Chapter One

Introduction and literature review

1.1 General Introduction:

Chronically elevated levels of glucose in the blood may contribute to the development of micro vascular complications of diabetes mellitus, such as diabetic retinal damage, as well as macro vascular complications such as cerebrovascular (Smith, et al., 2004).

Suggestion that is non enzymatic glycation of proteins in vascular tissue alters the structure and functions of these proteins. A protein exposed to chronically increased levels of glucose will bind glucose, form cross linked protein within the vascular and, may cause narrowing of the luminal diameter (Smith, et al., 2004).

In diabetes mellitus, platelets become more reactive and their mean platelet volume (MPV) is increased. The increased platelet size may be one factor of vascular complications. Hence, MPV is a useful prognostic marker of vascular complications in diabetes (Kodiattie, et al., 2012).

Although the underlying mechanism of higher MPV in diabetic subjects is incompletely understood, but it is due to osmotic swelling as a result of hyperglycemia (Martyn, et al., 1986).

Alternatively, increased platelet size may reflect the presence of high platelet turnover and younger platelets (Guthikonda, et al., 2008).
1.2 literature review:

1.2.1 Haematopoiesis

Is the formation of blood cellular components. All cellular blood components are derived from haematopoietic stem cells. In a healthy adult person, approximately $10^{11} - 10^{12}$ new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation (Tao, et al., 2010).

Haematopoietic stem cells (HSCs) reside in the medulla of the bone (bone marrow) and have the unique ability to give rise to all of the different mature blood cell types and tissues. HSCs are self-renewing cells (Morrison and Judith Kimble, 2006).

1.2.1.1 Blood cells are divided into three lineages:

Erythroid cells are the oxygen carrying red blood cells, lymphocytes are the cells of adaptive immune system, myelocytes which include granulocytes, megakaryocytes and macrophages and are derived from common myeloid progenitors, are involved in innate immunity, adaptive immunity, and blood clotting (Fernández and Alarcón, 2013).

1.2.1.2 Sites of haematopoiesis (human) in pre- and postnatal periods:

In developing embryos, blood formation occurs in aggregates of blood cells in the yolk sac, called blood islands. As development progresses, blood formation occurs in the spleen, liver and lymph nodes. When bone marrow develops, it eventually assumes the task of forming most of the blood cells for the entire organism (Fernández and Alarcón, 2013).

However, maturation, activation, and some proliferation of lymphoid cells occurs in secondary lymphoid organs (spleen, thymus, and lymph...
nodes). In children, haematopoiesis occurs in the marow of the long 
bones such as the femur and tibia. In adults, it occurs mainly in the pelvis, 
cranium, vertebrae, and sternum (Fernández and Alarcón, 2013).

1.2.1.3 Extramedullary hemopoiesis:

In some cases, haematopoietic function occur in the liver, thymus, and spleen if necessary. This is called extramedullary haematopoiesis. It may cause these organs to increase in size substantially (Georgiades, et al., 2002).

During fetal development, since bones and thus the bone marrow develop later, the liver functions as the main haematopoetic organ. Therefore, the liver is enlarged during development (Georgiades, et al., 2002).

1.2.2 Erythropoiesis:

Is the process by which red blood cells (erythrocytes) are produced. In the early fetus, erythropoiesis takes place in the yolk sac. By the third or fourth month, erythropoiesis moves to the liver. After seven months, erythropoiesis occurs in the bone marrow (Pali and Segel, 1998).

1.2.2.1 Erythrocyte differentiation

The following stages of development all occur within the bone marrow: Hemocytoblast a multipotent hematopoietic stem cell, becomes a common myeloid progenitor or a multipotent stem cell, and then a unipotent stem cell, then a pronormoblast, also commonly called an proerythroblast or a rubriblast (Tao, et al., 2010).

This becomes a basophilic or early normoblast, also commonly called an erythroblast, then a polychromatophilic or intermediate normoblast, then
an orthochromatic or late normoblast. At this stage the nucleus is expelled before the cell becomes a reticulocyte (Tao, et al., 2010).

1.2.2.2 Regulation of erythropoiesis

A feedback loop involving erythropoietin helps regulate the process of erythropoiesis so that, in non-disease states, the production of red blood cells is equal to the destruction of red blood cells and the red blood cell number is sufficient to sustain adequate tissue oxygen levels (Michael, et al., 2008).

Erythropoietin is produced in the kidney and liver in response to low oxygen levels. In addition, erythropoietin is bound by circulating red blood cells; low circulating numbers lead to a relatively high level of unbound erythropoietin, which stimulates production in the bone marrow (Michael, et al., 2008).

Recent studies have also shown that the peptide hormone hepcidin may play a role in the regulation of hemoglobin production, and thus affect erythropoiesis. The liver produces hepcidin (Nicolas, et al., 2002). Hepcidin controls iron absorption in the gastrointestinal tract and iron release from reticuloendothelial tissue. Iron must be released from macrophages in the bone marrow to be incorporated into the heme group of hemoglobin in erythrocytes (Nicolas, et al., 2002).
1.2.3 Hemoglobin (Hb)

Is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (with the exception of the fish family Channichthyidae) (Sidell, et al., 2006).

1.2.3.1 Synthesis of hemoglobin:

Hemoglobin (Hb) is synthesized in a complex series of steps. The heme part is synthesized in a series of steps in the mitochondria and the cytosol of immature red blood cells, while the globin protein parts are synthesized by ribosomes in the cytosol (Steinberg, 2001)

Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. Then the nucleus is lost. Even after the loss of the nucleus in mammals, residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loses its RNA soon after entering the vasculatur (Steinberg, 2001)

1.2.3.2 Structure of hemoglobin:

In most vertebrates, the hemoglobin molecule is an assembly of four globular protein subunits. Each subunit is composed of a protein (globin fold) chain tightly associated with a non-protein hemegroup (Hardison, 1996).

A heme group consists of an iron (Fe) ion (charged atom) held in ring, known as a porphyrin. This porphyrin ring consists of four pyrrole molecules linked together (by methine bridges) with the iron ion bound in the center (Hardison, 1996).
The iron ion, which is the site of oxygen binding, coordinates with the four nitrogens in the center of the ring. The iron ion may be either in the Fe\(^{2+}\) or in the Fe\(^{3+}\) state, but ferrihemoglobin (methemoglobin) (Fe\(^{3+}\)) cannot bind oxygen (Linberg, et al., 1998).

**1.2.3.3 Types of hemoglobin:**

In the embryo: Gower 1 (\(\zeta_2\varepsilon_2\)), Gower 2 (\(\alpha_2\varepsilon_2\)), hemoglobin Portland I (\(\zeta_2\gamma_2\)), hemoglobin Portland II (\(\zeta_2\beta_2\)). In the fetus: Hemoglobin F (\(\alpha_2\gamma_2\)), in postnatal hemoglobin A (\(\alpha_2\beta_2\)) – The most common with a normal amount over 95% ; hemoglobin A\(_2\) (\(\alpha_2\delta_2\)) – \(\delta\) chain synthesis begins late in the third trimester and in adults it has a normal range of 1.5–3.5% (Huisman. 1958).

Some well-known hemoglobin variants are responsible for diseases like hemoglobin S, C, D, bart H and E (Huisman. 1958).

**1.2.3.4 Degradation of hemoglobin:**

When red cells reach the end of their life due to aging or defects, they are broken down in spleen. The other major final product of heme degradation is bilirubin. Globulin is metabolised into amino acids that are then released into circulation (Kikuchi, et al., 2005).

**1.2.3.5 Glycated hemoglobin (hemoglobin A\(_\text{1c}\)):**

Is a form of hemoglobin that is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose (Larsen, et al., 1990).
Normal levels of glucose produce a normal amount of glycated hemoglobin, plasma glucose increases, the fraction of glycated hemoglobin increases, this serves as a marker for average blood glucose levels over the previous months prior to the measurement (Larsen, et al.,1990).

In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels (Larsen, et al.,1990).

1.2.3.5.1 Historical background:

Hemoglobin A1c was first separated from other forms of hemoglobin by Huisman and Meyering in 1958 using a chromatographic column (Huisman, et al., 1958).

Its increase in diabetes was first described in 1969 by Samuel Rahbar (Rahbar, et al.,1969).

The use of hemoglobin A1c for monitoring the degree of control of glucose metabolism in diabetic patients was proposed in 1976 by Anthony Cerami, Ronald Koenig and coworkers (Koenig, et al., 1976).

1.2.3.5.2 Bio synthesis of hemoglobin A1c:

Hemoglobin A1c, the most abundant minor hemoglobin component in human erythrocytes, is formed by the condensation of glucose with the N-terminal amino groups of the beta-chains of HbA (Bookcin and Gallop,1968).

suggesting that the formation of Hb A1c is a post translational modification (Gallop and Paz,1975).
Hb A1c is slowly formed during the 120-day life-span of the erythrocyte, probably by a non enzymatic process, patients with shortened erythrocyte life-span due to hemolysis had markedly decreased levels of HbA1c (Trivelli, et al., 1971).

There were international study are now reported hypothesis that there is a strong linear relationship between mean blood glucose and hemoglobinA1C (Nathan, et al., 2008).

The cause of the increased glycated hemoglobin in diabetes, which was predominantly the A1C component, was a result of excess non enzymatic glycation that occurred throughout the lifespan of red cells and in an essentially irreversible process (Bunn, et al. 1976).

Many other investigators confirmed a strong association between A1C and glycemic control and that the measurement had clinical utility (Gabbay, et al., 1977).

Thorough biochemical experiments performed in the 1970s and 1980s, most notably by Mortensen and Christophersen, demonstrated that the fraction of A1C in a sample depends on the glucose levels over a previous period, along with red cell turnover, reaching a steady state sometime between 4 and 12 weeks (Mortensen, et al., 1983).

1.2.3.5.3 Measurement of A1C:

Immunoassays, Affinity chromatography, Ion-exchange chromatography, Electrophoresis Separation Isoelectric focusing, high-pressure liquid chromatography (HPLC) (Bishop, et al., 2010)
HbA\textsubscript{1c} is to be reported in the International Federation of Clinical Chemistry (IFCC) units, and Diabetes Control and Complications Trial (DCCT) (Geistanger, et al., 2008).

1.2.3.5.4 Normal Result of hemoglobin A1C:

Normal: Less than 5.7%, Pre-diabetes: 5.7% to 6.4%, Diabetes: 6.5% or higher (Buse, et al., 2011).

1.2.3.5.5 Interpretation of results of hemoglobin A1c:

Results can be unreliable in many circumstances, such as after blood loss, blood transfusions, anemia, or high erythrocyte turnover, in the presence of chronic renal, liver disease, after administration of high-dose vitamin C and erythropoietin treatment (Nathan, et al. 2008).

Recent results from large trials suggest that a target below 53 mmol/mol (7 %) may be excessive because the intensive glycemic control required to reach this level leads to an increased rate of dangerous hypoglycemic episodes (Lehman, et al., 2009).

The risks of the main complications of diabetes (retinopathy, nephropathy, neuropathy and macrovascular disease) decreased with approximately 3% for every 1 mmol/mol decrease in hemoglobin A\textsubscript{1c} (Shubrook, 2010).

Lower-than-expected levels of HbA\textsubscript{1c} can be seen in people with shortened red blood cell lifespan, such as with glucose-6-phosphate dehydrogenase deficiency, sickle-cell disease, or any other condition causing premature red blood cell death (Kilpatrick, et al., 2009).
Conversely, higher-than-expected levels can be seen in people with a longer red blood cell lifespan, such as with Vitamin B₁₂ or folate deficiency (Kilpatrick, et al., 2009).

1.2.4 Platelets (Thrombocyte)

1.2.4.1 Definition of platelet:

Platelets are small anucleate cell fragments that have a characteristic discoid shape and range from 1 to 3 µm in diameter. Platelets are formed from the cytoplasm of megakaryocytes (MKs), their precursor cells, which reside in the bone marrow (Pease, 1956).

1.2.4.2 Megakaryocytes (MKs) and Platelet Production:

Platelets are derived from bone marrow megakaryocytes, which are large cells with multilobated nuclei and abundant finely granular light gray-blue cytoplasm. With the size (50–100 µm) and account for ~0.01% of nucleated bone marrow cells (Nakeff and Maat, 1974).

To assemble and release platelets, MKs become polyploid by endomitosis (DNA replication without cell division) and then undergoes a maturation process, into multiple long processes called proplatelets. An MK may extend 10–20 proplatelets, then fragments of cytoplasm break off into platelets (Richardson, et al., 2005).

As platelets develop, they receive their granule and organelle content as streams of individual particles transported from the MK cell body (Italiano, et al., 1999).

This process is regulated mainly by thrombopoietin (produced predominantly in the liver and have critical role in megakaryocytic growth and differentiation)(Kern, 2002).
1.2.4.3 Ultrastructure of resting platelet:

1.2.4.3.1 Peripheral zone:


1.2.4.3.2 Sol-Gel zone:

Responsible for contraction and support microtubule system. Contains the connecting system called the open canalicular system and the dense tubular system (McNicoland Israels, 1999).

1.2.4.3.3 Organelle zone:

Contains the dense body system, non-metabolic ADP, serotonin, catecholamines, calcium, alpha granules; platelet factor 4, platelet mitogenic factor, fibrinogen, beta thromboglobulin, lysosomal granules, mitochondria and glycogen granules (White, 1998).

1.2.4.4 Function of platelet:

The main function of platelets is to contribute to hemostasis, the process of stopping bleeding at the site of interrupted endothelium. They gather at the site and unless the interruption is physically too large, they plug the hole (Weyrich and Zimmerman, 2004).

In addition to being the cellular effector of hemostasis, platelets are rapidly deployed to sites of injury or infection, and potentially modulate
inflammatory processes by interacting with leukocytes and by secreting cytokines, chemokines, and other inflammatory mediators. Platelets also secrete platelet-derived growth factor (PDGF)(Wagner and Burger, 2003).

1.2.4.5 Process of platelet in hemostasis:

1.2.4.5.1 Platelet adhesion:

Endothelial cells are attached to the subendothelial collagen by von Willebrand factor (vWF). when the endothelial layer is disrupted, collagen and vWF anchor platelets to the subendothelium. Platelet GP1b-IX-V receptor binds with vWF and GPVI receptor binds with collagen(Dubois, et al., 2006).

1.2.4.5.2 Platelet activation:

1.2.4.5.2.1 Platelet induction:

Platelet activation begins seconds after adhesion occurs. It is triggered when collagen from the subendothelium, and/or tissue factor from the media and adventitia bind with their respective receptors on the platelet(Dubois, et al., 2006).
1.2.4.5.2.2 Role of platelet in coagulation:

Move of the negatively charged phospholipids from the inner to the outer platelet membrane surface. These phospholipids then bind the tenase and prothrombinase complexes, two of the sites of interplay between platelets and the coagulation cascade. Calcium ions are essential for the binding of these coagulation factors (Dubois, et al., 2006).

1.2.4.5.2.3 Platelet shape change:

Intraplatelet calcium concentration increases, stimulating the interplay between microtubule/actin filament complex with the platelet cell membrane and open canalicular system (OCS) (Matarrese, et al., 2009)

The continuous changes in shape from the unactivated to the fully activated platelet is best seen on scanning electron microscopy. Activated platelets secrete the contents of their granules through their canalicular systems to the exterior (Matarrese, et al., 2009)

1.2.4.5.2.4 GPIIb/IIIa activation

Thromboxane A₂ synthesis increases during activation it is secreted and acts on both its own thromboxane receptors (the so-called "out-in" mechanism), and those of other platelets. These receptors trigger intraplatelet signaling, which converts GPIIb/IIIa receptors to their active form to initiate aggregation (Yip, et al., 2005).

1.2.4.5.3 Platelet aggregation:

Aggregation begins minutes after activation, and occurs as a result of turning on the GPIIb/IIIa receptor, which allows these receptors to bind
with vWF or fibrinogen, there are 50–100 of these receptors per platelet(Yip, et al., 2005).

When any one or more of at least nine different platelet surface receptors are turned on during activation, intraplatelet signaling pathways cause existing GpIIb/IIIa receptors to change shape and thus become capable of binding(Yip, et al., 2005).

1.2.4.5.4 Platelet-coagulation factor interactions

In addition to interacting with vWF and fibrin, platelets interact with thrombin, Factors X, Va, VIIa, XI, IX, and prothrombin to complete clot formation via the coagulation cascade, many studies suggested platelets express tissue factor(Ahmad, et al., 1992).

1.2.4.6 Symptoms of platelet disorders:

Spontaneous and excessive bleeding can occur because of platelet disorders. This bleeding can be caused by deficient numbers of platelets, dysfunctional platelets(Murakawa, et al., 1992).

All of the following suggest platelet bleeding, not coagulation bleeding: the bleeding from a skin cut such as a razor nick is prompt and excessive, but can be controlled by pressure; spontaneous bleeding into the skin which causes a purplish stain named by its size: petechiae, purpura, ecchymoses(van Genderen, et al., 1996).

Bleeding into mucous membranes causing bleeding gums, nose bleed, and gastrointestinal bleeding; menorrhagia, intraretinal, and intracranial bleeding(van Genderen, et al., 1996).
1.2.4.7 Disorder of platelet:

1.2.4.7.1 Thrombocytopenia

Is defined as a platelet count less than the lower limit of the reference range (Kern, 2002).

1.2.4.7.1.1 Causes of Thrombocytopenia:

1.2.4.7.1.1.1 Inherited Thrombocytopenia:
Thrombocytopenia–absent radii (TAR) syndrome, wiskott-Aldrich syndrome, may-Hegglin anomaly, bernard-Soulier syndrome, Gray platelet syndrome (Laidlaw, et al., 2012)

1.2.4.7.1.1.2 Congenital non-inherited Thrombocytopenia:
Intrauterine viral infection, maternal drugs or medications: (thiazide diuretics), maternal ITP or other immunologic diseases, neonatal alloimmunethrombocytopenia (Kern, 2002).

1.2.4.7.1.1.3 Acquired Thrombocytopenia:
Immune: Idiopathic, Infections: viruses (EBV, CMV, HIV), bacteria, rickettsiae, Mycoplasma, Lymphoproliferative disorders, Autoimmune (collagen vascular) diseases Post-transfusion purpura (Kern, 2002).
Non-immune: Infections, Disseminated intravascular coagulation (DIC), Thrombotic thrombocytopenic purpura (TTP), Hemolytic-uremic syndrome (HUS), Preeclampsia/eclampsia and the HELLP syndrome Massive transfusion, Gestational thrombocytopenia (Kern, 2002).

1.2.4.7.1.1.4 Platelet sequestration in the spleen:
Hypersplenism: usually associated with anemia and/or leucopenia (Kern, 2002).
1.2.4.7.2 Thrombocytosis (Thrombocythemia):
Thrombocytosis is defined as a platelet count exceeding the upper limit of the reference range. Either primary thrombocythemia (may be associated with thrombosis or bleeding) or thrombocytosis secondary to some other condition (reactive thrombocytosis) and is not associated with an increased risk of thrombosis or other complication (like infection, inflammation, iron deficiency anemia) (Kern, 2002).

1.2.4.8 Measurement of platelet:
Platelet count is measured either manually using a hemacytometer, or by placing blood in an automated platelet analyzer using electrical impedance (Girling, 1962).

The normal range (99% of population analyzed) for platelets in healthy individual is 150,000 to 400,000 per cubic millimeter (a mm$^3$ equals a microliter) or 150–400 × 10$^9$ per liter (Ross, et al., 1998).

Platelet function is evaluated by bleeding time test, platelet aggregation test (Duke, 1910).

1.2.4.9 Transfusion therapy with platelet:

1.2.4.9.1 Indications of platelet transfusion:
Platelet transfusion is most frequently used to correct unusually low platelet counts, either to prevent spontaneous bleeding (typically at counts below (10–15)×10$^9$/L) or in anticipation of medical procedures that will necessarily involve some bleeding. For example, in patients undergoing surgery, a level below 50×10$^9$/L is associated with abnormal surgical bleeding (Roback, et al., 2011).
Platelets may also be transfused when the platelet count is normal but the platelets are dysfunctional, such as when an individual is taking aspirin or clopidogrel. Finally, platelets may be transfused to address severe hemorrhage. Platelet transfusion is contraindicated in thrombotic thrombocytopenic purpura (TTP) (Roback, et al., 2011).

1.2.4.9.2 Collection of the platelet:

Platelets are either isolated from collected units of whole blood and pooled to make a therapeutic dose, or collected by platelet apheresis (Högman, 1992).

Apheresis platelets are collected using a mechanical device that draws blood from the donor and centrifuges the collected blood to separate out the platelets and other components to be collected. The remaining blood is returned to the donor (Högman, 1992).

1.2.4.9.3 Storage of platelets:

Platelets collected by either method have a very short shelf life, typically five days. Platelets are stored under constant agitation at 20–24 °C (68–75.2 °F) (Högman, 1992).
1.2.4.9.4 Platelet Delivery to recipients

Platelets do not need to belong to the same A-B-O blood group as the recipient or be cross-matched to ensure immune compatibility between donor and recipient unless they contain a significant amount of red blood cells (RBCs) (Schoenfeld et al., 2006).

Prior to issuing platelets to the recipient, they may be irradiated to prevent transfusion-associated graft versus host disease or they may be washed to remove the plasma (Schoenfeld et al., 2006).

The change in the recipient's platelet count after transfusion is termed the "increment" and is calculated by subtracting the pre-transfusion platelet count from the post-transfusion platelet count. When recipients fail to demonstrate an adequate post-transfusion increment, this is termed platelet transfusion refractoriness (Schoenfeld et al., 2006).

1.2.4.10 Role of platelet in Wound healing:

Platelets release platelet-derived growth factor (PDGF), a potent chemotactic agent; and TGF beta, which stimulates the deposition of extracellular matrix; fibroblast growth factor, insulin-like growth factor 1, platelet-derived epidermal growth factor, and vascular endothelial growth factor. Local application of these factors in increased concentrations through Platelet-rich plasma (PRP) is used as an adjunct in wound healing (Gawaz and Vogel, 2013).
1.2.5 Diabetes Mellitus (DM)

1.2.5.1 Definition of DM:

Diabetes mellitus is a metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action or both (Unger and Grundy, 1985).

Prediabetes is an intermediate stage, in which the fasting glucose is increased above normal limits but not to the level of diabetes, has been named impaired fasting glucose (IFG). Use of the term impaired glucose tolerance (IGT) to indicate glucose tolerance values above normal but below diabetes levels (Bishop, et al., 2010).

Also pre diabetic refer to glycated hemoglobin (A1C) of 6.0% to 6.4%, each of which places individuals at high risk of developing diabetes and its complications (Santaguida and Balion, 2005).

1.2.5.2 Classification of Diabetes

1.2.5.2.1 Type 1 diabetes:

encompasses diabetes that is primarily result of pancreatic beta cell destruction. This form includes cases due to an autoimmune process and those for which the etiology of beta cell destruction is unknown (Turner, et al., 1997)

This disease is usually initiated by an environmental factor or viral infection in individuals with genetic predisposition and causes destruction of beta cells of the pancreas lead to decreased production of insulin (Bishop, et al., 2010)

Characteristics of type 1 diabetes include abrupt onset, insulin dependence, and ketosis tendency. (Bishop, et al., 2010)
1.2.5.2.2 Type 2 diabetes:
Is characterized by hyperglycemia as a result of an individual’s resistance to insulin with an insulin secretory defect. This resistance results in relative insulin deficiency (Bishop, et al., 2010).
This type of diabetes often goes undiagnosed for many years and is associated with a strong genetic predisposition, [usually occur in adult increase risk with obesity, and lack of physical exercise(Bishop, et al., 2010).

1.2.5.2.3 Gestational diabetes mellitus:
Refers to glucose intolerance with onset or first recognition during pregnancy (Turner, et al., 1997).
Glucose intolerance during pregnancy due to metabolic and hormonal changes,frequently return to normal postpartum (Bishop, et al., 2010).
Distinguishing between type 1 and type 2 diabetes is important because management strategies differ (Patel, 2010).

1.2.5.3 Diagnosis of diabetes:
A fasting plasma glucose (FPG) level of 7.0 mmol/L correlates most closely with a 2-hour plasma glucose (2hPG) value of ≥11.1 mmol/L in a 75 g oral glucose tolerance test (OGTT). (Engelgau, et al. 1997).
Random plasma glucose. RPG ≥11.1 mmol/L, glycated hemoglobin A1C ≥6.5% (in adults) (Forouhi and, 2006)
A1C values also are affected by age, rising by up to 0.1% per decade of life (Davidson, 2010).
1.2.5.4 Three way to diagnosis diabetes mellitus:

Symptoms of diabetes plus a random plasma glucose level of $\geq 200$ mg/dL, or fasting plasma glucose of $\geq 126$ mg/dL, or an oral glucose tolerance test (OGTT) with a 2-hour postload (75-g glucose load) level $\geq 200$ mg/dL, each of which must be confirmed on a subsequent day by any one of the three methods (Bishop, et al., 2010).

The combination of an fasting plasma glucose of 6.1 to 6.9 mmol/L and an glycated hemoglobin A1C of 6.0% to 6.4% is pre diabetic state and predictive of 100% progression to type 2 diabetes over a 5-year period (Heianza, et al., 2012).

1.2.5.5 Diagnosis of gestational diabetes:

Performance of a 3-hour OGTT (oral glucose tolerance test) and is diagnosed when any two of the following four values are met or exceeded: fasting, $\geq 95$ mg/dL; 1 hour $\geq 180$ mg/dL; 2 hours $\geq 155$ mg/dL; or 3 hours $\geq 140$ mg/dL (Bishop, et al., 2010).

1.2.5.6 Signs and symptoms of type 1 and type 2 diabetes include:

Increased thirst, Frequent urination, Extreme hunger, Unexplained weight loss, Presence of ketones in the urine, Fatigue, Blurred vision, Slow-healing sores, High blood pressure, Frequent infections such as gums or skin infections and vaginal or bladder infections (Longo, 2012).
1.2.5.7 Complications of DM

1.2.5.7.1 Cardiovascular disease associated DM:
Including coronary artery disease with chest pain (angina), heart attack, stroke and narrowing of arteries (atherosclerosis) (Bergenstal, et al., 2010)

1.2.5.7.2 Nerve damage (neuropathy) associated DM:
Excess sugar can injure the walls of the tiny blood vessels (capillaries) that nourish your nerves, especially in the legs called diabetic septic foot (Bergenstal, et al., 2010)

1.2.5.7.3 Kidney damage (nephropathy) associated DM:
The kidneys contain millions of tiny blood vessel clusters (glomeruli) that filter waste from your blood. Diabetes can damage this delicate filtering system. Severe damage can lead to kidney failure or irreversible end-stage kidney disease (Bainbridge, 2008)

1.2.5.7.4 Eye damage (retinopathy) associated DM:
Diabetes can damage the blood vessels of the retina (diabetic retinopathy), potentially leading to blindness. Diabetes also increases the risk of other serious vision conditions, such as cataracts and glaucoma. (Bergenstal, et al., 2010).

1.2.5.8 Treatment:
An important part of managing all types of diabetes is maintaining a healthy weight through a healthy diet and exercise plan:
1.2.5.8.1 Insulin:

Most people with type 1 diabetes need insulin therapy to survive. Some people with type 2 diabetes also need insulin therapy, either rapid-acting insulin, long-acting insulin and intermediate options (Elleri, et al., 2012).

Insulin decreases plasma glucose levels by increasing the transport entry of glucose in muscle and adipose tissue by way of nonspecific receptors. It also regulates glucose by increasing glycogenesis, lipogenesis, and glycolysis and inhibiting glycogenolysis (Bishop, et al., 2010).

1.2.5.8.2 Oral hypoglycemic agent:

Some diabetes medications stimulate pancreas to produce and release more insulin, Others inhibit the production and release of glucose from your liver, or block the action of stomach or intestinal enzymes that break down carbohydrates or make tissues more sensitive to insulin (Elleri, et al., 2012).

1.2.5.8.3 Transplantation of pancreas:

For type 1 diabetes, a pancreas transplant may be an option (Elleri, et al., 2012).

1.2.6 Association between platelet activity and diabetic:

DM is a complex metabolic syndrome characterized by chronic hyperglycemia resulting in complications affecting the peripheral nerves, kidneys, eyes, and micro- and macro vascular structures (Demirtunc, et al., 2009).

Diabetes and its vascular complications can cause a financial havoc, become a burden to a country’s national economy and dent its growth.
MPV can be used as a simple economical test in the monitoring of DM and thereby help in reduce the morbidity and mortality (Zuberi, et al., 2008).

Type 2 DM is characterized mainly by impaired insulin secretion and increased tissue insulin resistance (Demirtunc, et al., 2009).

Sustained hyperglycemia leads to a series of interrelated alterations that can cause evident endothelial dysfunction and vascular lesions in diabetic complications (Bae, et al., 2003).

In response to stimuli generated by the endothelium of blood vessels, platelets change shape, adhere to subendothelial surfaces, secrete the contents of intracellular organelles, and aggregate to form a thrombus (Mitchell, et al., 2010).

These pro-aggregatory stimuli include thrombin, collagen, epinephrine, ADP (dense storage granules), and thromboxane A2 (activated platelets) (Mitchell, et al., 2010).

Thus, platelets may assume an important role in signaling of the development of advanced atherosclerosis in diabetes (Colwell, et al., 2003).

MPV is an indicator of the average size and activity of platelets. Larger platelets are younger, more reactive and aggregable. Hence, they contain denser granules, secrete more serotonin and β-thromboglobulin, and produce more thromboxane A2 than smaller platelets (Chang, et al., 2010).

All these can produce a pro-coagulant effect and cause thrombotic vascular complications. This suggests a relationship between the platelet function especially MPV and diabetic vascular complications thus
indicating changes in MPV reflect the state of thrombogenesis (Hekimsoy, et al., 2004)

High MPV is emerging as a new risk factor for the vascular complications of DM of which atherothrombosis plays a major role (Zuberi, et al, 2008).

Thus, diabetes mellitus has been considered as a “prothrombotic state” with increased platelet reactivity (Jindal, et al., 2011).

Although the underlying mechanism of higher MPV in diabetic subjects is incompletely understood, but thought to be due to osmotic swelling as a result of hyperglycemia (Martyn, et al., 1986)

Hyperglycemia can increase platelet reactivity by inducing nonenzymatic glycation of proteins on the surface of the platelet, by the osmotic effect of glucose and activation of protein kinase C Such glycation decreases membrane fluidity and increases the propensity of platelets to activate (Vinik, et al., 2001)

Alternatively, increased platelet size may reflect the presence of high platelet turnover and younger platelets (Guthikonda, et al., 2008)

Platelet function is directly regulated by insulin via a functional insulin receptor (IR) found on human platelets. In vivo experiments have confirmed that insulin inhibits platelet interaction with collagen and attenuates the platelet aggregation effect of agonists in healthy nonobese individuals. This experiments done by (Vinik, et al., 2001) and (Kakouros, et al., 2011)

Platelets from patients with diabetes express more surface P-selectin and glycoprotein (GP) IIb/IIIa receptors and are more sensitive to agonist
stimulation than platelets from patients without diabetes (Yngen, et al., 2006).

Although several measurements of platelet activity have emerged as potential contributors to atherothrombosis, many of these measurements are time-consuming, expensive, uses high sample volume, or require specialty training (Michelson, 2009).

Alternatively, Mean Platelet Volume (MPV) is a marker of platelet size that is easily determined on routine automated hemograms and routinely available at a relatively low cost (Jakubowski, et al., 1983).

1.2.7 Previous studies:

Study done by (Hekimsoy, et al., 2004; Papanas, et al., 2004; Zuberi, et al., 2008; Atea, et al., 2009; Demirtunc, et al., 2009; Jindal, et al., 2011) concluded that MPV was significantly higher in diabetic group compared to non-diabetic subjects.

The mean platelet count was significantly lower in diabetic compared to non-diabetic which obtained by (Hekimsoy, et al., 2004). Other studies done by (Zuberi, et al., 2008; Demirtunc, et al., 2009) observed the opposite finding with higher platelet counts in the diabetic group compared with non-diabetic healthy subjects.

Study conducted by (Sonali, et al., 2011) concluded that platelet distribution width was significantly higher in diabetic patients compared to non-diabetic.

Study conducted by (Kodiattte, et al., 2012) conclude that mean platelet volume was significantly higher in patients with hemoglobin A1c>6.5%
than in patients with hemoglobin A1c <6.5%, these result disagreed with study done by (Papanas, et al., 2004)

Study done by (Pradeepm, et al., 2013) concluded that MPV was significant higher in diabetic patients on oral hypoglycemic therapy than on insulin therapy.

Study conducted by (Kodiatte, et al., 2012) showed positive correlation between MPV and fasting glucose level.

Study done by (Sonali, et al., 2011) concluded that PDW was significant higher in diabetic compared to non diabetic.

Study done by (Hekimsoy, et al., 2004; Demirtunc, et al., 2009) concluded no correlation between MPV and duration of diabetes.
1.3 Rationale:

Altered platelet morphology and function have been reported in patients with diabetes mellitus and associated with the risk of vascular disease (Colwell, et al., 2003).

The prevalence of diabetes in the world was 346 million according to World Health Organization (WHO) survey, in Sudan the prevalence of diabetes was 12% according to survey done by Mohamed Ali Altom, prevalence of microvascular disorder which include retinopathy 43%, nephropathy 22%, neuropathy 37%. Prevalence of the macrovascular disease (cardiovascular disease 28%, peripheral vascular disease 10%, cerebrovascular accident 5.5%).

Previous study was done in Sudan by Dalia Dafallah (2013) concluded that higher mean platelet volume and platelet distribution width in diabetic patient compare to non diabetic healthy individual, and lower platelet count in diabetes. My study could determine the relationship between diabetes and platelet count, mean platelet volume (MPV) and platelet distribution width (PDW). So MPV could be used as simple and cost effective less parameter to evaluate the diabetic vascular event.
1.4 Objectives:

1.4.1 General objective:

To measure platelets count, mean platelet volume, platelet distribution width and glycosylated hemoglobin (A1c) in diabetic patients.

1.4.2 Specific objectives:

- To estimate the mean platelet volume and platelet distribution width, platelet count and hemoglobin A1c in diabetics compared to non diabetics.
- To determine the relationship between mean platelet volume and fasting plasma glucose level, glycosylated hemoglobin (HbA1c) and correlation with duration of disease in the diabetic patients.
- To determine the variation in MPV with diabetic treatment.
Chapter Two
Material and Method

2.1 Study design:
This is case-control analytical study conducted from February to May 2014. At Sharg Alneel Hospital in hematology and clinical department. Aimed to measured platelet count, mean platelet volume, platelet distribution width, fasting blood glucose and hemoglobin A1cin diabetic patients (case) and non diabetic (control).

2.2 Study population:
The study includes 82 diagnosed diabetic patients (based on RBG ≥ 200 + symptom of diabetes then OGTT) and 50 healthy individuals as control group.

2.2.1 Inclusion criteria:

- Confirmed cases of type 2DM, who are on oral hypoglycemic treatment.
- Confirmed cases of type 2 DM, who are on insulin therapy.
- Non diabetic as control group for comparing.

2.2.2 Exclusion criteria:

- Type 1 Diabetes mellitus.
- Patients with abnormal platelet counts (thrombocytosis/thrombocytopenia).
- Patients with hypertension, obesity, renal failure, blood transfusion, blood loss and anemia.
2.3 Sample collection:
Blood sample were collected from superficial vein from case and control groups under sterile condition using sterile disposable syringe and 2.5 ml blood drained into EDTA vaccontainer and 2.5 ml drained into fluoride oxalate vaccontainer.

2.4 Data collection:
Data collected through questionnaires (sex, age, duration of the disease, therapy type and others disease)

2.5 Methodology:
2.5.1 Measurement of platelet count, MPV and PDW by Sysmex KX-21N:

2.5.1.1 Principle:
Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both side of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood size is detected as electric pluses. Blood cell size is calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes. Also, analyzing ahistogram makes it possible to obtain various analysis data. (Sysmex, 1999).

2.5.1.2 Reagent:
Cell pack
Stromatolyser-W

2.5.1.3 Method:
Well mixed EDTA blood was aspirated from the sample probe into the sample rotar valve, then 4μl of blood measured by sample rotor valve was
diluted into 1:50 with 1.996ml of diluted and brought to the mixing chamber as diluted sample, then out of the 1:500 dilution sample, 40μl is measured by the sample rotor valve, diluted into 1:25000 with 1.960ml of diluent, then transferred to the RBC\PLT transducer chamber. Then 250μl from this was aspirated through the aperture. Then was counted by detection method.

2.5.1.4 Result calculation:

\[ MPV(\text{fl}) = \frac{\text{PCT(platelet-crit)}}{\text{PLT}(\times 10^3 \mu\text{L})} \times 1000 \]

PDW(fl): is distribution width on 20% frequency level with the peak taken as 100%

2.5.1.5 Reference range:

PLT count = 150-450 x 10^3 μl

MPV = 8.5-12.5 fl

PDW = 11.0-18.5 fl

2.5.2 Measurement of hemoglobin A1c by Roche/Hitachi cobas c systems:

2.5.2.1 Test principle:

This method uses Tetradecyl tri methyl ammonium bromide (TTAB) as the detergent in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). All hemoglobin variants which are glycated at the β-chain N-terminus and which have antibody-recognizable regions identical to that of HbA1c are determined by this assay.

Hemoglobin A1c: determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood.

Sample and addition of R1 (buffer/antibody) glycohemoglobin (HbA1c) in the sample reacts with anti-HbA1c antibody to form soluble antigen-antibody complexes.
Addition of R2 (buffer/polyhapten) and start of reaction, the polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be determined turbidimetrically. Hemoglobin liberated in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum which is measured bichromatically during the pre incubation phase (sample + R1) of the above immunological reaction. A separate Hb reagent is consequently not necessary.

The final result is expressed as mmol/mol HbA1c or % HbA1c and is calculated from the HbA1c/Hb ratio as follows:


2.5.2.2 Reagents:

R1 Antibody Reagent
MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2; HbA1c antibody (ovine serum): ≥ 0.5 mg/mL; detergent; stabilizers; preservatives

R2 Polyhapten Reagent
MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2; HbA1c polyhapten: ≥ 8 μg/mL; detergent; stabilizers; preservatives

2.5.2.3 Method:

Well mixed EDTA blood (in vacutainer tube) was putted in the cobas system then the system take 5μl from the sample, 120μl from R1, 24μl from R2 and 120μl from diluted hemolyzing reagent to start the work.

2.5.2.4 Result:

Protocol 1 (mmol/mol HbA1c to International Federation of Clinical IFCC):

HbA1c (mmol/mol) = (HbA1c/Hb) × 1000

Protocol 2 (% HbA1c to Diabetes Control and Complications Trial DCCT):
HbA1c (%) = (HbA1c/Hb) \times 91.5 + 2.15.

2.5.2.5 Reference range:
4.8-5.9 % by DCCT or 29-42 mmol/mol by IFCC (Junge, et al., 2003)

2.5.3 Measurement of glucose level by Roche/Hitachi cobas c systems:

2.5.3.1 Test principle:
Enzymatic reference method with hexokinase. Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP. Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically (Kunst, et al., 1984; Tietz, 2006)

2.5.3.2 Reagents:

R1 MES buffer: 5.0 mmol/L, pH 6.0; Mg2+: 24 mmol/L; ATP: ≥ 4.5 mmol/L; NADP: ≥ 7.0 mmol/L; preservative
R2 HEPES buffer: 200 mmol/L, pH 8.0; Mg2+: 4 mmol/L; HK (yeast): ≥ 300 μkat/L; G6PDH (E. coli): ≥ 300 μkat/L; preservative

2.5.3.3 Method:
Centrifuged fluoride oxalated blood (in vacutainer tube) was putted in the cobas system then the system take 2 μl from the sample 28 μl from R1 and 10 μl from R2 to start the work.

2.5.3.4 Result:
Express in mmol/L or mg/dL.

2.5.3.5 Reference range:
4.11-6.05 mmol/L (74-109 mg/dL) (Thomas, et al., 2005)
2.6 Ethical consideration:
The research approved at the level of Hematology Department Research committee of Post Graduate Studies (SUST).
All patient had been informed(oral informed consent) about research before collection of samples.

2.7 Data analysis:
Was be analyzed by SPSS computer program, version 11.5. The significant level set at ($\leq 0.05$), was used one way ANOVA test between the mean platelet volume and therapy, correlation test between mean platelet volume and duration of diabetes and independent sample T-test for other tests done.
Chapter Three

Result

Table (3-1) show 45 diabetic patients (55%) were male and 37 diabetic patient (45%) were female, and 25 control (50%) were male and 25 control (50%) were female.

Table (3-2) show 13 diabetic patient (16%) were less than 40 years, 69 (84%) were more than 40 years.

Table (3-3) show 59 (72.0%) on oral hypoglycemic agent, 12 (14.6%) on insulin therapy and 11 (13.4%) on combination therapy.

Table (3-4) show that there was significance difference in these parameter between diabetic and non diabetic. (p < 0.05).

Table (3-5) show that there was insignificance difference in these parameter between therapy group. (p > 0.05).

Table (3-6) show that insignificant different between diabetic with fasting plasma glucose less than 126 mg/dl and diabetic with fasting plasma glucose more than 126 mg/dl (P > 0.05).

Table (3-7) show that in significant different between diabetic with A1c less than 6.5% and diabetic with A1c more than 6.5% (P-value > 0.05).

Figure (3-8) no correlation was observed between mean platelet volume and duration of diabetic disease (p = 0.28 , r = -0.120)
Characteristics data:

Table(3-1) Distribution of diabetic patient according to the gender:

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>Female</td>
<td>37</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table(3-2) Distribution of diabetic patient according to the age:

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤40years</td>
<td>13</td>
<td>16%</td>
</tr>
<tr>
<td>&gt;40years</td>
<td>69</td>
<td>84%</td>
</tr>
</tbody>
</table>

Table(3-3) Distribution of diabetic according to therapy type:

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Diabetic patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral hypoglycemic agent</td>
<td>Count 59</td>
</tr>
<tr>
<td></td>
<td>% of total 72.0%</td>
</tr>
<tr>
<td>Insulin</td>
<td>Count 12</td>
</tr>
<tr>
<td></td>
<td>% of total 14.6%</td>
</tr>
<tr>
<td>Combination</td>
<td>Count 11</td>
</tr>
<tr>
<td></td>
<td>% of total 13.4%</td>
</tr>
<tr>
<td>Total</td>
<td>Count 82</td>
</tr>
<tr>
<td></td>
<td>% of total 100%</td>
</tr>
</tbody>
</table>
Table (3-4) Distribution of fasting plasma glucose and A1c among diabetic patients:

<table>
<thead>
<tr>
<th>Test</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FPG mg(\text{dl})</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\leq 126)</td>
<td>30</td>
<td>36.3%</td>
</tr>
<tr>
<td>(&gt; 126)</td>
<td>52</td>
<td>63.4%</td>
</tr>
<tr>
<td><strong>A1c%</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\leq 6.5)</td>
<td>13</td>
<td>15.9%</td>
</tr>
<tr>
<td>(&gt; 6.5)</td>
<td>69</td>
<td>84.1%</td>
</tr>
</tbody>
</table>

# American diabetic association (ADA) proposed that 126mg\(\text{dl}\) and 6.5% is cutoff between diabetic and non diabetic for FPG and A1c respectively

Laboratory data:

Table (3-5) Mean of platelet count, mean platelet volume and platelet distribution width among diabetic patients and non diabetic.

<table>
<thead>
<tr>
<th>Items</th>
<th>Sample</th>
<th>Mean</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet countx10^9 C/L</td>
<td>Diabetic</td>
<td>248±67.7</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Non diabetic</td>
<td>272±44.9</td>
<td></td>
</tr>
<tr>
<td>MPV(\text{fl})</td>
<td>Diabetic</td>
<td>9.7±.96</td>
<td>.00</td>
</tr>
<tr>
<td></td>
<td>Non diabetic</td>
<td>9.3±.64</td>
<td></td>
</tr>
<tr>
<td>PDW(\text{fl})</td>
<td>Diabetic</td>
<td>12.4±1.9</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>Non diabetic</td>
<td>11.8±1.1</td>
<td></td>
</tr>
</tbody>
</table>

*\(p\leq0.05\) significant
Table (3-6) Mean of mean platelet volume in diabetic patients according to therapy type.

<table>
<thead>
<tr>
<th>Items</th>
<th>Therapy</th>
<th>Mean</th>
<th>P_value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPV(\mu l)</td>
<td>Oral</td>
<td>10±.99</td>
<td>0.149</td>
</tr>
<tr>
<td></td>
<td>Insulin</td>
<td>9.7±.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Combination</td>
<td>9.4±.99</td>
<td></td>
</tr>
</tbody>
</table>

*≤0.05 significant

Table (3-7) Mean of mean platelet volume (MPV) according to fasting plasma glucose level in diabetic patients.

<table>
<thead>
<tr>
<th>Test</th>
<th>Fasting plasma glucose</th>
<th>Mean</th>
<th>P_value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean platelet volume(\mu l)</td>
<td>≤126 mg(dl)</td>
<td>10.0±.91</td>
<td>.236</td>
</tr>
<tr>
<td></td>
<td>&gt;126 mg(dl)</td>
<td>9.8±.99</td>
<td></td>
</tr>
</tbody>
</table>

*≤0.05 significant

Table (3-8) Mean of mean platelet volume (MPV) according to hemoglobin A1c in diabetic patients.

<table>
<thead>
<tr>
<th>Test</th>
<th>Glycosylated hemoglobin</th>
<th>Mean</th>
<th>P_value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean platelet volume(\mu l)</td>
<td>≤6.5%</td>
<td>10.3±1.1</td>
<td>.073</td>
</tr>
<tr>
<td></td>
<td>&gt;6.5%</td>
<td>9.8±.9</td>
<td></td>
</tr>
</tbody>
</table>

*≤0.05 significant
Figure(3-1) Correlation between mean platelet volume and duration of the disease among diabetic patients.
Chapter Four

Discussion, Conclusion & Recommendation.

4.1 Discussion

Diabetes mellitus (DM) is a major global health problem. Altered platelet morphology and function have been reported in patients with diabetes mellitus and associated with the risk of vascular disease (Colwell, et al., 2003).

In this study MPV was significantly higher in diabetic group compare to nondiabetic subjects, this agree with the findings seen in studies done by (Hekimsoy, et al., 2004; Papanas, et al., 2004; Zuberi, et al., 2008; Atea, et al., 2009; Demirtunc, et al., 2009; Jindal, et al., 2011; Dalia Dafallah, 2013), although the underlying mechanism of higher mean platelet volume in diabetic subjects is incompletely understood, but suggestion that is due to osmotic swelling as a result of hyperglycemia or increased platelet size may reflect the presence of high platelet turnover and younger platelets (Martyn, et al., 1986).

Diabetic who are on insulin therapy and in combination therapy had insignificant lower MPV compared with patient who are on oral hypoglycemic drugs, this disagrees with the study done by Pradeeppm, et al. (2013) who found significant higher MPV in oral hypoglycemic therapy than in insulin therapy. Although the lower mean platelet volume in diabetic take insulin therapy was insignificant but this may be related to low sample size of patient taking insulin therapy in this study or may be this patients had insulin resistance.

The MPV was insignificantly different between patients with hemoglobin A1c >6.5% and patients with hemoglobin A1c <6.5% which was in on

Mean platelet volume had insignificantly different between patients with fasting blood glucose >126mg/dl and in patients with fasting blood glucose <126mg/dl which was in opposite result obtain by( Kolidatte, et al.,2012)who found correlation between FBG and MPV,I related that to the FBG level changes day by day in same patient and mean platelet volume is relatively stable.

The mean platelet count is significant lower in diabetic compare to non diabetics that was similar to the studies done by (Hekimsoy, et al.,2004; Dalia Dafallah. 2013). Other studies done by (Zuberi,et al.,2008;Demirtunc, et al.,2009) observed the opposite finding with higher platelet counts in the diabetic group compared with non diabetic healthy subjects. The platelet count could be dependent on several variables, that is, mean platelet survival, platelet production rate, and turnover rate in diabetes mellitus.

There is a significant higher in platelet distribution width in diabetic compare to non diabetic, agrees with result obtained by(Sonali, et al.,2011; Dalia Dafallah. 2013), this finding supported that there were active large platelet in diabetic patients.

No correlation between MPV and duration of diabetes, Similar finding found by other reasearchers(Hekimsoy, et al.,2004; Demirtunc, et al.,2009). But this findings were in contrast to the finding of (Ates, et al.,2009).Isuggested that if the vascular damage was only due to increased number of large and reactive platelets, then the rate of damage would have been constant for the duration, but platelet reactivity alone cannot explain the progression of vascular complications in diabetes since
there are other vascular risk factors that may lead to vascular complication. This was supported by the insignificant statistical correlation between mean platelet volume and duration of diabetes in this study.
4.2 Conclusions

This study concluded that:

- Platelet count was significantly lower in diabetic patients compared with non-diabetic subjects.
- MPV and PDW were significantly higher in diabetic patients compared with non-diabetic subjects.
- Mean platelet volume was not affected by the type of diabetic therapy.
- Mean platelet volume did not affect by fasting plasma glucose and hemoglobin A1c.
- No correlation was found between mean platelet volume and duration of disease in diabetic patients.
4.3 Recommendation:

This study recommended:

- Consider the MPV is one of essential indicative parameter for vascular event in diabetes mellitus.
- Further study with large sample size on the effect of insulin in mean platelet volume.
- Other research in mean platelet volume in diabetic hypertensive patient.
Reference


Georgiades, CS; Neyman, EG; Francis, IR; Sneider, MB; Fishman, EK (Nov 2002). Typical and atypical presentations of extramedullary hemopoiesis. American journal of roentgenology 179 (5): 1239–43


Sonali Jindal; Shilpa Gupta; Ruchika Gupta; Ashima Kakkar; Harsh V Singh; Kusum Gupta; Sompal Singh (2011) Platelet indices in diabetes mellitus: indicators of diabetic microvascular complications Volume 16 Issue 2, pp. 86-89.


Unger S (1985). Grundy Hyperglycemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: implications for the management of diabetes Diabetologia 28 119 121.


Appendices
Appendices(1): Questionnaire

بدم الله الرحمن الرحيم

Sudan University of Science & Technology

College of Post Graduate Studies

Medical Laboratory master program

Department of Hematology

Questionnaire

Data: \1\2014. Sample serial NO( )

Name: ................................................................. ...

Gender: ................................................................. ...

Age: ................................................................. ...

Duration of diabetes: ...........................................

Therapy type: insulin ........ oral hypoglycemic agent:...

Combination......

Blood transfusion: ........

Hypertension: ............

Renal disease: ............

Hemolytic anemia: ......

Blood loss: ..............

Obesity: .................

Other: .................

Glucose level: .................................
Hemoglobin A1C level: .................................................

Complete blood count (CBC): ......................................

Date: ..............

بعد فهم محتويات هذا الاستبيان والغرض من إجراء البحث أنا ............ (أو من ينوب عنه)

وافق على اخذ العينة.

Signature: ...........