Determination of Correlation between Platelet Parameters and Complications of Type2 Diabetes Mellitus in Sudanese Patients in Khartoum State

A dissertation submitted in partial fulfillment for the requirements of M.Sc. degree in medical laboratory sciences (Hematology and Immunohematology)

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بسم الله الرحمن الرحيم

(و بسَلَّمْك عَن الْرَّحْلِ الْرَّحْلَةِ وَأَرْجَعُ صِبْحَةً وَأَوْتِيْتُمُ الْعُلْمَ الْأَقْلِيْلَ)

صدق الله العظيم

سورة الإسراء الآية 85
Dedication

To the candle which burn to light my life

   My mother

To the soul of my dear father

   To my dear uncle

To those who will find it beneficial work
Acknowledgement

First and foremost, my thanks to the most beneficent and merciful, the almighty Allah, for giving me the power and health to complete this work. Next, I would like to express my sincere gratefulness and respect to Dr. Hisham Nour aldayem Altayeb, for his valuable guidance, kind supervision and great help. Words are not sufficient to express my gratitude to my friends especially whom provide me with love and support throughout the work and help me greatly in the completion of this work.
Abstract
This case control study was conducted to determine the association between platelet parameters and complications of type 2 diabetes mellitus in Sudanese patients in Khartoum state during the period from August to November 2018. 150 participants of age between 28-65 years were selected randomly (50 of them complain from diabetes mellitus, 50 have diabetic complications and the others are apparent healthy participate as controls). Ethyline Diamine Tetra Acetic acid anticoagulated venous blood sample (3ml) was collected from each subject. Platelet parameters were measured using automated hematological analyzer (Mindry BC2800) in Nourin’s medical center. The data analyzed using statistical package of social science programme, the statistical results revealed that there is significant difference in mean platelet volume (8.4±1.3fl vs. 7.9±0.7fl p=0.025) and platelet distribution width (14.1±1.9fl vs.15.4±0.2fl p=0.000) between diabetic patients and healthy participants. The mean of platelet count, mean platelet volume and plateletcrit were significantly increased in diabetic patients with complications as compared to diabetic patients without complication and control group (294±77×10^9/L vs.257±67×10^9/L p=0.003, 9.1±1.4fl vs.7.8±0.7fl p=0.000 and 0.266±0.06 vs.0.201±0.05 p=0.000 respectively) while platelet distribution width was significantly decreased (13.0±2.1fl vs.15.4±0.2fl p=0.000) By the end of this study, it was revealed that platelet indices (mainly the mean platelet volume and plateletcrit could be used as a prognostic markers for early detection of diabetic complications.
مستخلص البحث

أجريت هذه الدراسة للحالات والضوابط لتحديد العلاقة بين معلمات الصفائح ومضاعفات مرض السكري من النوع الثاني لمرضى السودانيين بولاية الخرطوم في الفترة من أغسطس إلى نوفمبر 2018.

تم اختيار 150 مشارك من عمر 28-65 عاما عشوائيا (50 مشارك من مرض السكري فقط، 50 مشارك من مشاكل مرض السكري من النوع الثاني و البقية أصحاب ظهوريا شاركوا كضوابط). سُجِّيت عينة دموي (3مل) من كل مشارك في مادة منع التنجلط (ثنائي أمين الإثيلين رباعي حامض الخليك). تم قياس معلمات الصفائح باستخدام محلل الدم الآلي (مندري بي سي-2800) في مركز نورين الطبي.

حللت البيانات باستخدام برنامج الحزم الإحصائية الاجتماعية. كشفت النتائج الإحصائية عن هناك اختلاف كبير في متوسط حجم الصفائح الدموية (8.4±7.9 مقابل 9.7±0.7) وعريس توزيع الصفائح (p=0.025) بين مرضى السكري والمشاركين الأصحاء.

متوسط عدد الصفائح الدموية،متوسط حجم الصفائح و حجم الصفائح المعيا زادا بشكل كبير في مرضى السكري الذين لديهم مضايقات بالمقارنة مع مرضى السكري من دون مضايقات ومجموعة الضبط (9.0±10 9 مقابل 7.8±10 9، p=0.003 و 0.0000 7.1±0.06 مقابل 0.266±0.06، p=0.0000) على التوالي (في حين انخفض عريس توزيع الصفائح بشكل ملحوظ (p=0.0000 15.4±13.0 مقابل 2.1±0.2، p=0.0000) بنهاية هذه الدراسة تم التوضيح أن مؤشرات الصفائح (بشكل رئيسي متوسط حجم الصفائح وحجم الصفائح المعينة) يمكن أن تستخدم كمؤشرات تكنية للكشف المبكر لمضاعفات السكري.

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Chapter One
Introduction
Chapter one

1.1. Introduction:
Diabetes mellitus is a metabolic disorder of multiple etiology characterize by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (WHO).

Type 2 DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia, insulin resistance and relative insulin deficiency (Kumar et al., 2005). This type result from interaction between genetic, environmental and behavioral risk factors (Riaz, 2009; Olokoba et al., 2012). People living with this type are more vulnerable to various forms of both short and long term complications which often lead to their premature death (Olokoba et al., 2012).

This tendency of increased morbidity and mortality is seen in patients with type 2 because of the commonest of this type of DM, it is insidious onset and late recognition especially in resource-poor developing countries like Africa (Azevedo and Alla, 2008).

Type 2 diabetes mellitus patients have two-four folds increase in risk of atherosclerosis (Forbes and Cooper, 2013). Eighty percent of diabetic mortality rate is due to -thrombotic events. While 75% of these mortalities belonged to cardiovascular problems and 25% reminder due to peripheral vascular events as well as cerebrovascular complication (Yamagashi et al., 2007).

Platelet indices are biomarkers of platelet activation. They allow clinical investigations focusing on the diagnostic and prognostic values in a variety of settings without bringing extra costs. Among these platelet indices are group of platelet parameters determine together in automatic CBC profile. They are related to platelet morphology and proliferative kinetic (Budak et al., 2016).
DM has been considered as a prothrombotic state (Akinsegun et al., 2014; Chen et al., 2017) with enhanced platelet reactivity. Researchers found the morphological changes of platelet and increased platelet activity occurred in diabetic patients (Chen et al., 2017). This raised in platelet activity demonstrated by mean platelet volume, the average volume of platelets a parameter in full blood count measures platelet size distribution and is not influenced by glycemic control (Ansari et al., 2017).

Increased platelet activity is emphasized to play a role in the development of vascular complication of the metabolic disorder (Dubey et al., 2017). Current study aims to clarify the relationship between platelet volume indices and diabetes mellitus and their clinical prognostic value.
1.2. Rationale:

Diabetes mellitus is one of popular common chronic disease. 80% of diabetic mortality is due to cardiovascular disorder these due to hypercoagulability state during the disease (Riaz, 2009). Platelet volume indices could help in the prediction of complication of the disease, so suitable intervention and decision could be taken from earlier stages and cases could be managed at the right time. Although platelet indices are very important, they are neglected and not used as they should. Current study will clarify the importance of these indices as a prognostic marker in diabetic patients.
1.3. Objective:

General objective:
-To detect association between platelet parameters and diabetes mellitus complication in Sudanese patients in Khartoum state.

Specific objectives:
-To estimate platelet and platelet indices.
-To compare between normal and diabetic participants.
-To compare between diabetic participants who have complication with those who are have not.
Chapter Two
Literature review
Chapter two

2. Literature review

2.1. Diabetes mellitus:

2.1.1. Definition

Diabetes mellitus is a metabolic disorder of multiple etiology characterize by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (WHO).

2.1.2. Root causes of diabetes mellitus:

Most cases begin with one of two processes:

A. Metabolic process: Un-healthy life style factors such as: over eating, physical inactivity and obesity can impair the body’s ability to use insulin. This is called insulin resistance. Uncontrollable risk factors including genetic, family history and age can also be involved. Metabolic form includes gestational diabetes and type 2 diabetes.

B. Autoimmune: The body’s immune system can mistakenly destroy the insulin producing beta cell of the pancreas. The causes of autoimmune diabetes are poorly understood, but genetic and family history play a role and viruses and other environmental factors are believed to figure in. It includes type 1 and latent autoimmune diabetes of adulthood (Riaz, 2009).

2.1.3. Types of diabetes mellitus:

A. Type 1(insulin dependent):

Hyperglycemia occurs due to complex disease process where genetic and environmental factors lead to autoimmune response that remains to be not fully elucidated. During this process the pancreatic beta cell are destroyed resulting in individual with this condition relying on exogenous insulin administration for
survival. (Forbes and Cooper, 2013). Although a subgroup has significant residual C-peptide production (Keenan et al., 2010).

B. Type 2 (non-insulin dependent):
This type is the majority of diabetes burden, comprising 85% of cases. In this form of disease, peripheral insulin resistance and compensatory hyper secretion of insulin from the pancreatic islets may precede the decline in islet secretory function (Forbes and Cooper, 2013).

C. Gestational diabetes:
Hormonal changes contribute to this condition which can develop in any previous non diabetic woman during pregnancy especially those who are over-weight. This type ends when pregnancy does (Riaz, 2009).

D. Secondary diabetes:
This type result from another disease such as: pancreatitis, or from medical treatment including pancreatectomy or certain medication. Some cases are temporary (Cefalu et al., 2007).

E. Double diabetes:
This condition occurs in some individuals with autoimmune diabetes who over-eat are sedentary gain weight or have certain gene can like people with metabolic form and develop insulin resistance (Gluffrida et al., 2016).

2.1.4. Pathogenesis and pathophysiology of diabetes mellitus:
There is a direct link between hyperglycemia and physiological and behavioral responses. Whenever there is hyperglycemia, the brain recognizes it and sends a message through nerve impulses to pancreas and other organs to decrease its effect (Patidar, 2011).

In type2 DM, these mechanisms break down, with the consequence that the two main pathological defects in type 2 are impaired insulin secretion through a dysfunction of the pancreatic beta cells and impaired insulin action through insulin
resistance. In situation where resistance to insulin predominates, the mass of beta cells undergoes a transformation capable of increasing the insulin supply and compensating for the excessive and anomalous demand. In absolute terms, the plasma insulin concentration usually is increased, although ‘relative’ to the severity of insulin resistance, the plasma insulin concentration is insufficient to maintain normal glucose homeostasis. Keeping in mind the intimate relationship between the secretion of insulin and the sensitivity of hormone action in the complicated control of glucose homeostasis, it is practically impossible to separate the contribution of each to the etiopathogenesis of type 2 DM (Baynest, 2015).

Type2 normally results the progressive development of insulin resistance and subsequent dysfunction of pancreatic beta cells. The fact that about 80% of people with type2 DM are obese highlights a clear association between type2 and obesity (abdominal obesity in particular). Abdominal fats are resistant to the anti-lipolytic effects of insulin which cause the release of excessive amounts of free fatty acids. High levels of free fatty acids cause insulin resistance in the liver and muscle cells. This leads to increase gluconeogenesis in the liver and inhibition of insulin mediated glucose uptake by muscle cells, resulting in increased levels of circulating glucose in addition if adipocyte get too large they become unable to store any more fat. As an alternative, Fat is stored in muscle, liver and pancreatic cells which worsen insulin resistance in these organs (Hackett and Jacques, 2009).

2.1.5. Diabetic symptoms:
Symptoms of type2 diabetes are usually insidious because insulin production decreases over time. Common symptoms include:

- Polyuria, increased thirst and nocturia due to hyperglycemia.
- Fatigue due to the inability to use glucose as an energy source.

These symptoms are sometimes accompanied by a rapid unhealthy weight loss due to the breakdown of protein and fat as an alternative energy source.
Some patients also get infections especially *Candida* spp and urinary tract infections because raised serum glucose impairs phagocyte function and provides a growth medium in which microorganisms can flourish (Hackett and Jacques, 2009).

2.1.6. Diabetic complications:
Diabetic complications may be classified based on whether the manifestation is physical such as: lipo-hypertrophy or metabolic as: hyper and hypoglycemia. However, diabetic complication can be classified into microvascular complications (neuropathy, nephropathy and retinopathy) concern in type 1 and type 2 patients, and macrovascular complications (cardiovascular, cerebrovascular and peripheral vascular disease) which are common in type 2 patients (Dunning, 2014; Ojo, 2016).

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

2.1.6.2. Diabetic retinopathy:
Is characterized by a spectrum of lesions within the retina and is the leading cause of blindness among adult (Hirral *et al.*, 2011). These include changes in vascular permeability, capillary microaneurysms, capillary degeneration and excessive neovascularization. The neural retina is also dysfunctional with death of some cells, which alters retinal electrophysiology and results in an inability to discriminate between colors (Frank, 2004).

2.1.6.3. Diabetic neuropathy:
It is a syndrome which encompasses both the somatic and autonomic divisions of the peripheral nervous system. There is a growing appreciation that damage to the spinal cord (Selvarajah *et al.*, 2006) and the higher central nervous system can also
occur (Wesseles et al., 2006) and that neuropathy is a major factor in the impaired wound healing, erectile dysfunction and cardiovascular dysfunction seen in diabetes (Obrosova, 2009).

2.1.6.4. Cardiovascular diseases:
Cardiovascular disorders in diabetes include premature atherosclerosis, manifest as myocardial infarction and stroke as well as impaired cardiac function, predominantly diastolic dysfunction (Okon et al., 2005). Cardiovascular disease account for more than half of the mortality seen in the diabetic population (Laing et al., 2003).

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

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

2.2. Platelet:
2.2.1. Structure and function:
Platelet is small a nucleated cell fragment has discoid shape maintained by a band of parallel microtubules. It has two tubular systems: the dense tubular system and open canalicular system. Also contain two types of granule alpha and delta. Platelet play role in managing and regulating hemostasis (Ghoshal and Bhattacharyya, 2014).
2.2.2. Platelet indices:
They are biomarkers of platelet activation. They allow extensive clinical investigations focusing on the diagnostic and prognostic values in a variety of setting. Among these platelet indices, plateletcrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW) are a group of platelet parameters determine together in automatic CBC profile, they are related to platelet morphology and platelet kinetics (Budak et al., 2016).

2.2.2.1. Mean platelet volume (MPV):
It is a measurement that describes the average size of platelet in the blood. This parameter provides an indicator as to whether the bone marrow is manufacturing platelets normally or there is some kind of production pressure from periphery. MPV normal range: 7.4-10.4 fl.

2.2.2.2. Platelet distribution width (PDW):
This parameter compares the uniformity and heterogeneity of platelet size. PDW normal range: 9-13 fl.

2.2.2.3. Plateletcrit (PCT):
It is the volume percentage that platelets take on a total blood volume, and it is directly related to the number of platelets and mean platelet volume.1 PCT normal range: 0.110-0.280 (Lokwani, 2013).

2.3. Previous studies:
The incidence of diabetes mellitus has soared worldwide in recent year and is expected to keep growing with the greatest increase seen in metabolic forms of diabetes notably type 2. This is blamed largely on the rise of obesity and the global spread of western style habits: physical inactivity along with a diet that is high in calories, processed CHO and saturated fats and insufficient in fiber rich whole food (Riaz, 2009).
According to world diabetes atlas, an estimated 415 million people had diabetes worldwide in 2015 with type 2 making up about 90% of the cases (IDF, 2015; Yuankai and Frank, 2014). Due to gravity of this disease many studies were done in order to predict the complication and to decrease severity of the complication using platelet indices.

In 2016 Tetikoglu and et al., were associated between MPV and diabetic macular edema in patient with type 2 DM they were found that there is significant difference in MPV (8.6±0.96Vs.8.3±0.9  p=0.011) and PCT(0.216±0.58Vs. 0.202±0.52 p=0.038) between diabetic patient and healthy individual. also they found that MPV value of patients with diabetic macular edema was significantly higher than those of diabetic patients without macular edema.

Another study was carried out in 2017 by Chen and his colleagues they were found that PDW and mean platelet count were not significantly different between diabetic and non-diabetic group (16.0% Vs. 16.0 p=0.88, and 194×10^9/L Vs.196×10^9/L p=0.05 respectively) .While MPV was significantly higher in diabetic participants (9.3fl Vs.9.3fl p=<0.05).

Bashir and Ali found that MPV (9.9±1.06fl Vs.10.4±0.99fl p=0.013) and PDW (13.07±2.2fl Vs.13.9±2.3fl p=0.034) were significantly decreased in diabetic septic foot patients than normal participants in 2017.

In the same year Buch found that MPV was significantly increased in diabetic patients with complication as compared to diabetic without complication and non-diabetic groups(11.3±1.9fl ,9.9±1.9fl and 8.4±1.01fl respectively)(p-value=0.0001).PDW also showed statistically significant difference between diabetic with and without complications and non-diabetic(p=0.0001). Significant correlation of MPV with diabetic retinopathy (p=0.000), nephropathy (p=0.005) and diabetic foot (p=0.046=8). PDW was significantly increased in diabetic retinopathy (p=0.035) and nephropathy (p=0.007).
Shilpi and Potekan found that MPV and PDW and were significantly higher in diabetics compared to non-diabetics (11.3±1.0fl vs. 9.0±0.6fl, 14.2±2.5 vs. 10.7±0.7fl) and significantly increased among those with complications compared to those without complications in 2018.

Also significant raised in MPV (9.97fl p=0.001) and PDW (12.54fl p=0.010) in diabetic patients in compared with non-diabetic individuals (mean MPV 9.1fl and mean PDW 11.89fl) were found by Elkalifa in 2016.

In 2016 Alhadas evaluated platelet parameters in patient with type 2 diabetes mellitus, and their research results were that all platelet parameters (MPV: 8.69±1.2fl vs. 8.2±1.2fl p=0.039, PCT: 0.210±0.054% vs. 0.200±0.045% p=0.020 and PDW: 17.8±1.06 vs. 17.5±0.87 p=0.039) were significantly higher among patients with complication of type 2 diabetes mellitus.

In 2014, Akinsegun conducted a study that revealed a higher mean platelet count for diabetic patients than non-diabetic control (235±76×10^9/L versus 211±66×10^9/L p=0.038). While mean platelet volume was lower in cases than control (8.69±0.67fl versus 8.91±0.80fl p=0.593).

Gaur and his colleagues conducted similar study in 2016, and they found that there is a higher significance in mean platelet volume among type 2 diabetic patients when compared to the control group (9.16±0.84 Vs. 4.89±0.65 p=0.001) while the mean platelet count and PDW were not showed statistically differences between two groups (248±78×10^9/L Vs. 270±79×10^9/L p=0.373, 19.2±14.1 Vs. 16.8±1.54 p=0.347 respectively).

In 2017, Ansari and his colleagues compare platelet volume indices and lipid profile of diabetic cases and non-diabetic control. They found significant increase in platelet volume indices through the diabetic patients (MPV=14.4±4.5fl versus 8.8±1.1fl p=0.001, PDW= 15.6±3.2 versus 9.5±1.08 p=0.001).
High statistical significance in platelet count and mean platelet volume among type 2 diabetic patients when compared to non-diabetic group (277±81×10⁹/L versus 269±78×10⁹/L p=0.025, 8.29±0.74fl versus 7.47±0.73fl p= 0.001 respectively) were revealed by Thomas in 2012.
Chapter Three
Materials and Methods
Chapter Three

3. Materials and Methods:

3.1. Study Design:

This is a case control study.

3.2. Study population:

150 samples were collected, 50 of type2 DM with complications, 50 of type2 DM without complications arrived to Nourin’s medical center and 50 non diabetic control of age between 28-65years

3.3. Study area and duration:

This study was conducted in Khartoum state, Sudan, during the period from August to November (2018).

3.4. Inclusion and exclusion criteria:

A. Inclusion criteria for cases:

Any patients have type2 DM (with and without complication) of age between in Khartoum state

B. Exclusion criteria for cases:

Subjects with any of these situations will be excluded:

• Non diabetic patients.

Patients who have one of the other type of DM.

• Receiving antiplatelet medication

• Insulin dependent and other type of diabetes

• Other chronic disease

• Malaria.

C. Inclusion criteria for control:

Any non-diabetic apparently healthy adult Sudanese in Khartoum state.

3.5. Data collection:

Samples were collected randomly.
A structured questionnaire was used to collect demographic and clinical data and it was full filled by subjects themselves.

3.6. Methodology:

3.6.1. Samples collection:

A total of 3ml of venous blood sample was collected in ethylene diamine tetraacetate (EDTA) evacuated tube for each participant.

3.6.2. Samples collection procedure:

- A sterile, dry, preferably plastic syringe of the capacity required 3ml was selected.
- The tourniquet was applied to the upper arm of the participant.
- Using the index finger, a suitable vein was felt.
- The puncture site was cleaned with 70% ethanol and allowed to dry.
- The venipuncture was made with the bevel of the needle directed upwards in the line of the vein. The piston of the syringe was withdrawn slowly and no attempt made to withdraw blood faster than the vein is filling
- After obtaining the blood and releasing the tourniquet, the needle was removed and carefully the container was filled with the required volume of blood
- Anti-coagulated specimens were mixed by inverting the containers several times.

3.6.3. Samples analysis:

Full blood count was performed using Mindray BC-2800 a three part hematological auto analyzer.

3.6.3.1. Mindray BC-2800:

This automated hematological analyzer able to run 19 parameters per sample. It measures the cell counts using direct current (DC) detection method and measures hemoglobin concentration by Non-Cyanide method.

3.6.3.2. Direct Current detection method:

Blood samples were aspirated, measured to predetermined volume, diluted at the specified ratio, and then fed into each transducer. The TD chamber has a minute
hole called the aperture. On both sides of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes.

As direct current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses.

**3.6.3.3. Procedure (Whole blood mode):**

- Blood is aspirated from the sample probe into the SRV.
- 6 µL of blood measured by the SRV is transferred to the WBC TD chamber along with 1.994 mL of diluent. At the same time, 1.0 mL of WBC/Hb lyse is added to prepare 1:500 diluent sample.

When the solution is made to react in this status for approximately 10 seconds, RBC is hemolyzed and platelets shrink, with membrane held as they are. At the same time, hemoglobin is converted into red colored methemoglobin.

- Of the diluted/hemolyzed sample in the WBC TD chamber, approximately 1 mL is transferred to the Hb flow cell.
- 500 µL of sample in the WBC transducer is aspirated through the aperture. The pulses of the blood cells when passing through the aperture area counted by the DC detection method.
- In the Hb flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) is applied to the sample in the Hb flow cell. Concentration of this sample is measured as absorbance. This absorbance is compared with that of the diluent alone was measured before addition of the sample, thereby calculating Hb (hemoglobin value).

**3.6.3.4. Quality control:**

The reliability of this instrument and reagents is monitored by quality control. By use 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 (Mindray, 2004)

**3.7. Data analysis:**
Data was analyzed by using statistical package of social sciences (SPSS) programme (version 16.0). The continuous variables were given as mean ± standard deviation. One way ANOVA test was used to test for association between discrete variable. P-value was considered to be significant when ≤ 0.05.

**3.8. Ethical consideration:**
All participants who gave informed consent and satisfied the study inclusion criteria were recruited into this study

Privacy and confidentiality for each participant were guaranteed.

Permission to carry out the study was obtained from the college of graduate studies.
Chapter Four
Results
Chapter Four

Result

4. Result:
A total of 100 diabetic patients (with and without complication) and 50 healthy individuals (controls) males of age between 28-65 years were selected in this study. The group of diabetic patients with complication was divided into three groups according to certain complication (25 members complain from nephropathy, 15 have retinopathy and 10 complain from diabetic septic foot) the differences in platelet indices between them were clarified in figure (4-1). Independent T-test was used to compare the platelet count and it’s indices between controls and cases (with and without complication), and to compare between diabetic patients with complication and other participants (table 4-1,4-2).

Table (4-1): Comparison of platelet and it’s indices between cases and controls:

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO.</th>
<th>Platelet</th>
<th>MPV</th>
<th>PDW</th>
<th>PCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Cases</td>
<td>100</td>
<td>266</td>
<td>81</td>
<td>8.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Controls</td>
<td>50</td>
<td>276</td>
<td>51</td>
<td>7.9</td>
<td>0.7</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.344</td>
<td>0.025</td>
<td>0.000</td>
<td>0.619</td>
</tr>
</tbody>
</table>

Table (4-2): Comparison of platelet and it’s indices between diabetic patients with complication and other participants:

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO.</th>
<th>Platelet</th>
<th>MPV</th>
<th>PDW</th>
<th>PCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Diabetic with c/o</td>
<td>50</td>
<td>294</td>
<td>77</td>
<td>9.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Other participants</td>
<td>100</td>
<td>257</td>
<td>67</td>
<td>7.8</td>
<td>0.7</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.003</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>
One way ANOVA test was used also to clarify the differences between the three groups separately (figure 4-2).
The statistical results revealed that platelet count is significantly decreased in diabetic patients who have no complications (p=0.006) and insignificant among the patients who have complications (p=0.193) (table 4-3).
While mean platelet volume is significantly increased in the patients with complications (p=0.000) and there no statistical significance among those who have no complications (p=0.204) (table 4-4).
The platelet distribution width is significantly decreased in patients with complications (p=0.000) and insignificant among patients without complications (p=0.806) (table 4-5).
The plateletcrit parameter is significantly increased in the diabetic patients who have complications (p=0.000) and it is significantly decreased in the patients who have no complications (p=0.001) (table 4-6).

**Table (4-3): Difference in platelet count between diabetic patients with complication, diabetic patients without complication and controls:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Compared with</th>
<th>Mean Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>D. without complication</td>
<td>38.720</td>
<td>.006</td>
</tr>
<tr>
<td></td>
<td>D. with complication</td>
<td>-18.060</td>
<td>.193</td>
</tr>
<tr>
<td>D. without complication</td>
<td>Control</td>
<td>-38.720</td>
<td>.006</td>
</tr>
<tr>
<td></td>
<td>D. with complication</td>
<td>-56.780</td>
<td>.000</td>
</tr>
<tr>
<td>D. with complication</td>
<td>Control</td>
<td>18.060</td>
<td>.193</td>
</tr>
<tr>
<td></td>
<td>D. without complication</td>
<td>56.780</td>
<td>.000</td>
</tr>
</tbody>
</table>
Table (4-4): Difference in mean platelet volume between diabetic patients with complication, diabetic patients without complication and controls:

<table>
<thead>
<tr>
<th>Group</th>
<th>Compared with</th>
<th>Mean Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>D. without complication</td>
<td>.2620</td>
<td>.204</td>
</tr>
<tr>
<td></td>
<td>D. with complication</td>
<td>-1.1880</td>
<td>.000</td>
</tr>
<tr>
<td>D. without complication</td>
<td>Control</td>
<td>-.2620</td>
<td>.204</td>
</tr>
<tr>
<td></td>
<td>D. with complication</td>
<td>-1.4500</td>
<td>.000</td>
</tr>
<tr>
<td>D. with complication</td>
<td>Control</td>
<td>1.1880</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>D. without complication</td>
<td>1.4500</td>
<td>.000</td>
</tr>
</tbody>
</table>
Table (4-5): Difference in platelet distribution width between diabetic patients with complication, diabetic patients without complication and controls:

<table>
<thead>
<tr>
<th>Group</th>
<th>Compared with</th>
<th>Mean Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>D. without complication</td>
<td>.0620</td>
<td>.806</td>
</tr>
<tr>
<td>Control</td>
<td>D. with complication</td>
<td>2.4560</td>
<td>.000</td>
</tr>
<tr>
<td>D. without complication</td>
<td>Control</td>
<td>-.0620</td>
<td>.806</td>
</tr>
<tr>
<td>D. without complication</td>
<td>D. with complication</td>
<td>2.3940</td>
<td>.000</td>
</tr>
<tr>
<td>D. with complication</td>
<td>Control</td>
<td>-2.4560</td>
<td>.000</td>
</tr>
<tr>
<td>D. with complication</td>
<td>D. without complication</td>
<td>-2.3940</td>
<td>.000</td>
</tr>
</tbody>
</table>
Table (4-6): Difference in plateletcrit between diabetic patients with complication, diabetic patients without complication and controls:

<table>
<thead>
<tr>
<th>Group</th>
<th>Compared with</th>
<th>Mean Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>D. without complication</td>
<td>.037200</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>D. with complication</td>
<td>-.046160</td>
<td>.000</td>
</tr>
<tr>
<td>D. without complication</td>
<td>Control</td>
<td>-.037200</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>D. with complication</td>
<td>-.083360</td>
<td>.000</td>
</tr>
<tr>
<td>D. with complication</td>
<td>Control</td>
<td>.046160</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>D. without complication</td>
<td>.083360</td>
<td>.000</td>
</tr>
</tbody>
</table>
Figure (4-1): Comparison of platelet indices between control and complication groups.
Figure (4-2): Comparison of platelet indices between cases and control
Chapter Five
Discussion, Conclusion and Recommendations
Chapter Five
Discussion, Conclusion and Recommendation

5.1. Discussion:
Diabetes is a growing health problem associated with increased risk of micro and macro-vascular complications. With the easy availability of various blood tests such as platelet indices, effort are made to identify and prove their utility to act as biomarkers for early detection of diabetic complication (Buch et al., 2017).
This study was conducted to associate between platelets count and platelet indices and complications of diabetes mellitus type 2 in Sudanese patients in Khartoum state. The study included 100 diabetic patients (50 with and 50 without complications) and 50 apparent healthy individuals as controls aged between 28-65 years.
The results show significant difference in MPV (8.4±1.3fl vs. 7.9±0.7fl p=0.025) and PDW (14.1±1.9fl vs.15.4±0.2fl p=0.000) between the cases and controls, while platelet count (p=0.344) and PCT (p=0.619) have no statistical significance. And when the diabetic patients who have complications compared to the remaining participants they show statistical significance in the four parameters (platelet count (p=0.003), MPV (p=0.000), PDW (p=0.000) and PCT (p=0.000)).
When the parameters are compared between each group separately, the statistical results revealed that platelet count is significantly decreased in diabetic patients who have no complications (p=0.006) and insignificant among the patients who have complications (p=0.193). While mean platelet volume is significantly increased in the patients with complications (p=0.000) and there no statistical significance among those who have no complications (p=0.204). The platelet distribution width is significantly decreased in patients with complications (p=0.000) and insignificant among patients without complications (p=0.806). The plateletcrit parameter is significantly increased in the diabetic patients who have
complications (p=0.000) and it is significantly decreased in the patients who have no complications (p=0.001).

In this study the mean platelet volume has a significant difference between cases and controls (8.4±1.3fl vs. 7.9±0.7fl p=0.025) and it is significantly increase in the patients with complications than those who are haven’t. This finding is agree with the findings of Tetikoglu (8.6±0.96Vs.8.3±0.9 p=0.011), Chen (9.3fl Vs.9.3fl p=<0.05), Buch (11.3±1.9fl vs.8.4±1.01fl p=0.0001), Shilpi (11.3±1.0fl vs.9.0±0.6fl), El-Khalifa (9.97fl p=0.001), Alhadad (8.69±1.2fl vs. 8.2±1.2fl p=0.039), Gaur (9.16±0.84 Vs.4.89±0.65 p=0.001), Ansari (14.4±4.5fl vs. 8.8±1.1fl p=0.001) and Thomas (8.29±0.74fl versus 7.47±0.73fl p= 0.001). While it is disagree with Akinsegun’s (8.69±0.67fl versus 8.91±0.80fl p=0.593) and Bashier’s(9.9±1.06fl Vs.10.4±0.99fl p=0.013) results.

The statistical tests revealed that the mean of the platelet count has no significant difference between cases and controls (266±81×10^9/L vs. 276±51×10^9/L p=0.344) which is consistent with Chen’s result (194×10^9/L Vs.196×10^9/L p=0.05) and opposite to the findings of Akinsegun and Thomas (235±76×10^9/L vs. 211±66×10^9/L p=0.038, 277±81×10^9/L vs.269±78×10^9/L p=0.025 respectively).

The platelet distribution width is significantly different between cases and controls (14.1±1.9fl vs.15.4±0.2fl p=0.000) and it is significantly decrease in the diabetic patients with complications (p=0.000), this result is consistent with Buch’s (p=0.0001) and Bashier’s (13.07±2.2fl Vs.13.9±2.3fl p=0.034) results and it is completely different from Chen’s, Shilpi’s, Gaur’s and El-Khalifa’s results (16.0% Vs. 16.0 p=0.88, 14.2±2.5 vs. 10.7±0.7fl, 19.2±14.1 Vs.16.8±1.54 p=0.347 and 12.54fl p=0.010 respectively).

The plateletcrit is significantly higher in patients with complications (p=0.000) and decreased in patients who have no complication (p=0.001) while it has no statistical difference between cases and controls(0.224±0.071 vs.0.220±0.038
p=0.619), this result is agreed with Alhadas’s (0.210±0.054% vs. 0.200±0.045% p=0.020) and disagreed with Tetikoglu’s results (0.216±0.58Vs. 0.202±0.52 p=0.038).

These differences in the results may be due to ethnic differences between participants or due to using variable methods and machines in determination of the platelet indices.
5.2. Conclusion:
The study found that platelet indices have significant differences between diabetic patients and healthy individuals.
There is marked increase in the mean platelet volume and plateletcrit among the diabetic patients with complications.
By the end of this study we conclude that platelet indices (mainly MPV and PCT) could be concerned as prognostic markers for diabetic complications.
5.3. Recommendations:
The mean platelet volume and plateletcrit could be used as early prognostic marker as follow up.
The relationship between platelet indices and other types of diabetes should be clarified by more studies.
The relationship between platelet indices and each type of diabetic complications should be clarified separately.
Sample size should be increased.
References
5. Reference:
Budak, Y.U., Polat, H., Huysal, K.,(2016). The use of platelet indices: platelet crit, mean platelet volume and platelet distribution width in emergency non-


Appendices
Appendix (1)

Sudan University of Science and Technology

Collage of Graduate Study

Detection of Correlation between Platelet Parameters and Complication of
Type2 Diabetes Mellitus in Sudanese Patients in Khartoum State

Questionnaire for research

- Date………………………… - Sample number…………………………
- Gender……………………… - Age……………………………………
- Occupation………………

-Presence of disease:-

- Hypertension Yes [ ]  No [ ]
- Malaria Yes [ ]  No [ ]
- Heart disease Yes [ ]  No [ ]
- Other…………………..

-Medication:

- Aspirin Yes [ ]  No [ ]
- Clopidogrel Yes [ ]  No [ ]
- Hypertensive drug Yes [ ]  No [ ]
- Other…………….

-Presence of complication:

- Nephropathy Yes [ ]  No [ ]
- Neuropathy Yes [ ]  No [ ]
- Retinopathy Yes [ ]  No [ ]
- Peripheral vascular disease Yes [ ]  No [ ]
- Other…………….
- Signature……………..
Appendix (2)
Sudan University of Science and Technology
Collage of Graduate Study
Detection of Correlation between Platelet Parameters and Complication of Type2 Diabetes Mellitus in Sudanese Patients in Khartoum State

Informed Consent

اننا توبية عثمان إبراهيم اقوم بإجراء البحث التكميلي لنيل درجة الماجستير بجامعة السودان للعلوم والتكنولوجيا. اجري هذا البحث لإيجاد العلاقة بين الصفائح و مؤشراتها و مضاعفات مرض السكري من النوع الثاني و ادعوك لتكون جزءا من هذا البحث.

ساقوم بسحب عينة دم ورئي (3مل) لإجراء فحص تعداد الدم الكامل. ستسحب العينة بواسطة حقينة جديدة ومعقمة، قد تتعاني من بعض الاحماض في موقع دخول الأبرة وان احتمال حدوث التهاب في موقع الأبرة أمر نادر الحدوث.

*قد قرأت المعلومات السابقة (أو قرأت لي) واتبعت لفرصة لطرح أسئلة حول الموضوع ووافق.

طاوعية على المشاركة كمشارك في هذا البحث وهذا اقرار مني بالموافقة.

اسم المشارك: ..........................................................
التوقع: ..........................................................
التاريخ: ..........................................................