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COLLEGE OF HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of platelet parameters among Type 2 Diabetes Mellitus Patient at Addis Ketema Woreda 3 Health Center, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Hanna Abebe, entitled: **"Assessment of platelet parameters among Type 2 diabetes mellitus patient at Addis ketema woreda 3 health center, Addis Ababa, Ethiopia."** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards concerning originality and quality.

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Abbreviations

BMI	Body Mass Index
CBC	Complete Blood Count
DM	Diabetes Mellitus
EDTA	Ethylene Diamine Tetra acetic Acid
FBS	Fasting Blood Sugar
fl	Femtoliters
gm	Gram
Hct	Hematocrit
Hgb	Hemoglobin
IDF	International Diabetes Federation
LY	Lymphocyte percent
LY#	Lymphocyte absolute number
mtDNA	Mitochondrial deoxyribonucleic acid
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MPV	Mean Platelet Volume
NE#	Neutrophil absolute number
NE%	Neutrophil percent
PCT	Plateletcrit
P-LCR	Platelet Large cell Ratio
RBC	Red Blood Cell count
RDW-CV	Red Cell Distribution Width, Coefficient variation
RDW-SD	Red Cell Distribution Width, Standard deviation
SOP	Standard Operational Procedure
WBC	White Blood Cell count
WHO	World Health Organization

Abstract

Background: Diabetes Mellitus has been growing as a worldwide health problem. Platelet indices such as platelet count, mean platelet volume, platelet distribution width and plateleticrit are indicators of platelet hyperactivity and can be used as early markers for diabetic complications.

Objective: To assess platelet parameters among Type II Diabetic Mellitus patient at Addis Ketema Woreda 3 health center, Addis Ababa, Ethiopia, from June to December 2021.

Method: A comparative cross-sectional study was used and involving 255 participants consisting of 85 diabetic and 170 non-diabetic controls was conducted from June to December/2021 G.C and convenient sampling technique used. About 3-4ml blood sample from each participant was collected into a test tube (purple cap) containing EDTA for complete blood count measurement on Mindray BC3000 hematology analyzer. Data was collected, entered, & analyzed using SPSS version 25 software. The mean and standard deviation was used to summarize platelet parameters. One-way ANOVA (analysis of variance) were done to compare the platelet parameters difference across the two groups (DM Type II & Control), and A P-value of <0.05 is considered as statistically significant.

Result: A total of 255 study participants was involved on the study. Out of the total 85 case were included in the study among them 47/85 (55.3%) were female and the mean age of participant was 47.4±13 years. Based on HbA1c values the pattern of glycemic control among diabetic patients was determined, majority of participant 75.3% had poor glycemic control. The platelet count, mean platelet volume, and plateletcrits decreased between patients with diabetic mellitus than control group (P=0.001). Platelet count, mean platelet volume, and plateletcrits parameters significantly different and correlated were case group of participant with HbA1c >6.5% (P<0.05).

Conclusion: Majority of diabetic subjects are poor glycemic control or HbA1c level greater than 6.5% were 75.3%. In this study platelet count, mean platelet volume, and plateletcrits significantly differences between diabetic's subject compared to non-diabetics subjects but a platelet parameter value there is no difference between Diabetic with complications and without complication participant.

Key words: Type 2, Diabetes Mellitus, platelet count, Mean Platelet Volume, Platelet Distribution Width, Plateleticrit, Platelet parameters, Ethiopia.

1. Introduction

1.1. Background

Diabetes Mellites (DM) is a serious, chronic disease that occurs either when the pancreas does not produce enough insulin or for using regulation of blood sugar level elevating amount produced hormone that regulates blood sugar, or glucose), another ways when the body cannot effectively use the insulin it produces or the cells are insulin resistance [1].

Diabetes is an important public health problem, one of four priority Non-Communicable Diseases (NCDs) targeted for action by world leaders. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades. In 2012, there were an estimated 370 million people with diabetes worldwide and nearly 5 million deaths due to diabetes and diabetes-related illnesses [2].

According to World Health Organization (WHO) classification are 3 main types, those are Type 1 diabetes (previously known as insulin-dependent, juvenile or childhood-onset diabetes) is characterized by deficient insulin production in the body. Type 2 diabetes (formerly called non-insulin-dependent or adult-onset diabetes) results from the body's ineffective use of insulin. Gestational diabetes (GDM) is a temporary condition that occurs in pregnancy and carries long-term risk of type 2 diabetes [3].

Type II DM is characterized by insulin insensitivity as a result of insulin resistance, declining insulin production, and eventual pancreatic beta-cell failure [4]. Insulin acts as a moderator of glucose level in the bloodstream and works as an antagonist of platelet hyperreactivity. Platelet morphology and functions have changed, because of its hyperactive behavior in type II DM patient [5, 6].

Moreover, this prolonged insulin resistance state causes a damaging effect on pericytes and vascular endothelium which make type II DM patient more susceptible to diseases due to metabolic changes such as atherogenic dyslipidemia, hypertension, glucose intolerance, and prothrombotic state that enhance thrombosis and suppress thrombolysis [7, 8, 9]. Generally, the injurious effects of hyperglycemia are separated into macrovascular complications (Coronary Artery Disease, Peripheral Arterial Disease, Stroke) and microvascular complications (Diabetic Retinopathy, Diabetic Neuropathy) [10].

Platelet indices are the major morphological representative parameters for platelets, it indirectly signifies both the morphological and as well as the functional status of the platelet. The most used platelet indices are the Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Plateletcrit (PCT), Platelet Large cell Ratio (P-LCR) and platelet count and those platelet parameters can be measured by automated hematology analyzers as a routine hematological procedure [11,12].

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) and cerebral ischemia. Other platelet markers such as PDW, PLCR, and 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 [13].

1.2 Statement of the problem

DM encompasses a heterogeneous group of disorders characterized by hyperglycemia associated with multiple disorders including metabolic, cellular, and blood disturbances leading to vascular complications [14].

The number of people suffering from type II DM has been increasing due to the aging population, urbanization, and low physical activity. According to the International Diabetes Federation (IDF) estimate of 2013, 382 million (8.3%) adults had diabetes worldwide. The number has been increasing by two fold over the past 20 years, and 80% of the people with diabetes particularly live in low- and middle-income countries [15]. Ethiopia experiences a heavy burden of communicable infectious diseases and nutritional deficiencies. Currently, it is also being faced by the rising magnitude of non-communicable diseases like DM. In Ethiopia, although a nationwide surveillance on occurrence of DM has not been documented, the 2012 IDF report indicated an estimated DM prevalence of 3.32% [16].

Altered platelet morphology and function have been reported in patients with diabetes mellitus and associated with the risk of vascular disease. Accelerated atherosclerosis and the increased risk of thrombotic vascular events in diabetes may result from dyslipidemia, endothelial dysfunction, platelet hyperactivity, an impaired fibrinolytic balance, and abnormal blood flow. Long-term complications of DM 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 [17].

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 [7]. Patients with DM have evidence of increased oxidative stress and inflammation compared with healthy subjects. DM is associated with an overproduction of reactive oxygen and nitrogen species and potent radicals, such as hydrogen peroxide and superoxide anion, that can directly lead to platelet activation [18].

Since platelet parameters are obtained from a complete blood count from a hematology analyzer, it’s simple, effective, and cheap tests that may be used to predict DM type 2 complications so DM patient at the beginning of each month check level of blood glucose for follow up with testing

parallel order platelet count effective diagnostic value tell a patients risk factor for DM complication. It has been shown in several studies conducted in different place of the world that some of the platelet profiles variations were observed between control groups and DM patients. The platelets play a vital role in the pathological changes in diabetes leading to complications caused by macro-vascular and micro-vascular disease. Platelet parameters being indicators of platelet activity, may be useful predictive markers of these complications. These can help in prevention of progression of diabetic complications. This study will be carried out to see if there is a significant relationship between platelet parameters with DM patients. This study aims to assess platelet parameters in DM type 2 complication.

1.3 Significance of the study

This study will be carried out to see if there is a significant relationship between platelet parameter with Diabetic Mellitus Type II. It could help to suggest that platelet parameters have diagnostic and prognostic value in certain diseases like Diabetic Mellitus complication.

This study will also contribute in proving evidence based information of using platelet parameters as useful parameters for early identification of Diabetic complication for clinicians working in hospitals as well as other health care facilities which provide Diabetic follow unit. By doing so, the findings of this study could play an important role in decreasing morbidity and mortality of Diabetic Mellitus patient as a result of complication. Develop explanatory scientific evidence about relationship between platelet parameters with diabetics disorder for Federal Ministry of Health (FMoH) can use the findings of this research for developing a more effective treatment modality of service adaptation thereby contributing to platelet indices collaborate with fasting blood sugar and urinalysis for minimizing Diabetic complication in the healthcare system. Furthermore, this study could be used as a reference or a bench mark study for related studies. The research findings can also initiate other researchers to further study the platelet parameters of similar affected patient in other parts of the country.

2. Literature review

A case control study was conducted in India by Joshi A et al in 2018 to evaluate mean platelet volume and platelet distribution width in Type 2 Diabetes Mellitus. A total of 120 patients were enrolled as a part of the study from which 60 were cases and 60 were controls. The mean MPV in the cases was 7.27(±2.42) fL, while in the controls it was 10.03(±1.16) fL which was statistically significant. The mean PDW in the cases was 16.66(±2.98) fL while in the controls it was 11.78(±2.44) fl. Which was also statistically significant. MPV values were lower in cases than controls. In controls, MPV shows a negative correlation with fasting blood sugar while in cases it shows a positive correlation with fasting blood sugar. MPV showed no association with HbA1c. PDW was high in cases than controls. In controls, negative correlation between PDW and FBS was observed, while in cases, a positive correlation was observed. A strong association between PDW and HbA1c was noted. At a cut off 9.5 fL, MPV had a sensitivity of 78.33%, specificity of 70% and a positive predictive value of 72.30%. At a cut off 15fL, PDW showed a sensitivity of 81.66%, specificity of 91.66% and a positive predictive value of 90.74%. Joshi and his colleagues showed strong association between PDW and HbA1c and so it can be used as an indicator of impending vascular events. Of all the platelet parameters obtained by hematology cell counters, Platelet count is only widely used as investigative tool. Platelet indices can be used as simple and cost-effective bio markers for predicting diabetic vascular complications [20].

In India, Swaminathan A et al conducted a cross-sectional study to evaluate the mean platelet volume and other platelet parameters in subjects with Type-2 diabetes mellitus. 100 Type-2 diabetic patients aged between 30 and 60 years and 100 age-matched non-diabetic subjects as controls were included in this study. In the result MPV was significantly increased among diabetics when compared to non-diabetics. MPV and PDW showed a significant increase in diabetics with HbA1c >7%, and MPV was increased in diabetics with >10 years duration of diabetes. Finally, they concluded that MPV was found to be higher in subjects with Type-2 diabetes and significantly increased in diabetics with poor glycemic control and having and having a longer duration of diabetes. MPV can be used as a prognostic marker of vascular complications in patients with DM [21].

Gowtham A et al conducted a case control study to assess platelet Indices in type II Diabetes Mellitus in India in 2018. 60 Type 2 diabetics and 60 nondiabetic healthy controls were involved in the study. PLT, MPV and PCT were significantly higher in diabetics compared to non-diabetics. There was a statistically significant correlation between MPV and PPBS. No significant correlation

was observed between MPV and HbA1c, FBS, BMI. Similarly, the correlation between PDW and HbA1c, FBS, PPBS, BMI was statistically insignificant. MPV or PDW was not significantly associated with duration of DM. No statistically significant differences were observed between platelet indices and occurrence of vascular complications. Finally, they conclude that platelet indices are a simple, easily available and cost-effective tool which can aid in the early detection of diabetes, but have a little role to play in detection of complications [22].

In 2017 Rajagopal L et al in India performed a cross-sectional study to analyze platelet indices with glycemic status in type-2 DM patients and to establish the correlation between MPV, PDW, FBS and HbA1c values. A total of 450 subjects (150 controlled and 150 uncontrolled diabetics, 150 non-diabetics) were recruited in this study. The observations derived from the present study revealed that diabetics had higher mean platelet count when compared to non-diabetics. Among diabetic patients, those with glycemic status under control showed lower mean platelet count (273.95±7.54) than those with poor glycemic status (276.01±7.23), however these differences were not found to be significant. The mean PDW among uncontrolled diabetics (11.86±0.12) was much higher than controlled diabetics (9.93±0.04) and non-diabetics (8.83±0.04) which was found to be statistically significant (p=0.0001). Similarly, the mean MPV among uncontrolled diabetics (10.85±0.05) was much higher than controlled diabetics (8.67±0.03) and non-diabetics (7.61±0.03) which was found to be statistically significant (p=0.0001). Thus, this study shown a significant stepwise increase in MPV from a non-diabetic population to controlled diabetics and then further to uncontrolled diabetic population. The mean Platelet Larger Cell Ratio (P-LCR) value among uncontrolled diabetics was lower (24.63±1.41) compared to controlled diabetics which in turn was lower (26.82±0.59) than non-diabetic subjects (27.88±0.64). The mean Plateletcrit (PCT) values were similar in all these three study groups. They observed a statistically significant difference (p=0.0001) in mean HbA1c percentage levels in non-diabetics (5.98±0.08), controlled diabetics (7.33±0.14) and uncontrolled diabetics (10.34±0.18). They conclude that MPV and PDW are increased in diabetics. They are simple and cost-effective tools that can be used as a good indicator of platelet activation and an independent predictor of impending vascular complications in DM [23].

A prospective analytical case–control study was conducted by Bhanukumar M et al in india in 2016. The objective of the study was to determine the mean platelet volume (MPV) and platelet distribution width (PDW) in subjects with type 2 diabetes mellitus (type 2 DM) compared to subjects without type 2 DM and their correlation with fasting blood glucose, glycosylated hemoglobin (HbA1c), and duration of type 2 DM respectively. Mean platelet volume in type 2

DM subjects was 9.372 (SD 0.677) fl, whereas in controls it was 8.634 (SD 0.778) fl. Mean PDW in type 2 DM subjects was 12.19 (SD 1.19), whereas in controls it was 11.27 (SD 1.06). MPV was significantly higher in type 2 DM compared to controls, p-value being 0.000 ($p < 0.005$) and 95% CI of –0.98 to –0.44. PDW was significantly higher in cases compared to controls, p-value being 0.000 ($p < 0.005$) and 95% CI of –1.37 to –0.471. The mean platelet count in the case group was $281x10^3$ /mm3 (SD 0.92), whereas in controls it was $259x10^3$ /mm3 (SD 0.71). There was no significant difference between platelet counts in type 2 DM subjects compared to controls, p-value being 0.143 ($p > 0.005$). MPV was not linearly correlated with the duration of type 2 DM as the correlation coefficient was 0.086 and p-value being 0.553. Similarly, PDW was also not correlated with the duration of type 2 DM, correlation coefficient being 0.183 and p-value 0.204. This study concludes that MPV and PDW are significantly increased in patients with type 2 DM compared to patients without type 2 DM. Platelet volume indices are an important, simple, and cost-effective tool that should be used and explored extensively for predicting the possibility of impending acute vascular events in patients with type 2 DM [24].

In Turkey Hekimsoy Z et al in 2012 conducted a case control study to evaluate Mean platelet volume in Type 2 diabetic patients. 145 consecutive Type 2 diabetic patients and 100 non-diabetic control subjects were included. In their result MPV was significantly higher and the mean platelet counts were significantly lower in diabetics compared to age- and sex-matched non-diabetic healthy controls [10.62+/-1.71fl vs. 9.15+/-0.86fl (P=.000), 260.38+/-68.65 x 10(9)/l vs. 292.33+/-79.19 x 10^9/l (P=.001)], respectively. This result shows significantly higher MPV in diabetic patients than in the non-diabetic controls. This suggests that platelets may play a role in the micro- and macro-vascular complications of diabetic patients [25].

A prospective hospital based study of platelet parameters MPV, PDW and P-LCR was carried out on 280 cases diagnosed with Type 2 diabetes Mellitus and 280 controls with normal blood glucose levels. The mean duration of diabetes was 4.7 ± 2.5 years. MPV, PDW and P-LCR were significantly higher in diabetics compared to non-diabetics (11.3 ± 1.0 vs. 9.0 ± 0.6, 14.2 ± 2.5 vs. 10.7 ± 0.7 fl, 35.0 ± 8.1 vs. 23.0 ± 2.4%). Among the diabetics, MPV, PDW and P-LCR were higher in those with complications as compared to those without complications, which was not statistically significant. The higher values of MPV, PDW and P-LCR indicates that they serve as better risk indicator of initial vascular complications in diabetes mellitus patients [26].

In 2011 Sonali J. et al the study conducted on 75 subjects with DM (50 with one or more micro-vascular complications) and 50 non-selected patients from the hospital as controls. The result

showed MPV, PDW and platelet-large cell ratio were all significantly higher in diabetic patients compared to the control subjects (P<0·05). Among the diabetics, PDW was higher in those with complications as compared to those without (P = 0·006). On stepwise discriminant analysis using age, duration of diabetes, platelet count and platelet indices, approximately 78.6% of patients with diabetic complications were accurately classified. Platelet indices, especially PDW, are different between diabetics and controls as well as between diabetics with and without micro-vascular complications. Discriminant analysis using PDW and MPV could classify majority of patients with diabetic complications [27].

A retrospective study conducted by Wu M in 2019 on 1,729 Type II DM patients from May 2017 to February 2018. These patients were divided into two subgroups depending on their platelet volume indices and HbA1c levels. The result shows Mean platelet volume (MPV), platelet distribution width (PDW) and platelet-large cell ratio (P-LCR) were positively correlated with HbA1c levels (all $p < 0.01$), but not the thrombocytocrit (PCT). The platelet, MPV, PDW, P-LCR, and glucose levels were significantly higher in the higher HbA1c subgroup (≥ 6.5%) than that in lower subgroup (< 6.5%) ($p < 0.01$). The platelet, MPV, P-LCR, PCT, HbA1c, and glucose levels were significantly higher in higher PDW subgroup (≥ 17 fL) than that in lower subgroup (< 17 fL) ($p < 0.01$). In the higher MPV subgroup (≥ 12 fL), the platelet, PDW, P-LCR, PCT, HbA1c, and glucose levels were significantly higher than that in lower subgroup (< 12 fL) ($p < 0.01$). These result finalized platelet volume indices were positively correlated with HbA1c in T2DM patients [28].

Activated platelet levels and platelet-activating capacity are well recognized as useful index parameters for the physiological and pharmacological prediction of thrombotic events. The relation of physiological factors, such as sex, aging, and laboratory tests, to platelet functions has not been well documented. We conducted a blood analysis of people with normal/pre-metabolic syndrome and patients with type 2 diabetes mellitus to clarify the pathological factors. Statistical analyses indicated significantly high basal-activated platelet in pre-metabolic syndrome, and basal-activated platelet was positively associated with hyperlipidemia and hepatic damage. Platelet-activating capacity was significantly low in aging and hyperlipidemia, but high in hyperglycemia, and was negatively associated with hyperlipidemia and hepatic damage. Aging and high nutrient condition impaired platelet functions [29].

In 2018 western Algeria by Kachekouche Y. et al the study conducted on a sample of 1852 subjects, 1059 with type 2 diabetes and 793 witnesses, shows the Hematological profile associated

with type 2 diabetes mellitus retained the mean corpuscular hemoglobin concentration over the normal ratio, lower platelets blood ratio, basophils ratio and sedimentation rate at one hour. As regards to the platelets blood ratio, subjects with a ratio lower are five times more exposed to type 2 diabetes compared to subjects with a normal ratio (OR = 5.01; 95% CI = 1.78-14.13, P < 0.002) [30].

A comparative cross-sectional study was conducted at Gondar University Hospital from February to April 2015 by Biadgo B et al to determine hematological indices and their correlation with fasting blood glucose level and anthropometric measurement in type 2 DM patients in comparison with healthy controls. A total of 296 participants (148 cases and 148 healthy controls) were selected using systematic random sampling technique. The result showed a platelet indices, mean platelet volume (10.4±1.1 fL vs 9.9±1.1 fL) and platelet distribution width (14.5±2.1 fL vs 13.4 ±2.1 fL) were found to be significantly increased in the diabetic patients (P, 0.05). Anthropometric measurements significantly correlated with white blood cell and platelet indices. They suggested the routine hematological profile checking of patients with T2DM may help to prevent complications associated with aberrations in hematological values [19].

3. Objectives

3.1 General objective

- To assess platelet parameters among Type II Diabetic Mellitus patient at Addis Ketema Woreda 3 health center, Addis Ababa, Ethiopia from June to December 2021.

3.2 Specific objective

- To compare the platelet parameters between diabetics and non-diabetics groups
- To determine the relationship between platelet parameters with fasting blood sugar and hemoglobinA1c
- To evaluate the correlation between platelet parameters and diabetic complications

4. Materials and methods

4.1. Study area

This study was conducted at Addis Ababa city Administration, Health bureau, Addis Ketema woreda 3 health center. Which are found in Addis Ababa City. Addis Ababa is the capital city of Ethiopia, and is in the central part of Ethiopia. The Addis Ketema woreda 3 health center commenced to giving different OPD, ANC, Delivery service, Pharmacy service, Laboratory service, under 5 years clinic, TB service, ART. The health center has more than 77 health professionals and 66 Administrative and supporting staff in the various areas of health centers.

4.2. Study design and period

- A comparative cross-sectional study was conducted from June to December/2021 G.C.

4.3. Population

4.3.1. Source population

- All patient diagnosed with Diabetes Mellitus attending at Addis Ketema Woreda 3 health center.

4.3.2. Study population

- All patient diagnosed with Type II Diabetes Mellitus who fulfills the eligibility criteria.

4.4. Eligibility criteria

4.4.1. Inclusion criteria

- All Type-II Diabetic Mellitus patient attending AKW-3 health center laboratory services during the study period and time.

4.4.2. Exclusion criteria

- A patient, who are diagnosed Type I & gestational DM, and also taking insulin injection hormone.
- For male patient hgb level below 13 g/dl and for female hgb level below 12 g/dl.
- Subject on Anti-platelet drugs such as Aspirin.
- Patient, who are diagnosed with any hematogical malignancy.

4.5. Study variables

4.5.1. Dependent variable

- Platelet parameters

4.5.2. Independent variables

- Socio-demographic data (Sex, & Age)
- Duration of Diabetes
- Diabetic complications
- Level of HbA1c
- Value of FBS
- Glycemic control drug

4.6. Measurement and Data collection

4.6.1. Sample size determination

Two population mean formulae is used to calculate the sample size by considering the following assumptions: 95% confidence interval (two-sided), 80% power, and ratio of cases to control group was 1:2. Taking the mean and standard deviation (SD) of MPV for type II DM and control group from a study conducted in Ethiopia, 10.4 and 1.2 for type 2 DM group and 9.9 and 1.2 for control group [19].

Standard deviation S1, S2

Level of significance which is usually set to a level of 0.05; respective Z value is 1.96.

Power of the test: 100(1- *B*) %, which is usually set to 80%, which is equal to 0.84.

Sample size can be estimated using the formula:

$$\frac{\mathbf{2}\propto^{\mathbf{2}}[\boldsymbol{Z_1}-\frac{\propto}{\mathbf{2}}+\boldsymbol{Z_1}-\boldsymbol{\beta}]^{\mathbf{2}}}{(\boldsymbol{\mu_1}-\boldsymbol{\mu_2})^{\mathbf{2}}}$$

$$\boldsymbol{n_1}=\frac{\mathbf{2(1.21)[1.96+0.84]^2}}{\mathbf{(10.4-9.9)^2}}=\frac{\mathbf{18.97}}{\mathbf{0.25}}=\mathbf{75.88}\approx\mathbf{76}$$

76 + (10% for contingency) = 85

The minimum sample size required for group 1, n1 = 85

To evaluate the clinical accuracy of platelet parameters in Type 2 DM, it is possible to make the number of controls (Apparently healthy) similar of the cases (Type 2 DM) and multiply by 2x. The sample required for group 2, n2 = 1(n1) = 85 x 2 = 170

$$N = n_1 + n_2 = 170 + 85 = 255$$

The total number of participants required for this study is 255.

4.6.2. Sampling method

- Convenient sampling technique was used to select sample population.

4.6.3. Data collection procedure

Voluntary Diabetic Mellitus Type II patient after getting an informed consent was recruited for this study. Data of age, sex, result of FBS, duration of diabetes and complications were collected from the patient's history card. Blood sample was collected from the study participants by a qualified laboratory technologist. About 6-8 ml blood sample from each patient were collected into a test tube (purple cap) containing EDTA for complete blood count and HbA1c measurement and SST (yellow cap) for blood glucose determination.

4.6.4. Laboratory analysis

CBC was analyzed on blood sample collected in EDTA tubes and platelet parameter values are taken from the CBC result then measured HbA1c by using **Finecare™** HbA1c Rapid Quantitative Test. FBS was done on **Mindray, BS120** chemistry analyzer from the blood that has been collected in serum separator tubes.

Mindray, BC3000 performs speedy and accurate analysis of 19 parameters including a 3-part WBC differential plus histograms for RBC, PLT and WBC in blood (WBC, LYM%, MXD%, NEUT%, LYM#, MXD#, NEUT#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, PDW, MPV, and PCT). The analyzer will be employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the Direct current (DC) detection method. The RBC count and platelets are taken by the RBC detector block, also using the DC detection method. The HGB detector block measures the hemoglobin concentration using the non-cyanide hemoglobin method [user manual]

4.7. Data Quality Assurance

Blood sample quality was ensured by collecting and processing according to the standard operating procedures (SOPs).

Pre-analytic

Patient identification and labeling was made with great care and samples are properly collected and transported without delay. Samples are checked whether they are in the acceptable criteria like; hemolysis, clotting, volume and checked for collection time. Safety procedure concerning specimen handling was strictly followed.

Analytical

Three levels of commercially prepared hematology cell controls (Normal, Low and High) for Mindray, BC3000 analyzer is run. Analysis was performed by following standard operating procedure (SOP) after running and passing of controls.

Post analytical

To avoid any clerical error, printout results that was generate by the analyzers was used but there is no print out results recorded by using hand shows from the screen of the analyzers. Then the results are documented and recorded using SPSS version 25 with great care.

4.8. Data analysis and interpretation

The data obtained from study was entered and analyzed by using Statistical Package for Social Science (SPSS) version 25. The mean and standard deviation was used to summarize platelet parameters. Analysis of variance (ANOVA) and independent sample *t*-test were used to test the difference between means. To verify the association between fasting blood glucose and platelet parameters, applied a Pearson correlation, considering a statistically significant result when $p < 0.05$. Tables & figures are used for the description of the data.

4.9. Ethical considerations

The study was conducted after it was ethically reviewed and approved by the Department of Medical Laboratory Science research and ethical review committee (DRERC), College of Health Science, Addis Ababa University. By getting permission from Addis Ababa health bureau, ethical clearance was also obtained from Addis Ketema 3 health center. Then permission was obtained from the health center administration office and a letter informing each department was written.

The study aims, risks, benefits and right for withdrawal anytime from the study was explained from the study participants and informed consent was obtained. Samples was coded, and confidentiality of patient data was maintained throughout the study by locking hard copies and password protecting electronic files.

4.10. Dissemination of the result

The finding of this study will be presented and submitted to Addis Ababa University, College of Health Science Medical Laboratory Science Department, and a copy of this material will be given to Addis ketema woreda 3 health center. The finding will also be communicated to Ministry of Health, Addis Ababa Health Bureau, and respective hospitals. The result will also be disseminated through publication in peer reviewed local and international journals and through presenting it in relevant workshop, seminars and scientific conferences.

4.11. Operational definitions

Platelet indices: are the major morphological representative parameters for platelets, it indirectly signifies both the morphological and as well as the functional status of the platelet. Include list of the parameters (PLT count, MPV, PCT, PDW, and P-LCR).

Type-II Diabetes Mellitus: Too much sugar in the blood (high blood glucose) or fasting blood sugar level greater than 140mg/dl.

Poor glycemic control: means HbA1c level ≥6.5%.

Good glycemic control: means HbA1c level <6.5%.

5. Work flow

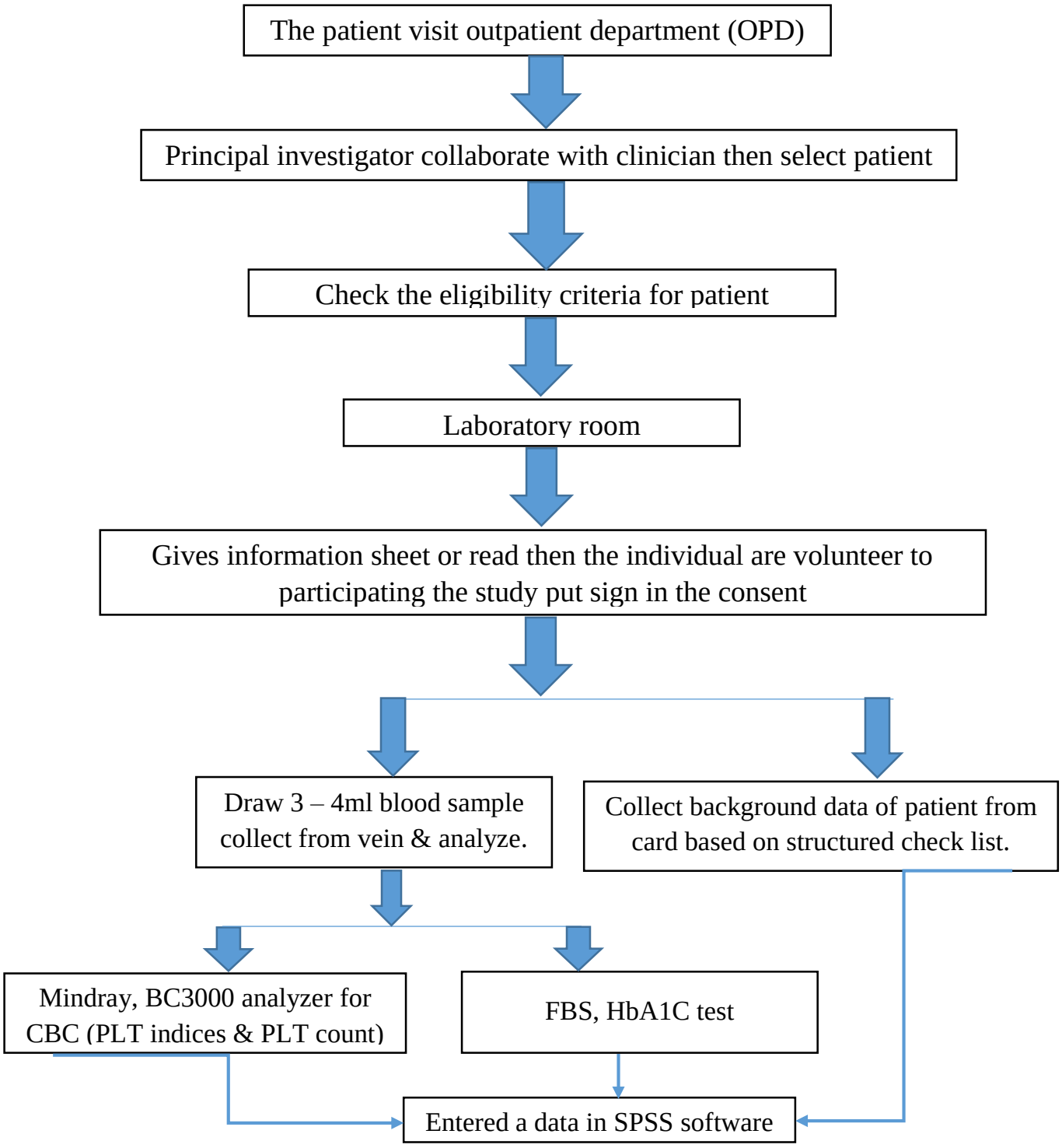

Figure 1: - Workflow

6. Result

6.1. Socio-demographic characteristics of participant

Out of the total 255 (85 case (with DM) and 170 control (without DM)) study participants was involved. This study included 2 groups those are Case and Control: first group included 85 participant affect with Type-II diabetic mellitus and second group included 170 participant non-affect with Type-II diabetic mellitus.

Out of the total 85 case participant were included in the study among them 47/85 (55.3%) were female from Diabetic group and the age range of the participant were 23 to 80 years and the (mean±SD) age was 47.4±13 years, educational status of 35/85 (41.2%) were unable write & read, majority of participant 65/85 (76.4%) were married and also occupational status of merchant and other were as 30/85 (35.3%) **(Table 1).**

Table 1: - Socio-demographic characteristics of assess platelet parameters among Diabetic Mellitus Type-II patient at AKW-3 HC, Addis Ababa, Ethiopia, 2021 (n=255).

Independent Variables	Category	Case group (n=85)		Control group (n=170)	
		Frequency	%	Frequency	%
Education	Unable write & read	35	41.2	5	2.9
	Able write & read	10	11.8	-	-
	Primary school	25	29.4	45	26.5
	Secondary school	10	11.8	100	58.8
	College & above	5	5.8	20	11.8
Marital status	Single	10	11.8	50	29.4
	Married	65	76.4	105	61.8
	Divorced	5	5.9	10	5.9
	Widowed	5	5.9	5	2.9
Occupation	Government employee	15	17.6	80	47.1
	Private employee	10	11.8	45	26.5
	Merchant	30	35.3	10	5.9
	Others	30	35.3	35	20.6
Total		**85**	**100.0**	**170**	**100.0**

6.2. Clinical characteristics of participant

From a total of 85 diabetic mellitus case participant be alive with Type-II diabetic mellitus majority of respondent 30/85 (35.3%) are above 10 years duration then followed 23/85 (27.1%) were as 6 to 10 years duration with diabetic mellitus disorder, and also 32/85 participant (37.6%) are developed diabetic mellitus related complication from those 18/32 (56.2%) are developed nephropathy and 10/32 (12.5%) respondent are retinopathy disease developed related with DM complication, and all diabetic patient was taking metformin glycemic control drug **(Table 2).**

Table 2: - Frequency distribution of Diabetics related variables for participant with DM group at Addis Ketema Woreda 3 health center, Addis Ababa, Ethiopia, June-December 2021 (n=85).

Variable	Category	Frequency	Percent
Duration of DM	Less than 1 year	17	20
	1 – 5 year	15	17.6
	6 – 10 year	23	27.1
	Above 10 year	30	35.3
DM related complication	Yes	32	37.6
	No	53	62.4
Type of Complication	Nephropathy	18	56.2
	Neuropathy	4	31.3
	Retinopathy	10	12.5
Glycemic control drug	Metformin	85	100

6.3. Status of glycemic control

The mean HbA1c of 8.9% (SD±2.1; median 9.0%) for female and 9.1% (SD±1.8; median 9.05%) for male with diabetic mellitus participant. Based on HbA1c values the pattern of glycemic control among diabetic patients was determined, and 24.7% (21/85; (12 female and 9 male)) patients had good/Ideal glycemic control i.e. HbA1c 6.5 %, and, while majority of patients 75.3% (64/85; (35 & 29 were female & male participant respectively)) had poor glycemic control (HbA1c value was more than 6.5%). The percentage of males with poor glycemic control (29/38, 76%) was higher compared with percentage of females with poor glycemic control (35/85; 74%) (Fig 2).

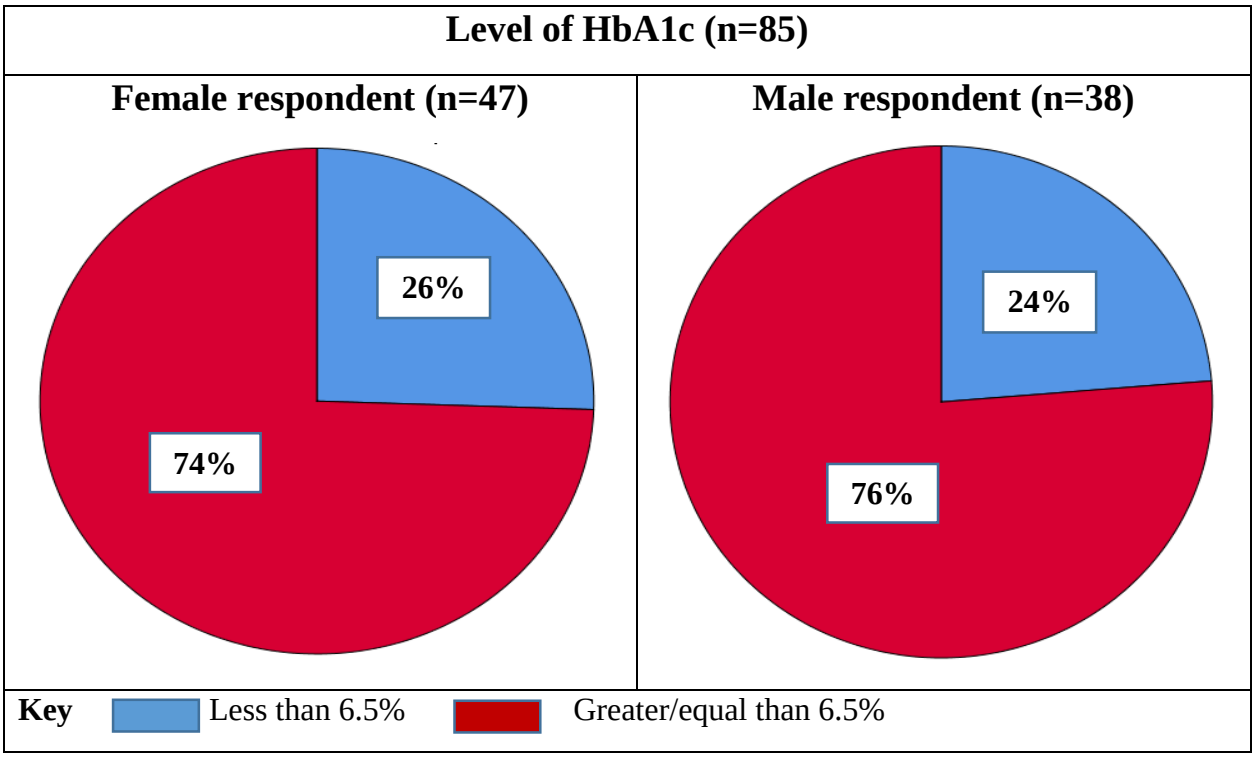

Figure 2:- Degree of glycemic control based on HbA1c value among patients with diabetes at AKW-3 HC, Addis Ababa, Ethiopia, 2021 (n=85).

6.4. Comparison of selected hematological parameter of participant

A total of 255 (85 & 170) participant, patient group with DM & control group without DM or health population respectively of both gender participated in this study. Calculate descriptive statistical analysis the central tendency of data calculated mean, median & standard deviation of the total participant categorized case and control group set a result. Additionally set a range (lower and upper data) of all hematological parameters, and also all hematological parameters except WBC, hgb, HCT, MCH, & MCHC are significantly mean difference between case and control group of subject (p value less than 0.05) (**Table 3**).

Table 3: - Mean±SD of hematological parameters between diabetics and non-diabetics groups at AKW-3 HC, Addis Ababa, Ethiopia, 2021 (n=255).

Variables	Case group (n=85)				Control group (n=170)				P-value
	N	Mean± SD	Range	Median	N	Mean± SD	Range	Median	
WBC (10^3/µl)	85	6.87±2.1	3.29-14.8	6.81	170	7.01±1.9	2.2-8.34	6.9	0.453
Neutrophil (10^3/µl)	85	59.2±10.8	35.5-88.6	58.1	170	58.2±13.3	29.4-89.3	60.5	**0.001***
Lymphocyte (10^3/µl)	85	33.3±10.4	11.7-53.9	34.2	170	30.8±11.7	4.8-61.9	29.1	**0.001***
Mix (10^3/µl)	85	7.9±2.1	3.5-14.8	7.8	170	11.1±4.2	3.2-29.2	10.4	**0.001***
RBC (10^6/µl)	85	5.2±1.6	1.24-6.4	5.1	170	4.8±0.6	3.0-8.08	4.8	**0.012**
Hemoglobin (g/dl)	85	14.2±2.2	16.2-28.3	14.3	170	13.8±1.9	10.3-25.3	13.8	0.919
Hematocrit (%)	85	42.3±8.9	18.8-62.7	43.4	170	41.3±5.4	31.2-71.6	41.2	0.981
MCV (fl)	85	85.7±7.1	50.6-103	86.3	170	86.7±6.2	68.7-107.8	86.7	0.051
MCH (Pg)	85	28.7±4.5	15.7-54.5	28.4	170	29.1±2.7	19.4-38.3	29.3	0.109
MCHC (g/dl)	85	33.4±4.4	30.4-59.6	32.5	170	33.6±1.3	28.2-36.5	33.6	0.389
RDW-CV (%)	85	12.5±1.02	11.0-18.4	12.3	170	13.6±1.6	11.3-21.5	13.1	**0.001***
Platelet (10^3/µl)	85	235±58	113-408	239	170	294±79	105-625	279	**0.001***
Plateletcrits (%)	85	0.18±0.4	0.08-0.31	0.19	170	0.3±0.07	0.13-0.50	0.28	**0.001***
PDW-SD (fl)	85	11.5±5.6	7.6-40.5	10.5	170	11.4±2.3	7.2-23.4	11.0	**0.001***
MPV (fl)	85	8.1±0.8	6.7-10.2	8.1	170	10.1±1.06	7.9-13.7	10.0	**0.001***
P-LCR (%)	85	26.8±6.8	15.0-44.4	26.4	170	25.7±8.4	8.7-52.4	25.2	**0.001***

Independent sample t-test compare mean difference of hematological parameters between case & control $p<0.05$

6.5. Comparison of platelet parameters across a group

Variations in platelet parameters within two groups of participant are described in Table 3. The mean±SD platelet counts value were 235±58x10^9/L and 294±79x10_9/L, in patients with diabetic and non-diabetics (control) groups, respectively. The platelet parameters except PDW-SD & P-LCR were significantly different among the group of participant between participants with diabetic mellitus (case) than non-diabetic participant (control) group (P=0.001).

Table 4: - Comparison of platelet parameters between diabetics and non-diabetics groups at AKW-3 health center, Addis Ababa, Ethiopia, 2021 (n=255).

Variables	Case group (n=85)	Control group (n=170)	p-value
	Mean± SD	Mean± SD	
Platelet (10^3/µl)	235±58	294±79	**0.001***
MPV (fl)	8.1±0.8	10.1±1.06	**0.001***
Plateletcrits (%)	0.18±0.4	0.3±0.07	**0.001***
PDW-SD (fl)	11.5±5.6	11.4±2.3	0.879
P-LCR (%)	26.8±6.8	25.7±8.4	0.276

Mean (Standard Deviation); One way-Anova for mean difference between diabetics and non-diabetics groups; MPV-Mean Platelet Volume; PDW-Platelet Distribution Width; PLCR-platelet large cell ratio. (P-value < 0.05).

6.6. Comparison of platelet parameters with HbA1c and FBS

In table 5 shows FBS & HbA1c value increase between patients with diabetic mellitus (case) than control group (P=0.001) (**Table 5**).

In comparison of platelet parameters with HbA1c with different cut-off value and FBS level of case group. Most of parameters significantly different and correlated were case group participant (P=0.001). HbA1c value less than 6.5% higher value PDW-SD parameter (F=167.7, p-value 0.001), and also HbA1c value greater than 6.5% higher value on PLT, MPV, and PCT parameter with statically significantly p-value less than 0.05 (**Table 6**).

Table 5: - Comparison of Fasting blood sugar and HbA1c between diabetics and non-diabetics groups at AKW-3 health center, Addis Ababa, Ethiopia,2021 (n=255).

Variables	Case group (n=85)	Control group (n=170)	p-value
	Mean± SD	Mean± SD	
FBS (mg/dl)	191.7±72.2	90.8±14.8	**0.001***
HbA1c (%)	8.98±1.9	4.33±0.76	**0.001***

Mean (Standard Deviation); One way-Anova for mean difference between diabetics and non-diabetics groups; FBS-Fasting Blood Sugar; HbA1c-Hemoglobin A1C. (P-value <0.05)

Table 6: - Comparison of platelet parameters between HbA1c and FBS value at AKW-3 HC, Addis Ababa, Ethiopia, 2021 (n=85).

Variables	Case group (n=85)								
	HbA1c result						Fasting blood sugar result		
	Value < 6.5%			Value > 6.5%			Mean(SD)	F	P-value
	Mean(SD)	F	P-value	Mean(SD)	F	P-value			
Platelet (10^3/µl)	232(53)	1.114	0.453	236(60)	2.098	**0.027**	235(58)	0.863	0.666
MPV (fl)	7.9(0.6)	0.808	0.643	8.2(0.9)	2.238	**0.008**	8.1(0.8)	0.760	0.756
Plateletcrits (%)	0.18(0.04)	1.838	0.197	0.19(0.05)	2.386	**0.012**	0.18(0.4)	1.064	0.504
PDW-SD (fl)	12.6(8.8)	167.7	**0.001***	11.07(4.08)	0.379	0.995	11.5(5.6)	6.892	**0.002**
P-LCR (%)	25.2(5.2)	0.808	0.643	27.4(7.1)	0.873	0.654	26.8(6.8)	0.727	0.785

Mean (Standard Deviation); One way-Anova for mean difference platelet indices and HbA1c & FBS groups, (P-value is<0.05).

6.7. Comparison of platelet parameters with and without Type-II diabetic mellitus complication

Most of the diabetic patients (53/85) without diabetic complication or there is no related diabetic disease complication, whereas the remaining diabetic patients (32/85) with diabetic complication, out of 32 patient were as Nephropathy #18, Neuropathy #4 and Retinopathy #10 patient. And also there is no relationship between platelet indices with diabetic complication p value greater than 0.05. These means platelet indices is not using as clinical parameters for predicting value to developing or developed diabetic related complication **(Table 7).**

Table 7: - Comparison of platelet parameters with diabetics with and without complication at AKW-3 HC, Addis Ababa, Ethiopia, 2021 (n=85).

Variables	DM with complications (n=32)	DM without complications (n=53)	P value
	Mean± SD	Mean± SD	
Platelet (10^3/μl)	231±61	238±56	0.683
MPV (fl)	8.1±0.8	8.1±0.8	0.391
Plateletcrits (%)	0.18±0.04	0.19±0.04	0.134
PDW-SD (fl)	10.8±5.3	11.8±5.8	0.452
P-LCR (%)	25.9±6.3	27.3±7.0	0.317

7. Discussion

The aimed of these study was assessment of platelet parameters among Diabetic Mellitus Type II patient at Addis Ketema Woreda 3 health center, Addis Ababa, Ethiopia. In our study, we compared platelet parameters between 85 diabetic subjects and 170 non-diabetic control subject involved in this study. There is significant difference in the platelet parameters like platelet count were 235±58 & 294±79x10^3/µl, MPV were 8.1±0.8 & 10.1±1.06fl, and Plateletcrits were 0.18±0.4 & 0.3±0.07% between diabetics and non-diabetics groups and the two group values were comparable but most of platelet parameters are decrease in the diabetics than non-diabetic control group was statistically significant (p value <0.05).

In this study the mean platelet volume value were 8.1±0.8fl and 10.1±1.06fl and PDW were 11.5±5.6 & 11.4±2.3% on a patients with diabetic and non-diabetics (control) groups respectively. The MPV decreased compare between participants with diabetic mellitus (case) than non-diabetic participant (control) group, while increased P-LCR with group of patients with diabetic mellitus (case), but on PDW value of both group of participant there is no difference. These finding consistent with study conducted in Turkey by illhan et al, platelet parameters was lower among type II DM patients than the control group [31]. These finding contrast with the study conducted in India by Shilpi K et al was reported diabetics, MPV, PDW and P-LCR were significantly higher in diabetics compared to non-diabetics [26]. In the present study contrast with other study because of glycemic control drug effect (metformin) demonstrates capable of antiplatelet agents that are highly effective at inhibiting platelet activation by decreasing the release of free mtDNA [32], and also study participant and study design maybe influence on a subject different platelet parameter value because the participant test often varies from day to day and from person to person.

In our study, the MPV in the cases was 8.1(±0.8)fl, while in the controls it was 10.1(±1.06)fl which was statistically significant. These result consistent with study conducted in India by Joshi A et al in 2018, the mean value of MPV in the cases was 7.27(±2.42) fl, while in the controls it was 10.03(±1.16) fL which was statistically significant. MPV values were lower in cases than controls [20], and also the study conducted in Turkey by Dolasık et al. MPV was lower among type II DM patients than the control group after starting metformin treatment [31]. Different result reported in platelet parameters between with & without Type-2 diabetic mellitus by Hekimsoy Z et al the result shows significantly higher MPV in diabetic patients than in the non-diabetic controls [25]. In the present study difference with other study because a pharmacological effect of metformin are

highly effective at preventing platelet activation by reducing the release of free mtDNA, which encourages platelet activation in a DC-SIGN-dependent routine [32], and also study participant was not hospital admitted related with diabetic complication and study design maybe influence on a subject different platelet parameter value.

In these study PLT & PCT was showed strong association with poor glycemic control HbA1c value greater than 6.5. These finding consistent with study conducted by Wu M et al. the result show the platelet, MPV, and P-LCR levels were significantly higher on HbA1c subgroup (≥ 6.5%) than lower subgroup (< 6.5%) ($p< 0.01$) [28]. and different result reported in Turkey by illhan et al, there is no correlation between MPV and HbA1c values [31]. A possible reason positive correlation in platelet parameters on people with type 2 diabetes particularly those with poor glycemic control was increased platelet reactivity. Hyperglycemia contributes to greater platelet reactivity through direct effects and by promoting glycation of platelet proteins. Hypertriglyceridemia increases platelet reactivity [34].

The finding of this study showed there is no relationship between platelet indices with diabetic complication. These means platelet indices not for predicting value to developing or developed diabetic related complication. These result contrast with study conducted by Sonali J. et al, shows the platelet indices, especially PDW and MPV are different between diabetics and controls as well as between diabetics with and without micro-vascular complications. [27], the study was conducted by Hekimsoy Z et al the result shows significantly higher MPV in diabetic patients than in the non-diabetic controls. This suggests that platelets may play a role in the micro- and macro-vascular complications of diabetic patients [25], the study conducted in India by Shilpi K et al was reported diabetics, MPV, PDW and P-LCR were higher in those with complications as compared to those without complications, which was not statistically significant [26]. In this result compare with other study report different because thrombosis and its complications are the leading cause of death in patients with diabetes. However, whether metformin can effectively prevent both venous and arterial thrombosis with no significant prolonged bleeding time by inhibiting platelet activation and extracellular mitochondrial DNA (mtDNA) release [32]. Some glucose-lowering agents have also demonstrated antithrombotic effects in observational studies. The potential benefit on platelets may be related to the normalization of glycemic control, but other additional direct antithrombotic and anti-inflammatory mechanisms may be involved [33].

8. Strength and Limitation of the study

8.1. Strength

- Data and sample collection was strictly adhere on SOPs.
- Analyses of laboratory tests were done with full-automated instruments.

8.2. Limitation

- Use a little number and old reference use.
- The sample size was small making it difficult to generalize the findings.
- This study was limited by cross-sectional study, which could not provide a well- established association between glycemic control and its potential predictors.
- It was done only among type 2 diabetes patients who were on follow up at outpatient clinic which may not be representative of the overall type 2 diabetes population.

9. Conclusion & Recommendation

9.1. Conclusion

Most of diabetic subjects are poor glycemic control. In this study significantly differences between platelet parameters (PLT, MPV, & plateletcrits) was decrease on diabetic's subject compared to non-diabetics (control) subjects. Also, hemoglobin A1C, and fasting blood sugar level with platelet parameters increase in the diabetic's subject was statistically significant. Finally, based on our finding the platelet parameters (PLT, MPV, & PCT) value have no difference between a participant with diabetic related complications and without complication.

9.2. Recommendation

- Platelet parameters are easily obtained together with the CBC report; thus, clinicians should evaluate platelet parameters (PLT, MPV, & PCT) when assessing progress of glycemic control.
- Further research including more study participants would further help us to assess the complication of diabetic mellitus or not diabetic related complication can be predicted in the admitted patient of diabetics in a similar way with outpatient with longitudinal study design.

References

1. WHO Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part 1: Diagnosis and Classification of Diabetes Mellitus (WHO/NCD/NCS/99.2). Geneva: World Health Organization; 2020.
2. World Health Organization, Global Burden of Disease Database 2008. Available at http://www.who.int/healthinfo/global_burden_disease/projections/en/index.html.
3. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet. 2009; 373:1773-79.
4. Robertson RP. Antagonist: diabetes and insulin resistance–philosophy, science, and the multiplier hypothesis. J Lab Clin Med 1995 May; 125(5): 560-64.
5. Keating FK, Sobel BE, Schneider DJ. Effects of increased concentrations of glucose on platelet reactivity in healthy subjects and in patients with and without diabetes mellitus. Am J Cardiol 2003; 92:1362-5.
6. Kodiatte TA, Manikyam UK, Rao SB, Jagadish TM, Reddy M, Lingaiah HK, et al. Mean platelet volume in Type 2 diabetes mellitus. J Lab Physicians 2012; 4:59.
7. Buch A, Kaur S, Nair R, Jain A. Platelet volume indices as predictive biomarkers for diabetic complications in Type 2 diabetic patients. J Lab Physicians. 2017 9(2):84-88.doi: 10.4103/0974-2727.199625. PMID: 28367021; PMCID: PMC5320886
8. Reaven GM. Role of insulin resistance in human disease. Diabetes 1988; 37:1595-607.
9. Grundy SM. Hypertriglyceridemia, atherogenic dyslipidemia, and the metabolic syndrome. Am J Cardiol 1998; 81:188-258
10. Fowler MJ. Microvascular and macrovascular complications of diabetes. Clin Diabetes. 2008;26:77-82
11. Subramanian S, Green SR. A review of relationship between platelet indices and microvascular complications in type 2 diabetic patients. Int J Adv Med. Rev. 2020;7(4):714-719.
12. Dwivedi T, Davangeri. Variation of Platelet Indices among Patients with Diabetes Mellitus. J Clin Diag Res, 2018;12(11):22-26.
13. Davi G, Patrono C. Platelet Activation and Atherothrombosis. N Engl J Med. Rev. 2007; 357:2482-94.
14. Elalamy I, Chakroun T, Gerotziafas GT, Petropoulou A, Robert F, Karroum A et al. Circulating platelet-leukocyte aggregates: a marker of microvascular injury in diabetic

patients. Thromb Res. 2008;121(6):843-8. doi: 10.1016/j.thromres.2007.07.016PMID: 17825880.

15. Rizvi A, Sanders MB. Assessment and monitoring of glycemic control in primary diabetes care: monitoring techniques, record keeping, tests of average glycaemia, and point of care evaluation. J Am Acad Nurse Pract. 2006; 18:11–21. doi.org/10.1111/j.1745-7599.2006.00092.
16. International Diabetes Federation Atlas. 5th ed. 2012. Available from http://www. Indiaenviromentalportal.org. accessed on December 2020.
17. Colwell JA, Nesto RW. The platelet in diabetes: focus on prevention of ischemic events. Diabetes Care. 2003;26(7):2181-8.doi: 10.2337/diacare.26.7.2181. PMID: 12832332.
18. Yilmaz T, Yilmaz A. Relationship between Altered Platelet Morphological Parameters and Retinopathy in Patients with Type 2 Diabetes Mellitus", Journal of Ophthalmology, 2016, https://doi.org/10.1155/2016/9213623
19. Biadgo B, Melku M, Mekonnen S, Abebe M. Hematological indices and their correlation with fasting blood glucose level and anthropometric measurements in type 2 diabetes mellitus patients in Gondar, Northwest Ethiopia. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 2016;9:91-99.
20. Joshi AA, Jaison J. Study of Platelet Parameters- Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) in Type 2 Diabetes Mellitus. Annals of Pathology and Laboratory Medicine. 2019;6(8): 408-13.DOI: 10.21276/APALM.2467
21. Swaminathan A, Amitkumar K, Ganapathy S, Ayyavoo S. Evaluation of mean platelet volume and other platelet parameters in subjects with Type-2 diabetes mellitus. Natl J Physiol Pharm Pharmacol 2017;7(1):51-54.
22. Gowtham A, Sowmya B, Nayak R. A study on platelet indices in type II Diabetes Mellitus. PUMRJ. 2019; 2(1): 48-54.
23. Rajagopal L, Arunachalam S, Abdullah Sm, Ganesan V, Kathamuthu K, Ramraj B. Can Mean Platelet Volume and Platelet Distribution Width be used as Predictive Markers for Impending Diabetic Vascular Complications? J Clin Diagn Res. 2018; 12(2): 01-05.
24. Bhanukumar M, Ramaswamy P, Peddi NK, Menon NB. Mean Platelet Volume and Platelet Distribution Width as Markers of Vascular Thrombosis in Type 2 Diabetes Mellitus. Jpmer. 2016; 50(3):127-31.
25. Hekimsoy Z, Payzin B, Ornek T, Kandoğan G. Mean platelet volume in Type 2 diabetic patients. J Diabetes Complications. 2004;18(3):173-6. doi: 10.1016/S1056-8727(02)00282-9. PMID: 15145330

26. Shilpi K, Potekar RM. A Study of Platelet Indices in Type 2 Diabetes Mellitus Patients. Indian J Hematol Blood Transfus. 2018 Jan;34(1):115-120. doi: 10.1007/s12288-017-0825-9. Epub 2017 May 8. PMID: 29398809; PMCID: PMC5786619.
27. Sonali Jindal, Shilpa Gupta, Ruchika Gupta, Ashima Kakkar, Harsh V Singh, Kusum Gupta & Sompal Singh (2011) Platelet indices in diabetes mellitus: indicators of diabetic microvascular complications, Hematology, 16:2, 86-89, DOI: 10.1179/102453311X12902908412110 To link to this article: https://doi.org/10.1179/102453311X12902908412110.
28. Wu M, Xiao L, Yang X. Positive Relationship of Platelet Volume Indices with HbA1c in Unselected Type-2 Diabetes Mellitus Patients. Clin Lab. 2019 Aug 1;65(8). doi: 10.7754/Clin.Lab.2019.190101. PMID: 31414737.
29. Okano K, Shitamoto K, Araki M, Kawamoto C, Kawano R, Nogaki H. Influencing factors in quantitative measurement using activated platelet levels and platelet-activating capacity for the assessment of thrombosis in pre-metabolic syndrome and type 2 diabetes mellitus. Nurs Health Sci. 2018 Mar;20(1):69-78. doi: 10.1111/nhs.12389. Epub 2017 Dec 12. PMID: 29235231.
30. Kachekouche Y, Dali-Sahi M, Benmansour D, Dennouni-Medjati N. Hematological profile associated with type 2 diabetes mellitus. Diabetes Metab Syndr. 2018 May;12(3):309-312. doi: 10.1016/j.dsx.2017.12.015. Epub 2017 Dec 21. PMID: 29287841.
31. Dolasık, Ilhan & Sener, Selcuk & Celebı, Koray & Aydın, Zekı & Korkmaz, Ugur & Canturk, Zeynep. The effect of metformin on mean platelet volume in diabetic patients. 24.10.3109/09537104.2012.674165.
32. Xin, G., Wei, Z., Ji, C. *et al.* Metformin Uniquely Prevents Thrombosis by Inhibiting Platelet Activation and mtDNA Release. *Sci Rep* **6,** 2016. https://doi.org/10.1038/srep36222
33. Nusca A, Tuccinardi D, Pieralice S, Giannone S, Carpenito M, Monte L, et al. Platelet Effects of Anti-diabetic Therapies: New Perspectives in the Management of Patients with Diabetes and Cardiovascular Disease. Frontiers in Pharmacology, 2021.
34. Schneider DJ. Factors contributing to increased platelet reactivity in people with diabetes. *Diabetes Care*. 2009;32(4):525-527. doi:10.2337/dc08-1865

Annex

Annex I - Participant information sheet (English Version)

Principal Investigator: Hana Abebe (BSc, MSc candidate) AAU, CHS

Introduction: You are being asked to take part in research study on assessment of platelet indices in type 2 Diabetes mellitus.

The study has been approved by Addis Ababa University Medical Laboratory Science department research ethics committee.

Purpose: The purpose of this study is to evaluate platelet indices in type 2 diabetes mellitus in comparison with control group

Procedures to be carried on: you are invited to participate in the study after giving your consent by giving blood samples if CBC and FBS is not ordered by your doctor.

Risks associated with the study: There is no risk & serious invasive procedure at the beginning as well as at the end of the study, there is no additional time required from you to stay during study.

Benefits of the study: There is not any financial benefit to you. But the result of the study will be used for your clinical care as well as plays a role if platelet parameters could use for diagnosis and prediction of DM and will play a role in minimizing mortality and morbidity rate. There is no compensation for using your blood sample.

Confidentiality of your information: The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and the responsible physician. There will be no personal information to be attached to your data.

Termination of the study: We will respect your decision if you later change your mind. Your withdrawal of consent will not affect your right to receive medication. Also, you have the right to have question about the study. I will be glad to answer your questions about this study at any time.

You may contact me at e-mail address hannaab8@gmail.com, mobile +251991847387 /+251924778646

Department of Medical Laboratory Science research ethics office +251 11 275 5170

Annex II - Participants information sheet (Amharic version)

ስለ ጥናቱ ለተሳታፊዎች መረጃ የሚሰጥ አማርኛ ቅጽ

ስሜ- ሀና አበበ (የማሰተርስ ዲግሪ እጩ ተመራቂ)

እርስዎ በዚህ የምርምር ሥራ ለመሳትፍ ፍቃደኛ ኖት? ከአዲስ አበባ ዩኒቨርስቲ በሕክምና ሳይንስ ኮሌጅ የላቦራቶሪ ሳይንስ ትምህርት ቤት የምርምር ሥራ ሥነ-ምግባር ኮሚቴ ፈቃድ ያገኘ ሲሆን ከጥናቱ የሚገኘዉ ዉጤት ለማወቅ ይጠቅማል፡፡

የጥናቱ አላማ

በደም ውስጥ የሚገኙትን ፕላትሌት የሚባሉ የደም ሕዋሳቶችን በስኳር ታማሚዎች ላይ ያላቸውን ለዉጥ ለማወቅ ይረዳናል ወይ የሚለዉን ለማወቅ ነው። በዚህ የጥናት ሥራ ለመሳተፍ ፈቃደኛ ከሆኑ ከ3-4 ሚሊ ሊትር የደም ናሙና ይሰጣሉ፡፡

በዚህ ጥናት በመሳተፍዎ በሚሰጡት የደም ናሙና ሙሉ የደም ምርመራ (የፕላትሌት)ና የስኳር መጠን ውጤትዎን ለማወቅ ዕድል ይሰጠዎታል፡፡

ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት

ጥናቱ በርሶ ላይ የሚያመጣዉ ጉዳት የሌለ ሆኖ ለጥናቱ የሚያጠፉት ተጨማሪ ጊዜ ኣይኖርም።

ከጥናቱ የምያገኙት ጥቅም

ምንም ኣይነት የገንዘብ ጥቅም ባይኖረውም ከጥናቱ በሚገኘዉ ዉጤት ለማወቅ እና ተጠቃሚ እንዲሆኑ ይጠቅማል ኣላማው በስኳር ህመም የሚመጣውን ሞት ለመቀነስ ይረዳል ::

ከርስዎ የምናገኘዉ መረጃ እና ሚስጥራዊነቱ

በሰጡት ደም ላይ የሚደረገው የምርመራ ውጤት ሙሉ በሙሉ ሚስጥራዊነቱ እንደተጠበቀ ሆኖ እና ለጥናቱ ዓላማ ብቻ ጥቅም ላይ እንደሚውል ላረጋግጥልዎ እወዳለሁ፡፡በዚህ ጥናት ላይ ያለዎትን ጥያቄ በማንኛውም ሰዓት ሊጠይቁና ምላሺን ሊያገኙ ይችላሉ፡፡ በጥናቱ ላይ ያለመሳተፍም ሆነ በመሀል የማቋረጥ ሙሉ መብት አለዎት፡፡

በማንኛውም ሰዓት በጥናቱ ላይ ያለዎትን ጥያቄ ለመመለስ ደስተኛ ነኝ!!! በሚቀጥለው አድራሻ ሊያገኙን ይችላሉ፡፡

ሞባይል ስልክ፡- +251991847387/+251924778646

ኢሚይል: - hannaab8@gmail.com

የህክምና ላቦራቶሪ ትምህርት ክፍል የምርምር ሥነ-ምግባር ቢሮ ስልክ ቁጥር +251 11 275 5170

Annex III - Informed consent form (English version)

By signing below, you are agreeing that: (1) you have read and understood the participant information sheet, (2) questions about your participation in this study have been answered satisfactory, (3) you are taking part in this research study voluntarily (without any coercion).

Participant's ID

Participant's signature

Date

Name of person obtaining consent

Signature of person obtaining consent & date

Annex IV Informed consent form (አማርኛ ቅጽ)

እርስዎ በጥናቱ ለመሳተፍ ሙሉ ፈቃደኛ መሆኖን የሚገልጽበት ቅጽ

ከዚህ በታች ያለውን በጥናቱ ለማሳተፍ ፈቃደኛ መሆኑዎን እንዲያረጋግጡ በሚጠይቀው ቅጽ ላይ ሲፈርሙ ነው፡፡

1. ከላይ የተሰጠዎትን ስለጥናቱ መረጃ የሚሰጠውን ጽሑፍ ማንበብዎን ና መረዳትዎን
2. በጥናቱ ላይ ላነሱት ጥያቄ አጥጋቢ ምላሽ እንዳገኙ
3. በጥናቱ ለመሳተፍ የወሰኑት በራስዎ ሙሉ ፈቃደኝነት ና ምንምዓይነት ተጽዕኖ ወይም ግፊት ሳይደረግብዎ መሆኑን ያረጋግጣሉ፡፡

……..

የተሳታፊው መለያ ቁጥር

…… ……

የተሳታፊው ፊርማ ቀን

…… . ……..

ስምምቱን የተቀበለው ሰው ስም ፊርማ እና ቀን

Annex V – Check list

Patient Background data check list		
Fill only by Health professional		
No	**Question**	**Response**
1	Sex	Male ☐ Female ☐
2	Age	……..…… years
3	Duration of Diabetic	……..……..
4	Fating blood sugar result	……..…mg/dl
5	What type of glycemic control was taking?	…… (Without metformin exclude)
5	DM related complication	1. Ketoacidosis 2. Retinopathy 3. Neuropathy 4. Angiopathy 5. Nephropathy 6. Infection 7. Other specific ……
6	HbA1C level	_____%
7	Result of peripheral morphology **(Selected blood sample)**	

Annex VI - Standard Operating Procedures

SOP for Blood collection

Equipment's

- 21 gauge needle for each participant with closed vacutainer system
- Blood collection tubes for each participant
- Tourniquet
- Disposable gloves
- Alcohol wipes
- Cotton balls/swabs
- Bandages
- Pillow/pad for raising arm to comfortable elevation
- Safety box's
- Pen/pencil

Procedure for drawing blood

Step 1 – Assemble equipment

Step 2 – Identify and prepare the patient

Step 3 – Select the site

Step 4 – Perform hand hygiene and put on gloves

Step 5 – Disinfect the entry site

Step 6 – Take blood

Step 7 – Fill the laboratory sample tubes

Step 8 – Draw samples in the correct order

Step 9 – Clean contaminated surfaces

SOP for Mindray BC3000 plus and reagents (CBC)

Specimen requirements

- About 3-4 ml of venous blood collected into EDTA tubes.

Procedure

1. Turn ON the power switch on the right side of the unit. Self-check, auto rinse, and background check will be automatically performed, and the "Ready" (ready for analysis) will appear.
2. When auto rinse and background check are normally completed, "Ready" is displayed.
3. Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material.
4. If the result of quality control in acceptable range run the blood samples.
5. Press [SAMPLE No.] key in the Ready status.
6. Entering patient ID, sample ID, Patient name, etc
7. Press [ENTER] key, this will fix the sample No. and the status becomes ready for analysis.
8. Mix the sample sufficiently before analysis.
9. Set the tube to the sample probe, and in that condition, press the start switch.
10. When the LCD screen displays "Analyzing," remove the tube.
11. After that, the unit executes automatic analysis and displays the result on the LCD screen.
12. Analysis result can be printed out on the built-in printer

Reagents

Diluent: is ready to use for impedance and photoelectrical analysis of whole blood, its ingredients are: sodium chloride, boric acid, sodium tetra borate, EDTA-2K.

Lyze: is ready to use lysing reagent to analyze the leucocytes by lysing the RBC and left the WBC Free and easy to count; whole blood sample by resistance measurement and photometric measurement, and its ingredients are: non-ionic surfactant, organic quaternary ammonium salt

Rinse: is a strong alkaline detergent to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of Mindray analyzer. Ingredients: sodium hypochlorite.

SOP for Blood Smear by Wright Staining

Specimen requirements

About 3-4 ml of venous blood collected into EDTA tubes.

Procedure

1. Place a drop of blood, about 2mm in diameter approximately inch from the frosted area of the slide.
2. Place the slide on a flat surface, and hold the narrow side of the non-frosted edge between your left thumb and forefinger.
3. With your right hand, place the smooth clean edge of a second (spreader) slide on the specimen slide, just in front of the blood drop.
4. Hold the spreader slide at a 30 angle, and draw it back against the drop of blood.
5. Allow the blood to spread almost to the edges of the slide.
6. Push the spread forward with one light, smooth, and fluid motion. A thin film of blood in the shape of a bullet with a feathered edge will remain on the slide.
7. Label the frosted edge with participant code # and date.
8. Allow the blood film to air-dry completely before staining.
9. Place the air dried smear film side up on the staining rack
10. Cover the smear with Wright stain and leave for 5 minutes
11. Dilute with buffer for 5 minutes.
12. Wash the smear with tap water
13. Air dry the smear
14. Count the platelets under 100x objective

Quality control

1. Prepare one differential slide daily using a patient sample with a normal MCV, MCH, MCHC and total white count. Stain the slide as indicated in procedure section. Review the slide for Color, Precipitation and Contamination.
2. If the color does not meet the specifications, identified in the precipitation and/or contamination are present, the quality is determined to be unsatisfactory. Replace the stain solution.

SOP for Finecare™ HbA1c Rapid Quantitative Test

Analyzer name - Finecare™

Specimen - Whole blood is only applicable for this test kit. Blood collected into EDTA tubes.

Principle – The Finecare™ HbA1c Rapid Quantitative Test is based on fluorescence immunoassay technology. The Finecare™ HbA1c Rapid Quantitative Test uses a sandwich immunodetection method to measure percentage of HbA1c in human blood. After mixing with sample and buffer, sample mixture is added to the sample well of the Test Cartridge, the fluorescence-labeled detector HbA1c antibody binds to HbA1c in blood specimen. As the sample mixture migrates on the nitrocellulose matrix of test strip by capillary action, the complexes of detector antibody and HbA1c are captured to HbA1c antibody that has been immobilized on test strip. The fluorescence-labeled detector Hb antibody binds to Hb in blood specimen; the complexes are captured to Hb antibody that has been immobilized on test strip. Signal intensity of fluorescence is proportional to concentrations of HbA1c and Hb in blood specimen. The ratio between inflorescent signals of HbA1c and Hb is the ratio between HbA1c and Hb.

MATERIALS

- Test Cartridge
- Detector buffer
- Finecare™ FIA Meter
- Transfer Pipette Set (10μL, 100μL size)
- Specimen Collection Containers
- Sterile syringe
- Alcohol Pads
- Timer

STORAGE AND STABILITY

1. Store the detector buffer at 4～30℃. The buffer is stable up to 24 months.
2. Store Finecare™ HbA1c Rapid Quantitative Test Cartridge at 4～30℃, shelf life is up to 24 months.
3. Test Cartridge should be used within 1 hour after opening the pack.

Procedure

Refer to Finecare™ FIA Meter Operation Manual for the complete instructions on use of the Test. The test should be in room temperature.

Step1: Preparation

Check/insert ID Chip into the instrument.

Step2: Sampling

Draw 10 μL of whole blood with a transfer pipette and add it to the buffer tube.

Step3: Mixing

Mix well the specimen with buffer for 1minute by tapping or inverting the tube.

Step4: Loading

Take 75 μL of sample mixture and load it onto the sample well of the Test Cartridge.

Step5: Testing

1. Finecare™ FIA meter:

Standard test: Insert the Test Cartridge onto the Test Cartridge Holder and click **"Test".** 5 minutes later, the result will show in the display and print out when click **"Print".**

Quick test: Put the Test Cartridge on the operation platform. 5 minutes later, insert the Test Cartridge onto the Test Cartridge Holder and click **"Test"**. The result will show in the display and print out when click **"Print".**

2. Finecare™ multi-channel FIA meter:

Insert the Test Cartridge onto the Test Cartridge Holder. 5 minutes later, the result will show in the display and print out when click "Print".

Quality control

- Each Finecare™ HbA1c Rapid Quantitative Test Cartridge contains internal control that satisfies routing quality control requirements.
- This internal control is performed each time a patient sample is tested. This control indicates that the Test Cartridge was inserted and read properly by Finecare™ FIA Meter. An invalid result from the internal control causes an error message on Finecare™ FIA Meter indicating that the test should be repeated.

Annex VII - Checklist

Result reporting form

Code Nº	Age	Sex	Diabetic complications if any	Duration of DM	Drug	PLT count	MPV	PCT	PDW	P-LCR	FBS	HbA1C

Code Nº	Peripheral morphology result Remark: - CBC result (High or Low) circle it the result

Annex VIII - Dummy table

Variables	Diabetic group (n =)	Control group (n =)	F-test	P value
Male				
Female				
PLT count				
PDW				
MPV				
PCT				
P-LCR				
FBS				
HbA1C				

Parameters	Complications present	Complications absent	P-value
PLT count			
PDW-SD			
PCT			
MPV			
P-LCR			

Declaration

Assurance of Principal Investigator

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

Name of the student

MSc candidate **Hanna Abebe (BSc)**

Date: - _____ Signature: - _____

Approval of Advisors

Fikadu Urgessa (MSc, Ph.D. fellow)

Date_____ Signature _____

Melatwork Tibebu (MSc, Ph.D. fellow)

Date _____ Signature _____