ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of the relationship between some hematological parameters and blood glucose level and its role as predictors of glycemic control among diabetes mellitus patients attending at ALERT hospital, Addis Ababa, Ethiopia

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A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Science (Hematology and Immunohematology track).

May, 2021

Addis Ababa Ethiopia

Addis Ababa University
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This is to certify that the thesis prepared by Wubalem Biresaw, entitled:
Assessment of the relationship between HbA1c and blood glucose level with hematological parameters and the role of some hematological parameters as predictors of glycemic control among diabetes mellitus patients attending at ALERT hospital, 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 with respect to originality and quality.

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ACKNOWLEDGEMENT

First and above all I would like to thank the almighty God for his unreserved support throughout my life. And I wish to express my sincere gratitude and appreciation to my advisors Mr. Zemenu Tamir and Mr Moges wordofa for their exhaustive, guidance and support from the beginning to the whole process of my thesis. Moreover, Addis Ababa University Department of medical laboratory science deserve duly acknowledgement for giving me golden opportunity to prepare this thesis. I would also like to forward my indisputable gratefulness to Dr. Beruk Kebede for guiding and supporting me, phlebotomists and nurses of medical outpatient department of ALERT Hospital sister Dehab and Sister Aster deserve gratitude for their help in the whole process of sample collection, and also ALERT Laboratory staffs for their good understanding, supporting, encouraging and cooperating with me for the whole process of my research.

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ABREVIATIONS

ALERT: All African Leprosy Rehabilitation and Training

BMI: Body Mass Index

BP: Blood Pressure

CI: Color Index

EDTA: Ethylene Diamine Tetraacetic Acid

FBG: Fasting Blood Sugar

GDP: Gross Domestic Product

Hb: Hemoglobin

HbA1c: Hemoglobin A1c

HCT: Hematocrite

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean Corpuscular Hemoglobin Concentration

MCV: Mean Cell Volume

MPV: Mean Platelet Value

NLR: Neutrophil to Lymphocyte Ratio

PCT: Plateletcrit

PCV: packed cell volume

PDW: Platelet Distribution Width

PLCR: Platelet Large Cell Ratio

PLR: Platelet to Lymphocyte Ratio

RBC: Red Blood Cell

RDW: Red Cell Distribution Width

SD: Standard Deviation

SI: Saturation Index

T2DM: Type 2 Diabetic mellitus

TTAB: Tetradecyltrimethylammonium

WBC: White Blood Cell

WHO: World Health Organization

ABSTRACT

Introduction: Good glycemic control is the main recommendation to prevent the development of diabetic complications. Recently, hematological parameters became renewed interest in the clinical diagnosis and management of diabetes mellitus.

Objective: the objective of this study was to assess selected hematological parameters as predictors of glycemic control and its correlation with blood glucose level among diabetes mellitus patients at ALERT hospital, Addis Ababa, Ethiopia.

Methods: A cross-sectional study was conducted between February to August, 2020 among 422 diabetic patients attending at ALERT hospital to assess the utility of selected hematological parameters as indicators of glycemic control association with FBS. Diabetic patients fulfilling the inclusion criteria were selected using convenient sampling technique. Cell-Dyn Ruby and Cobasc331 analyzer were used. Data were analyzed using SPSS version 25 and Chi-square test was used to assess association of dependent and independent variables. ROC was employed to evaluate the utility of hematological parameters for glycemic control.

Result: Of the 422diabetic patients enrolled in the study, 242(57.4%) were females and 180 (42.6%) were males. ROC analysis result for MCH (AUC=0.647 (95% CI=0.575-0.719)), HCT(AUC=0.599 (95% CI=0.508-0.689)), and MCV(AUC=0.662 (95% CI=0.588-0.736)), cut points were >29.55pg, >45.05%, and >90.25fl for MCH, HCT, and MCV, respectively.HCT(r=0.15), MCV(r=0.189),and RDW (r=0.156) showed positive correlation with HbA1C. Correlation of hematological parameters with FBS indicated that RBC (r=0.147), HCT(r=0.112), and MPV (r=0.134) showed positive correlation with FBS.

Conclusion: HCT, RDW, MCH, and MCV had positive correlation with HbA1C; whereas, RBC, Hb, HCT, and MPV had positive correlation with FBS. HCT, MCV and MCH are identified as the most sensitive and specific predictors for glycemic control.

Key words: glycemic control, HbA1c, diabetes mellitus

1. INTRODUCTION

1.1 BACKGROUND

Diabetes mellitus is not a single disorder. It represents a series of metabolic conditions associated with hyperglycemia and caused by defects in insulin secretion and insulin action. Insulin allows glucose to move out of the blood into cells throughout the body where it is used for fuel. People suffered diabetics either do not produce insulin or cannot use insulin properly [1].

A single classification system for diabetes mellitus would facilitate the clinical care, epidemiology and studying the natural history of the disease. However, this is difficult because of limited availability of resources and current state of knowledge. Currently, most literatures categorized diabetes mellitus in to two types. These include, type 1diabetes, which is caused by an absolute deficiency of insulin secretion. Individuals with type 1 diabetes mellitus are often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers. The second much more common category is known as, type 2 diabetes; the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretor response [1, 2]. In addition to type 1 and 2 diabetics, gestational diabetes mellitus can also exist temporarily during pregnancy but still below those diagnostic of diabetes [3].

Due to building up of glucose in the blood instead of going to the cell, blood glucose level remains high in the blood stream. This not only starves all the cells that need glucose for fuel, but also harm certain organs and tissues that exposed to the high glucose level. This causes health complications like heart disease, nerve and kidney damage. Kidney failure impairs hematopoiesis. White blood cell and platelets production also affected due to failures of various organs [1].

In recent years, hematological parameters became renewed interest in the clinical diagnosis and management of diabetes mellitus. When plasma glucose elevated over

time, small amount of hemoglobin A are non-enzymatically glycated to form (HbA1c).HbA1c has a glucose attached to terminal valine in each B chain. Reduced erythrocyte life span increase blood glucose concentration. The Hb concentration depends not only on erythrocyte mass but also on plasma volume: hence, it is sensitive to variations in hydration and renal, liver and cardiac function. Once Hb concentration affected, glycemic level also affected [2].

In addition, elevated (WBC) is a classical inflammatory marker and is also associated with several cardiovascular disease risk factors and diabetes. Peripheral white blood cell count has been shown associated with insulin resistance, type 2diabetes, Coronary arteries disease, stroke, and diabetes micro and macro vascular complication [3]. The associations of increased MPV, platelet distribution width (PDW), plateletcrit (PCT) and platelet count with diseases related to endothelial dysfunction and inflammation as metabolic syndrome and diabetes have been shown to be markers of various non-communicable diseases such as diabetes mellitus [4].

Platelet hyper reactivity is a well-known contributing factor to the prothrombotic state in diabetics,

hence causing increased coagulation, impaired fibrinolysis, and endothelial dysfunction. These hyperactive platelets play a critical role in the pathophysiology of the thrombotic events leading to diabetic complications.MPV is a parameter used to assess platelet size, and it is a potential biomarker of platelet reactivity. It has been shown that larger platelets are more reactive than smaller ones. PDW can directly measure the variability in platelet size, and its high values suggest increased production of larger reticulated platelets [5].

HbA1c is when the sugar builds up in the blood; it binds to the hemoglobin in red blood cell. Hemoglobin (Hb) consists of four protein subunits, each containing a heme moiety, and is the red- pigmented protein located in the erythrocytes. Its main function is to transport oxygen and carbon dioxide in blood. Among this heterogeneous group of hemoglobin HbA1c is one of the glycated hemoglobin, a sub-fraction formed by the attachment of various sugars to the Hb molecule. HbA1c is formed in two steps by the

non-enzymatic reaction of glucose with the N-terminal amino group of the β-chain of normal adult Hb (HbA). In the erythrocytes, the relative amount of HbA converted to stable HbA1c increases with the average concentration of glucose in the blood. The conversion to stable HbA1c is limited by the erythrocyte's life span of approximately 100 to 120 days. As a result, HbA1c reflects the average blood glucose level during the preceding 2 to 3 months. HbA1c is thus suitable to monitor long-term blood glucose control in individuals with diabetes mellitus. Glucose levels closer to the time of the assay have a greater influence on the HbA1c level [6].

Factor that associated with good glycemic control for diabetic patient should be consistently advised to restrict sugar intake, exercise, stop smoking, adequate protein intake, weight management, increase glucose monitoring and adhere to medication instruction [1].

1.2 STATEMENT OF THE PROBLEM

According to the World Health Organization report on the global burden of diabetes mellitus, it was estimated that, over 422 million adults were living with diabetes in 2019; compared to 108 million in 1980. The global prevalence (age-standardized) of diabetes has been nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. By 2035, the prevalence is estimated to rise to 15.2%. Diabetes caused 1.5 million deaths in 2012, which is 2.2 million higher than the death caused by cardiovascular and other diseases and 43% of deaths occur before the age of 70 years. The percentage of deaths that occur before age 70 is higher in low- and middle-income countries than in high-income countries [7]. Furthermore, diabetes inflicts an economic burden on the global healthcare system and the wider global economy [8]. This burden can be attributed to medical costs, indirect costs associated with productivity loss, premature mortality, and the negative impact of diabetes on nations' gross domestic product (GDP) [9, 10].

In 2010, 12.1 million people were estimated to be living with diabetes in Africa and estimated to increase to 23.9 million by 2030 [11, 12]. In Sub-Saharan Africa, apart from the emerging communicable disease, diabetes mellitus has become considerably increasing. Accordingly, type 2 diabetes accounts for over 90% of diabetes in Sub-Saharan Africa, and population prevalence proportions ranged from 1% in rural Uganda to 12% in urban Kenya. Reported type 1 diabetes prevalence was low and ranged from 4 per 100,000 in Mozambique to 12 per 100,000 in Zambia. Gestational diabetes prevalence varied from 0% in Tanzania to 9% in Ethiopia. The review also revealed that the proportions of patients with diabetic complications ranged from7-63% for retinopathy, 27-66% for neuropathy, and 10-83% for microalbuminuria [13]. Furthermore, diabetes mellitus inflict significant economic loss in Africa, according to the finding on the economic burden of diabetes, resulted from a total economic loss of $25.51 billion, this translated into a total economic loss of Int$11,431.6, Int$4,770.6 and Int$ 2,144.3 per diabetes case per year in the groups 1, 2 and 3countries respectively [13].

A systematic review of diabetes in Ethiopia from 2000-2016 revealed that the prevalence of diabetes varied across cities/towns, ranging from 0.3% at Debre Berhan Referral Hospital to 7.0% at Harar town. In addition, the prevalence across urban and rural revealed that all studies found a higher prevalence in urban than rural areas [14].

Since diabetes is a worldwide disease it is important to monitor it without causing any complications. One of the parameters used to monitor diabetes is doing an HbA1c test. Few studies indicated the association of some hematological parameters with glycemic control. A study in turkey evaluates all hematological parameters in type 2 diabetes mellitus (T2DM) with inappropriate blood glucose management (HbAlc value >7 %). They investigate white blood cell, neutrophil, and lymphocyte counts and PDW were higher in type 2 diabetic patients with inappropriate glycemic management [15]. Additionally, a study in India revealed to estimate HbA1c using CBC parameters. Hb, erythrocyte sedimentation rate, PCV, red blood cells count, mean corpuscular volume, and mean corpuscular Hb exhibited a statistically significant difference at the level ($p<0.05$) between the normal and diabetic groups. [16]. A study in Sudan also shows a significant positive correlation between HbA1c value with Hb, HCT, and MCHC. No significant correlation between HbA1C and other RBCs parameters was observed. [17]

However, there have been no enough reports of hematological results report influence on the determination of HbA1C result. Even though some research showed a significant association of some hematological parameters with glycemic control, hematological parameters are not used to indicate the glycemic status of the patients.

Despite an increase in the epidemiology of diabetes mellitus in Ethiopia, studies are limited and most of them were focused on its magnitude. Published articles regarding the association of glycemic control and hematological parameters among Ethiopian diabetic patients are limited. Therefore, this study was intended to assess the relationship between HbA1C and hematological parameters and the status of some hematological parameters as predictors of glycemic control.

1.3 SIGNIFICANCE OF THE STUDY

Studies and experiences in the past have identified that the mortality, morbidity and economic impact of diabetes mellitus have been increasing steadily. Good glycemic control is advised for diabetic patients to prevent the complications of diabetes and better quality of life.

This study is aimed to assess the relationship of some hematological parameters with fasting blood sugar and Hemoglobin A1c as well as the status of some hematological parameters as predictors of glycemic control. Hematological parameters can be done at any time of the day without requiring special preparations like fasting, relatively cheap and widely available. This will be helpful for patients and attending physicians in order to prevent unwanted complications due to poor glycemic control. Besides, the finding from this study will be useful for policymakers and health officials to improve protocols and guidelines related to management and control of diabetes mellitus and it gives researchers insight to conduct further large-scale studies.

2. LITERATURE REVIEW

The Literature highlighted that high blood glucose level in type 2 diabetes mellitus contributes to disturbance of blood cells and its indices such as total WBC count, MCV, Red cell count, platelet count, and hemoglobin level. [6]

2.1 Platelet parameters among diabetic patients

A study conducted in India among 141 diabetic patients between January 2016 to May 2016 revealed that the platelet indices such as platelet distribution width (PDW), Mean Platelet Volume (MPV), Platelet Count (PLT) and platelet large cell ratio (PLCR) showed significant increment among patient with poor control of diabetes mellitus than patient with good control of diabetes mellitus. Furthermore, patients with poor control of diabetics and hypertension showed higher values of platelet parameters than those with only poor control of diabetic Mellitus [18].

A case-control study was conducted on 300 Type 2 diabetics and 200 non-diabetics in India from 2015 to 2016-to assess the status of platelet parameters comparatively. Accordingly, Platelet count was significantly decreased in diabetics ($P = 0.005$) than non-diabetics, and MPV was significantly increased in diabetic patients with complications as compared to diabetics without complications and non-diabetic group ($P < 0.0001$). PDW showed a statistically significant difference between diabetics with and without complications and nondiabetics ($P < 0.0001$). However, no statistically significant difference was observed in platelet‐large cell ratio (P‐LCR) among all three study groups. We found statistically significant correlation of MPV with diabetic retinopathy ($P = 0.000$), nephropathy ($P = 0.005$), and diabetic foot ($P = 0.048$). PDW was significantly increased in diabetic retinopathy ($P = 0.035$) and nephropathy ($P = 0.007$). P‐LCR had no statistically significant correlation with diabetic complications [5].

Similarly, a retrospective study had done in turkey shows that from 102 patients with type 2 diabetes, out of which 51 receiving insulin treatment and 51 receiving oral antidiabetics

(OAD). Hemogram data of insulin and OAD treated groups were compared. Platelet counts were 27866.67 ± 77693 109/L before treatment and 258941.18 ± 69068.2109/L in the OAD group at six months, p: 0.015 whereas; 293011.76 ± 73711.21 109/L before treatment and 289492.86 ± 82631.49 109/L in the insulin group at six months p: 0.821. The result of the study shows that glucose control effects decrease blood indices HbA1C, basophils, eosinophils, platelets, and lymphocyte counts [19].

Additionally, a study in Turkey was carried out in 65 patients with type 2 DM and 40 non-diabetic subjects. All diabetic patients were divided into two groups according to their HbA1c levels also showed that some of the platelet indices such as the Mean Platelet Volume (MPV), and Platelet Distribution Width (PDW) were significantly increased in the diabetic group compared to the non –diabetic control, and the association was statistically significant ($p<0.05$)[23,24]. Contrary to the above-discussed literature, a case-control study done in India revealed that the platelet indices had no significant difference between the diabetic cases non-diabetic controls. [22].

A case-control study done in Nigeria showed that the mean platelet count was significantly higher among a total of 100 consecutively recruited confirmed T1D patients. Subject included 52 male (52%) and 48 females (48%). The age range and the mean range were 25-60 years. [20, 21].

Another study from Ethiopia was conducted at the chronic illness clinic of Gondar university hospital from February to April 2015. The study aimed 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 comparative cross-sectional study was conducted in a total of 296 patients (148 cases and 148 healthy controls) who were selected using a systematic random sampling technique. Fasting blood glucose levels and hematological indices were determined by using Biosystems A25 and Sysmex-KX 21N analyzers, respectively. Independent sample t-test, Mann–Whitney U-test, and correlation statistics were used. A P-value, 0.05 was- considered statistically significant. There was a significant difference in red blood cell distribution width (47.3±2.6 fL vs 45.2±3 fL) between diabetic patients and control patients. Total white

blood cell in 103/uL. (6.59±1.42 fL vs 5.56±1.38). Absolute lymphocyte count in 103/uL (2, 6±0.7fL vs 2.04±0.63) increased significantly in the diabetic patient compared with control. Among platelet indices, mean platelet volume (10.4±1.1 fL vs 9.9±1.1fL) and platelet distribution width (14.5±2.1fL vs. 13.4±2. 1fL) were found to be significantly increased in the diabetic patients ($p<0.05$).[36].

2.2. Total WBC count among diabetic patients

Evidence suggested that inflammation is risk factors for many chronic non-communicable diseases including diabetes mellitus [23], for example, a study done in India to assess the relationship between WBC count and diabetic complications revealed that, among a total of 130 individuals with type 2 diabetes 56% had Total White Blood Cell Counts of the upper normal level side of the normal range furthermore, the differential count revealed that, the proportion of Polymorphs were also on the higher side of the normal range [24].

Similarly, the case-control study was conducted in India showed that the periodontal and hematological manifestations in diabetic and non-diabetic patients. Periodontal evaluations were assessed by probing pocket depth, clinical attachment level, and Russell's periodontal score. There was a statistically significant ($p=0.000$) difference in the diabetic group than the control group with Hb, HbA1c levels, differential counts, total leukocyte count, clinical attachment level [25].

A study from China also revealed 50 patients with diabetic ketosis (DK), 50 non-DK diabetic patients with stable glycemic control, and 50 normal controls were enrolled. Their total and differential leukocyte counts were measured and evaluated at admission and after treatment significant correlation between diabetic ketosis and leukocytosis accompanied with neutrophilia [26]

Similarly, a study from Nigeria from a total of 100 consecutively recruited confirmed T1D patients constituted. Subjects included 52 males (52%) and 48 females (48%). The age range and mean range were 25-60 years and 42.45 years ± 12.23 years respectively. Forty

age and gender-matched non-diabetics were monitored as controls also showed a significant association between type 1 diabetes mellitus and increased level of WBC was obesereved [21].

2.3 Red cell indices among diabetic patient

Several data demonstrated the effect of hyperglycemia on various body tissues and subsequent glycation of different proteins. Bone marrow is one of the hemophilic tissues that produce all the different types of blood cells regularly, including red blood cells through a process called erythropoiesis. The continuous raising of glycosylated hemoglobin as a result of diabetes-related diabetes mellitus affects the structure and function of the hemoglobin molecule, it also affects the cytoplasmic viscosity within each cell, and subsequently could have a noticeable effect on any of the red blood cell indices. [27].

A Prospective study done in India including 141 patients in the Department of Medicine, Mayo Institute of Medical Sciences between January 2016 to May 2016 was done. Diabetic patients were divided into those with hypertension and those without hypertension. Patients were also divided as good control (HbA1c <7%), poor control (HbA1c between 7-9%), and uncontrolled (HbA1c>9 and also study from Iran showed all the red cell indices were elevated among the diabetic patients[18,28,29].

Similarly, a cross-sectional study did in a Saudi hospital-based study of 1000 type 2 Saudi diabetic patients without any hematological diseases. Patients were fully evaluated clinically and biochemically with full blood hematological parameters assessment. The study showed that a resultant increase of all the red cell indices including the red blood cells count, mean corpuscular volume (MCV)(r=0.07,p= 0.053), mean corpuscular hemoglobin (MCH)(r= 0.08,p=0.021), mean corpuscular hemoglobin concentration (MCHC)(r= 0.05,p= 0.175), among the diabetic cases with noticeable hyperglycemia. However, the Red blood cell distribution width (RDW) (r=-0.18,p= < 0.001) was not linearly associated with hyperglycemia [30].

A comparative cross-sectional study in India among men with type 2 diabetes and healthy men in the study the authors compared the two groups red cell indices, and the prevalence of anemia among the diabetic men was 26%, in addition, besides the peripheral red cell morphology also showed various abnormalities such as microcytic, hypochromic red cell morphology and with MCV of more than 100 [31].

Opposing to the findings aforementioned litterateurs a study from Iraq revealed the hemoglobin concentration and PCV percentage were found to have no difference among diabetic and non-diabetic health control [32]. Similarly, a study from India also revealed a similar finding that Red cell count, PCV and, MCV was lower among the diabetic case as opposed to the non –diabetic controls [22]. Likewise, a study in Iraq revealed a Correlation between some Blood Parameters and Diabetic Mellitus Type 2 among women and the result showed a significant decrease of some of the red cell indices such as MCH, MCV, MCHC color index (CI) and, saturation index(SI) among the diabetic women unlike that of the non-diabetic control [33].

A cross-sectional study was done in El-Beida Hospital Libya in 103 Libyan types 2 diabetic patients (79 males + 24 females) and 39 healthy non-diabetic subjects (29 males and 10 females) acted as controls to assess the effect of diabetics in hematological parameteres. Accordingly, hematological studies in diabetic patients showed significantly lower HCT values, hemoglobin content, RBCs count, and MCV concentration than in the controls [34].

A Hospital-based, cross-sectional study was conducted on people with T2DM in the Federal police Specialized Hospital, Addis Ababa, and Ethiopia. This study involved 70 people with T2DM (male/females, 47/23) and 70 age and sex-matched Healthy people without T2DM (Male/females, 46/24). Male people with T2DM were characterized by significantly elevated levels of mean cell hemoglobin (MCH), red blood cell distribution width (RDW) as compared with healthy people without the T2DM group. Similarly, female people with T2DM were characterized by significantly elevated levels of HCT, MCHC, RDW, ($P<0.05$ as compared with non-diabetic female control [35].

A comparative cross-sectional study was conducted at the chronic illness clinic of Gondar University Hospital from February to April 2015. A total of 296 participants (148 cases and 148 healthy controls) were selected using a systematic random sampling technique. There was a significant difference in red blood cell distribution width (47.3±2.6 fL vs 45.2±3 fL) between diabetic patients and controls. Total white blood cells in 103/μL (6.59±1.42 vs 5.56±1.38), absolute lymphocyte count in 103/μL (2.60±0.70 vs 2.04±0.63), and absolute neutrophil count in 103/μL (3.57±1.46 vs 3.11±1.04) increased significantly in diabetic patients compared with controls, respectively. Among 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) [36].

2.4 HbA1c and fasting blood sugar

A cross-sectional analysis was carried out in a sample of 87,284 non-diabetic Koreans without anemia who participated in comprehensive health check-ups between January and December 2009 at the Kangbuk Samsung Hospital. The study revealed that HbA1c was increased linearly with increasing fasting blood sugar levels among men and women participated in the study, with a significant increase of HbA1c among women than men [37].

Similarly, a cross-sectional study was done to determine the HbA1c variability and diabetic peripheral neuropathy in type 2 diabetic patients revealed that among the recruited diabetic patients, 18.1% were found to have diabetic peripheral neuropathy, and also showed a higherHbA1c values than the patients without diabetic peripheral neuropathy [40].

Similarly, a cross-sectional study in Cameroon and Guinea involving 1267 people (61% women) with type 2 diabetes (mean age 58 years) was recruited across health facilities. Predictors of poor glycemic control (HbA1c ≥7.0% (53mmol/mol)) were investigated via logistic regressions. The mean body mass index was 27.4 ± 5.8 kg/m2, and 74% of patients had poor glycemic control. Predictors of poor glycemic control in multivariable regression

models were recruitment in Guinea [odd ratio: 2.91 (95% confidence interval 2.07 to 4.11)], age <65 years [1.40 (1.04 to 1.88)], diabetes duration ≥3 years [2.36 (1.74 to 3.21)], Poor control of blood glucose is common in patients with type 2 diabetes in these two countries. Limited access to HbA1c appears to be a key factor associated with poor glycemic control in Guinea [38].

A retrospective chart review study was conducted at the diabetes clinic of Addis Ababa's public teaching hospitals among a randomly selected sample of 685 charts of patients with T2DMwho were on follow-up from January 1, 2013, to June 30, 2017. The Median time to first optimal glycaemic control among the study population was 9.5 months. Factors that affect time to first optimal glycaemic control were age group, for 60–69 years and > = 70 years), diabetes neuropathy, more than one complication, hypertension dyslipidemia, cardiovascular disease and, hospital patient being treated. The Median time to first optimal glycaemic control among T2DM patients is longer than expected which might imply that patients are being exposed to more risk of complication and death [39].

2.5 Complete Blood Count in Diabetes Mellitus with Glycemic Control

Several components of complete blood count were investigated and be higher in diabetic patients. A cross-sectional study in turkey evaluate white blood cell (WBC), neutrophil, lymphocyte and, platelet counts and, red cell distribution width (RDW), mean platelet volume (MPV) and, platelet distribution width (PDW) in type 2 diabetes mellitus (T2DM). 135 type 2 diabetic patients with inappropriate blood glucose management (HbAlc value >7 %) despite using insulin therapy for at least 3-month and 121 healthy subjects were included in the study. A t-test was performed to compare the differences between two independent groups. WBC, neutrophil, lymphocyte and, monocyte counts were higher in the DM group ($p<0.0001$). In this study white blood cell, neutrophil and, lymphocyte counts and PDW were higher in type 2 diabetic patients with inappropriate glycemic management [15].

A cross-section study in India revealed that a total of 83 subjects (mean age: 52.8±9.0 years) were involved in the study, among which 39 (mean age: 49.1±8.8 years) were normal and 44 (mean age: 56±7.8 years) were diabetic. The stepwise linear regression model was used to determine the empirical formula to estimate HbA1c using the CBC parameters. The Student's t-test was performed to identify the group differences. A Negative correlation was observed for Hb ($r=-0.35^{**}$, $p<0.001$) and packed cell volume (PCV) ($r=-0.23^{**}$, $p<0.05$) against HbA1c. The CBC parameters Hb, erythrocyte sedimentation rate, PCV, red blood cells count, mean corpuscular volume, and mean corpuscular Hb exhibited a statistically significant difference at the level ($p<0.05$) between the normal and diabetic groups. The empirical formula derived to estimate HbA1c could be useful in the prediction of diabetes with an appreciable accuracy [16].

A Cross-sectional survey was done in South District in the central part of Ghana. A total of 691 adults between 18 and 59 years the resident were randomly selected using the Kintampo Health and Demographic Surveillance System and enrolled in this study. Out of these, 625 adults made up of 316 males and 309 females were assessed by a clinician to be healthy. Reference values established include hemoglobin 113–164 g/L for males and 88–144 g/L for females; total white blood cell count 3.4–9.26109 /L; platelet count 88–3526109 /L for males and 89–4036109 /L for females. .Using the hematological reference values based on the package inserts would have screened out up to 53% of potential trial participants and up to 25% of the population using the biochemical parameters [40].

2.6 HemoglobinA1c values and hemoglobin level

A cross-sectional study done in Korea was carried out in a sample of 87,284 non-diabetic Koreans without anemia who participated in comprehensive health check-ups between January and December 2009 at the Kangbuk Samsung Hospital. They categorized men and women separately according to fasting plasma glucose and hemoglobin level. Women had a lower mean hemoglobin value compared with men, and women had higher HbA1c% levels at a given fasting glucose level consistently across the fasting glucose deciles. There was

also a gender-specific association between age and HbA1c (P< 0.001 for interaction). HbA1c values were affected by hemoglobin level and gender in nonanemic Koreans. Thus, hemoglobin level and gender should be considered in the diagnosis of diabetes using HbA1c [35].

This prospective study was carried out in the Obstetrics and Gynaecology Hospital of Fudan University china from November 2014 to February 2015. In total, 690 pregnant women between 20 and 35 years old were included in this study. One hundred seven women were diagnosis with GDM at 24–28 gw. An HbA1c cutoff value < 4.55% at 12–16 gw showed adequate sensitivity to exclude GDM (85.0%) but low specificity (17.3%), while an HbA1c cutoff value ≥ of 5.25% presented adequate specificity (96.6%) but low sensitivity (13.3%) in diagnosing GDM. The area under the receiver operating characteristic curve for HbA1C (12–16 gw) detection of GDM was 0.563 (95% confidence interval [CI], 0.50–0.625). When combined HbA1c with HCT (> 38.8%) for the screening of GDM, the area under the receiver operating characteristic curve was 0.604 (95% [CI] 0.509, 0.701) [41].

Cross-section study done in India Fifty patients confirmed to have iron deficiency anemia were enrolled in this study. HbA1c and absolute HbA1c levels were measured both at baseline and 2 months after treatment and these values were compared with those in the control population. The mean baseline HbA1c level in anemic patients (4.6%) was significantly lower than that in the control group (5.5%, $p<0.05$). A significant increase was observed in the patients' absolute HbA1c levels at 2 months after treatment (0.29 g/dL vs. 0.73 g/dL, $p<0.01$). There was a significant difference between the baseline values of patients and controls (0.29 g/dL vs. 0.74 g/dL, $p<0.01$). The study showed that HbA1c levels and absolute HbA1c levels increased with the treatment of iron deficiency anemia [42].

A facility-based comparative cross-sectional study was conducted on 174 diabetic patients (87 with IDA and 87 without IDA) from April to July 2016 in TikurAnebessa hospital. Socio-demographic data and clinical conditions were collected using a structured questionnaire. Mean hemoglobin (Hb), hematocrit (HCT), Mean cell volume (MCV), mean

cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) were lower in the IDA group compared to non-IDA diabetic patients. HbA1c (%) level was significantly lower in the IDA group (6.18 ± 1.57) compared with the non-IDA diabetic patients (7.74 ± 1.81) ($p < 0.05$). HbA1c is significantly lower in diabetic patients with IDA compared to non-IDA diabetic patients [43].

2.7 Glycated hemoglobin and red blood cell indices

A cross-sectional study done in India shows a correlation between Hb1Ac and RDW in diabetic individuals using a random sampling technique. In this study, 50 patients with type two diabetes mellitus were selected. This study shows a significant correlation between RDW and HbA1c in males (r = 0.400), whereas in females it was a weak correlation (r = 0.04). This agrees with Lippi et al in 2014(13) who also suggested that in the entire study population, HbA1c was significantly associated with RDW values (r = 0.11; p < 0.001), even after adjustment for age and gender [44].

This cross-sectional study was conducted on diagnosed type II diabetic patients visiting the outpatient department of medicine at Al-Tibri Medical College Hospital Pakistan from July 2017 to January 2018, A total of 119 patients were eligible for the study with a mean age of 48.63±12.462 (range 24-76)years; The mean hemoglobin of patients was 11.59±1.315 gm/dl. The mean corpuscular volume (MCV) was 76.65±11.121 fl and the mean RDW was found to be 18.287±4.352, with the highest value of 30.20. The mean MCH was 30.223±23.873 pg, with the highest value of 38.4pg. The mean cell hemoglobin concentration (MCHC) was 28.214±4.7498 mg/dl. The correlation of HbA1c with RDW turned out to be significant statistically (p-0.035) while other RBCs and/or hematological parameters, such as MCV, hemoglobin, and platelets, revealed no significant correlation [45].

the cross-sectional study was carried- out at the antenatal care of Saad Abu Elela Hospital, Khartoum, Sudan Obstetrics history was gathered using a questionnaire, and body mass

index was calculated. Fasting blood sugar, at one hour, at two hours postprandial, and HbA1c were investigated. Complete blood count parameters of RBCs count performed, including, hematocrit, Hb, RBCs indices of MCV, MCH, and MCHC. One hundred twenty-three women were enrolled. The mean age of the participants was 28±5.6 years and the mean body mass index was 27.65±6.8 k/m2. There was significant positive correlation between HbA1c and Hb (r=0.174, P=0.037), HCT (r=0.174, P=0.037), and MCHC (r=0.180, P=0.031). A negative correlation between HbA1c and the platelet index PDW (r= –0.198, P=0.017) was documented. In linear regression analysis, HbA1c correlated positively with Hb (P=0.044) and HCT (P=0.047). The present study shows a significant positive correlation between HbA1c value with Hb, HCT, and MCHC. No significant correlation between HbA1c and other RBCs parameters was observed [17].

3. OBJECTIVES

3.1. GENERAL OBJECTIVE

Assessment of the relationship between some hematological parameters and blood glucose level and its role as predictors of glycemic control among diabetes mellitus patients attend at ALERT hospital, Addis Ababa, Ethiopia 2019/2020.

3.2. SPECIFIC OBJECTIVE

- To evaluate the correlation of hematological parameters with HbA1c.
- To assess the correlation of hematological parameters with blood glucose level.
- To determine the utility of selected hematological parameters as indicators of glycemic control.
- To determine associated factors with glycemic control among diabetes mellitus patients.

4. MATERIAL AND METHOD

4.1. STUDY AREA

The study was conducted at ALERT hospital in Addis Ababa, Ethiopia. Addis Ababa is the Capital City and the seat of the Federal Government and Parliaments. [47].The city is divided into ten sub-cities and 99 Kebeles. According to the 2007 census report, the Addis Ababa City administration had a population of 3,147,000, of which,1,511,000 are men and 1,636,000 women, The city has 39 hospitals, 41 Health Center, and 359 clinics [46].

Alert hospital is one of the government hospitals located in kolfekeraniyo sub-city. The hospital was opened in 1934 called princess Zenebework. A memorial hospital dedicated entirely to people with leprosy. Since 2000 the ministry of health of the federal republic of Ethiopia took over to integrate leprosy management into the general health service. The hospital bed capacity is 367. The hospital has 1600 total staff. Out of this, 760 are permanent health care providers and 840 of them are supportive staff. The hospital gives different services like dermatological, ophthalmology, ART, trauma management, plastic and reconstructive surgery, general chronic medical services including diabetic and hypertensive patients. Around 750 patients are getting permanent outpatient services on diabetic cases. Of this number, 700 are patients between the age of 25-82 years old and 50 of them are under 20years [47].

4.2. STUDY DESIGN AND PERIOD

A cross-sectional study was conducted on diabetic Mellitus patients attending at ALERT hospital during the study period (February to August 2020).

4.3 SOURCE POPULATION

All diabetic patients attending medical outpatient department (OPD) side in ALERT hospital during the study period.

4.3.1 STUDY POPULATION

Diabetic patients above the age of 18 years and who are attending medical OPD in ALERT Hospital during the study period and fulfilling the inclusion criteria of the study.

4.4 INCLUSION AND EXCLUSION CRITERIA

4.4.1 INCLUSION CRITERIA

- All adult diabetic patients who were attending ALERT hospital during the study period and willing to participate were included in the study.

4.4.2 EXCLUSION CRITERIA

- Diabetic patient with concomitant anemia
- Pregnant women
- HIV patient
- TB patient
- Patient with Renal Failure
- Patient with malignancy
- Patient with a hematological abnormality
- Patient previously diagnosed with chronic disease

4.5 STUDY VARIABLE

4.5.1 DEPENDENT VARIABLE

- Blood glucose value
- Status of glycemic control
- HgbA1C
- All hematological parameters (WBC, RBC, and PLT).

4.5.2 INDEPENDENT VARIABLE

- Socio-demographic variables such as age, sex, educational status, marital status, residence, and occupational status

- Blood pressure, BMI, alcoholism, smoking, and type of diabetes, exercise, and medication.

4.6 SAMPLE SIZE AND SAMPLING METHOD

4.6.1 Sampling size and Sample technique

Sample size

Single population proportion formulae were used to calculate the sample size using by considering the following assumptions: 95% confidence interval, the sample size was determined to be 422. A convenient sampling technique was employed to select the study participants.

Sample size calculation formula

$$n = \frac{(z1-\alpha/2)2pq}{D2}$$

- n= minimum sample size required
- P= proportion of prevalence 0.5
- q =1-0.5
- d= the margin of sampling error tolerated = 0.05
- z1-α/2= the standard normal value at 5% error is 1.96

$$n= \frac{1.96^2 X (0.5)(0.5)}{(0.05)^2}$$

n=384

Add10% non-respondent thus final sample size (nf) will be

nf =384+ (384*10%)

nf =384+38

nf =422

Therefore, the final sample size(nf) was 422

4.6.2 Sampling technique

A convenient sampling technique was used to select diabetic patients who fulfill the eligibility criteria. The following framework was applied to select the patient. First, patients with chronic illness were identified from the patient registration book, and then all diabetic patients on follow-up were selected again from the registration book. Finally, by reviewing their medical records eligible participants were selected as the right patient for this study.

4.7 MEASURMENT AND DATA COLLECTION

4.7.1 Data collection procedure

After a brief explanation about the aim of the study and obtaining informed consent, 9 milliliters of blood was collected by experienced phlebotomist Socio-demographic data was collected by a senior nurse using a pre-tested, structured questionnaire originally developed in English and translated to Amharic. Four milliliters (4 ml) sample collected with Ethylene diamine tetraacetic acid (EDTA) anticoagulant tube used for hematological tests and HbA1c. Five milliliters (5 ml) sample collected with serum separator clot activator tubes used for fasting blood sugar. After the samples were collected, the phlebotomist shipped the samples to the laboratory with sample shipment universal precaution technique. Tests were analyzed using Cell-Dyn Ruby for hematological and Cobasc331 analyzer for) fasting blood sugar tests and HbA1c determination. The results for all hematological parameters, HbA1c, and fasting blood sugar were recorded on the prepared worksheet. Physical measurements like weight and height for BMI measurement and blood pressure of the patient were measured and recorded on the prepared socio-demographic worksheet.

4.7.2 LABORATORY ANALYSIS

All samples shipped to the laboratory as soon as possible using safety precaution procedure by a trained phlebotomist. After the samples reached the laboratory, sample segregations were done. Sample with EDTA anticoagulant was used for hematological and HbA1c test. Hematological test analyzed using Cell-Dyn Ruby analyzer and HbA1c test analyzed using Cobasc331analyzer. Samples with serum separator gel were analyzed using Cobasc331analyzer. Samples were processed as soon as possible using LAB SOP strictly.

4.7.3 CELL-DYN RUBY Quality control and sample analysis principle and procedure

The Cell-Dyn Ruby uses the same principle for quality control and the patient sample process. It uses flow Cytometry and impendence technique to analyze RBC/PLT and WBC and Colorimetric for hemoglobin detection. Flow cytometry is a standard laser-based technology that is used in the detection and measurement of a physical and chemical characteristic of the cell or particle in a heterogeneous fluid mixture. A particle's size, granularity or internal complexity, and fluorescence intensity are detected based on light scattered and fluorescence intensity in a stream through a laser beam. Cell-Dyn Ruby after pipe the sample, the sample pass to the ultrasonic and Led sensor located downstream of the shear valve that checks the sample stream to ensure the proper amount of sample has been transferred through the shear valve. The shear valve rotates to separate; 20µl for WBC dilution, 1.67µl for RBC/PLT dilution, and 12µl for the Hb dilution. Hb is measured using the colorimetric determination method. The detector selectively converts heme into a stable chemical complex that absorbs maximally at 575 nm. The intensity of the color is directly proportional to the total concentration of hemoglobin in the sample [48, 49].

For reliable performance that leads to quality results, three levels of commercial quality controls were used that were low, normal, and high, and in-house quality control was also used. In the morning quality control was done daily by following laboratory procedures

strictly. Once the quality control sample passed the acceptable criteria using the Levey-Jennings control chart, the Patient sample proceeds [48, 49].

Preparation: - quality control samples are ready to use and store at 2-8 °c. Remove the quality control from the refrigerator and put it at room temperature for 15 minutes. After the quality control becomes normal like the patient sample, process using Cell-Dyn Ruby analyzer.

4.7.4 COBAS c 331 QC AND SAMPLE PROCESSING PRINCIPLE AND PROCEDURE

QC and sample analysis used the same principles which use photometric analysis and ion-selective electrode measurements and uses serum/plasma and whole blood sample for analyzing. For serum/plasma samples like fasting blood sugar; the sample is mixed with the appropriate reagent to produce a reaction that results in color. The concentration of the analyte determines the strength of color produced.

The photometer shines the light of the appropriate wavelength at the sample and measures the amount of light absorbed, which is directly correlated to the concentration of the analyte in the sample. An ion-selective electrode (ISE) is a sensor that determines the concentration of ions in a solution by measuring the current flow through an ion-selective membrane [50, 51].

To test glucose enzymatic reference method with hexokinase used. Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP. Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically [50, 51].

Whole blood samples like HbA1c use the turbidometric inhibition immunoassay technique. Tetradecyltrimethylammonium bromide (TTAB) is the detergent in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). Sample pre-treatment to remove labile HbA1c is not necessary. All

hemoglobin variants which are glycated at the β- chain N-terminus and which have antibody-recognizable regions identical to that of HbA1c are measured by this assay. Consequently, the metabolic state of patients having uremia or the most frequent hemoglobinopathies (HbAS, HbAC, and HbAE) can be determined using this assay.

Hemoglobin A1C: The HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood.

1. Sample and addition of R1 (buffer/antibody reagent): Glycohemoglobin (HbA1c) in the sample reacts with anti-HbA1c antibody to form soluble antigen-antibody complexes. Since the specific HbA1c antibody site is present only once on the HbA1c molecule, formation of insoluble complexes does not take place.
2. Addition of R 3 (buffer/polyhapten reagent) and start of reaction: The polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be determined turbidimetrically.

Hemoglobin: Liberated hemoglobin in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum which is measured bichromatically during the pre-incubation phase (sample + R1) of the above immunological reaction. A separate Hb reagent is consequently not necessary [50, 51].

QC materials use two controls, which was preipath u plus (in the pathological range or at the normal/pathological threshold)) and precicontrol clinchem multi 1(in the normal range or at the normal/pathological threshold. Dispense the required volume into a sample cup and analyze in the same way as a patient sample. The control runs daily in parallel with the patient sample and after every calibration control. Control interval adapted to individual laboratory's requirements.

Preparation - Carefully open one bottle1, by avoiding the loss of lyophilizate and pipette in exactly 3.0 ml of diluents (bottle 2). Carefully close the bottle and dissolve the contents completely by occasional gentle swirling within 30 minutes. Avoid the formation of foam. The enclosed bar-coded labels are intended exclusively for the Roche/Hitachi Modular analyzers and Cobasc systems to identify the control. Attach the

bar-coded labels to the tubes carrying the sample cups containing the control materials. The control store at 2-8°c [50, 51]

4.8 DATA QUALITY ASSURANCE

To achieve good data quality data collectors were recruited based on their profession. Qualification for that reason, senior nurse was involved in the data collection and previous experience of data collection was considered. Before the data collection begins orientation was provided to the data collector on objectives of the study, steps, and approach for interviewing, proper sample collection, processing, and testing and recording of the result. Vague points and other problems encountered about the questionnaire were explained and clarified before the data collection.

During the time of data collection firm supervision was undertaken by the investigator to ensure good quality of data and appropriateness of the blood sample collection. At the end of each data collection, the questionnaire was crosschecked daily by the principal investigator, and problems faced were discussed overnight with data collectors.

The sample analyzed strictly adhere to laboratory SOP, the results obtained from quality control samples were within the mean ± 2 standard deviations (SD) of the given Levy Jennings's (LJ) chart. Therefore, to ensure the quality of the final results, pre-analytical, analytical, and post-analytical precautions were verified before the assay was taken. Finally, the samples were processed within 1 hour of specimen collection in the hematology and Cobasc331 machine respectively.

4.8.1 Pre-analytical: in this phase, the investigator checked the samples quality and its accuracy by checking the patient request with full information and proper labeling, handling, and transportation of the sample .based on sample acceptance and rejection criteria the sample evaluated for clot, hemolysis and lipemic and any sample with that problem rejected and also patient request that was not properly filled with necessary patient information and miss-match of sample in identification was rejected.

4.8.2 Analytical phase: in this phase, the patient specimen is prepared for testing and ends when the test result was interpreted and verified. Doing this the specimen was rechecked for its correct labeling and identifications again by the personnel who analyze the sample. After checking the sample accuracy the personnel checked the machine which is Cell-Dyn Ruby and Cobasc331 by doing daily, weekly or as-needed maintenance. After doing the maintenance the personal processed the daily QC. The QC was passed, the result plotted on LJ graph and rechecked all the daily work by section supervisor before the sample is processed .So it's verified that the sample is ready for processing.

4.8.3 Post-analytical phase Is the final phase of the testing process and involves evaluation of laboratory test results, the release of the test results in a timely promptly, particularly critical results, and modification of results for the doctors by LIS (Laboratory Information System). In this phase critical result was informed the clinic by phone call and delivering the result as soon as possible through the system. Transcription error, result review, flagging was rechecked and retested by senior laboratory personnel.

4.9 DATA ANALYSIS AND INTERPERTATION

Data were entered into a computer using Epi data version 3.1 and double-entry verification was made. Then data were exported into SPSS version 25 statistical software followed by data cleaning and analysis. SPSS Chi-square and correlation statistics were used to assess the association between dependent and independent variables. Receiver operator curve analysis was employed to evaluate the utility of hematological parameters for glycemic control. A P-value of <0.05 used to assess statistical significance.

4.10 OPERATIONAL DEFINITION

Good glycemic control: glycated hemoglobin (HbA1c) ≤7%.

Poor glycemic control: glycated hemoglobin (HbA1c) > 7%.

Glycemic control: maintain blood glucose level in the normal range, that for fasting blood sugar (70-99 mg/dl) and ≤ 7% for HbA1c.

Hyper glycemia: is high blood glucose which is >99 mg/dl.

4.11 ETHICAL CONSIDERATION

The study was ethically approved by the Department Research ethics Review committee of Medical laboratory Sciences, College of Health Sciences, Addis Ababa University. Letter of permission to conduct the study was obtained from the Hospital. Informed consent was sought from each study participant to participate in the study. All personal identifiers were removed and only codes were used throughout the study. Any abnormal findings were communicated to the attending physician for further management of the patient.

4.12 PLAN FOR DISSEMINATION OF THE RESULT

The findings of this study will be disseminated to the Addis Ababa Health office Department include the Department of medical laboratory's main campus Addis Ababa University presented at different national and international conferences, Sent for international peer-reviewed journals for publication. The findings were also disseminated to different health organizations that have a contribution to improve the utilization of hematological parameters for diabetic control services. The findings will also present in the Department of Medical Laboratory Sciences in ALERT hospital.

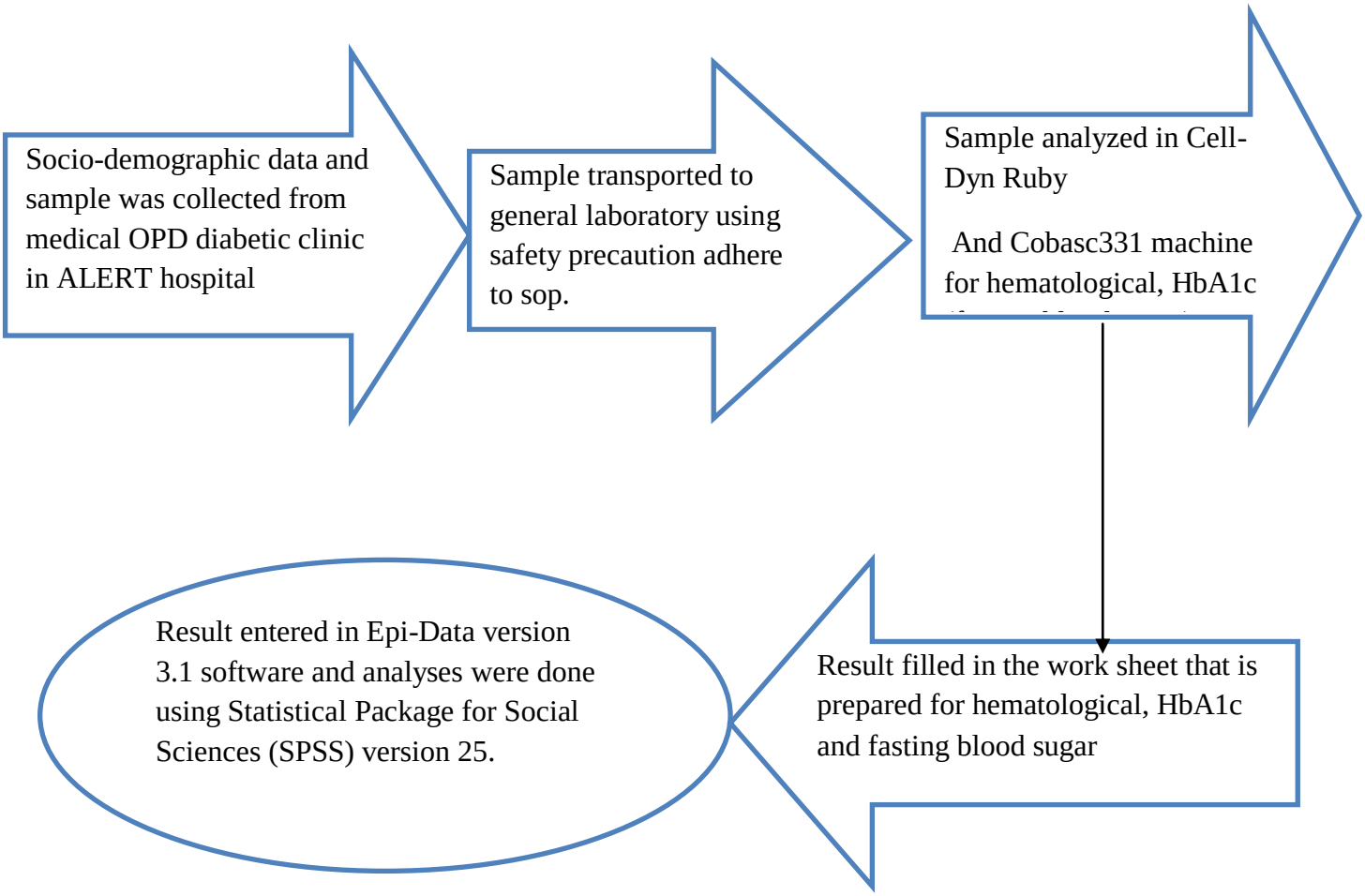

Fig.1WORK FLOW

5. RESULTS

5.1. Socio-demographic characteristics

Of the 422 diabetic patients who are enrolled in the study, 242(57.5%) were females and the rest, 180 (42.5%) were males. Majority of the participants were in the age group of 41-60 years (49.7 %). Most of the patients were married, 322(76.3%). Regarding the educational status of the patients, 100(23.7%) were uneducated, 221(52.4%) primary level, 76(18%) secondary level and 25(5.9%) higher education level. In terms of the place of residence, 411(97.4%) of patients were urban dwellers. Concerning occupation, majority 261(61.9%) of them were unemployed. Majority of the study participants (85.5%) were within the normal range of BMI (18.5-24.9 Kg/m^2). Regarding physical activity 379(89.8%) was not performing any physical activity. Furthermore, 65.5%of the patients did not use alcohol. (Table 1)

Table 1. Socio-demographic characteristics of diabetic patients (n=422) attending ALERT Specialized Hospital, Addis Ababa, Ethiopia November, 2020.

Variables	N (%)
Sex	
Male	180(42.5)
Female	242(57.5)
Age in years	
18-40	163(38.6)
41-60	210(49.7)
>60	49(11.7)
Marital status	
married	322(76.3)
single	77(18.3)
Divorced	23 (5.4)
Educational level	
Illiterate	100(23.7)
Primary	221(52.4)
Secondary	76 (18)
Higher education	25(5.9)
Occupation	
Student	10(2.4)
Unemployed	75(17.8)
Retired	66(15.6)
Housewife	110(26.1)
Employed/Business	161(38.1)
Residence	
Urban	411(97.4)
Rural	11(2.6)

BMI	
<18.5 kg/m^2	10 (2.5)
18.5-24.9 kg/m^2	361 (85.5)
25-29.9 kg/m^2	50 (11.8)
>30	1 (0.2)
Blood pressure	
Diastolic	
110-120	372(89)
>120	50(11)
Systolic	
70-80	360(86.1)
>80	62(13.6)
Alcohol consumption	
Alcohol used	147(34.5)
Alcohol not used	275(65.5)
Physical activity	
Not performing	379(89.8)
Rarely	38(9.0)
Always	5(1.2)

5.2. Correlation of HbA1C with some Hematological parameters.

From a total of 422 patients with HbA1c≤7% were 56 (12.4%) and those with HbA1c>7%were 366 (87.6%).Absolute magnitude of the observed correlation coefficient 0 up to 0.1 interpreted as negligible correlation,0.1up to 0.39 means weak correlation,0.4up to 0.69 means moderate correlation, 0.7 up to 0.89 means strong correlation, 0.9 upto1 means very strong correlation[53].The Bivariate correlation analysis of HbA1c with hematocrit (r=0.15, P=0.01), mean cell volume(r=0.189, P=0.01)),mean corpuscular hemoglobin(r= 0.147, P=0.01and red cell distribution width(r= 0.156, P= 0.01) revealed significant and weak positive correlation.(Table 2).

Table 2. Correlation of HbA1c with hematological parametersamong diabetic patients attending ALERT hospital,Addis Ababa,Ethiopia (n=422).

Variable	Pearson correlation(r)	P-value
HCT	0 0.15	0.01
MCV	0.189	0.01
MCH	0.147	0.01
RDW	0.156	0.01

The association of FBS with some hematological parameters showed positive correlation. A Pearson's correlation analysis revealed a weak positive correlation of red blood cell (r=0.147, p=0.01), Hemoglobin (r=0.133, P=0.01), HCT (r=0.112, P=0.05) and Mean platelet volume(r=0.134, P=0.01) with fasting blood sugar. (Table 3).

Table 3. Correlation of FBS with hematological parameters among diabetic patients attending ALERT hospital,Addis Ababa,Ethiopia (n=422).

Variable	Pearson correlation(r)	P –value
RBC	0.147	0.01
HGB	0.133	0.01
HCT	0.112	0.05
MPV	0.134	0.01

5.3. Utility of hematological parameters for prediction of glycemic control

Receiver operating characteristics (ROC) was used to determine the cut-off for different hematological parameters representing the best sensitivity and specificity to predict HbA1c of < 7%. The sensitivity and specificity of HCT, MCH and MCH for prediction of glycemic control:-For HCT 62.5% sensitivity and 65% specificity with optimal cutoff >45.5%, For MCH 70.6% sensitivity and 67.2% specificity with optimal cutoff >29.55pg and for MCV 66.7% sensitivity and 64.2% specificity with optimal cutoff >90.25fl [Table4]. For a continuous-scale diagnostic test, the optimal cutoff point for the positive disease is the cutoff point leading to the maximization of the sum of sensitivity and specificity [Figs 2-4]

For HCT

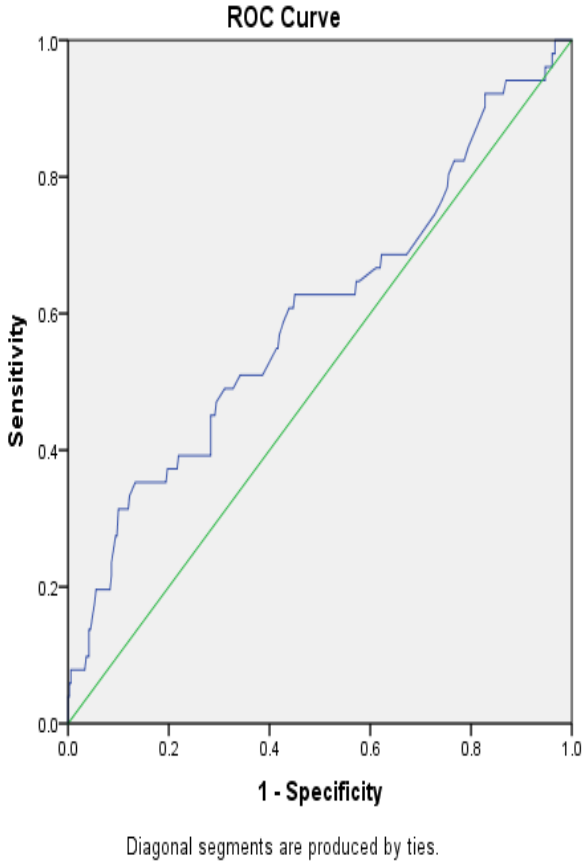

Fig 2. ROC curve with sensitivity and 1-specificity of HCT cutoff of >45.05% identifying a HbA1c count of ≤7% (AUC = 0.599) CI=95% (0 .508-0.689).

For MCH

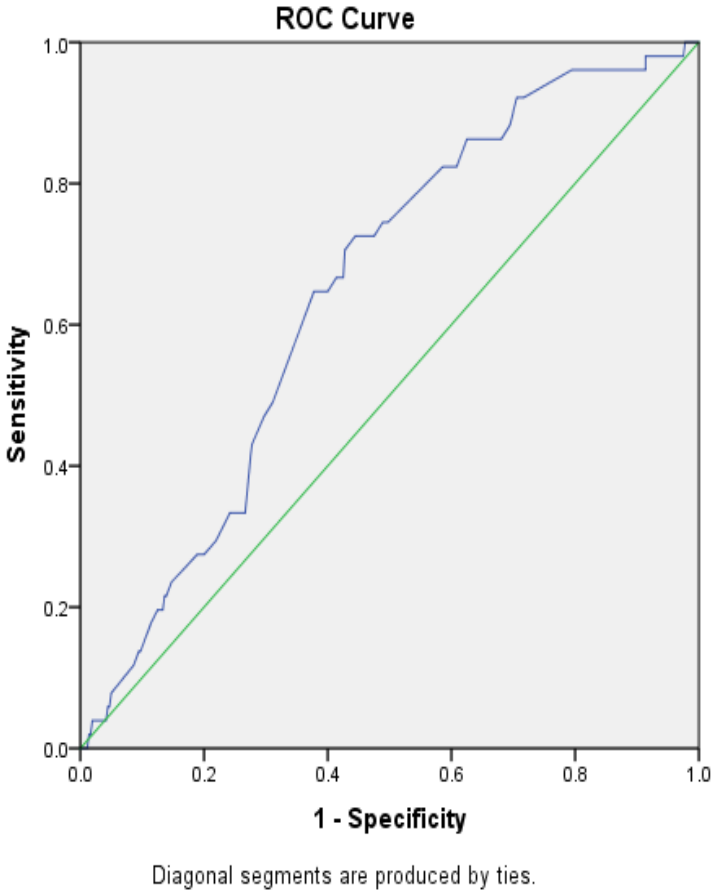

Fig 3. ROC curve with sensitivity and 1-specificity of MCH cutoff of >29.55 pg identifying a HbA1C count of ≤7% (AUC = 0.647) CI=95% (0 .575-0.719).

For MCV

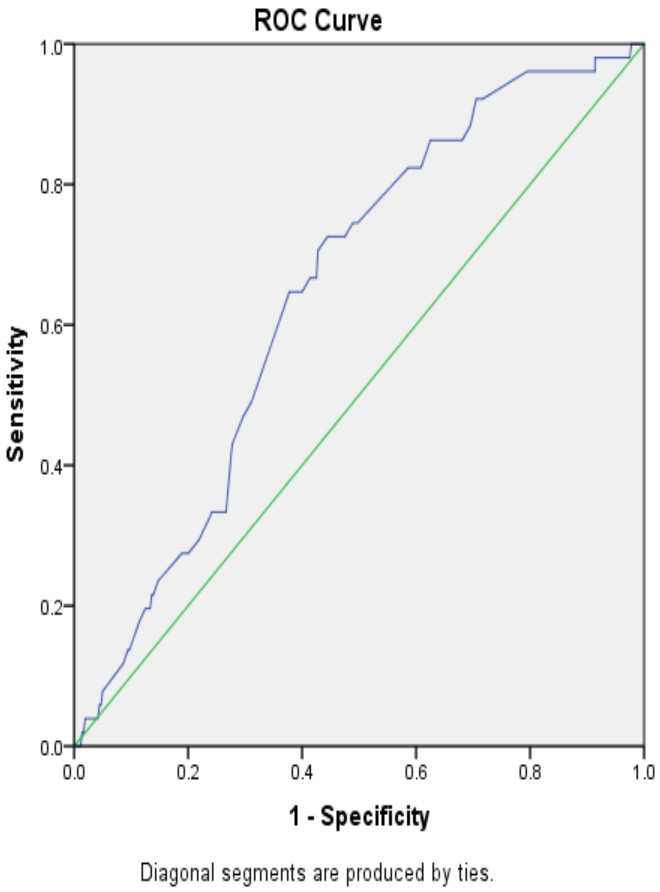

Fig 4. ROC curve with sensitivity and 1-specificity of MCV cutoff of >90.25 fl identifying a HbA1c count of ≤7% (AUC = 0.662), CI=95% (0 .588-0.736)

Table 4. Sensitivity and specificity of different hematological parameters representing the best sensitivity and specificity to predict HbA1C of ≤ 7% among diabetic patients attending ALERT hospital,Addis Ababa,Ethiopia (n=422).

Hematological parameters	Cutoff	Sensitivity (%)	Specificity (%)
	44.85	62.7	58.6

HCT (%)	44.95	62.7	59.2
	45.05	**62.7**	**65**
	45.15	60.8	66.3
	45.25	60.8	66.4
MCH (pg)	29.25	72.5	62.5
	29.35	72.5	64.2
	29.45	72.5	65.6
	29.55	**70.6**	**67.2**
	29.65	66.7	67.5
MCV (fl)	**90.25**	**66.7**	**64.2**
	90.35	66.7	64.4
	90.5	65.7	64.7
	90.65	60.8	65.3
	90.75	58.8	66.7

5.4. Factors associated with glycemic control among DM patient.

Of 422 DM patients, 61(14.45%) had good glycemic control. Amongst a good glycemic control,13.9% and 12.1% were males and females, respectively, and gender was not statistically associated glycemic control (P=0.349).A proportion of good glycemic control among age group of 18-40 years,41-60 years and >60 years were 12.9%,12.1% and

12.2%,respectively and age was not statistically associated with glycemic control (p=0.430). The finding of this study showed that, none of socio-demographic and clinical characteristics except blood pressure (systolic pressure=0.014 and diastolic pressure=0.045) of diabetic patients were associated with good glycemic control.

Table5. Association of behavioral, socio-demographic and clinical characteristics with glycemic control among DM patients at ALERT hospital, Addis Ababa, Ethiopia (n=422)

Variable	Glycemic control		P-value
	Yes, n(%)	No, n(%)	
Gender Male	23 (37.7)	157 (43.5)	0.349
Female	38 (62.2)	204 (56.5)	
Age			
18- 40	23 (37.7)	135 (37.3)	0.43
41-60	32 (52.4)	180 (49.8)	
>60	6 (9.8)	46 (12.7)	
Educational level			0.467
Illiterate	16 (26.2)	84 (23.3)	
Primary	20 (32.7)	196 (54.3)	
Secondary	15(24.5)	66(18.3)	
Higher education	10(16.4)	15(4.15)	
Physical activity			0.61
Not performing	43(70.5)	332 (89.2)	
Rarely	14(22.9)	28 (7.8)	
Always	4(6.55)	1(0.1)	
Occupation			
Student	6 (9.8)	14 (3.9)	0.9
Unemployed	13 (21.3)	72 (19.9)	
Retired	10(16.4)	56(15.5)	
Housewife	20(32.8)	100(27.7)	
Employed/business	12(19.7)	119(32.3)	

Alcohol consumption			0.27
Intake	23(37.7)	128 (35.5)	
Not take	38(62.3)	233 (64.5)	
Marital status			0.708
Single	42 (68.8)	280 (77.6)	
Married	11 (18)	70 (18.4)	
Divorced	8 (13.11)	11 (3.0)	
Residence			0.509
Urban	60 (98.4)	354 (98.0)	
Rural	1 (1.6)	7 (1.9)	
Body mass index			0.58
<18.5 kg/m^2	3 (4.9)	7 (1.9)	
18.5-24.9 kg/m^2	52(85.2)	309 (85.4)	
25-29.9 kg/m^2	6 (9.8)	44 (12.2)	
>30	0(0)	1 (0.3)	
Blood pressure			0.041
Systolic			
<70 mm/Hg			
70 mm/Hg	3 (4.9)	7 (1.9)	
80 mm/Hg	13 (21.3)	42 (11.6)	
>80 mm/Hg	41 (67.2)	258 (71.5)	
90 mm/Hg	4 (6.5)	8 (2.2)	
	-	46 (12.7)	
Diastolic			0.045
<110 mm/Hg	5 (8.2)	7 (1.9)	
110 mm/Hg	13 (21.3)	40 (11.1)	
120 mm/Hg	43 (70.5)	270 (74.8)	
<130 mm/Hg	-	44 (12.2)	

6. DISCUSSION

Diabetes mellitus is a metabolic disease, the deficiency or decreased responsiveness of tissues to insulin, and is associated with derangements in the metabolism of carbohydrates, lipids, and proteins. Hence, insulin is a very important hormone in metabolic homeostasis. Persistent hyperglycemia resulting from insulin deficiency leads to the devastating and life-threatening complications of diabetes [1]. In recent years, evaluation of hematological parameters along with HbA1C played a significant role in the clinical diagnosis and management of diabetes mellitus [2]. This study aimed to determine the correlation of selected hematological parameters with HbA1c and FBS and investigated the utilization of those hematological parameters for glycemic control among diabetic patients in ALERT Hospital.

In this study, a total of 422 diabetic patients were included and 61 (14.45%) had HbA1c≤7% while 361 (85.5%) had HbA1c>7%. The present study showed that hematocrit, mean corpuscular volume, and red cell distribution width showed a positive weak correlation with HbA1c.

The persistent elevation of glycosylated hemoglobin as a result of diabetes-related hyperglycemia is associated with the structural and functional changes in the hemoglobin molecule, the osmotic disturbance, and the cytoplasm viscosity within each cell. All these changes could have an imposing effect on any of the red blood cell indices, which include the RBCs count, HCT, MCV, MCH, mean MCHC, and the cell shape and deformability represented by RDW [30]

In Diabetes mellitus, the life span of RBC may be decreased due to disturbances in the hematopoietic milieu, such as chronic hyperglycemia [2]. RDW is also one of the parameters of RBCs, which is reported along with a simple routine laboratory investigation of the CBC and can be defined as a marker of the variation in cell volume within the red cell population that is reported as an index of heterogeneity in the size of circulating erythrocytes [1]. In our study, we found a statistically significant correlation of RDW with HbA1c. ($r=0.156$, $p<0.01$), and the same observations were reported by

Lippi et al, in their study (r=-0.11, p<0.001) [43]. Another study by Bhutto A. et al. has also shown a positive correlation between RDW and HbA1c r=0.193 (p-0.035) [17].

Since hematocrit has a direct relation with red blood cell, if red blood cell volume and life span are affected hematocrit level will also be affected. Anemia and glycosylation increased red cell turnover that has a direct effect on hematocrit and HbA1c consecutively [5]. This study also showed that HCT is significant correlated with HbA1c (r=0.15, P<0.05). The finding is in line with studies by Kui Wu1 et al. [41] and Abass AE et al. [17].

In this study, MCV had a significant and weak positive correlation (r=0.189, p<0.01) with HbA1C. Supporting this finding, Farooqui.R et al (r=0.07, P<0.053)) [28] and Abass AE et al. (r =0.95, P<0.256)) showed positive correlation of MCV with HbA1C. [17].

The finding of the current study revealed that RBC, HCT, and MPV showed a weak positive correlation with fasting blood sugar. Similarly, studies from Saudi Arabia by Farooqui. R et al [30] and Ethiopia by Biadgoet al. showed a positive correlation of FBS with RBCs, HCT, and Hb among diabetic patients [36].

This study also showed that hematocrit had a weak positive correlation (r=0.112, P<0.01) with FBS. The result was similar to findings from a study by Belete Biadgo et al. that achieved a significant but weakly positive correlation with RBC count, HCT, and Hb in DM groups [36]. Study done by Farooqui et al= [30] also illustrated a significant correlation (r=0.23, p< 0.001) of HCT with FBS [34].

In this study (MPV) showed positive correlation (r=0.134, p<0.01) with FBS. MPV is a parameter used to assess platelet size, and it is a potential biomarker of platelet reactivity. These hyperactive platelets play a critical role in the pathophysiology of the thrombotic events leading to diabetic complications it has been shown that larger platelets are more reactive than smaller ones. (PDW) can directly measure the variability in platelet size, and its high values suggest increased production of larger platelets. The study done by Buch, et al supports MPV as a statistically significant difference between diabetics with and without complications and non-diabetics (P < 0.0001) [5].A study done in turkey by

Vinik et al Mean Platelet Volume (MPV), and (PDW) were significantly increased in the diabetic group compared to the non –diabetic control, and the association was statistically significant ($p<0.05$ ($p<0.05$[20].

The Receiver operation curve (ROC) analysis of MCH, HCT, and MCV among 422 diabetic patients revealed the potential predictive role of those hematological parameters for glycemic control.

MCH was able to predict glycemic control with >29.55 pg optimal cut off having 70.6% sensitivity and 67.6% specificity. HbA1c has been increasingly used as a diagnostic criterion for diabetes with a cut-off point of <7% in non-pregnant persons. For a continuous-scale diagnostic test, the optimal cutoff point for the positive disease is the cutoff point leading to the maximization of the sum of sensitivity and specificity[41].Farooqui .R et al showed the correlation of MCH with HbA1C (r= 0.08,p=0.021)[30].Even though it didn't assess the utility of MCH for glycemic control. This study revealed that mean corpuscular hemoglobin with cut-off>29.55 pg had a 70.6% sensitivity and 67.6% specificity to predict with good glycemic control (HbA1C cut-off <7%).

HCT (AUC = 0.599 (95% CI: 0 .508-0.689)) predicted glycemic control with >45.05% optimal cut off having 62.7% sensitivity and 65 %specificity. This finding was supported by Kui Wu et al reporting the role of HCT > 38.8% for the screening of GDM with AUC=0.604 (95% [CI] 0.509, 0.701)[41].A study from Libya also revealed the association of HCT with glycemic control[34].Study done on Sudan strengthen the finding that there was significant positive correlation between HbA1c with Hb (r=0.174, P=0.037), HCT (r=0.174, P=0.037), and MCHC (r=0.180, P=0.031).The study also agrees with the current study that HCT is used for utilization of glycemic control with a cut point 41.9 % [17].

MCV (AUC = 0.662 (95% CI: 0 .588-0.736)) was able to predict glycemic control with >90.25% optimal cut off having 66.7% sensitivity and 64.2% specificity.

Among the 422 DM patients enrolled in this study, 12.4% had good glycemic control. In this study factors such as gender, age marital status, residence, the habit of alcohol consumption, BMI level, and habit of physical exercise were not found statistically associated with glycemic control ($p > 0.05$). In line with this, the current study showed that diastolic and systolic blood pressure were statistically associated with glycemic control ($P<0.05$). This finding was supported by Leulseged et al which revealed that hypertension is one of the factors that affect time to first optimal glycaemic control [39].

The study was done in Cameron also revealed that poor glycemic control ≥7.0% (53mmol/mol)) showed the mean body mass index was 27.4 ± 5.8 kg/m2, and 74% of patients had poor glycemic control.[odd ratio: 2.91 (95% confidence interval 2.07 to 4.11)] [38]. A study done in Cameron strength this study, body mass index for this study was 25-29.9 kg/m2 (88 %) had poor glycemic control while 74% in Cameron. Even if BMI is not significant in this study which is p-value 0.58, that may be due to sampling size variation. In Cameroon, the study participants were 1267 compared to this study which was 422.

7. STRENGTH OF THE STUDY AND LIMITATION

7.2. Strength of the study

This study has strengths in such a way that since the study was not much more done in our country, it will open eyes for physicians that hematological parameters could be used as an indicator of glycemic control. All laboratory analysis was done in an accredited laboratory and with competent personnel that gives more reliable and accurate results.

7.1. Limitation of the study

- Not included DM patients with age < 18 years old. If all it will be more representative.
- The study was done in one hospital, it was better to include much more hospital in a different location so that can get a different way of living so the socio-demographic data will be more variety than wills strengthen the study.

8: CONCLUSION AND RECOMMENDATION

8.1. Conclusion

This research aimed to identify hematological parameters correlation with HbA1C and FBS and the utilization of hematological parameters for glycemic control. The study has found that 12.4% of adult DM patients attending ALERT hospital and participated in this study had good glycemic control. Based on quantitative and qualitative analysis of hematological parameters with diabetic patients HBA1C and FBS results, it can be concluded that:-HCT, RDW, and MCV are some of the parameters that showed a weak positive correlation with HbA1C.

Similarly, RBC, HCT, and MPV are the parameters that showed a weak positive correlation with FBS. Besides this, the study has found that the predictive potential of some hematological parameters for glycemic control such as HCT, MCV, and MCH which relatively sensitive and specific for glycemic control by checking their cutoff and taking the measurement values. It is also indicated that systolic and diastolic blood pressure indices are associated with glycemic control of DM patients.

8.2. Recommendation

From the findings of this study, the following recommendations can be forwarded to different stakeholders.

- ✓ The majority of the study participants had poor glycemic control so that interventions are necessary to improve glycemic control to prevent diabetic complications and improve the quality of life of diabetic patients.
- ✓ Some of the routine hematological parameters which are relatively widely available, cost-effective, and did not require special patient preparation such as fasting could be potential inputs for the management and control of diabetic patients in facilities where sensitive tests such as HA1C are not available.
- ✓ Further controlled studies incorporating different factors are required to better characterize the utility of hematological parameters for glycemic control.

9. REFERENCE

1. Milosevic D, Panin VL. Relationship between hematological parameters and glycemic control in type 2 diabetes mellitus patients. Journal of Medical Biochemistry. 2019; 38(2):164.
2. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2013; 36 1):67-74.
3. Roglic G. WHO Global report on diabetes: A summary. International Journal of Noncommunicable Diseases. 2016; 1(1):3.
4. Demirtas L, Degirmenci H, Akbas EM, Ozcicek A, Timuroglu A, Gurel A, Ozcicek F. Association of hematological indices with diabetes, impaired glucose regulation and microvascular complications of diabetes. International Journal of Clinical and Experimental Medicine. 2015; 8(7):14-20.
5. Buch A, Kaur S, Nair R, Jain A. Platelet volume indices as predictive biomarkers for diabetic complications in Type 2 diabetic patients. Journal of Laboratory Physicians. 2017 ;9(2):84
6. Roche Tina-quant Hemoglobin A1c Gen.3 – Hemolysate and Whole Blood Application package insert.
7. World Health Organization. Global report on diabetes: executive summary. World Health Organization; 2016.
8. Martinez RE, Quintana R, Go JJ, Villones MS, Marquez MA. Use of the WHO Package of Essential Noncommunicable Disease Interventions after Typhoon Haiyan. Western Pacific Surveillance Response Journal. 2015;6: Suppl 1, 18–20.
9. Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. PLoS Medicine. 2008; 5 :(7) e152.
10. Riza AL, Pearson F, Ugarte-Gil C, Alisjahbana B, van de Vijver S, Panduru NM, et al. Clinical management of concurrent diabetes and tuberculosis and the implications for patient services. Lancet Diabetes Endocrinology. 2014;2:(9)740–753

11. Sicree R, Shaw J, Zimmet: The Global Burden: Diabetes and Impaired Glucose Tolerance. Diabetes Atlas, IDF. 4 editions. International Diabetes Federation: Brussels; 2009.
12. Hall V, Thomsen RW, Henriksen O, Lohse N. Diabetes in Sub Saharan Africa 1999-2011: epidemiology and public health implications. A systematic review. BMC Public Health. 2011; 11(1):564.
13. Kirigia JM, Sambo HB, Sambo LG, Barry SP. Economic burden of diabetes mellitus in the WHO African region. BMC International Health and Human Rights. 2009; 9(1):6.
14. Abebe N, Kebede T, Addis D. Diabetes in Ethiopia 2000-2016–prevalence and related acute and chronic complications; a systematic review. African Journal of Diabetes Medicine. 2017; 25(2).
15. Su JB, Zhao LH, Zhang XL, Cai HL, Huang HY, Xu F, Chen T, Wang XQ. HbA1c variability and diabetic peripheral neuropathy in type 2 diabetic patients. Cardiovascular Diabetology. 2018; 17(1):47.
16. Vinupritha, M, Hariharan, Dhandapani, Kathirvelu, S, Chinnadurai (Estimation of hemoglobin A1C using the complete blood count measures in the diagnosis of diabetes)Asian Journalof Pharmaceutical and ClinicalResearch.10.22159/ajpcr.2017.10i9.19407
17. Abass AE, Musa IR, Rayis DA, Adam I. Glycated hemoglobin and red blood cell indices in non-diabetic pregnant women. Clinics and practice. 2017; 7(4).
18. Shukla DK, Chandra KP, Pawah AK. Study of hematological indices in patients with diabetes mellitus and hypertensive diabetes mellitus. International Journal of Medicine Research. 2016;1(4):28-31
19. Varım C, Kaya T, Nalbant A, Uçar A, Tamer A. Effects of glucose control on hematological indices in patients with diabetes mellitus. Macedonian Journal of Medical Sciences. 2014; 7(1):83-8.
20. Vinik AI, Erbas T, Park TS, Nolan R, Pittenger GL. Platelet dysfunction in type 2 diabetes. Diabetes Care. 2001; 24(8):1476-85.
21. Uko EK, Erhabor O, Isaac IZ, Abdulrahaman Y, Adias TC. Some hematological parameters in patients with type-1 diabetes in Sokoto, North-Western Nigeria. Journal of Blood & Lymph. 2013;3(110):2165-7831

22. Hernández-Aguilera A, Rull A, Rodríguez-Gallego E, Riera-Borrull M, Luciano-Mateo F, Camps J, Menéndez JA, Joven J. Mitochondrial dysfunction: a basic mechanism in inflammation-related non-communicable diseases and therapeutic opportunities. Mediators of inflammation. 2013;2013
23. Naredi M. Study of the relationship between WBC count and diabetic complications. International Journal of Advances in Medicine. 2017; 4(4):1128
24. Xu W, Wu HF, Ma SG, Bai F, Hu W, Jin Y, Liu H. Correlation between peripheral white blood cell counts and hyperglycemic emergencies. International journal of medical sciences. 2013;10(6):758
25. Ilango P, Vummidi AV, Suresh V, Chandran V, Bharathy VS. Evaluation of Periodontal and Hematological Findings in Diabetes Patients-A Case-Control Study. Dentistry. 2019;9(535):2161-1122.
26. Twig G, Afek A, Shamiss A, Derazne E, Tzur D, Gordon B, Tirosh A. White blood cells count and incidence of type 2 diabetes in young men. Diabetes Care. 2013 ;36(2):276-82
27. Alamri BN, Bahabri A, Aldereihim AA, Alabduljabbar M, Alsubaie MM, Alnaqeb D, Almogbel E, Metias NS, Alotaibi OA, Al-Rubeaan K. Hyperglycemia effect on red blood cells indices. European Review for Medical and Pharmacological Sciences. 2019;23(5):2139-50
28. Ziaee A, Ghorbani A, Kalbasi S, Hejrati A, Moradi S. Association of hematological indices with prediabetes: A cross-sectional study. Electronic physician. 2017 ;9(9):5206
29. Bharathi K. Study of hematological profile and its significance in type 2 diabetes mellitus patients. Journal of Diagnostic Pathology and Oncology. 2016; 1(1):14-7.
30. Farooqui R, Afsar N, Afroze IA. Role and Significance of Hematological Parameters in Diabetes Mellitus Annals of Pathology and Laboratory Medicine, 6, Issue 3, 2019.
31. World Health Organization. Use of glycated hemoglobin (HbA1c) in the diagnosis of diabetes mellitus: abbreviated report of a WHO consultation. Geneva: World Health Organization; 2011
32. Esmaeel AK, Hammoud HJ, Abbas EF, Al-Harbby HJ. A Study of Correlation between some Blood Parameters and Diabetic Mellitus Type 2 in Diabetic Women

33. Kizilgul M, Sencar E, Ucan B, Beysel S, Ozcelik O, Ozbek M, Cakal E. Components of the Complete Blood Count in Type 2 Diabetes Mellitus with Inadequate Glycemic Control. Dicle Tıp Dergisi. 2018;45(2):113-20(35)
34. Al Salhen KS, Mahmoud AY. Hematological Profile of Patients with Type 2 Diabetic Mellitus in El-Beida, Libya. Ibnosina Journal of Medicine & Biomedical Sciences. 2017; 9(3).
35. Olana C, Seifu D, Menon M.K.C, and Natesan G. Abnormal hematological indices and anthropometric parameters associated with type 2 Diabetes. International Journal of Biomedical and Advance Research 2019; 10(11): e5296
36. Biadgo B, Melku M, Abebe SM, 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.
37. Bae JC, Suh S, Jin SM, Kim SW, Hur KY, Kim JH, Min YK, Lee MS, Lee MK, Jeon WS, Lee WY. Hemoglobin A1c values are affected by hemoglobin level and gender in non-anemic Koreans. Journal of Diabetes Investigation. 2014 ;5(1):60-5
38. Camara A, Baldé NM, Sobngwi-Tambekou J, Kengne AP, Diallo MM, Tchatchoua AP, Kaké A, Sylvie N, Balkau B, Bonnet F, Sobngwi E. Poor glycemic control in type 2 diabetes in the South of the Sahara: the issue of limited access to an HbA1c test. Diabetes Research and Clinical Practice. 2015; 108(1):187-92.
39. Leulseged TW, Ayele BT. Time to optimal glycaemic control and prognostic factors among type 2 diabetes mellitus patients in public teaching hospitals in Addis Ababa, Ethiopia. PloS One. 2019; 14(7).
40. Dosoo DK, Kayan K, Adu-Gyasi D, Kwara E, Ocran J, Osei-Kwakye K, Mahama E, Amenga-Etego S, Bilson P, Asante KP, Koram KA. Hematological and biochemical reference values for healthy adults in the middle belt of Ghana. PloS One. 2012; 7(4).
41. Wu K, Cheng Y, Li T, Ma Z, Liu J, Zhang Q, Cheng H. The utility of HbA1c combined with hematocrit for early screening of gestational diabetes mellitus. Diabetology & Metabolic Syndrome. 2018; 10(1):14.

42. Sinha N, Mishra TK, Singh T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. Annals of Laboratory Medicine. 2012; 32(1):17-22.
43. Solomon A, Hussein M, Negash M, Ahmed A, Bekele F, Kahase D. Effect of iron deficiency anemia on HbA1c in diabetic patients at Tikur Anbessa specialized teaching hospital, Addis Ababa Ethiopia. BMC Hematology. 2019; 19(1):2.
44. Salimon AH, Patil HA. Correlation of Red Blood Cell Distribution Width (RDW) and Haemoglobin A1C (HbA1C) Levels in Diabetic Individuals
45. Bhutto A, Abbasi A, Abro A (August 30, 2019) Correlation of Hemoglobin A1c with Red Cell WidthDistribution and Other Parameters of Red Blood Cells in Type II Diabetes Mellitus. Cureus 11(8): e5533.DOI 10.7759/cureus.5533
46. Ministry of Information, Fact about Ethiopia, 2011. 3. Ethiopia Statistics Authority. Ethiopia Census 2007 report, Addis Ababa revised on June 2012.
47. https://www.addismap.com/index.php/a204776351/alert-hospital
48. CELL-DYN Ruby automated hematology analyzer 9140548E operators, manuals, 2014
49. Berte LM, Nevalainen DE. Quality systems for the laboratory. ASCP Press; 2000.
50. Occupational Safety and Health Administration. Occupational safety and health standard: bloodborne pathogens. Federal Register
51. Anonymous. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16 (1) of Directive 89/391/EEC).
52. Schober P, BoerC,Schwarte LA. Correlation coefficients: appropriate use and interpretation. Anesthesia & Analgesia. 2018 1; 126(5):1763-8.ia. 2018 1; 126(5):1763-8.

10. ANNEX

Annex 1: Information sheet

Addis Ababa University College of Health Sciences Department of Medical Laboratory Sciences

You are invited to participate in a study to be conducted by an MSc student at Addis Ababa, College of Health Sciences, and Department of Medical Laboratory Science. Please read the following statements and ask for any unclear points before you agree to participate.

Introduction

The topic of this study "Assessment of the relationship between some hematological parameters and blood glucose level and its role as predictors of glycemic control among diabetes mellitus patients attending at ALERT hospital, Addis Ababa, Ethiopia).The aim of the study is by using the hematology test we will assess Hematological Parameter for Glycemic Control among Diabetic Patients. If you are not interested to participate or if you once decide to participate and withdraw at any time, there will be no consequences and you will get all the services provided in the hospital with no problems. If you decide to participate, you have to sign the consent form and you may obtain a copy of this information sheet.

What is expected from me as a participant in the study?

As a participant of this study, you are expected to participate in giving a blood sample

In addition, you are expected to give answers to some questions about your health and socio-demographic conditions.

You need to know that your results might be discussed with other appropriate individuals out of this hospital. But your name, address, and phone number will not be disclosed, and rather than

Identification code will be used in such conditions.

How much time will I spend participating in this study?

You will spend 20-25 minutes until the specimen is collected, the consent form is signed.

How my information is to be kept secret?

All information that you give and the results from your sample will be used for this study only, only limited numbers of professionals will have access to the information.

All the information will be encoded in a computer and saved with password protection.

What are the benefits of participation?

Since this study is MSc student research, there will not be payments for participants. But your

Participation is important for the establishment good assessment of platelet count.

Please direct any questions or problem you may encounter during this study to:

Wubalem Biresaw

Mob: +2519-61-31-85-76

Email: wubelem.biresaw@gmail.com

Advisors: Mr.Zemenu Tamer zemenut266@gmail.com

Mr modes word of email heranmakmow@gmail.com For additional information, please contact Department of Medical Laboratory Sciences, Addis

Ababa University, Institutional Review Board (IRB) office;

Tel: +2511911107099

P.O Box: 9086, Addis Ababa, Ethiopia

Agree to participate? Yes No

Annex 2: Informed consent (in English) (>18 years)

Name of main researcher: Wubalem Biresaw GSE/2294/10, MLS; AAU.

Advisors/Co-investigators: Zemenu Tamir (MSc, PhD fellow, Assistant Professor) and

Moges woredofa (MSc)

Beruk Kebede (PhD)

Name of institute: AAU and ALERT hospital

Funded by: SELF AND ALERT HOSPITAL

Reviewed by: DREC (AAU), AAREC and NRERC

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I would participate in this study.

To allow the researcher for collecting my blood and be a participant in this study and understand that I have the right to withdraw from the study at any time .

Print name of participant, date and signature or thumb impression of participant

_____ __ /____ /____ (dd/mm/yy) _____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)_____ ____/____/_____ (dd/mm/yy) _____

Phone number

Print name of researcher, date and signature of researcher____ _____/____/_____ (dd/mm/yy) ----------

Annex 3: Informed consent form (Amharic version) (>18 years)

የተሳታፊዎች፡መረጃ፡ቅጽ

ጥናቱን፡ የሚያጠናዉ፡ዉባለም፡ቢረሳዉ

በአዲስ፡አበባዩኒቨርሲቲ፡ጤና፡ሳይንስ፡ኮሌጅ፡የህክምና፡ላቦራቶሪ፡ሳይንስ፡ዲፓርትመንት

የጥናቱ፡አላማ

የተከታታይ የ3 ወር ስኳር መጠን ክትትል እና ጥናቱ በተደረገበት ሰዓት ያለው የስኳር መጠን ከጠቅላላ የደም ህዋሳት ቁጥር ጋር ያለውን ተዛምዶ እና የጠቅላላ የደም ህዋስ ቁጥር በተከታታይ 3 ወር የስኳር መጠንን በጠቅላላ የደም ህዋስ ቁጥር አማካኝነት ለመገመት እንዲያስችል በአለርት አዲስ አበባ ኢትዮጵያ የተደረገ ጥናት

ጥናቱ፡በአዲስአበባ፡ከተማ፡ዐለርት፡የጥናት፡ማዕከል፡፡

በጥናቱ፡ወቅት፡ከእርስዎ፡የሚጠበቀው፡በጥናቱ፡ለመሳተፍ፡ፈቃደኛ፡ከሆኑ፡የደም፡ናሙና፡መስጠት፡ነው፡፡

ለጥናቱ፡ተሳታፊዎች፡ያለው፡ልዩ፡ጥቅም

በጥናቱ፡ለሚሰተፉ፡ፍቃደኛ፡ተሳታፊዎች፡ምንምአይነት፡የገንዘብ፡ክፍያ፡የለውም፡፡ነገርግን፡ከጥናቱ፡የሚገኘው፡ውጤት፡ለርስዎ፡ህክምና፡ተጨማሪ፡መረጃ፡ለማግኘትና፡የጎንዩሽ፡ጉዳቶችን፡ለመቀነስ፡ይጠቅማል፡፡

በጥናቱ፡ተሳታፊዎች፡ላይ፡ያለው፡ጉዳት

በጥናቱ፡መጀመሪያም፡ይሁን፡መጨረሻ፡በዚህ፡ጥናት፡ላይ፡፡በመሳተፍዎ፡ሊደርስብዎ፡የሚችል፡አንድም፡ጉዳት፡አይኖርም፡፡በጥናቱ፡ምክንያት፡የሚያባክኑት፡ተጨማሪ፡ጊዜም፡አይኖርም፡፡

የመረጃ፡ሚስጥራዊ፡አጠባበቅ

የሚሰጡት፡መረጃ፡በጥናቱ፡ወቅትም፡ሆነ፡ከዚያ፡በኋላ፡ባሉት፡ጊዜያት፡ሙሉ፡በሙሉ፡ሚስጥራዊቱ፡የሚጠበቅና፡መረጃውም፡የሚያዘዉ፡በስም፡ሳይሆን፡በመለያ፡ቁጥር፡ይሆናል፡፡

በጥናቱ፡ላይ፡ያለምሳተፍ፡መብት፡አለዎት፡፡

ይህ፡መረጃ፡በጥንቃቄ፡የሚያዝ፡ይሆናል፡፡በተጨማሪም፡የጥናቱ፡ዉጤት፡ለሚመለከተዉ፡አካል፡ ለጥናቱ፡አላማና፡ለህክምና፡ባለሙያዎች፡ብቻ፡የሚገለፅ፡ይሆናል፡፡

ያስታውሱ፤ስለዚ፡ህጥናት፡ማንኛውም፡ጥያቄ፡ካለዎት፡በማንኛውም፡ጊዜ፡ከዚህ፡በታች፡ በተጠቀሱት፡አድራሻዎች፡መጠየቅይችላሉ፡፡

እኔም፡የጥናቱ፡ተሳታፊ፡ይህንን፡በመገንዘብ፡ጥናቱ፡ላይ፡ለመሳተፍ፡ተስማምቼያለሁ፡፡

ፊርማ ----------

መረጃውንየሰበሰበውግለሰብስም----------

ፊርማ ----------

የዋና፡ተመራማሪው፡አድራሻ፤

ዉባለም፡ቢረሳዉ፤የሕክምና፡ላቦራቶሪ፡ቴክኖሎጂ፡ዲፓርትመንት፣የጤናሳይንስ፡ኮሌጅ፣አዲስ፡ አበባ፡ዩኒቨርሲቲ- አዲስአበባ፣ኢትዮጵያ

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ይህመረጃ: በጥንቃቄ:የሚያዝ: ይሆናል፡፡በመጨረሻም: የጥናቱ: ውጤት :ለሚመለከተዉ :አካል: ለጥናቱ: አላማና:ለህክምና:ባለሙያዎች: ብቻ: የሚገለፅ:ይሆናል፡፡

ያስታውሱ፤ስለዚህ:ጥናት:ማንኛውም: ጥያቄ: ካለዎት: በማንኛውም: ጊዜ: ከዚህ: በታች: በተጠቀሱት; አድራሻዎች; መጠየቅ: ይችላሉ፡፡

እኔምየ:ጥናቱ: ተሳታፊ: ይህንን: በመገንዘብ: ጥናቱ: ላይ: ለመሳተፍ:ተስማምቼያለሁ::

ፊርማ ----------

መረጃውን:የሰበሰበው፡ግለሰብ፡ስም----------

ፊርማ ----------

የዋና: ተመራማሪው; አድራሻ፤

- ዉባለምቢረሳዉ፤
- የሕክምና፡ላቦራቶሪ፡ቴክኖሎጂ፡ዲፓርትመንት፣የጤና፡ሳይንስ፡ኮሌጅ፣አዲስ፡አበባ፡ዩኒቨርሲቲ-አዲስአበባ፣ኢትዮጵያ
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Annex 3: DIABETES QUESTIONNAIRE

Patients' clinical diagnosis

Diagnosis	Positive	Negative
TB		
HIV		
Malignancy		
Renal failure		
Hematological abnormality		
Anemia		
Pregnancy		

1. Participant code :_____
2. MRN: _____Age_____
3. Sex: Male_____

   Female _____ pregnant _____Not pregnant _____Not known_____

4. Maritalstatus:_____Married_____Single_____Divorced_____Widow
5 Education level

   Illiterate _____Primary _____Secondary_____ Higher education

6 Alcohol consumption _____Yes _____No
7 Physical activity

   Not performing _____ Rarely _____ Always _____

8 Residence _____Urban _____Rural
9 Blood pressure: diastolic _____mm/Hg

   Systolic _____mm/Hg

10 BMI_____CM.

Annex 4: Worksheet for data extraction for socio-demographic data and laboratory test result

S.N.	Age	Sex	MRN	BMI	BP	Glu	HbA1c

Annex: 5Worksheet for data extraction for CBC test result

S.N	MRN	WBC	RRC	Hb	HCT	PLT	MPV	MCH	MCHC	MCV	RDW	Gra	Lym	Mon

Annex 6: Standard Operating Procedures

Laboratory standard operating procedure for QC and sample processing on Cell-Dyn Ruby machine

1. Cell-Dyn Ruby Machine for QC processing.

Purpose

This procedure provides instruction for how to analyzer quality control running procedure on Cell-Dyn Ruby.

Principle

The same principle used by Cell-Dyn Ruby to analyze hematological parameters is used to analyze quality control materials.

Reagents	Disinfectant	Detergent	Supplies	Equipment
• Diluent/sheath (20L) • Cn-Free Hb/ Noc Lyse • Wbc Lyse • Reagents preparation: • Ready for use Reagents stability and storage: Reagent Stability: Up-to expiry date ReagentStorage:15^{o}c-25^{o}c	Bleach -To prepare 10% bleach add 10ml of bleach to 90ml of distilled water	Enzymatic Cleaner	Lab coats Gloves Distiled water 10% Bleach Ethanol (70%) Testtube rack Plastic spensing	Cell-Dyn Ruby Automated hematology Analyzer Computer(data station) - barcode reader -Ups

Materials

When to run Quality control:-

❖ Quality Control specimens should be run and results confirmed to be within acceptable limits prior to reporting patient results. Controls should also be run:

- After a reagent lot number change
- After maintenance, component replacement, or a field service action
- After a software change
- Following calibration
- According to your laboratory's quality control program
- According to regulatory requirements

Control material guide line:-

Use the following guidelines for proper handling of control material:

- Check the condition of incoming control material. Be sure the tubes are at the
- Proper temperature and are not leaking. Check for gross Hemolysis.
- Check the shelf-life and open-tube stability dating. Do not use products longer than

Program Operation.

QCID Files

QC result data and statistics are stored in QCID Files. Three QCID subtypes available on the Cell-Dyn Ruby are:

- Commercial
- Whole Blood
- Background and RETC_Background

A QCID File is assigned to each level of commercial control and each whole blood patient control. There is a maximum of 500 QCID files that can be set up and created on the System.

PROCEDURE:

Creating a commercial quality control ID (QCID)

1. Select Setup from the menu bar and QCID Setup from the pull-down menu. The QCID Status: View dialog box opens (default view displays QCID: Background).
2. Select Create and the QCID Setup: Basics dialog box opens.
3. Enter the new Quality Control ID or scan the bar code (if one is present) in the New QCID field. Select the control type from the pull-down menu in the control field.
4. To upload control assay values from the disk:
5. Click Continue and the QCID Setup: Create New dialog box opens with the Control Data tab as the default.
6. Select the QC Limits tab and the QC Limits page opens.
7. Enter or confirm assay values:
8. Click OK and the QC Limits dialog tab opens.
9. Using the control assay sheet, confirm that the assay values displayed on screen are correct for the appropriate level.
10. Remove the disk and store it in a safe place in case it is needed to reload data for this control lot.
11. Select the Rule or Rules, if any, and click OK. The QCID Setup: View dialog box opens, reflecting the newly created QCID information.

Rejecting/Accepting Specimens

1. To reject a QC specimen, proceed as follows:

2. From the QC View, highlight the specimen record from the log.

3. Select F8 – QCID L-J Plots, then select F8 – QCID Data.

4. From the QCID data view, highlight the specimen record and select F5 –

Preparing to run specimen

1. Prime the analyzer to insure that background counts are within acceptable limits before running quality control or patient sample.

2. Inter, check, or change operator ID.

3. Check waste container level and empty waste container as needed.

4. Check the level of the reagent and change reagents as needed.

5. Check for any maintenance due in maintenance view.

6. Check QC status region and review specific QCID files and moving average programs.

7. Verify background count.

8. Prepare run and verify controls.

9. Verify specimen acceptability for processing (ID, volume, temperature e.t.c)

Running specimen

Open mode analysis

1. Verify analyzer status indicates the ready state and is in the open mode. (select F11- select open to switching from the closed mode
2. Wand or inter the specimen ID in the specimen ID or QCID field. If the specimen is quality control, the specimen type and test selection will be automatically based on the QCID selection setup. If the specimen has a matching pending order, the test selection will be automatically selected based on the order.
3. Select the MORE SPEC INFO button to verify, add or change specimen demographic information in the next tube entry (DETAILED) dialog box.
4. With the stopper in the sample tube gently mixes the sample.
5. Open the sample tube and place it under the open mode sample prob. Raise the tube until the end of the probe is deeply immersed in the sample. (do not allow the

probe to touch the bottom of the tube as it may affect aspiration and produce an erroneous result.

6. Press the touch plate to activate aspiration.
7. Remove the tube when the beep sound and replace the cup.
8. When the cycle is finished, the results post to the data log and are displayed in the RUN view. Analyzer

Reference intervals (normal population reference ranges) were developed for Cell-Dyn Ruby analyzer using normal individuals. The ranges for each parameter, WBC, RBC, Hemoglobin, Hct,

MCV, MCH, MCHC, RDW-CV, RDW-SD, Platelet, PDW*, MPV, P-LCR*, Lym% and #, Mxd% and #, and Neut% and #were determined and displayed in Table.

- For Investigational Use Only in the United States of America.
- Not a reportable parameter.

	Parameters	**Range for females n=133**	**Range for males n=182**
Reference value	WBC	3.1-10.3	2.6 - 8.8
	Neu%	43.7 - 77.1	38.3 – 69
	Lymph%	15 - 45.8	17.5 - 47.9
	Mix%	1.3 - 25.9	1.9 - 24.6
	Neut#	1.6 - 6.9	1.2 - 5.3
	Lymph#	0.9 - 2.8	0.8 - 2.7
	RBC	3.2 - 4.6	3.6 - 5.3
	HGB	9.9 -13.6	11.3 - 15.7
	HCT	30.2 - 42.3	32.6 - 47.5
	MCV	78.6 - 102.2	80.3 - 103.4
	MCH	25.2 - 34.7	26 - 34.4

	MCHC	31.3 - 35.4	31.8 - 36.3
	RDW-SV	10.6 - 15.7	10.8 - 14.9
	RDW-SD	35.3 - 48.9	33.4 - 49.2
	PLT	128 - 434	134 - 377
	MPV	8.5 - 12.4	8.1 - 12.4

Normal Population Reference Ranges

NOTE: The age range for females was **17 - 66** years with a mean age of 33.4.

The age range for males was **17 - 72** years with a mean age of 42.2.

II. COBAS c 331 Machine for QC processing

Assay

- It uses two control, which is preipath u plus (in the pathological range or at the normal/pathological threshold)) and precicontrol clinchem multi 1(in the normal range or at the normal/pathological threshold.
- Dispense the required volume into a sample cup and analyze in the same way as the patient sample.
- The control should be run daily in parallel with the patient sample and after every calibration control. Control interval must be adapted to individual laboratory's requirements.

Preparation

Carefully open one bottle1, avoiding the loss of lyophilizate and pipette in exactly 3.0 ml of diluents (bottle 2)

Carefully close the bottle and dissolve the contents completely by occasional gentle swirling within 30 minutes. Avoid the formation of foam.

The enclosed bar-coded labels are intended exclusively for the Roche/Hitachi MODULAR analyzers and Cobas c systems to identify the control. Attach the bar-coded

labels to the tubes carrying the sample cups containing the control materials. The control store at 2-8 °c

COBAS c331 Machine for sample processing

Purpose

This procedure provides instruction for how to run the sample on COBAS c331 Analyzer

Principle

QC and sample analysis used the same principle which uses photometric analysis and ion-selective electrode measurements and uses serum/plasma and whole blood sample for analyzing. For serum/plasma samples like fasting blood sugar, a sample is mixed with the appropriate reagent to produce a reaction that results in color. The concentration of the analyte determines the strength of color produced. The photometer shines the light of the appropriate wavelength at the sample and measures the amount of light absorbed, which is directly correlated to the concentration of the analyte in the sample.

An ion-selective electrode (ISE) is a sensor that determines the concentration of ions in a solution by measuring the current flow through an ion-selective membrane.

Glucose use enzymatic hexokinase method. Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

Whole blood samples like HbA1c, the sample lysed with a hemolyzing solution which is TetradecylTrimethylAmmonium Bromide (TTAB).

Hemoglobin A1C – The HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood.

1. Sample and addition of R1 (buffer/antibody reagent): Glycohemoglobin (HbA1c) in the sample reacts with anti-HbA1c antibody to form soluble antigen-antibody complexes. Since the specific HbA1c antibody site is present only once on the HbA1c molecule, formation of insoluble complexes does not take place.
2. Addition of R 3 (buffer/polyhapten reagent) and start of reaction: The polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be determined turbidimetrically.

Hemoglobin – Liberated hemoglobin in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum which is measured bichromatically during the pre-incubation phase (sample + R1) of the above immunological reaction. A separate Hb reagent is consequently not necessary.

Procedure

Sample preparation

1. Sample and addition of R1 (buffer/antibody reagent): Glycohemoglobin (HbA1c) in the sample reacts with anti-HbA1c antibody to form soluble antigen-antibody complexes.
2. Since the specific HbA1c antibody site is present only once on the HbA1c molecule, the formation of insoluble complexes does not take place.
3. Addition of R 3 (buffer/polyhapten reagent) and the start of reaction: The polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be determined turbid metrically

Procedure

1. Specimens should be at room temperature before analysis.
2. Mix capped specimens by inversion. remove caps before placing specimens in racks with blue stickers.
3. If there are less than 1 ml of the specimen, transfer approximately 200 µl of the mixed specimen to a labeled Cobas cup and place the cup in position 5 of the #3 white QC rack.

4. Click on QC, status, and sort by the control column. Highlight both tests for the a1c-pt control.
5. Click select, save. after the analysis of the patient is complete,
6. Print the report.

Interpretation Of The Result: Results are reported to the nearest tenth in %.

AMR (Analytical Measurement Range): 4.3-18.8%

- Values below 4.3 are reported as <4.3%.
- Values below 18.8 are reported as >18.8%.

Reasons for Linearity Flags:

Linearity flags for Hb and/or HbA1c are typically caused by one of the following:

- <Test (Hb and/or HbA1c) due to abnormally low hemoglobin (Hb) levels in the whole blood sample. This could occur in very anemic patients or due to pre-analytical errors (e.g., when the blood is drawn from a central venous catheter, i.e., unintended dilution of the whole blood sample.

The calculated reported result for % HbA1c

The reported result for the % HbA1c is calculated on the analyzer from the HbA1c/Hb ration as follows:

$$\text{HbA1c (\%)} = (\text{HbA1c/Hb}) \times 91.5 + 2.15$$

Estimated Average Glucose

Based on the results of the A1c-Derived Daily Glucose (ADAG) study, the ADA recommends the use of a new term in diabetes management, estimated average glucose, or eAG. This new calculation is intended to help health care provides report HbA1c results to patients using the same units that patients see routinely in blood glucose measurements.

The following equation is used to calculate the eAG (mg/dL):

$$eAG\ (mg/dL) = (28.7 \times HbA1c) - 46.7$$

Interpretation:

- **Expected Values**: 4.0-5.6%. Hemoglobin A1c values of 5.7-6.4% indicate an increased risk for developing diabetes mellitus. Hemoglobin A1c values ≥6.5% are diagnostic for diabetes mellitus. In diabetic patients, HbA1c goals should be discussed with a healthcare provider.

Fasting blood sugar Glucose

SPECIMEN: Universal precautions apply. Li-heparin plasma is the preferred specimen. Serum is also acceptable (serum specimen must be separated immediately after clotting), as well as urine, body fluid, or cerebrospinal fluid. The only acceptable anticoagulant is heparin.

Plasma, Serum

The stability of glucose in specimens is affected by storage temperature, bacterial contamination, and glycolysis. Plasma or serum samples should be separated from the cells or clot within 30 minutes of being drawn. Specimens that cannot be separated from the cells within 30 minutes should be placed on ice or refrigerated.

When blood is drawn and stands uncentrifuged at room temperature, the average decrease in serum glucose is ~ 7% in 1 hour. This decrease is the result of glycolysis. (Even NaF does not prevent glycolysis within the first few hours when left at room temperature.) The rate of in vitro glycolysis is higher in the presence of leukocytosis or in patients with increased hematocrits.

Reagents/Materials:

- R1 MES buffer: 5.0 mmol/L, pH 6.0; Mg2+: 24 mmol/L; ATP: >4.5 mmol/L; NADP: >7.0 mmol/L; preservative.

- R2 HEPES buffer: 200 mmol/L, pH 8.0: Mg2+: 4 mmol/L; HK (yeast): >300 ukat/L; G-6-PDH (E.coli): >300 ukat/L; preservative

AMR (Analytical Measurement Range):

2-750 mg/dL

2 – 1500 mg/dL with automatic rerun

Report values of greater than 1500 as ">1500 mg/dL".

- The concentration of a diluted specimen must fall within the AMR of 2 – 750 mg/dL.

Calculations:

- Serum/Plasma/CSF are reported out in mg/dL.

Interpretation:

- Expected Range:
- Fasting Serum/Plasma: 70 – 99 mg/dL
- CSF: 45 – 75 mg/dL

11. DECLARATION

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.

M.Sc. candidate: wubalem Biresaw GSE/2294/10 (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor:

Zemenu Tamir (MSc, PhD fellow, Professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Moges wordofa (MSc, PhD candidate)

Signature: _____ Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Beruk kebede (PhD)

Signature: _____Date: _____

Place: Addis Ababa, Ethiopia.