ASSESSMENT OF HEMATOLOGICAL PARAMETERS IN ADULT PATIENTS WITH TYPE 2 DIABETES MELLITUS AT DEBRE BERHAN REFERRAL HOSPITAL, NORTHEAST ETHIOPIA: A COMPARATIVE CROSS-SECTIONAL STUDY

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A RESEARCH THESIS SUBMITTED TO SCHOOL OF MEDICAL LABORATORY SCIENCE, FACULTY OF HEALTH SCIENCE, INSTITUTE OF HEALTH, JIMMA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CLINICAL LABORATORY SCIENCE SPECIALTY IN HEMATOLOGY AND IMMUNOHEMATOLOGY

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ABSTRACT

Background: Diabetes is a global public health problem and associated with metabolic, cellular, and blood disturbances. Hematological changes have been reported in diabetes and play a major role in diabetes-associated complications. However, reports are contradicting and data on hematological parameters of type 2 diabetic patients in the study area are scarce.

Objective: To assess hematological parameters of type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia from May 01 to June 30, 2020.

Methods and materials: A comparative cross-sectional study was conducted on 268 (134 type 2 diabetic patients and 134 controls) study participants selected by systematic random sampling technique. Socio-demographic, behavioral, and clinical data were collected using a structured questionnaire and checklist. Ethical approval was obtained from Jimma University. All phase of quality assurance was maintained. Hematological parameters and blood glucose levels were determined using UniCel DxH 800 (Beckman Coulter, USA) and Biosystems A25 (Costa Brava, Spain) analyzers, respectively. Independent t-test, Mann–Whitney U-test, correlation, and logistic regression were used during data analysis. P-value <0.05 was considered as statistically significant.

Results: The current study found that total white blood cell count, absolute counts of neutrophil, lymphocyte, eosinophil, and basophil, red blood cell distribution width, platelet count, and mean platelet volume were significantly higher in type 2 diabetic patients as compared to the control group (P<0.05). On the other hand, the mean hemoglobin was significantly lower in type 2 diabetic patients than the control group (P=0.007). Anemia was found in 17.9% of type 2 diabetic patients. Longer duration of diabetes (AOR=3.05, 95% CI=1.12-8.34) and milk consumption (AOR=4.60, 95% CI=1.50-14.0) were significantly associated with anemia.

Conclusion: This study showed a statistically significant variation in some hematological parameters of type 2 diabetic patients compared to control group. Anemia among type 2 diabetic patients was found to be a mild public health problem. Therefore, routine screening of hematological parameters should be considered for proper management of type 2 diabetic patients. Close attention should also be given to the duration of diabetes and dietary practice.

Keywords: Ethiopia, Hematological parameters, Type 2 diabetes mellitus

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ABBREVIATIONS AND ACRONYMS

AGE:	Advanced Glycation End Products
AOR:	Adjusted Odds Ratio
BMI:	Body Mass Index
BP:	Blood Pressure
CBC:	Complete Blood Count
COR:	Crude Odds Ratio
CVD:	Cardiovascular Disease
DBP:	Diastolic Blood Pressure
DBRH:	Debre Berhan Referral Hospital
DM:	Diabetes Mellitus
EDTA:	Ethylene Di-amino Tetra Acetic acid
FBG:	Fasting Blood Glucose
HbA1c:	Glycated hemoglobin
HC:	Hip Circumference
Hct:	Hematocrit
Hgb:	Hemoglobin
IL-1:	Interleukin 1
IL-6:	Interleukin 6
IQR:	Inter Quartile Range
MCH:	Mean Corpuscular Hemoglobin
MCHC:	Mean Cell Hemoglobin Concentration
MCV:	Mean Corpuscular Volume
MPV:	Mean Platelet Volume
PLT:	Platelet
RBC:	Red Blood Cell
RDW:	Red blood cell Distribution Width
ROS:	Reactive Oxygen Species
SBP:	Systolic Blood Pressure
SD:	Standard Deviation
T2DM:	Type 2 Diabetes Mellitus
WBC:	White Blood Cell
WC:	Waist Circumference
WHO:	World Health Organization
WHR:	Waist to Hip Ratio

CHAPTER ONE: INTRODUCTION

1.1. Background

Diabetes mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycemia with abnormalities in the metabolism of carbohydrate, lipid, and protein resulting from defects in insulin secretion, action, or both (1). Diabetes is classified into two major types, type 1 and type 2 diabetes. Type 1 DM, which consists of about 5-10% of the cases, occurs as a result of an absolute deficiency of insulin secretion. On the other hand, type 2 diabetes is the majority of the DM burden, comprising 90–95% of cases and is characterized by peripheral insulin resistance or reduced production of insulin (1). Uncontrolled DM is associated with multiple disorders including metabolic, cellular, and blood disturbances leading to microvascular complications (retinopathy, nephropathy, and neuropathy) and macrovascular complications like cardiovascular disease (2).

Hematological parameters are measurable blood indices that can be used as a marker for the diagnosis and prognosis of certain physiological and pathological abnormalities. These parameters can be affected by disease conditions affecting hematopoietic milieu, or due to immunological response, and the clinical status of the tissue environment (3). Hematological and biochemical changes have been observed in patients with type 2 diabetes (T2DM). Hematological changes encountered in T2DM patients include changes in the function, structure, and metabolism of red blood cells (RBCs), white blood cells (WBCs), and platelet (PLT) (4). These changes may manifest as immunological and coagulation problems, and anemia characterized by a decrease in the RBC count, hemoglobin (Hgb) and hematocrit (Hct) level as compared to non-diabetic individuals (5).

Hematological changes in diabetes can be caused by several factors including increased production of reactive oxygen species (ROS) and the formation of advanced glycation end products (AGEs) as a result of the long-term hyperglycemia. Increased production of ROS resulting in oxidative stress, which is implicated in tissue damage and hematological changes such as RBC dysfunction, PLT hyperactivity, and endothelial dysfunction (6,7). These hematological changes may lead to complications such as anemia, and a state of hypercoagulability, and contribute to cardiovascular disease (CVD) in diabetic patients (8). Another mechanism is insulin resistance, which is associated with endothelial dysfunction, increased levels of inflammatory markers, and PLT hyperactivity, which accelerates vascular complications in T2DM patients (7).

There has been renewed interest in hematological parameters such as WBC, red blood cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width, and platelet count, have emerged as predictors of endothelial dysfunction and inflammation in T2DM (9,10). An increased WBC count is a classical marker of inflammation and evidence from epidemiological studies suggests an association between WBC count and diabetes risk (11). Chronic inflammation in diabetes is characterized by increased inflammatory cytokines such as interleukin 6 (IL-6), and interleukin (IL-1). These pro-inflammatory cytokines are thought to changes the sensitivity of erythroid progenitors to erythropoietin and promote apoptosis of immature RBCs and decrease the number of circulating RBCs resulting anemia of inflammation (12).

Platelets play a vital role in the integrity of normal homeostasis and MPV is the marker for its function. Diabetes is complicated by accelerated atherosclerosis and platelet activation plays a key role in inflammation and the atherothrombosis process contributes to the development of CVD in a patient with T2DM (13). Mean platelet volume reflects changes either in platelet stimulation or the rate of platelet production and increased MPV has been observed in diabetes patients with coronary heart disease, nephropathy, and retinopathy (14).

Generally, good glycemic control is the main recommendation in the prevention of the development of diabetic complications. It has been suggested that early normalization of glycemia may inhibit pathological processes that are closely related and induced by hyperglycemia such as increased oxidative stress and glycation of cellular proteins and lipids. Laboratory tests for management of diabetes patients are fasting blood glucose, glucose in the urine, glycated hemoglobin (HbA1c), and parameters of lipid status (2). Parameters obtained from hematologic analyzers can provide insight into changes that occur in hematological indices such as WBC, Hgb, Hct, RBC, PLT, RDW, MPV, and other parameters. Analysis of these parameters could contribute to the following-up of the development of degenerative complications in diabetes (15). Therefore, this study was aimed to assess the hematological parameters of type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia.

1.2. Statement of the Problem

Diabetes is an important global public health problem and one of the priority non-communicable diseases targeted for action by world leaders (16). The global burden of diabetes among adults has dramatically risen from 108 million in 1980 to 424.9 million in 2017 with an estimated 4.0 million deaths (16,17). Three-quarters of the global diabetes burden exists in low and middle-income countries and the number of people with diabetes by 2045 is estimated to rise to 628.6 million worldwide (17). Sub-Saharan Africa is experiencing a markedly increased prevalence of diabetes. According to the 2017 report of the International Diabetic Federation, 15.5 million (4.4%) adults in the Africa Region and 2.6 million (5.2%) adults in Ethiopia had diabetes (17).

The overall temporal burden of hyperglycemia is responsible for diabetes complications and adverse outcomes (16). Patients with T2DM have increased risk of CVD related to atherogenic dyslipidemia, coronary artery disease, and myocardial infarction, which is the leading cause of morbidity and mortality worldwide (18). Type 2 diabetes is a part of the metabolic syndrome that comprises dyslipidemia, obesity, hypertension, and changes in hematological parameters (19). Several blood components, including RBCs, WBCs, PLT, and the coagulation systems are affected in diabetes (20). These hematological changes may present as a manifestation of diabetes and contribute to severe problems such as anemia, infection, and state of hypercoagulability (21).

Anemia is a common hematological change in patients with T2DM and often unrecognized, and estimates of its prevalence vary widely. The prevalence of anemia among T2DM patients ranges from 18% to 41% in different ethnic populations worldwide (22–24). The etiology of anemia in T2DM is multifactorial and includes chronic hyperglycemia, inflammation, oxidative stress, AGEs, nutritional deficiencies, drugs, and hormonal changes in addition to kidney disease (12). As a consequence, anemia tends to occur early in diabetic patients, even in the absence of overt nephropathy, and it is more severe. Anemia is also a significant adverse prognostic factor to increase the risk of diabetic complications including nephropathy, retinopathy, and cardiovascular disease due to hypoxia-induced organ damage (25). Indeed, the constellation of these modifiable risk factors is often overlooked and if untreated, it is associated with poor outcomes, including poor quality of life, increased hospitalization, and all-cause mortality in diabetes populations (12).

Changes in WBCs indices have been reported in diabetes. Systematic review and meta-analysis of cross-sectional and prospective studies have shown that the number of peripheral WBCs such as granulocyte and lymphocytes are increased, with no change in the number of monocytes in patients with T2DM (26). Diabetic leukocytes especially neutrophils have been associated with impaired deformability, chemotaxis, phagocytosis, bactericidal activity, altered superoxide anion production, and increased apoptosis (27). All these factors act in concert and contribute to the high susceptibility to and severity of infections in diabetic patients. Additionally, different platelet functional and morphological abnormalities have been reported in vivo and in vitro in T2DM, altogether consistent with increased activity and reduced responsiveness to low dose of aspirin (28,29). Since vascular complications of diabetes are important causes of morbidity and medical expenditure; the change in platelet function affects the patient's quality of life.

Generally, hematological changes have been observed in T2DM patients, anemia is the most common and often unrecognized or overlooked, despite its contribution to morbidity and mortality. Current guidelines on the management of diabetes do not recommend periodic follow-up monitoring of hematological parameters. Although studies on the hematological parameters of T2DM patients have been done in developed and developing countries, they came up with a range of findings and inconsistencies. Moreover, there is limited information in Ethiopia particularly in the study area and the hematological parameters in T2DM are not well studied in our population in general. Therefore, the present study was aimed to assess hematological parameters of type 2 diabetic adult patients in comparison with screened blood donors at Debre Berhan Referral Hospital, Northeast Ethiopia.

1.3. Significance of the Study

The findings in the present study will help to generate information about the hematological parameter of type 2 diabetic patients. Early assessment of hematological parameters will have paramount significance for early detection and management of hematological disorders like anemia and lower the risk of developing diabetes-associated complications in diabetic patients at the primary care setting that reduce hospital admissions and maintain optimum health. The use of hematological parameters as a simple and cost-effective technique, that is routinely done to investigate the state of various indices of blood elements has an additive role with plasma glucose and HbAlc in the monitoring of diabetic patients. Furthermore, this study is important for governmental and non-governmental organizations and health care practitioners who seek information for evidence-based intervention and future policymaking. This study will also serve as a baseline for other studies.

CHAPTER TWO: LITERATURE REVIEW

Many studies have been documented the significant difference of hematological parameters between diabetic and non-diabetic subjects as well as investigated the correlation of these parameters with cardio-metabolic risk factors. A descriptive study was done in Brazil from February 2013 to January 2014 with a total of 200 subjects (100 T2DM patients and 100 control) to evaluate the platelet parameters in diabetic patients. In this study, a statistically significant increase was observed in MPV in the DM patients as compared to their respective control groups. However, the RBC, WBC, platelet counts, Hgb, and Hct were not showed a statistical difference between the groups. Regarding the correlation between fasting blood glucose (FBG) levels and platelet parameters, a weak positive and significant correlation was observed with MPV, indicating that patients with higher FBG levels tended to present higher values of MPV (30).

A study was conducted in 2018 in Turkey to evaluate components of the complete blood count on 135 T2DM patients and 121 controls. In this study, the author reported that the mean of WBC, neutrophil, lymphocyte, and monocyte counts was significantly higher in the DM group as compared to the controls. The respective values were 8.63 ± 2.00 vs 7.30 ± 1.70 for WBC count ($x10^3$/uL), 5.20 ± 1.55 vs 4.30 ± 1.40 for neutrophil count ($x10^3$/uL), 2.71 ± 1.00 vs 2.30 ± 0.60 for lymphocyte count ($x10^3$/uL), and 0.55 ± 0.162 vs 0.50 ± 0.20 for monocyte count ($x10^3$/uL). On the other hand, Hgb levels, PLT counts, RDW, and MPV were similar between groups. Additionally, WBC and Lymphocyte counts were positively correlated with systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), waist circumference (WC), FBG. Neutrophil counts were positively correlated with, FBG, and BMI (31).

Comparative analysis of biochemical and hematological parameters was also carried out in Bangladesh during the period 2014 on a total of 723 subjects (403 Diabetic and 320 non-diabetic). The result of hematological parameters showed that the mean value of total WBC count, neutrophil, and eosinophil was significantly higher in diabetic patients than in the non-diabetic subjects. On the other hand, monocyte and Hgb were significantly lower in the DM group as compared to the controls and lymphocytes were higher in the former group without showing any significant difference. Moreover, total WBC count and Hgb were correlated with FBG; but neutrophil, lymphocyte, monocyte, and eosinophil had no significant correlation (32).

A comparative study has been carried out in India during the study period of 2016 on 140 study subjects to determine the hematological profiles among T2DM patients. Overall, the results showed a significant decrease in the mean value of RBC, Hgb, Hct, and mean cell volume (MCV) in diabetics compared to the controls. Whereas mean cell hemoglobin concentration (MCHC), WBCs and lymphocytes were significantly higher in diabetics when compared to non-diabetics. No difference was observed for mean cell hemoglobin (MCH), neutrophils and PLT counts between groups. Among the diabetics, anemia was observed in 50 (71.4%) patients, with 34.3% had mild, 28.6% had moderate and 8.6% had severe anemia (33).

According to the study done in 2018 in Pakistan on 170 diabetic and 92 control subjects, a significant increase in all RBC indices, and MPV were observed in diabetic patients in comparison to control group. Parallelly the WBCs indices were evaluated and a significant increase was found in percentage and number of monocytes and a decrease in the percentage of granulocyte in diabetic patients as compared to their respective control group. The correlation studies indicated that the number of granulocytes and all PLT indices were positively correlated with FBG and whereas no significant correlation has existed between FBG and RBC indices in diabetic patients except MCH which was found negatively correlated with FBG (34).

A comparative cross-sectional study was conducted in Saudi Arabia in 2016 by involving 405 study subjects (205 T2DM and 200 control). The report of this study showed that RBC count, Hgb, MCV, MCHC, and MCH were significantly lower in T2DM compared to the control group. In contrast, the mean of RDW, MPV, WBCs, and differential leukocyte counts (neutrophils, lymphocytes, eosinophils, monocytes, and basophils) were significantly increased in diabetic patients compared to the control group. However, the mean PLT count was not significantly changed between the groups. The author concludes that hematological changes in T2DM patients may lead to the development of long-term complications and poor quality of life (35)

An analytical cross-sectional study was conducted in Sudan in 2012 on 90 study subjects (60 diabetic Vs 30 controls) to determine the blood parameters in patients with diabetes mellitus. Statistical analysis showed that the mean of total WBC and neutrophil were relatively higher in the diabetic group than the control whereas the MCH and MCHC were significantly lower in the former group. Significant variations were not observed between the groups in RBC, Hgb, Hct, MCV, PLT, and lymphocytes count. Among diabetics, 11 (18.3%) patients were anemic (36).

Furthermore, another cross-sectional study was carried out in Libya in 2017 to investigate the status of hematological parameters by including 103 patients with T2DM and 39 controls. This study showed that Hct, Hgb, RBCs, and MCV values were significantly lower in T2DM patients than controls. On the other hand, the mean values for MCHC, MCH, WBCs count, lymphocytes, and neutrophils counts were significantly increased in diabetic patients than controls. However, no significant differences were observed in PLT counts between diabetic patients and controls (37).

A Hospital-based comparative study was conducted in Nigeria during the period 2013 on 200 study subjects and in 2019 on 233 participants to determine the prevalence of anemia and variations in PLT parameters among patients with T2DM. The mean level of Hgb in diabetes patients was significantly lower compared to the controls (12.2 ±1.53 g/dl vs 13.0 ±1.38 g/dl). Among diabetics, anemia was observed in 70 (45.2%) of patients. Duration of DM remained independent predictors of anemia and no association with FBG and the use of medications (metformin, sulfonylureas). Regarding PLT parameters, the mean PLT count was significantly higher in diabetics compared to the controls, and a statistically significant difference was not observed for MPV between the two groups. On a correlation analysis, Hgb concentration had a significant negative relationship with the duration of DM but not with BMI; platelet count and MPV showed a statistically significant correlation with BMI (38,39).

In a cross-sectional study conducted in Dessie, Northeast Ethiopia in 2018 on 412 diabetic patients to investigate anemia, and 110 (26.7%) study subjects had anemia. Males were more likely to be anemic than females (35.5% vs 19.7%) (40). A comparative cross-sectional study was conducted in 2019 in Addis Ababa, Ethiopia by enrolling 140 study subjects (70 T2DM and 70 control). The mean of WBC count, platelet count, MCH, RDW, MCHC, and MPV were significantly higher in T2DM as compared with the healthy group. No significant difference was observed in regarding RBC count, Hgb, neutrophil, and lymphocyte between the groups (41).

A comparative cross-sectional study was conducted at Gondar University Hospital in 2015 on a total of 296 participants (148 T2DM and 148 healthy controls). According to this study, there was a significant increment in RDW, total WBC, absolute lymphocyte, absolute neutrophil count, and MPV in diabetic patients as compared with controls. On the correlation analysis, a significantly weak positive correlation was obtained in neutrophil count, RBC count, Hgb, and RDW values with BMI and Waist-hip ratio (WHR). Hematological parameters such as MCV, platelet count, lymphocyte count, and MPV showed a statistically significant correlation with SBP, while WBC and PLT count showed a significant negative correlation with DBP in T2DM groups. Additionally, FBG showed a significant positive correlation with total WBC count, absolute lymphocyte count, absolute neutrophil count, and MPV. Moreover, the duration of diabetes in T2DM patients achieved a significant positive correlation with MPV and platelet count (42).

From the above literatures, hematological changes have been observed in diabetic patients, with anemia being the most common. Studies regarding the hematological parameters of diabetic patients done in different areas came up with a range of contradictory reports. Some studies showed that no statistically significant difference between diabetic patients and healthy controls in RBCs indices (30,31,36), WBC count, and platelet count (10,30), while others showed that RBC, WBC, and PLT indices are significantly higher in diabetic patients than controls (34). Others reported that RBCs indices except RDW are significantly lower, whereas WBC and PLT indices are significantly higher in the diabetic group than the control group (35,42).

CHAPTER THREE: OBJECTIVES

3.1. General Objective

To assess hematological parameters of type 2 diabetic adult patients in comparison with screened blood donors at Debre Berhan Referral Hospital, Northeast Ethiopia from May 01 to June 30, 2020, G.C.

3.2. Specific Objectives

- To compare hematological parameters between T2DM patients and controls.
- To determine the prevalence of hematological abnormality in patients with T2DM.
- To assess the associated factors of hematological abnormality in patients with T2DM.
- To assess the correlation of hematological parameters with anthropometric measurement, blood pressure measurement, FBG level, duration of DM in patients with T2DM.

CHAPTER FOUR: MATERIALS AND METHODS

4.1. Study Area

This study was conducted at the Chronic Care Clinic of Debre Berhan Referral Hospital, (DBRH). The hospital is found at Debre Berhan town, located 130 km northeast of Addis Ababa, the capital city of Ethiopia at an average elevation of 3000 meters (43). In Debre Berhan, two hospitals, three health centers, and seventeen private clinics provide healthcare services. Debre Berhan Referral Hospital was established 76 years ago and provides comprehensive healthcare services to a catchment population of more than 2.4 million (44). As a referral hospital, it plays an important role in providing teaching, research, and community service. The Diabetes follow-up clinic at DBRH is providing service to more than 2,100 diabetic patients on regular follow–up currently.

4.2. Study Design and Period

A comparative cross-sectional study was conducted from May 01 to June 30, 2020, G.C.

4.3. Population

4.3.1. Source Population

All adult T2DM patients attending in chronic care clinic of DBRH and age and gender-matched blood donors at Debre Berhan blood bank were taken as a source population for the diabetic and control group, respectively.

4.3.2. Study Population

All adult T2DM patients attending in chronic care clinic of DBRH who fulfill the inclusion criteria during the study period were study population for the diabetic group. All age and gender-matched screened healthy blood donors at Debre Berhan blood bank during the study period were study population for the control group.

4.4. Sample Size Determination and Sampling Technique

4.4.1. Sample Size Determination

The sample size was calculated based on two population mean formula using G*-Power statistical free software version 3.1, by considering the following assumptions: 95% confidence level (2-tailed, α=0.05), 80% power (β=0.20), the ratio of sample size (T2DM/control) was 1:1, effect size (d) was 0.36 and 10% non-respondent rate. The mean and standard deviation (SD) [mean ± SD] of absolute neutrophils (10^3/μL) for T2DM and control groups were taken from a study conducted in Gondar (42), 3.57±1.46 for T2DM and 3.11±1.04 for the control group. The sample size was determined to be 134 for each group and a total of 268 study participants were included in this study. The formula hereunder can be used to calculate the sample size manually:

$$n = \frac{2\left(z\frac{\alpha}{2} + z\beta\right)^2}{d^2}$$

Where, n = minimum sample size for each group

d= effect size calculated by dividing the mean difference to pooled standard deviation

$z_{\alpha/2}$ = the value under the standard normal table for the given value of confidence level =1.96

zβ = the value under the standard normal table for the power of the study =0.84

4.4.2. Sampling Technique

A systematic random sampling technique was used. The total number of diabetic patients with regular follow–up in DBRH was 2,100 (900 type 1 DM and 1200 T2DM). An average of 50 T2DM patients attends the chronic care clinic per week. The total expected T2DM patients during the study period were estimated at 400. When the total population for the study period (N=400) was divided by the sample size of the diabetic group (134), the sample interval (K) was found 3. The first participant was selected randomly between one and K (3) using the lottery method, and the next participant was selected following the K value until the sample size was reached.

4.5. Inclusion and Exclusion Criteria

4.5.1. Inclusion Criteria

Adult T2DM patients who had follow-ups at the chronic illness clinic of DBRH during the study period and volunteer to participate were included in the diabetic group. Additionally, age and gender-matched screened nonremunerated blood donors at Debre Berhan blood bank during the study period were included in the control group.

4.5.2. Exclusion Criteria

Type 2 diabetic patients who have a chronic disease like cardiac, renal and liver disease, patients who were on hormone therapy like erythropoietin, insulin, taking hematin factors, anticoagulant therapy, statins, antihypertensive treatment, smoker, alcoholics, pregnant women, those patients below the age of 18 and above 65 years were not engaged in this study. The participants were excluded, due to any of the exclusion criteria, from the study following a critical review of their medical records and face to face interviewees.

4.6. Study Variables

4.6.1. Dependent Variable

Hematological parameters

4.6.2. Independent Variables

- Sociodemographic characteristics (gender, age, level of education, and residence)
- Clinical characteristics (duration of DM, type of anti-DM drug)
- Behavioral characteristics (physical exercise, dietary habits)
- Fasting blood glucose level (glycemic control)
- Anthropometric measurement (BMI and WHR)
- Blood pressure measurement (SBP and DBP)

4.7. Operational Definitions

Hematological parameters: Are hematological tests (i.e. RBC, WBC, PLT count, Hgb, Hct, RBC indices, WBC differential count, and MPV) (45).

Anemia: defined as a level of Hgb <13 g/dl for males and <12 g/dl for non-pregnant women (46).

Leukocytosis: corresponds to a level of WBC count higher than 10.2 $x10^3$/ul (45)

Leukopenia: corresponds to a level of WBC count below 3.10 $x10^3$/ul (45)

Neutrophilia: corresponds to a level of neutrophil count higher than 7.6 $x10^3$/ul (45)

Neutropenia: corresponds to a level of neutrophil count below 1.7 $x10^3$/ul (45)

Lymphocytosis: corresponds to a level of lymphocyte count higher than 3.2 $x10^3$/ul (45)

Lymphopenia: corresponds to a level of lymphocyte count below 1.0 $x10^3$/ul (45)

Monocytopenia: corresponds to a level of monocyte count below 0.3 $x10^3$/ul (45)

Eosinophilia: corresponds to a level of eosinophil count higher than 0.5 $x10^3$/ul (45)

Basophilia: corresponds to a level of basophil count higher than 0.1 $x10^3$/ul (45)

Thrombocytopenia: is a condition of low levels of PLT in the blood usually below 150 $x10^3$/ul (45)

T2DM Patients: T2DM adult patients who are taking oral glucose lowering agent plus lifestyle modifications.

Control group: apparently healthy screened blood donors.

Fasting blood sample: blood sample collected from T2DM patient after overnight fasting.

Good glycemic control: Mean fasting blood glucose level 70-130 mg/dl (47).

Poor glycemic control: Mean fasting blood glucose level ≥130 mg/dL (47).

Duration of DM: The time measured from a patient was diagnosed with DM until the date of the study.

BMI: Four categories of BMI can be identified as follows: underweight, <18.5 kg/m^2; normal, 18.5–24.9 kg/m^2; overweight, 25.0–29.9 kg/m^2; and obesity, >30 kg/m^2 (48)

Abdominal obesity: is defined as a WC of >94 cm for male and >80 cm female (48).

WHR: Values ≥0.8 for females and ≥0.9 for males were used to define overweight (48)

Hypertension: is defined as systolic BP≥140 mmHg and/or diastolic BP ≥90 mmHg.

4.8. Data Collection Materials

- Bio systems A25 automated clinical chemistry analyzer (Costa Brava, Spain)
- UniCel DxH 800 Haematology Analyzer (Beckman Coulter, USA)
- Serum separator tube
- Ethylene di-amino tetra acetic acid (EDTA) test tubes
- Centrifuge
- Plastic pipette and tips
- Digital BP apparatus(sphygmomanometer)
- Weight scale
- Tape meter
- Stadiometer
- Pen
- Pencil
- Pencil-sharpener
- Questionnaires
- A4 paper
- 70% alcohol
- Vacutainer tube with needle
- Cotton
- Tourniquet
- Glove
- Safety box

4.9. Data Collection Procedure

4.9.1. Socio-demographic and clinical data collection

Data related to socio-demographic, and behavioral characteristics were collected using a structured questionnaire through face-to-face interviews (Annex II). Clinical variables including duration of diabetes, type of anti-DM drug, and fasting blood glucose level for the last two months were abstracted from the diabetic patients' medical records using a checklist (Annex III). Participants fasting blood glucose reading for at least three months including the current reading was used for computing the average blood glucose level. Blood pressure (BP) was measured by clinical nurses from the upper arm of the participant after 5-minute resting using a sphygmomanometer. Anthropometric variables were measured according to the anthropometric measurements protocol (Annex VI). Before data collection, the data collectors were selected patients who full fill inclusion criteria and took informed consent. After an interview, review of records, anthropometric, and BP measurement was completed by trained clinical nurses, the study subjects were sent to a laboratory where a blood sample was collected for determination of FBG, and hematological parameters.

4.9.2. Laboratory Sample Collection and Analysis

Six-milliliter of venous blood sample (2ml in a serum separator tube and 4ml in EDTA tube) was collected from each T2DM patient by laboratory professionals after overnight fasting (Annex V). On the other hand, four milliliters of venous blood were collected into EDTA test tube from the control group (blood donor) at the time of the donation. A Serum prepared from a serum separator tube was used to determine FBG. Fasting blood glucose was estimated by following the glucose oxidase method (49), using Biosystems A25 (Costa Brava, Spain) automated chemistry analyzer according to the manufacturer's instructions (Annex VI). The blood sample collected in EDTA tube was gently mixed by inverting the tube 6-8 times to prevent clotting and used for hematological tests. Complete blood cell count (CBC) was analyzed using UniCel DxH 800 (Beckman Coulter, USA) automated hematology analyzer using the coulter counting, spectrophotometry, and VCSn technology (50) (Annex VII). Fasting blood glucose, hematological parameters (RBC, Hgb, Hct, MCV, MCH, MCHC, RDW, WBCs, absolute lymphocytes, monocyte, basophil, eosinophil, neutrophils, platelet count and MPV) were analyzed at DBRH laboratory and collected for each study participants using laboratory result registration form (Annex VIII).

4.10. Data Quality Assurance and Management

To ensure the quality of the data, preanalytical, analytical, and post-analytical precautions were taken. The English version of the questionnaire was translated into the local language, (Amharic) and re-translated to the English version for its accuracy and consistency. The questionnaire was pretested on 5% of the sample size at Enat Hospital and training was given for the data collectors before the actual data collection started. Manufacturer instructions and standard operating procedures were strictly followed during specimen collection and all other laboratory procedures. Anthropometric and BP measurements were taken twice and the average value was used for data analysis. Control reagents were used for the hematology analyzer and glucose analysis. The blood samples were processed within 2 hours of specimen collection. A blood film examination was performed for suspected flags in the analyzer. Completion, and clarity of the collected data were checked regularly and the results were properly recorded, transcribed, and reviewed.

4.11. Statistical Analysis

Data were checked for their completeness and consistency and entered into Epidata version 3.1 (Epidata Association, Odense Denmark) and exported to Statistical Package for Social Sciences (SPSS) version 25 software (IBM Corporation, USA) for analysis. The normality of data distribution was checked using histogram, Shapiro-Wilk test, and Kolmogorov-Smirnov test. The results were reported as frequency and percentages for categorical variables, mean ± SD for normally distributed continuous variable and median with interquartile range (IQR) for continuous variables with skewed distribution. Statistical differences between the groups were determined by the chi-square test for categorical variables. The comparison of hematological parameters between diabetic and control participants were done by independent t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data. The correlation of hematological parameters with independent variables was assessed by Pearson's correlation for normally distributed data and Spearman's correlation for non-normally distributed data. Bivariate and multivariate logistic regression analysis was conducted for categorical dependent variables. Hemoglobin was adjusted for an average altitude of 3000 meters with a factor of 1.9 based on the World Health Organization (WHO) recommendation for the diagnosis of anemia (46). Crude odds ratios (COR) and adjusted odds ratios (AOR) with 95% confidence intervals (CI) were used to see associations of predictors and outcomes. In any condition, P-value <0.05 was considered as statistically significant. The results were presented by tables.

4.12. Ethical Consideration

Ethical clearance was obtained from the Institutional Review Board (IRB) of Jimma University, Institute of Health with letter protocol number IRB000138/2020. A support letter from the School of Medical Laboratory Science of Jimma university was submitted to DBRH. Permission was also obtained from DBRH and Debre Berhan blood bank. Written informed consent was obtained from each participant after a clear explanation of the purpose, the procedure, benefits, possible discomfort of the study, and the right to voluntary participation was given (Annex I). To ensure confidentiality of data, the study participants were identified using codes instead of individual identifiers and unauthorized persons were not able to access the collected data. Results with hematological abnormalities were communicated to the physicians who were working in the

chronic care clinic for proper management of the patients. For the sake of ethics, the control groups (blood donors) were screened in Debre Berhan blood bank.

4.13. Dissemination of the Result

The finding of this study will be submitted to Jimma University, Institute of Health, Faculty of Health Sciences, School of Medical Laboratory Science, and DBRH. The findings of this study will be also published in peer-reviewed scientific journals. It will also be presented on different scientific forums both in Ethiopia and abroad.

CHAPTER FIVE: RESULT

5.1. Demographic, Anthropometric, and Clinical Characteristics of Study Participants

A total of 268 (134 T2DM patients and 134 controls) study participants were included in this study. The T2DM patients and control groups did not differ in terms of age and gender ($p>0.05$). Majority of the study participants 85 (63.4%) were males for both T2DM patients and controls. The mean age (mean ±SD) was 43.08±9.3 for T2DM patients and 42.71±8.6 years for controls. Out of the total study participants, around 63 (47.0%) and 76 (56.7%) have a higher educational level for T2DM and controls, respectively. About 113 (84.3%) and 107 (79.9%) were from urban residences for T2DM and controls, respectively. Regarding the anthropometric and clinical characteristics of the study participants, statistically higher values of WHR ($p<0.001$), WC ($p<0.001$), BMI ($p<0.001$), systolic BP ($p<0.001$), and diastolic BP ($p=0.002$) were observed in T2DM patients compared to controls. The median of FBG levels was 159.2 (136.0-185.0) and the median duration of DM since diagnosis was 7.0 (4.0-9.0) years in T2DM patients (Table 1).

Table 1: Socio-demographic, anthropometric, and clinical characteristics of type 2 diabetic adult patients and control group, at Debre Berhan Referral Hospital, Northeast Ethiopia, 2020 (n=268).

Variables		**T2DM (n=134)**	**Control (n=134)**	**P-value**
Age (years), mean ± SD		43.08±9.3	42.71±8.6	0.734
Gender, n (%)	Male	85 (63.40)	85 (63.4)	1.00
	Female	49 (36.60)	49 (36.6)	
Educational status, n (%)	Unable to read and write	13 (9.70)	5 (3.7)	0.028
	Able to read and write	21 (15.70)	10 (7.5)	
	Primary school	37 (27.60)	43 (32.1)	
	High school and above	63 (47.0)	76 (56.7)	
Residence, n (%)	Urban	113 (84.3)	107 (79.9)	0.141
	Rural	21 (15.7)	27 (20.1)	
BMI (kg/m^2), median (IQR)		24.5 (22.1-27.7)	22.5 (21.1-24.1)	<0.001
WC (cm), median (IQR)		79.0 (74.0-85.0)	75.0 (70.0-79.0)	<0.001
WHR, mean ± SD		0.88±0.1	0.86±0.1	<0.001
Systolic BP (mmHg), median (IQR)		120 (110-130)	120 (110-120)	<0.001
Diastolic BP (mmHg), median (IQR)		80 (70-80)	70 (70-80)	0.002
FBG level (mg/dl), median (IQR)		159.2 (136.0-185.0)	_	
Duration of DM, median (IQR)		7.0 (4.0-9.0)	_	

Note: p-value <0.05 is considered as statistically significant.

Abbreviations: DM, diabetes mellitus; T2DM, type 2 diabetes mellitus; BMI, body mass index; WHR, waist to hip ratio; FBG, fasting blood glucose; BP, blood pressure; WC, waist circumference; SD, standard deviation, IQR, Interquartile range.

5.2. Comparison of Hematological Parameters of the Study Participants

Among the WBC indices, statistically higher values of total WBC (*P*=0.004), absolute neutrophil (*P*=0.02), absolute lymphocyte (*P*<0.001), absolute eosinophil (*P*<0.001), and absolute basophil counts (*P*<0.001) were observed in T2DM patients as compared to the control group. Regarding the RBC indices, the mean Hgb in patients with T2DM patients (15.7±1.2) was significantly lower than the control (16.2±1.3) group (P=0.007). The median (IQR) of RDW was significantly increased in T2DM groups than the control group (P<0.001). Additionally, there were significantly higher mean platelet count (P=0.013) and mean MPV (P=0.010) in the T2DM group than the control group (Table 2).

Table 2: Comparison of hematological parameters of type 2 diabetic adult patients and control group, at Debre Berhan Referral Hospital, Northeast Ethiopia, 2020 (n=268).

Parameters	**T2DM (n=134)**	**Control (n=134)**	**p-value**
WBC (10^3/µl), mean ± SD	7.01±1.74	6.50±1.34	0.004*****
Neu (10^3/µl), mean ± SD	4.14±1.51	3.80±0.96	0.020*****
Lymph (10^3/µl), mean ± SD	2.07±0.62	1.86±0.54	<0.001*****
Mon (10^3/µl), median (IQR)	0.6 (0.4-0.7)	0.5 (0.4-0.7)	0.150******
Eos (10^3/µl), median (IQR)	0.2 (0.1-0.3)	0.1 (0.1-0.2)	<0.001******
Bas (10^3/µl), median (IQR)	0.1 (0.0-0.1)	0.0 (0.0-0.0)	<0.001******
RBC (10^6/µl), mean ± SD	5.10±0.45	5.2±.0.5	0.100*****
Hgb (g/dl), mean ± SD	15.7±1.2	16.2±1.3	0.007*****
HCT (%), median (IQR)	46.4 (43.5-48.6)	46.6 (43.6-49.2)	0.206******
MCV (fl), median (IQR)	90.1 (88.4-94.0)	90.8 (87.2-93.6)	0.917******
MCH (Pg), median (IQR)	31.0 (29.8-32.2)	31.1 (30.2-32.3)	0.307******
MCHC (%), mean ± SD	34.2±1.0	34.5±1.0	0.052*****
RDW (%), median (IQR)	14.0 (13.4-14.7)	13.5 (13.1-14.0)	<0.001******
Platelet(10^3/µl), mean ± SD	262.8±57.2	247.3±43.1	0.013*****
MPV (fl), mean ± SD	8.5±1.1	8.2±0.8	0.010*****

Note: p-value <0.05 is considered as statistically significant.

Abbreviations: T2DM, type 2 diabetes mellitus; WBC, white blood cell; Neu, neutrophil; Lymph, lymphocyte; Mon, monocyte; Bas, basophil; Eos, eosinophil; RBC, red blood cell; Hgb, hemoglobin; HCT, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red blood cell distribution width; MPV, mean platelet volume; SD, standard deviation, IQR, Interquartile range.

5.3. Prevalence of Hematological Abnormality in Type 2 Diabetic Adult Patients

The prevalence of anemia in the current study among T2DM patients was 17.9%. Leukocytosis was observed in 5 (3.7%) of T2DM patients. Additionally, out of 134 study subjects, 2 (1.5%) had neutrophilia and 5 (3.7%) had neutropenia. On the other hand, lymphocytosis and lymphopenia have occurred in 3 (2.2%) and 2 (1.5%) of T2DM patients, respectively. Moreover, 5 (3.7%) patients had monocytopenia, 6 (4.5%) had eosinophilia, 7 (5.2%) had basophilia, and 2 (1.5%) had thrombocytopenia (Table 3).

Table 3: Prevalence of hematological abnormality among Type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia, 2020 (n=134).

Abnormality	**n (%)**	**Cutoff value**
Anemia	24 (17.9)	<13 g/dl (male) & <12 g/dl (female)
Leukocytosis	5 (3.7)	>10.2 $x10^3$/ul
Neutrophilia	2 (1.5)	>7.6 $x10^3$/ul
Neutropenia	5 (3.7)	<1.7 $x10^3$/ul
Lymphocytosis	3 (2.2)	>3.2 $x10^3$/ul
Lymphopenia	2 (1.5)	<1.0 $x10^3$/ul
Monocytopenia	5 (3.7)	<0.3 $x10^3$/ul
Eosinophilia	6 (4.5)	>0.5 $x10^3$/ul
Basophilia	7 (5.2)	>0.1 $x10^3$/ul
Thrombocytopenia	2 (1.5)	<150 $x10^3$/ul

5.4. Factors Associated with Anemia Among Type 2 Diabetic Adult Patients

Categorical variables were created from hemoglobin after the hemoglobin was adjusted for an average altitude of 3000 meters with a factor of 1.9 based on the World Health Organization (WHO) recommendation (46). Associations of anemia with the predictor variables were evaluated using univariable and multivariable logistic regression analysis in diabetic individuals. In the bivariable logistic regression analysis gender, duration of DM, anti-DM drug, and milk consumption was associated with anemia with a p-value of less than 0.2. Variables with a p-value of less than 0.2 were included in the multivariable regression model, duration of diabetes, and milk consumption were significantly associated with anemia. Patients who had been diagnosed with diabetes for 7 years or above were 3 times more likely to have anemia compared to those who have had it for less than 7 years (COR = 2.61, 95 % CI= 1.00-6.8), and (AOR = 3.05, 95 % CI = 1.12-8.34). Type 2 diabetic patients with milk consumption were 4.6 times more likely to have anemia compared to that of no milk consumption (COR = 3.4, 95 % CI= 1.2-9.8), and (AOR = 4.60, 95 % CI = 1.50-14.0) (Table 4).

Table 4: Factors associated with anemia among type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia, 2020 (n=134).

Variables	Categories	Anemia		COR (95% CI)	P-Value	AOR (95% CI)	P-Value
		Yes (%)	No (%)				
Age	18-41	11 (45.5)	49 (44.5)	1.05 (0.43-2.55)	0.90		
	42-65	13 (54.5)	61 (55.5)	1.00			
Gender	Male	18 (75)	67 (60.9)	1.00		1.00	
	Female	6 (25)	43 (39.1)	0.5 (0.20-1.42)*	0.20	0.35 (0.12-1.03)	0.06
Residence	Urban	22 (91.7)	94 (85.5)	1.00			
	Rural	2 (8.3)	16 (15.5)	0.53 (0.11-2.50)	0.42		
BMI	Normal	12 (50.0)	60 (54.5)	1.00			
	Abnormal	12 (50.0)	50 (45.5)	1.2 (0.49-2.90)	0.69		
Glycemic control	Good control	8 (33.3)	48 (43.6)	1.00			
	Poor control	16 (66.7)	62 (56.4)	1.55 (0.61-3.92)	0.36		
Anti-DM drug	Metformin	19 (79.2)	99 (90)	1.00		1.00	
	Metformin+ Sulfonylurea	5 (20.8)	11 (10)	2.40 (0.73-7.6)*	0.14	2.40 (0.58-7.04)	0.27
Duration of DM	< 7 years	7 (29.2)	57 (51.8)	1.00		1.00	
	≥ 7 years	17 (70.8)	53 (48.2)	2.61 (1.00-6.8)*	0.05	3.05 (1.12-8.3)*	0.03
Physical exercise	No	14 (58.3)	37 (33.6)	1.00			
	Yes	10 (41.7)	73 (66.4)	0.36(0.14-0.90)	0.25		
Vegetable consumption	No			1.09 (0.33-3.60)	0.88		
	Yes			1.00			
Egg consumption	No	11 (45.8)	51 (46.4)	1.00(0.40-2.37)	0.96		
	Yes	13 (54.2)	59 (53.6)	1.00			
Meat consumption	No	7 (29.2)	42 (38.2)	0.67(0.26-1.74)	0.41		
	Yes	17 (70.8)	68 (61.8)	1.00			
Milk consumption	No	5 (20.8)	52 (47.3)	1.00		1.00	
	Yes	19 (79.2)	58 (52.7)	3.4 (1.2-9.8)*	0.02	4.6 (1.50-14.0)*	0.008
Tea/coffee consumption	No	3 (12.5)	26 (23.6)	1.00			
	Yes	21 (87.5)	84 (76.4)	2.17 (0.60-7.84)	0.24		

Note: COR: Crud Odds Ratio, AOR: Adjusted Odds Ratio, CI: Confidence Interval, *: Statistically significant association

5.5. Correlations of Hematological Parameters with Anthropometric and Clinical Variables Among Type 2 Diabetic Adult Patients

In the correlation analysis, RBC count (p=0.04) and RDW (p=0.003) showed a statistically significant correlation with the duration of diabetes. Lymphocyte count showed significant and weak positive correlation with SBP (p=0.02) and DBP (p=0.01). Basophil count (p=0.03) and MCH (p=0.03) also showed a weak positive and significant correlation with DBP. Platelet count achieved a statistically significant negative correlation with BMI (p=0.02) and WHR (p=0.01). Additionally, MPV (*P*=0.02) achieved a statistically significant correlation with BMI. In the current study, a statistically significant correlation was not observed between hematological parameters and fasting blood glucose levels (Table 5).

Table 5: Correlations of hematological parameters with anthropometric and clinical variables among type 2 diabetic adult patients, at Debre Berhan Referral Hospital, Northeast Ethiopia, 2020 (n=134).

Parameters	**BMI**	**WHR**	**DM duration**	**FBG**	**SBP**	**DBP**
	rho(p)	**rho(p)**	**rho(p)**	**rho(p)**	**rho(p)**	**rho(p)**
WBC	-0.11(0.28)	0.09(0.29)$	-0.01(0.91)	0.004(0.961)	0.12(0.16)	-0.04(0.65)
Neutrophil	-0.10(0.50)	0.04(0.67)$	0.06(0.47)	0.008(0.929)	0.04(0.61)	-0.04(0.64)
Lymphocyte	0.10(0.36)	0.07(0.44)$	-0.04(0.66)	-0.02(0.76)	0.20**(0.02)	0.22**(0.01)
Monocyte	0.02(0.80)	0.05(0.57)	0.008(0.924)	0.13(0.12)	-0.02(0.79)	-0.06(0.51)
Eosinophil	-0.01(0.91)	0.03(0.70)	-0.04(0.62)	0.03(0.71)	0.14(0.10)	0.02(0.82)
Basophil	-0.01(0.840)	0.10(0.22)	0.03(0.71)	0.06(0.49)	0.16(0.06)	0.18**(0.03)
RBC	-0.06(0.50)	-0.11(0.17)$	-0.18**(0.04)	-0.15(0.08)	0.06(0.44)	-0.13(0.12)
Hemoglobin	-0.02(0.86)	0.01(0.89)$	-0.17(0.06)	-0.04(0.64)	0.07(0.34)	-0.01(0.87)
Hematocrit	-0.06(0.45)	-0.05(0.51)	-0.20(0.01)	-0.17(0.05)	0.04(0.66)	-0.08(0.40)
MCV	0.15(0.09)	0.16(0.06)	-0.04(0.67)	0.06(0.50)	0.00(0.99)	0.17(0.06)
MCH	0.12(0.19)	0.13(0.14)	0.01(0.85)	0.13(0.15)	0.05(0.60)	0.19**(0.03)
MCHC	-0.04(0.59)	0.10(0.25)$	0.03(0.70)	0.17(0.06)	0.05(0.51)	0.06(0.46)
RDW	0.12(0.15)	0.09(0.29)	0.260**(0.003)	0.02(0.85)	0.17(0.06)	0.15(0.08)
Platelet	-0.19**(0.02)	-0.20**(0.01)$	0.008(0.930)	-0.014(0.869)	-0.02(0.78)	-0.08(0.33)
MPV	0.19**(0.02)	0.04(0.64)$	0.028(0.746)	0.019(0.831)	0.14(0.10)	0.16(0.07)

Note: ** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); $ P-value derived from Pearson's correlation coefficient; rho is spearman's correlation coefficient; p is p-value; p-value <0.05 is considered as statistically significant.

Abbreviations: DM, diabetes mellitus; BMI, body mass index; WHR, waist to hip ratio; FBG, fasting blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell; RBC, red blood cell; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red blood cell distribution width; MPV, mean platelet volume.

CHAPTER SIX: DISCUSSION

Recently, it has been recognized that hematological change is a common complication of DM and represents a significant and under-recognized burden in these patients (20). In the present study, there was a statistically significant increment in total WBC count, absolute count of neutrophil, lymphocyte, eosinophil, and basophil, platelet count, Hgb, RDW and MPV) of T2DM patients compared to control. The mean RBC count was lower in T2DM patients as compared to the control group, but the difference was not statistically significant. This finding is in coherence with the report of studies conducted in India (33), Libya (37), Sudan (36), and Addis Ababa, Ethiopia (41). The possible explanation for decreased RBCs count might be that persistent hyperglycemia causes increased production of ROS and nonenzymatic glycosylation of Hgb and RBC membrane proteins leading to reduced deformability, increased aggregation, and accelerated aging of RBCs (6,51,52). These changes in RBCs are also shown to markedly increase blood viscosity that adversely affects the microcirculation in diabetes, leading to microangiopathy (51). In contrary to our findings, studies carried out in Pakistan (34) and Gondar, northwest Ethiopia (42) reported higher RBC count and Hgb concentration in T2DM patients than controls. This might be explained by the effect of insulin resistance, which is associated with the stimulation of erythroid progenitors increasing RBC count, and increased levels of Hgb and HCT (53).

Regarding RDW, the present study revealed that RDW values were significantly higher in T2DM patients than control groups. This finding is in harmony with the findings of previous studies in Pakistan (34), Saudi Arabia (35), Addis Ababa, Ethiopia (41), and Gondar, northwest Ethiopia (42). Higher RDW indicates the presence of heterogeneity among the circulating RBCs, which is related to the impairment of erythropoiesis and degradation of RBCs (54). Chronic inflammation and increased level of oxidative stress are common in diabetes and they are known to reduce RBCs' survival that results in variation in RBCs size and decreased RBCs count (55).

In the current study, we observed that the Hgb as an index of anemia was significantly lower among T2DM patients as compared to controls. This finding is supported by previous studies carried out in Bangladesh (32), India (33), Libya (37), and Nigeria (38) that have been reported significantly lower Hgb in T2DM patients than the control group. The prevalence of anemia was 17.9% (95% CI: 11.5-24.5), which is comparable with studies conducted in Saudi Arabia 22% (5),

Australia 17.8% (22), and Sudan 18.3% (36). In contrast, our prevalence estimate was lower than those reported by studies conducted in India 71.4% (33), Nigeria 45.2% (38), and Dessie, northeast Ethiopia 26% (40). This discrepancy might be due to a difference in the characteristics of the study population and sample size variation.

According to the WHO classification of anemia public health significance in populations (46), the current study showed a mild prevalence of anemia and a public health problem among T2DM. Anemia in diabetic patients has been attributed to reduced responsiveness of erythroid progenitors to erythropoietin (12). Other factors could contribute to depressed RBC production and accelerated destruction in diabetes including decreased renal function, nutritional deficiency, low testosterone, chronic inflammation, oxidative stress, advanced glycation, and antidiabetic drugs (12,52). Whatever the cause, the consequences of anemia complicating diabetes appear adverse, including evidence of increased all-cause and cardiovascular mortality (12,22)

On logistic regression analysis, the duration of DM was significantly associated with anemia similar to the studies from Nigeria (38). Patients who had DM for more than 7 years were 3 times more likely to have anemia compared to those who had DM for 7 years or less. Evidence suggested that the longer the duration of the disease the higher the inflammatory process, resulting in increased IL-6 with anti-erythropoietic effect, causing a decrease in the number of circulating RBCs and consequently causing a reduction of circulating hemoglobin (12). Milk consumption (AOR = 4.60, 95 % CI = 1.50-14.0) was significantly associated with anemia. Milk consumption might bring this effect by altering the absorption of iron due to its high calcium and casein contents as well as the low content of iron and folate in milk.

Regarding WBC indices, the present study demonstrated that total WBC count, absolute neutrophil count, and absolute lymphocyte count were significantly higher in the T2DM group compared to the control group. This is in corroboration with the report of previous studies conducted in Turkey (31), Bangladesh (32), Libya (37), and Gondar, northwest Ethiopia (42). Absolute eosinophil and basophil count also showed a significant increase in the diabetic group than the control group in consonance with the study conducted in Saudi Arabia (35) and Bangladesh (32). The pattern of WBC disturbances in T2DM patients are not widely available in the literature; in the current study 3.7% had leukocytosis, 1.5% had neutrophilia, 4.5%) had eosinophilia, and 5.2% had basophilia.

The mechanism underlying this increase of the total and differential WBC counts in T2DM patients might be explained by the effect of hyperglycemia and the pathogenesis of T2DM. The available biological data have strongly suggested that T2DM is an inflammatory disease (26). Increased WBC count is a classical marker of inflammation and evidence from epidemiological studies suggests an association between WBC count and diabetes risk (11). Although defects in insulin action on the peripheral tissues lead to a chronic low-grade inflammatory state and induce the secretion of proinflammatory cytokines, which promote differentiation and maturation of leukocytes (11,56). Additionally, in hyperglycemic state leukocytes are activated by AGEs, oxidative stress, and cytokines that increase the state of inflammations and the development of vascular complications in diabetes (8,52). Neutrophils and monocyte also are suggested to be a marker of inflammation, which is associated with the progression of complications (31,52).

In the present study, the differential white cell counts showed that majority of 127 (94.8%) of T2DM patients had a normal neutrophil count and 5 (3.7%) had neutropenia. Diabetic neutrophils have been associated with impaired deformability, chemotaxis, phagocytosis, bactericidal activity, and they also die sooner than normal (27), which might also explain the neutropenia in the present study. Regarding absolute monocyte count, the present study showed that there was no significant difference between the diabetic and control group. This is in agreement with the study conducted in Ethiopia (41,42) and contrary to a study conducted in Turkey (31) and Bangladesh (32). Additionally, 5 (3.7%) of T2DM patients had monocytopenia. The reason for this might be that several stimuli, including pro-inflammatory as well as metabolic stimuli, increase the recruitment of monocytes to peripheral tissues, where they differentiate to macrophages and dendritic cells. The destination of monocytes is therefore not the bloodstream and hence peripheral enumeration is not representative of monocyte tissue presence (26).

In this study, analysis of the platelet indices demonstrated that MPV and platelet counts were significantly higher in T2DM compared to the controls. Similar to this study, studies conducted in Nigeria (39) and Ethiopia (41) found that platelet counts were significantly higher in the diabetes group as compared to the controls. The increased MPV among T2DM patients in this study is also in agreement with several studies (34,35,41,42). The reason might be that platelet counts and MPV are indicators of thrombotic potential and risk of vascular complications in diabetes. There might be a release of S100A8/A9 by neutrophils which triggers IL-6 production and thrombopoietin

synthesis from hepatocytes, leading to bone marrow stimulation to recruit greater numbers of reticulated platelets, which are associated with both atheroprogression and atherothrombosis (57).

The other reason might be due to differences in platelet function between diabetic and control individuals. Evidence suggests that platelets from patients with T2DM have increased reactivity and baseline activation compared to healthy controls (58). The function of platelet and its size are said to be related and large circulating platelets are reflected by higher MPV which is the marker of the average size, and platelet activity (59). Diabetic platelets are larger with denser granules and they are enzymatically and functionally hyperactive to produce more prothrombotic factors like thromboxane A2, platelet factor 4, serotonin, and P-selectin than smaller platelets and hence cause an increased tendency to thrombotic events (60). Platelet hyperactivity in diabetics patients is also attributed to a multitude of factors including insulin resistance, oxidative stress, endothelial dysfunction, inflammation, and hyperglycemia (7,58,61).

Additionally, the present study assessed the correlation of hematological parameters with several cardio-metabolic risk factors. In our study, RBC and RDW were correlated with the duration of diabetes. This relationship might be explained by the effect of chronic hyperglycemia in which lower RBC count and elevated RDW are frequently observed in diabetics with a long duration of disease and vascular complication (62). Lymphocyte count showed significant and weak positive correlation with SBP and, similar to the study conducted in Turkey (31) and Gondar, Ethiopia (42). Lymphocyte, and basophil count, and MCH significantly correlated with DBP.

The association between blood pressure and hematological parameters may be due to the development of endothelial dysfunction and hypertension in diabetes mellitus. Evidence suggested that hyperglycemia triggers damage to the vascular bed by several cellular mechanisms including accumulation of ROS and AGEs; creating an imbalance between vasodilators and vasoconstrictors (7). This condition can lead to increased vasoconstriction and vascular remodeling ultimately affecting the blood cells. In respect to the correlation of hematological parameters with body adiposity, platelet count was statistically correlated with BMI and WHR. Moreover, MPV achieved a statistically significant correlation with BMI. A similar study on adult Nigerian people with T2DM found that platelet count and MPV were statistically correlated with BMI. The observed relationship might be due to that obesity is associated with systemic inflammation that could play a role in platelet activation and the production of larger platelets (63).

CHAPTER SEVEN: STRENGTH AND LIMITATIONS OF THE STUDY

According to our extensive literature, there has been no comprehensive study assessing the hematological parameters in type 2 diabetes patients; which is addressed by the current study. Despite its strength, our study has some limitations. One limitation of this study is that we cannot determine a cause-effect relationship due to the cross-sectional nature of our study design. Also, morphological and coagulation profile study were not assessed. Another limitation is that it was a single Hospital-based study, thus the observed prevalence of anemia may not reflect its actual burden among patients with type 2 diabetes in the general community.

CHAPTER EIGHT: CONCLUSION AND RECOMMENDATIONS

8.1. Conclusion

In the present study, there was a statistically significant variation in the hematological parameters (total WBC count, absolute count of neutrophil, lymphocyte, eosinophil, and basophil, platelet count, Hgb, RDW and MPV) of T2DM patients compared to control. Thus, inflammation and tendency to coagulation and thrombosis can be detected with these easily accessible parameters. This study also highlights that anemia was a common hematological change among T2DM patients and it was a mild public health problem in our clinical practice. Of the examined patients, nearly one out of five diabetic patients were anemic. Longer duration of diabetes and milk consumption increased the likelihood of anemia. Platelet count and MPV were significantly correlated with anthropometric measurements. Red blood cells count and RDW were correlated with the duration of diabetes. Thus, the prediction of the hematological changes enables the clinician to establish an effective and early therapeutic intervention to prevent the occurrence of major complications.

8.2. Recommendations

To the clinicians

Regular screening of hematological parameters should be considered for proper management of type 2 diabetic patients. Close attention should also be given to the duration of diabetes and dietary practice with respect to hematological abnormality.

To the researchers

A longitudinal study should be conducted using a large sample size. Also, morphological and coagulation profile study should be considered. It might be very important to have other studies on newly diagnosed diabetic patients to provide rational care for the patient.

To the Policymakers

Guidelines for the management of diabetes should consider to incorporating screening of hematological parameters into the routine assessment of diabetic complications to initiate early detection and treatment of hematological changes and hence improve the overall care of patients with diabetes.

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ANNEXS

Annex I: Participant Information Sheet and Consent Form

English version of the participant information sheet and consent form

Participant information sheet for T2DM group

Dear participant:

Hematological changes have been reported in diabetes and they play a major role in diabetes-associated complications. In view of this issue, an MSc student from the graduating school of Jimma University is conducting a study to assess the hematological parameter of diabetic patients. This study has been approved by the Research and Ethical Review Committee/Institutional Review Board (IRB) of Jimma University, institute of health. The information sheet is prepared for explaining the aim of the research that you are asked to join as a research participant. Please read or listen when it is read for you about the general information of the study. If you have any questions regarding the study you can ask freely.

Title of the Research: Hematological parameters of type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia: A comparative cross-sectional study

Name of Principal Investigator: Mesay Arkew

Advisors: Dr. Tilahun Yemane (MD, MSc) and Mr. Girum Tesfaye (MSc, Ph.D. Candidate)

Name of the Organization: Jimma University, Institute of Health, Faculty of Health Sciences, School of Medical Laboratory Sciences.

Aim of the study: To assess the hematological parameter of type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia from May 01 to June 30, 2020, G.C.

Procedure: You are kindly requested to give the correct information about yourselves and the necessary measurements will be performed. Then 6ml of blood samples will be collected for the determination of blood glucose and hematological parameter.

Benefits: There is no direct benefit (financial incentives or other inducements). However, the cost for your medical examination will be covered and if the medical examination reveals any abnormalities that need immediate treatment, your doctor will be notified about your result. Most importantly, your participation is likely to help us to evaluate the possible change of hematological parameters in diabetes; you are indirectly benefiting other patients and the society in this respect.

Risk and discomfort: The risk of being participating in this study is very minimal, and will not cause more discomfort than as required you could go through for routine examination. Only taking a few minutes from your time and there will be minimum pain during blood sample collection.

Right of participants: You have the full right to withdraw from participating in the study at any time before and after consent without explaining the reason and not respond to some or all the questions. Your decision will not affect your right to get health service you are supposed to get otherwise.

Confidentiality: All of the responses in the study will be recorded anonymously. The data collected will be kept confidential, and no one except your physician and the principal investigator will have access to them. However, the results generated from the data will be published so that the scientific community, control programs, and other people can utilize them.

Contact Address

If you have any question, you can contact Mesay Arkew at any time using the following address: Mesay Arkew, Hematology and Immunohematology MSc student at Jimma University, Institute of Health, Faculty of Health Sciences, School of Medical Laboratory Sciences.

Tel: 09-26-67-87-40 Email: mesayarkew12@gmail.com Jimma, Ethiopia

Consent Form

I undersigned confirm that, as I give my consent to participate in the study, it is with a clear understanding of the objectives of the study entitled "Hematological parameters of type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia". I am also informed that all information contained within the questionnaire is to be kept confidential. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The necessary information about the research is explained to me in the appropriate language I understand. Therefore, I would like to give you my agreement and take part in the study by giving the following declaration: I have been given detailed information about this study, I had the opportunity to discuss any question with the researcher, I understood that my participation in the study is voluntary and I agree to take part in this study. I _____hereby give my consent forgiving of the requested information and specimen for this study.

Participant code _____Participant signature _____date_____

Name of the data collector and signature _____ date_____

Name of the Researcher and signature _____ date_____

Amharic Version of Participant Information Sheet and Consent form

ለጥናቱ ተሳታፊዎች የሚሰጥ መረጃ ቅፅ ለሰኳር ታካሚዎች

ውድ የጥናቱ ተሳታፊ

ይህ የማብራሪያ ቅፅ አሁን እርስዎ እንዲሳተፉ የምንጠይቀዎትን ምርምር ጥናት የሚያብራራ ነው፡፡ በዚህ ጥናት ለመሰሳተፍ ከመወሰንዎ በፊት ይህንን ቅፅ መረጃ ሰብሳቢዎቹ በሚያነቡበት ጊዜ በጥሞና በማድመጥ ጥያቄ ካለዎት በመጠየቅ ትክክለኛዉን መልስ ይመልሱ፡፡ በዚህ ጥናት መሳተፍ ከጀመሩ በኃላ በማንኛውም ጊዜ ጥያቄ ካለዎት መጠየቅ ይችላሉ፡፡

የጥናቱ ርዕስ: የደም ሴሎች (ህዋስ) ልኬት (hematological parameter) የስኳር ህመም ባለባቸዉ እና የስኳር ህመም በሌለባቸዉ ሰወች ላይ በደብረ ብርሀን ሪፈራል ሆስፒታል ፣ ሰሜን-ምስራቅ ኢትዮጵያ፡፡

የጥናቱ ባለቤት፡ መሳይ አርቀዉ

አማካሪ፡ ዶ/ር ጥላሁን የማነ (MD, MSc) እና አቶ ግሩም ተስፋየ (MSc, PhD Candidate)

የተቋሙ ስም፡- ጅማ ዩኒቨርሲቲ፣ ጤና ኢንስቲትዩት ፣ ህክምና ላቦራቶሪ ትምህርት ቤት

የጥናቱ ዓላማ፡ የደም ሴሎች (ህዋስ) ልኬትን የስኳር ህመም ባለባቸዉ እና የስኳር ህመም በሌለባቸዉ ሰወች ላይ በደብረ ብርሀን ሪፈራል ሆስፒታል፣ ማጥናት ነዉ፡፡

የጥናቱ ሂደት እና የጥናቱ ተሳታፊ ድርሻ፡ በዚህ ጥናት ለመሳተፍ ፍቃደኛ ከሆኑ ከጤናዎ ሁኔታ ጋር የተያያዙና ሌሎች የግል መረጃዎችን እንዲሰጡ ይጠየቃሉ፡፡ በመቀጠልም የሰዉነት ክብደተዎን እና የደም ግፊተዎን እንዲለኩና 6 ሚሊ መጠን ያለው የደም ናሙና በመውሰድ በደምዎ ዉስጥ ያለዉን የስኳር መጠንና አጠቃላይ የደም ህዋስ ቆጠራ ይደረግልዎታል፡፡

በጥናቱ የመሳተፍ ጥቅም፡ ከጥናቱ የሚያገኙት ምንም ዓይነት የገንዘብ ጥቅም ባይኖረውም እርስዎ በዚህ ጥናት ላይ በመሳተፍዎና ከጥናቱ የሚገኘው ውጤት ከስኳር ህመም ጋር ተያይዘዉ ለሚከሰቱ ችግሮች በዋናነት የደም ሴሎች ልኬት ችግር ተጋላጭነትን ለመቀነስ ይጠቅማል፡፡

ከጥናቱ ጋር የተያያዘ ጉዳት/አለመመቸት፡ እርስዎ በዚህ ጥናት ውስጥ በመሳተፈዎ ለከፋ ጉዳት የሚጋለጡበት ሁኔታ አይኖርም፡፡ ምርመራው እርስዎ ላይ ምንም ችግር እንደማይፈጥርም እናረገግጣለን፡፡ ለዚህ ጥናት የሚያገለግል ናሙና የሚወሰድ ሲሆን ከመጠነኛ ስሜት በስተቀር በጤናዎ ላይ ምንም ጉዳት አይደርስም፡፡

የጥናቱ ተሳታፊዎች መብት፡ በጥናቱ ላይ ለመሳተፍ ባይስማሙ ምንም አይነት ቅጣት የማያስከትል ሲሆን ማንኛውም እርስዎ ሊያገኙ የሚገባውን ህክምናና ተያያዥ መብት የማያሳጣ መሆኑን እናረጋግጣለን፡፡

የጥናቱ መረጃዎች ምስጥራዊነት፡ እርስዎን በተመለከተ የምንናገኘውን መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኃላ ባሉት ጊዜያት እንዲሁም ከጥናቱ የተገኘው መረጃ ሚስጥራዊነት የሚጠበቅ ሲሆን መረጃዎቹም የሚያዙት በስም ሳይሆን በልዩ ምስጥራዊ ቁጥር ነው፡፡ ይኸው መረጃ በጥንቃቄ የሚያዝና የተፈቀደለት ተመራማሪ እና ለህክምና ባለሙያዉ ብቻ ይህም እጅግ አስፈላጊ በሆነ ጊዜ ብቻ ካልሆነ በስተቀር ለሌላ ለማንም ሰው አይሰጥም፡፡ ማንኛውም ከርስዎ ጋር የተያያዘ ውጤት በልዩ ኮድ ብቻ የሚያዝ ሲሆን ውጤቱም ለሳይንሳዊ ዓላማ ብቻ ስም በማይገልፅ ሁኔታ እንዲታተም ይደረጋል፡፡

ስለጥናቱ መረጃ ማግኘት ቢፈልጉ፡ ጥናቱን በተመለከተ ግልጽ ያልሆነ ማንኛዉንም ጥያቄ ካለዎት ነፃ ሆነው ከዚህ በታች ባለው አድራሻ መጠየቅ ይችላሉ፡፡

መሳይ አርቀዉ፣ በጅማ ዩኒቨርሲቲ፣ ጤና ኢንስቲትዩት፣ ህክምና ላቦራቶሪ ሳይንስ ትምህርት ቤት በሄማቶሎጅና ኢሚኖሄማቶሎጅ ትምህርት አይነት የሁለተኛ ዲግሪ ተማሪ፡፡
ስልክ: 09-26-67-87-40 ኢ-ሜይል: mesayarkew12@gmail.com ጅማ, ኢትዮጵያ

የስምምነት መጠየቂያ ቅጽ ለሰኳር ታካሚዎች

በተሰጠው መረጃ መሰረት የጥናቱን ዓላማና ጥቅም በግልጽና በሚገባኝ ቋንቋ ተረድቻለሁ፡፡ በማንኛውም ጊዜ ከጥናቱ ያለምንም ችግርና መንገላታት መውጣት እንደምንችልም ተገልፆልኛል፡፡ ከዚህ በተጨማሪም በጥናቱ በመሳተፌ የሚከፈለኝ ክፍያ እንደሌለ አውቂያለሁ። በዚህ መሠረት የጥናት ቡድኑ አባላት ተፅዕኖ ሳያሳድሩብኝ በእኔው ሙሉ ፈቃደኝነት በዚህ ጥናት ውስጥ በመሣተፍ የሚጠበቅብኝን አስተዋፅዎ ለማበርከት በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊው/ዋ የሚስጥር ቁጥር _____ የተሳታፊው/ዋ ፊርማ _____ ቀን _____

የመረጃ ሰብሳቢው ስምና ፊርማ _____ ቀን_____

የተመራማሪው ስምና ፊርማ _____ ቀን_____

Participant information sheet for the control group

Dear participant:

Hematological changes have been reported in diabetes and they play a major role in diabetes-associated complications. In the view of this issue, MSc student from graduating school of Jimma University is conducting a study to assess the hematological parameter of diabetic patients by comparing with blood donors. This study has been approved by the Research and Ethical Review Committee/Institutional Review Board (IRB) of Jimma University, institute of health. The information sheet is prepared for explaining the aim of the research that you are asked to join as a research participant. Please read or listen when it is read for you about the general information of the study. If you have any questions regarding the study you can ask freely.

Title of the Research: Hematological parameters of type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia: A comparative cross-sectional study

Name of Principal Investigator: Mesay Arkew

Advisors: Dr. Tilahun Yemane (MD, MSc) and Mr. Girum Tesfaye (MSc, Ph.D. Candidate)

Name of the Organization: Jimma University, Institute of Health, Faculty of Health Sciences, School of Medical Laboratory Sciences.

Aim of the study: To assess the hematological parameter of type 2 diabetic adult patients in comparison with screened blood donors at Debre Berhan Referral Hospital, Northeast Ethiopia from May 01 to June 30, 2020, G.C.

Procedure: You are kindly requested to give the correct information about yourselves and the necessary measurements will be performed. Then 4ml of blood samples will be collected from the blood you have donated for determination of hematological parameter.

Benefits: There is no direct benefit (financial incentives or other inducements). However, the cost for the laboratory test will be covered and if your result reveals any abnormalities you will be notified about your result. Most importantly, your participation is likely to help us to evaluate the possible change of hematological parameters in diabetes by comparing with your result; you are indirectly benefiting other patients and the society in this respect.

Risk and discomfort: The risk of being participating in this study is very minimal, and will not cause more discomfort. Only taking a few minutes from your time.

Right of participants: You have the full right to withdraw from participating in the study at any time before and after consent without explaining the reason and not respond to some or all the questions.

Confidentiality: All of the responses in the study will be recorded anonymously. The data collected will be kept confidential, and no one except the principal investigator will have access to them. However, the results generated from the data will be published so that the scientific community, control programs, and other people can utilize them.

Contact Address

If you have any question, you can contact Mesay Arkew at any time using the following address: Mesay Arkew, Hematology and Immunohematology MSc student at Jimma University, Institute of Health, Faculty of Health Sciences, School of Medical Laboratory Sciences.

Tel: 09-26-67-87-40 Email: mesayarkew12@gmail.com Jimma, Ethiopia

Consent Form

I undersigned confirm that, as I give my consent to participate in the study, it is with a clear understanding of the objectives of the study entitled "Hematological parameters of type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia". I am also informed that all information is to be kept confidential. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The necessary information about the research is explained to me in the appropriate language I understand. Therefore, I would like to give you my agreement and take part in the study by giving the following declaration: I have been given detailed information about this study, I had the opportunity to discuss any question with the researcher, I understood that my participation in the study is voluntary and I agree to take part in this study. I _____hereby give my consent forgiving of the requested information and specimen for this study.

Participant code _____Participant signature _____date_____

Name of the data collector and signature _____ date_____

Name of the Researcher and signature _____ date_____

Amharic Version of Participant Information Sheet and Consent form

ለጥናቱ ተሳታፊዎች የሚሰጥ መረጃ ቅፅ (የስኳር ህመም ለሌለባቸዉ)

ውድ የጥናቱ ተሳታፊ

ይህ የማብራሪያ ቅፅ አሁን እርስዎ እንዲሳተፉ የምንጠይቀዎትን ምርምር ጥናት የሚያብራራ ነው፡፡ በዚህ ጥናት ለመሰሳተፍ ከመወሰንዎ በፊት ይህንን ቅፅ መረጃ ሰብሳቢዎቹ በሚያነቡበት ጊዜ በጥሞና በማድመጥ ጥያቄ ካለዎት በመጠየቅ ትክክለኛዉን መልስ ይመልሱ፡፡ በዚህ ጥናት መሳተፍ ከጀመሩ በኃላ በማንኛውም ጊዜ ጥያቄ ካለዎት መጠየቅ ይችላሉ፡፡

የጥናቱ ርዕስ: የደም ሴሎች (ህዋስ) ልኬት (hematological parameter) የስኳር ህመም ባለባቸዉ እና የስኳር ህመም በሌለባቸዉ ሰወች ላይ በደብረ ብርሀን ሪፈራል ሆስፒታል ፣ ሰሜን-ምስራቅ ኢትዮጵያ፡፡

የጥናቱ ባለቤት፡ መሳይ አርቀዉ

አማካሪ፡ ዶ/ር ጥላሁን የማነ (MD, MSc) እና አቶ ግሩም ተስፋየ (MSc, PhD Candidate)

የተቋሙ ስም፡- ጅማ ዩኒቨርሲቲ፣ ጤና ኢንስቲትዩት ፣ ህክምና ላቦራቶሪ ትምህርት ቤት

የጥናቱ ዓላማ፡ የደም ሴሎች (ህዋስ) ልኬትን የስኳር ህመም ባለባቸዉ እና የስኳር ህመም በሌለባቸዉ ሰወች ላይ በደብረ ብርሀን ሪፈራል ሆስፒታል፣ ማጥናት ነዉ፡፡

የጥናቱ ሂደት እና የጥናቱ ተሳታፊ ድርሻ፡ በዚህ ጥናት ለመሳተፍ ፍቃደኛ ከሆኑ ከእርስዎ ጋር የተያያዙና የግል መረጃዎችን እንዲሰጡ ይጠየቃሉ፡፡ በመቀጠልም የሰዉነት ክብደተዎን እና የደም ግፊተዎን እንዲለኩና 4 ሚሊ መጠን ያለው የደም ናሙና ከእርስዎ ከለገሱት ደም ላይ በመውሰድ አጠቃላይ የደም ህዋስ ቆጠራ ይደረግልዎታል፡፡

በጥናቱ የመሳተፍ ጥቅም፡ ከጥናቱ የሚያገኙት ምንም ዓይነት የገንዘብ ጥቅም ባይኖረውም እርስዎ በዚህ ጥናት ላይ በመሳተፍዎና ከጥናቱ የሚገኘውን እርስዎን ውጤት ከስኳር ህመምተኞች የደም ሴሎች ልኬት ጋር በማነፃፀር የስኳር ህመምተኞችን የደም ሴሎች ልኬት ለዉጥ ለማወቅ ይጠቅማል፡፡

ከጥናቱ ጋር የተያያዘ ጉዳት/አለመመቸት፡ እርስዎ በዚህ ጥናት ውስጥ በመሳተፈዎ ለከፋ ጉዳት የሚጋለጡበት ሁኔታ አይኖርም፡፡ ከራስዎ ሰዓት ላይ የተወሰኑ ደቂቃወችን ከመዉሰድ ዉጪ ምርመራው እርስዎ ላይ ምንም ችግር እንደማይፈጥርም እናረገግጣለን፡፡

የጥናቱ ተሳታፊዎች መብት፡ በጥናቱ ላይ ለመሳተፍ ባይስማሙ ምንም አይነት ቅጣት የማያስከትል ሲሆን ለመሳተፍ ከተሰማሙ በኃላም በማንኛዉም ሰዓት ከጥናቱ እራስዎን ማግለል እንደሚችሉ እናረጋግጣለን፡፡

የጥናቱ መረጃዎች ምስጥራዊነት፡ እርስዎን በተመለከተ የምንናገኘውን መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኃላ ባሉት ጊዜያት እንዲሁም ከጥናቱ የተገኘው መረጃ ሚስጥራዊነት የሚጠበቅ ሲሆን መረጃዎቹም የሚያዙት በስም ሳይሆን በልዩ ምስጥራዊ ቁጥር ነው፡፡ ይኸው መረጃ በጥንቃቄ የሚያዝና የተፈቀደለት ተመራማሪ ብቻ ካልሆነ በስተቀር ለሌላ ለማንም ሰው አይሰጥም፡፡ ማንኛውም ከርስዎ ጋር የተያያዘ ውጤት በልዩ ኮድ ብቻ የሚያዝ ሲሆን ውጤቱም ለሳይንሳዊ ዓላማ ብቻ ስም በማይገልፅ ሁኔታ እንዲታተም ይደረጋል፡፡

ስለጥናቱ መረጃ ማግኘት ቢፈልጉ፡ ጥናቱን በተመለከተ ግልጽ ያልሆነ ማንኛዉንም ጥያቄ ካለዎት ነፃ ሆነው ከዚህ በታች ባለው አድራሻ መጠየቅ ይችላሉ፡፡

መሳይ አርቀዉ፣ በጅማ ዩኒቨርሲቲ፣ ጤና ኢንስቲትዩት፣ ህክምና ላቦራቶሪ ሳይንስ ትምህርት ቤት በሄማቶሎጅና ኢሚኖሄማቶሎጅ ትምህርት አይነት የሁለተኛ ዲግሪ ተማሪ፡፡

ስልክ: 09-26-67-87-40 ኢ-ሜይል: mesayarkew12@gmail.com ጅማ, ኢትዮጵያ

የስምምነት መጠየቂያ ቅጽ (የስኳር ህመም ለሌለባቸዉ)

በተሰጠው መረጃ መሰረት የጥናቱን ዓላማና ጥቅም በግልጽና በሚገባኝ ቋንቋ ተረድቻለሁ፡፡ በማንኛውም ጊዜ ከጥናቱ ያለምንም ችግርና መንገላታት መውጣት እንደምንችልም ተገልፆልኛል፡፡ ከዚህ በተጨማሪም በጥናቱ በመሳተፌ የሚከፈለኝ ክፍያ እንደሌለ አውቂያለሁ። በዚህ መሠረት የጥናት ቡድኑ አባላት ተፅዕኖ ሳያሳድሩብኝ በእኔው ሙሉ ፈቃደኝነት በዚህ ጥናት ውስጥ በመሣተፍ የሚጠበቅብኝን አስተዋፅዎ ለማበርከት በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊው/ዋ የሚስጥር ቁጥር _____ የተሳታፊው/ዋ ፊርማ _____ ቀን _____

የመረጃ ሰብሳቢው ስምና ፊርማ _____ ቀን_____

የተመራማሪው ስምና ፊርማ _____ ቀን_____

Annex II: Questionnaire

English version Questionnaire for T2DM group

Data collection questionnaire designed to conduct a study on hematological parameters of type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia

Instructions: This questionnaire contains a question, which is pertinent to the research objectives. You are kindly requested to answer all the questions as much as possible.

Does the patient fulfill all inclusion criteria and no exclusion criteria? 1. No 2. Yes

Identification code_____

S. No	Questions	Possible answers
	Part I. Sociodemographic variables	
101.	Age (in years)	_____
102.	Gender	1. Male 2. Female
103.	Educational level	1. Unable to read & write
		2. Can read and write
		3. Elementary school
		4. High school & above
104.	Residency	1. Urban 2. Rural
105.	Occupation	1. Government employee 2. Private employee 3. Private worker 4. Other
106.	Average monthly income (in birr)	_____
	Part II. Anthropometric and BP measurements	
201.	Weight	_____kg
202.	Height	_____m
203.	BMI	_____ kg / $(m)^2$

204.	Waist Circumference (WC)	_____cm	
205.	Hip Circumference (HC)	_____cm	
206.	WHR	_____	
207.	Blood pressure (BP)	Systolic_____mmHg Diastolic_____ mmHg	
	Part III: Behavioral variables		
301.	Do you perform physical exercise	1. No 2. Yes	
302.	Do you consume green leafy vegetables?	1. No 2. Yes	If 1, skip to Q304
303.	If yes for Q302. How many times per week?	1. One time 2. Two-three times 3. Above 3 times	
304.	Do you consume eggs?	1. No 2. Yes	If 1, skip to Q306
305.	If yes for Q304. How many times per week?	1. One time 2. Two-three times 3. Above 3 times	
306.	Do you consume red meat	1. No 2. Yes	If 1, skip to Q308
307.	If yes for Q306. How many times per week?	1. One time 2. Two-three times 3. Above 3 times	
308.	Do you consume milk products?	1. No 2. Yes	If 1, skip to 310
309.	If yes for Q308. How many times per week?	1. One time 2. Two-three times 3. Above 3 times	
310.	Do you have a habit of drinking coffee or tea after a meal?	1. No 2. Yes	
Data collector name _____ Date _____Signature_____			

Amharic Version Questionnaire for T2DM group

የደም ሴሎች (ህዋስ) ልኬት የስኳር ህመም ባለባቸዉ እና የስኳር ህመም በሌለባቸዉ ሰወች ላይ በደብረ ብርሀን ሪፈራል ሆስፒታል ለማጥናት የተዘጋጀ መረጃ መሰብሰቢያ መጠይቅ፡፡

መመሪያ፡ ይህ መጠይቅ በውሰጡ ከጥናቱ ዓላማ ጋር የተያያዙ ጥያቄዎችን ይዟል እርሰዎም ትክክለኛዉን መልስ እንድሰጡን በትህትና እነጠይቅዎታልን በተቻለዎ መጠን ተገቢ የሆኑትን መልሶችን ይናገሩ፡፡

የጥናቱ ተሳታፊ ሁሉንም የማካተቻ መስፈርቶች አሟላተዋል? 1. አላሟሉም? 2. አዎ ያሟላሉ

የተሳታፊ መለያ ቁጥር_____

ተ. ቁ	መጠይቆች		አማራጮች
	ክፍል 1፡ የማህበራዊና ስነ-ህዝብ ባህሪያት መረጃ		
101.	እድሜ		_____
102.	ጾታ		1. ወንድ 2. ሴት
103.	የትምህርት ደረጃ		1. ማንበብና መጻፍ የማይችሉ
			2. ማንበብና መጻፍ የሚችሉ
			3. አንደኛ ደረጃ
			4. ሁለተኛ ደረጃና ከዚያ በላይ
104.	የሚኖሩበት አካባቢ		ነ. ከተማ 2. ገጠር
105.	የስራ አይነት		1. የመንግስት መስሪያ ቤት ሰራተኛ 2. የግል መስሪያ ቤት ሰራተኛ 3. የግል ስራ 4. ሌላ ካለ ይገለፅ----------
106.	የወር ገቢ		_____ብር
	ክፍል 2፡ የሰዉነት አቋምና የደም ግፊት ልኬት		
201.	ክብደት		_____ኪግ
202.	ቁመት		_____ሜ
203.	የሰዉነት አቋም ልኬት		_____ ኪግ / (ሜ)^2
204.	የወገብ ዙሪያ ልኬት		_____ሴሜ
205.	የዳሌ ዙሪያ ልኬት		_____ሴሜ
206.	የወገብ ልኬት ለዳሌ ልኬት ማመዛዘን		_____
207.	የደም ግፊት ልኬት		ሲስቶሊክ _____mmHg ዳያስቶሊክ_____ mmHg
	ክፍል 3፡ የአካል ብቃት እቅስቃሴና የአመጋገብ ሁኔታ ጋር የተያያዙ መረጃዎች		
301.	የአካል ብቃት እቅስቃሴ ይሰራሉ	ነ. አይሰሩም 2. አዎ	
302.	አረነጓደ ቅጠላቅጠሎችን ይመገባሉ	1. አልመገብም 2. አዎ እመገባለሁ	መልስዎ 1 ከሆነ ወደ ጥያቄ 304 ይዝለሉ
303.	መልስዎ አዎ ከሆነ በሳምንት ስንት ጊዜ ይመገባሉ	ነ. 1 ጊዜ 2. 2-3 ጊዜ 3. ከ 3 ጊዜ በላይ	

304.	እንቁላል ይመገባሉ	1. አልመገብም 2. አዎ እመገባለሁ	መልስዎ 1 ከሆነ ወደ ጥያቄ 306 ይዝለሉ
305.	መልስዎ አዎ ከሆነ በሳምንት ስንት ጊዜ ይመገባሉ	1. 1 ጊዜ 2. 2-3 ጊዜ 3. ከ 3 ጊዜ በላይ	
306.	ስጋ ይመገባሉ	1. አልመገብም 2. አዎ እመገባለሁ	መልስዎ 1 ከሆነ ወደ ጥያቄ 308 ይዝለሉ
307.	መልስዎ አዎ ከሆነ በሳምንት ስንት ጊዜ ይመገባሉ	1. 1 ጊዜ 2. 2-3 ጊዜ 3. ከ3 ጊዜ በላይ	
308.	ወተትና የወተት ተዋፅዖዎችን ይጠቀማሉ	ነ. አልጠቀምም 2. አዎ	መልስዎ 1 ከሆነ ወደ ጥያቄ 310 ይዝለሉ
309.	መልስዎ አዎ ከሆነ በሳምንት ስንት ጊዜ ይመገባሉ	ነ. 1 ጊዜ 2. 2-3 ጊዜ 3. ከ 3 ጊዜ በላይ	
310.	ከምግብ በኋላ ሻይ ወይም ቡና ይጠጣሉ	1. አልጠጣም 2. አዎ	
የመረጃ ሰብሳቢዉ ስምና ፊርማ_____ ቀን _____			

English version questionnaire for the control group (blood donors)

Does the participant fulfill all donors' criteria? 1. No 2. Yes

Identification code_____

S. No	**Questions**	**Possible answers**
	Part I. Sociodemographic variables	
101.	Age (in years)	_____
102.	Gender	1. Male 2. Female
103.	Educational level	1. Unable to read & write
		2. Can read and write
		3. Elementary school
		4. High school & above
104.	Residency	1. Urban 2. Rural
105.	Occupation	1. Government employee 2. Private employee 3. Private worker 4. Other
106.	Average monthly income (in birr)	_____
	Part II. Anthropometric and BP measurements	
201.	Weight	_____kg
202.	Height	_____m
203.	BMI	_____ kg / $(m)^2$
204.	Waist Circumference (WC)	_____cm
205.	Hip Circumference (HC)	_____cm
206.	WHR	_____
207.	Blood pressure (BP)	Systolic_____mmHg Diastolic_____ mmHg
Data collector name _____ Date _____Signature_____		

Amharic Version Questionnaire for the control group

የጥናቱ ተሳታፊ ሁሉንም የደም ለጋሾች ማካተቻ መስፈርቶች አሟላተዋል? 1. አላሟሉም? 2. አዎ ያሟላሉ

የተሳታፊ መለያ ቁጥር_____

ተ. ቁ	መጠይቆች	አማራጮች
	ክፍል 1፡ የማህበራዊና ስነ-ህዝብ ባህሪያት መረጃ	
101.	እድሜ	_____
102.	ጾታ	ነ. ወንድ 2. ሴት
103.	የትምህርት ደረጃ	1. ማንበብና መጻፍ የማይችሉ
		2. ማንበብና መጻፍ የሚችሉ
		3. አንደኛ ደረጃ
		4. ሁለተኛ ደረጃና ከዚያ በላይ
104.	የሚኖሩበት አካባቢ	ነ. ከተማ 2. ገጠር
105.	የስራ አይነት	1. የመንግስት መስሪያ ቤት ሰራተኛ 2. የግል መስሪያ ቤት ሰራተኛ 3. የግል ስራ 4. ሌላ ካለ ይገለፅ----------
106.	የወር ገቢ	_____ብር
	ክፍል 2፡ የሰዉነት አቋምና የደም ግፊት ልኬት	
201.	ክብደት	_____ኪግ
202.	ቁመት	_____ሜ
203.	የሰዉነት አቋም ልኬት	_____ ኪግ / (ሜ)^2
204.	የወገብ ዙሪያ ልኬት	_____ሴሜ
205.	የዳሌ ዙሪያ ልኬት	_____ሴሜ
206.	የወገብ ልኬት ለዳሌ ልኬት ማመዛዘን	_____
207.	የደም ግፊት ልኬት	ሲስቶሊክ _____mmHg ዳያስቶሊክ_____ mmHg
የመረጃ ሰብሳቢዉ ስምና ፊርማ_____ ቀን _____		

Annex III: Checklist

Checklist prepared to collect clinical variables from the medical record of type 2 diabetic patients at Debre Berhan Referral Hospital northeast Ethiopia.

Identification code_____

S.no	Clinical variables	Possible options
101.	Oral hypoglycemic therapy used	1. Metformin 2. Sulfonylureas 3. Metformin+ Sulfonylureas
102.	Duration of oral hypoglycemic therapy used	_____
103.	Duration of DM since diagnosis (in years)	_____
104.	Fasting blood glucose level in the last 2 months	1. Month1_____mg/dl 2. Month2____mg/dl
Data collector name _____ Date _____Signature_____		

Amharic Version of Checklist

በደብረ ብርሀን ሪፈራል ሆስፒታል የስኳር ህክምና ከሚከታተሉ ሰወች መዝገብ ላይ ከህክምና ጋር የተያያዙ መረጃዎች ለመሰብሰብ የተዘጋጀ ማጣሪያ ዝርዝር ፡፡

የተሳታፊ መለያ ቁጥር_____

101.	የሚወስዱት የመድሀኒት አይነት	1. Metformin 2. Sulfonylureas 3. Metformin+Sulfonylureas
102.	መድሀኒት መዉሰድ ከጀመሩ ምን ያህል ጊዜ ሆነዉ	_____
103.	የስኳር ህመም እንዳለባቸው ከታወቀ ምን ያህል ጊዜ ሆነው	_____
104.	ባለፉት ሁለት ወራት የታካሚዉ/ዋ የስኳር መጠን	ነ. ወር 1_____ሚግ/ዲል 2. ወር 2_____ሚግ/ዲል
የመረጃ ሰብሳቢዉ ስምና ፊርማ_____ ቀን _____		

Annex IV: Anthropometric and Blood Pressure Measurement

Weight measurement: Before the study participants weight is measured, the weight scale was placed on a firm, flat surface, turn to zero and the participant removes any coats, heavy sweaters, shoes, keys, or heavy pocket contents. Again, the participant was asked to stand in the middle of the scale's platform with the body weight equally distributed on both feet. Weigh the participant in kilograms to the nearest 0.1 kg (100 grams) (64).

Height measurement: Height was measured using a height measure scale (Infiniti Med Lab Pvt. Ltd., India). Participants stand erect on the floorboard of the stadiometer with their back to the vertical backboard of the stadiometer. The heels of the foot are placed together with both heels touching the base of the vertical board. Foot pointed slightly outward at a 60-degree angle. The buttocks, scapulae, and head are positioned in contact with the vertical backboard. During the height measurement, the participant's shoes and hats were removed. Recorded the height measurement to the nearest 0.1 cm (64).

BMI: After measuring the participant's height and weight, the BMI was calculated by dividing weight in (kg) by height squared (m^2).

WC measurement: The WC was measured after the participant's shirt removed and at midway between the lower margin of the last palpable rib and the top of the iliac crest of the hip bone using a non-stretch, pliable tape measure to evaluate abdominal obesity. The measurement record is to the nearest 0.1 cm (64).

HC measurement: was taken around the widest point of buttocks while the participant wears only nonrestrictive underwear. The recording of the measurement is to the nearest 0.1 cm (64).

WHR: The WHR was calculated by dividing WC by HC.

Blood Pressure (BP) measurement: BP was measured by clinical nurses using an analog sphygmomanometer (Omron Health Care, Japan) after the participant was rested for at least 5 minutes. Two separate measurements were obtained on the upper arm of the seated participant using a cuff of an appropriate size and the average readings of SBP and DBP were taken as the BP of the participant. The 2^{nd} measurement was taken five minutes after the 1^{st} measurement.

Annex V: Procedure for Venous Blood Sample Collection

Blood Sample Collection from T2DM patients

Supplies and Equipment

1. Test requisition
2. Tourniquet and disposable gloves
3. Alcohol (70%) and gauze square or alcohol wipes
4. Sterile vacutainer tube with needles
5. EDTA and serum separator test tube

Method (procedure)

1. Assemble the necessary materials and equipment
2. Identify the patient and allow him/her to sit comfortably preferably in an armchair stretching his/ her arm.
3. Visually inspect both arms and select the arm that has not been repeatedly used for venipunctures and one that is free of bruises, abrasions, and sites of infection. In the arm, there are three veins and the median cubital is commonly used for venipuncture.
4. Applying a tourniquet at a point about 6-8cm above the bend of the elbow making a loop in such a way that a gentle tug on the protruding ends will release it
5. Prepare the arm by swabbing using a cotton ball saturated with 70% alcohol or an alcohol pad saturated with 70% alcohol, cleanse the skin in the area of the venipuncture site. Using a circular motion, clean the area from the center, and move outward. Do not go back over an area once it has been cleansed.
6. Allow the site to dry.
7. Grasp the back of the patient's arm at the elbow and anchor the selected vein by drawing the skin slightly taut over the vein.
8. Using the assembled vacutainer tube with a needle, enter the skin first and then the vein. The test tube is plugged into the back of the vacutainer tube and the blood starts to draw from the vein to the test tube.

9. After the desired amount of blood has been drawn (6ml), into EDTA tube (4ml) for hematological parameter and serum separator tube (2ml) for glucose analysis, apply cotton to the puncture site and gently withdraw the needle. Instruct the patient to press on the cotton until the bleeding has stopped. Do not let the patient go until the bleeding stops.
10. Discard the used needle into an appropriate safety container.
11. If possible, the patient elevates the entire arm and presses on the gauze pad with the opposite hand. If the patient is unable to do this, apply pressure until bleeding ceases. Note: Failure to apply sufficient pressure to the venipuncture site could result in a hematoma (a collection of blood under the skin that produces a bruise).
12. Mix blood with anticoagulant by inverting the tubes several times (5-8 times).
13. Label all test tubes as required by the laboratory.
14. Clean up supplies from the work area, remove gloves, and wash hands. Note: If the patient is an outpatient, wait a few minutes after the venipuncture is complete, and check to be sure that the patient does not feel dizzy or nauseated before discharge. Discard all contaminated supplies in a biohazard disposal bag.

Blood Sample Collection from the control group (blood donor)

Blood sample for the hematological test was collected in the following methods

1. The phlebotomist who works in the blood bank collected blood from the blood donors by following the strict procedures.
2. After the completion of the procedure, the EDTA tube was plugged into the tip (needle) of the pilot tube and the blood starts to draw from the pilot tube of blood bag to the EDTA tube.
3. After 4ml of blood has been drawn into the EDTA tube for the hematological test, the tops of the collecting pilot tube were sealed.
4. Then, mix blood with anticoagulant by inverting the tubes several times (5-8 times).
5. Label all test tubes with the donors' code.
6. A blood sample was placed in a specimen bag with ice and transported to the DBRH laboratory.
7. The hematological analysis was performed after the blood sample has been warmed at room temperature.

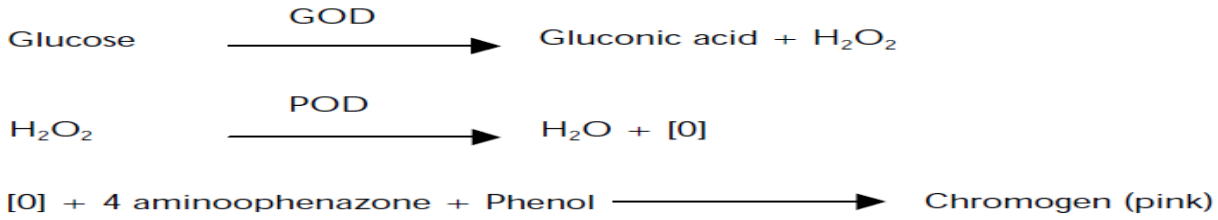
Annex VI: Biosystem A25 Chemistry Analyzer (SOP for Glucose Measurement)

General principle

Biosystem A25 Chemistry Analyzer is a random-access instrument that automatically determines the concentrations of the analytes based on optical absorbance measurements. It has been designed as a processing and reading unit, connected to an internal built computer where the application runs. To measure the concentration of a certain analyte in a sample, the analyzer uses a pipette to take a specific volume of the sample and the corresponding reagent, quickly thermostatises them in the needle itself and dispenses them into the reaction's rotor. The very dispensing speed together with the geometry of the reaction well causes the mixture to be shaken and the chemical reaction begins. In the bi-reagent modes, the reaction begins when the analyzer later dispenses the second reagent in the same reaction well. The reactions can be biochemical or turbidimetric. In both cases, the reaction or the chain of reactions produced generates substances that attenuate certain wavelengths, either by absorption or by dispersion. Comparing the light intensity of a certain wavelength that crosses a well when there is a reaction and when there is not a reaction can determine the concentration of the corresponding analyte. This comparison is quantified with the physical magnitude called absorbance.

Method for glucose determination: glucose oxidase method

Glucose is a reducing monosaccharide that serves as the principal fuel of all the tissues. It enters the cell through the influence of insulin and undergoes a series of chemical reactions to produce energy. Glucose Oxidase is a highly specific enzyme for glucose (beta D – glucose) and does not react with other blood saccharides. Glucose present in the blood sample is oxidized by the enzyme glucose oxidase (GOD) to gluconic acid with the liberation of hydrogen peroxide, which is converted to water and oxygen by the enzyme peroxidase (POD). 4 Aminophenazone, an oxygen acceptor, takes up the oxygen and together with phenol forms a pink-colored chromogen which can be measured at 515nm. The intensity of the color measured as absorbance is proportional to the amount of glucose present in the sample.



Specimen Requirements

- Serum sample that collected in serum separator tube
- The amount required is 0.3mL
- Serum glucose levels have been checked to be quite stable for 6 hours at room temperature (25 -35^{0}C) and 24 hours at storage temperature (2-8^{0}C).

Equipment

- Biosystem A25 clinical chemistry analyzer
- Centrifuge
- Micropipette (50-200 µl, 200-1000 µl)
- Refrigerator with two compartments (2-8^{0}C and -20^{0}C).

Reagents and supplies

- Biosystem or human reagent for glucose
- Control N
- Control P
- Calibrator
- Micropipette tips (200 µl, 1000 µl yellow and blue tips)
- Sample cup
- Reaction wells/cuvettes
- Reagent bottles

Glucose determination or analysis procedure

1. After proper collection of the sample, whole blood will be stored for 30 minutes and serum will be separated from the clotted blood by centrifugation for 3 minutes at 3,400 revolutions per minute.
2. Place the labeled test tube that contains a serum sample in to sample rack in the analyzer.
3. Mix the sample and the reagent in the Cuvvet automatically
4. After reagent mix with the sample, absorbance is measured

N.B A normal and abnormal quality control sample (assayed or commercially manufactured) should be analyzed with patient samples to detect analytical errors.

Annex VII: SOP for UniCel DxH 800 Hematology Analyzer

Purpose: The UniCel DxH 800 analyzer is a quantitative, automated hematology analyzer for in-vitro diagnostic use in screening patient populations in clinical laboratories. The UniCel DxH 800 analyzer provides the following: CBC, Leukocyte 5-Part Differential (Diff), Reticulocyte (Retic), and Nucleated Red Blood Cell (NRBC) on whole blood.

Principle: The UniCel DxH 800 CBC analysis is based on electrical impedance counting, absorption spectrophotometry methods, and VCSn technology. Electrical impedance is used to count and size WBCs, RBCs, and platelet. This method counts and sizes cells by detecting and measuring changes in electrical resistance (electrical current) when a cell suspended in a conductive liquid pass through a small aperture. The change produces a measurable electrical pulse and the number of pulses is proportional to the volume and size of the cell that produced it. The system counts the individual cells and provides cell size distribution. The size of the blood cell is detected as electric pulses and the number of blood cells is calculated by counting the pulses. Each pulse is amplified and compared to internal reference voltage channels. These channels are delineated by calibrated size discriminators to accept only pulses of certain amplitude.

Absorption Spectrophotometry is the method used to measure Hgb. Methemoglobin chromogen is formed and measured when sample is mixed with a cyanide-free lytic reagent. An LED light source and photodetector are used to detect the chromogen at 525 nm. The Hgb concentration is directly proportional to the light absorption of the sample. Initial blank reading is made on reagents only, and then a comparison of the blank and sample readings determines the Hgb concentration.

The VCSn module in DxH 800 system uses the Multi-Transducer Module (MTM), to all Diff, NRBC, and Retic analysis by measuring additional multiple angles of light scatter, a major improvement over the single light scatter measured by conventional flow cytometry. The VCSn module is responsible for controlled sample preparation and delivery of the prepared sample to the flow cell for analysis of the WBC differential, reticulocyte, and NRBC. The VCSn module includes the Air Mix and Temperature Control (AMTC) and the Multi-Transducer Module (MTM). In the flow cell, low-frequency direct current measures volume, while high-frequency (RF) current senses cellular internal content through measuring changes in conductivity.

Specimen Requirements

- Whole blood collected in an EDTA tube.
- The instrument aspirates 165 μL of the patient sample.
- Samples are stable at room temperature for eight hours. But sample for analysis of hematological parameters in this study does not exceed 2hrs.
- Cause for rejection: hemolysis, clotted specimen, insufficient volume, unlabeled specimen.

Reagents

- Coulter DxH diluent (store at 2 - 40° C)
- Coulter DxH Lyse reagent (store at 2 - 40° C)
- Coulter DxH Diff Pack (store at 2–25° C)
- Coulter DxH cleaner (store at 2–25° C)

NB. All reagents are cyanide-free and stable for about 60 days after opening except the cleaner, it is stable for 90 days.

Equipment: UniCel DxH 800 Coulter Cellular Analysis System.

Procedure for running blood samples by cassette presentation

1. Select the default test order as automatic (specimen – cassette presentation)
2. Click on the system status screen or the details status screen and place the specimen processing module (SPM) online to run samples by pressing the start button.
3. Ensure that the specimens have been collected and stored properly.
4. Load the specimens into the cassette
5. Place the cassettes into the input buffer to the right of the SPM. The SPM automatically begins cycling the cassettes.
6. In each cycle, the result were displayed in the review dialog box.
7. Click on review and enter specimen number and patient name in the MRN and patient name boxes using the keyboard.
8. Send the result to the release dialog box.
9. Press PRINT REPORT for a hardcopy of the report.

Procedure for running blood samples by a single-tube presentation

1. Select the default test order as manual (specimen – single-tube presentation)
2. Place the specimen on the bar-code reader platform of the Single-tube Presentation Station with the bar code facing the SPM to allow the Single-Tube Presentation Bar Code Reader to scan the specimen label. OR type/scan the specimen identifier. Place the cursor at the end of the last character of the Specimen ID and press enter.
3. Verify the Specimen Identifier and Test request. Follow the prompts on the screen.
4. Mix the specimen by inverting the tube 8 times.
5. Place the specimen into the correct Single-tube position.
6. Click on run the test.
7. Click on review and enter specimen number and patient name in the MRN and patient name boxes using the keyboard.
8. Send the result to the release dialog box.
9. Press PRINT REPORT for a hardcopy of the report

Quality control

Quality control checks are performed daily according to the laboratory's protocol. Commercial controls materials are properly warmed and mixed according to the manufacturers' recommendations. The controls are handled according to the manufacturers' recommendations and the laboratory's protocol.

Preparation of thin blood film

Materials

- Clean microscope slides
- Well-mixed EDTA blood sample
- Pipette
- pencil
- Gloves
- Waste and sharps disposal containers

Procedures

- Place a small drop of well-mixed EDTA blood 1.0 cm far from the frosted end of the slide
- The spreading slide is placed in front of the drop of blood at an angle of about 30^{o} -40^{o} to the slide and then is moved back to make contact with the drop
- The drop will spread out quickly along the line of contact of the spreader with the slide
- The spreader is advanced with a smooth steady motion so that a thin film of blood is spread over the slide
- Allow the smear to air-dry
- Label with patients ID

Wright staining and examination

Procedure

- Place the air-dried smear film side up on a staining rack
- Cover the smear with undiluted filtered stain and leave for 1 minute
- Add an equal volume of distilled water (i.e., the same number of drops as the stain)
- Mix by blowing until a metallic sheen appears.
- Allow the diluted stain to act for 3-5 minutes
- Wash off the stain with running tap water
- Wipe the back of the slide clean and stand it in a rack for the smear to dry.
- Examine gross morphology by 40x and use the 100x objective for studying the fine details of the cell morphology

HCG Test

Principle of the test

- Most of the urine pregnancy test kits are based on lateral-flow technology. Most of them qualitatively detect the presence of hCG in urine specimens at the sensitivity of 25 mIU/mL. The test uses two lines to indicate results. The test line utilizes a combination of antibodies including a monoclonal hCG antibody to selectively detect elevated levels of hCG. The control line is composed of goat polyclonal antibodies and colloidal gold particles.
- The assay is conducted by adding a urine sample to the sample well of the test device and observing the formation of colored lines. The sample migrates via capillary action along the membrane to react with the colored conjugate. Positive samples react with the specific antibody-hCG-colored conjugate to form a colored line at the test line region of the membrane. The absence of this colored line suggests a negative result. A colored line will always appear in the control line region if the tests have been performed properly.

The procedure of the test

1. Allow the Pregnancy Test Strip and urine sample to reach room temperature (15-30°C) before opening the foil pouch.

2. Remove the Pregnancy Test Strip from the pouch and use it as soon as possible.

3. Place the test device on a clean and level surface. Hold the dropper vertically and transfer 3 full drops to the specimen well and start the timer. Avoid air bubble formation.

4. Wait for the colored line(s) to appear. Read the result after 5 minutes. Do not read the result after 15 minutes.

Interpretation

- Positive: Two colored lines appear. One line should be in the Control region (C) and another line should be in the test region (T). This means there is a strong possibility that the patient is pregnant.

Annex VIII: Laboratory Result Registration Form

Laboratory registration for the T2DM group.

Laboratory registration sheet for collecting fasting blood glucose and hematological parameter results of study participants at Debre Berhan Referral Hospital, Northeast Ethiopia from May 01 to June 30, 2020, G.C.

Code	Age	Gender	FBG (mg/dl)	WBC (10^3/µl)	Neu (10^3/µl)	Lymph (10^3/µl)	Mon (10^3/µl)	Eos (10^3/µl)	Bos (10^3/µl)	RBC (10^6/µl)	Hgb (g/dl)	HCT (%)	Platelet(10^3/µl)	MCV (fl)	MCH (Pg)	MCHC (%)	RDW (%)	MPV (fl)
01																		
02																		
03																		
04																		
05																		
134																		

Name and signature of laboratory technologist: _____

Laboratory registration form for the control group.

Laboratory registration sheet for collecting hematological parameter results of study participants at Debre Berhan Referral Hospital, Northeast Ethiopia from May 01 to June 30, 2020, G.C.

Code	Age	Gender	WBC (10^3/µl)	Neu (10^3/µl)	Lymph (10^3/µl)	Mon (10^3/µl)	Eos (10^3/µl)	Bos (10^3/µl)	RBC (10^6/µl)	Hgb (g/dl)	HCT (%)	Platelet(10^3/µl)	MCV (fl)	MCH (Pg)	MCHC (%)	RDW (%)	MPV (fl)
01																	
02																	
03																	
04																	
05																	
134																	

Name and signature of laboratory technologist: _____

DECLARATION

I the undersigned, declare that the thesis entitled with: **Assessment of Hematological Parameters in Adult Patients with Type 2 Diabetes Mellitus at Debre Berhan Referral Hospital, Northeast Ethiopia: A Comparative Cross-Sectional Study 2019/2020** is my work and has never been presented for any degree or other purposes at Jimma University or any other institution of higher learning. I also declare that, when other people work has been used, it has been carefully acknowledged and referenced per the requirements. Therefore, I agree to accept responsibility for the scientific, ethical, and technical conduct of this research thesis.

Principal investigator: Mesay Arkew

Signature: _____ Date: _____/_____/_____

Jimma University

School of Graduate Studies

This is to certify the thesis prepared by Mesay Arkew entitled with: **Assessment of Hematological Parameters in Adult Patients with Type 2 Diabetes Mellitus at Debre Berhan Referral Hospital, Northeast Ethiopia: A Comparative Cross-Sectional Study 2019/2020** and submitted for partial fulfilment of the requirements for the degree of Master of Science in Clinical Laboratory Science Specialty in Hematology and Immunohematology complies with regulations of the university and meets the accepted standards with respect to originality and quality.

Signed by the examining committee

Advisors:

1. Dr. Tilahun Yemane (MD, MSc).
   Signature: _____Date: _____/_____/_____
2. Mr. Girum Tesfaye (MSc, PhD candidate)
   Signature: _____ Date: _____/_____/_____

Examiners:

1. External examiner: _____
   Signature: _____ Date: _____/_____/_____
2. Internal examiner: _____
   Signature: _____Date: _____/_____/_____

Department head: _____

Signature: _____ Date: _____/_____/_____