Sudan University of Science and Technology

College of Graduate Studies

Measurement of Mean Platelet Volume and Platelet Distribution Width among Uncontrolled Type 2 Diabetic Sudanese Patients

قياس حجم الصفائح الدموية وعرض توزيع الصفائح الدموية عند المرضى السودانيين بداء السكر من النوع الثاني غير المنضبط

A Dissertation Submitted in Partial Fulfillment of the Requirements for M.Sc. Degree in Medical Laboratory Science (Hematology and Immunohematology)

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الاية

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(وَلَسَوْفَ يُعْطِيكَ رَبُّكَ فَتَرْضَى (5))

صدق الله العظيم

(سورة الضحى: الاية 5)

I

Dedication

I dedicate my dissertation to:

My……

Beloved parents

Beloved husband

Beloved Sisters & brothers

Lovely kids Mohamed, Ahmed, Omer & Ammar

&

Those who played a great role in my life

Acknowledgment

Thanks, granted to the most beneficial and merciful, Almighty Allah, for giving me the power, health, strength, and patience to start and complete this work.

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Abstract

The morphological difference in mean platelet volume (MPV) and platelet distribution width (PDW) of platelet imply assessing the function of platelets. MPV and PDW generally reported high in uncontrolled type 2 diabetes mellitus patients.

This study is a hospital-based non-intervention analytical case-control study aimed to determine and compare MPV and PDW in uncontrolled type 2 diabetes mellitus patients in comparison with non-diabetic individuals among Sudanese during the period from January to July 2019.

Three ml of EDTA anticoagulated blood was taken from 200 participants who selected conviniously (100 are type 2 diabetic patients with uncontrolled glycemic status (HbA1c> 7%) as cases and 100 healthy non-diabetic individuals (HbA1c between 4.0%−5.6%) as controls). MPV and PDW measured by the automated hematological analyzer (CBC Sysmex XP-300). The data obtained was analyzed by the SPSS computer program.

The mean age of uncontrolled type 2 diabetes mellitus patients was (57.05 ± 14.262) years and in the non-diabetic individuals was (50.42 ± 13.723) years. The percentage of gender in uncontrolled type 2 diabetes mellitus patient's male was (41%), and female was (59%), and in non-diabetic individual's male was (49%), and female was (51%). The mean of HbA1c in uncontrolled type 2 diabetes mellitus patients was ($9.433\pm2.0113\%$), and in the non-diabetic individuals was ($5.106\pm0.4000\%$) with a statistically significant difference between cases and controls (P.value= 0.000). The mean of MPV in uncontrolled type 2 diabetes mellitus patients was ($10.067\pm1.2100fl$), and in the non-diabetic individual was ($9.966\pm1.1328fl$) with a statistically insignificant difference between cases and controls (P.value= 0.543). The PDW in uncontrolled type 2 diabetes mellitus patients was ($12.857\pm2.4368fl$), and in the non-diabetic individuals was ($12.725\pm2.2530fl$) with a statistically insignificant difference between cases and controls (P.value=0.689). In cases the correlation between HbA1c and PDW showed strong positive correlation (P.value= 0.028, r=0.781), and showed no correlation between HbA1c and MPV (P.value=0.182, r=0.135).

In conclusion, PDW and MPV are not affected by uncontrolled type 2 diabetes mellitus. There is a strong positive correlation between HbA1c and PDW and no correlation between HbA1c and MPV among uncontrolled type 2 diabetes mellitus patients.

مستخلص الدراسة

الفرق الشكلي في متوسط حجم الصفائح الدموية وعرض توزيع الصفائح الدموية له تاثير كبير علي تقييم وظيفة الصفائح الدموية عند مرضى السكر غير المنضبط. هذه دراسة حالة وحالة ضابطة تحليلية غير تداخلية مستشفوية هدفت الى تحديد ومقارنة حجم الصفائح الدموية و عرض توزيع الصفائح الدموية في مرضى السكر من النوع الثاني غير المنضبط في المرضى السودانيين خلال الفترة من يناير الى يوليو 2019.

تم اخذ 3 مل من الدم في مادة مانعة للتجلط (ثنائى امين الاثيلين رباعي حمض الخليك) من 200 مشارك تم اختيارهم عشوائيا (100منهم مصابين بمرض السكر من النوع الثاني غير المنضبط كعينة دراسية (HbA1C > 7%) و100 مشارك سليم غير مصاب بمرض السكر(HbA1c 4%-5.6%)كعينة ضابطة. تم قياس متوسط حجم الصفائح الدموية وعرض توزيع الصفائح بواسطة محلل امراض الدم الالي وتم تحليل البيانات باستخدام برنامج الحزمة الاحصائية للعلوم الاجتماعية .

وجد ان متوسط العمر لدى مرضى السكر من النوع الثاني غير المنضبط كان (57.05±14.262) سنة وفي غير المصابين بالسكر كان (50.42±13.723) سنة . وكانت النسبة المئوية للجنس في مرضى السكر من النوع الثاني غير المنضبط من الرجال (41%) ومن النساء (59%) وفي العينة الضابطة من الرجال (49%) ومن النساء (51%).

كان متوسط HbA1c عند مرضى السكر من النوع الثاني غير المنضبط (9.433 ±2.0113%) وعند العينة الضابطة (5.106±0.4000)% مع وجود فروق ذات دلالة احصائية بين العينات الدراسية والعينات الضابطة (القيمة الاحتمالية = 0.000) .ووجد ان متوسط حجم الصفائح الدموية في مرضى السكرمن النوع الثاني غير المنضبط (10.067±1.21) وفي الاصحاء (9.966±1.328) مع عدم وجود فروق ذات دلالة احصائية بين العينات الدراسية والعينات الضابطة (القيمة الاحتمالية= 0.543) .وكان عرض توزيع الخلايا في مرضى السكر من النوع الثاني غير المنضبط (12.857±2.4369) وفي الاصحاء (12.275±2.253) مع عدم وجود فروق ذات دلالة احصائية بين العينات الدراسية والعينات الضابطة (القيمة الاحتمالية =0.689) .في العينات الدراسية كانت هناك علاقة موجبة قوية بين HbA1c وعرض توزيع الخلايا (القيمة الاحتمالية=0.028 ,r=0.781) ولاتوجد علاقه بين HbA1c ومتوسط حجم الخلايا (القيمه الاحتمالية= 0.182 ,r=0.135).

خلصت الدراسة الى عدم تأثر عرض توزيع الخلايا ومتوسط حجم الخلايا بعدم انضباط السكر لدى مرضى السكر السودانيين من النوع الثاني ووجود علاقة قوية موجبة بين عرض توزيع الخلايا و HbA1cعند مرضى السكر من النوع الثاني غير المنضبط وعدم وجود علاقة بين متوسط حجم الخلايا و HbA1c لديهم.

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Chapter I

Introduction

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Chapter I

Introduction

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1.1 Introduction:

Diabetes Mellitus is one of the oldest diseases known to man, and it was first reported in Egyptian manuscripts about 3000 years ago (Ahmed, 2002). Diabetes Mellitus is a significant health problem in Africa and worldwide, and the prevalence of diabetes is expected to increase in Africa at a rapid rate. (Elmadhoun *et al*., 2016).

Diabetes mellitus is a disease of carbohydrate metabolism where glucose not utilized, leading to hyperglycemia, and as the disease progress, individuals become at risk for specific complications development (Burtis *et al*., 2008).

The most widely ranging complication that is due to chronic elevation of blood glucose levels which lead to blood vessels damage(angiopathy) is long-term vascular complications either macrovascular diseases (due to damage in arteries) or microvascular diseases (due to damage in small blood vessels) (Forbs and Cooper, 2013).

Diabetes complications are including retinopathy (which may lead to blindness), renal failure, neuropathy (nerve damage), atherosclerosis and at the extreme condition it may result in stroke, gangrene, or coronary artery disease (Burtis *et al*., 2008).

Diabetes mellitus is a significant health problem in Sudan and is leading to cause morbidity and mortality that showed there is a high prevalence of complications of type 2 diabetes especially in patients with uncontrolled diabetes for a long time (Awadallah *et al*., 2017).

Risk factors for type 2 diabetes mellitus include family history, the age that the prevalence increases with it, and lifestyle, which includes increased body mass index (BMI), physical inactivity, hypertension, poor nutrition, smoking, and distribution of body fats especially increased waist to hip ratio, all these factors increase the risk factors for diabetes (Deshpande *et al.,* 2008).

Glycosylated Hb is widely used for monitoring of long -term glycemic control and measure the risk of diabetes complications development (Burtis *et al*., 2008). Diabetes mellitus (DM) considered as a prothrombotic state that enhanced platelet activity (Jindal *et al*., 2011).

Platelet indices (platelet, mean platelet volume (MPV), and platelet distribution width (PDW)) are the indicators to detect platelet activity. MPV and PDW found to be predictive biomarkers for the detection of diabetic complications early. Also, both are more statistically significant in microvascular complications in comparison to macrovascular complications (Buch *et al*., 2017).

1.2 Rationale:

Diabetes mellitus is a chronic systemic disease that has multiple complications that vary from microvascular to macrovascular diseases, in-depth glycemic control consistently associated with decreased microvascular complications (Teleb *et al.,* 2016). Type 2 Diabetes Mellitus patients have a high risk of developing micro-and macro complications that lead to a decrease in the quality of life and increase morbidity, Hence platelet indices (MPV and PDW) have been available in the laboratory routine using blood cell counters ,these indices could alert us regarding microvascular complications (Walinjkar *et al.*, 2019). Diabetes is associated with a hypercoagulable state (Hughes *et al.,* 1983). Platelet indices (MPV and PDW) are neglected in diagnosis, although their increase is a known marker of platelet activation (Buttarello and Plebani, 2008). This study has been performed to determine and compare the MPV and PDW values to verify if it can predict and help in early diagnosis of microvascular complication in the individual with uncontrolled diabetes mellitus.

1.3 Objectives:

1.3.1 General objective:

To measure mean platelet volume and platelet distribution width in uncontrolled type 2 diabetic patients.

1.3.2 Specific objectives:

1-To estimate HbA1c in all study populations.

2-To estimate MPV and PDW in all study populations.

3-To compare mean of MPV and PDW between uncontrolled type 2 diabetes mellitus patients and non-diabetic individuals.

4-To correlate HbA1c with MPV and PDW among uncontrolled type 2 diabetic patients.

Chapter II

Literature review

Chapter II

Literature review

2.1 Diabetes mellitus background:

Diabetes mellitus (DM) constitute a global health problem with increasing in it in low and middle-income countries' ongoing epidemiological transition, including Sudan (Eltom *et al*., 2018). Diabetes mellitus is characterized by hyperglycemia, which results from defects in insulin secretion, insulin action, or both (Deshpande *et al.,* 2008).

World Health Organization (WHO) guidelines recommend the following categories of diabetes: Type 1 diabetes which characterized by inappropriate hyperglycemia as a result of pancreatic islet B-cell destruction and a tendency to ketoacidosis. The second type is Type 2 diabetes, which includes hyperglycemia cases that result from insulin resistance with an insulin secretory defect. Other specific type of diabetes (An intermediate stage), in which fasting glucose increased above normal limits but not to the level of diabetes, named impaired fasting glucose. Also, gestational diabetes mellitus (GDM), which usually retained for women who develop glucose intolerance during pregnancy (Bishop *et al.,* 2010).

2.1.1 Type 2 diabetes:

Type 2 diabetes (> 80% of DM cases) is a slow onset of heterogeneous disorder due to the interaction between environmental factors and polygenetic inheritance (Buch *et al*., 2017).Type 2 diabetes is a multifactorial disease due to environmental and genetic risk factors that it has many environmental risk factors include environmental risk factors which cause pathogenesis (lifestyles like physical inactivity, age, diet smoking, and alcohol consumption), internal environmental factors (inflammatory factors, adipocytokines, and hepatocyte factors) and external environmental factors (as environmental endocrine disruptors) (Bi *et al.,* 2012).

2.1.2 Diabetes complications:

In patients with chronic hyperglycemia, many cellular components of blood are damaged causing several vascular complications that lead to many pathophysiological disturbances, which are among the leading causes of morbidity and mortality (Ziaee *et al.,* 2017).

Diabetes affects many parts of the body and leads to severe conditions that classified to macrovascular complications that include cardiovascular disease, stroke, and also a peripheral vascular disease which may lead to injuries that may not heal, gangrene, and then amputation, and microvascular complications which includes nervous system damage (neuropathy), eye damage (retinopathy), and renal system damage (nephropathy) (Deshpande *et al.,* 2008).

One of the leading causes of the increased rate of disease and health care costs is microvascular complications of DM so an early indication of the presence of complications would help in reducing microvascular complications (Koltai *et al.,* 2006).

For diagnosis of diabetic mellitus, there are three possible ways to diagnose diabetes which in the absence of unambiguous hyperglycemia have to be confirmed on a subsequent day by any method of the three methods in (table 2.1), using of HbA1c is not recommended at this time according to American Diabetes Association in ("Diagnosis and Classification of Diabetes Mellitus," 2010) .

Table 2.1: Criteria for the diagnosis of diabetes:

1	FPG $\geq$126 mg/dl (7.0 mmol/l). Fasting define as no caloric intake for at least 8 h.
	OR
2	Symptoms of hyperglycemia and a casual plasma glucose $\geq$200 mg/dl (11.1 mmol/l). Casual define as any time of day without regard to time since last meal. The classic symptoms of hyperglycemia include polyuria, polydipsia, and unexplained weight loss.
	OR
3	2-h plasma glucose $\geq$200 mg/dl (11.1 mmol/l) during an OGTT. The test should perform as described by the World Health Organization, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.

In the absence of unequivocal hyperglycemia, criteria for diabetes diagnosis should be confirmed by repeat testing on a different day.

2.1.3 Glycosylated hemoglobin (HbA1c):

Prevention of diabetic complications can achieve under reasonable long-term glycemic control, many extensive large-scale clinical studies have valued HbA1c as an indicator of glycemic control (HbA1c result from hemoglobin glycation and represent two months glycemia) (Sato, 2014). Glycated protein is formed post-transitionally through the slow, non-enzymatic reaction between glucose and amino group on proteins. HbA1c is the gold standard for diabetic control evaluation because its levels are correlated with the development

of diabetic complications (to avoid complications it should not be more than 7%) (Farcet *et al.*, 2016).

According to American Diabetes Criteria (2013), diabetes mellitus type 2 can be either controlled diabetes with HbA1c ≤ 7% or uncontrolled diabetes with HbA1c > 7% (Radha and Selvam, 2016). Non-diabetes usually falls within the 4.0%–5.6% HbA1c range (Sherwani *et al.*, 2016). Some reports indicate that HbA1c is not a suitable marker to determine glycemic control in all patients because it influenced by changes in the erythrocytes life span and they take glycemic albumin (GA) as an alternative indicator of glycemic control in diabetic patients (Yazdanpanah *et al.*, 2017).

2.2 Platelet:

Platelets are derived from large cells (megakaryocytes) in the bone marrow which arises from differentiation from hemopoietic stem cell and undergo fragmentation of their cytoplasm to produce platelet under the control of humoral agents like thrombopoietin (Kamath *et al.*, 2001).

Platelets are a tiny, disc-shaped, non-nucleated structure with this in mind the morphological difference in MPV and PDW of platelet have an essential role in assessing the functional expressions of platelets (Radha and Selvam, 2016).

Large platelets more active than small platelets (Mangalpally *et al.*, 2010).In general, more giant platelets are younger, more reactive and aggregable than older platelets, consequently the younger platelets contain more granules, secrete more serotonin and β-thromboglobulin, and produce more thromboxane A2 than smaller platelets these can produce pro-coagulant effect which lead to thrombotic vascular complications, in brief changes in (MPV) reflect the state of thrombogenesis (Kodiatte *et al.*, 2012).

2.2.1 Platelet indices:

Platelet indices are biomarkers of platelet activation. They allow extensive clinical investigations focusing on the diagnostic and prognostic values in a variety of settings. These parameters are (platelet count, mean platelet volume MPV, platelet distribution width PDW, and plateletcrit PCT), are used to measure platelets amount, platelet morphology and proliferation kinetics (table 2.2) (Budak *et al.*, 2016).

Mean platelet volume (MPV) measures the average size and activity of platelets found in blood while platelet distribution width (PDW) is an indicator of platelet's size variation (Guclu *et al.*, 2013). Usually, high MPV and high PDW associated with more severe illness (Zhang *et al.*, 2015).

Table 2.2: **Platelet indices:**

Parameter	Description	Unit
Mean platelet volume (MPV)	Analyzer-calculated measure of thrombocyte volume	femtoliters (fL)
Platelet volume distribution width (PDW)	Indicator of volume variability in platelets size	femtoliters (fL)

Usually, platelet indices apply in hematological system disease diagnosis, in addition to this recently it has been discovered that these indices have a relation with the severity of illness and patient's prognosis (Zhang *et al.,* 2015).

MPV and PDW can be taken as an early biomarker that detects complications and more statistically significant in microvascular complication when compared to macrovascular complication (Buch *et al.,* 2017).

Increased MPV indicates increased platelet diameter, which can be used as a marker of platelet activation hence, during activation platelet's shape change from biconcave discs to spherical, and formation of pseudopod occurs that leads to increase MPV during activation (Budak *et al.,* 2016).

MPV is found to be significantly increased in patients with high HbA1c (Agrawal *et al.,* 2017). MPV is associated with the severity of diabetes strongly and independently (Shah *et al.,* 2012).

PDW is an indicator of volume variability in platelet size and is increased in the presence of platelet anisocytosis (Osselaer *et al.,* 1997).

PDW can be as a predictor of diabetic macrovascular complications onset that the high RDW indicates inflammation and increase the levels of oxidative stress both of them are signs of type 2 diabetic (Ziaee *et al.,* 2017). Also, PDW is a more specific marker for platelet activation, which causes morphologic changes of platelets, among both the spherical shape and pseudopodia formation since it does not increase during simple platelet swelling. Platelets with an increased number and size of pseudopodia differ in size, possibly affecting platelet distribution width (PDW) (Vagdatli *et al.,* 2010).

Platelet indices serve as a better risk indicator of initial vascular complications in diabetes mellitus patients also can be used as a simple and cost-effective tool to assess vascular events (Shilpi and Potekar, 2018).

Type 2 diabetes mellitus patients have higher MPV and PDW values in comparison with subjects without it (Zaccardi *et al.,* 2015).

The reference intervals (RIs) for:

mean platelet volume (MPV): 8.9-11.8 fL,

Platelet distribution width (PDW): 9.6-15.3 fL (Maluf *et al.,* 2015).

Ninety-five percent of the individuals had an MPV between 7.2 and 11.7 fL (Demirin *et al.,* 2011).

2.2.2 Complete blood count (CBC):

Platelet indices are obtained from automated complete blood cell count to approve that platelets have a diagnostic and prognostic value in certain diseases (Budak *et al.,* 2016).

This automated hematological analyzer can run 20 parameters per sample. It measures the cell counts using direct current (DC) detection method and measures hemoglobin concentration by the Non-Cyanide method.

Complete blood count (CBC) is requested to give information about the cells in the patient's blood to diagnose a medical condition and monitor medical conditions or treatment (VERSO, 1964). Blood count of various types of blood cells has been used for clinical purposes since the 19th century and using automated equipment for CBC was developed in 1959s and1960s (Buttarello and Plebani, 2008).

2.3 Diabetic and coagulation:

It was approved the concept of association between diabetes and hypercoagulable state Hughes *et al*., 1983).

Diabetes mellitus recognized as a prothrombotic tendency with increased platelet reactivity, which plays a role in microvascular complications of diabetes (Koltai *et al.,* 2006). Many factors cause this prothrombotic condition as increasing coagulation, impaired fibrinolysis, endothelial dysfunction, and platelet hyperreactivity (Keating *et al.,* 2003).

Hyperglycemia cause troubles in cellular metabolism because it enhances the generation of reactive oxygen species (ROS) and non-enzymatic glycation that lead to change in cellular structure and function, and organization of advanced glycation end-products (AGEs) (Gerner *et al.,* 2013).

Early detection of platelet and endothelial disorder refine the screening of patients at high risk that lead to early diagnosis, suitable treatment, and successful prevention of complications of diabetes mellitus (Kubisz *et al.,* 2015).

Platelets considered in the pathogenesis of vascular complications because in the diabetic patients it shows increased adhesiveness and an exaggerated aggregation, which is caused by

altered exposure and plenty of glycoprotein receptors to agonists and adhesive proteins on the platelet surface, decreased membrane fluidity and increased binding to fibrinogen. Altered platelet metabolism and changes in intraplatelet signaling pathways also the altered biophysical state of platelet membrane components in diabetes melitus may be one of the significant determinants of platelet hypersensitivity, hyperfunction and may contribute to impairments in various metabolic pathways (Sobol and Watala, 2000).

Early hypothesis approved that the availability of platelet membrane receptors may change accordingly to the alteration of the membrane lipid micro viscosity and this leads to platelet hypersensitivity, which results from non-enzymatic glycosylation-induced loss in membrane fluidity (Watala *et al.,*1996).

Membrane fluidity is essential to platelets because it modulates the cell function and associated with hypersensitivity to thrombin in platelets from diabetic patients (Winocour, 1992). Platelets aggregate and adhere to vascular endothelium more readily in type 2 diabetic individuals than those in healthy people, this as a result of losing sensitivity to the usual self-control by prostacyclin and nitric oxide which are generated by vascular endothelium presents as a significant defect in platelet function. Insulin one of the natural antagonists of platelet hyperactivity it enhances the endothelial generation of prostacyclin and nitric oxide and sensitizes the platelet to prostacyclin in all these defects in insulin action in diabetes create an environment of platelet activity disorders leading to vascular events (Vinik *et al.,* 2001).Hence, several mechanisms may account for the increased platelet activity in diabetes (Kim *et al.,* 2013).

There is a strong relationship between poor glycemic control and increased platelet activity in type 2 diabetes mellitus, in other words, platelet activity recovered with the improvement of glycemic control (Demirtunc *et al.,* 2009).

Platelet indices serve as a better risk indicator of initial vascular complications in diabetes mellitus patients also can be used as a simple and cost-effective tool to assess vascular events (Shilpi and Potekar, 2018).

Chapter III

Materials and Methods

Chapter III

Materials and methods

3.1 Methods

3.1.1 Study design

This study is a non-intervention analytical case-control study aimed to determine MPV and PDW in uncontrolled type 2 diabetic patients compared to non-diabetic individuals.

3.1.2 Study population

Two hundred subjects were collected. One hundred were uncontrolled type 2 diabetic patients, and one hundred were non-diabetic individuals.

3.1.3 Study area and duration

This study conducted in Khartoum state (Sudan) during the period from January to July 2019.

3.2 Inclusion and exclusion criteria

3.2.1 Inclusion criteria

Individuals with type 2 diabetes mellitus with uncontrolled glycemic status (HbA1c >7%) were included in this study.

3.2.2 Exclusion criteria

Individuals with any of these situations like non-diabetic, anemic, platelet and coagulation problems were excluded.

3.3 Data collection

Samples were collected conviniously. A structured non-self- administered questionnaire was used to collect demographic and clinical data.

3.4 Methodology

3.4.1 Sample collection

Three ml whole blood was collected in EDTA vacutainer from each participant under aseptic technique.

3.4.2 Sample analysis

3.4.2.1 HbA1c estimation

i-CHROMA™, which is an immunoassay system for quantitative measurement of hemoglobin A1c in human blood with i-CHROMA™ reader was used.

3.4.2.2 i-CHROMA™ principle

i-CHROMA™ HbA1c based on the fluorescence immunoassay technology, precisely the competition immune-detection method. Whole blood was added to the mixture of hemolysis buffer and detection buffer, which results in hemolysis of red blood cells. The mixture which contains HbA1c from the hemolyzed red blood cells and fluorescence-labeled HbA1c peptides from the detection buffer was loaded onto the sample well of the cartridge. The mixture then migrates through the nitrocellulose matrix of the test strip by capillary action. HbA1c from the blood competes with fluorescence-labeled HbA1c peptides for binding sites on HbA1c antibodies fixed on the nitrocellulose matrix. As a result, the higher concentration of HbA1c produces a lower fluorescence-labeled HbA1c peptide for binding sites on HbA1c antibodies fixed on the nitrocellulose matrix, and as a result, the higher concentration of HbA1c produces a lower fluorescence signal from HbA1c-peptides. The signal interpreted, and the result appears on i-CHROMAT™ reader in a unit of percentage (i-CHROMA TM HbA1c Procedure Guide Boditech Med Inc, n.d.).

3.4.2.3 MPV and PDW estimation

3.4.2.4 Automated procedure of CBC

blood cells are counted by using direct current detection methods with coincidence correction. Automatic discriminators separate the cell population based on complex algorithms. The intensity of the electronic pulse from each analyzed blood cell is proportional to the cell volume. The Sysmex cell counter analyzes WBCs, RBCs, and PLTs with uncompromised precision and accuracy.

In Direct Current detection method Blood samples were aspirated, measured to a 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 detected as electric pulses. Calculation of the blood cell is by counting the pulses.

MPV and PDW can influence by platelet concentration – the analysis of platelet size distribution becomes problematic in thrombocytopenic samples. The lack of standardization and the dependence of results on preanalytical variables and the measurement method used requires different reference intervals and allows for poor comparison of clinical studies carried out in non-standard conditions (Bossche *et al.,* 2002).

3.4.2.5 Quality control

The reliability of the instruments and reagents were monitored by quality control.

3.5 Ethical consideration

All participants were informed verbally and satisfied with the study objectives recruited in this study. Privacy and confidentiality for each participant were guaranteed.

3.6 Data analysis

Data collected was analyzed by statistical package for social science for a computer program (IBM- SPSS). Mean and standard deviation were calculated for quantitative variables. Frequencies were calculated for qualitative variables; independent sample t-test was used to compare qualitative and quantitative variables. A correlation test was used to compare two quantitative variables. P.value was considered to be significant when less than 0.05.

Chapter IV

Results

Chapter IV

Results

4 Results:

The study includes 200 subjects (100 diabetic individuals with HbA1c>7%, and 100 known healthy non-diabetic (HbA1c between 4%-5.6%)).

The mean of the age of uncontrolled type 2 diabetes mellitus patients was (57.05±14.262), and in the non-diabetic was (50.42±13.723). In uncontrolled type 2 diabetes mellitus patients, there was (41% male and 59% female), and in non-diabetic individuals (49% male and 51% female) Table 4.1.

HbA1c mean in uncontrolled type 2 diabetes mellitus patients (9.433± 2.0113), and in non-diabetic individual (5.106 ±0.40) P.value was significant (P.value= 0.000). MPV in uncontrolled type 2 diabetes mellitus patients (10.067± 1.2100), and in non-diabetic individuals (9.966± 1.1328) the P.value was insignificant (P.value=0.543), and the PDW in uncontrolled type 2 diabetes mellitus patients (12.857± 2.4368), and in non-diabetic individuals (12.275± 2.2530) the P.value was insignificant(P.value=0.689) as indicated in table 4.2.

The correlation between HbA1c and PDW was strong positive (sig=0.028, r=0.781) as indicated in (fig 4.1) and we found no correlation between HbA1c and MPV (sig=0.135, r=0.182) as indicated in (fig 4.2) in uncontrolled type 2 diabetes mellitus patients.

Table 4.1: Demographic data of study population:

Demographic data	Uncontrolled type 2 DM patients N (100)		Non-diabetic controls N (100)	
Age (mean±sd)	57.05±14.262		50.42±13.723	
Sex	Male N (%)	Female N (%)	Male N (%)	Female N (%)
	41	59	49	51

Table 4.2: The relation between HbA1c, MPV and PDW in uncontrolled type 2 DM and non-diabetic controls:

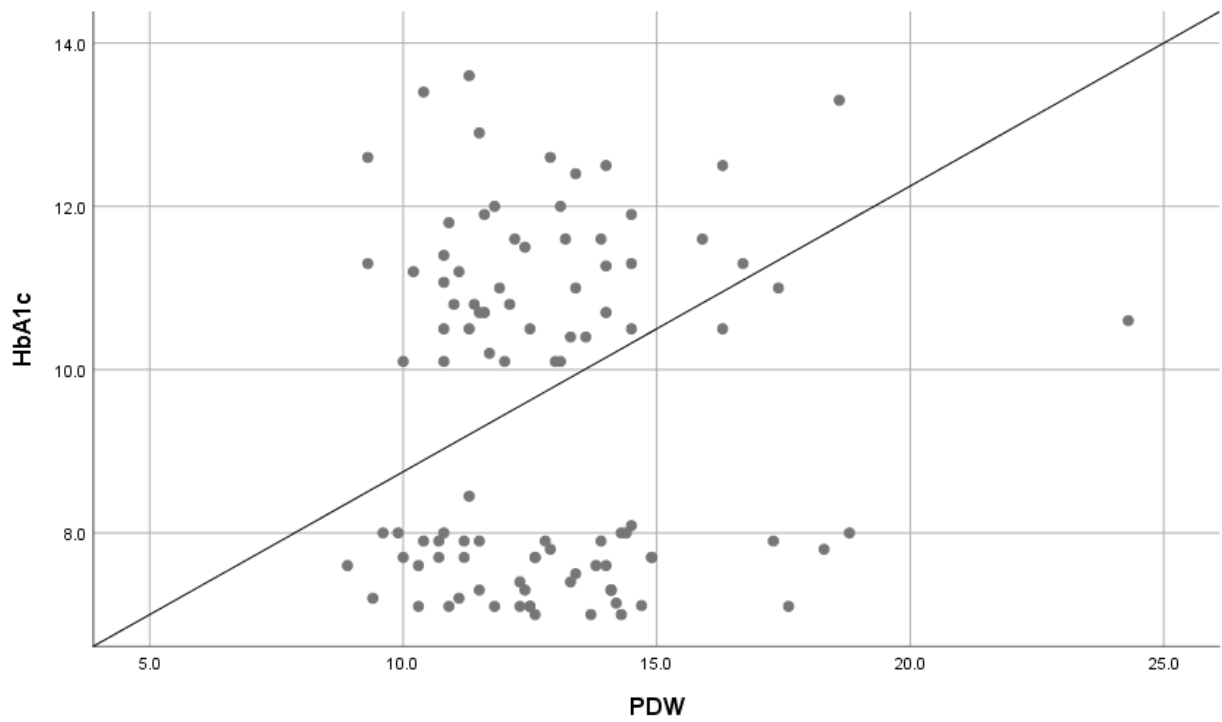| Parameter	Uncontrolled type 2 DM patients (mean ±sd)	Non-diabetic controls (mean±sd)	P.value
HbA1c	9.433± 2.0113	5.106 ±0.4000	0.000
MPV	10.067± 1.2100	9.966± 1.1328	0.543
PDW	12.857± 2.4368	12.275± 2.2530	0.689

Figure 4.1: The correlation between HbA1c and PDW among uncontrolled type 2 DM patients (sig=0.028, r=0.781).
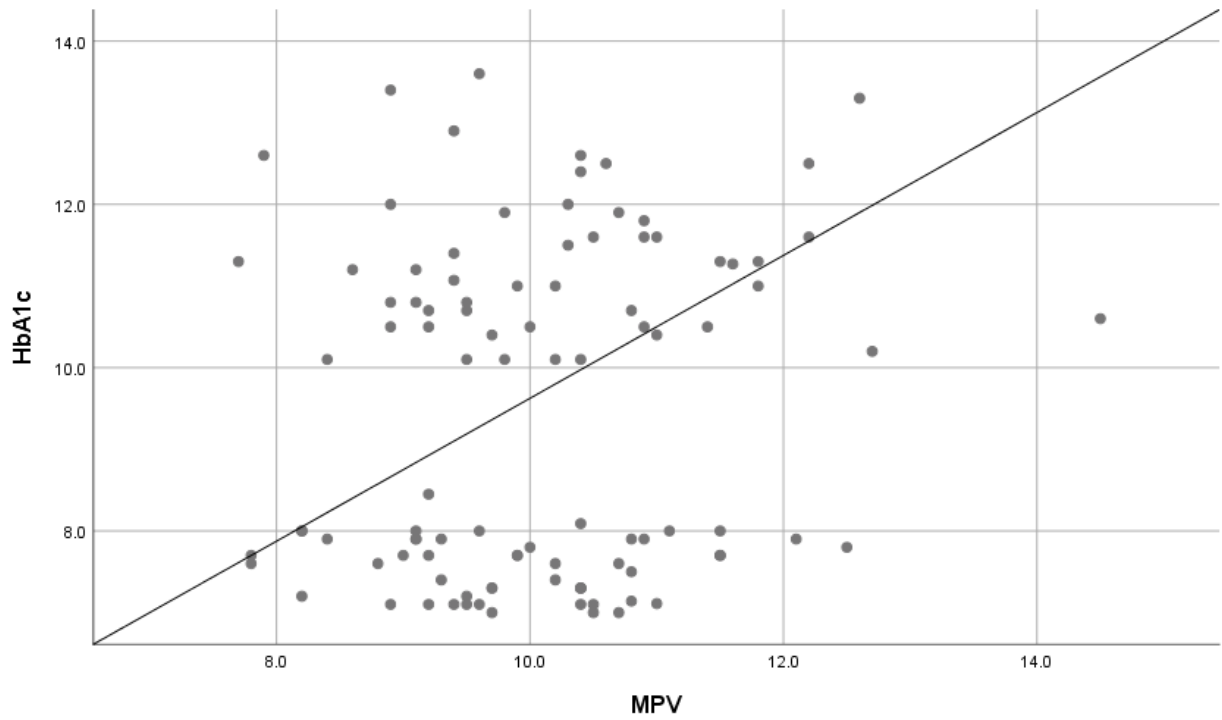
Figure 4.2: The correlation between HbA1c and MPV among uncontrolled type 2 DM patients (sig=0.135, r=0.182).

Chapter V

Discussion, Conclusion and Recommendations

Chapter V
Discussion, conclusion and recommendations

5.1 Discussion:

The present study aimed to determine and compare the MPV and PDW values to verify if it can predict and help in early diagnosis of microvascular complication in the individual with uncontrolled diabetes mellitus.

Regarding the age of study subjects we found that the mean of age in uncontrolled type 2 diabetes mellitus patients is 57.05 years, this result is compatible with the Centers for Disease Control (CDC, 2017) reported that , adults between 45 and 64 years of age receive the majority of new diabetes diagnoses in the U.S, and to (Radha and Selvam, 2016) study who reported that the mean of age in type 2 DM patients was 51.63years.

The study revealed that most uncontrolled type 2 diabetes mellitus patients were females (59%), this result agrees with (Kautzky-Willer *et al.,* 2016) who reported that type 2 diabetes mellitus is more common in women.

HbA1c in uncontrolled type 2 diabetes mellitus patients was significantly high. This result agrees with (Sato, 2014) who reported that HbA1c is an indicator to identify rapid changes in hyperglycemia.

We found that MPV in uncontrolled type 2 diabetes mellitus patients was not changed, this result agrees with (Akinsegun *et al*, 2014) who elicited that no significant difference between the mean platelet volume in diabetics and healthy individuals, and contradicted with (Zuberi *et al*., 2008) who concluded that MPV significantly increased in the impaired fasting glucose patients , and also disagree with (Yilmaz and Yilmaz, 2016) who reported that MPV lower in both type1 and type 2 diabetes mellitus patients vs control subjects,

PDW in uncontrolled type 2 diabetes mellitus patients was not altered, this result agrees with (Chen *et al.,* 2017), he found PDW is not significantly different between the diabetic group and non-diabetic group and contradicted with (Shilpi and Potekar,2018) who reported that PDW significantly higher in diabetics individuals compared to non-diabetics.

The study showed that there is no correlation between HbA1c and MPV in uncontrolled type 2 diabetes mellitus patients compared to non-diabetic Sudanese, this result agree with the results of (Ünübol *et al*., 2012) who reported that there is no correlation between poor glycemic control and MPV in diabetic patients, and contradicted with the results of (Radha and Selvam, 2016) (Demirtunc *et al*., 2009), (Jindal *et al.,* 2011), (Zaccardi *et al.,* 2015),

(Vernekar and Vaidya, 2013), and (Kodiatte *et al.,* 2012) which showed strong correlation between MPV and uncontrolled type 2 diabetes mellitus patients.

We found a strong positive correlation between HbA1c and PDW in uncontrolled type 2 diabetes mellitus patients. The increase of PDW in hyperglycemic patients could be the osmotic swelling due to raised levels of some glucose metabolites. This result agrees with (Demirtas *et al.,* 2015), who reported that PDW levels were significantly increased in type 2 DM patients with HbA1c ≥ 7%.

5.2 Conclusion:

From these results, we conclude that:

MPV and PDW were not affected by uncontrolled type2 DM when compared to a healthy non-diabetic individual.

There is a strong positive correlation between HbA1c with PDW and no correlation with MPV among uncontrolled type 2 DM Sudanese patients.

5.3 Recommendations:

On the basis, we recommend the following:

•   More studies should clarify the relationship between platelet indices and other types of diabetes.

•   The sample size should increase.

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Appendix

Appendix

Questionnaire:

Sudan University of Science and Technology

College of Graduate Studies

Measurement of Mean Platelet Volume and Platelet Distribution Width Among Uncontrolled Type 2 Diabetic Sudanese Patients

Sample number……………………………………

Name…………………………………………………

Age……………………………………………………

Gender………………………………………………

Occupation…………………………………………...……