بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Alteration in Platelet Count and Platelets Indices in Type 2 Diabetes Mellitus in Sudanese Patients -Khartoum State

ومؤشرات الصفائح الدموية لدي مرضي السكري السودانيين من تعداد التغير في النوع 2- ولاية الخرطوم

A thesis submitted in partial fulfillment for degree of the requirements for the award of the degree of (M.SC) in medical laboratory science

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الآية

قال تعالى:

(إِنَّ الْمُتَّقِينَ فِي جَنَّاتٍ وَنَهَرٍ (54) فِي مَقْعَدِ صِدْقٍ عِندَ مَلِيكٍ مُّقْتَدِرٍ (55))

صدق الله العظيم

سورة القمر الآيتان ("54،55")

Dedication

I dedicate this research to

My father

My mother

My brothers

My friends and colleagues

And

Everyone who facilitated this work

Acknowledgement

First of all thanks for **Allah** Almighty for helping me to complete this work.

And thanks to my dear supervisor Prof. Shadia Abdalati

And aspecial thank for

Al-Ribat Medical Specialize Complex

My great full appreciation to my friends who helped me during the preparation of this work .

And for all the Diabetic people from whom the blood samples had been collected.

Abstract

This is a case control study carried out in Al-ribat Medical Specialize Complex-Khartoum State during February 2017 to April 2017,, to evaluate the alteration in platelets count, mean platelet volume (MPV/ fl), platelet distribution width (PDW/fl) plateletcrit(PCT/%) and platelet large cells ratio(P-LCR%),in Type 2 Diabetes Mellitus Sudanese patients .Sixty diabetics and sixty healthy individuals were enrolled in the study after taking their verbal consent.

Venous blood (2.5ml) was collected from the participants in EDTA containers and gently mixed. Platelets count and Platelets indices were determined by an automated sysmix (KX-21) technique and HbA1c by Ichroma.

The obtained data were analyzed by both Student's Independent T test and One Way ANOVA test using SPSS version 16 computer program.

The platelet count C/cmm, MPV/fl and PCT% did not vary with T_2DM.

T_2DM caused a significant (P =0.01) increase of mean PDW in the cases(13.6 ±4.5fl) compared with the control(11.6 ±2.3fl). The diabetics recorded significantly (P =0.005) higher values for P-LCR (12.7±4.2%) than the control(9.8%±3.2%).

The duration of DM type 2 did not (P ≤ 0.05) alter any of the studied parameters.

Diabetics individuals with poor glycemic control had significantly(P≤0.05) lower PCT value(0.21±0.05%) than good control group (0.28±0.09%) and fair control group(0.26±0.06%).

PLT count c/cmm , MPVfl , PDWfl and PLCR% did not vary with the level of glycemic control

It is concluded that Type 2 diabetes mellitus altered some of the platelets indices.

It is concluded that Type 2 DM altered some of the platelets indices. Further studies with a large sample size using more advanced techniques are recommended.

مستخلص البحث

هذه دراسة تحليلية حالة وحالة ضابطة في ولاية الخرطوم في مجمع الرباط الطبي التخصصي خلال الفترة من (فبراير 2017 إلى أبريل 2017) لتقييم التغير في تعداد ومؤشرات الصفائح الدمويه لدي المرضى الذين يعانون من داء السكري من النوع الثاني في السودان ، 60 حالة و 60 أصحاء كمجموعة ضابطه تم اخد الاستبيان شفاهته. ثم اخذ2.5مليلتر من الدم الوريدي من الحالات والضوابط وتم وضعها في وعاء يحتوي على مانع تجلط (EDTA) لقياس تعداد ومؤشرات الصفائح الدمويه باستخدام (Automated sysmix KX-21) وقياس مستوي السكر التراكمي باستخدام جهاز(Ichroma) .

تم تحليل النتائج باستخدام اختبار الفرق بين المتوسطين غير المعتمدين في برنامج الحزم الإحصائية للعلوم الاجتماعية المحوسب.

بصوره عامه لا يوجد تغير في(تعداد الصفائح الدموية(MPV,PCT لدي مرضي السكري من النوع الثاني عند المقارنة بلمجموعة الضابطة .

الاصابه بالنوع الثاني من داء السكري ادت الي زياده في متوسط PDW(بدلاله احصائيه=0.01)في المجموعه الاختباريه (4.5±13.6) بالمقارنه مع المجموعه الضابطة (2.3±11.6).

بينما كان لمرضي السكري معدلات اعلي من ال P-LCR(بدلاله احصائيه=0.005)في المجموعه الاختباريه (12.7±4.2)بلمقارنه مع المجموعه الضابطة (9.8±3.2).

مدة الاصابه لم يكن لها اي تاثير علي المتغيرات تحت الدراسه.

كان لمرضي السكري الغير متحكمين علي نسبة السكر في الدم معدلات اقل من الPCT(بدلالة احصائيهP≤0.05) مقارنه مع المتحكمين بصوره عاليه (0.28±0.09%)والمتحكمين بصوره وسط (0.26±0.06%).

بينما لم يتفاوت تعداد الصفائح الدمويه وMPV/fl وPDW/flو P-LCR/%مع معدل التحكم في مستوي السكر في الدم.

خلصت الدراسه الي ان الاصابه بالنوع الثاني من داء السكري قد غيرت بعض من مؤشرات الصفائح الدموية. كما يوصي بادراج عينات اكبر وستخدام اجهزه متقدمه في دراسات لاحقه .

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List of Abbreviation

Abbreviation	Full text
AA	Arachidonic acid
ADA	American Diabetes Association
ADP	Adenosine diphosphate
CAD	Cardiovascular disease
DKA	Diabetic keto acidosis.
DR	Diabetic retinopathy
EASD	European Association for the Study of diabetes
EPI	Epinephrine
FBG	Fasting blood glucose
GP	Glycoprotein
HbA1c	Glycosylated hemoglobin
HPLC	High-performanceliquid chromatography
IDDM	Insulin dependent diabetes mellitus
IDF	International Diabetes Federation
MI	Myocardial infarction
MPI	Mean platelets indices
MPV	Mean platelets volume
PCT	Plateletcrit
PRP	platelets rich plasma
PLCR	Platelet large cell ratio
T2DM	Type 2 diabetes mellitus
TPO	Thrombopoietin
VWF	Von Willebrand Factor

CHAPTER ONE

Introduction

Type 2 diabetes mellitus (T2DM) is an endocrine disease characterized by impaired insulin excretion by the pancreas and insulin resistance of body tissues Chronic hyperglycemia leads to micro- and macrovascular complicationsin patients with T2DM; diabetic retinopathy (DR) is the most common and the specific microangiopathy. Abnormal insulin activation in patients with T2DMmay increase platelet activation and precipitate microvascular complications. Some authors have emphasized the importance of platelet dysfunction in macrovascular (cardiovascular disease [CVD], stroke, and peripheral artery disease [PAD]) and microvascular (nephropathy, neuropathy, and retinopathy) complications, which lead to increased morbidity and mortality in T2DM. (Vinikand Macagni ,2001).

Various parameters reflect the condition of platelets, including platelets count, plateletcrit, and mean platelet indices (MPI) (mean platelet volume [MPV], platelet distribution width [PDW] and platelet large cell ratio [PLCR]).MPV reflects the average size of platelets. It is a marker that indicates subclinical platelet activation and maybe increased in some vascular conditions such as myocardial infarction (MI), coronary artery disease (CAD), cerebral ischemia, and PAD . Other platelet markers such as PDW, PLCR, and plateletcrit (PCT), which reflect platelet morphology, are also important in vascular events such as atherosclerosis and thrombosis. PDW gives an indication of the distribution of platelet size .PLCR indicates the ratio of younger platelet group that has the largest volume, and PCT gives the total mass of platelets. (Dav`ı and Patrono,2007).

Literature Review

1.1 Haemostasis:

Haemostasis is one of a number of protective processes that have evolved in order to maintain a stable physiology. It has many features in common with (and to some extent interacts with)other defense mechanisms in the body, such as the immune system and the inflammatory response (Hoffbrand , Danial and Edward.,2016).

1.1.2 Haemostatic response:

1.1.2.1 Vasoconstriction:

An immediate vasoconstriction of the injured vessel and reflex constriction of adjacent small arteries and arterioles is responsible for an initial slowing of blood flow to the area of injury. When there is a wide spread damage this vascular reaction prevents exsanguination. The reduced blood flow allows contact activation of platelets and coagulation factors. The vasoactive amines and thromboxane A2 liberated from platelets, and the fibrin peptides liberated during fibrin formation, also have vasoconstrictive activity (Hoffbrand, *et al.,* 2016).

1.1.2.2 Platelet reactions and primary hemostatic plug formation:

Platelets are small cell fragments (average size 3–4 µm) that are important for hemostasis and coagulation. The normal platelet count is between 150,000 and 450,000/µL. Platelets are derived from megakaryocytes, which are very large cells with a large, multi-lobulated nucleus. The mean DNA content of megakaryocytes is at least eight times that of other somatic cells. One megakaryocyte can produce at least several thousands of platelets. The formation and release of platelets is related to a preformed structure in the cytoplasm of megakaryocytes, the so-called

demarcation membrane system. Megakaryocytes are derived from megakaryocyte progenitors, which in turn originate in the hematopoietic stem cell. Megakaryocytes are mainly found in the bone marrow but can transit to many organs, including the lung, where part of the platelet release occurs. The maturation of megakaryocytes and the production of platelets occur under the influence of thrombopoietin (TPO). TPO acts, together with certain other cytokines like IL-6 and IL-11, on early megakaryocyte progenitors as well as mature megakaryocytes. Under physiological conditions, the serum levels of TPO are low at normal or elevated platelet counts and high in individuals with low platelet counts (Wintrobe, Lippincott, and Wilkins.,2009).

Following a break in the endothelial lining, there is an initial adherence of platelets to exposed connective tissue, potentiated by VWF. Collagen exposure and thrombin produced at the site of injury cause the adherent platelets to release their granule contents and also activate platelet prostaglandin synthesis leading to the formation of thromboxaneA 2. Released ADP causes platelets to swell and aggregate. Additional platelets from the circulating blood are drawn to the area of injury. This continuing platelet aggregation promotes the growth of the hemostatic plug which soon covers the exposed collective tissue. The unstable primary hemostatic plug produced by these platelet reactions in the first minute or so following injury is usually sufficient to provide temporary control of bleeding. and smooth muscle cells in the vessel wall adjacent to the area of damage, is important in limiting the extent of the initial platelet plug (Wintrobe ,*et al.,* 2009).

1.1.2.3 Stabilization of the platelet plug by fibrin:

Definitive hemostasis is achieved when fibrin formed by blood coagulation is added to the platelet mass and by platelet-induced clot retraction compaction. Following vascular injury, the formation of extrinsic Xase (VIIa, TF, PL and Ca2+) initiates the coagulation cascade. Platelet aggregation and release reactions accelerate the coagulation process by providing membrane phospholipid. Thrombin generated at the injury site converts soluble plasma fibrinogen into fibrin, potentiates platelet aggregation and secretion and also activates factor XI and XIII and cofactors V and VIII. The fibrin component of the hemostatic plug increases as the fused platelets autolysis and after a few hours the entire hemostatic plug is transformed into a solid mass of cross-linked fibrin. Nevertheless, because of incorporation of plasminogen and tPA , this plug begging to auto digest during the same time frame (Wintrobe ,*et al.,* 2009).

1.1.3 Activation:

 The complex process of converting inactive platelets into a platelet plug, is essential. There are three phases to platelet function :-

*Adhesion :-

Endothelial cells are attached to the sub-endothelial collagen by von Wille brand factor (VWF) . VWF is also stored in the Weibel-Palade bodies of the endothelial cells and secreted constitutively into the blood. Platelets store vWF in their alpha granules .when the endothelial layer is disrupted, collagen and VWF anchor platelets to the sub-endothelium. Platelet GP1b-IX-V receptor binds with VWF; and GPVI receptor and integrin alpha2 - beta1 bind with collagen.(Dubois*, et al.,* 2006)

*Morphology change:-

Mitochondria hyperpolarization is a key event in initiating changes in morphology. Intraplatelet calcium concentration increases, stimulating the interplay between microtubule/actin filament complex. The continuous changes in shape from the un activated to the fully activated platelet. This dramatic increase in surface area comes about with neither stretching nor adding phospholipids to the platelet membrane.(Matarrese, *et al.,*2009).

*Granule secretion:-

Platelets contain dense granules, lambda granules and alpha granules. Activated platelets secrete the contents of these granules through their canalicular systems to the exterior. Simplistically, bound and activated platelets degranulate to release platelet chemotactic agents to attract more platelets to the site of endothelial injury. (Yip, *et al.*,2005).

1.1.4 Platelet disorders:

*Thrombocytopenia according to the classification of Geddis,(2013).

- Immune thrombocytopenias (ITP) – formerly known as immune thrombocytopenic purpura and idiopathic thrombocytopenic purpura
- Splenomegaly
- Chemotherapy
- Thrombotic thrombocytopenic purpura
- Hemolytic-uremic syndrome
- Pregnancy associated
- Aplastic anemia
- Transfusion associated
- Pseudo-thrombocytopenia
- idiopathic thrombocytopenic purpura

*Thrombocytosis and thrombocythemia according to the classification of Laidlaw *et al*.,(2012*).*

- Chronic infection
- Chronic inflammation
- Malignancy
- Hyposplenism (post-splenectomy)
- Iron deficiency
- Acute blood loss
- Myeloproliferative neoplasms – platelets are both elevated and activated
  - Essential thrombocytosis
  - Polycythemia vera
- Congenital.

* Altered platelet function according to the classification of Laidlaw *et al*.,(2012*).*

Congenital

- Disorders of adhesion
  - Bernard-Soulier syndrome
- Disorders of activation
  - Disorders of granule amount or release
  - ADP Receptor defect
  - Decreased cyclooxygenase activity
  - Storage pool defects, acquired or congenital
- Disorders of aggregation
  - Glanzmann's thrombasthenia

Acquired

- Disorders of adhesion
  - Paroxysmal nocturnal hemoglobin uria
  - Cancer
  - Malaria

1.1.5 Platelets indices:

1.1.5.1 Mean platelet volume (MPV):

Is a measure of the average size of platelets, which are cells that help your blood clot. with normal rang (7.4 _ 10.4 fl). A high MPV is usually a sign that there are more young platelets circulating in your bloodstream. If you have had a procedure such as major surgery, your body is using up platelets to repair the cuts to the blood vessels. In response, your bone marrow releases more of the young, larger platelets, and your MPV rises, a low MPV along with a low platelet count can point towards disorders affecting the bone marrow that slow down or decrease the production of platelets, such as a condition called aplastic anemia. In addition, a low MPV can be seen with high, low or normal platelet counts in sepsis (a life-threatening reaction to infection in the body), splenomegaly (enlarged spleen), chronic kidney failure, or treatment with drugs that suppress blood production (Chandrashekar, (2013).

1.1.5.2 Platelet distribution width(PDW):

A measure of the variation in the size of platelets found in the circulating blood. With normal range (10.0 -14.0/fl).
Platelets recently released from bone marrowtend to be larger and to contain more RNA than older, smaller platelets, which discard their endoplasmic reticulum as th ey mature. medical dictionary,The volume is determined by a machine and a Complete Blood Profile, known as a CBC. This reading determine if a patient's body is producing larger than average platelets, indicative of platelet destruction or bone marrow diseases (Chandrashekar, (2013).

1.1.5.3 Platelet-crit (PCT):

Is a measure of total platelet mass. Values vary depending on mean platelet volume resulting in overlap between normal platelets, thrombocytopenia and thrombocytosis .with normal range(0.10_0.28%) (Chandrashekar.,(2013).

1.1.5.4 Platelet large cell ratio(P-LCR):

Means Platelet large cell ratio with normal range (13.0_43.0%) and it's calculated in automated blood analyzers .Increased percentage of large platelets (P-LCR) is observed in patients with Hyper-lipidaemia and suggest possible risk of thrombosis. an increase in P-LCR + MPV + PDW has been observed in autoimmune thrombocytopenic purpura. (Chandrashekar , (2013).

1.1.6 Laboratory evaluations:-

1.1.6 .1 Platelet count test:

Principle blood is diluted 1 in 20 in a filtered solution of ammonium oxalate reagent which lyses the red cells, platelets are counted microscopically using an improved neighbor ruled counting chamber and the number of platelet per liter of blood calculated .may be requested to investigate abnormal skin and mucosal bleeding .also performed when patients are being treated with cytotoxic drugs or other drugs which may cause thrombocytopeni (Hoffbrand, *et al* .,2016).

1.1.6.2Bleeding time:

Principle bleeding time is defined as the time taken for a standardized skin wound to stop bleeding. It is measures the ability of platelets to arrest bleeding and therefore, measures platelet number and function (Hoffbrand, *et al*.,2016).

1.1.6.3 Platelet aggregation :-

Aknown platelet aggregating factor such as collagen, ADP or thrombin is added to a suspension of the platelets under test and the degree of aggregation measured by decrease in turbidity of the suspension.(Hoffbrand, *et al.,* 2016).

1.2 Diabetes Mellitus (DM):

1.2.1Definition and types:-

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes. diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. There are three main types of diabetes mellitus. (Whitcomb,*et al* ,2005).

- Type 1 DM results from the pancreas's failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown. (Kitabchi ,*et al*,2009).
- Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly. as the disease progresses a lack of insulin may also develop. This form was previously referred to as "non insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The most common cause is excessive body weight and not enough exercise.(Kitabchi ,*et al,*2009).

- Gestational diabetes is the third main form and occurs when pregnant women without a previous history of diabetes develop high blood sugar levels (Whitcomb,*et al*.,2005).

1.2.2 Signs and symptoms.

The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 DM, while they usually develop much more slowly and may be subtle or absent in type 2 DM.Several other signs and symptoms can mark the onset of diabetes although they are not specific to the disease. In addition to the known ones above, they include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes (Whitcomb,*et al* ,2005).

1.2.3 Complications.

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20) but may be the first symptom in those who have otherwise not received a diagnosis before that time. the major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease. Other "macrovascular" diseases are stroke, and peripheral artery disease (Whitcomb,*et al* ,2005).

The primary complications of diabetes due to damage in small blood vessels include damage to the eyes, kidneys, and nerves. Damage to the eyes, known

as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and blindness. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplantation .Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle atrophy and weakness (Whitcomb,*et al* ,2005).

1.2.4 Pathophysiology.

Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells of the body, especially liver, adipose tissue and muscle, except smooth muscle, in which insulin acts via the IGF-1. Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus (Lee, *et al.*,2014).

The body obtains glucose from three main places: the intestinal absorption of food; the breakdown of glycogen, the storage form of glucose found in the liver; and gluconeogenesis, the generation of glucose from non-carbohydrate substrates in the body. Insulin plays a critical role in balancing glucose levels in the body. Insulin can inhibit the breakdown of glycogen or the process of gluconeogenesis, it can stimulate the transport of glucose into fat and muscle cells, and it can stimulate the storage of glucose in the form of glycogen (Gardner,*et al.*, 2007*).*

Insulin is released into the blood by beta cells (β-cells), found in the islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically

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after eating. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Lower glucose levels result in decreased insulin release from the beta cells and in the breakdown of glycogen to glucose. This process is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin (Kim*, et al.,*2012).

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or insulin resistance), or if the insulin itself is defective, then glucose will not be absorbed properly by the body cells that require it, and it will not be stored appropriately in the liver and muscles. The net effect is persistently high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis. (Gardner ,*et al.*, 2007*).

When the glucose concentration in the blood remains high over time, the kidneys will reach a threshold of reabsorption, and glucose will be excreted in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst (polydipsia) (Gardner,*et al.*, 2007*).

1.2.5 Diagnosis of Diabetes.

Diabetes mellitus is characterized by recurrent or persistent high blood sugar, and is diagnosed by demonstrating any one of the following:

- Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)

- Plasma glucose $\geq$ 11.1 mmol/l (200 mg/dl) two hours after a 75 g oral glucose load as in a glucose tolerance test.

- Symptoms of high blood sugar and casual plasma glucose $\geq$ 11.1 mmol/l (200 mg/dl)

- Glycated hemoglobin (HbA_{1C}) $\geq$ 48 mmol/mol ($\geq$ 6.5 DCCT %).

A positive result, in the absence of unequivocal high blood sugar, should be confirmed by a repeat of any of the above methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test. According to the current definition, two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) is considered diagnostic for diabetes mellitus.

Fasting glucose levels from 6.1 to 6.9 mmol/l (110 to 125 mg/dl) are considered to have impaired fasting glucose. people with plasma glucose at or above 7.8 mmol/l (140 mg/dl), but not over 11.1 mmol/l (200 mg/dl), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two prediabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus, as well as cardiovascular disease. The American Diabetes Association since 2003 uses a slightly different range for impaired fasting glucose of 5.6 to 6.9 mmol/l (100 to 125 mg/dl). Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause (Weiss and Sumpio,2006).

1.3 Rationale:

Type 2 Diabetes Mellitus results from resistance to insulin action associated with a relative deficiency of this hormone, has an insidious development and is often diagnosed due to the presence of microvascular or macrovascular complications.

Long-term complications of diabetes mellitus are a leading cause of death in people with diabetes. Recent studies suggest that platelets with altered morphology could be associated with an increased risk for developing vascular complications in diabetes.

This study will be focus on change in Platelet count and Platelet indices in type 2 D.M.

1.4 Objectives

1.4.1 General objectives:

To evaluate the alterations in Platelets count and indices in Type 2 Diabetes Mellitus patients.

1.4.2 Specific objectives:

1.To evaluate platelets count, platelets indices (MPV,PDW,PCT and P-LCR) and HbA1c.

2.To compare the PLT count and indices in diabetic group with healthy individual.

3.To assess the effect of DM duration on PLT count and indices.

4.To assess the effect of glycemia control level on PLT count and indices.

CHAPTER TWO

Materials and methods

2.1 Study Design:

This a case control study conducted in Khartoum State during (February 2017 to April 2017).

2.2 Study Area:

Al-rebat Medical Specialize Complex.

2.3 Study Population:

sixty Samples from Sudanese people were diagnosed type 2 diabetes mellitus. And sixty samples was collected from healthy individuals as control.

2.4 Inclusion criteria:

- Diagnosed Type 2 D.M patients.
- Healthy individuals as control group for comparison.

2.5 Exclusion criteria:

Any patient diagnosed as Type 2 D.M with any factors which may affect the study line such as blood or platelet transfusion, history of smoking, alcohol consumption, chronic disease and other type of diabetes .

2.6 Data Collection:

Collected after having verbal consent by a coded questionnaire .

2.7 Sample collection:

Venous blood was collected using sterile disposable plastic syringes after cleaning the vein puncture area with 70% ethanol ,the blood was add to the anticoagulant at ratio of 2.5 to 1.5 of 0.1%EDTA solution and gently mixed .

2.8 Methodology:

2.8.1 Test components of Ichromia:-

1/ID chip.

2/Detection buffer tube.

3/Hemolysis buffer vial.

4/Capillary tube

5/ Test cartridge.

6/i-Chamber and ichroma Reader.

*Quality control:-

1.Quality control tests are a part of the good testing practice to confirm the expected result and validity of the assay and should be performed at regular intervals.

2.The control test should be performed immediately after opening a new test lot to ensure the test performance is not altered.

3.Quality control tests should also be performed whenever there is any question concerning the validity of the test results.

2.8.2 CBC (Automated sysmix (KX-21) technique):

2.8.2.1 Methods of sysmix:

Blood is aspirated from the sample probe into the sample rotor valve:

1. 4.0 μl of blood measured by the sample rotor valve is diluted into 1:500 with 1.996 μl of diluents and brought to the mixing chamber as diluted sample (1st step dilution).

2. Out of the 1:500 dilution sample 40 μl is measured by the sample rotor valve, diluted into 1:25000 with 1.960 μl of diluent then transferred to the RBCs/plt transducer chamber (2nd step dilution).

250 μl of the sample in the RBCs/plt transducer chamber is aspirated through the aperture. At this time RBCs and plt are counted by the DC detection method. At the same time, hematocrit (Hct) value is calculated by RBCs pulse height detection method.

*Calibration test:

1.Perform quality control test under whole blood mode and pre-diluent mode respectively.

2.The test result should be in accordance with reference value.

3.Each mode test for 10 times, test result should accord with accuracy and precision requirement.

2.9 Ethical consideration:

Ethical approval for conducting the research was obtained from the College of Laboratory Medial Science. The participants were provided with information about the study and assured that all the obtained information

will be kept highly confidential and will not be used for any other purpose than for this study.

2.10 Data analysis:

Values were given as mean ± SD. using independent T test (to compare the mean of Plt count and indices between the study group and control group) .and using one way ANOVA (to compare the mean of parameters result between the study group in different degree of glycemic control) The level of significance was set at($p = 0.05$). all statistical analyses were performed using SPSS version 16 computerize.

CHAPTER THREE

Chapter Three

Results

3.1. The comparison between the diabetics and non-diabetics in the platelets count and indices:

This is presented in Table (1) as follows:

No significant (P≤0.05) variation were observed between the diabetics and non-diabetics with regard to the mean platelet count ($290.55 \times 10^3 \pm 80.5$C/cumm vs $301.55 \times 10^3 \pm 64.2$C/cumm), MPV ($9.8 \pm 2.3$ fl vs 8.9 ± 0.8 fl) or PCT ($0.25 \pm 0.08\%$ vs $0.25 \pm 0.08\%$).

Diabetics registered significantly higher (P. value=0.01) PDW values (13.6 ± 4.5 fl)than the non-diabetics(11.6 ± 2.3 fl).

A significant (P. value=0.00) increase in mean P-LCR value was observed in the diabetics ($12.7 \pm 4.2\%$) compared with the non-diabetics ($9.8 \pm 3.2\%$).

3.2. The effect of type 2 diabetes duration on platelets count and indices:

Table (2) shows that duration of type 2 diabetes did not exert any significant variation in the mean PLT-count(P. value=0.18), MPV(P. value =0.09),PDW (P. value =0.94) ,PCT (P. value =0.27) or P-LCR(P. value = 0.27) .

Table (1)Comparison of PLT count and PLT indices between diabetics and non-diabetics.

Parameter	Sample	Mean±SD	P.Value
PLT count×10^3/cumm	Case	290.5±80.5	0.55
	Control	301.5±64.2	
MPV/fl	Case	9.8±2.3	0.09
	Control	8.9±0.8	
PDW/fl	Case	13.6±4.5	0.01
	Control	11.6±2.3	
PCT%	Case	0.25±0.08	0.96
	Control	0.25±0.08	
P-LCR%	Case	12.7±4.2	0.00
	Control	9.8±3.2	

* Significant level at (P-value ≤ 0.05).

Table (2) The effect of type 2 diabetes duration on platelets count and indices:-

Parameters	Duration groups	Mean±SD	P.value
PLT count×10³/cumm	More than 10	311.11±89.0	0.18
	Less than 10	281.6±75.5	
MPV/fl	More than 10	9.14±2.6	0.09
	Less than 10	10.21±1.4	
PDW/fl	More than 10	12.8±0.4	0.94
	Less than 10	12.2±2.0	
PCT%	More than 10	0.27±0.08	0.27
	Less than 10	0.24±0.07	
P-CLR %	More than 10	15.4±3.2	0.27
	Less than 10	11.1±3.6	

* Significant level at (P-value ≤ 0.05).

3.3 The effect of glycemia level on PLT count and PLT indices :

Table (3) Shows insignificant(P. value=0.32) difference in mean platelet count result between cases groups in different degree of glycemic control.

Table (3) Shows insignificant(P. value=0.71) difference in mean MPV result between cases groups in different degree of glycemic control.

Table (3) Shows insignificant(P. value=0.79) difference in mean PDW result between cases groups in different degree of glycemic control.

Table (3) Shows significant(P. value =0.01) increase in mean of PCT result between the Good control and uncontrolled ,significant(P. value=0.05) increase in mean of PCT result between the Fair control and uncontrolled and insignificant(P. value =0.53) difference in mean of PCT result between the Good control and fair control .

Table (3) Shows insignificant (P. value=0.35) difference in mean P-LCR result between cases groups in different degree of glycemic control.

Table(3) The effect of glycemia control on PLT count and PLT indices:-

Parameter	Mean±SD Good control	Mean±SD Fair control	Mean±SD Uncontrolled
PLT count×10^3 C/cumm	312.95±93.5	283.15±59.3	276.75±84.0
MPV/fl	10.23±3.4	9.65±1.6	9.74±1.3
PDW/fl	14.29±5.8	13.74±4.2	14.86±4.8
PCT%	0.28±0.09[a]	0.26±0.06[a]	0.21±0.05[b]
P-CLR %	12.16±4.1	12.91±4.2	13.26±4.4

[a/b]Means within the same row followed by different super script are significantly different at (P. value≤ 0.05).

CHAPTER FOUR

Discussion

Type 2 diabetes mellitus is a serious public health problem, considering its epidemic prevalence level and high morbidity and mortality rate. This type of diabetes that results from resistance to insulin action associated with a relative deficiency of this hormone, has an insidious development and is often diagnosed due to the presence of microvascular or macrovascular complications. The mean of platelet count , MPV or PCT did not show any significant differences between the diabetics and non-diabetics which is on line with the findings of (Yenigun,*et al*., 2014) and (Tejeswini ,2016).

There is a discrepancy in the effect of diabetes on the platelet count as(Buch *et al*.,(2017) recorded a decrease in the PLT count, This discrepancy in the effect of T_2DM on the PLT count may be attributed to many factors in T_2DM which can affect it such as mean platelet survival, mean platelet production rate and turn over.

(Gupta, *et al*., 2016) reported an increase in MPV which contradicts the findings of this work. There is a debate whether PLT activation plays a pathogenic role in the development of diabetic vascular complications.

(Yenigun*, et al*.,2014) and (Hassan, *et al*.,2016) observed higher MPV values in diabetics with vascular complications than those without complications and according to that they suggested PLT play a role in the development of vascular complications and the raised MPV cannot be caused by T_2DM alone.

The contradiction in the MPV values of this work with finding of (Sharma,2016) ; can be attributed to the diabetics in this study have no history of vascular complications, variation in the used anticoagulant or the time between the sample collection and MPV measurement as PLT are able to change their volume after

blood collection (Yenigun ,*et al*.,2014). Another aspect that deserves attention is the platelet size seems to be related to their function. Studies have shown that platelets with greater volume are more reactive and aggregable, have a greater amount of dense granules, and present increased thrombotic potential when compared with smaller and less active platelets .Hyperglycemia is also a factor that contributes to an increase in platelet reactivity, since it exerts direct effects on these cells and promotes glycosylation of platelet proteins. Therefore, large circulating platelets are reflected by increase in MPV, and the elevation of this parameter is considered an independent risk factor for thromboembolism, stroke and acute myocardial infarction. In diabetic patients, a high MPV is an important finding and could predict an increased risk for thrombosis and chronic complications. The activated platelets differ in size from non-activated ones mainly due to a change from a discoid to a spherical shape and pseudopodia formation, leading to a change in the PDW, as observed by (Buch ,*et al*.,2017).

The duration of DM type 2 did not alter PLT count or PLT indices which is consistent with (Buch ,*et al*.,2017).

The diabetic individuals with uncontrolled glycaemia exhibited significantly lower mean values of PCT than the Good and the Fair glycaemic control , Which is consistent with (Berger, *et al*.,2010).

Conclusion

- The platelet count, MPV and PCT did not vary with T_2DM.

- T_2DM caused significant increase of mean PDW and P-LCR .

- The duration of T_2DM did not alter any of the studied parameters.

- Diabetic individuals with poor glycemic control had significantly lowered the PCT value .

Recommendations

1. More studies should be conducted with a large sample size:

 a.To confirm the state of the PLT such as platelets aggregation, coagulation profile, Thrombin time, D.Dimer.

b. To assess Platelet count and indices as risk factors for cardiovascular complications in T_2DM patients.

c. Using more advanced technique for platelets function as the confirmatory test.

2. Regular checkup of hematological parameters for T_2DM patients should be performed to avoid the sudden crises which may occur.

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Appendices

Appendix 1:-

Sudan University of science &technology

Collage of Graduate studies

Assessment of platelet indices among type 2 diabetic Sudanese patients on Khartoum state

Questionnaire

- Name: ……………………………………… ID: ……………………………
- Age:……………………………….
- Sex : M …… F …….
- Duration of disease ……………………………………………
- Do you have previous a blood or platelet transfusion? Yes……….. No ………
- Do you have taken any Treatment of anticoagulant? Yes……. No……….
- If any History of smoking and alcohol consumption? Yes………. No………..
- Do you suffer from a chronic disease? Yes……………. No ……….

*Investigation :

1- Hb A1c………………………………………………………….

2-CBC …………………………………………………………..

*Platelet count…………………………………………………………

*PCT………………………………………………………………..

*MPV……………………………………………………………….

*PDW……………………………………………………………….

*P-LCR………………………………………………………..

*Signature…………………………………*Date..

Appendix 2:-

Principle of sysmix (KX-21):

Particles suspended in an isotonic diluents, when drawn through an aperture which has an electric current flowing through it will cause a measurable drop in voltage which is proportional to the size of the particle passing through the aperture is constant the particle can be quantified per unit volume. This is also called electrical impedance.(Abass, *et al*,2016).

Appendix 3:-

Principle of Ichroma HbA1c:

The test uses a sandwich immune detection method, the detector antibody in buffer binds to antigen in sample, forming antigen –antibody complexes, and migrates on to nitrocellulose matrix to be captured by the other immobilized –antibody on test strip. The more antigen in sample forms the more antigen –antibody complex and leads to stronger intensity of fluorescence signal on detector antibody .Instrument for ichroma test displays the of glycated hemoglobin in terms of percent of the total hemoglobin in blood . (Jalali, *et al* ,2016).