Sudan University of Science &Technology

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Measurement of Fibrinogen Level, Platelet Count and Mean Platelet Volume of Diabetic Patient with Retinopathy

قياس مستوى الفيبرينوجين و عدد الصفائح الدموية ومتوسط حجم الصفائح الدموية لدي مرضي السكري المصابين بإعتلال الشبكية

A Dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc. Degree in Hematology & Immunohematology

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الآيـة

بسم الله الرحمن الرحيم

فَتَبَسَّمَ ضَاحِكًا مِنْ قَوْلِهَا وَقَالَ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ (١٩)

صدق الله العظيم

سورة النمل الآية (19)

Dedication

To the heart of my life, father and mother

To my brothers

To my sisters

To my colleagues

And to myself

I dedicate this work

Walaa . . . .

Acknowledgment

First grateful thanks to Allah …..

Then I am so indebted my supervisor:

Dr / Khalda Mirghani Hamza and all members of medical laporatory department for their supervision, supporting and motivation throughout this work.

A lot of thanks to the members of my family and all my friends…

Walaa…

Abstract

This is an analytical descriptive study which carried out in the City of Khartoum at Fedail Hospital in the period from June 2013 to October 2013 to measure the fibrinogen level, platelets count and the mean platelet volume on the diabetic patient with retinopathy. 50 diabetic patients were selected,50 diabetic patients with retinopathy and 50 non diabetic as control group.5 ml of venous blood was drawn from each patient and was divided into 2.5 ml in a Trisodum citrate container then centrifuged to get the platelet poor citrated plasma to measure the fibrinogen level. Stogo coagulation analyzer used to measure the fibrinogen level .The remaining 2.5 ml in EDTA container to measure the platelet count and the mean platelet volume, Sysmex KX21 auto analyzer used to measure the platelet count and the mean platelet volume. The results were obtained and the mean of each parameter was calculated they were as followed: For diabetic patients Fibrinogen level: 300 mg/dl, compared to 371mg/dl for diabetic patients with retinopathy and 278mg/dl for the control group. Platelet count for diabetic patients: 282.000/ml, compared to 237.000/ml for diabetic patients with retinopathy and 255.000/ml for the control group. Mean platelet volume for diabetic patients 10.2 fl , 11.2 for diabetic patients with retinopathy and 10.1 for the control group.

This study showed that there is an increase in the fibrinogen level in patients with diabetes and retinopathy when compared to the control group , which indicates the existence of a significant relation between high fibrinogen level and an increased risk of having retinopathy for patients with diabetes type II. This study also showed no significant relation between platelet count and the retinopathy, while there was an increase in the mean platelet volume in patients with retinopathy, which indicated the existence of a significant relation between mean platelet volume and diabetic retinopathy.

مستخلص الدراسة

هذه دراسة وصفية تحليلية تم تنفيذها في مدينة الخرطوم في مستشفى فضيل في الفترة من يونيو 2013 إلى أكتوبر 2013 لقياس مستوى الفيبرينوجين ، عدد الصفائح الدموية و متوسط حجم الصفائح الدموية على مرضي السكري مع اعتلال الشبكية . وقد تم اختيار 50 مريضا بالسكري و50 مرضى السكري مع اعتلال الشبكية السكري و 50 شخص كمجموعة مقارنة..

تم سحب 5 مل من الدم الوريدي من كل مريض و قسمت إلى 2.5 مل في حاوية Trisodumcitrate ثم تحصلنا على البلازما فقيرة الصفائح الدموية لقياس مستوى الفيبرينوجين ،تم استخدام جهاز Stago coagulation analyzerلقياس مستوى الفيبرينوجين . و 2.5 مل المتبقية في حاوية EDTA لقياس عدد الصفائح الدموية و و متوسط حجم الصفائح الدموية ، تم استخدام Sysmex KX21 autoanalyzer , لقياس عدد الصفائح الدموية و متوسط حجم الصفائح الدموية. و قد كانت النتائج على النحو التالي:بالنسبه لمرضى السكري: مستوى الفيبرينوجين : 300 ملغ / ديسيلتر ، مقارنة ب371 ملغ / ديسيلتر لمرضى السكري مع اعتلال الشبكية و 278 ملغ / بالنسبة لمجموعة المقارنة. عدد الصفائح الدموية لمرضى السكري : 282.000/مل ،مقارنة ب237.000/مل لمرضى السكري مع اعتلال الشبكية و 255.000/مل بالنسبة لمجموعة المقارنة.متوسط حجم الصفائح الدموية لمرضى السكري المرضى 10.2 فمتوليتر ، 11.2 *فمتوليتر* لمرضى السكري مع اعتلال الشبكية و 10.1 *فمتوليتر* لمجموعة المقارنة.وأظهرت هذه الدراسة أن هناك زيادة في مستوى الفيبرينوجين لدي المرضى الذين يعانون من مرض السكري و اعتلال الشبكية عند مقارنتهم مع مجموعة المقارنة، والذي يدل على وجود علاقة حقيقية بين ذيادة مستوى الفيبرينوجين و الاصابة باعتلال الشبكية لمرضى السكري من النوع الثاني.و بالنسبة لعدد الصفائح الدموية ،فقد أظهرت الدراسة عدم وجود علاقة حقيقية بين عدد الصفائح الدموية و اعتلال الشبكية ، في حين أن هناك زيادة في متوسط حجم الصفائح الدموية في المرضى الذين يعانون من اعتلال الشبكية ، مما يدل على وجود علاقة حقيقية بين متوسط حجم الصفائح الدموية و اعتلال الشبكية لدي مرضي السكري .

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Abbreviations

BFU-MK: Burst-Forming Unit-Megakaryocyte

CFU-MK: Colony-Forming unit-Megakaryocyte

CHD: Coronary Heart Disease

CVD: Cardiovascular Disease

DCCT: Diabetes Control Complication Trail

GDM: Gestational Diabetes Mellties

GP: Mean Platelets Volume

HMWK: High Molecular Wight Kalekren

MPV: Mean Platelets Volume

NCIA: Nonsteroidal Anti-inflammatory Agent

NBDR: Nonproliferative Diabetic Retinopathy

PDR: Proliferative Diabetic Retinopathy

PLA2: Phospholipase A2

P PIP2: Phosphatidylinositol-4,5-Biphosphate

PGI2: Prostglandin12

PKC: Protein kinase C

PLC-g: Phospholipase C-G

PMNs: Polymorpho Nuclear Leukocytes

SCCS: Surface-Connected Canalicular System

TXA2: Thromboxane A2

UKPDS: U.K. Prospective Diabetes Study

VWF: Von Willbrand Factor

Chapter one

Introduction and Literature review

1.3 General introduction

Diabetes mellitus is not a single disease entity, but rather a group of metabolic disorders sharing the common underlying feature of hyperglycaemia. Worldwide, over 140 million people suffer from diabetes making this one of the most common diseases (Inzussh *et al.* 2003).

Researchers have suspected that fibrinogen is involved in the increased rate of cardiovascular disease in patients with non-insulin dependent diabetes mellitus (Dilli J 2010).

Altered platelet morphology and function have been reported in patients with DM, and MPV was found to be significantly higher in diabetic patients (Zuberi *et al* 2008).

1.4 Literature review

1.2.1 Blood physiology (constituent and function)

1.2.1.1 Plasma

The fluid portion of the blood, the plasma, is a remarkable solution containing an immense number of ions inorganic molecules, and organic molecules that is in transit to various parts of the body or aid in the transport of other substances. The normal plasma volume is about 5% of body weight, or roughly 3500mL in a 70-Kg man (Ganong1981).

1.2.1.2 Cellular elements

The cellular elements of the blood –white blood cells, red blood cells and platelets- are suspended in the plasma. The normal total circulating blood volume is about 8% of the body weight (5600mL in a 70-Kg man). About 55%of is plasma (Ganong1981).

1.2.1.2.1 Red blood cells

In order to carry haemoglobin into close contact with the tissues and for successful gaseous exchange, the red cell, 8 /lm in diameter, must be able: to pass repeatedly through the microcirculatioi1 whose minimum diameter is 3.5 /lm, to maintain haemoglobin in a reduced (ferrous) state and to maintain osmotic equilibrium despite the high concentration of protein (haemoglobin) in the cell. Its total journey tlu-oughout its 120-day lifespan has been estimated to be 480 km (300 miles). (Hoffbrand *et al* 2006).

1.2.1.2.2White blood cells

There are normally 4000-11000 white blood cells per microliter of human blood .Of these the granulocytes (polymorphonuclear leukocytes, PMNs) are the most numerous. Most of them contain neutrophilic granules (neutrophils), but a few contain granules that stain with acidic dyes (eosinophils), and some have basophilic granules (basophils). The other 2 celltypes found normally in peripheral blood are lymphocytes, which have large round nuclei and scanty cytoplasm, and monocytes, which have abundant agranular cytoplasm and kidney-shaped nuclei. Acting together, these cells provide the body with powerful defenses against tumors and viral, bacterial, and parasitic infections. (Hoffbrand *et al* 2006**).**

1.2.1.2.3 Platelets

1.2.1.2.3.1 Definition:

Platelets are versatile fragments of cytoplasm whose major function is to arrest bleeding (Zucker and Nachmias 1985).

1.2.1.2.3.2 Platelet production, circulation and their life span

Each day the adult human produces approximately 1×10^{11} platelets, a level of production can increase 10- to 20- fold in times of increased demand (Ganong1981).

Production of platelets depends on the proliferation and differentiation of a hemopoietic stem and progenitor cells to a cell committed to the large megakaryocyte lineage, its maturation to a large, polyploid megakaryocyte, and its final fragmentation into platelet (Bonnefoy *et al* 2001).The external influences that impact megakaryopoiesis and thrombosis are a supportive marrow stroma consisting of endothelial and other cells, matrix, glucoseaminoglycans, and a family of protein hormones and cytokines, including thrombopoitein, stem cell factor, interleukin-6, interleukin-11and stromal-cell derived factor-1.(Bennett *et al* 2009). Thrombocytes production starts in the yolk sac, then, shifts to the fetal liver and then to the marrow at the time of gestation.Two colony morphologies, colony-forming unit–megakaryocyte(CFU-MK) and burst-forming unit–megakaryocyte (BFU-MK) are thought to represent primitive and mature progenitor cells restricted to the megakaryocyte lineage. CFUs-MK is a cell that develops into a simple colony containing 3 to 50 mature megakaryocytes.

BFUs-MK develops into larger, more complex colonies that include satellite collections of megakaryocytes and contain up to several hundred cells. (White 1974).Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakarocytes. The precursor of the megakarocyte the megakaryoblast arises by a process of differentiation from haemopoietic stem cells. The megakarocytes mature by endomitotic synchronous nuclear replication, enlarging cytoplasm volume as the number of nuclear lobes increases in multiples of two. At variable stages in development most commonly at eight nucleus stage the cytoplasm become granular and platelet are liberated. Platelet production follows formation of micro-vesicles in the cytoplasm of the cell which coalesce to form platelet demarcation membrane. Each megakarocyte is responsible for the production of about 4000 platelets.(Hoffbrand *et al* 2005). The circulating half-life of platelet is approximately 10 days in human with normal platelet count, but is somewhat shorter in patients with moderate (7 days) to severe (5 days) thrombocytopenia as a higher proportion of the total body platelets mass is consumed in the day-to-day function of maintaining vascular integrity. In steady state, when platelet production equals destruction, platelet turnover has been estimated at 1.2 to 1.5 $\times$ 10^{11} cells per day. The sites for platelet removal appear to be the spleen, the liver, and the bone marrow The platelet count varies among the healthy population (1.5 to 3.5 ∞ 10^5/μl) but remains within a fairly narrow range in any given individual (Greer *et al* 2004).

1.2.1.2.3.3 Platelet structure

under light microscopy, platelets appear as small, anucleate (i.e., lacking a nucleus) fragments with occasional reddish granules, measuring approximately 2 μm in diameter with a volume of approximately 8 fl .By scanning electron microscopy, circulating platelets appear as flat discs with smooth contours, rare spiny filopodia, and random openings of a channel system, the surface-connected canalicular system (SCCS), which invaginates throughout the platelet and is the conduit by which granule contents exocytose after stimulation. They are roughly discoid in shape and contain cytoplasmic organelles, cytoskeletal elements, invaginating open-canalicular membrane systems, and platelet-specific granules called alpha and dense granules. Platelets have numerous intrinsic glycoproteins attached to the outer surface of their plasma membrane that are receptors for such ligands as fibrinogen, collagen, thrombin, and thrombospondin to vonWillebrand factor and fibronectin (White 1974). In describing detailed platelet anatomy,

most information is derived from transmission electron microscopy, and platelet structure is classified into four general areas:

1. The platelet surface.
2. The membranous structure.
3. The cytoskeleton (sol-gel-zone).
4. The granules (Greer *et al* 2004).

1.2.1.2.3.3.1 Platelet surface

The platelet surface includes the plasma membrane that separates intra- from extra cellular regions, it is exceptionally complex in composition, distribution and function, incorporating a number of glycoproteins and lipids into is phospholipids bilayer and integrating a variety of extra- and intraplatelet events such as permeability, agonist stimulation, and platelet adhesion, activation\secretion, and aggregation; and the glycocolyx that is a fuzzy layer of lipids, sugars, and proteins, coats the outside surface of the platelet plasma membrane, including the surface-connected tubular system, and interacts with both the plasma and the cellular components of the blood and blood vessels(Greer *et al* 2004).

Integrins are a large family of receptors which are constitutively expressed on the surface of almost all cells. They consist of transmembraneaß heterodimers and can bind extracellular matrix proteins as well as immunoglobulin-like adhesion molecules. The major physiological role of the GPIb/IX/V glycoprotein complex is to mediate the initial adhesion of circulating platelets to the exposed subendothelium or to intact proinflammatory endothelium under high shear stress and the most important ligand of the GIb/IX/V complex is vWF GPVI is the main platelet collagen receptor.In addition to GPIb/IX/V and GPIIb/IIIa, GPVI isan important receptor in thrombus growth underhigh shear stress. The absence of GPVI on the surfaceof platelets is associated with a mild bleedingpredisposition and impaired adhesionand thrombus formation.The Glycoprotein IIb/IIIa integrin constitutes the most abundant platelet adhesion receptor, the GIIb/IIIa receptor is an important molecule for the aggregation of platelets and platelet-neutrophil-interaction. Upon activation, platelet GPIIb/IIIa binds soluble extracellular adhesion molecules, such as vWF, fibrinogen, fibronectin, and thrombospondin. Furthermore, GPIIb/IIIa is responsible for the formation of fibrin bridges among platelets and is involved in platelet cohesion and thrombus growth following ligand binding; ‘outside-in’ signals influence platelet function (spreading and

contraction) and the expression of adhesion molecules. The absence of GPIIa/IIIb in Glanzmann thrombasthenia is associated with a severe bleeding due to defective platelet aggregation and clot retraction. (Zarbock *et al* 2007).

1.2.1.2.3.3.2 Platelet membranous system

The platelet membranous systems consists of surface connected canalicular system (SCCS) that is also called the open canalicular system, is fenestrated and continuous with surface plasma membrane, weaving through the entire platelet cytoplasm in a tortuous fashion; the dense tubulr system that is a closed-channel system consisting of narrow membrane-limited tubules. It is residual smooth endoplasmic reticulum from the megakaryocytes (Greer *et al* 2004).

1.2.1.2.3.3.3 Platelet cytoskeleton

The platelet cytoskeleton is subdivided into membrane skeleton that is a short actin filaments that connect surface receptors with the bulk of cytoplasmic actin filaments, cytoplasmic actin and intermediate filaments that connect between the membrane skeleton and microtubules, and microtubules that they are circumferential and they support the discoid form of platelet (Greer *et al* 2004).

1.2.1.2.3.3.4 Platelet granules

Considering platelet granules and organelles, platelet process secretary granules and mechanisms that serve these purpose by releasing additional stimulatory materials previously sequestered within the resting platelet, into the environment for developing a haemostatic or thrombotic mass.Two main secretary granules, the al granules and dense bodies, appear to be the main effectors with their highly reactive and readily available contents. The role of these other platelet granules (lysosomes, peroxisomes) and organelles such as mitochondria is less dramatic than those of the alpha granules and dense bodies (Greer *et al* 2004).

1.2.1.2.3.4 Platelet function and activation

The main function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury. In the absence of platelets spontaneous leakage of blood through small vessels may occur. Central to their fuction are platelets activation, adhesion,

secretion, aggregation, fusion and procoagulant activity (Hoffbrand *et al* 2001). Physiologic stimuli that can activate platelets both in vivo and in vitro are amazingly diverse. Substances shown to produce this reaction include materials as diverse as proteolytic and structural proteins (such as thrombin and collagen), vasoactive material (such as serotonin and epinephrine), nucleotides (such as ADP), polypeptides hormones (such as vasopressin), and non biologic surfaces (such as glass and latex particles). Other stimuli arise under pathologic conditions. Among these are antigen-antibody complexes or aggregated gamma globulin that react with an Fc receptor on human platelets and a complement receptor on rabbit platelets. The responses to these stimuli are initiated when the agonists bind to specific receptors on the plasma membrane(Zucker and Nachmias 1985).Most stimuli cause a change in shape and this change involves first the formation of very fine pseudopodia (i.e., filopodia) from the rim of the disc, followed by a general "rounding up" of the platelet so that it becomes a spiny sphere, often with much broader pseudopodia (Zucker and Nachmias 1985).

Shape change with or without secretion causes the microtubule bundle that lies beneath the rim of the disk to become centralized and surround the platelet granules, which are consequently concentrated toward the center of the platelet (White 1974).The adhesion of platelets to the collagen exposed on endothelial cell surfaces is mediated by von Willebrand factor. The function of vWF is to act as a bridge between a specific glycoprotein on the surface of platelets (GPIb/IX) and collagen fibrils. In addition to its role as a bridge between platelets and exposed collagen on endothelial surfaces.Adhesion to this surface is associated with loss of granules (i.e., secretion), which is not inhibited by nonsteroidal anti-inflammatory (NSAI) agents (Tracy 2001).Collagen exposure or thrombin action results in the secretion of platelet granule content. The initial activation of platelets is induced by thrombin binding to specific receptors on the surface of platelets, thereby initiating a signal transduction cascade. The thrombin receptor is coupled to a G-protein that, in turn, activates phospholipase C-g (PLC-g). PLC-g hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) leading to the formation of inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 induces the release of intracellular Ca^{2+} stores, and DAG activates protein kinase C (PKC). The collagen to which platelets adhere as well as the release of intracellular Ca^{2+} leads to the activation of phospholipase A_2 (PLA_2), which then hydrolyzes membrane phospholipids, leading to liberation of arachidonic acid (Theroux P et al. 2000).Arachidonic acid release leads to an increase in the production and subsequent release of

thromboxane A_2 (TXA_2). TXA_2 is a potent vasoconstrictor and inducer of platelet aggregation by lowering platelet cAMP. The release reaction is inhibited by substances which increase the levels of cAMP. One such substances is the prostaglandin prostacyclin (PGI_2) which is synthesized by vascular endothelial cells. It is a potent inhibitor of platelet aggregation and prevents their deposition on normal vascular endothelium (Newby *et al* 2001). ADP, thrombin, serotonin, vasopressin, and epinephrine initiate aggregation within a few seconds. Fluid-phase calcium or magnesium is necessary for aggregation.5657 In addition, fibrinogen is also required. With ADP or epinephrine, this protein must be present in the suspension medium, whereas with thrombin or collagen it is secreted from the alpha granules (Zucker and Grant 1978).Once platelet aggregates are formed, there is a tendency for the fibrin threads to be laid on them to form a clot. This process is facilitated by the platelets, possibly via more than one mechanism. Phospholipids on the platelet membrane support the intrinsic pathway of coagulation, which results in the formation of thrombin from prothrombin by activated factor X. The platelet surface also prevents active coagulation factors from inactivation by their natural inhibitors. Considering platelet release reactions, platelet factor 4 looks to possess antiheparin activity, and fibrinogen that is released from platelet granules may potentially contribute further to the formation of the thrombus. Also, P-selectin expression could result in platelet–leukocyte interaction making fibrin deposition by the leukocytes to form thrombus.The simultaneously activation of coagulation FV that located in the alpha granules of the platelets by thrombin and convulxin (an activator of the collagen receptor glycoprotein VI), becomes membrane bound when activated simultaneously with two agonists, making the factor membrane bound. These platelets are referred to as convulxin and thrombin induced Factor V platelets and they are capable of generating more prothrombinase activity than any other physiological agent and thus, are effectively more procoagulant. (Kamath *et al* 2003).

Other functions for platelets are also known:

- Platelets cause leucocytes to accumulate around the platelet plug; that is, they may release chemotactic substances.
- Platelets do release vasoactive amines.

- Platelets may release hydrolytic and proteolytic enzymes directly into the intimal and subintimal structures provoking changes that may eventually lead to atheroma.
- Platelets act to transport serotonin from sites of synthesis to other sites of function (Hemker et al. 1983).

1.2.1.2.3.5. Mean platelet volume and platelet activation

Mean platelet volume (MPV) is an indicator of the average size and activity of platelets. MPV is measured as the part of the complete blood count. Under normal circumstances larger platelets are younger and exhibit more activity .The platelets have an important role in the pathogenesis of vascular diseases. MPV is the indicator of the size and activity of platelets. Increased values of MPV have been shown as a risk factor for stroke. Relationship between increased MPV with deep venous thrombosis, acute myocardial infarction, and acute ischemic cerebrovascular events was also reported. Since larger platelets store and release larger amounts of serotonin and β-thromboglobulin and produce more thromboxane A_2, they are more reactive and prone to aggregation. (Sahin *et al* 2013). Platelets have an important role in the pathogenesis of thrombo-occlusive disease such as retinopathy and MPV is an indicator of platelet size and has been known to be a marker of platelet activity. Large platelets are more reactive than small platelets and produce more thromboxane A2, express more glycoprotein Ib and glycoprotein IIb/IIIa receptors, and aggregate more easily (Lioudaki and Ganotakis 2010).

1.2.1.2.3.6 Platelet abnormalities in Type 2 diabetes

The abnormalities described in patients with diabetes are listed here:

- Increased production of thromboxane A2 from arachidonic acid.
- Increase in platelet-dependent thrombin generation.
- Increased expression of platelet surface adhesion molecules such as CD31, CD49b, CD62P, and CD63, leading to increased platelet activation.
- Increased platelet surface receptors such as P-selectin, GP Ib, and GP IIb/IIIa.
- Reduced vascular synthesis of the anti-aggregants PGI2 and NO, shifting balance towards aggregation and vasoconstriction.

- Disordered calcium homeostasis that affects platelet shape change, secretion, aggregation, and thromboxane formation.
- Decreased platelet insulin receptor number and affinity and failure to reduce platelet responses to the agonists ADP, collagen, thrombin, arachidonate, and PAF.Glycation of circulating LDL rendering platelets hypersensitive.
- Glycated LDL causes an increase in intracellular calcium concentration and platelet NO production, as well as inhibition of the platelet membrane Na+/K+-ATPase activity (Halushka and Mayfield 1981).

1.2.2. Coagulation pathway and physiology

The coagulation process is a complex series of enzymatic reactions involving the proteolytic activation of circulating coagulation factors (zymogens) and activity of cofactors (V, VIII), leading to production of thrombin which converts soluble plasma fibrinogen into fibrin (table 1). The fibrin enmeshes the platelet plug, forming a stable thrombus which prevents further blood loss from the damaged vessel (Cheesbrough 2000).

Table (1) Blood clotting factors, their role and function (Cheesbrough 2000).

Factor	Source and function
Fibrinogen I	Plasma protein made in the liver. Converted to fibrin
Prothrombin II	Plasma protein made in the liver with help of vitamin K. converted to thrombin
Tissue factor III (Thromboplastin)	Released from damaged tissue. Essential in activating in vivo coagulation
Calcium ions IV	Inorganic ions in plasma, derived from diet or bone. Essential for the coagulation process.
Labile factor V	Plasma protein made in the liver. Also released from platelets. Co-factor involved in converting prothrombin to thrombin.

Note, there is no factor VI	
Proconvertin VII	Plasma protein made in the liver with the help of vitamin K. In vivo activates factor IX
Antihaemophilic factor VIII	Globulin made in the liver. Co-factor, involved in activating factor X
Christmas factor IX	Plasma protein made in the liver with help of vitamin K.Involved in activating factor X
Stuart-Power factor X	Plasma protein made in the liver with help of vitamin K. Involved in converting prothrombin to thrombin
Plasma thromboplastin antecedent, XI	Plasma protein made in the liver. Important at major site of trauma
Hageman factor XII	Plasma protein made in the liver. Involved in converting plasminogen to plasmin and activating factor XI. In vitro, it intiates the clotting process
Fibrin stabilizing Factor, XIII	Plasma protein made in the liver and present in platelets. Converting fibrin polymer to stable insoluble fibrin

Three proteins, factor XII, prekallikrein, and HMWK, are required for activity of the contact or accessory pathway. Factor XII and prekallikrein are zymogens that are activated to generate serine proteases, and HMWK is a nonenzymaticprocofactor and may also be involved in the promotion of thrombus stability (Danish *et al* 2005). The accessory pathway may also be important in cardiopulmonary bypass due to contact between blood components and synthetic surfaces. The primary pathway of coagulation involves the vitamin K–dependent zymogens and serine proteases, cofactor proteins, and Ca^{2+} ions assembled on anionic phospholipid membranes. The complexes display reaction rates 10^5 to 10^6 times greater than the respective serine proteases alone (Kleinschnitz *et al* 2006).

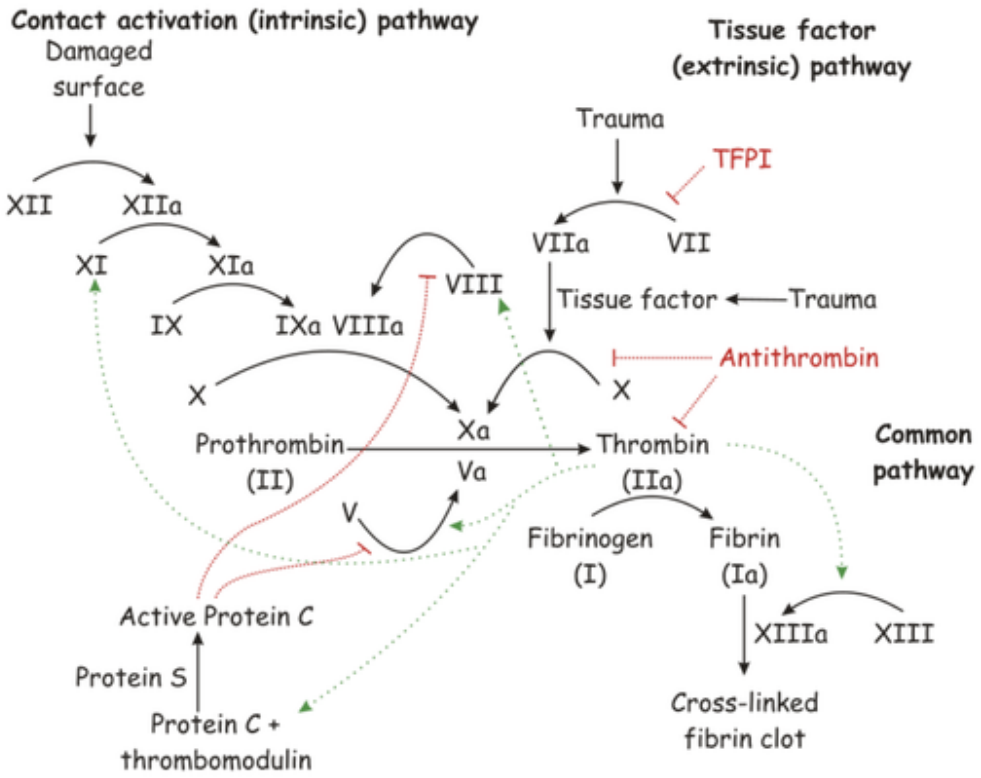

Fig (1) Coagulation cascade (Cheesbrough 2000).

The three complexes of the primary pathway (extrinsic tenase, intrinsic tenase, and prothrombinase) function in tandem to achieve a rapid burst of α-thrombin generation. The primary pathway of coagulation is initiated or triggered by the interaction of circulating factor VIIa with its cofactor tissue factor. In general, the serine proteases associated with hemostasis circulate in their zymogen or inactive forms; however, low levels of circulating factor VIIa are present in blood (Nesheim *et al* 1979). This factor VIIa binds to tissue factor and initiates the procoagulant response. Although low levels of factor VIIa are in continuous circulation, initiation of coagulation is a very specific response. Free factor VIIa is a poor enzyme with virtually no proteolytic activity and does not react with circulating inhibitors in the absence of tissue factor (Krishnaswamy 1990).Tissue factor, an integral membrane protein that is not normally expressed on vascular cell surfaces, is constitutively expressed on extravascular cellular surfaces and thus becomes exposed on damage to the endothelial cell layer. Tissue factor is also

expressed on peripheral blood cells and endothelial cells stimulated by inflammatory cytokines (Butenas and Mann 1996).

On interaction of circulating factor VIIa and injury-exposed tissue factor, the extrinsic tenase complex is formed and initiates coagulation by activating factors IX and X. The extrinsic tenase also activates additional factor VII. Factor IXa forms a complex with its cofactor, factor VIIIa, to generate the intrinsic tenase complex, and factor Xa combines with factor Va to form the prothrombinase complex. The factor VIIIa–factor IXa complex serves to activate factor X to factor Xa, providing a more robust source of the enzyme component of the prothrombinase complex. Prothrombinase activity subsequently leads to a burst of α-thrombin generation and propagation of the procoagulant response (Mann *et al* 1992).

1.2.3 Fibrinogen

1.2.3.1 Fibrinogen in hemostasis

Fibrinogen (factor I) is a soluble, 340 kDa plasma glycoprotein, that is converted by thrombin into fibrin during blood clot formation. Fibrinogen is synthesized in the liver by the hepatocytes. The concentration of fibrin in the blood plasma is 200-400 mg/dL (normally measured using the Clauss method).During normal blood coagulation, a coagulation cascade activates the zymogen prothrombin by converting it into the serine protease thrombin. Thrombin then converts the soluble fibrinogen into insoluble fibrin strands. These strands are then cross-linked by factor XIII to form a blood clot. FXIIIa stabilizes fibrin further by incorporation of the fibrinolysis inhibitors alpha-2-antiplasmin and TAFI (thrombin activatable fibrinolysis inhibitor, procarboxypeptidase B), and binding to several adhesive proteins of various cells. Both the activation of Factor XIII by thrombin and plasminogen activator (t-PA) are catalyzed by fibrin. Fibrin specifically binds the activated coagulation factors factor Xa and thrombin and entraps them in the network of fibers, thus functioning as a temporary inhibitor of these enzymes, which stay active and can be released during fibrinolysis (Muszbek *et al* 2008).In its natural form, fibrinogen can form bridges between platelets, by binding to their GpIIb/IIIa surface membrane proteins; however, its major function is as the precursor to fibrin. Fibrinogen, the principal protein of vertebrate blood clotting, is a hexamer, containing two sets of three different chains (α, β, and γ), linked to each other by disulfide bonds. The N-terminal sections of these three chains contain the cysteines that participate in the cross-linking of the chains. The C-terminal parts of the α, β and γ chains contain a domain of about 225 amino-acid residues,

which can function as a molecular recognition unit. In fibrinogen as well as in angiopoietin, this domain is implicated in protein-protein interactions. In lectins, such as mammalian ficolins and invertebrate tachylectin 5A, the fibrinogen C-terminal domain binds carbohydrates. On the fibrinogen α and β chains, there is a small peptide sequence (called a fibrinopeptide). These small peptides are what prevent fibrinogen from spontaneously forming polymers with itself. The conversion of fibrinogen to fibrin occurs in several steps. First, thrombin cleaves the N-terminus of the fibrinogen alpha and beta chains to fibrinopeptide A and B respectively. (Blomback *et al* 1978). The resulting fibrin monomers polymerize end to end to from protofibrils, which in turn associate laterally to form fibrin fibers.[12] In a final step, the fibrin fibers associate to form the fibrin gel.(Lorand *et al* 1977). Fibrinogen and its metabolites may lead to endothelial dysfunction through various mechanisms. Several atherosclerotic lesions contain large amounts of fibrin, either in the form of wall thrombus in the intact surface of the plaque or scattered diffusely all over the plaque. This phenomenon is associated with a decrease in fibrinolytic activity and plasminogen concentrations, states that are observed in CAD. It has been found that fibrin (intima) triggers cell proliferation, contributing to cell migration, and bonds fibronectin, which triggers cell migration and adhesion. Fibrinogen and products of its decomposition mediate the transportation of adhesion molecules in the surface of endothelium and their further migration to the intima. The decomposition products located in the inner layer can trigger mitogenesis and synthesis of collagen, attract leukocytes, and enhance permeability as well as vascular tone (Papageorgiou *et al.* 2010). Fibrinogen is a key component of the blood coagulation system and an acute phase reactant. Fib is produced by the liver and is a large soluble glycoprotein found in the plasma, consisting of two identical subunits. Each subunit is comprised of three polypeptide chains alpha, beta and gamma linked to each other by disulfide bonds. Serum plasma fibrinogen levels are determined by both genetic and environmental factors. Advancing age, smoking, obesity, hypertension, dyslipidemia, physical inactivity, menopause, low socio-economic status, oral contraception, female sex and black race are associated with elevated fibrinogen levels .On the other hand there are some factors related to lower fibrinogen Fib levels such as moderate alcohol consumption, hormone replacement therapy, regular exercise and weight reduction (Kamath *et al* 2003).

1.2.3.2 Fibrinogen and cardiovascular diseases

Fibrinogen is the major coagulation protein in blood by mass, the precursor of fibrin and an important determinant of blood viscosity and platelet aggregation. Because fibrinogen level can be reduced considerably by life style interventions that also affect levels of established risk factors (such as regular exercise, smoking cessation, and moderate alcohol consumption)

there is possibility that measurement (or modification) of fibrinogen may help in disease prediction or prevention. (Danish *et al* 2005).

Elevated fibrinogen levels were first reported to correlate with CVD and coronary heart disease (CHD) in particular, almost 60 years ago. During the following decades, numerous epidemiologic studies showed a definite relation of Fib to CVD .The causality still remains uncertain. The first meta-analysis which recognized fibrinogen levels as an independent CV risk factor included six prospective epidemiologic studies. Elevated levels were associated with subsequent myocardial infarction (MI) or stroke [odds ratio in the upper vs. lower tertile varying between 1.8 (95% CI 1.2-2.5) and 4.1(95% CI 2.3-6.9). A recent meta-analysis of 31prospective studies including 154, 211 apparently healthy individuals showed that long-term increases in plasma fibrinogen levels of 1 g/L are associated with an approximate doublingof risk of major CVD. (James *et al* 2000). Among blood viscosity major determinants, fibrinogen emerged as the only remaining significantly associated with CV events and total mortality after adjustment for conventional risk factors. Finally, some studies have reported that fibrinogen levels also correlate with the severity of the underlying CVD (Lioudaki and Ganotakis 2010). Fibrinogen plays a central role in hemostasis1 and recent data indicate that high circulating levels of fibrinogen are associated with the occurrence of thrombotic episodes. Except for its role in the coagulation cascade being the precursor of fibrin, fibrinogen exhibits a number of other functions. It stimulates platelet aggregation, increases blood viscosity, promotes smooth muscle proliferation and migration and regulates cell adhesion and chemotaxis .These may be the mechanisms through which fbrinogen participates in vascular disease. Furthermore, the effect of elevated fibrinogen levels on atherosclerosis may be mediated by inflammation through its role as an acute phase reactant (Giovanni *et al* 1986).

1.2.4 Blood coagulation, fibrinogen and diabetes

The factors implicated in diabetic microangiopathy include changes in physical and functional properties of red blood cells, alterations in platelet aggregation and adhesiveness, prostaglandin metabolism and changes in clotting factors and blood viscosity .Some of these changes may precede the development of retinopathy and may be related to the severity of the disease. As the concentration of fibrinogen rises, it causes increased blood viscosity and the blood flow to the retina decreases leading to deposition of platelets and fibrin with subsequent thrombus

formation. (Rema *et al* 1995). persons with type 2 diabetes mellitus are at increased risk for cardiovascular-related illness and death, but this excess risk is not completely explained by an increased prevalence of the major conventional cardiovascular risk factors (for example, smoking, hypertension, hypercholesterolemia and obesity). Researchers have suspected that fibrinogen is involved in the excessive rate of cardiovascular disease in patients with non-insulindependent diabetes mellitus. Clinic-based studies reported that plasma fibrinogen levels were higher in diabetic patients than in controls and in diabetic patients with microalbuminuria than in diabetic patients with normoalbuminuria. Because microalbuminuria has been recognized as a powerful predictor of cardiovascular-related illness and death, fibrinogen level may be considered a potential additional risk factor in patients with diabetes. (Dilli *et al* 2010)

1.2.5 Diabetes Mellitus

1.2.5.1 Definition and incidence

Diabetes mellitus is not a single disease entity, but rather a group of metabolic disorders sharing the common underlying feature of hyperglycaemia. Worldwide, over 140 million people suffer from diabetes making this one of the most common diseases (Inzucch *et al* 2003). In the Western population the prevalence of DM has been estimated to be 3-5% and the incidence is rapidly growing up and will be more than doubled within 15 years Type II DM accounts for more than 80 % cases of DM and is slow-onset, heterogeneous disorder, resulting from interactions between environmental factors and polygenetic inheritance. Recent estimates indicate there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030. (Wild *et al* 2004).

1.2.5.2 Classifications

Assigning a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis, and many diabetic individuals do not easily fit into a single class For example, a person with gestational diabetes mellitus (GDM) may continue to be hyperglycemic after delivery and may be determined to have, in fact, type 2diabetes. (Inzucch *et al* 2003).

1.2.5.2.1 Type 1 diabetes

This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulindependent diabetes, type 1 diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the _-cells of the pancreas Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. ((Inzucch *et al* 2003).

1.2.5.2.2 Type 2 diabetes

This form of diabetes, which accounts for _90–95% of those with diabetes, previously referred to as non–insulindependent diabetes, type 2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. (Inzucch *et al* 2003).

1.2.5.2.3 Gestational diabetes mellitus

For many years, GDM has been defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Although most cases resolve with delivery, the definition applied whether or not the condition persisted after pregnancy and did not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy. (Inzucch *et al* 2003).

1.2.5.3 Complications of diabetes mellitus

Long-standing diabetes mellitus inevitably leads to the development of vascular, renal and other pathologies. Some common long-term effects of DM include vascular complications (such as atherosclerosis, ischemic heart disease, ischemia and gangrene of the foot andmicroangiopathy), diabetic nephropathy (glomerular damage and renal failure), eye change (diabetic retinopathy and cataract), and nervous defects (peripheral neuropathy and autonomic insufficiency diarrhea)

(Saboor 2012). Diabetes is associated with acceralated rates of thrombosis, circulatory dysfunction, and atherosclerosis. Most of the morbidity and mortality seen in patients with diabetes mellitus, especially in type II ('non-insulin dependent') diabetes, is the result of micro- and macro-vascular occlusive disease in which thrombosis plays an important part. (Rebort *et al* 1998).

1.2.5.4 Risk factors for diabetic retinopathy

Diabetic retinopathy is one of the leading causes of blindness in the world that increases the chance of losing the sight about 25 times higher compared to normal individuals. Diabetic retinopathy is the most frequent cause of new cases of blindness among adults aged 20 –74 years. During the first two decades of disease, nearly all patients with type 1 diabetes and _60% of patients with type 2 diabetes have retinopathy.Diabetic retinopathy progresses from mild nonproliferative abnormalities, characterized by increased vascular permeability, to moderate and severe nonproliferative diabetic retinopathy (NPDR), characterized by vascular closure, to proliferative diabetic retinopathy (PDR), characterized by the growth of new blood vessels on the retina and posterior surface of the vitreous. Macular edema, characterized by retinal thickening from leaky blood vessels, can develop at all stages of retinopathy (Fong *et al* 1988).

1.2.5.4.1 Duration of DM

The duration of diabetes is probably the strongest predictor for development and progression of retinopathy. Among younger-onset patients with diabetes in the WESDR, the prevalence of any retinopathy was 8% at 3 years, 25% at 5 years, 60% at 10 years, and 80% at 15 years. The prevalence of PDR was 0% at 3 years and increased to 25% at 15 years. The incidence of retinopathy also increased with increasing duration. The 4-year incidence of developing proliferative retinopathy in the WESDR younger-onset group increased from 0% during the first 5 years to 27.9% during years 13–14 of diabetes. After 15 years, the incidence of developing PDR remained stable. (Klein *et al* 1984)

1.2.5.4.2 Glycemic control

The Diabetes Control and Complications Trial (DCCT) investigated the effect of hyperglycemia in type 1 diabetic patients, as well as the incidence of diabetic retinopathy, nephropathy, and

neuropathy. The protective effect of glycemic control has also been for confirmed patients with type 2 diabetes. The U.K. Prospective Diabetes Study (UKPDS) demonstrated that improved blood glucose control reduced the risk of developing retinopathy and nephropathy and possibly reduces neuropathy. The overall rate of microvascular complications was decreased by 25% in patients receiving intensive therapy versus conventional therapy. Epidemiological analysis of the UKPDS data showed a continuous relationship between the risk of microvascular complications and glycemia, such that for every percentage point decrease in HbA1c (e.g., from 8 to 7%), there was a 35% reduction in the risk of microvascular complications. (Robert *et al* 1998).

1.2.5.4.3 Blood pressure control

The UKPDS also investigated the influence of tight blood pressure control. With a median follow-up of8.4 years, patients assigned to tight control had a 34% reduction in progression of retinopathy and a 47% reduced risk of deterioration in visual acuity of three lines in association with a 10/5 mmHg reduction in blood pressure. In addition, there were reductions in deaths related to diabetes and strokes (Robert *et al* 1998).

1.3 Rationale

80% of patients with diabetes mellitus die due to thrombotic death. 75% of these deaths are due to cardiovascular complications and the remainder is due to cerebrovascular events and peripheral vascular complications. Patients are considered to have a hypercoagulable state if they have laboratory abnormalities associated with increased risk of thrombosis. Plasma levels of many clotting factors including fibrinogen, factor VII, factor VIII, factor XI, factor XII, kallikrein, and vWF are elevated in diabetes.(Ritu *et al* 2010).

Platelets play a key role in the development of atherothrombotic events. Platelets are essential for primary hemostasis and repair of the endothelium, but they also play a key role in the development of acute coronary syndromes and contribute to cerebrovascular events. (Davi and Patrono 2007). Large platelets are more thrombogenic and thus put the patient at a higher risk status. Mean platelet volume (MPV) is a determinant of platelet functionality and increased MPV is associated with increased risk for myocardial infarction, stroke and transient ischaemic attacks. (Zuberi *et al* 2008).

This study aimed to measure the role of fibrinogen, platelet count and mean platelet volume in type II DM and its contribution to the progression of the diabetic retinopathy. This may have a significant role in management of diabetic patients and prevention of cardiovascular complications.

1.4. Objective of the study

1.4.1 General objective

The aim of this study is to measure the fibrinogen levels, platelet count and MPV of Sudanese diabetic patient with retinopathy.

1.4.2. Specific objectives

1. To compare the levels of fibrinogen, platelet count and MPV between diabetic patients and non-diabetic individuals**.**
2. To compare the fibrinogen level, platelet count and MPV between retinopathy diabetic patients with retinopathy and diabetic patients.
3. To correlate between the fibrinogen level, platelet count and MPV, and the diabetic retinopathy.

Chapter Two

Material and Methods

Chapter Two

Materials and Methods

2.1 Study design:

This is a retrospective analytical case control study carried out in the period from June 2013to the end of October 2013.

2.2 Sample size:

50 diabetic patients with retinopathy,50 diabetic patients and 50 non diabetic patients.

2.3 Study area:

This study was conducted in Khartoum state at Fedail Hospital.

2.4 Inclusion criteria:

Sudanese patients known to have type II diabetes mellitus of both genders who aged between 40-69 years were participated in this study, after medical cheekup to confirm absence of any systemic diseases.

2.5 Ethical consideration

This study will be passing no risk to subject. Each participant was assigned a unique number, after accepting the contribution in this study.

2.6 Tool of data collection:

Data was collected using a questionnaire designed to provide personal and medical information about the patients (appendix I) and results of blood sample tests .

2.7 Method of sample collection:

2.7.1 Requirements:

1. Stago coagulation analyzer for quantitative determination of serum fibrinogen level.
2. Automated Hematological analyzer sysmex KX21N for determination of complete blood count.
3. Cotton, tourniquet, syrings and 70% ethanol.
4. EDTA and Tri Sodium Citrate anti-coagulant containers.

2.7.2 Collection of samples:

1. Each enrolled subject was either sat or laid down right on examination table.
2. The skin was cleaned with 70%ethanol and allowed to dry.
3. Personal details were checked up on the forms.
4. A tourniquet was applied to the arm, tight sufficiently to distend the vein, but not tightly to cause discomfort.
5. 5ml of blood samples were taken from the superficial vein of the forearm.
6. Each blood sample was divided into two parts: 2.5 ml in Tri Sodium Citrate anti-coagulant containers for fibrinogen, 2.5 ml EDTA containers for platelet count and MPV determination.

2.8 Method

2.8.1 Measurement of fibrinogen level

Quantitative determination of the fibrinogen level in the plasma by Clauss method. Diluted plasma is clotted with a strong thrombin solution; the plasma was diluted to give a low level of any inhibitors (e.g., FDPs and heparin). A strong thrombin solution was used so that the clotting time over a wide range is independent of the thrombin concentration. The Fibrinogen assay was determined using a commercially available kit (Diagnostica Stago /France).

2.8.1.1 Procedure

0.2ml Tri Sodium Citrate plasma (obtained by centrifugation of Tri Sodium Citrate blood for 10 minutes at 2000-2500g) incubated at 37°C for 2 minute. Then 0.1 ml reagent 1'added prewarmed at 37°C.Time for sample to clot is recorded and Using log -log paper, clotting times

(seconds) were plotted on theY-axisand their corresponding fibrinogen level was plotted on the X-axis, then the best fit calibration line was drawn .

2.8.2 Measurement of MPV and platelet count

MPV and platelet count were measured using Sysmex KX21 autoanalyzer that uses aperture-impedance principle to size platelets on the red blood cell/platelet channel that produces the following parameters: PLT (109/L) and MPV (fL) .In addition to this, cells are hydrodynamically focused through a small aperture, and a voltage pulse is generated that is proportional in size to the volume of the cell. Mobile "autodiscriminators" distinguish between machine noise at the lower end and red blood cells at the upper end of each individual platelet volume distribution. MPV is calculated by the following formula:

MPV (fL)=Pct (%)x1000÷Plt (x103/µL), where Plt is the platelet count and is the number of particles between the upper and lower discriminators, Pct is the platelet crit and is calculated electronically from the raw histogram data (O'Malley et al. 1995).

Complete blood count including platelet indices was measured within 1 hour of collection to minimize variations due to sample aging. Complete Blood Count including platelet indices was performed following manufacturer instructions .

2.8.2.1 procedure

The whole blood mode (WB) was selected to analyze the whole blood sample without pre-dilution. The sample number was entered before each sample. This procedure was as followed:

- A well mixed anticoagulated sample was set to the sample probe, and the start switch was pressed till the aspirating process was finished. (Volume aspirated approx. 50ML).
- The sample was removed straight down and the sample probe was automatically cleaned.
- The aspirated sample was then automatically suspended into the different detector blocks and different parameters were measured.
- The results of platelete count and MPV were then viewed on the screen and subsequently printed out (Sysmex KX-21 1998).

2.9 Data analysis

SPSS software program used to obtain mean, standard deviation and P value by Ttest and one way ANOVA and mean data is presented in form of tables and figures.

Chapter Three

Results

Chapter Three

Results

150 subjects were included in this study, 50 healthy individuals as control group, 50 diabetic patients and 50diabetic patients with retinopathy. The mean age of control group was 53.3±7.9years, diabetic group was 54.8±8.5 years, and for the diabetic patients with retinopathy the mean age was 52.7±7.5 years. Table (1). The control group consist of: 26 males (52%) and 24 females (48%), the diabetic group consist of: 26 males (52%) and 24 females (48%) and the diabetic patients with retinopathy group consist of: 32 males (64%) and 18 females (36%).Table (2). Diabetic patients were divided into three groups according to the duration of diabetes mellitus <10 years, 10- 20 years and > 20 years. More than 50% of diabetic patients were in the duration period of <10 years, and exactly 50% of the diabetic patients with retinopathy were in in the duration period of <10 years.Fig(1). Fibrinogen level, platelet count and MPV were compared between the three groups and the results showed a significant increase in fibrinogen level between groups (p value= 0.00). (Fig2). While there were no significant differences in the platelet count between the groups (p value = 0.14).Fig (3). The MPV was significantly increased between diabetic group = 10.2±0.88 fl, diabetic with retinopathy group = 11.28±0.66 fl and the control group = 10.11±0.80 fl. (P value = 0.00) (Fig 4) .

Table (1): Distribution of study group according to age

	<50 years(n)	50-60 years(n)	>60years(n)	Total
Control	21	18	11	50
Diabetic patients	20	16	14	50
Diabetic patients with retinopathy	24	16	10	50

Table (2): Distribution of study group according to sex

	Male(n)	Female (n)	Total (n)
Control	26	24	50
Diabetic patients	26	24	50
Diabetic patients with retinopathy	32	18	50

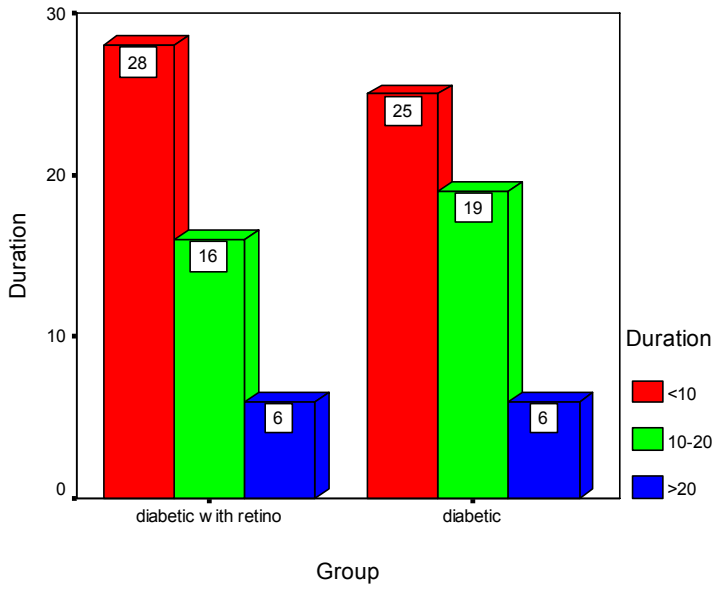

Fig (1): Distribution of patients according to the duration of the diabetes mellitus

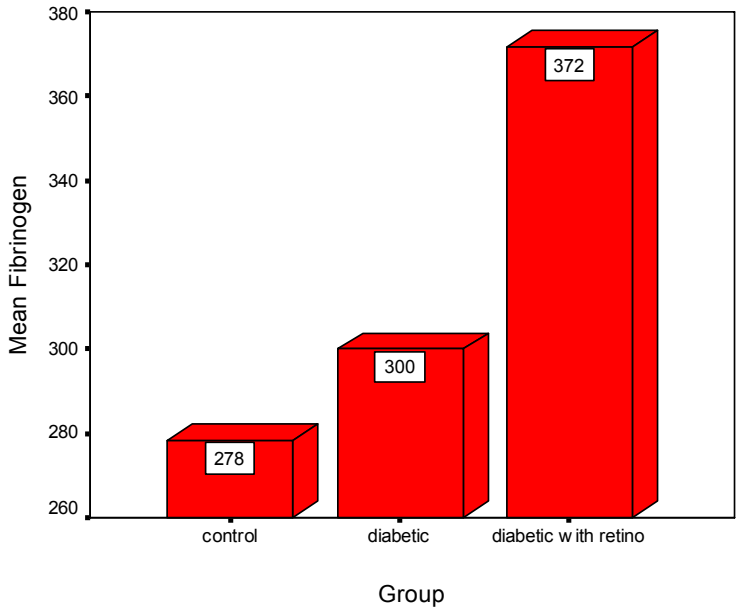

Fig (2): Fibrinogen level of study group

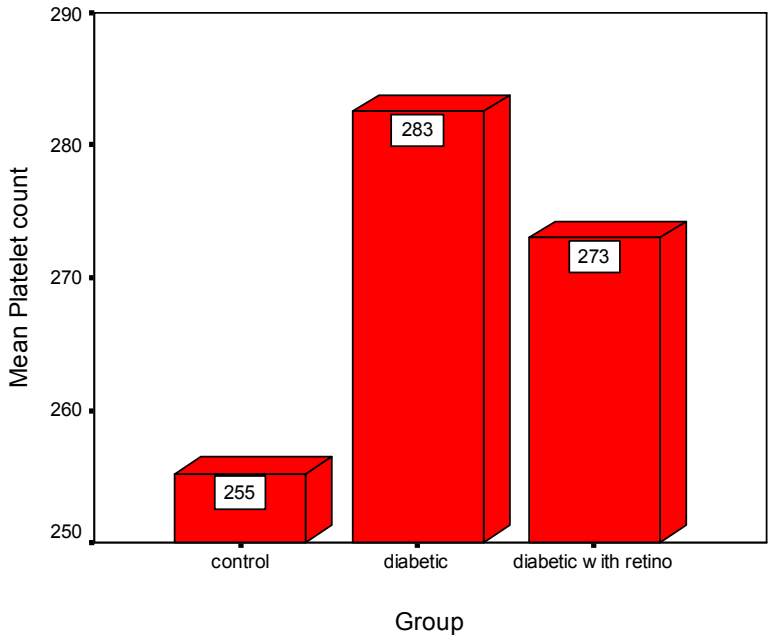

Fig (3): Platelet count of study group

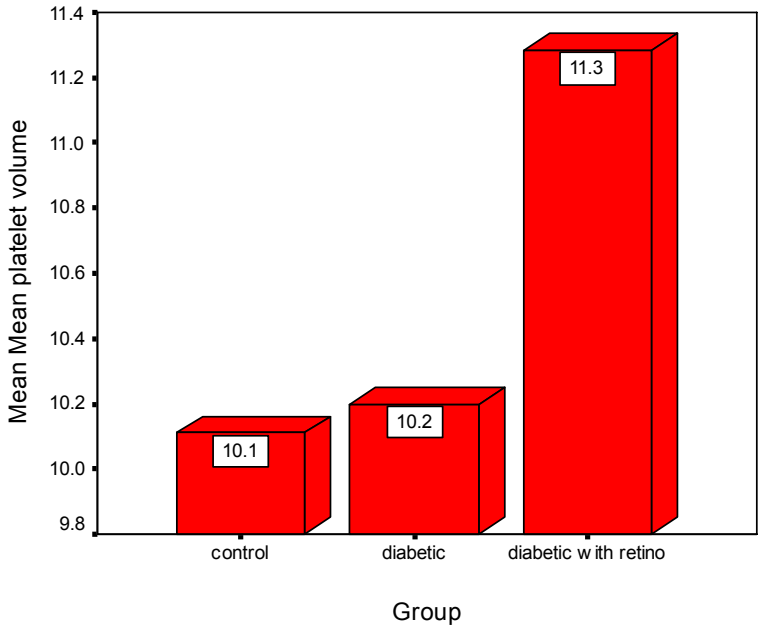

Fig (4): Mean Platelet Volume of study group

Chapter four

Discussion, Conclusion and Recommendation

Chapter four

Discussion, Conclusion and Recommendation

4.1 Discussion

The aim of this study is to measure the fibrinogen level, platelet count and mean platelet volume in diabetic patients with retinopathy. 150 subjects were included in this study, 50 healthy individuals as control group, 50 diabetic patients and 50 diabetic with retinopathy were included in this study which conducted at Fedal Hospital. A significant elevation in fibrinogen levels were found in type II diabetic patients with retinopathy compared to control and diabetic patients without retinopathy. Previous studies were carried out to determine the association of diabetic retinopathy with fibrinogen levels and platelet activation. Dilli (2010) reported that the results from their study showed fibrinogen significantly higher in diabetic patients who also had coronary artery disease than those who had only coronary artery disease or only diabetes. Our present study also showed that fibrinogen significantly higher in patients with diabetes than the control. James *et al* (2000) found fibrinogen higher in diabetic patients than the control,he also showed fibrinogen higher in patients with coronary artery disease compared to diabetic patients without the coronary artery disease. There was no differences between the study groups in the platelet count in this study. Saboor *et al (2012)* Studies on platelet survival in patients with diabetes mellitus have produced conflicting results, some studies have shown decreased platelet survival in patients with diabetes mellitus with overt vascular complications, other researcher however did not find any difference in platelet survival and vascular complications, they failed to demonstrate any relationship between platelet survival and vascular complications in patients with diabetes mellitus compared to normal healthy

controls. Figure (4) showed significant elevation in mean platelet volume in type II diabetic patients with retinopathy when compared to control and diabetic patients without retinopathy. A previous study investigated the association of MPV with the retinopathy in patients with retinal vein occlusion; their results demonstrated that the MPV values were significantly higher in patients with retinal vein occlusion, suggesting that larger platelets may contribute to the pathogenesis of the retinal vein occlusion (Sahin *et al 2013)*.

4.2 Conclusions

1. The mean age of the study group is more than 50 years, males are more than females and the dominant duration period is more than 10 years.
2. Higher fibrinogen levels were significantly found in type II diabetic patients with retinopathy compared to control and diabetic patients without retinopathy.
3. There is no significant difference between the platelets count between the study groups. While MPV was significantly increase in type II diabetic patients compared to control and further in diabetic patients with retinopathy compared to diabetic patients without retinopathy.

4.3 Recommendations

1. Estimation of plasma fibrinogen is simple and cost effective procedure that if established as a marker of retinopathy and would be valuable in controlling of the diabetic retinopathy
2. The occurrence of hyper fibrinogenemia and platelet activation in diabetes type II is highly predictive of retinopathy.
3. Diabetic patients who have high levels of fibrinogen must have close ophthalmologic follow up.

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Appendices

Appendix (I)

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies and Scientific Research

Questionnaire

Name: ……..

Age: ……..

Sex: Male ( ) Female ( )

Duration of D.M: ……..

Retinopathy: ……..

Other disease : ……

RESULTS:

Fibrinogen :……mg\dl

Platelet count :……*10^3\ml

MPV: ……fl

Appendix (II)

بسم الله الرحمن الرحيم

جامعة السودان للعلوم و التكنولوجيا

كلية الدراسات العليا برنامج الماجستير- مختبرات طبية

تخصص علم الدم و مبحث المناعة الدموية

براءة اخلاقية

الاسم :.....

سوف يتم اخذ عينة من الدم (5 مل) من الوريد بواسطة حقنة طعن و ذلك بعد مسح منطقه اخذ العينه بواسطة المطهر . كل الادوات المستخدمة معقمه و متبع فيها وسائل السلامة المعمليه .

و انا اقر بان هذة العينات سوف يتم تحليلها فقط لطلب البحث.

اوافق انا المزكور اعلاه اخذ عينه لاجراء الدراسة.

الإمضاء .....

التاريخ.....

Appendix (III)

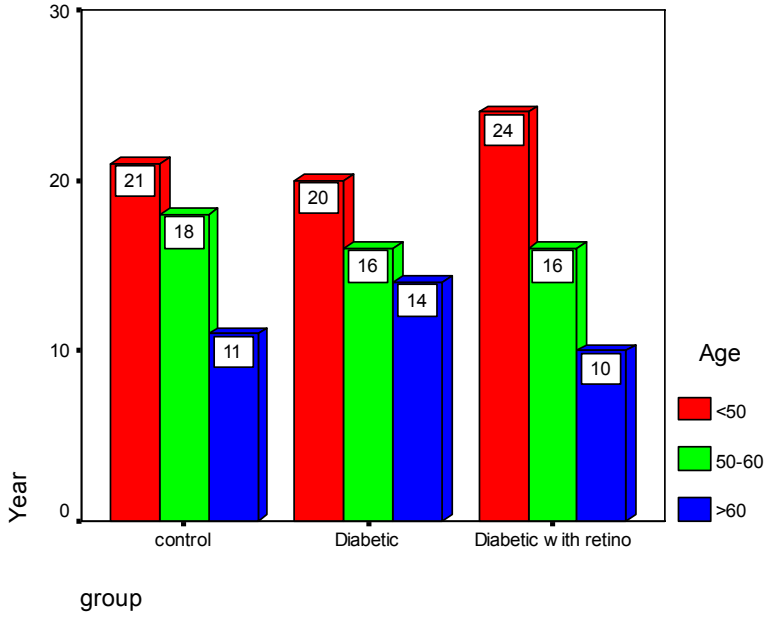

Fig (1): Distribution of study group according to Age

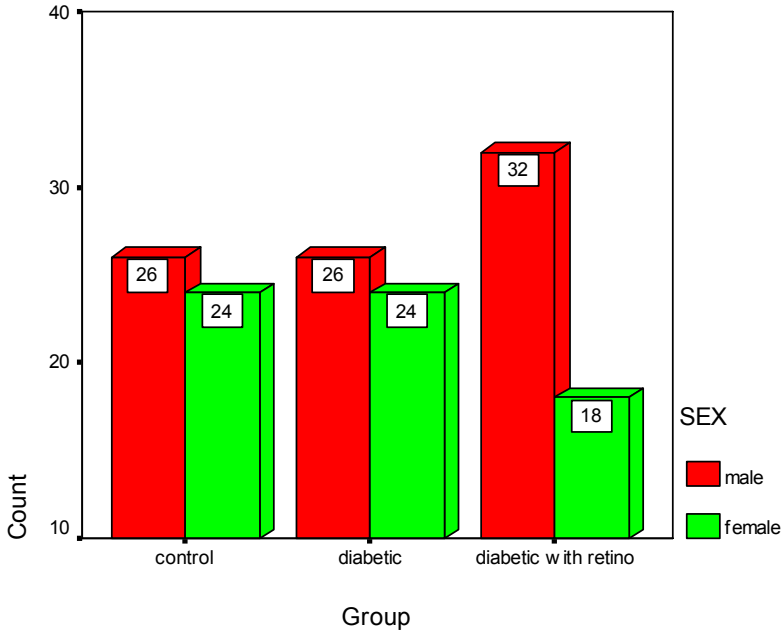

Fig (2): Distribution of study group according to sex

Appendix (III)

Table (1): Distribution of patients according to the duration of the diabetes mellitus

	<10years(n)	10-20 years(n)	>20 years(n)	Total
Diabetic patients	25	19	6	50
Diabetic patients with retinopathy	28	16	6	50

Table (2): Fibrinogen level of study group

	Fibrinogen level± STD (mg/dl)	P value
Control	278.26±48.42	0.00
Diabetic patients	300±56.13	
Diabetic patients with retinopathy	371.64±56.57	

Table (3): Platelet count of study group

	Platelet count ± STD (X 10^3 /ml)	P value
Control	255.16±74.58	0.14
Diabetic patients	282.54±68.38	
Diabetic patients with retinopathy	273.02±69.34	

Table (4): Mean platelet volume of study group

	MPV ± STD (fl)	P value
Control	10.11±0.80	0.00
Diabetic patients	10.20±0.88	
Diabetic patients with retinopathy	11.28±0.66	

Appendix (IV)

Master Sheet

NO	Sample	Sex	Age Year	Duration	Fibrinogen Mg\dl	Platelet Count *10^3\ml	MPV Fl
1	Diabetic	female	47	4	390.00	296.00	10.20
2	Diabetic	male	42	6	320.00	265.00	9.00
3	Diabetic	male	55	11	210.00	201.00	9.10
4	Diabetic	female	49	8	350.00	236.00	11.30
5	Diabetic	female	61	21	390.00	254.00	8.20
6	Diabetic	male	67	20	328.00	142.00	8.80
7	Diabetic	male	56	12	290.00	347.00	10.10
8	Diabetic	female	64	20	430.00	201.00	9.80
9	Diabetic	male	48	4	259.00	250.00	9.10
10	Diabetic	female	44	6	197.00	243.00	9.50
11	Diabetic	male	56	12	247.00	265.00	9.90
12	Diabetic	female	54	13	298.00	264.00	9.80
13	Diabetic	male	67	22	311.00	218.00	10.30
14	Diabetic	male	45	7	345.00	256.00	10.20
15	Diabetic	female	53	12	278.00	480.00	12.00
16	Diabetic	male	47	7	240.00	198.00	12.20
17	Diabetic	female	62	21	320.00	286.00	10.80
18	Diabetic	male	57	11	289.00	295.00	10.30
19	Diabetic	female	65	13	342.00	256.00	9.80
20	Diabetic	female	68	23	268.00	347.00	9.70
21	Diabetic	male	67	21	280.00	301.00	9.80
22	Diabetic	male	65	22	246.00	254.00	9.50

23	Diabetic	female	44	7	266.00	265.00	10.30
24	Diabetic	male	49	7	340.00	201.00	11.00
25	Diabetic	female	65	12	356.00	306.00	9.20
26	Diabetic	female	54	11	345.00	350.00	9.90
27	Diabetic	male	47	6	318.00	360.00	9.80
28	Diabetic	male	64	4	249.00	158.00	10.90
29	Diabetic	female	60	15	377.00	225.00	10.90
30	Diabetic	male	42	3	269.00	380.00	10.40
31	Diabetic	female	53	12	297.00	283.00	10.20
32	Diabetic	male	54	10	347.00	295.00	8.60
33	Diabetic	female	59	13	244.00	337.00	9.50
34	Diabetic	male	46	7	428.00	224.00	10.50
35	Diabetic	female	55	12	370.00	301.00	10.80
36	Diabetic	male	53	3	268.00	246.00	11.20
37	Diabetic	female	42	5	260.00	254.00	9.70
38	Diabetic	male	52	8	311.00	290.00	9.60
39	Diabetic	male	45	6	234.00	470.00	10.80
40	Diabetic	female	61	18	319.00	380.00	10.80
41	Diabetic	female	60	21	200.00	295.00	11.20
42	Diabetic	male	64	19	238.00	312.00	10.40
43	Diabetic	male	45	6	344.00	159.00	8.50
44	Diabetic	female	50	12	324.00	305.00	9.90
45	Diabetic	male	40	3	312.00	283.00	10.60
46	Diabetic	female	66	3	239.00	265.00	11.50
47	Diabetic	female	45	2	278.00	354.00	10.90
48	Diabetic	male	69	25	284.00	326.00	11.10
49	Diabetic	female	56	15	340.00	311.00	10.90
50	Diabetic	male	44	7	216.00	337.00	11.50
51	D +Rtin	female	47	4	355.00	150.00	12.00
52	D +Rtin	male	42	6	365.00	243.00	10.10

53	D +Rtin	male	55	11	478.00	264.00	10.10
54	D +Rtin	female	49	8	356.00	347.00	11.80
55	D +Rtin	female	61	20	356.00	243.00	11.50
56	D +Rtin	male	67	21	367.00	236.00	11.30
57	D +Rtin	male	56	12	323.00	460.00	10.20
58	D +Rtin	female	64	20	410.00	158.00	11.00
59	D +Rtin	male	57	4	367.00	296.00	11.20
60	D +Rtin	female	44	6	450.00	377.00	9.90
61	D +Rtin	male	56	12	314.00	243.00	11.90
62	D +Rtin	female	54	13	456.00	312.00	11.20
63	D +Rtin	male	67	22	390.00	327.00	10.90
64	D +Rtin	male	45	7	300.00	318.00	11.10
65	D +Rtin	female	53	12	320.00	244.00	12.10
66	D +Rtin	male	47	7	450.00	283.00	10.50
67	D +Rtin	female	62	21	278.00	142.00	11.10
68	D +Rtin	male	57	11	290.00	347.00	11.80
69	D +Rtin	female	65	13	390.00	343.00	12.00
70	D +Rtin	female	68	23	365.00	216.00	11.20
71	D +Rtin	male	67	21	387.00	364.00	11.40
72	D +Rtin	male	65	22	325.00	296.00	12.50
73	D +Rtin	female	44	7	430.00	188.00	11.60
74	D +Rtin	male	49	7	398.00	286.00	11.30
75	D +Rtin	female	56	12	518.00	322.00	11.80
76	D +Rtin	female	45	11	423.00	328.00	10.10
77	D +Rtin	male	44	6	435.00	236.00	10.50
78	D +Rtin	male	68	4	356.00	317.00	11.00
79	D +Rtin	female	60	15	366.00	343.00	10.80
80	D +Rtin	male	42	3	377.00	142.00	12.10
81	D +Rtin	female	53	12	356.00	347.00	11.60
82	D +Rtin	male	54	18	328.00	266.00	11.00

83	D +Rtin	female	59	14	344.00	258.00	12.60
84	D +Rtin	male	46	7	380.00	243.00	11.00
85	D +Rtin	female	55	12	265.00	296.00	10.90
86	D +Rtin	male	43	3	290.00	265.00	11.00
87	D +Rtin	female	42	5	310.00	264.00	10.80
88	D +Rtin	male	52	8	456.00	377.00	11.20
89	D +Rtin	male	45	6	476.00	218.00	11.10
90	D +Rtin	female	61	18	367.00	256.00	12.30
91	D +Rtin	female	60	21	360.00	212.00	11.50
92	D +Rtin	male	64	19	356.00	243.00	11.90
93	D +Rtin	male	45	6	340.00	286.00	10.30
94	D +Rtin	female	50	12	367.00	327.00	11.50
95	D +Rtin	male	40	3	380.00	318.00	10.80
96	D +Rtin	female	44	3	356.00	256.00	10.90
97	D +Rtin	female	58	2	356.00	244.00	12.30
98	D +Rtin	male	69	25	410.00	142.00	11.40
99	D +Rtin	female	56	16	421.00	312.00	11.90
100	D +Rtin	male	44	7	269.00	150.00	12.20
101	Control	female	46	----	360.00	160.00	11.00
102	Control	male	52	----	335.00	343.00	10.00
103	Control	male	65	----	320.00	396.00	10.10
104	Control	male	49	----	393.00	165.00	8.80
105	Control	female	61	----	298.00	364.00	9.10
106	Control	female	57	----	266.00	247.00	10.80
107	Control	male	56	----	293.00	301.00	9.00
108	Control	male	64	----	312.00	360.00	9.30
109	Control	male	44	----	370.00	154.00	8.20
110	Control	male	44	----	278.00	226.00	8.80
111	Control	female	56	----	276.00	143.00	10.50
112	Control	male	54	----	190.00	162.00	10.10

113	Control	male	57	----	230.00	247.00	9.40
114	Control	female	45	----	267.00	301.00	9.00
115	Control	male	53	----	327.00	246.00	10.30
116	Control	male	47	----	334.00	150.00	9.00
117	Control	male	62	----	323.00	260.00	10.10
118	Control	male	57	----	210.00	258.00	10.00
119	Control	male	55	----	255.00	224.00	9.90
120	Control	male	48	----	290.00	199.00	10.20
121	Control	female	67	----	188.00	165.00	9.80
122	Control	male	65	----	211.00	212.00	11.00
123	Control	female	64	----	214.00	327.00	9.90
124	Control	male	69	----	254.00	180.00	10.10
125	Control	male	56	----	268.00	156.00	10.10
126	Control	female	54	----	290.00	180.00	10.20
127	Control	male	47	----	220.00	188.00	11.00
128	Control	male	48	----	284.00	233.00	11.90
129	Control	male	50	----	256.00	188.00	11.20
130	Control	female	42	----	342.00	195.00	10.80
131	Control	female	53	----	356.00	323.00	11.00
132	Control	male	45	----	290.00	347.00	10.90
133	Control	male	59	----	190.00	328.00	11.10
134	Control	male	46	----	245.00	267.00	10.30
135	Control	male	55	----	320.00	234.00	10.10
136	Control	female	43	----	280.00	357.00	10.80
137	Control	male	42	----	246.00	321.00	9.20
138	Control	male	52	----	266.00	326.00	9.30
139	Control	female	45	----	210.00	324.00	9.10
140	Control	male	62	----	265.00	155.00	9.80
141	Control	male	60	----	250.00	183.00	10.40
142	Control	male	64	----	278.00	422.00	11.10

143	Control	male	55	----	290.00	247.00	10.80
144	Control	male	50	----	245.00	301.00	9.60
145	Control	male	40	----	248.00	266.00	10.10
146	Control	female	46	----	298.00	250.00	11.00
147	Control	male	55	----	277.00	360.00	9.20
148	Control	female	69	----	295.00	258.00	11.00
149	Control	male	46	----	345.00	243.00	9.90
150	Control	male	44	----	265.00	316.00	11.20

**** D +Rtin =Diabetic with Retinopathy