



بسم الله الرحمن الرحيم

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Evaluation of Platelet Count and Platelets Indices in Type 2

Diabetes Mellitus in Sudanese Patients –Qadarif State

تقييم تعداد ومؤشرات الصفائح الدموية لدى مرضى السكري السودانيين من النوع الثاني –

ولاية القضارف

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الآية

قال تعالى :

[فَتَبَسَّمْ ضَاحِكًا مِنْ قَوْلِهَا وَقَالَ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ وَأَنْ أَعْمَلَ

صَالِحًا تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ (١٩)]

صدق الله العظيم

سورة النمل الآية " 19 "

Dedication

To the soul of my father

To my loved mother

To my loved husband

To my loved sister

Acknowledgment

First and foremost I am grateful to Allah for providing me health and strength to conduct the present study.

And thanks to my dear supervisor **Dr. Mubark Elsaed Elkarsany** and a special thank for **Al Faw Teaching Hospital**. 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

Background: Diabetes mellitus (DM) has been considered as a prothrombotic state with enhanced platelet reactivity. Platelets indices have been altered in patients with DM type 2.

Objectives: The aim of this study to evaluate the platelets indices among Sudanese patients with type 2 diabetes mellitus.

Materials and methods: This is a case control study carried out in AL-Faw Teaching hospital - Al Qadarif State during May 2018 to July 2018, 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.

Result: The results were showed a significant increase in mean platelets volume(MPV) among diabetic patients when compare with non-diabetics (9.45 ± 1.42 vs 7.976 ± 1.249 / fl), PDW / fl (13.97 ± 1.63 vs. 11.74 ± 1.62 / fl), P-LCR% (14.98 ± 0.91 vs. 12.240 ± 1.82 %) and significant decrease for platelets count (258.50 ± 32.164 vs. 284.12 ± 1.249) x 10^9 /L, PCT% did not vary with T2DM. The duration of DM type 2 did not ($P \leq 0.05$) alter any of the studied parameters. PLT count c/cmm, MPV fl, PDW fl, PCT % and PLCR% did not vary with the level of glycemic control.

Conclusion: the study was observed that Type 2 diabetes mellitus altered some of the platelets indices and count.

المستخلص

خلفية:

يعتبر داء السكري كحالة تجلطية مع تفاعل معزز للصفائح الدموية. مؤشرات الصفائح الدموية تتغير عند المصابين بداء السكري النوع الثاني.

الهدف من الدراسة:

تهدف هذه الدراسة لقياس مؤشرات الصفائح الدموية للمرضى السودانيين المصابين بداء السكري النوع الثاني.

المواد و الطرق:

هذه دراسته مراقبة اجريت في مستشفى الفاو التعليمي – ولاية القضارف, خلال الفترة من مايو 2018 حتى يوليو 2018, لتقييم التغير في عدد الصفائح الدموية, متوسط حجم الصفائح, عرض توزيع الصفائح, نسبة الصفائح الدموية, ونسبة الخلايا الصفيحية الكبيرة في المرضى السودانيين المصابين بداء السكري النوع الثاني. تم تسجيل 60 مريضا بالسكري النوع الثاني و 60 شخصا سليما في هذه الدراسة بعد اخذ موافقتهم شفويا.

النتيجة:

اظهرت النتائج زيادة كبيرة في متوسط حجم الصفائح الدموية و عرض توزيع الصفائح الدموية ونسبه الخلايا الصفيحية الكبيرة بين مرضى السكري عند مقارنتها مع غير المصابين بالسكري و انخفاض كبير في عدد الصفائح الدموية ونسبة الصفائح الدموية. لم تغير المده الزمنية لمرض السكري النوع الثاني من هذه المؤشرات المدروسة.

الخلاصة:

قد لاحظت الدراسة ان داء السكري من النوع الثاني قد يغير بعض مؤشرات و عدد الصفائح الدموية.

Abbreviation

ADP	Adenosine diphosphate
ATP	Adenosine tri phosphate
DR	Diabetic retinopathy
DIC	Disseminated intravascular coagulation
EDTA	Ethylene diamine tetra acetic acid
FBG	Fasting blood glucose
FL	Femto-liter
GP	Glycoprotein
HbA1c	Hemoglobin A1c(glycosylated hemoglobin)
HELLP	Hemolysis, elevated liver enzymes, and low platelets.
HPLC	High-performanceliquid chromatography
HUS	Hemolytic-uremic syndrome
IDDM	Insulin dependent diabetes mellitus
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
ITP	Immune thrombocytopenic purpra
MI	Myocardial infarction
MPV	Mean platelet Volume
OCS	Open canalicular system
OGTT	Oral glucose tolerance test
PCT	Plateletcrit
PDW	Platelet distribution width
PLCR	Platelet large cell ratio
SPSS	Statistical package of social science
TAR	Thrombocytopenia-absent radii
T2DM	Type 2 diabetes mellitus
TTP	Thrombotic thrombocytopenic purpura
vWF	Von willebrand Factor

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Chapter One

Introduction and literature review

Chapter One

Introduction and literature review

1. Introduction

Type 2 diabetes mellitus (T2DM) and its complications, which lead to increased morbidity and mortality in T2DM. (Vinikand and 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`1 and Patrono,2007)

2. literature review

1.2. Platelets (Thrombocyte)

1.2.1. 1 Definition of platelet

Platelets are small anucleate cell fragments that have a characteristic discoid shape and range from 1 to 3 μm in diameter, are formed from the cytoplasm of megakaryocytes (MKs), their precursor cells, which reside in the bone marrow (Pease, 1968).

1.2.1.2 Megakaryocytes (MKs) and Platelet Production

Platelets are derived from bone marrow megakaryocytes, which are large cells with multilobated nuclei and abundant finely granular light gray blue cytoplasm. With the Size (50–100 μm) and account for $\sim 0.01\%$ of nucleated bone marrow cells (Nakeff and Maat, 1976).

Megakaryocytes become polyploid by endomitosis (DNA replication without cell division) and then undergoes a maturation process, into multiple long processes called proplatelets. An MK may extend 10–20 proplatelets, then fragments of cytoplasm break off into platelets (Richardson, *et al.*, 2005).

As platelets develop, they receive their granule and organelle content as streams of individual particles transported from the MK cell body (Italiano, *et al.*, 2019).

This process is regulated mainly by thrombopoietin (produced predominantly in the liver and have critical role in megakaryocytic growth and differentiation)(Kern,2002).

1.2.1.3 Ultrastructure of resting platelet:

1.2.1.3.1 Peripheral zone:

Peripheral zone Responsible for adhesion and aggregation, Consists of fluffy glycocalyx coat, cytoskeleton and platelet membrane. Contains absorbed coagulation factors I, V, VIII, XI, XII, receptors for ADP, thrombin, vWF, collagen, fibrinogen, fibrin, fibronectin, epinephrine, thrombospondin, thromboxane A₂, prostacyclin, epinephrine, serotonin and glycosyltransferase(Escolar and white,2018).

1.2.1.3.2 Sol-Gel zone:

Responsible for contraction and support microtubule system. Contains the connecting system called the open canalicular system and the dense tubular system (McNicol Israels,1999).

1.2.1.3.3 Organelle zone:

Contains the dense body system, non-metabolic ADP, serotonin, catecholamines, calcium, alpha granules; platelet factor 4, platelet mitogenic factor, fibrinogen, beta thromboglobulin, lysosomal granules, mitochondria and glycogen granules(White,2017).

1.2.1.4 Platelet Function:

The main function of platelets is to contribute to hemostasis, the process of stopping bleeding at the site of interrupted endothelium. They gather at the site and unless the interruption is physically too large, they plug the hole (Weyrich and Zimmerman, 2004).

In addition to being the cellular effector of hemostasis, platelets are rapidly deployed to sites of injury or infection, and potentially modulate inflammatory processes by interacting with leukocytes and by secreting cytokines, chemokines, and other inflammatory mediators. Platelets also secrete platelet-derived growth factor (PDGF)(Wagner and Burger, 2003).

1.2.1.5 Process of platelet in hemostasis:

1.2.1.5.1 Platelet adhesion:

Endothelial cells are attached to the subendothelial collagen by von Willebrand factor (vWF). when the endothelial layer is disrupted, collagen and vWF anchor platelets to the sub endothelium. Platelet GPIbIX -V receptor binds with vWF and GPVI receptor binds with collagen (Dubois, *et al.*,2006).

1.2.1.5.2 Platelet activation:

1.2.1.5.2.1 Platelet induction:

Platelet activation begins seconds after adhesion occurs. It is triggered when collagen from the sub endothelium, and/or tissue factor from the media and adventitia bind with their respective receptors on the platelet (Dubois, *et al.*,2006).

1.2.1.5.2.2 Role of platelet in coagulation:

Move of the negatively charged phospholipids from the inner to the outer platelet membrane surface. These phospholipids then bind the tense and prothrombinase complexes, two of the sites of interplay between platelets and the coagulation cascade. Calcium ions are essential for the binding of these coagulation factors (Dubois, *et al.*,2006).

1.2.1.5.2.2 Platelet shape change:

Intraplatelet calcium concentration increases, stimulating the interplay between microtubule/actin filament complex with the platelet cell membrane and open canalicular system. The continuous changes in shape from the unactivated to the fully activated platelet is best seen on scanning electron microscopy. Activated platelets secrete the contents of their granules through their canalicular systems to the exterior (Matarese, *et al.*, 2009)

1.2.1.5.2.3 GPIIb/IIIa activation

Thromboxane A₂ synthesis increases during activation it is secreted and acts on both its own thromboxane receptors (the so-called "out-in" mechanism), and those of other platelets. These receptors trigger intraplatelet signaling, which converts GPIIb/IIIa receptors to their active form to initiate aggregation (Yip, *et al.*, 2005).

1.2.1.5.3 platelet aggregation:

Aggregation begins minutes after activation, and occurs as a result of turning on the GPIIb/IIIa receptor, which allows these receptors to bind with vWF or fibrinogen, there are 50–100 of these receptors per platelet. When any one or more of at least nine different platelet surface receptors are turned on during activation, intraplatelet signaling pathways cause existing GpIIb/IIIa receptors to change shape and thus become capable of binding (Yip, *et al.*, 2005).

1.2.1.5.4 Platelet-coagulation factor interactions:

In addition to interacting with vWF and fibrin, platelets interact with thrombin, Factors X, Va, VIIa, XI, IX, and prothrombin to complete clot formation via the coagulation cascade, many studies suggested platelets express tissue factor (Abbas, *et al.*, 2003).

1.2.1.6 Symptoms of platelet disorders:

Spontaneous and excessive bleeding can occur because of platelet disorders. This bleeding can be caused by deficient numbers of platelets, dysfunctional platelets (Murakawa, *et al.*, 2002).

All of the following suggest platelet bleeding, not coagulation bleeding:

the bleeding from a skin cut such as a razor nick is prompt and excessive, but can be controlled by pressure; spontaneous bleeding into the skin which causes a purplish stain named by its size: petechiae, purpura, ecchymoses (van Genderen, *et al.*, 2003).

Bleeding into mucous membranes causing bleeding gums, nose bleed, and gastrointestinal bleeding; menorrhagia, intraretinal, and intracranial bleeding (van Genderen, *et al.*, 2003).

1.2.1.7 Disorder of platelet:

1.2.1.7.1 Thrombocytopenia

Is defined as a platelet count less than the lower limit of the reference range (Kern, 2002).

1.2.1.7.1.1 Causes of Thrombocytopenia:

1.2.1.7.1.1.1 Inherited Thrombocytopenia:

Thrombocytopenia-absent radii (TAR) syndrome, wiskott-Aldrich syndrome, may-Hegglin anomaly, Bernard-Soulier syndrome, Gray platelet syndrome (Laidlaw, *et al.*, 2012)

1.2.1.7.1.1.2 Congenital nonhereditary Thrombocytopenia:

Intrauterine viral infection, maternal drugs or medications: (thiazide diuretics), maternal ITP or other immunologic diseases, neonatal alloimmune thrombocytopenia.

1.2.1.7.1.1.3 Acquired Thrombocytopenia:

Immune: Idiopathic, Infections: viruses (EBV, CMV, HIV), bacteria, rickettsiae, Mycoplasma, Lymphoproliferative disorders, Autoimmune (collagen vascular) diseases Post-transfusion purpura.

Non-immune: Infections, Disseminated intravascular coagulation (DIC), Thrombotic thrombocytopenic purpura (TTP), Hemolytic-uremic syndrome (HUS), Preeclampsia/eclampsia and the HELLP syndrome

Massive transfusion, Gestational thrombocytopenia

1.2.1.7.1.1.4 Platelet sequestration in the spleen

Hypersplenism: usually associated with anemia and/or leucopenia .

1.2.1.7.2Thrombocytosis (Thrombocythemia)

Thrombocytosis defined as a platelet count exceeding the upper limit of the reference range. either Primary thrombocythemia(may be associated with thrombosis or bleeding) or Thrombocytosis secondary to some other condition (reactive thrombocytosis) and is not associated with an increased risk of thrombosis or other complication.(like infection, inflammation, iron deficiency anemia) (Kern, 2002).

1.2.1.8Measurement of platelet

Platelet count is measured either manually using a hemacytometer, or by placing blood in an automated platelet analyzer using electrical impedance(Girling,2018).

The normal range (99% of population analyzed) for platelets in healthy individual is 150,000 to 400,000 per cubic millimeter (a mmequals a microliter) or $150\text{--}400 \times 10^9$ per liter (Ross ,*et al.*,2019).

Platelet function is evaluated by bleeding time test, platelet aggregation test (Duke, 2009).

1.2.1.9Platelets indices

The mean platelet volume is an indication of platelet size. Normal MPV ranges are approximately 7 to 11 fl (Corcoran and Marchant 2002).

Clinical Value of MPV

Thromboembolic diseases are among the major cause of mortality in developed countries. Early diagnosis of progressive activation of coagulation can help manage

these diseases successfully. A significant list of reliable markers have been investigated recently, concerning activation of coagulation, such as prothrombin fragment, thrombin-antithrombin complex (TAT), and platelet activation, such as β -thromboglobulin (β -TG) or soluble platelet P-selectin¹. However, laboratory measurement of these indices is laborious and expensive. Additionally, the above mentioned indices cannot be included in routine laboratory tests (Vagdatli *et al.*, 2010) the MPV can be an indication of platelet turnover because younger platelets tend to be larger. A spectrum of platelet sizes is seen in patients with rapid turnover (Corcoran and Marchant 2002).

The Largest platelets are more reactive and release a greater quantity of thrombogenic factors as they contain more dense granules and produce more thromboxane A₂. Increased MPV has been associated with greater *invitro* aggregation in response to ADP and collagen. Platelet volume seems to be correlated with megakaryocyte ploidy as, the increase of MPV in conditions with increased platelet turnover is probably mediated by several cytokines (interleukins 6 and 11 and thrombopoietin) that affect megakaryocyte ploidy and result in the production of larger and more reactive platelets (Hou *et al.* 2015). Several experimental and clinical studies have demonstrated that platelet size and function correlate since large platelets are hemostatically more reactive than platelets of normal size. Elevated MPV levels have been identified as an independent risk factor for thrombotic diseases (Buttarelo and Plebani, 2007).

1.2.1.9.2 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 marrow tend to be larger and to contain more RNA than older, smaller platelets, which discard their endoplasmic reticulum as they 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).

1.2.1.9.3 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).

1.2.1.9.4 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) isobserved in patients with Hyper-lipidaemia and suggest possible risk of thrombosis. an increase in P-LCR + MPV + PDW has been observed inautoimmune thrombocytopenic purpura (Chandrashekar , 2013).

1.2.1.10 Laboratory evaluations

1.2.1.10.1Bleeding 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).

1.2.1.10.2 Platelet aggregation

A known 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).

1.2.2. Diabetes Mellitus (DM)

1.2.2.1 Definition of DM

Diabetes mellitus is a metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action or both (Unger and Grundy, 2011).

Prediabetes is an intermediate stage, in which the fasting glucose is increased above normal limits but not to the level of diabetes, has been named impaired fasting glucose (IFG). Use of the term impaired glucose tolerance (IGT) to indicate glucose tolerance values above normal but below diabetes levels (Bishop, et al., 2010), also pre-diabetic refer to glycated hemoglobin (A1C) of 6.0% to 6.4%, each of which places individuals at high risk of developing diabetes and its complications (Santaguida and Balion, 2005).

1.2.2.2 Classification of Diabetes

1.2.2.2.1 Type 1 diabetes:

Encompasses diabetes that is primarily result of pancreatic beta cell destruction. This form includes cases due to an autoimmune process and those for which the etiology of beta cell destruction is unknown (Turner, *et al.*, 2016)

This disease is usually initiated by an environmental factor or viral infection in individuals with genetic predisposition and causes destruction of beta cells of the pancreas lead to decreased production of insulin (Bishop, *et al.*, 2010)

Characteristics of type 1 diabetes include abrupt onset, insulin dependence, and ketosis tendency (Bishop, *et al.*, 2010)

1.2.2.2 Type 2 diabetes:

Is characterized by hyperglycemia as a result of an individual's resistance to insulin with an insulin secretory defect. This resistance results in relative insulin deficiency (Bishop, *et al.*, 2010).

This type of diabetes often goes undiagnosed for many years and is associated with a strong genetic predisposition, usually occur in adult increase risk with obesity, and lack of physical exercise (Bishop, *et al.*, 2010). q

1.2.2.3 Gestational diabetes mellitus:

Refers to glucose intolerance with onset or first recognition during pregnancy (Turner, *et al.*, 2016).

Glucose intolerance during pregnancy due to metabolic and hormonal changes, frequently return to normal postpartum (Bishop, *et al.*, 2010).

Distinguishing between type 1 and type 2 diabetes is important because management strategies differ (Patel, 2010).

1.2.2.3 Diagnosis of diabetes:

A fasting plasma glucose (FPG) level of 7.0 mmol/L correlates most closely with a 2-hour plasma glucose (2hPG) value of ≥ 11.1 mmol/L in a 75 g oral glucose tolerance test (OGTT). (Engelgau, *et al.* 2012).

Random plasma glucose. RPG ≥ 11.1 mmol/L, glycated hemoglobin A1C $\geq 6.5\%$ (in adults) (Forouhi and, 2006)

A1C values also are affected by age, rising by up to 0.1% per decade of life (Davidson and Schriger 2010).

1.2.2.4 Diagnosis of gestational diabetes:

Performance of a 3-hour OGTT (oral glucose tolerance test) and is diagnosed when any two of the following four values are met or exceeded: fasting, ≥ 95 mg/dL; 1 hour ≥ 180 mg/dL; 2 hours ≥ 155 mg/dL; or 3 hours ≥ 140 mg/dL (Bishop, *et al.*, 2010).

1.2.2.5 Signs and symptoms of type 1 and type 2 diabetes include:

Increased thirst, Frequent urination, Extreme hunger, Unexplained weight loss, Presence of ketones in the urine, Fatigue, Blurred vision, Slow healing sores, High blood pressure, Frequent infections such as gums or skin infections and vaginal or bladder infections (Longo, 2012).

1.2.2.6 Complications of DM

1.2.2.6.1 Cardiovascular disease associated DM:

Including coronary artery disease with chest pain (angina), heart attack, stroke and narrowing of arteries (atherosclerosis) (Bergenstal, 2010)

1.2.2.6.2 Nerve damage (neuropathy) associated DM:

Excess sugar can injure the walls of the tiny blood vessels (capillaries) that nourish your nerves, especially in the legs called diabetic neuropathy (Bergenstal, 2010)

1.2.2.6.3 Kidney damage (nephropathy) associated DM:

The kidneys contain millions of tiny blood vessel clusters (glomeruli) that filter waste from your blood. Diabetes can damage this delicate filtering system. Severe damage can lead to kidney failure or irreversible end stage kidney disease (Bainbridge, 2008)

1.2.2.6.4 Eye damage (retinopathy) associated DM:

Diabetes can damage the blood vessels of the retina (diabetic retinopathy), potentially leading to blindness. Diabetes also increases the risk of other serious vision conditions, such as cataracts and glaucoma. (Bergenstal,2010).

1.2.2.7Treatment:

An important part of managing all types of diabetes is maintaining a healthy weight through a healthy diet and exercise plan:

1.2.2.7.1Insulin:

Most people with type 1 diabetes need insulin therapy to survive. Some people with type 2 diabetes also need insulin therapy, either rapid-acting insulin, long-acting insulin and intermediate options (Elleri,2012).Insulin decreases plasma glucose levels by increasing the transport entry of glucose in muscle and adipose tissue by way of nonspecific receptors, It also regulates glucose by increasing glycogenesis, lipogenesis, and glycolysis and inhibiting glycogenolysis(Bishop, *et al.*,2010)

1.2.2.7.2Oral hypoglycemic agent:

Some diabetes medications stimulate pancreas to produce and release more insulin, Others inhibit the production and release of glucose from your liver, or block the action of stomach or intestinal enzymes that break down carbohydrates or make tissues more sensitive to insulin (Elleri,2012)

1.2.2.7.3Transplantation of pancreas:

For type 1 diabetes, a pancreas transplant may be an option (Elleri, *et al.*,2012).

1.2.2.8 Association between platelet activity and diabetic:

DM is a complex metabolic syndrome characterized by chronic hyperglycemia resulting in complications affecting the peripheral nerves, kidneys, eyes, and micro- and macro vascular structures (Demirtunc, *et al.*,2009).

Diabetes and its vascular complications can cause a financial havoc, become a burden to a country's national economy and dent its growth.

MPV can be used as a simple economical test in the monitoring of DM and thereby help in reduce the morbidity and mortality(Zuberi, *et al.* ,2008).

Type 2 DM is characterized mainly by impaired insulin secretion and increased tissue insulin resistance(Demirtunc, *et al.*,2009).

Sustained hyperglycemia leads to a series of interrelated alterations that can cause evident endothelial dysfunction and vascular lesions in diabetic complications(Bae, *et al.*,2003).

In response to stimuli generated by the endothelium of blood vessels, platelets change shape, adhere to subendothelial surfaces, secrete the contents of intracellular organelles, and aggregate to form a thrombus(Mitchell, *et al.*,2010).

These pro-aggregatory stimuli include thrombin, collagen, epinephrine, ADP (dense storage granules), and thromboxane A₂ (activated platelets) (Mitchell, *et al.*,2010).

Thus, platelets may assume an important role in signaling of the development of advanced atherosclerosis in diabetes (Colwell and Nesto ,2003).

MPV is an indicator of the average size and activity of platelets. Larger platelets are younger, more reactive and aggregable. Hence, they contain denser granules, secrete

more serotonin and β -thromboglobulin, and produce more thromboxane A₂ than smaller platelets(Chang, *et al.*,2010).

All these can produce a pro-coagulant effect and cause thrombotic vascular complications. This suggests a relationship between the platelet function especially MPV and diabetic vascular complications thus indicating changes in MPV reflect the state of thrombogenesis(Hekimsoy, *et al.*,2004)

High MPV is emerging as a new risk factor for the vascular complications of DM of which atherothrombosis plays a major role(Zuberi, *et al.*, 2008).

Thus, diabetes mellitus has been considered as a “prothrombotic state” with increased platelet reactivity(Jindal, *et al.*,2011).

Although the underlying mechanism of higher MPV in diabetic subjects is incompletely understood, but thought to be due to osmotic swelling as a result of hyperglycemia (Martyn, *et al.*,2017)

Hyperglycemia can increase platelet reactivity by inducing nonenzymaticglycation of proteins on the surface of the platelet, by the osmotic effect of glucose and activation of protein kinase C Such glycation decreases membrane fluidity and increases the propensity of platelets to activate(Vinik, *et al.* ,2001)

Alternatively, increased platelet size may reflect the presence of high platelet turnover and younger platelets (Guthikonda, *et al.*,2008)

Platelet function is directly regulated by insulin via a functional insulin receptor (IR) found on human platelets.

In vivo experiments have confirmed that insulin inhibits platelet interaction with collagen and attenuates the platelet aggregation effect of agonists in healthy nonobese individuals. This experiments done by (Vinik, *et al.*,2001) and(Kakouros, *et al.*,2011)

Platelets from patients with diabetes express more surface P-selectin and glycoprotein (GP) IIb/IIIa receptors and are more sensitive to agonist stimulation than platelets from patients without diabetes(Yngen, *et al.*,2006)

Although several measurements of platelet activity have emerged as potential contributors to atherothrombosis, many of these measurements are time-consuming, expensive, uses high sample volume, or require specialty training (Michelsonm , 2009).

Alternatively, Mean Platelet Volume (MPV) is a marker of platelet size that is easily determined on routine automated hemograms and routinely available at a relatively low cost(Jakubowski, *et al.*,1983)

1.2.3 Glycated hemoglobin (hemoglobin A1c):

Is a form of hemoglobin that is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose(Larsen, *et al.*,2015).

1.2.3.1 Measurement of A1C:

Immunoassays, Affinity chromatography, Ion-exchange chromatography, Electrophoresis Separation Isoelectric focusing, high-pressure liquid chromatography (HPLC) (Bishop, *et al.*,2010) HbA1c is to be reported in the International Federation of Clinical Chemistry (IFCC) units, and Diabetes Control and Complications Trial (DCCT) (Geistanger,*et al.*,2008).

1.3. Rationale:

Altered platelet morphology and function have been reported in patients with diabetes mellitus and associated with the risk of vascular disease (Colwell, and Nesto , 2003).

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 type2 D.M.

1.4. Previous studies

Hekimsoy, Papanas (2004) were performed a study and found a significant decrease of mean platelet volume (MPV) in patients who had a diabetes mellitus type2, it was incompatible with a study performed by Zuberi (2008), Demirtunc (2009), were found a significant increase of MPV.

Sonali (2011), was found a significant increase in platelet distribution width (PDW) in diadetic group when compare with non-diabetic group.

Yenigun (2014), Tejeswini (2016), did not show any significant differences of PCT between the diabetics and non-diabetics groups.

A study done by Dalia Dafallah (2013) showed that PLC-R was significantly increase in patients who had a diabetes mellitus.

Buch (2017), studied a correlation between the duration of DM type 2 and platelet indices levels, he wasn't found any correlation between them.

1.5. Objectives:

1.5.1 General objectives:

To evaluate the Platelets, count and indices in Type 2 Diabetes Mellitus patients and control in Al-faw.

1.5.2 Specific objectives:

- 1.To evaluate platelets count, platelets indices (MPV, PDW, PCT and P-LCR) and HbA1c among study volunteers.
- 2.To compare the PLT count and indices in diabetic group with healthy individual (control).
- 3.To assess the effect of DM duration on PLT count and indices and the effect of glycemia control level on PLT count and indices.

CHAPTER TWO

Materials and methods

CHAPTER TWO

Materials and Methods

2.1 Study Design and duration

This was case control study conducted in Al-faw town during (May 2018 to July 2018).

2.2 Study Area

Al-faw Teaching Hospital, Gadarif state.

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 a patient consent by a coded written questionnaire.

2.7 Sample collection

Blood sample were collected from vein from case and control groups 2.5 ml.

Blood drained into EDTA vaccontainer and 2.5 ml drained into fluoride oxalate vaccontainer.

2.8 Methods

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

2.8.2.1 Principle of sysmex: - automated Cell Counter:

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

1. 4.0 μ l of blood measured by the sample rotor valve is diluted into 1:500 with 1.996 μ l of diluents and brought to the mixing chamber as diluted sample (first step dilution).
2. Out of the 1:500 dilution sample 40 μ l is measured by the sample rotor valve, diluted into 1:25000 with 1.960 μ l of diluent then transferred to the RBCs/plt transducer chamber (second step dilution).

250 μ l 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. quality control test was performed 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 Medical 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:

The statistical analysis of the results was performed by using the Statistical Package for Social Sciences (SPSS) version 16 using independent T-test for testing difference significance (r value as the coefficient). P value < 0.05 was considered statistically significant.

Chapter Three

Results

Chapter Three

Results

3.1 The baseline characteristics of study subjects

This study included 60 patients with type II DM and 60 control Subjects.

The diabetic group was composed of 31 (51.7%) males and 29 (48.3%) Females and the control group was composed of 25 (41.66%) males and 35

(48.33%) females. The mean age of diabetic patients was 60.52 ± 1.70 (years)

And the mean age of the control group was 50.70 ± 10.69 (years).

Diabetic patients were divided into two groups according to the duration of DM \leq 10 years: 22 (36.7%) and >10 years: 38 (63.3%).

according to HbA1c values, 24 (40%) were good controlled patients

While 36(60 %) were poorly controlled patients.

Table (3-1): Frequency and gender distribution among study volunteers.

Parameters	Diabetic	Non-diabetics
Frequency	60	60
Male	31	25
Female	29	35

Table (3-2): Distribution of Diabetic patients according to age, HA1c and Duration.

Parameters	Frequency	Percentage
Age group		
≤ 40 Years	5	8.3%
> 40 Years	55	91.7%
HA1c		
≤ 6.5	24	40%
> 6.5	36	60%
Duration		
≤ 10 Years	22	36.7%
> 10 Years	38	63.3%

3.2. The comparison between the diabetics and non-diabetics in the Platelets count and indices:

This is presented in Table (3-5) as follows:

Diabetics registered significantly higher (P. value = 0.000) MPV value (9.453 ± 1.427 fl) than the non – diabetic (7.976 ± 1.249), significantly higher (P. value=0.000) PDW values (3.971 ± 1.630 fl) than the non-diabetics (11.741 ± 1.627 fl)

and significantly higher (P. value=0.000) P-LCR values (14.980± 0.915%) than the non-diabetics (12.240 ± 1.826) .

A significant (P. value=0.011) decrease Platelet account $\times 10^3$ /cumm value was observed in the diabetics (258.50 ±32.164%) compared with the non-diabetics (284.12 ±69.125%).

No significant (P value = 0.317) variation were observed between the diabetics and non-diabetics with regard to PCT (0.287 ± 0.178 % vs 0.25 ±0.166%)

Table (3.3) Comparison of PLT count and PLT indices between Diabetics and non-diabetics.

arameter(Mean ±SD)	Case	control	<i>P. Value</i>
PLT count $\times 10^3$ /cumm	258.50 ±32.164	284.12 ±69.125	0.011
MPV / fl	9.4533 ±1.42787	7.9767 ±1.24973	0.000
PDW / fl	13.9717 ±1.63075	11.7417 ±1.62744	0.000
PCT %	.2827 ±.17867	.2510 ±.16665	0.317
P-LCR %	14.9800 ±.91500	12.2400 ±1.82638	0.000

* Significant level at (P-value \leq 0.05).

3.3 Associations of platelet indices with control status according to HbA1c among the diabetic group.

This is presented in Table (3-6) as follows:

Control status of diabetes was not associated with platelet count ($267.58 \times 10^3/\mu\text{L}$ vs. $252.44 \times 10^3/\mu\text{L}$, $P = 0.69$). There is no difference in mean values of MPV ,PDW, PCT and P-LCR between patients with HbA1c < 6.5% and those with HbA1c > 6.5%, (9.558 (fl) vs. 10.4 (fl), ($P = 0.646$) (14.412 (%) vs. 13.67 (%), ($P = 0.095$) , (0.30% vs 0.27%) and (14.82% vs 15.08) respectively.

Table (3.4) Associations of platelet indices with control status according to HbA1c among the diabetic group.

Parameter(Mean \pm SD)	Good Control	Poor Control	<i>P. Value</i>
	Mean \pm SD HbA1c \leq 6.5	Mean \pm SD HbA1c >6.5	
PLT count $\times 10^3$ /cumm	276.58 \pm 29.717	252.44 \pm 32.700	0.069
MPV / fl	9.55 \pm 1.99	9.38 \pm 1.621	0.046
PDW / fl	14.412 \pm 1.693	13.677 \pm 1.541	0.065
PCT %	0.3017 \pm 0.185	0.27 \pm 0.175	0.511
P-LCR %	14.825 \pm 0.925	15.833 \pm 0.906	0.288

* Significant level at (P-value \leq 0.05).

3.4 Type 2 diabetes duration on platelets count and indices: This is presented in Table (3-7) as follows:

Table (2) shows that duration of type 2 diabetes did not exert any significant variation in the mean PLT-count (*P. value*=0.131), MPV (*P. value* =0.443), PDW (*P. value* =0.229), PCT (*P. value* =0.743) or P-LCR (*P. value* = 0.318).

Table (3.5) No association between platelets count , platelets indices and type 2 diabetes duration

Parameter(Mean ±SD)	> 10 years	≤ 10 years	
PLT count ×10 ³ /cumm	250.23 ± 24.158	263.29±35.41	0.131
MPV / fl	9.640 ± 1.470	9.644± 1.644	0.443
PDW / fl	13.636± 1.588	14.165 ± 1.644	0.229
PCT %	0.292 ± 0.134	0.271±0.201	0.743
P-LCR %	15.136±0.833	14.898 ± 0.957	0.318

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

CHAPTER FOUR

Discussion, Conclusion and Recommendation

4.1 Discussion

Diabetes mellitus (DM) is a major global health problem. Altered platelet morphology and function have been reported in patients with diabetes mellitus and associated with the risk of vascular disease. (Colwell, *et al.*,2003)

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. (Buch, *et al.*,2017).

A great number of studies appeared in the literature review exposing the relationship between alteration of platelet indices and diabetes mellitus type 2, in this study there was high statistically difference in the mean platelet count and indices between the study group and the control group. MPV was significantly increase in test group when compared with reference one. which agree with study performed by Hekimsoy (2004), Papanas (2004), Zuberi (2008).

The mean platelet count is significantly decrease in diabetic group when compare to non-diabetic group that was similar to the studies done by Jindal (2011), but other studies done by Zuberi (2008), Demirtunc (2009) were observed opposite finding.

Sonali (2011), Dalia Dafallah (2013), were performed a study compatible with this study in platelet distribution width (PDW), it was significantly increase.

Dalia Dafallah (2013) was found a significant increase in PLC-R and PDW that is similar to this study.

No association between platelet count, MPV or PDW with poor or sufficient diabetic controls. This is in agreement with a previous study performed by Papanas (2004).

This study came to the conclusion that there is no a significant association between the duration of DM type 2 and platelet count and indices, that is compatible with study performed by Buch (2017).

4.2Conclusions

- Platelet count was significantly lower in diabetic patients compared with non-diabetic subjects
- MPV, PDW and P-LCR were significantly higher in diabetic patients compared with non-diabetic subjects.
- Platelet count and platelet indices did not affected by HA1c status.
- The duration of T2DM did not alter any of the studied parameters

4.3 Recommendations

- Regular checkup of hematological parameters for T2DM patients should be performed to avoid the sudden crises which may occur.
- Platelet function using standards methods should be used platelet induced aggregation tests and floctometry method with platelet indices should be investigated among type II DM.
- Large studies needed to determine the contribution of platelet hyper activation to each type of Cardio Vascular disease, e.g. nephropathy, retinopathy, and neuropathy is needed and using large sample size.
- Using more advanced technique for platelets function as the confirmatory test.

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Appendix

Appendix 1:-

Sudan University of science & technology

Collage of Graduate studies

تقييم تعداد ومؤشرات الصفائح الدموية لدى مرضى السكري السودانيين من النوع

الثاني – بمستشفى الفاو

Questionnaire

- ❖ Name:
- ❖ Age:.....
- ❖ Sex : M F
- ❖ Duration of disease

*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).

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.

Appendix 4:-

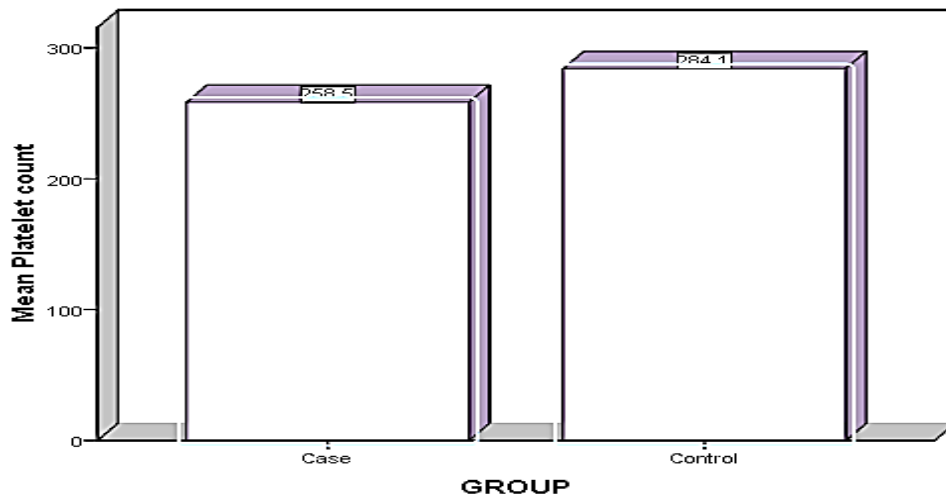


Fig (1): Mean Platelet count of study group

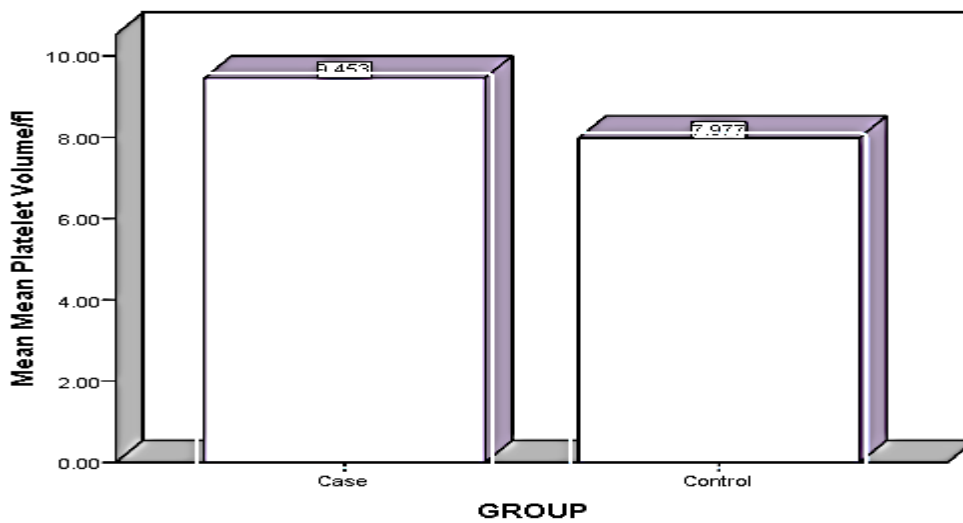


Fig (2): Mean Platelet Volume of study group

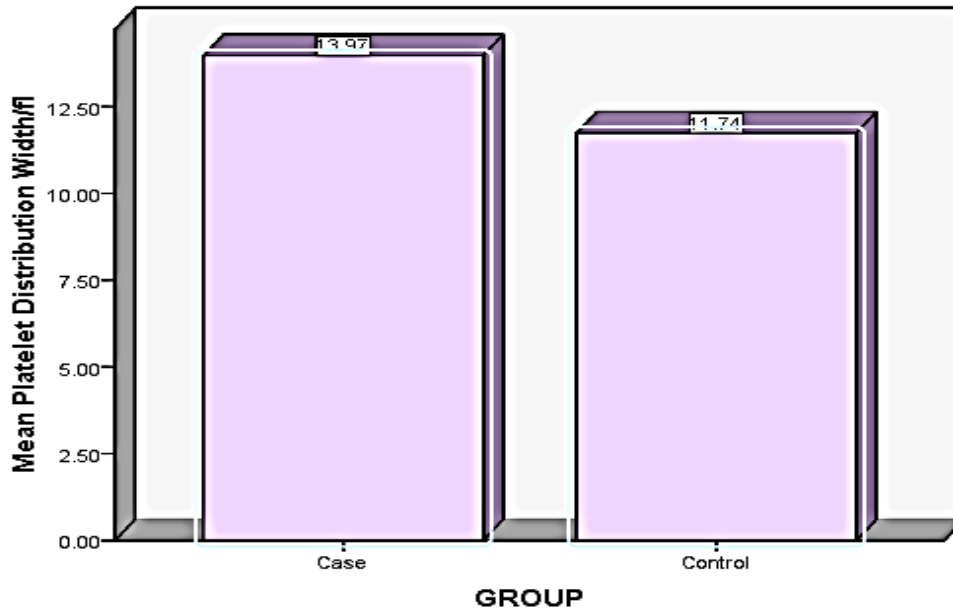


Fig (3): Mean Platelet Distribution Width of study group

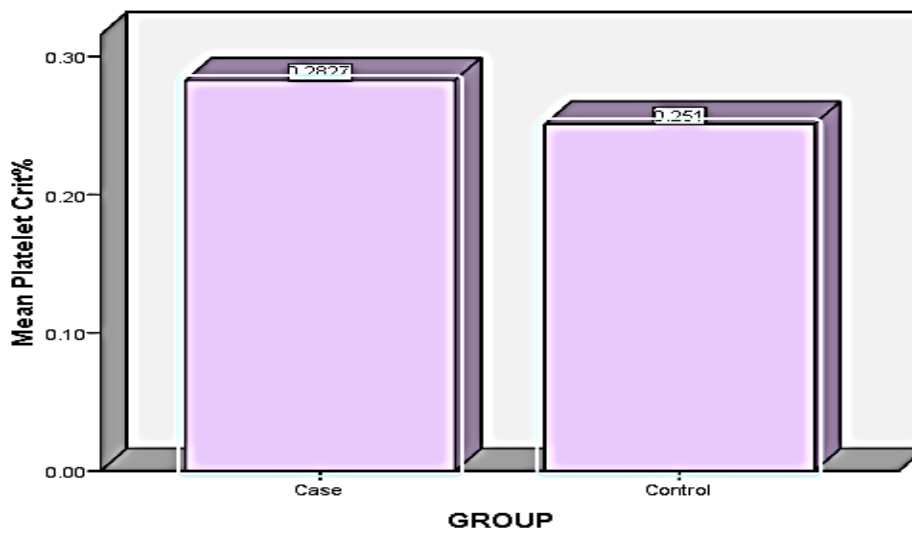


Fig (4): Mean Platelet Crit of study group

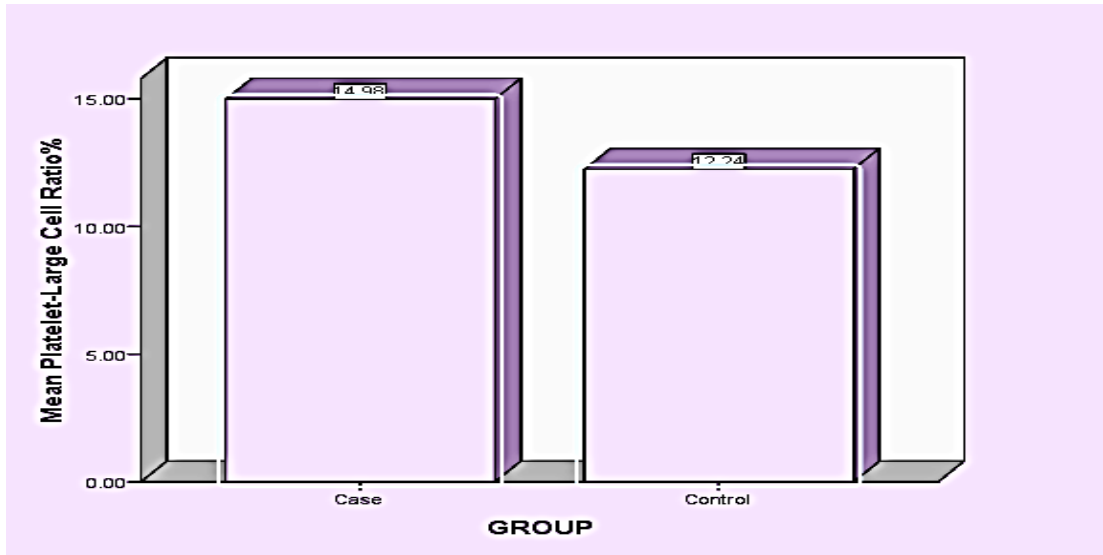


Fig (5): Mean Platelet-Large Cell Ratio of study group