hemostatic mechanism. In general terms, a “plug” of platelets is covered over by a “net” of fibrin, resulting in the formation of a clot. The resultant clot normally leads to cessation of bleeding. Bleeding occurs when there is a precipitant such as direct traumatic injury to the endothelium or in the case of menstruation hormonal-induced shedding of the endothelium, so “injuring” the endothelium. The understanding of hemostasis can be simplified into two steps: first (step 1) the formation of the platelet “plug” at the initial site of injury and second (step 2) the formation of a “net” of fibrin covering the platelet plug (Christine *et al.*,2009).

Formation of thrombin via a series of reactions within the coagulation system is central in coagulation activity. Coagulation is initiated *in vivo* mainly through exposure of tissue factor, TF, on damaged tissue or endothelium. Activated monocytes can also expose TF, e.g. in sepsis. TF binds and activates FVII. The TF-FVIIa (a = activated) complex initiates coagulation by activating FIX and FX, and they transform prothrombin into thrombin. The process continues, mainly as surface connected enzymatic reactions, where activated platelets probably offer the phospholipid surface to which coagulation factors (enzymes as well as co-factors) can bind e.g. by means of Ca^{+2} bridges. Moreover, the coagulation inhibitors (antithrombin, APC) quickly react with non-connected enzymes and co-factors, which helps to limit the spread of fibrin formation. Thrombin cleaves off fibrinopeptides A and B to form fibrin monomers, which then polymerize and cross-link to form an insoluble fibrin network. The formation of thrombin is accelerated initially by a positive feedback, whereby the thrombin activates FVIII and FV in order to produce more thrombin. Thrombin also promotes coagulation by activating platelets and endothelium. The thrombin specificity is modified by its binding to the endothelial receptor thrombomodulin (TM). The TM–thrombin complexes then

activate protein C into active protein C (APC), which then decomposes FVIIIa and FVa. So, thrombin both stimulates and inhibits its self-formation. A model for cell associated blood coagulation has also been proposed where the reaction sequence has been divided into three stages:

• The initiation phase where a small amount of thrombin is generated via the extrinsic pathway to activate platelets and coagulation co-factors V and VIII to their activated forms.

• The priming phase where coagulation factors bind to receptors and phosphatidyl serine enriched surfaces on activated platelets.

• The propagation phase where thrombin is formed both via the intrinsic and extrinsic pathways in order to generate large amounts of thrombin that will transform fibrinogen to fibrin. Screening analyses undertaken in hemostatic contexts usually include CB/B-PLT, P-APT time, CB/P-PT (INR), P-fibrinogen and, if applicable, Pt-bleeding time. If disseminated intravascular coagulation (DIC) is suspected, usually P-fibrin D-dimer and P-antithrombin are also measured (P-factor VIII can also be determined) (Antovic and Blomback., 2010).

2.2.3.5.1 Prothrombin Time and Internatinal Normalization Ratio:

PT is a widely used test to evaluate secondary hemostasis. In this test, platelet poor plasma from a patient (collected in a blood collection tube containing sodium citrate) is mixed with thromboplastin and calcium, and then clotting time is determined at 37°C using a variety of methods, including photooptical and electromechanical. Automated coagulation analyzers are commercially available for measuring PT along with other coagulation parameters. PT is a functional measure of the extrinsic pathway and the common pathway, and the reference range is 8.8_11.6 sec. Therefore, PT is a useful test to detect inherited or acquired defects in coagulation related to the extrinsic pathway. However, often PT is reported in the form of the international normalized ratio (INR). The thromboplastin used may vary from

laboratory to laboratory and from country to country. However, reporting results as INR ensures results are comparable between different laboratories. INR is calculated as follows: INR 5½patient PT=mean normal PT_ISI where ISI is the international standardized index, which is available from the reagent package insert. The normal value of INR is 0.8_1.2 (Dasgupta and Wahed, 2014).

Normal values depend on the thromboplastin used, the exact technique and whether visual or instrumental endpoint reading is used. With most rabbit thromboplastins the normal range of the PT is between 11 and 16 s; for recombinant human thromboplastin, it is somewhat shorter (10–12 s). Each laboratory should establish its own normal range (Bain *et al*., 2011).

2.2.3.5.2. Activated Partial Thromboplastin Time:

PTT (also known as activated partial prothrombin time: (APTT or aPTT) is another useful test for evaluation of secondary homeostasis. In this test, a patient's platelet-poor plasma (citrated plasma, but oxalate can also be used), surface activating agent (silica, kaolin, celite, or ellagic acid), calcium, and platelet substitute (crude phospholipid) are mixed, and clotting time is usually determined using an automated coagulation analyzer. It is a functional measure of the intrinsic pathway as well as the common pathway and can detect hereditary or acquired defects of the coagulation factors XII, XI, X, IX, VIII, V, prothrombin, and fibrinogen. PTT or APTT derives its name from the absence of tissue factor (thromboplastin) in the tests. The normal value varies from laboratory to laboratory, but it is usually between 25 and 39 secs (Dasgupta and Wahed, 2014). The normal range is typically 26–40 s. The actual times depend on the reagents used and the duration of the preincubation period, which varies in manufacturer's recommendations for different reagents. These variables also greatly alter the sensitivity of the test to minor or moderate deficiencies of the contact activation system. Laboratories can choose appropriate

conditions to achieve the sensitivity they require. Each laboratory should calculate its own normal range (Bain *et al*., 2011).

2.2.3.6. D-dimer:

D-dimer is a fibrin degradation products (FDPs), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. It is so named because it contains two D fragments of fibrin protein joined by a cross-link. D-dimer concentration may be determined by a blood test to help diagnosis of thrombosis. Since its introduction in 1990s, it has become an important test performed in patients with suspected thrombotic disorders. A negative result practically rules out thrombosis, while a positive result may indicate thrombosis but does not rule out other potential causes. Its main use, therefore, is to exclude thromboembolic disease where the probability is low. Moreover, it has been used in the diagnosis of the blood disorder Disseminated Intravascular Coagulation (DIC) (Elbadri *et al*., 2020).

An indirect marker of thrombotic activity, dimer is the final fragment of degradation of cross-linked fibrin that was mediated by fibrin. The elevated D-dimer level was detected at the onset of thrombosis, and the negative level was used to exclude the venous thromboembolism. D-dimer was increased in diabetic patients under most studies, and when there is a hypercoagulable state, hyperfibrinolysis will occur, subsequently elevation of D-dimer. Due to hypercoagulable and hypofibrinolytic states, diabetes was considered important risk factor for thrombosis.One of the marker is D-dimer, when elevated showed hyperactivation of hemostatic system (Widjaja and Syahputa, 2020).

Fibrinolysis is mediated by plasmin, which degrades fibrin clots into D-dimers and fibrin degradation products. Assays for D-dimer and FDPs are semiquantitative or quantitative immunoassays: Latex agglutination assays are also available for D-dimers. Various automated and quantitative versions of this assay are commercially available for D-dimers, in which the agglutination is detected turbidimetrically by a

coagulation analyzer rather than visually by a technologist. Enzyme-linked immunosorbent assay (ELISA): Quantitative ELISAs are also available for FDP and D-dimers. The traditional ELISA method is accurate but is not useful due to a long analytical time. An automated, rapid ELISA assay for D-dimers is also available, the reference value is 0.0 _ 0.4 ug/ml (Turgeon, 2012, Dasgupta and Wahed, 2014).

2.3. Previous studies:

The result of study conducted in Sudan by (Bayoumi *et al*, 2018) involved 90 diabetics and 100 non-diabetics to determine altered platelets morphological parameters in type 2 diabetes mellitus**.** Showed that MPV and PDW higher in diabetic than non-diabetic statistically significant, platelet count was higher in diabetic than non-diabetic but not statistically significant.

The result of study conducted in India by (Kodiatte *et al*, 2012) subjected on 300 diabetics and 300 non-diabetics to show MPV in type 2 diabetes mellitus. Means platelet count and MPV were higher in diabetic compared to non-diabetic and statistically significant.

In 2018 (Shilpi and Potekar, 2018) study platelet indices in type 2 diabetes mellitus in India. This study carried out on 280 cases and 280 controls. MPV, PDW and P-LCR were significantly higher in diabetic compare with non-diabetic.

In 2011 (Jindal *et al*, 2011) in India study platelet indices in diabetes mellitus included 75 with diabetic and 50 non-diabetic. MPV, PDW and P-LCR were statistically significantly higher in diabetic compare with non-diabetic.

(Alhadas *et al*, 2016) in France determined the evaluation of diabetes mellitus by estimate the platelet indices. This study subjected on 100 diabetics and 100 non-diabetics. PCT, MPV and PDW were statistically significant higher among patients with diabetes than non-diabetic.

In 2016 (Yilmaz and Yilmaz, 2016) in Turkey consider the releationship between altered platelet morphological parameters in type 2 DM, subjected on 85 as control and 262 as case. Showed that MPV, PDW and P-LCR were statistically significantly higher in diabetic compare with non-diabetic.

(Brown *et al*, 2018) in London study platelet changes in diabetic patients subjected on 63 patients. Their result showed platelet count was not significantly change among group and MPV was statistically significant higher in group.

(Citrik *et al*, 2015) in Turkey study on 140 patients and 40 health subject to evaluate MPV and platelet activation in diabetic patients. MPV was statistically significantly higher in diabetic compare with non-diabetic whereas PDW and PCT were not significantly change among groups.

In 2016 (Elkhalifa *et al*, 2016) in Sudan study MPV in type 2 diabetes mellitus conducted on 40 patients and 10 healthy individuals. MPV and PDW were statistically significantly higher in diabetic compare with non-diabetic.

The result of (Hasan *et al*, 2016) in India study for assessment of mean platelet type 2 diabetes mellitus and pre-diabetes, included 77 with type 2 diabetic, 25 pre-diabetics and 38 healthy subjects. MPV was not statistically significantly in diabetic when compare to non-diabetic.

(Elbadri *et al*, 2020) in Sudan evaluate platelet parameters and D-dimer level in long standing type 2 Diabetes mellitus, included 40 participants were diabetic and 40 were non-diabetic and found out that Platelets count was statistically insignificant while MPV, PDW, PCLR, and D-dimer Level were statistically significantly increased.

In 2020 (Widjaja and Syahputa, 2020) study the correlation of D-dimer in diabetic patients, 60 samples were including in this study and realize D-dimer was significantly increase in diabetes individuals.

In India (Sherin *et al*, 2020) compare coagulation profile in type 2 diabetic patients, collaborate in this study 84 individuals, and obtain that APTT was significantly increase in type 2 diabetics and no significant changes were noted in PLT count, PT and D-dimer.

This study was done in China by (Cheng *et al*, 2022) submitted on 1976 patients with diabetes and found that D-dimer has correlation with age, sex and form of treatment.

(Kanani *et al*, 2017) in India study the association of D-dimer in type 2 D.M and the total number included in this study is 90 and concluded that patients had significantly higher plasma D-dimer levels.

(Ebrahim *et al*., 2021) showed that PT, INR are reduced in type 2 DM compared with healthy control, and PDW, MPV are significantly increase in type 2 DM, the total of participants is 180 and done in Ethiopia.

Plasma coagulation were measured in 297 type 2 DM patients, this study was done in China by (Pan *et al*., 2018) and the result showed that APTT was shortened and platelet was decrease and D-dimer was increase compared with control.

(Agaewal *et al*., 2018) in India showed that APTT significantly lower when compare it in diabetics and non-diabetics and this applied on 60 diabetics and 30 control.

In Nigeria (Toyosi *et al*., 2021) study the assessment of coagulation and fibrinolytic factors among patients with type 2 DM, the number of cases was 78 and control was 78 and realized that no significant differences were observed in platelet count, APTT and INR.

Hemostatic abnormality and associated factors in diabetic patients were evaluated in Ethiopia by (Asrat *et al*., 2019) on 119 diabetics and 119 healthy individuals and showed the PT was not statistically significant, APTT was significant differences and PLT count was significant differences.

Chapter Three

Materials and Method

CHAPTER III

Materials and methods

3.1. Study design:

This is an analytical case control study conducted in Khartoum state in the period from August to October 2022.

3.2. Study population and sample size:

One hundred individual were participated in this study and classified into two groups: 50 individuals were type 2 diabetic, and 50 individuals were non diabetic as control group with matched sex and age. Sample size equation: $n = z^2 (p*q) / d^2$

n= sample size, p= prevalence of problem, q= 1_ p, d= acceptable error, z= 1.96 (standard normal deviate). According to economic situation of the country 100 individual were participated.

3.3. Inclusion criteria:

Case group were type 2 DM and must be free from other diseases that affect parameters under testing.

3.4. Exclusion criteria:

Previous history of hypertension, renal problems, and cardiovascular diseases and other diseases that affect parameters under testing were excluded.

3.5. Ethical consideration:

The specimens and information that collected from the participants were under privacy and confidentially. The aim of the research was explained for the type 2 diabetic patients under the study in simple language and verbal concept was obtained and they understood the research idea.

3.6. Data collection:

The data were collected by the direct interview through designed questionnaire.

3.7. Data analysis:

The data were computed and analyzed to obtain the platelet count, indices, coagulation profile and D-dimer using Statistic Package for Social Science (SPSS) program version16. The statistical analysis was performed by using Independent Sample T test and One-way ANOVA test.

3.8. Methods:

3.8.1. Method of blood sample collection and preparation:

3.8.1.1. Requirements of blood collection:

- Ethylene diamine tetra acetic acid (EDTA. K3) containers.
- Tri sodium citrate containers
- Cotton.
- Alcohol (70%).
- Syringe and tourniquet.

3.8.1.2. Procedure:

Sterile, dry, preferably plastic syringe was selected. A tourniquet was applied to the upper arm of the patient but not applied tightly or more than 2 minutes. The patient was asked to make a tight fist which will make the veins more prominent, sufficiently large straight vein was selected after been felt by index finger. The puncture site was cleaned with 70% ethanol and allowed to dry. The venipuncture was made while the bevel of the needle directed upwards in the line of the vein, the plunger of the syringe was steadily withdrawal when sufficient blood has been collected, the tourniquet was released and the needle removed from the vein. Then it is removed from the syringe and carefully the container(s) was filled with the required volume of blood. The blood was mixed immediately in an EDTA, Tri sodium anticoagulants (Cheesbrough, 2006).

3.8.1.3. Preparation of platelet poor plasma:

Most routine coagulation investigations are performed on platelet-poor plasma (PPP), which is prepared by centrifugation at 2000 g for 15 min at 4°C. The sample should be kept at room temperature if it is to be used for PT tests, lupus anticoagulant (LAC) or factor VII assays and it should be kept at 4°C for other assays; the testing should preferably be completed within 2 h of collection. Care must be taken not to disturb the buffy coat layer when removing the PPP. Samples for platelet function tests, LAC and the activated PC resistance (APCR) test should not be centrifuged at 4°C. These samples should be prepared by centrifugation at room temperature to prevent activation of platelets and release of platelet contents such as phospholipid and factor V. For LAC testing and APCR it is very important that the number of platelets and the amount of platelet debris in the samples are minimized. The platelet count should be below 10 _ 109/L. This is best achieved by double centrifugation or filtration of the plasma through a 0.2 mm filter (Bain *et al*., 2011).

3.8.2. Automatic hematology analyzer:

The mission HA-360 3-Diff Automatic hematology analyzer determine the WBC data by impedance method, determining the RBC and PLT data by sheath flow impedance method, determining the HGB by colorimetric method. This analyzer can process two types of blood cells samples and pre-diluted samples, dilute the blood sample to separate the cells so that they can pass through the aperture one by one, dilaution process also create a conductive environmental for cell counting, usually use number of red blood cells is 1,000 times bigger than the number of white blood cells, red blood cells can be eliminated using lyse solution before WBC counting (Instruction manual). (See appendix 2).

3.8.2.1. Reagents:

3.8.2.1.1. Diluent:

Cyto-diluent is a filtered isotonic solution with specific electrical conductivity, offering a stable environment for blood cell counting (Instruction manual). (See appendix 2).

3.8.2.1.2. Stomatolyser:

Cyto-lyser can dissolve red blood cell and combine with hemoglobin, which can be used for determining hemoglobin and for counting and sizing leukocyte (Instruction manual). (See appendix 2).

3.8.2.1.3. Cleaner:

Mi-Po Cleaner was used for cleaning pool and hydraulic cycle regularly (Instruction manual). (See appendix 2).

3.8.2.1.4. Quality control:

There is risk that the error may occur after long period of time usage of the analyzer. This error may cause the incorrect or unreliable analysis result. Quality control program offers an effective method to detect the potential error. In order to ensure the reliable of the analysis results, we recommended users run the QC program daily with low, normal and high level controls. A new lot of control should be tested in parallel with the current lot twice a day for five days prior to their expiration dates. The results should be within the expected ranges as described in the control insert. The Mission HA-360 3-Diff Automatic Hematology Analyzer provides two QC programs: L-J QC and X-mean QC. (See appendix 2).

3.8.3. COATRON Instrument for measurement of PT and APTT:

3.8.3.1. Theory of operation:

The COATRON M1 is a highly sensitive signal channel photometer. A very intensive laser LED-Optic at 400 nm ensures accurate and precise results. The receiver signal is detected and converted to an electrical current. During the actual test the system

is searching for the best signal amplification. The software algorithms are based on optical density. Plasma/blood and reagent absorb the transmitted laser light. The rate of absorbency is obtained by the detector and sent to the micro controller. Here a program analyses the signal and send the result to the display (Operation manual). (See appendix 3).

3.8.3.2. Prothrombin time test:

3.8.3.2.1. Principle of PT:

The one stage Prothrombin time measures the clotting time of test plasma after the addition of thromboplastin reagent containing calcium chloride. The reagent supplies a source of tissue thromboplastin, activating factor VII, and is therefore sensitive to all stage II and III factors (Operation manual). (See appendix 4).

3.8.3.2.2. Reagents:

LIQUPLASTIN is a novel, highly sensitive, low opacity, ready to use liquid calcified liquiplastin reagent, which is derived from rabbit brain (EGY-CHEM for lab technology). (See appendix 4).

3.8.3.2.3. Procedure:

25 ul of plasma were added into cuvette and prewarmed plasma foe 1 minute then transferred to measuring position and activate optic and 50 ul of prewarmed thromboplastin were added and start optic. The result was displayed in second and INR (Operation manual). (See appendix 4).

3.8.3.2.4. Quality control:

Control plasma should be tested in conjunction with patient samples. It is recommended that at least one normal and one abnormal be run at least each shift and a minimum of once per 20 patient samples. A control range should be established by the laboratory to determines the allowable variation in day to day performance of each control plasma. (See appendix 4).

3.8.3.2.5. International normalization ratio:

Calculated from the formula: INR= (Patient PT/ Normal PT) ^{ISI} (See appendix 5).

3.8.3.3. Activated partial thromboplastin time:

3.8.3.3.1. Principle of APTT test:

The APTT test measures the clotting time test plasma after addition of APTT reagent, then allowing an activation time, followed by the addition of calcium chloride. Deficiencies of approximately 40% and lower of factors VIII, IX, XI and XII wll result in a prolonged APTT (Operation manual). (See appendix 6).

3.8.3.3.2. Reagents:

LIQUCELINE-E is liquid ready to use activated cephaloplastin reagent for the determination of APTT. It is a phospholipids preparation derived from rabbit brain with ellagic acid as an activator (EGY-CHEM for lab technology). (See appendix 6).

3.8.3.3.3. Procedure:

25 ul of plasma were added into cuvette and 25 ul of APTT reagent were added to plasma then incubated for 3 minutes and transferred cuvette to measuring position and activate optic then 25 ul of prewarmed calcium chloride were added and start optic. The result was displayed in second and ratio (Operation manual). (See appendix 6).

3.8.3.3.4. Quality control:

Control plasma should be tested in conjunction with patient samples. It is recommended that at least one normal and one abnormal be run at least each shift and a minimum of once per 20 patient samples. A control range should be established by the laboratory to determines the allowable variation in day to day performance of each control plasma. (See appendix 6).

3.8.4. Biotime device for D-dimer measurement:

3.8.4.1. Principle:

This test kit is based on fluorescent lateral flow immunoassay. While the sample and the buffer are mixed and applied into the test cartridge, the D-dimer in the sample and the mouse anti-D-dimer monoclonal antibody labeled with fluorescent microsphere form a reaction intermediate complex. During lateral flow, the intermediate complex moves along with the nitrocellulose membrane to a detection line (T-line: coated with D-dimer specific monoclonal antibodies). The intermediate complex will be captured by T-line to form final reaction compound sandwich. Thus the fluorescent signal on detection line is positively correlated with the concentration of D-dimer in human blood. The fluorescent signal from microspheres of compound sandwich will be detected and calculated according to the calibration curve (in SD card provided with the reagents) to represent the concentration of D-dimer in human whole blood or plasma (Xiamen Biotime Biotechnology Co.,Ltd.). (See appendix 7).

3.8.4.2. Components:

Test cartridge, detection buffer, SD card and incubator (Xiamen Biotime Biotechnology Co.,Ltd.). (See appendix 7).

3.8.4.3. Procedure:

The test should be operated at room temperature. SD card was checked into the equipment. One tube of buffer was taken from refrigerator and balance it to room temperature. 50 ul of plasma or 80 ul of whole blood were added into the buffer tube mixed well the specimen with buffer by tapping or inverting the tube, 80 ul of sample mixture were taken and load it into the well of the test cartridge this step should be completed within 1 minute to insure the accuracy of the test.

When the mixure is dropped into the well of test cartridge, immediately countdown reaction time 3 minute with a timer, when time is up, inserted the test cartridge into

test cartridge inlet immediately, the test result will be showed on screen and printed automatically (Xiamen Biotime Biotechnology Co.,Ltd.). (See appendix 7).

3.8.4.4. Quality control:

D-dimer control materials with two different concentrations were tested by every batch of test cartridges, and the deviation were within ± 15%. (Xiamen Biotime Biotechnology Co.,Ltd.). (See appendix 7).

Chapter Four

Results

CHAPTER IV

Results

4.1. Results:

One hundred (100) venous blood samples were collected from the participants; 2.5 ml placed in EDTA anticoagulant tubes and 1.8 placed in Tri sodium citrate anticoagulant tubes; 50 of each were diabetics whereas 50 of each were non-diabetics. Platelet count, platelets indices were measured by Mission HA-360 3-Diff Automatic hematology analyzer, D-dimer was measured by BIOTIME and coagulation profile was evaluated by COARTON IM instruments.

This study is subjected on 51 males and 49 females, and the age of participants is arranged into 3 categories which are: 20-40 years and those are 46, 41-60 years were 35 and 60-81 years were 19 individulas.

Figure (1): Gender distribution:

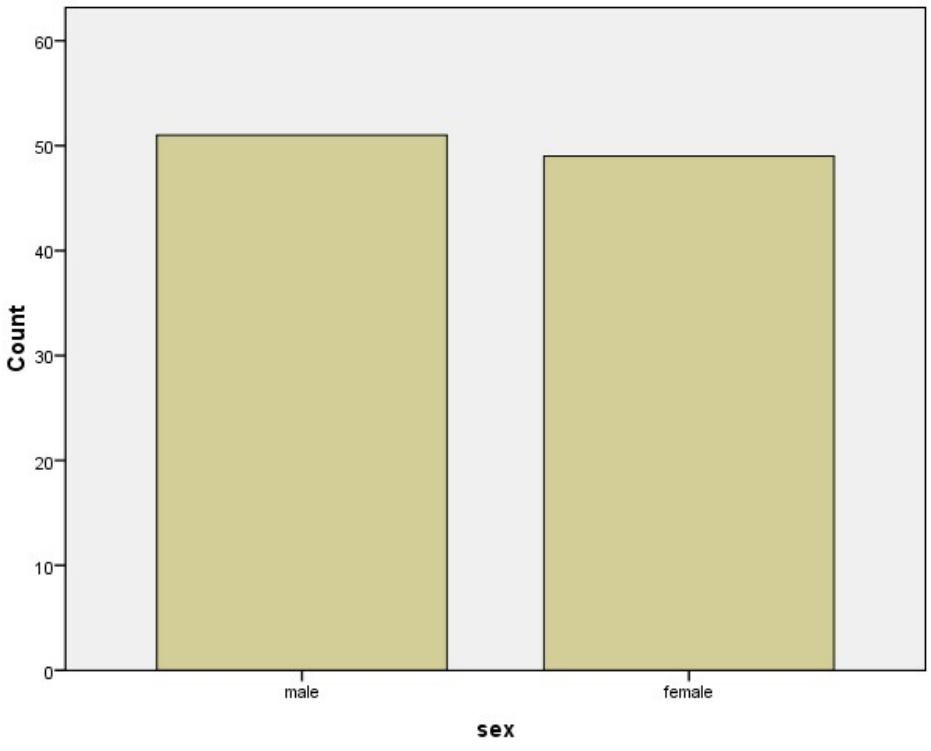


Figure (2): Age distribution:

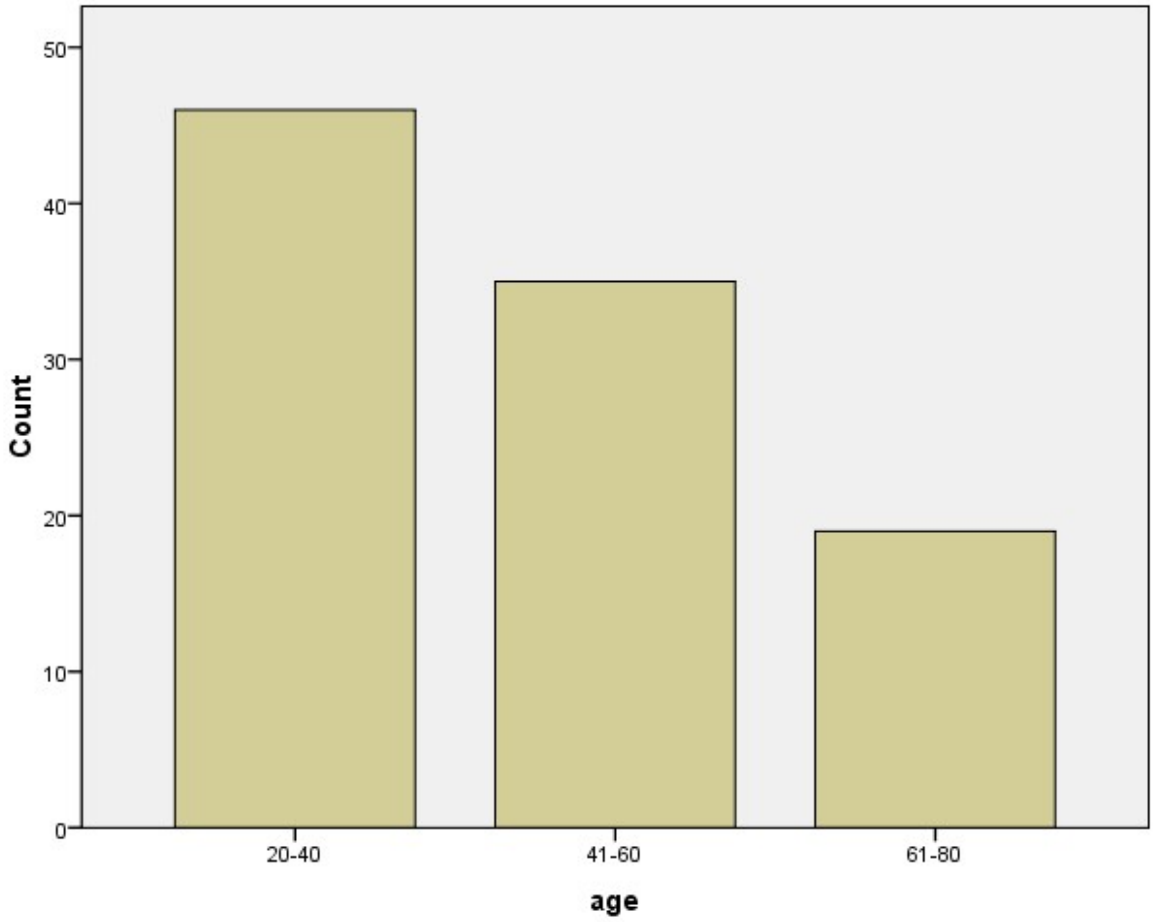


Table No. (1): Type 2 diabetic patients and control group with PLT count and indices:

Study group / Parameters	Diabetic patients Means ±SD	Control group Means ±SD	*P* value
Number	50	50	
PLT count	252.3±78.24	255.1±64.37	0.22
MPV	10.8±1.06	10.3±1.03	0.72
PDW	14.4±2.59	14.2±2.09	0.44
P-LCR	18.7±6.90	14.9±4.42	0.02
P-LCC	39.0±10.43	35.1±9.42	0.94
PCT	0.3±0.08	0.3±0.06	0.04

This table show there was significant changes in P-LCR and PCT with *p* values (0.02 and 0.04) respectively and no significant changes in PLT count, MPV, PDW and P-LCC with p values (0.22, 0.72, 0.43 and 0.94) respectively. And the mean of PLT count was lower in case than in control groups, the mean of P-LCC was higher in diabetics than in non-diabetics and there was no change in means of MPV and PDW.

Table No. (2): Type 2 diabetic patients and control group with coagulation profile and D-dimer:

Study group / Parameters	Diabetic patients Mean ±SD	Control group Mean ±SD	*P* value
Number	50	50	
PT	16.5±4.69	17.0±1.92	0.46
INR	1.2±0.37	1.1±0.27	0.06
APTT	33.5±0.37	40.0±42.67	0.29
D-dimer	0.5±0.54	0.1±0.06	0.00

In this table there was significant change in D-dimer with *p* value (0.00), and no significant changes in PT, INR and APTT with *p* values (0.46, 0.06 and 0.29) respectively. And there was mild increase in means of PT, INR and D-dimer, and increase in mean of APTT in control group.

Table No. (3): **Type 2 diabetic patients with PLT count and indices according to duration of disease:**

Duration of DM / Parameters	<1 year Mean ± SD	1-5 years Mean ± SD	6-10years Mean ± SD	>10 years Mean ±SD	*P* value
Number	3	19	17	11	
PLT count	238.3±44.39	283.7 ± 71.60	231.8 ± 42.94	248.9 ±110.41	0.38
MPV	10.4± 0.52	10.8± 1.00	11.1± 0.70	10.8 ±1.50	0.49
PDW	13.4± 1.02	14.3± 2.32	14.4± 1.18	15.3± 3.80	0.30
P-LCR	16.1± 4.51	20.5± 8.00	20.5± 8.20	18.1± 6.55	0.35
P-LCC	34.4± 8.63	44.2± 10.57	39.6± 10.02	37.9± 10.76	0.12
PCT	0.3±0.06	0.3± 0.06	0.3± 0.06	0.3±0.09	0.14

Here in this table there was no statistically significant changes in PLT count, MPV, PDW, P-LCR, P-LCC and PCT with *p* values (0.38, 0.49, 0.30, 0.35, 0,12 and 0.14) respectively. And the mean of PLT count was higher in duration 1-5 years, mean of MPV was mild increase in duration 6-11 years, mean of PDW was slightly increase in duration >10 years, the duration 1-5 and 6-10 years were higher in means of P-LCR than others, the duration of 1-5 years show increase in mean of P-LCC and slightly increase in mean of PCT in duration 6-10 years.

Table No. (4): Type 2 diabetic patients with coagulation profile and D-dimer according to duration of disease:

Duration of DM / Parameters	<1 year Mean ± SD	1-5 years Mean ± SD	6-10years Mean ± SD	>10 years Mean ±SD	*P* value
Number	3	19	17	11	
PT	13.8±1.56	15.4±4.69	18.1±4.90	16.7±4.48	0.26
INR	1.0±0.10	1.1±0.34	1.4±0.39	1.2±0.37	0.89
APTT	30.3±0.46	33.7±8.14	33.8±7.77	33.4±5.57	0.11
D-dimer	0.6±0.36	0.7±0.60	0.64±0.60	0.5±0.22	0.81

Here in this table there was no statistically significant changes in PT, INR, APTT and D-dimer with *p* values (0.256, 0.892, 0.099 and 0.81) respectively. And the means of PT and INR were increase in duration 6-10 years, and the mean of APTT was decrease in duration < 1year and the mean of D-dimer was decrease in duration > 10 years.

Chapter Five

Discussion, Conclusion and Recommendations

CHAPTER V

Discussion, conclusion and recommendations

5.1. Discussion:

This is an analytical case control study that aimed to evaluate some hematological parameters in type 2 diabetic patients. This study was conducted on one hundred individuals, 50 are diabetics whereas 50 are non-diabetics, 100 venous blood samples were collected 2.5 ml put in EDTA anticoagulant tubes and 1.8 ml put in Tri sodium citrate anticoagulant tubes, to evaluate the effect of diabetes mellitus on platelet count, platelet indices, D-dimer and coagulation profile. Platelet count and indices were measured by Mission HA-360 3-Diff Automatic hematology analyzer, whereas D-dimer was measured by BIOTIME and coagulation profile was evaluated by COARTON IM instruments.

In this study the P-LCR was significantly increased in diabetic when compared with non-diabetic (18.7±6.90 vs 14.9±4.42, *p* value= 0.03) this result agree with (Shilpi K and Potekar, 2018) in India, (Yilmaz and Yilmaz, 2016) in Turkey and (Elbadri *et al*., 2020).

The PCT was significantly higher in diabetic than in non-diabetic (0.3±0.08 vs 0.3±0.06, *p* value= 0.05) which agree with (Alhadas *et al*., 2016) in France and disagree with (Citrik *et al*., 2015) in Turkey they found PCT was no statistically significant in diabetic patients.

The D-dimer was statistically significant higher in diabetics when compared with non-diabetics (0.5±0.05 vs 0.1±0.06, *p* value= 0.00) and this result was compatible with the result of (Elbadri *et al*., 2020) in Sudan, (Widjaja and Syahputa, 2020) in Indonesia, (Kanani et al., 2017) in India and (Pan *et al*., 2018) in China. And not compatible with (Sherin *et al*., 2020) in India found out there is no statistically significant change in D-dimer in patients with diabetes.

There was no significant change in platelet count among diabetic and non-diabetic (252.3±78.23 vs 255.1±64.37, *p* value= 0.22) which agree with (Bayoumi *et al*., 2018), (Elbadri *et al*., 2020) in Sudan, (Brown *et al*., 2018) in London, (Sherin *et al*., 2020) in India (Pan *et al*., 2018) in China, (Toyosi *et al*., 2021) in Nigeria and (Ephraim *et al*., 2017) in Ghana. And disagree with (Asrat *et al*., 2019) in Ethiopia.

There was no significant change in MPV between diabetic and non-diabetic (10.8±1.06 vs 10.3±1.03, *p* value= 0.72) which is compatible with (Hasan, 2016) in India and incompatible with (Bayoumi *et al*, 2018) in Sudan, (Papanas *et al*, 2009) in Greece, (Elkhalifa, 2016), (Elbadri *et al*., 2020) in Sudan and (Kodiatte, 2012) in India they found that MPV significantly increased in diabetic patients.

The PDW was show no significant change between diabetic and non-diabetic (14.4±2.59 vs 14.2±2.09, *p* value= 0.44) which is corresponding with (Citirik *et al*., 2014) in Turkey and not corresponding with (Jindal, 2011) in India and (Elbadri *et al*., 2020) in Sudan revealed PDW was statistically increased in case group.

The P-LCC had no significant change when compared with control group (39.0±10.43 vs 35.0±9.42, *p* value= 0.95).

The result of PT in this study was not statistically significant (16.5±4.69vs 17.0±1.92, *p* value= 0.46) and this is match with (Sherin *et al*., 2020) in India, (Ebrahim et al., 2021) in Ethiopia, (Asrat *et al*., 2019) in Ethiopia. And not match with the result of (Ephraim *et al*., 2017) in Ghana this show significant shortened in PT.

INR show there is no statistically significant change (1.2±0.37 vs 1.1±0.27, *p* value= 0.06) and this is corresponding with the result of (Toyosi *et al*., 2021) in Nigeria, (Ephraim *et al*., 2017) in Ghana and (Ebrahim *et al*., 2021) in Ethiopia.

APTT reveal there is no significant change when compare the case with control (33.5±0.37 vs 40.0±42.67, *p* value= 0.29) and this is show similarity with (Toyosi *et al*., 2021) in Nigeria, (Agarwal *et al*., 2018) in India, (Pan *et al*., 2018) in China.

And show no similarity with (Asrat *et al*., 2019) in Ethiopia and (Asrat *et al*., 2019) in Ethiopia.

There are no differences in means of MPV and PDW (10.6±1.06 vs 10.3±1.03, *p* value= 0.72 and 14.4±2.59 vs 14.2±2.09, *p* value= 0.44) respectively.

The means of P-LCC, PT and INR were higher in diabetic than in non-diabetic but statistically insignificant (39.0±10.43 vs 35.0±9.42, *p* value=0.95, 16.5±4.69 vs 17.0±1.92, *p* value= 0.46 and 1.2±0.37 vs 1.1±0.27, *p* value= 0.06) respectively.

The means of APTT and PLT count were lower in case compared with control and not statistically significant (252.3±78.23 vs 255.1±64.37 *p* value= 0.22 and 33.5±0.37 vs 40.04±42.67, *p* value= 0.29) respectively.

The means of PLT count and P-LCC were higher in duration (1-5 years) diabetics patients than other groups of duration but not statistically significant (283.7±71.60 *p* value= 0.38 and 44.2±10.57 *p* value= 0.12) respectively.

The MPV, PDW, P-LCR, PCT, PT, INR, APTT and D-dimer were not influenced by duration of disease and statistically not significant with (*p* values= 0.49, 0.30, 0.35, 0.14, 0.26, 0.89, 0.11 and 0.81) respectively.

5.2. Conclusion:

The summation from the study that P-LCR and PCT were show significant changes in case group when compared with control group, so the complete blood count is effective and not cost to monitor the diabetic health situation for management. The D-dimer was also show significant change in case group.

In summary, patients with and type 2 DM are prone to thrombotic events based on a series of disorders, including platelet hypersensitivity, coagulation factor modifications and hypofibrinolysis. Studies on the altered coagulation in DM suggest that hyperglycemia, insulin resistance and other comorbidities contribute to the hypercoagulable state.

5.3. Recommendations:

- Estimation of other hematological and coagulation parameters in type1, type 2 and gestational diabetes, because this research found out some parameters were change in type 2 DM.
- Study the association of complication and parameters change.
- Other studies including other blood cells parameters and coagulation studies in diabetic patients to monitor the complication of disease and control it.
- Increase area of population.
- Management of the enhanced thrombogenicity in DM requires comprehensive treatments of existing prothrombotic factors along with antithrombotic therapy.

In summey, routine coagulation tests should be part of tests among diabetic patients and advanced coagulation tests should also be considered to identify specific markers so as to pinpoint the particular problem.

References

Agarwal, C., Bansal, K., Pujani, M., Singh, K., Chauhan, V., Ranmicrova, D. and **Lukhmana, Sh.** (2018). Association of coagulation profile with microvascular complication and glycemic control in type 2 DM. Hematology Transfusion Cell Therapy, **42**(1): 31-36.

Alhadas, KR., Santos, BN., Freitas, MMS., Viana, SMSA., Ribero, LC. and **Costa, MB**. (2016). Are platelet indices useful in evaluation of type 2 diabetic patients. *J Bas patol med lab*, **52**(2): 96-102.

American diabetes association (ADA). (2007). Diagnosis and classification of diabetes mellitus. *Diabetes care*, **30**(1): P 42-47.

Anderson, JV. Diabetes mellitus and other disorders of metabolism, in: **Kumar**, **P**. and **Clark, M**. (2012). 8th ed. Clinical medicine. USA, New York: Saunders Elsevier, p 564-1006.

Asrat, D., Tesfaye, G., Gedefaw, L., Addisu, W. and **Yemane, T**. (2019). Hemostatic Abnormality and Associated factors in Diabetic patients at Jimma university specialized hospital, Ehiop J Health Sci, **29**(2): 251-258.

Bain, BJ., Bates, I., Laffan, MA. and **Lewis, SM**. (2011). Dacie and Lewis practical haematology. 11th ed. USA: Elsevier, p 47.

Bain, BJ., Clark, DM. and **Wilkins, BS**. (2010). Bone marrow pathology. 4th ed. UK: Wiley-Blackwell, p 20-23.

Balla, **SA., Abu Ahmed, H**. and **Awadelkareem, MA.** (2014). Prevalence of Diabetes, Knowledge and Attitude of Rural Population towards Diabetes and Hypoglycemic Event, Sudan 2013. *American Journal of Health Research*, **2**(6): 356-360.

Bashir, BA. Dirar, HH. and **Badaneen, MA**. (2017). Platelet indices among sudanses pregnant women with medical disorder association; cross-sectional study in Port Sudan city. *International journal of science*, **6**(6): 71-75.

Bayoumi, **M**., **Mourtadaa, S**. and **Elbager, S**. (2018). Altered platelets morphological parameters in obese adults with type 2 diabetes mellitus in sudan. *American journal diabetes, obesity and metabolism*, **4**(1): 17-24.

Budak, **YU**., **Polat**, **M**. and **Huysal, K**. (2016). The use of platelet indices, plateletcrit, mean platelet volume and platelet distribution width in emergency non-troumatic abdominal surgery, *Biochemia Medica*, **26**(2): 178-193.

Castollone, D. Hemostasis and Disorders of Coagulation, in: **Ciesla**, **B**. (2012). 2nd ed. Hematology in practice. USA: F.A. Davis compony, p 236-237.

Cheesbrough, M. (2006). District laboratory practice in tropical countries. 2nd ed. USA: Cambridge university press, p 297-298.

Citirik, **M**., **Beyazildiz, E., Simsek, M., Beyazildize, O**.and **Haznedaroglu, K**. (2014). MPV may reflect subcinical platelet activation in diabetic patients with and without diabetic retinopathy. *Npg*, **29**(3): 376-397.

Ciulla, AP. and **Lehman, DC**. (2010). Succsess in clinical laboratory science. 4th ed. USA: Peason education, P 379-380.

Dasgupta, **A**. and **Wahed**, A. (2014). Clinical chemistry, immunology and laboratory quality control. USA: Saunders Elsevier, p 107-112.

Dunning, T. (2003). Care of people with diabetes: A manual of nursing practice, New Jersy, USA: Wiley Black well, p 121-127.

Ebrahim, H., Asrie, F. and **Gentaneh, Z.** (2021) Basic coagulation profiles and platelet parameters among adult type 1 and type 2 diabetes patients at Dessie Referral hospital, northeast Ethiopia. Journal of Blood medicine, **12**: 33-42.

Egberg,N. Schematic presentation of the hemostatic system, in: **Antovic,JP** and **Blomback,M**.(2010).1st , Essential Guide to Blood Coagulation.US: Wiley-Blackwell, p 3-5.

Elbadri,A., Abdaluahafeez,I., Ali,N., Osman,Sh., Mohammed,t., Hamad,m., Ali,E., Ibrahim,A. and **Nimir,A**. (2020). Measuerment of Platelet

Parameters and D-dimer Level in Sudanese Patients with Long Stand Diabetes Mellitus Type 2. EC Diabetes and Metabolic Research, **4**: 1-13.

Elebute, Mo. and **Kesse-Adu, R**. Acquired haemolytic anaemias, in: **Hoffbrand**, **AV**., **Higgs, DR., Keeling, DM.** and **Mehta, AB**. (2016). 11th ed. Postgraduate haematology. UK: Jhanwiley and sons Ltd, p 152.

Elkhalifa, **H**., **Saad, R., Ali, M., Alzubair, H.** and **Nimar, A**. (2016). Platelet volume indices of patients with type 2 diabetes mellitus in Soba University Hospital-Khartoum Sudan, Journal of hypertension, **34**: 11-37.

Ephraim,RKD., Awuku,YA., Adu,P., Ampomah,LTW., Adoba,P., Panford,S., Ninnoni,JPK. and **Agbodzakey,H**. (2017). High risk of coagulopathy among tye 2 diabetes mellitus client at a ministry hospital in Ghana. Original Article, **51**(3): 101-107.

Frank, R.N. (2004). Diabetic retinopathy, *N Engl J Med* **350**: 48-58.

Freeman, **VS**. Carbohydrates, in: **Bishop**, **ML., Fody, EP.** and **Schoeff, LE**. (2013). 7th ed. Clinical chemistry principles, techniques and correlation. USA: Lippincott Williams and Wilkins, p 297-570.

Gad Allah, **NE**. (2009). Serum level of urate and creatinine in Sudanese patients with type 2 diabetes mellitus. MSc thesis. Sudan University of Science and Technology.

Greer, **PJ**., **Arber, DA., Glader, B., List, AF., Paraskeras, F**. and **Rodgers, GM**. (2014). Wintrobe's clinical hematology. 13th ed. USA: Lippincott Williams and wilkins, p 827.

Harris, **MI**. (1993). Undiagnosed NIDDM; clinical and public health issues. *Diabetes care*, **16**(4): 642-652.

Haruban, **RH**. Pancreas, in: **Mitchell**, **RN., Kumar, V., Abbas AK., Fausto, N. And Aster, JC**. (2012). 8th ed. Pathologic basis of disease. USA, New York: Saunder Elsevier, p 575-648.

Harvey, RA. and **Ferrier, DR.** (2011).5th ed. Biochemistry. USA: Lippincott Williams and wikins, p 340-344.

Hasan, Z., Hegde, S., Uday, I., Jayakumar, NM. and Anatharjaiah, PH. (2016). Assessment of MPV in type 2 diabetes mellitus and prediabetes. *National journal of laboratory medicine*, **5**(3): 54-57.

Hiller,E. Basic Principles of Hemostasis, in: **Munker,R., Hiller,E., Glass,J.** and **Paquette,R.** (2007). Second edition, Modern Hematology Biology and Clinical Magement. New Jeresy: Human Press, p 331.

Hirral, F.E., Tielsch, J.M., Klien, B.E. and **Klein, R. (2011)**. Ten years change in vision-related quality of life in type 1 diabetes: Wisconsin epidemiologic study of diabetic retinopathy, *Ophthalmology* **118**: 353-358.

Hoffbrand, **AV**., **Moss, PAH.** and **Pelit, JE**. (2006). Essential hematology. 5th ed. UK: Black well puplishing Ltd, p 264.

Howard,M. and **Hamilton,PJ**. (2013). Haematology An Illustrated colour text. 4th ed. London: Elsevier Churchill Livingstone. p 13.

Hubbard, JD. (2010). A concise review of clinical laboratory science. 2nd ed. USA: Lippincott Williams and wilkins, p0 58-64.

Jindal, **S**., **Gupta, S., Gupta, R., Kakkar, A., Singh, HV., Gupta, K.** and **Singh, S**. (2011). Platelet indices in diabetes mellitus: indicator of diabetic microvascular complication. *Hematology*, **16**(2): 86-90.

Jindal,S., Gupta,Sh., Gupta,R., kakkar,A., Singh,H., Gupta,K. and **Singh,S**. (2011). Platelet Indices in Diabetes Mellitus: Indicator of Diabetic Microvascular Complication, Hematology, **16**(2): 86-89.

Kanani,D., Vaidehipatel., Kiracganhan. and **Haridas,N**. (2017).Association of D-dimer in type 2 diabetes mellitus, International Journal of Advanced Research, 5(2): 2139-2145.

Kawthalkar, SM. (2013). Essentials of haematology. 2nded. London: Jaypee Brother medical pupilshers (p) Ltd, p 37-38.

Kodiatte, **TA**., **Manikyam, VK., Rao, SB., Jagadish, TM., Reddy, M., Lingaiah, HK**. and **Lakshmaiah, V**. (2012). MPV in type 2 diabetes mellitus. *J lab physicians*, **4**(1): 5-9.

Kodiatte, TA., Udaya, KML. and **Venkataswamy, L**. (2012). Mean platelet volume in type 2 diabetes mellitus. *Journal of laboratory physicians*, **4**(1): 59.

Kodiatte,Th., Manikyam,U., Rao,S., Jagadish,Th., Reddy,M., Lingaiah,H. and **Lakshmaiah,V**. (2021).Mean Platelet Volume in Type 2 Diabetes Mellitus, Journal of Laboratory and Physician , **4**(1): 5-9.

Laing, S.P., Swerdlow, A.J., Slater, S.D., Burden, A.C., Morris, A. and **Waugh, N.R.** (2003). Mortality from heart disease in a cohort of 23000 patients with insulin treated diabetes, *Diabetologia* **46**: 760-765.

Li, X., Weber,N., Cohon,D., Hollmann,M., Devries,J., Hermanides,J. and **Perckel,B**. (2021). Effects of Hyperglyciemia and Diabetes Mellitus on Coagulation and Hemostasis, Journal of Clinical Medicine, p 10:1-14.

Lim, A. KH. (2014). Diabetic nephropathy complications and treatment, *International journal of nephrology and renovascular disease* **7**: 361-381.

Obrosova, I.G. (2009). Diabetic painful and insensate neuropathy: pathogensis and potential treatment, *Neurotheraputics* **6**: 638-647.

Ojo, O. (2016). An over view of diabetes and its complication, *Diabetes Res Open J* **2**(2): 4-6.

Okon, E.B., Chung, A.W., Rauniyar, P., Padilla, E., Tejerina, T. and **Mc Manus, B.M.** (2005). Compromised arterial function in human type2 diabetic patients, *Diabetes* **54**: 2415-2423.

Olokoba, **AB., Obateru, OA**. and **Olokoba, LB**. (2012). Type 2 diabetes mellitus: A review of current trends. *Oman medical journal*, **27**(4): 269-273.

Pan, L., Ye, Y., Wo, M., Bao, D., Zhu, F., Cheng, M. and **Ni, X.** (2018). Clinical significance of hemastatic parameters in prediction of type 2 DM. Research Article, **2018**: 1-7.

Papanas, N., Nena, **E., Steiropoulos, P., Zikidou, P., Zaroqoulidis, P., Pita, E., Constantinidis, TC., Maltezos, E., Mikhailidis, DP**. and **Bouros, D**. (2012). Mean platelet volume and platelet distribution width in diabetic subject. *Taylor and francis online*, **23**(6): 447-454.

Pletcher, P. (2016). Glucose tolerance test. [Online]. Available at www.healthline.com. [Accessed 2-7-2018].

Porwit. A., McCullough, J. and **Erber, WN**. (2011). Blood and bone marrow pathology. 2nd ed. USA: Elsevier, p 24.

Riaz, S., (2009). Diabetes mellitus, *Scientific research and Essay* **4***(*5): 367-373.

Rodak, **BF**., **Fritsma, GA**. and **Keohane, EM**. (2012). Hematology clinical principles and applications. 4th ed. USA: Saunders, an imprint of Elsevier Inc, p 627.

Sacks, **DB**. Carbohydrates, in: **Burtis**, **CA., Ashwood, ER**. and **Bruns, DE**. (2008). 6thed. Tietz fundamental of clinical chemistry. USA, New York: Saunders Elsevier, p 374-383.

Selvarajah, D., Wilkinson, D., Emerg, C.J., Harris, N.D., Show, P.J. and **Witte, D.R.** (2006). Early involvement of the spinal cord in peripheral neuropathy, *Diabetes care* **29**: 2664-2669.

Sherin,B., **Ramamurthy,BD., Sudalaimthu,M**. and **Ganapathy,S.** (2020). Comparison of Coagulation Profile in Type 2 Diabetic Patients with good Glycaemic control and poor Glycaemic control, Journal of Clinical and Diagnostic Research, **14**(7): 6-8.

Shilpi, **k**. and **Poteker, RM**. (2018). A study of platelet indices in type 2 diabetes mellitus pateints. *Indian j hematol blood transfuse*, **34**(1): 115-120.

Thiruvoipati, T., Kielhorn, C.E. and **Armstrong, E.J.,** (2015). Peripheral artery disease in patient with diabetes: Epidemiology, mechanism and outcome, *World J Diabetes* **6**(7): 961-969.

Toyosi, OJ. and **Joyce, OAK**. (2021). Assessment of coagulation and fibrinolytic factors among patients with type 2 DM in university of IIorin teaching hospital, IIorin, Nigeria. Annals of clinical and Biomedical Reseach 2021, **2**: 121.

Turgeon, ML. (2012). Clinical hematology theory and procedure. 5th ed. USA: Lippincott Williams and wilkins, p 406-527.

Vasudevan, **DM., Sreekumari, S**. and **Vaidyanathan, K**. (2011). Text book of biochemistry for medical students. 6th ed. USA, St Louis: Jaypee Brothers medical publishers, P 285-288.

Wahed, A.and **Dasgupta, A**. (2015). Hematology and coagulation, 1sted. UK: Elsevier, p 232.

Weitz,JI., Fredenburgh,JC. and **Eikelborn,JW. (**2017). A Test in context: D-dimer. Journal of American College of Cardiology. **70**(19). 2411-2420.

Wesseles, A.M., Rombouts, S.A., Simsek, S., Kuijer, J.P., Kostense, P.J. and **Barkhof, F. (2006).** Microvascular disease in type1 diabetes alter brain activation: a functional magnetic resonance imaging study*, Diabetes* **55**: 334-340.

White, JG. Morphology and ultrastructure of platelets, in: **Gresele**, **P**., **Page, C., Fuster, V.** and **Vermylen, J.** (2002). 1st ed. Platelets in thrombotic and non-thrombotic disorders. USA: Cabmrdige, p 43.

Widjaja,S. and **Syhputa,N. (**2020). Correlation of Glycated Hemoglobin and D-dimer in Diapetic Patients, Science and Technology, **10**: 648-651.

Yilmaz, **T**. and **Yilmz, A**. (2016). Releationship between altered platelet morphological parameters in pateints with type 2 diabetes mellitus. *Journal of Ophthalmology*, **2016**: 1-5.

Zhou, H., Zhang, X. and **Lu, J.,** (2014). Progress on diabetic cerebrovascular disease, *Bons J Basic Med Sci* **14**(4): 185-190.

APPENDIX (1)

Sudan University of Science and Technology

College of Graduate Studies

Medical Laboratory Science Department of Hematology

Questionnaire about:

Estimation of Platelet Count, Platelet Indices, D-dimer and coagulation profile among Type 2 Diabetic Patients in Khartoum State.

Serial number: ☐☐

Sex: Male ☐ Female ☐

Age: 20-40 ☐ 41-60 ☐ 61-80 ☐ > 80 Years ☐

Duration of disease: <1 ☐ 1-5 ☐ 6-10 ☐ >10 Years ☐

Result:

1. PLTs count and indices indices:

PLTs count	MPV	PDW	P_LCR	PCT	P_LCC

2. Bleeding profile:

PT	**INR**	**APTT**

3. D-dimer:

☐

APPENDIX (2)

3. Principles

3.1 Introduction

The *Mission*® HA-360 3-Diff Automatic Hematology Analyzer determines the WBC data by impedance method, determining the RBC and PLT data by sheath flow impedance method, determining the HGB by Colorimetric method.

3.2 Aspiration

This analyzer can process two types of blood samples – whole blood samples and pre-diluted blood samples.

For whole blood mode (WB)

Present the whole blood sample to the sample probe directly and presses the aspirate key to aspirate 14 μL of the sample into the analyzer.

For Predilute Mode (PD)

This mode is used to analyze a small amount of blood. Dilute the whole blood sample first (dilute 20 μL of capillary blood sample by 120 μL of diluent) and then present the pre-diluted sample to the sample probe and press the aspirate key to aspirate 54 μL of the diluted sample into the analyzer.

3.3 Dilution

Dilute the blood sample to separate the cells so that they can pass through the aperture one by one. Dilution process also create a conductive environment for cell counting. Usually the number of red blood cells is 1,000 times bigger than the number of white blood cells, the red cells can be eliminated using lyse solution before WBC counting. The *Mission*® HA-360 3-Diff Automatic Hematology Analyzer can works in two analysis modes: Whole Blood Mode (WB) and Prediluted Mode (PD).

2.5.1 Reagents

***Mission*® 3D Hematology Reagent (Cyto-Diluent)**

Mission® 3D Hematology Reagent (Cyto-Diluent) is a filtered isotonic solution with specific electrical conductivity, offering a stable environment for blood cell counting.

***Mission*® 3D Hematology Reagent (Cyto-Lyser)**

Mission® 3D Hematology Reagent (Cyto-Lyser) can dissolve red blood and combine with hemoglobin, which can be used for determining hemoglobin and for counting and sizing leukocyt.

***Mission*® 3D Hematology Reagent (Mi-Po Cleaner)**

Mission® 3D Hematology Reagent (Mi-Po Cleaner) is used for cleaning the counting pool and hydraulic cycle regularly.

7. Quality Control

7.1 Introduction

There's risk that the error may occur after a long period of time usage of the analyzer. This error may cause the incorrect or unreliable analysis results. Quality Control (QC) program offers an effective method to detect the potential error.

In order to ensure the reliable of the analysis results, we recommend users run the QC program daily with low, normal and high level controls. A new lot of controls should be tested in parallel with the current lot twice a day for five days prior to their expiration dates. The results should be within the expected ranges as described in the control insert.

The *Mission*® HA-360 3-Diff Automatic Hematology Analyzer provides two QC programs: L-J QC and X-mean QC.

APPENDIX (3)

5 Theory of operation

The *Coatron M1* is a highly sensitive single channel photometer. A very intensive laser LED-Optic at 400 nm ensures accurate and precise results, even when icteric or lipemic samples are used. The receiver signal is detected and converted to an electrical current. During the actual test the system is searching for the best signal amplification. The software algorithms are based on optical density (extinction), which absorbs outside light effects.

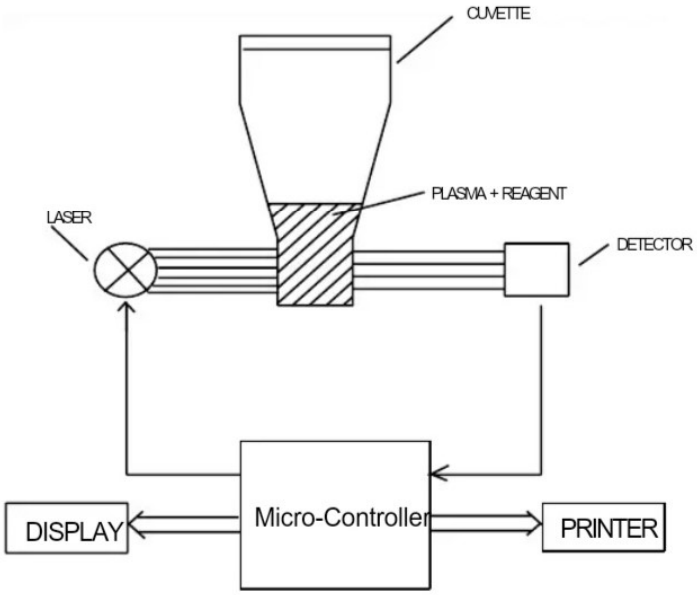


Figure 3 detection principle

Plasma/blood and reagent absorb the transmitted laser light. The rate of absorbency is obtained by the detector and sent to the micro controller. Here a program analyses the signal and send the result to the display and printer (optional).

APPENDIX (4)

7 PT – Determination

SUMMARY

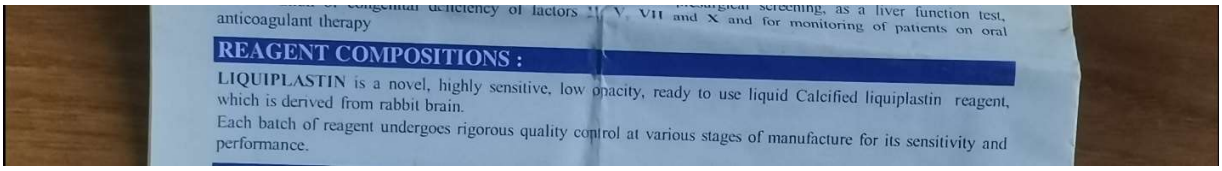
The Prothrombin Time test, as originally devised by Quick, has been widely used for a number of years as a pre-surgical screen for assessing certain coagulation factors and in monitoring oral anticoagulant therapy. All Stage II and III factors are necessary for normal results when performing the Prothrombin Time Test, so it is sensitive to reduced levels or deficiencies in Factors I, II, V, VII and X. Dicumerol and related drugs reduce the activity of the so-called "Prothrombin complex" Factors, II, VII, IX and X. Since the Prothrombin Time test is sensitive to deficiencies of all these Factors, except IX, it has proven useful in monitoring oral anticoagulant therapy. The Prothrombin Time test is also used in the quantitative determination (Factor Assays) of Factors II, V, VII and X.

PRINCIPLE

The one stage Prothrombin Time measures the clotting time of test plasma after the addition of Thromboplastin reagent containing Calcium chloride. The reagent supplies a source of "tissue thromboplastin", activating Factor VII, and is therefore sensitive to all Stage II and III Factors. Deficiencies of Stage I Factors (VIII, IX, XI, and XII) are not detected by the test.

PROCEDURE ON Coatron M1

1. Pipette **25µl plasma** finto cuvette
2. Prewarm plasma for 1 min
3. Transfer cuvette to measuring position
4. Activate optic (press **key „Optic“**)
5. Add **50 µl prewarmed Thromboplastin** and simultaneously start the optic. (press **key „Optic“ again**)
6. The instrument will read maximal 300 secs. If no clot is detected, the display will read **„+++.+s“**
7. The result is displayed in seconds and INR.



anticoagulant therapy

REAGENT COMPOSITIONS :

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

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

Enter both values into the **Coulton 1.1**

QUALITY CONTROL

Control plasma should be tested in conjunction with patient samples. It is recommended that at least one Normal and one Abnormal be run at least each shift and a minimum of once per 20 patient samples. A Control Range should be established by the laboratory to determines the allowable variation in day to day performance of each Control Plasma.

EXPECTED RESULTS OF CONTROLS:

Control Normal	0.8 - 1,3 INR
Control A1	2,0 - 3,5 INR
Control A2	5.0 - 8,0 INR

APPLICATION RECOMMENDATIONS

1. Don't use glass. Use only plastic.
2. Don't delay mixing of blood with anticoagulant.
3. Avoid extreme hemolysis or lipemic samples.
4. Avoid plasma contamination with tissue thromboplastin.
5. Avoid improper ratio of anticoagulant with blood.
6. Run patient samples in duplicate. At differences greater than 5 % repeat testing.
7. Run quality controls regularly to confirm reagent + instrument functionality.
8. Don't run a test if the green LED is off.

APPENDIX (5)

found if the INR=3), it had been used double reagent.

This is termed the International Normalized Ratio (INR), and is determined by:

$$INR = R^{ISI} = Ratio^{ISI} = \left(\frac{\text{Patient PT(s)}}{\text{Normal PT (s)}}\right)^{ISI}$$

Each PT Reagent of TECO is assigned an ISI value in relationship to the WHO Standardized Thromboplastin.

APPENDIX (6)

8 PTT – Determination

SUMMARY

From its origins through the work of Langdell and coworkers, and later modified by others, the Activated Partial Thromboplastin Time test has been widely used for a number of years as a pre-surgical screen for assessing certain coagulation factors and in monitoring Heparin therapy. All Factors of the Intrinsic Pathway are necessary for normal results when performing the APTT test. It is used principally, however, to detect deficiencies in the Stage I Factors, namely Factors VIII, IX, XI and XII, as well as Fletcher Factor. The APTT test is also used to monitor Heparin therapy, showing prolonged test results at approximately 0.1 units and above. The test is also used in the quantitative determination (Factor Assays) of Factors VIII, IX, XI, XII and Fletcher Factor.

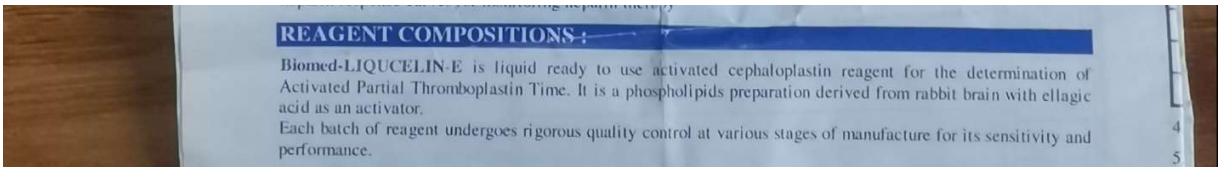
PRINCIPLE

The APTT test measures the clotting time of test plasma after the addition of APTT reagent, then allowing an "activation time", followed by the addition of calcium chloride. Deficiencies of approximately 40% and lower of Factors VIII, IX, XI and XII will result in a prolonged APTT. Heparin, in the presence of adequate amounts of AT-III will also result in a prolonged APTT.

Operation Manual **TECO GmbH**
Coatron M1 C 1.20 Rev. 12 Germany

PROCEDURE ON Coatron M1

1. Pipette **25µl plasma** into cuvette
2. Add **25 µl APTT** to plasma
3. Incubate exactly for **5 minutes**
4. Transfer cuvette to measuring position
5. Activate optic (press **key „Optic“**)
6. Add **25 µl prewarmed Calcium Chloride** and simultaneously start the optic. (press **key „Optic“ again**)
7. The instrument will read maximal 300 secs. If no clot is detected, the display will read **„+++.+s“**
8. The result is displayed in seconds and Ratio



REAGENT COMPOSITIONS :

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

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

QUALITY CONTROL

Control plasma should be tested in conjunction with patient samples. It is recommended that at least one Normal and one Abnormal be run at least each shift and a minimum of once per 20 patient samples. A Control Range should be established by the laboratory to determine the allowable variation in day to day performance of each Control Plasma.

EXPECTED RESULTS OF CONTROLS:

Control Normal	0.8 - 1,2 Ratio
Control A1	1,5 - 2,0 Ratio
Control A2	2,0 - 3,0 Ratio

APPLICATION RECOMMENDATIONS

1. Don't use glass. Use only plastic.
2. Don't delay mixing of blood with anticoagulant.
3. Avoid extreme hemolysis or lipemic samples.
4. Avoid plasma contamination with tissue thromboplastin.
5. Avoid improper ratio of anticoagulant with blood.
6. Run patient samples in duplicate. At differences greater than 5 % repeat testing.
7. Run quality controls regularly to confirm reagent + instrument functionality.
8. Don't run a test if the green LED is off.

APPENDIX (7)

BIOTIME®

Xiamen Biotime Biotechnology Co.,Ltd.

D-Dimer Rapid Quantitative Test

Catalog No.: BT2105

INTENDED USE

The Biotime D-Dimer Rapid Quantitative Test is intended to quantify the concentration of D-Dimer in human whole blood or plasma on Biotime FIA Analyzers by fluorescent immunoassay. It is used as an aid detection of PE and DVT.

-Fluorescent immunoassay

-PE and DVT

For in vitro diagnostic use only. For professional use only.

INTRODUCTION

D-Dimer is a fibrin degradation product (or FDP), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. D-Dimer doesn't normally present in human blood plasma, except when the coagulation system has been activated, for instance because of the presence of thrombosis or disseminated intravascular coagulation. It is regarded as a specific fibrinolytic process marker. D-Dimer concentration is used to suspected thrombotic disorders. While a negative result practically rules out thrombosis, a positive result can indicate thrombosis but does not rule out other potential causes. Its main use, therefore, is to exclude thromboembolic disease where the probability is low. In addition, it is used in the diagnosis of the blood disorder disseminated intravascular coagulation. Myocardial infarction, cerebral infarction, pulmonary embolism, venous thrombosis, surgery, cancer, disseminated intravascular coagulation, infection and tissue necrosis may lead to elevated concentration of D-Dimer. Increased concentration of D-Dimer may also be observed in elderly and hospitalized patients with abnormal coagulation caused by bacteremia[1-3].

PRINCIPLE

This test kit is based on fluorescent lateral flow immunoassay. While the sample and the buffer are mixed and applied into the test cartridge, the D-Dimer in the sample and the mouse anti-D-Dimer monoclonal antibody labeled with fluorescent microsphere form a reaction intermediate complex. During lateral flow, the intermediate complex moves along with the nitrocellulose membrane to a detection line (T-line: coated with D-Dimer specific monoclonal antibodies). The intermediate complex will be captured by T-line to form final reaction compound sandwich. Thus the fluorescent signal on detection line is positively correlated with the concentration of D-Dimer in human blood.

The fluorescent signal from microspheres of compound sandwich will be detected and calculated according to the calibration curve (in SD card provided with the reagents) to represent the concentration of D-Dimer in human whole blood or plasma.

PRECAUTIONS

1. This reagent is used for in vitro diagnosis only. Please do not use expired products.
2. All blood samples (including the remaining samples after testing), used reagents and waste should be treated as infectious materials.
3. The reagent is for single time use. Once the pouch is opened, it should be used within 30 minutes to avoid failure caused by the moisture absorption.
4. While using the test cartridge and instruments, vibration and strong electromagnetic environment should be avoided.
5. Lot number of buffers and test cartridges must be matched.
6. Do not insert the cartridges that are contaminated with blood or other liquids on the surface. Otherwise, it may cause damages to the instrument.

V. Components

1. Test cartridge 25 tests/kit
2. Detection buffer 25 tubes/kit
3. SD card 1 piece/kit
4. Instructions for Use 1 copy/kit

Material Required But Not Provided

1. Biotime FIA Analyzer
2. Transfer Pipette Set and pipette tips (range 10~100μL)
3. Specimen collection containers
4. Timer

STORAGE AND STABILITY

1. Store the detection buffer at 2-30°C, the shelf life is 24 months.
2. Store the test cartridge at 2-30°C, the shelf life is 24 months.
3. Test Cartridge should be used within 30 minutes after opening the pouch.

SPECIMEN COLLECTION AND PREPARATION

1. The test can be performed with whole blood or plasma.
2. The specimen collection container should be citric acid tube for plasma or whole blood.
3. The collection of the sample: the venipuncture for blood collection method referring to the National Clinical Laboratory Procedures, if the sample can't be detected in time, plasma can be stored in refrigerator at 2-8°C for no more than 7 days, or at -20°C for no more than 6 months and whole blood can only be stored at 2~8°C for no more than 48 hours. Samples must be recovered to the room temperature before test.
4. Separate the plasma from blood as soon as possible to avoid hemolysis.

TEST PROCEDURE

Please refer to operation manual of Biotime FIA analyzers for details.

The test should be operated at room temperature (~25°C).

Step 1: Preparation

Check/insert SD card into the equipment.

Take out one tube of buffer from refrigerator and balance it to room temperature.

Step 2: Sampling

Take 50μL of plasma or 80μL of whole blood with a transfer pipette and add it into the buffer tube.

Step 3: Mixing

Mix well the specimen with buffer by tapping or inverting the tube.

Step 4: Loading

Take 80μL of sample mixture and load it into the well of the test cartridge.

Note: Step 2 to step 4 should be completed within 1 minute to ensure the accuracy of the test results.

Step 5: Testing

Standard test: Click "Test", and then choose "Standard Test". Immediately insert test cartridge into test cartridge inlet and click "Start Test", the cartridge will be in the process of chromatography for 150s. After chromatography, the analyzer will count down time automatically. When the test is finished, the test result will be showed on screen and printed automatically.

Quick test: Click "Test" and then choose "Quick Test". When the mixture is dropped into the well of test cartridge, immediately count down reaction time(3 min) with a timer. When time is up, insert the test cartridge into test cartridge inlet immediately and then click "Start Test", the test result will be showed on screen and printed automatically.

REFERENCE INTERVAL

Normal reference interval: <0.5mg/L

Note: Individual reference range is suggested to be established for each laboratory.

LIMITATIONS OF PROCEDURE

1. The test sample should be whole blood or plasma.
2. Human anti-mouse antibody (HAMA) may be presented in patients who have received immunotherapy with a murine monoclonal antibody. This kit has been specially designed to minimize the effect of these antibodies on the test results. However, the test result must be carefully evaluated when patients are known to have these antibodies[4-5].
3. Other factors also can induce the false results, including the technology, operational error and other sample factors.

PERFORMANCE CHARACTERISTICS

Accuracy

D-Dimer control materials with two different concentrations were tested by every batch of Test Cartridges, and the deviations were within ±15%.

Assay Range: 0.1-10.0 mg/L

The Lowest Detection Limit: 0.1 mg/L

Linearity

A serial concentrations of D-Dimer reference materials at 0.1-10.0mg/L were tested, and the correlation coefficient (R) is ≥ 0.9900.

APPENDIX (8)

Automatic hematology analyzer:

APPENDIX (9)

BIOTIME advice:

Incubator:

APPENDIX (10)

COARTON MI: