Sudan University of Sciences and Technology

College of Graduate Studies

Estimation of Platelet Count and Indices in Type2 Diabetus mellitus Patients in Khartoum State

تقدير الصفائح الدموية ومعاملاتها في مرضى السكري من النوع الثاني في ولاية الخرطوم

A Thesis Submitted for partial fulfillment of the requirements of M.Sc. Degree in Medical laboratory sciences (Hematology and immunohematology)

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الآيـة

بسم الله الرحمن الرحيم

قال تعآلي:

"هُوَ الَّذِي جَعَلَ الشَمْسَ ضِيَآءً والقَمْرَ نُوراً وقَدَّرَهُ مَنَازِلَ لِتَعْلَمُواْ عَدَدَ السِّنِينَ والحِسَابَ

مَاخَلَقَ الله ذُلِكَ إِلَّا بِالحَقِّ يُفَصِّلُ الايَاتَ لِقَومٍ يَعْلَمُونَ"

صدق الله العظيم

سورة يونس الآية (5)

DEDICATION

To my … ***Father and Mother***

Who weaves me my happiness with strings from their merciful heart

To my … ***Brothers & Sisters***

Whose love flows in my veins, and my heart always remembers them

To my… ***best friends Saba Salah &NajlaKhalifa & Tasneem saif***

Who reworded to me their knowledge simply

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In the name of Allah the most merciful, the most compassionate all praise is to Allah, the lord of world, and prayers and peace be upon Mohammed his servant and messenger.

I thank Almighty God for giving me the courage and the determination as well as guidance in conducting this research study, despite all difficulties.

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Abstract

Dabetus Mellius is the main health problem worldwide with estimated 4.6 million death in 2011, diabetic patients are suffering from complication that with the long term could lead to death, one of the most important complication is the hematological changes.

This was cross sectional study that conducted in Khartoum state during the period between April to November 2019 in antalia medical center that aimed to measure platelet count and platelet indices in type 2 diabetic patients. This study is conducted on 100 subject 50 case and 50 controls. 2.5 ml of venous blood samples were collected in EDTA anticoagulant containers, blood samples were analyzed by sysmex XP-300 hematological analyzer and mindary to mesure Hb A1c. Data obtained were analyzed statistically by SPSS version 20 using independent T test and personal correlation. Study showed the following result: there is no significant differences in platelet count (case:288X10^9,control:296 X10^9) , MPV (case:9.2,control 9.4fl), PLCR was significantly increased in diabetic patients compared with control .the mean of case is(24.3%) and the mean of control (21.1%) with p value=(0.02) , (PDW) showed significant difference in case the mean is (13.6fl) compared with control (11.3fl)with P value =(0.00). PCT (case:0.26, control 0.27%) when compared the diabetics to non- diabetics, with P.value 0.7 , 0.2 and 0.4, respectively. The study concluded that P-LCR and PDW can be used as indicators for detection of diabetes type 2 complications.

المستخلص

مرض السكري من المشكلات الصحية الرئيسية على مستوى العالم وقد تسبب في 4.6 مليون حالة وفاة في عام 2011 .مرضى السكري يعانون العديد من المضاعفات التي قد تؤدي الى الوفاة مع مرور الوقت واحد اهم تلك المضاعفات هي التغييرات الدموية .هذه دراسة تحليلية اجريت في ولاية الخرطوم في مركز انطاليا الطبي في الفترة بين ابريل ونوفمبر في العام 2019 تهدف لقياس كمية الصفائح الدموية ومعاملاتها في مرضى السكري النوع الثاني .

اجريت هذه الدراسة على 50 مريض بالسكري النوع الثاني و50 من الاصحاء كفئة ضابطة . اخذت 2.5 مل من الدم الوريدي في وعاء يحتوي على مانع التخثر ايثلين ثنائي الامين رباعي حمض الخليك لتحليل الدم الكامل وحللت العينات باستخدام جهاز التحليل الالي سيسمك 300 -xp وجهاز ميندري لقياس السكر التراكمي . البيانات الناتجة حللت احصائيا باستخدام برنامج الخدم الاحصائية للعلوم المجتمعية النسخة العشرين وباستخدام الاختبار الفرضي المستقل والمقارنة الشخصية . وقد اظهرت الدراسة النتائج التالية :

اثبتت الدراسة وجود اختلاف في نسبة الصفائح الدموية الكبيرة بين المصابين 24.3 % والفئة الضابطة 21.1% مع وجود قيمة احصائية 0.02 كما اوضحت ان هنالك اختلاف في حجم الصفائح الدموية في المصابين بمتوسط 13.6و الفئة الضابطة بمتوسط 11.3 مع قيمة احصائية0.00 .

لا يوجد اختلاف في متوسط الصفائح الدموية بين المرضى 288×10^9 وبين الفئة الضابطة 296×10^9 كما لايوجد اختلاف في نطاق توزيع الصفائح الدموية 9.2 في المرضى و 9.2في الفئة الضابطة. وتركيز الصفائح الدموية الكبيرة بين المصابين 0.26 %وبين الفئة الضابطة 0.27 % مع قيمة احصائية 0.4 , 0.2 , 0.7 على التوالي

هذه الدراسه استنتجت ان الفرق في نسبة الصفائح الدمويه الكبيرة وحجم الصفائح الدموية المكدسة يمكن استخدامها كمؤشر للكشف عن مضاعفات مرض السكري النوع الثاني .

List of Contents

List of Tables

Lists of Figures

Lists of Abbreviation

Symbols	Meaning
ADA	American Diabetes Association
AGEs	advanced glycation end-products
BM	bone marrow
CFU-MK	megakaryocyte colony-forming unit
CSII	Continuous subcutaneous insulin infusion
Cx	Connexins
DME	Diabetic macular edema
DR	Diabetic retinopathy
FPG	Fasting Plasma Glucose
GDM	Gestational diabetes
GP	Glycoproteins
HLA	human leukocyte antigen
HPA	human platelet antigens
IDDM	insulin dependent diabetes mellitus
MPV	mean platelet volume
NIDDM	non-insulin dependent diabetes mellitus
OGTT	Oral Glucose Tolerance Test
OS	oxidative stress
PCT	Plateletcrit
PDW	Platelet distribution width
PLCR	Platelet large cell ratio
Plt	Platelets
SCOCS	surface-connected open canalecular system
TPO	Thrombopoietin
VEGF	vascular indothelial growth factor
vWF	Vonwillebrand factor

List of Appendices

CHAPTER I

INTRODUCTION

CHAPTER 1

1-INTRODUCTION

1.1 Introduction

Normal homeostasis is an amazing and complex process that keeps blood fluid in the circulation and then when an injury occur produce a clot to stop the bleeding ,keeps the clot confined to the site of injury ,and finally dissolves the clot and the wound heals. Thus haemostatic system represents delicate balance between pro-coagulant and anticoagulant mechanisms allied to a process for fibrinolysis, (Hoffbrand *etal .,2006 ;Rodak,etal*.,2012). The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels. Platelets are produced in bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. Platelets are extremely small and discoid 3.0×0.5 mm in diameter, with mean volume (7-11 fl). The normal platelet count is approximately 250×10^9 cell (range $150\text{-}400\times10^9$ cell/l) and the normal platelet life span is 7-10 days (Hoffbrand, 2006).

Haemostatic disorder associated with platelet counts are classified in two type less than the reference range is thrombocytopenia and greater than normal range thrombocytosis.

Haemostatic disorder involving quantitative(ex: chronic myeloproliferative disorder or reactive or postoperative or neoplasm) or qualitative (ex: decrease production or increased loss or destruction) platelet abnormalities can be either inherited or acquired .in some cases ,such as bernared-soulier syndrome ,thrombocytopenia and thrombocytopathy occur together (Turgeon, M L, 1993).

The platelet is responsible for initiation of the haemostatic mechanisms that repair injury to the vascular endothelium. The four major platelet functions include platelet adherence, platelet activation and secretion, platelet aggregation, interaction with coagulation factor (Deutsh and Tomer, 2006).

Beside platelet count found other parameters such as mean platelet volume (MPV) which is a machine –calculated measurement of the average size of platelet, it's a marker of platelet function and activation,is measured as MPV by hematology analyzers,(Kodiatte,2012).

its found that MPV increased in state of platelet destruction (ex: inflammatory bowel disease). Platelet distribution width (PDW) is a simple practical and specific marker of platelet activation of coagulation ,since it does not increase during simple platelet swelling. Plateletcrit (PCT) is

an effective screening tool for detecting platelet quantitative abnormality. Platelet large cell ratio (PLCR) is a blood test that measures the average number of platelet in blood.(j.keith fisher and M.D, 2019).(ex: thromboembolic ischemic event).

Diabetes mellitus is actually a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Classified into two broad categories: type 1, insulin dependent diabetes mellitus (IDDM); and type2, non-insulin dependent diabetes mellitus (NIDDM) (Bishop, *etal.* 2013) .

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

Diabetic patient have an increased risk of developing micro and macro-vascular disease, and platelet may be involved as a causative agent with respect to altered platelet morphology and function (kodiatte,2012). The increased platelet activity is emphasized to play a role in the development of vascular complication of this metabolic disorder.

1-2-Rationale:

Diabetes Mellitus (DM) is the main health problem worldwide with estimated 4.6 million death in 2011,The number of platelets with type 2 diabetes mellitus (DM) is increasing in every country, with 80% of people with DM living in low and middle-income countries .

Prevalence of diabetes rise from 9.3%in 2010 to 10.6% in four states in Sudan (Gadarif, River Nile, Gazera and Northern) (Balla ,*et al*., 2014). There are few studies about evaluation of platelets indices among diabetic patients were performed. The aim of our study is to determine if platelets are activated in diabetes by measuring platelets indices in the diabetics compared to the non-diabetics.

The main hemostatic complicatiom of DM is atherosclerosis, diabetic retinopathy and nephropathy.

Platelet indices such as P-LCR, PDW considered as early indicator to predict these complications.

This study was conducted to measure platelet count and indices in type 2 diabetic patients.

1-3- Objectives:

1-3-1- General Objectives

To estimate the platelets count and indices among type 2 diabetic patients in Khartoum state.

1-3-2- Specific Objectives

-To measure platelets count and indices in type two diabetic patients and non-diabetic individual by hematology analyzer

-To measure HbA1c in case.

-To compare between platelets count and platelets indices among case and control.

-To correlate platelets count, indices among case and control group according toHbA1c.

CHAPTER II

LITERATURE REVIEW

CHAPTER II

2-LITERATURE REVIEW

2. 1 Normal Homeostasis

Homeostasis is physiological process that stops bleeding at the site of an injury while maintaining normal blood flow elsewhere in the circulation. Blood loss is stopped by formation of a haemostatic plug .The endothelium in blood vessels maintains an anticoagulant surface that serves to maintain blood in its fluid state, but if the blood vessel is damaged component of the subendothelial matrix are exposed to the blood. Several of these components activate the two main processes of homeostasis to initiate formation of a blood clot, composed primarily of platelets and fibrin .This process is tightly regulated such that it is activated within seconds of an injury but must remain localized to the site of injury (Hoffbrand *etal*., 2006).

There are two main components of homeostasis. Primary homeostasis refers to platelet aggregation and platelet plug formation. Platelets are activated in a multifaceted process, and as a result, they adhere to the site of injury and to each other, plugging the injury. Secondary homeostasis refers to the deposition of insoluble fibrin, which is generated by the proteolytic coagulation cascade. This insoluble fibrin forms a mesh that is incorporated into and around the platelet plug .This mesh serves to strength and stabilizes the blood clot. These two processes happen simultaneously and are mechanistically intertwined. The fibrinolysis pathway also plays a significant role in the last few decades. Multiple anticoagulant mechanisms regulate and control these systems to maintain blood fluidity in the absence of injury and generate a clot that is proportional to the injury .the proper balance between pro-coagulant systems and anticoagulant systems is critical for proper homeostasis and the avoidance of pathological bleeding or thrombosis (Deice and Lewis, 2012).

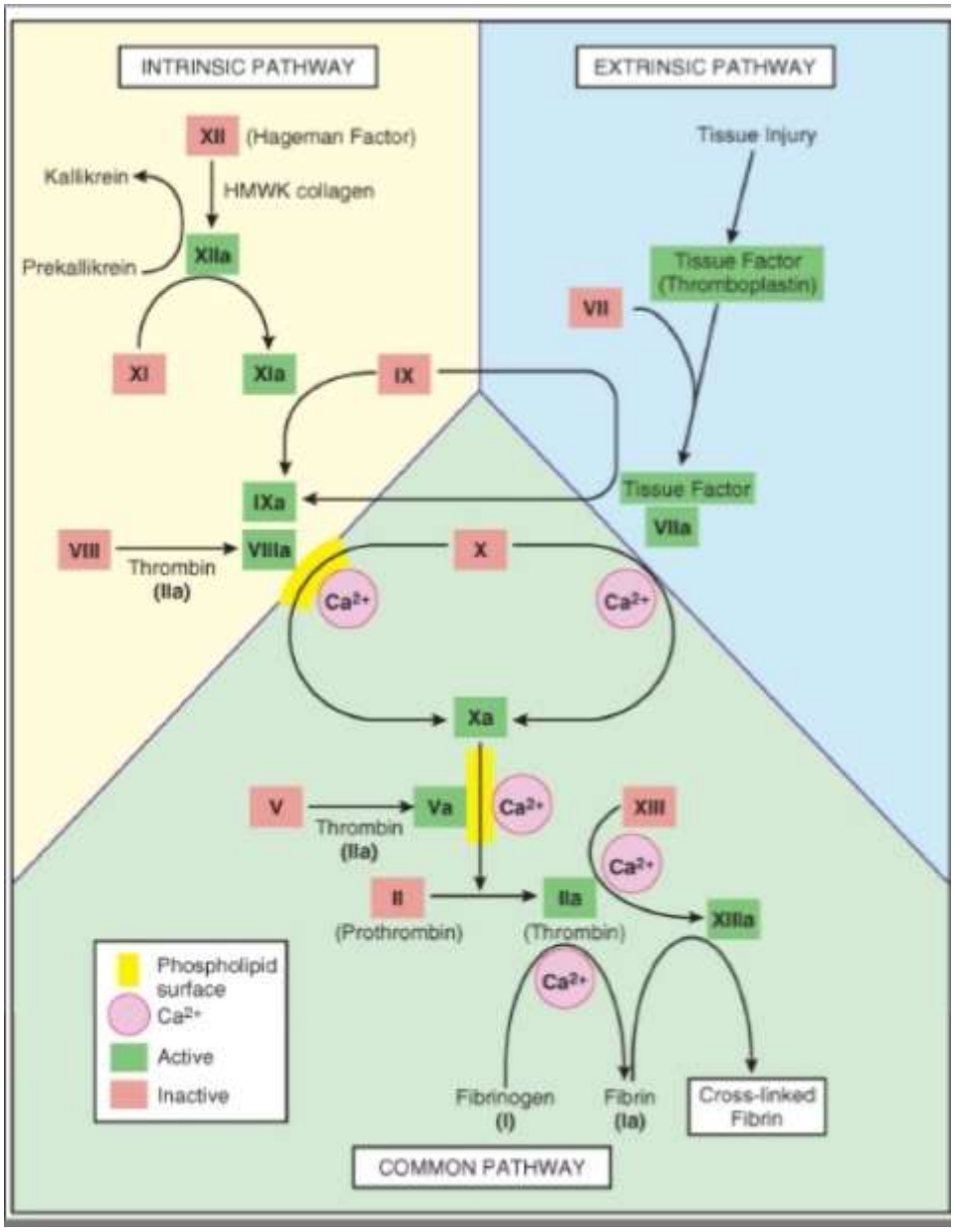

Figure2.1: Coagulation cascade

2-2 Platelets

Are small fragments of cytoplasm derived from megakaryocyte. On average, they are 1.5-3.5µm in diameter but may be larger in some disease states. They do not contain a nucleus and are bound by a typical lipid bilayer membrane. The outer membrane lies the marginal band of microtubules, which maintain the shape of the platelet and depolymerize when aggregation begins. The central cytoplasm is dominated by three type of platelet granules: the dense granules, α granules and lysosomal granules. Finally there exist the dense tubular system and the canalicular membrane system; the latter communicates with the exterior. (Deice and Lewis, 2012).

Platelet contains three morphologically distinct types of storage granules; Alpha granule, dense granules, lysosomes containing acid hydrolyses (Machlus, Thon, 2014).

2-2-1-Platelet production:

It is a process by which platelet produce known as megakaryopoiesis or thrombopoiesis is a process of development of megakaryocyte and platelet within the bone marrow (BM)(Powrit, *etal*.,2011).

The primary regulator of platelet production is thrombopoietin (TPO) it is protein produced by the liver with molecular weight of about 33kda,it is increased in thrombocytopenia and thrombocythemia. IL-6 and IL-11 also play role in generation of megakaryocyte colony-forming unit (CFU-MK).CFU-MK are a diploid cell population, in which DNA synthesis and nuclear division (karyoinesis) is followed by cell division (cytokines).

CFU-MK undergoes further maturation to megakaryoblasts (Bain, *etal*, 2011).

Four stages of megakaryocyte can be identified in Romanowsky stain BM smears, these are:

Megakaryoblast:

Proliferative cells with single, large and oval nucleus, not generally recognizable in normal BM. Have nucleoli, some degree of nuclear lobulation and azurophilic cytoplasmic granules with 2-6µmindiameter (Bain *etal*. 2010)(Kawthakar, 2013).

Promegakaryocyte:

Have strongly basophilic cytoplasm and very high nucleocytoplsmic ratio. It is larger than megakaryoblast and have 15-30µmin diameter, may contain azurophilic granules and have lobulated shape nucleus (Bainetal, 2010; Kawthalkar, 2013).

Granular Megakaryocyte:

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

Mature megakaryocyte:

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

Platelet:

Are small fragments of megakaryocyte cytoplasm with an average volume of 7-8fl. Have a diameter of 2-3micro meter .they have an irregular outline, stain light blue and contain a number of small azurophilic granules. The normal range for platelet count in peripheral blood is about $150\text{-}450\times10^9$/I. the life span of normal platelet is 7-10 days (Porwit, *etal*.,2011).

2-2-2-Platelet Structure:

Platelet divided into three regions; peripheral zone, sol-gel zone, and organelle zone (Hoffbrand *etal*, 2006).

Peripheral Zone:

The major structural elements of the plt peripheral zone are the cell surface and channels of the surface-connected open canalecular system(SCOCS). The peripheral zone is made up of the their structural domains :the exterior code, the unit membrane, the sub-membrane.

The exterior code is glycocalyx covering the outer surface of platelets. Many different glycol proteins have been defined by on the exterior coat, including glycoproteins(GP);

GP1a11a: allow platelet adhesion to collagen.

GP11b/111a: allow platelet to aggregate.

GP1b/1x: initial adhesion of plt to vWF and thrombin.

GPv: allow plt adhesion.

GP1v: plt adhesion to collagen.

GPV1: activation of collagen(Hoffbrand ,*etal*.,2006).

The sol-gel Zone:

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

approximately 30-50% of the total platelet protein. Masses as well as discrete particles of glycogen are distributed in the sol-gel matrix (Hoffbrand ,*etal*.,2006).

The organelle zone:

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

Platelets contain four main types of storage granules; dense granules, α-granules, lysosomes and peroxisomes, and several mitochondria. There are between five and nine dense granules in platelets,which contain high levels of ADP (increase adhesive of plts), ATP, polyphosphates, 5-hydroxtryptamine(serotonin)promote vasoconestruction and ca^{+2}. There are approximately 80 α-granules per platelet and this contain a rich diversity of protein and membrane receptors that support hemostasis, vascular repair, inflammation and host defence. Major component of α granules include clotting factor such as fibrinogen, VWF, factor(F), protein sand tissue factor pathway inhibitor (TFPI),the chemokines stromal cell derived factor/1 alpha (SDF/1α), plt factor 4(PF4) and β thromboglobiolin. The growth factor(PDGF) and vascular endothelial growth factor (VEGF) A and C. plt α-granules also express key transmembrane proteins including intigrin α 2bβ3, p/selictin(CD62) and CD40L that are only expressed on activated cells. Platelets are enriched in signaling and cytoskeletal proteins that enable to aggregate to withstand the high shear forces of the vasculature. Platelets have a network of intracellular membranes known as the dense tubular system that release intracellular ca^{+2} in response to the second messenger inositol 1,4,5-trisphosphate (1P3). They also have a network of investigations of the surface membrane, known as the surface-connected canalicular system (SCCS), which increase the surface area of the plasma membrane during platelet spreading. The SSCS also gives rise to membrane tethers that play a vital role in supporting adhesion (Hoffbrand, *etal*.,2016).

In addition to containing substantial quantities of the contractile proteins, including actomyosin (thrombosthenin), myosin, and filamin, the cytoplasm of the platelet contains glycogen and enzymes of the glycolytic and hexose pathways. Energy for metabolic activates and cellular contraction is derived fromaerobic metabolism in the mitochondria and anaerobic glycolysis utilizing glycogen stores. The platelet is very high-energy cell with a metabolic rate 10 times that of an erythrocyte. Based on energy availability and endogenous constituents, the platelet is

effectively equipped to fulfill the role of protecting the body against vascular trauma (Turgeon,2012).

2-2-3-Platelets antigens:

Several platelet surface proteins important antigens in platelet-specific autoimmunity and they have been termed human platelet antigens (HPA). In most cases, two different alleles exist, termed α or β alleles (e.g.HPA-1A). platelet also express ABO and human leukocyte antigen (HLA) class 1 but not class 11 antigens (Hoffbrand ,*etal*.,2006).

2-2-4- Platelet function:

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

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

Adhesion:

This means binding of platelet to non_ endothelial surface, particulary sub-endothelial which uncovered following vascular injury. Vonwillebrand factor (vwf) mediates adhesion of platelet to sub-endothelium via glycoprotein Ib (Gp1b) on the surface of platelet.

Congenital absence of glycoprotein receptor Gp1b (Bernared-souler syndrome) or von willebrand in plasma (von willebrands disease) cause defective platelet adhesion and bleeding disorder.Platelet normally circulatesas around to oval disc-like structures. Wth actvaton, platelets undergo shape change. i.e. they become more spherical and form pseudopodia. Ths shape change is due to recognztion of microtubules and contracton of actomysin of microfilaments (Kawthalkar, 2013)

Relase Reaction:

Immediately after adhesion and shape change, process of release reaction or secretion begins. In this process, contents of platelets organelles and released to the exterior. ADP released from dense granules promotes Plts aggregations. Plts factor 4 released from alpha granules neutralizes the anticoagulant activity of heparin while Plts-derived growth factor stimulate proliferation of vascular smooth muscle cell and skin fibroblasts and play a role in wound healing.

Activated platelet also synthesize and secrete thromboxane A2(TxA2). Platelet agonist such as ADP, epinephrine, low –dose thrombin bind to their specific receptors on plt surface, and activate phospholipase enzymes, which release arachidonic acid from membrane phosphor lipids. Arachidonic acid is converted to cyclic endo peroxides by the enzyme cycloxygenase. These are

then converted to thromboxane A2 by thromboxane synthetase. TxA2 has a very short half life and is degraded into thromboxane B2 which is biologically inactive. TxA2 also induces aggregation of other plts and local vasoconstriction (Kawchalkar,2013).

Platelet Aggregation:

This may be defined as binding of platelet to each other. ADP released from plt or from damaged cells binds to specific receptors on plt surface. This is cause inhabitation of adenylcyclase and reduction in the level of cyclic AMP in plts. A configurationally change in the membrane occur so that receptor for fibrinogen (Gp11b and Gp111a) become exposed on the surface. Binding of fibrinogen molecules to gp11b/ 111a receptors on adjacent plt causes plt aggregation. The activated plt release ADP and TxA2 leading to the formation of a platelet plug. Also thrombine causes plt aggregation (Kawthalker,2013).

2-2-5- Platelet Indices:

Are group of platelet parameter determine together with automated complete blood counter, are biomarkers of platelet activation. They allow extensive clinical investigations focusing on the diagnostic and prognostic values in a variety of settings without bringing extra cost (Budak *etal, 2016:* Bashir *etal, 2017*).

Platelet indices are potentially useful markers for the early diagnosis of thromboembolic diseases. Platelet indices is : mean platelet volume (MPV) ,platelet distribution width (PDW) and platelet crit (PCT) .An increase in both mean platelet volume (MPV) and platelet disturbing width (PDW)due to platelet activation , was suggested due to :platelet swelling ,pseudopodia formation .MPV and PDW are simple platelet indices ,which increase during platelet activation (Machlus *etal,*2014).

Mean Platelets Volume:

The mean platelets (MPV) is the calculated measurement of the average size of platelet found in blood .The normal range is given as 7.5-10.4fl it is measure of thrombocyte volume ,is determined directly by analyzing the platelets distribution curve .when platelets production is decreased, young platelets become bigger and more active and MPV level is increased. Low MPV is associated with low grade inflammation (Rheumatoid arthritis), MPV is increased in chronic disorders (Budak *etal*,2016). MPV reflects the average size of platelets present in a persons blood sample. It is a marker indicating subclinical platelet activation & is an independent risk factor for various vascular episodes such as acute myocardial infarction, coronary artery

disease cerebral ischemia & peripheral artery disease. Increased MPV has been found to be associated with increased risk of retinopathy in diabetic patients.

Platelet Distribution Width:

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

Platelet Large Cell Ratio:

Platelet large cell ratio (P-LCR) is an indicator of circulating larger platelets(more than 12fl) which is presented as percentage, the normal range is 15-35%, use to monitor platelet activity (Budak, *etal*,2016) .

Platelet Large Cell Concentration:

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

Plateletcrit:

Plateletcrit (PCT) is the volume occupied by platelet in blood as percentage and calculated according to the formula PCT=PLT count×MPV÷10000. Normal range is 0.22-0.24%. Higher with acute colitis and cholecystitis (Budak ,*etal*,2016). PCT is a measure of total platelet mass (Budak, *etal*,2016).

PDW &PCT may have an important role on vascular events, such as atherosclerosis &thrombosis (Budak, *etal*,2016).

2-3- Diabetes Mellitus:

Diabetes mellitus is a heterogeneous metabolic disorder characterized by the presence of hyperglycemia due to impairment of insulin secretion, defective insulin action or both (Punthakee, Goldenberg and Katz, 2018). DM is probably one of the oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 years ago. In 1936, the distinction between type 1 and type 2 DM was clearly made (Ahmed AM, 2002). Type2 DM was first described as a component of metabolic syndrome in1988 (PatlakM, 2002).

The worldwide prevalence of diabetes has continued to increase dramatically. Globally, as of 2011, an estimated 366 million people had DM, with type 2 making up about 90% of the cases (Jumpup, 2007; Chen L *et al*, 2014). The number of people with type 2 DM is increasing in every country with 80% of people with DM living in low- and middle-income countries. Literature search has shown that there are few data available on the prevalence of type 2 DM in Africa as a whole. Studies examining data trends within Africa point to evidence of a dramatic increase in prevalence in both rural and urban setting, and affecting both gender proportionally (DMICC, 2014). According to the World Fact book report in 2008, in Africa the prevalence of diabetes mellitus was 3.2%, and 40,895 persons (2.0%) was in Ethiopia (Central Intelligence Agency, 2008).

2-3-1- Classification of Diabetes

Diabetes mellitus can be broadly classified into 2 categories: type 1 diabetes and type 2 diabetes. Type 1 diabetes is primarily a result of pancreatic beta cell destruction with consequent insulin deficiency, which is prone to ketoacidosis (MaitraA& Abbas AK 2005). This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the b-cells of the pancreas. Markers of the immune destruction of the b-cell include islet cell auto-antibodies, auto-antibodies to insulin, auto-antibodies to GAD (GAD65), and auto-antibodies to the tyrosine phosphates IA-2 and IA-2b (Diagnosis and Classification of Diabetes Mellitus, 2013).

This form includes cases due to an autoimmune process and those for which the etiology of beta cell destruction is unknown. Type 2 diabetes may range from predominant insulin resistance with relative insulin deficiency to a predominant secretary defect with insulin resistance. Ketosis is not as common (Wild. S, *et a*l, 2004). Gestational diabetes (GDM) refers to glucose intolerance with onset or first recognition during pregnancy (Guidance for Industry, Diabetes Mellitus, 2008).

2-3-2- Clinical Features Of Diabetes Mellitus

Most of the symptoms are similar in both types of diabetes but they vary in their degree and develop more rapidly in type 1 diabetes and more typical (Baynest H., 2015).

Clinical Features Of Type I Diabetes

Some of the symptoms include weight loss, polyurea, polydipsia, polyphagia, constipation fatigue, cramps, blurred vision, and candidacies (Bearse MA Jr *et al*, 2004). Long lasting type 1 DM patients may susceptible to micro-vascular complications; (Hove MN *et al*, 2004) and macro-vascular disease (coronary artery, heart, and peripheral vascular diseases) (Pittas AG, 2009).

Clinical Features Of Type II Diabetes

Most cases are diagnosed because of complications or incidentally. Carries a high risk of large vessel atherosclerosis commonly associated with hypertension, hyper-lipidaemia and obesity. Most patients with type 2 diabetes die from cardiovascular complications and end stage renal disease (Craig. ME, *et al*, 2009).

2-3-3- Complications of Diabetes Mellitus

It include diabetic retinopathy, macular edema and cataract

Diabetic Retinopathy

Diabetic retinopathy (DR) is damage to the eye's retina that occurs with long-term diabetes. Diabetic retinopathy is the most common cause of blindness in most of the countries. It is commonly seen in both type 1 (40%) and type 2 DM (20%). There are two types of diabetic retinopathy. They are Non-proliferative which develops first, Proliferative is the more advanced and severe form of the disease. In patients with T2DM, involvement of fovea by edema and hard exudates or ischemia is the most common cause of visual impairment (Atul.K. *et al*, 2011). Hyperglycemia and the increased duration of diabetes are the major risk factors for DR. Other risk factors include hypertension, hyperlipidemia, pregnancy, and micro-albuminuria (Mohamed. QG, *et al*, 2007). Symptoms of diabetic retinopathy appears only after the damage occurs to eyes which include- blurred vision and slow vision loss over time, floaters, shadows or missing areas of vision, trouble seeing at night. The vascular commitment is the most serious and common condition in DM. The factors for vascular damage of DM include poor glycemic control, lipoprotein abnormalities, hypertension, oxidative stress (OS), inflammation and advanced glycation end-products (AGEs). Retinopathy is characterized by increased vascular permeability, by vascular closure mediated by the formation of new blood vessels-neovascularization, on the retina and posterior surface of the vitreous (Liu DT *et al*, 2011; Bradley J 2007). Generally, neovascularization results from occlusion of fragile capillaries and frequently originate pre-retinal and vitreous hemorrhage in case of vitreous detachment. Much attention has been focused on the role of OS in the pathogenesis of diabetic complications. The

retina is highly susceptible to OS and the oxidation products are toxic to the microvascular walls and therefore results in diabetic microvascular damage. Diagnosis of retinopathy is based on finding the diagnostic signs of retinopathy on eye exams by fundoscopy (Abougalambou.SSI, *et al*, 2011*).*

Diabetic Macular Edema

Diabetic macular edema (DME) is the leading cause of visual loss in patients with non-proliferative diabetic retinopathy. DME is the consequence of accumulation of fluid in the retina after dysfunction of the blood retinal barrier. Breakdown in blood retinal barrier at the level of the perifoveal vessels results in edema (Abdollahi .A, *et al*, 2011).

Cataract

Cataract develops at an earlier age in diabetic patients, which is characterized by clouding of the eye lens. In cataract, the lens becomes opaque, reducing the amount of light reaching the retina. Connexins (Cx) are a family of proteins that forms hemichannels that communicate the cytoplasm with the extracellular space. Under oxidative stress conditions such as diabetes, it is possible that Cx oxidation will contribute to cataract formation. Neurotrophic corneal ulcers may develop in patients with DM (Salman. AG, *et al*, 2010).

2-3-4- Diagnosis of Diabetes Mellitus

The 1997 American Diabetes Association (ADA) recommendations for diagnosis of DM focus on Fasting Plasma Glucose (FPG), while WHO focuses on the Oral Glucose Tolerance Test (OGTT) (Gillett MJ, 2009).

Diagnosis of Both Types of Diabetes

Random Plasma Test

The simplest test and does not require fasting before taking the test.

If 200 or more than 200 mg/dl of blood glucose it probably indicates diabetes but has to be reconfirmed.

Fasting Plasma Glucose Test:

There should be eight hours fasting before taking this test. Blood glucose more than 126 mg/dl on two or more tests conducted on different days confirms a diabetes diagnosis (Gillett MJ, 2009).

Oral Glucose Tolerance Test (GTT):

When random plasma glucose test is 160-200 mg/dl and the fasting plasma test is 110-125 mg/dl, then this test is conducted (Guidance for Industry, Diabetes Mellitus, 2008)

This blood test evaluates body's response to glucose. This test requires fasting at least eight but not more than 16 hrs.

Fasting glucose level is determined, and then gives 75 gm of glucose, 100 gm for pregnant women. The blood is tested every 30 minutes to one hr for two or three hrs.

This test is normal if your glucose level at two hrs is less than 140 mg/dl. A fasting level of 126 mg/dl or greater and two hour glucose level of 200 mg/dl or Higher confirms a diabetes diagnosis (Gillett MJ, 2009).

Glycated Proteins

Proteins react spontaneously in blood with glucose to form glycated derivatives. The extent of glycation of proteins is controlled by the concentration of glucose in blood and by the number of reactive amino groups present in the protein that are accessible to glucose for reaction. All proteins with reactive sites can be glycated and the concentration of the glycated proteins that can be measured in blood is a marker for the fluctuation of blood glucose concentrations during a certain period. From a clinical diagnostic point glycated proteins with a longer life time in blood are of interest, since they reflect the exposure of these proteins to glucose for longer periods (Baynest H., 2015).

Glycated Hemoglobin

The life span of hemoglobin in vivo is 90 to120 days. During this time glycated hemoglobin A forms, being the ketoamine compound formed by combination of hemoglobin A and glucose. Several subfractions of glycated hemoglobin have been isolated. Of these, glycated hemoglobin A fraction HbA1c is of most interest serving as a retrospective indicator of the average glucose Concentration. HbA1c is recommended as an essential indicator for the monitoring of blood glucose control. The blood HbA1c$\geq$ 6.5% is considered as diabetes (Selvin E, *et al*, 2010).

Fructosamine Test

Albumin is the main component of plasma proteins. As albumin also contains free amino groups, non-enzymatic reaction with glucose in plasma occurs. Therefore glycated albumin can similarly serve as a marker to monitor blood glucose. Glycated albumin is usually taken to provide a

retrospective measure of average blood glucose concentration over a period of 1 to 3 weeks. Reference interval: 205- 285 micro mol/L (Baynest H., 2015).

2-3-5-Treatment of Diabetes Mellitus

The treatment for diabetes mainly involves the regulation of blood sugar levels and to prevent diabetic complications. Medicines, diet, and exercise are included in treatment. Lifestyle modifications and oral anti-diabetic medications are recommended for initial treatment of DM (Esteghamati A, *et al*, 2011). Banting and Macleod first discovered the insulin hormone. Insulin therapy is required for T1D because cells cannot produce insulin. Although cells produce insulin hormone in T2D but they do not respond normally to insulin. In such cases, insulin therapy helps cells to overcome the resistance to insulin. Continuous subcutaneous insulin infusion (CSII) is useful therapy for brittle T1D worldwide. The frequency of hypoglycemia was decreased and improved glycemic variability was achieved with CSII therapy, which is beneficial to pregnant women with diabetes (Higuchi C, *et al*, 2010).

2-4- Previous studies:

The result of study conducted in India by (kodiatte,*etal*, 2012) subjected on 300 diabetic and 300 non-diabetic to show MPV in type diabetes mellitus. Mean platelet count and MPV were higher in diabetic compared to non-diabetic and statistically significant.

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

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

study conducted in Sudan involved 90 diabetic and 100 non-diabetic to determine altered platelets morphological parameters in type2 diabetes mellitus. Showed that MPV and PDW higher in diabetic than non-diabetic patient with p.value 0.05 (statistically significant), platelet count was higher in diabetic than non-diabetic was but not statistically significant.

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

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

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

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

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

3-Materials and methods

3.1 Study design

This is an analytical cross-sectional study conducted In Antalia medical center. In Khartoum state in the period from January 2019 to October 2020

3.2 Study Population and Sample Size

One hundred individual were participated in this study and classified into two groups: 50 individual were type 2 DM, and 50 individual were non-diabetic as control group.

3.2-1-Inclusion Criteria

Diabetic type 2 patients.

3.2-2- Exclusion Criteria

Previous history of hypertension, renal problems, and cardiovascular diseases, haemostatic disorder and other diseases were excluded.

3.3- Ethical Consideration

The specimens and information that collected from the participants were under privacy and confidentially. The aim of the research was explained for the subjects under the study in simple language and they understood the research idea.

3.4-Tools of Data Collection

The data were collected by the direct interview through designed questionnaire to the patients, including age, gender, and history of disease.

3.-5- Sample collection and preparation

One hundred samples were collected from patients and healthy volunteers, 2.5 ml of venous blood was collected from each one using disposable syringes and sprit (70%alcohol) is use to sterilizing the area of collection, the blood is drawn in EDTA containers, measurement of platelets count and indices was determined within two hours after collection of blood sample.

3.6- Methods

3.6.1. Estimation of platelet count and indices

Automated Hematology Analyzer

Determination of platelet counts and indices by auto hematology analyzer (sysmex):

BC-3000 plus auto hematology analyzer is a three part auto hematology analyzer able to run 19 parameters per sample including : hemoglobin level , packed cell volume , red cell concentration, mean corpuscular hemoglobin , mean cell volume, mean corpuscular hemoglobin concentration, white blood cells. Platelets count, mean platelet volume, platelet distribution width, plateletcrit.

The cells are counted and sized by the electrical impedance method; this method is based on the measurement of changes in electrical current.

The poorly conductive blood cells are suspended in conductive diluents, the diluents is passed through an electric field created between two electrodes, the diluents passed through small hole. The passage of each particle through the hole shortly increase in impedance creates a pulse that can be measured, the number of pulse generated is indicative of the number of particles that traversed the aperture. Each pulse is amplified and compared to internal reference voltage channels.

Calibrated size discriminators to accept only pulses of certain amplitude delineate these channels. Thus, the pulses are sorted in to various size channels according to their amplitude (Operation manual, 2015; Turgeon, 2012).

Result: Normal range of platelet indices

Platelet count:

MPV: 7.2-11.7fl

PDW: 8.3-56.6%

PLCR: 15-35%

PCT: 0.22-0.24%

Quality Control

The reliability of this instrument and reagents is monitored by controls and calibrators using of control or blood or control materials the stability of the measured value is monitored over a certain period of time, and problems can be detected early or prevented.

Control of CBC

quality control for CBC after every 50samples batch seems to fulfill both the health ministry order (HMO) 1301\2007 And International organization for standardization ISO 15189:2013 recommendation. All quality instrument must work together to assure better patient results and every laboratory should design its own control plan that is appropriate for better quality achievement.

HbA1c

In the first reaction, the concentration of hemoglobin is measured at absorbance of fixed wavelength, and simultaneously the fructosyl dipeptides are generated from the N-terminus amino groups of the beta-chain of HbA1c by the reaction of protease . In the second reaction , the reaction of fructosyl peptide oxidase (FPOX) with fructosyl dipeptides, generated hydroperoxide allows 10-(carboxymethylaminocarbonyl)-3,7-bis (dimethylamino) phenothlazine sodium salt to develop a color in the presence of peroxidase The change in absorbance is measured for HbA1c determination. The combined assay results for hemoglobin and HbA1c are used by the system to calculate and express HbA1c% by using of mindary.

3.7. Data analysis

The data was computed and analyzed to obtain the platelet count and indices using statistic package for social science (spss) program version 20.

3.7.1. Statistical analysis

The statistical analysis was performed by using independent sample T-test and person correlation test.

CHAPTER IV

RESULTS

CHAPTER IV

4. RESULTS

4.1 .Description of study population

One hundred Sudanese subjects were enrolled in this study, 50 patients with type 2 diabetes and 50 healthy volunteers as control group. Were males(%) and were females(%).The mean age of patients was (54years), the mean of duration of the disease about 8.9 years and the patients mean of hemoglobin A1C were 9.5 .(Table 3.1). % the study is represent 50% cases (26 male, 24 female) and 50% control (26 male, 24 female). .

4.2. Platelet count and indices result.

Current study showed the following results: platelet count mean in cases is 288.8×10^9 and in control is296.9×10^9. MPV (fl) in case is 902 and in control is 9.4, PLCR n case is 24.3 and n control is21.1, PDW (%) in case is 13.6 and n control s 11.3, PCT (%) in case is 0.26 and in control is 0.27%.

The study is represent 52 (52%) male and 48(48%) female .26(26%) of patients were male and 24 (24%) of patients were female. 26(26%) of control were male and 24 (24%) of control were female

.

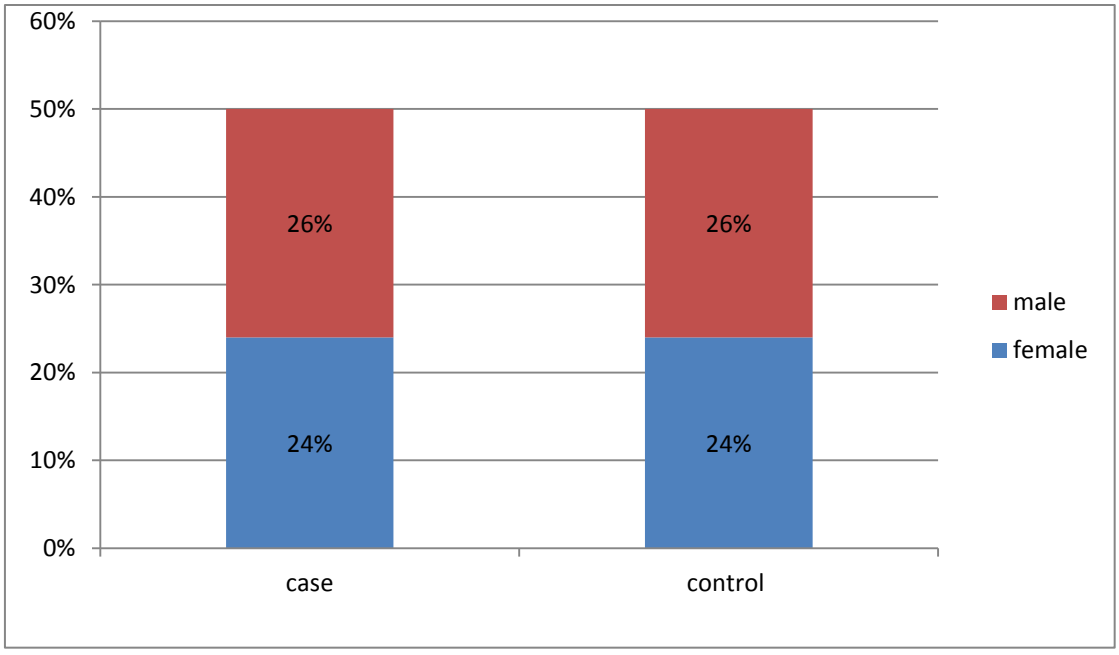

Figure 4-1: Gender distribution in study groups.

4.3. Comparison of platelet parameter between diabetic and non diabetic

The platelet profile investigate by CBC the result showed statistically insignificant difference in patient of type 2 diabetes in platelet count , platelet crit, (PCT) and mean platelet volume (MPV) when compared with control group. Platelet distribution width (PDW) and platelet large cell ratio (PLCR) of diabetic patients when compared with control group.

Table 1: Comparison mean of platelet profile between patients and control

Variable	Mean	p. value
Platelet count $\times 10^9$/l	Patient 288.8±113 Control 296.9±105	0.7
PDW%	Patient 13.6±2.5 Control 11.3±1.5	**0.00**
MPV fl	Patient 9.2±1.1 Control 9.4±0.8	0.2
PLCR	Patient 24.3±7.3 Control 21.1±6	**0.02**
PCT%	Patient 0.26±0.09 Control 0.27±0.08	0.4

p.value> 0.05 is statistically significant.

4.4. Correlation between platelet parameter and Hb.A1c

There was positive correlation between Hb.A1c and PLCR with p.value 0.01 and R 0.1, and PDW with p.value 0.07 and R 0.1

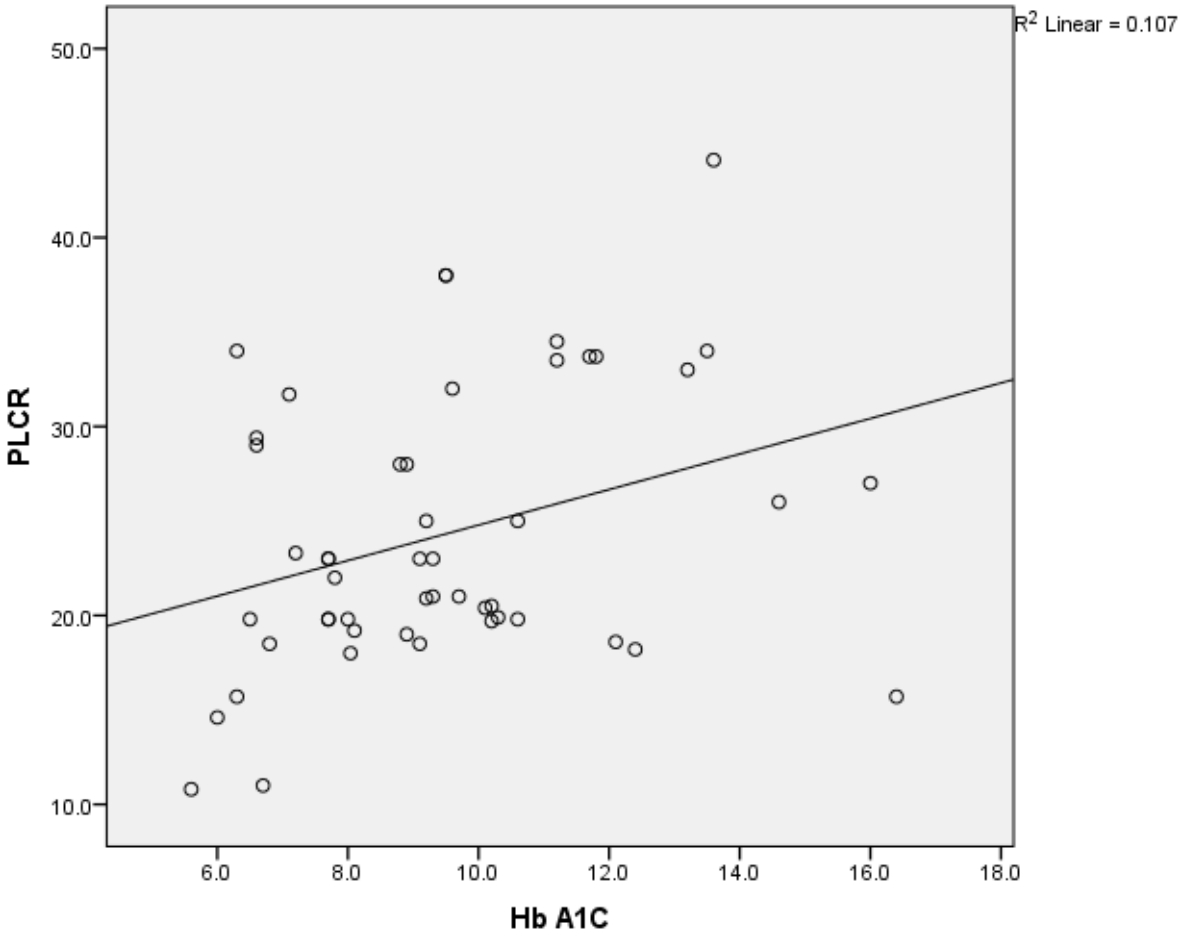

Figure 4-2: Correlation between HbA1c levels in diabetic patients and PLCR.

There is positive correlation between PLCR and HbA1c with p.value (0.01)

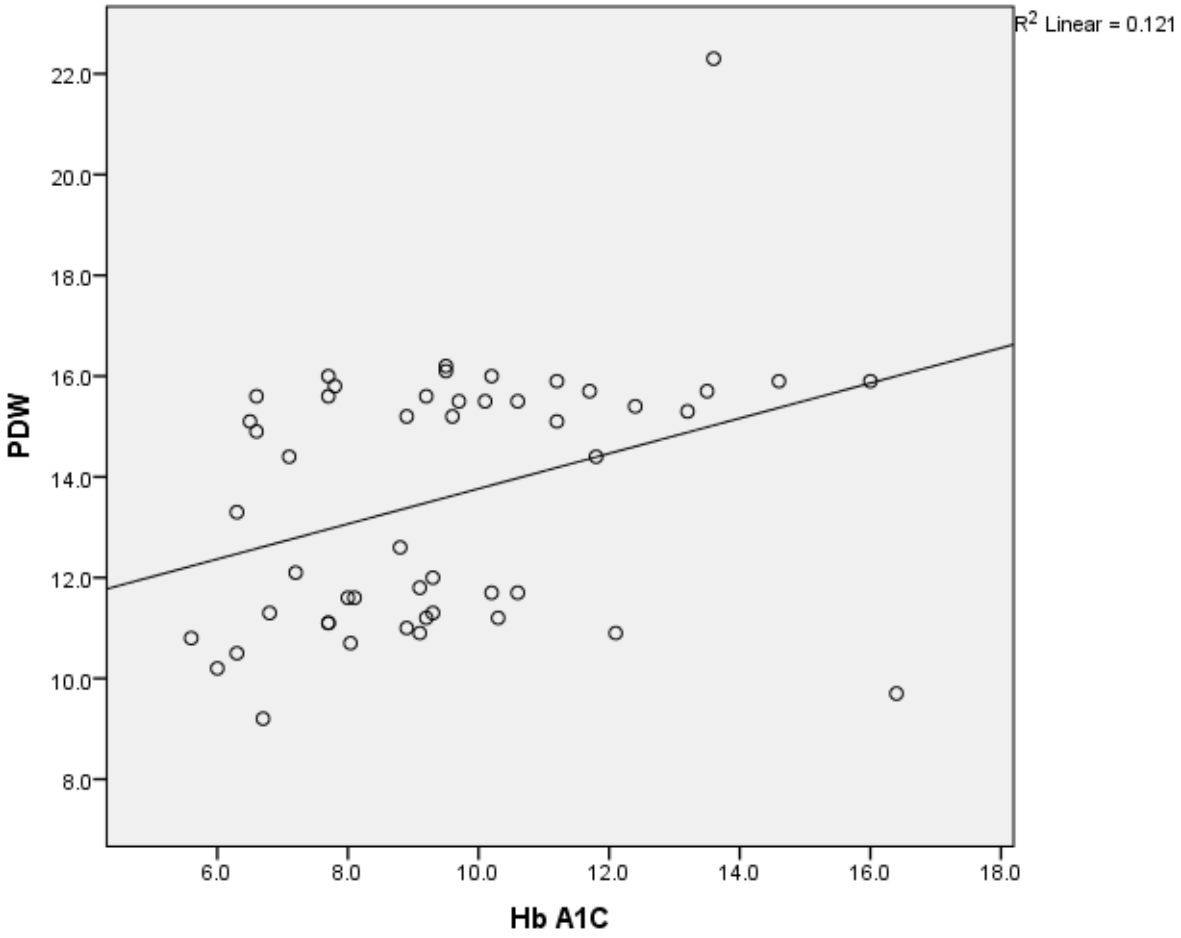

Figure 4-3: correlation between HbA1C levels in diabetic patients and PDW. PDW was higher .in diabetic patient, positive correlation P.value (0.07)

CHAPTER V

DISCUSSION, CONCULSIONS, RECOMMENDATIONS & APPENDICES

CHAPTER V

DISSCUSION, CONCULOSION, RECOMMENDATIONS

5.1 Discussion

This is an analytical cross sectional study that aims to measure platelet count and indices in diabetic type 2 patients. This study was conducted on hundred volunteers. 50 were diabetic (cases) and 50 were non-diabetic (control) to evaluate the effect of diabetes mellitus on platelet count and platelet indices. Platelet count and indices were measured by hematology analyzer.

In this study the mean platelet count was higher in diabetic than non-diabetic with p.value 0.7. The difference is statistically insignificant which agrees with study of Bayoumi(P-value(0.5)) in Sudan and Brown(P.value 0.5) in London. (Brown,*et al*, 1997) and (bayoumi ,*et al*, 2018).

MPV was not statistically significant in diabetic when compare to no-diabetic (P-value 0.2). This result agrees with a study conducted in India by Hassan and his colleagues (2016) the P.value was 0.5. and with bayoumi (2016) found P-value (0.05)

The study disagrees with other studies that found statistical significant in MPV like Jindal and etal the mean was (12.08)in case and (11.4) in control, with P-value 0.01 , (Jindal *et al*,2011) Alhadas and *et al* with P-value 0.015 , (Alhadas *et al* , 2016), (Citrik, *et al*, 2015)and (Elkhalifa, *et al*, 2016)with P-value (0.001).

P-LCR, PDW were significantly increased in diabetic when compare to non-diabetic patient P.value (0.02, 0.00) respectively. The result agree with study conducted in India by Shilpi, K. and Potekar that found incresd in PDW with p.value 0.003 in , and other study conducted in Turkey by Yilmaz and Yilmaz , their result show significantly increased of P-LCR (P.value 0.00), PDW(p.value 0.00) and MPV(p.value 0.00) in diabetic when compare with non-diabetic , but in this study the MPV at the normal range which show insignificant statistical changes.

Study in London conducted by Brown show that PCT insignificant which agree with this study that found the mean case value is 0.26 and control 0.27% and P-value 0.4.

Statistical evaluation was found by using student's unpaired T-test and person correlation test to compare between parameter.

This study correlates between HA1C and platelets count and indices .There was positive correlation between HbA1c and PLCR; the P.value was=0.01, regarding PDW. This study revealed a positive correlation with HbA1C,the p.value was 0.00.

There was negative correlation between HA1C and MPV P=0.2.and negative correlation with Plt count and PCT.

study conducted by kumara shilpi and R.M.Potekor found positive correlation between MPV, PDW and P-LCR with HbA1c with p.value(0.14).

Study conducted by Manoj ,etal show significantly high MPV with high HbA1c (0.001) in diabetic patients.

5.2 Conclusion:

In this study the P-LCR was showed significant change in case group.

There was also significant change in PDW when compared between diabetic patients and non-diabetic individuals, so the complete blood count is effective and not cost to monitor the diabetic health situation for management.

5.3 Recommendations

-Estimation of other hematological parameters in type 1, type2 and gestational diabetes, because we found some parameters were change in type2 DM.

-Study the association of complication and parameters change.

-Other studies including other blood cells parameters and coagulation studies in diabetic patients such as D-dimer to monitor the complication of disease and control it.

-Increasing the sample size to give accurate result.

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Appendix (1)

Reagents:

Diluents:

it is a kind of reliable isotonic diluents which can dilute white blood cells (WBC), red blood cells (RBC), platelet(PLT) and hemoglobin(HB), keep the shape of cells during test process, offer appropriate background value and clean WBC and RBC micro-aperture and tubes (Operation manule,2015).

Lyse:

Lyse is a new-type reagent without NaN3complex and cyanide which can dissolve RBC instantly with minimum ground substance complex, transform the membrane of WBC to diffuse cytoplasm, and then WBC shrinks to form membrane –bound nuclei, transform the hemoglobin to form hemo-compound which is suitable for the measurement in the condition of 540 n m wavelength and avoid the pollution caused by cyanide (Operation manual, 2015).

Appendix (2)

Questionnaire

Sudan University of Science and Technology

Collage of Graduates Studies

Department of Hematology and Immuno-hematology

Questionnaire About: Estimation of Platelet Count and Indices in Type 2 Diabetes millets Patients in Khartoum state.

Serial number:…….

Gender: Male ☐ Female ☐

Age:……years

History of platelet disease Yes No

Treatment: Antin-hyperglycemic agent Regulator

Duration of disease:……months

Result:

Platelet count	$\times 10^9$/L
PLCR	%
MPV	FL
PCT	%
PDW	Fl
HbA1C	%

Appendix(3)

Hematological analyzer instrument

Detergent:

Detergent contains the active enzyme which can dissolve the agglomerated protein in the WBC, RBC, cups and measurement circuit. Probe detergent contains effective oxide to dredge the stubbornly-(Operation manual, 2015).

Appendix (4)

Mindary BS-240