ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of hematological and biochemical alteration in type II diabetes mellitus patients attending Aksum St. Marry Hospital, Aksum, Northern Ethiopia

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

September, 2021

Addis Ababa, Ethiopia

Examiners' Approval Sheet

This is to certify that the thesis prepared by Kidane Yhdego, entitled "Alteration of Hematological and Biochemical parameters in type II diabetes mellitus patients attending Aksum St. Marry Hospital, Aksum, Northern Ethiopia", summited in partial fulfillment of the requirements for masters of science degree in Clinical hematology and Immunohematology complies with the regulations of the university and meets the accepted standard with respect to originality and quality.

Signed by the Examining committee

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Chairman of the Department of Graduate program coordinator

Acknowledgments

First, I would like to give my deepest gratitude to GOD for all the blessings and support granted to me. I would also like to express my deepest gratitude to Addis Ababa University, College of Health Science, and Department of Medical Laboratory Science for providing me the opportunity of conducting this thesis, and I would like to say thanks to my advisors Mr. Zemenu Tamir and Mrs. Rahel Alemu for their humble effort to help me to do my thesis. My deepest gratitude also goes to Dr. Bereket Aregawi who guided me every day when I need help regarding the clinical cases. I also would like to Thank Mr. Mulugeta Berhane who helped me to select the study cases and also helped me in measuring the anthropometric measurements. I would also to say thanks to my study participants. The last but not least, I would lexpress my acknowledgment to my friends who helped me by sharing their ideas/ experience and other necessary materials that helped me to accomplish this thesis.

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Abbreviations and acronyms

AAMI	US Association for the Advancement of Medical Instrumentation
AAU	Addis Ababa University
ALP	alkaline phosphate
ALT	Alanine Transaminase
AST	Aspartate Transaminase
BMI	Body mass index
CBC	Complete Blood Count
CI	Confidence Interval
DC	Direct Current
DM	Diabetes mellitus
DREC	Department Research Ethics Committee
ESH	European Society of Hypertension ESH
HCT	Hematocrit
HGB	Hemoglobin
IDF	International diabetes federation
ISO	International Organization for Standardization
K_2EDTA	Di-Potassium Ethylene Diamine Tetra Acetic Acid
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume

MPV	Mean Platelet Volume
PCV	Packed Cell Volume
PI	Principal Investigator
QC	Quality Controls
RBC	Red Blood Cell
RDW	Red Blood Cell Distribution Width
SD	Standard Deviation
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
T2DM	typeII diabetes mellitus
WBC	White Blood Cells
WHO	World Health Organization

Abstract

Background: Diabetes mellitus is a non-communicable disease that has a high prevalence worldwide. Altered level of many hematological and biochemical parameters have been observed in patients with diabetes. Although type II diabetes mellites alters hematological and biochemical parameters, so far, very little work has been done in Ethiopia linking hematological parameters and biochemical markers with type II diabetes mellites patients.

Objective: To assess alteration of hematological and biochemical parameters in type II diabetes mellitus patients attending Aksum St. Marry Hospital, Aksum, Northern Ethiopia, from December 2019-April 2020

Method: comparative cross-sectional study was conducted from December 2019 to April 2020 at Aksum St. Marry Hospital, Aksum, Northern Ethiopia. A total of 168 participants were enrolled using convenient sampling method. Socio-demographic, behavioral and physical measurement data were collected. Complete blood count was performed by sysmex KX-21N hematology analyzer. The biochemical parameters were analyzed by using BE200. Data was analyzed using SPSS 23. Student independent t-test and person's correlation statistical method employed to analyze data. P values < 0.05 was regarded as statistically significant at 95% confidence level.

Result: Among the hematological parameters WBC count, neutrophil count, lymphocytes count, platelet count, MPV significantly increased. Similarly, RBC count, hemoglobin and PCV showed significant decrease in T2DM patients compared to controls. With regard to biochemical parameters ALT, AST, ALP, Creatinine and urea showed significantly increasing in T2DM patients when compared to controls. Regarding correlation, WBC with BMI and MCHC with FBS had positive correlations.

Conclusion: There is alteration in total WBC, neutrophil, lymphocyte, eosinophil, basophil, RBC, hemoglobin and PCV. Also, from biochemical parameters ALT, AST, ALP, urea and creatinine altered in diabetes patients compared with control group.

Key word: Type II diabetes mellitus, hematological parameters, biochemical parameters, hyperglycemia, study area

1. INTRODUCTION

1.1. Background

Diabetes mellitus is defined as a group of metabolic disorders that are characterized by several defects in the regulation of carbohydrate, fat or protein metabolism. It is one of the non-communicable diseases and carbohydrate metabolism disorder that results persistently increased the level of blood glucose [1]. In 2012 there were 1.5 million deaths worldwide directly caused by diabetes. It was the eighth leading cause of death among both sexes and the fifth leading cause of death in women. The main cause for type two diabetes mellitus is the absence of insulin secretion due to either the progressive or inability of the β-Langerhans islet cells of the pancreas to produce sufficient insulin or due to defects in insulin uptake in the peripheral tissue. Diabetes mellites is broadly classified under two categories [2]. Type 1 diabetes most commonly considered as children disease, but it can sometimes also observe in adult age groups, especially those in their late thirties and early forties [3]. The main factor in the pathophysiology of type 1 diabetes is considered to be autoimmunity caused destruction of β-cells. Type 2 diabetes is described as a combination of low amounts of insulin production from pancreatic β-cells and peripheral insulin resistance [4].

Abnormal hematological parameters are observed in type two diabetes mellitus patients [4]. Alteration in many hematological parameters such as red blood cells, white blood cells (WBC), and the platelet function has been observed in patients with the diabetes mellites. Many studies have advocated the importance of raised level of WBC and RBC count in the diagnosis of metabolic syndrome [5]. Anemia is the most common hematological finding in diabetic patients [6]. Anemia is defined as a condition that decrease in the hemoglobin concentration in blood, which leads to reduces the oxygen-carrying capacity of red blood cells such that they are unable to meet the body's physiological needs for oxygen [7]. Many research studies have reported that anemia mostly occurs in patients with diabetes who also have renal insufficiency [8].

Patients with diabetes mellitus show a significant alteration in various hematological parameters [9]. In fact, several hematological alterations in white blood cells, platelet count or function and red blood cells, as well as coagulation factors are shown to be directly associated with T2DM [10]. Furthermore, findings of so many studies suggested that activated platelets may increase vascular complications in T2DM patients and alteration in platelet morphology and function can be indicated as a factor for risk of micro vascular and macro vascular diseases [12]. In addition, as it has demonstrated by many studies insulin resistance and hyperinsulinemia have been associated with the stimulation of erythroid progenitors in bone marrow and increased levels of inflammatory markers or cytokines [13]. Peripheral blood leucocytes are composed of granulocytes monocytes as well as lymphocytes. Leucocyte can be activated by advanced glaciation end products, oxidative stress and cytokines in the state of hyperglycemia [8, 14].

The association between some components of metabolic syndromes and leukocytes was observed in several epidemiological studies that showed correlation between total white blood cell count (WBC) and diabetes mellitus [14]. Leukocyte plays an important role during inflammation and also play some roles in the development of diabetes [12]. There are common findings of reduced hemoglobin concentrations in diabetic patients [13, 14].

Association between diabetes mellitus and liver injury is well established. Carbohydrate homeostasis is also mainly regulated by liver. In type two diabetes patients with uncontrolled glucose, intracellular glycogen is increased in hepatocytes in response to increase glycogen synthesis. In response to this, there is a mild to moderate increase in aminotransferases [18]. Individuals with type 2 diabetes have a higher incidence of liver function test abnormalities than individuals who do not have diabetes [16]. Mild chronic elevations of transaminases often reflect underlying insulin resistance [19]. Elevation of transaminases within three times the upper limits of normal is not a contraindication for starting oral anti diabetic or lipid modifying therapy [20]. In contrast, anti-diabetic agents have generally been shown to decrease alanine aminotransferase levels as tighter blood glucose levels are achieved [21].

The liver plays a major role in the regulation of carbohydrate metabolism, as it uses glucose as a fuel, it has the capability to store glucose as glycogen and also synthesize glucose from non-carbohydrate sources [22]. This key function of liver makes it susceptible to diseases in subjects with metabolic disorders, particularly diabetes mellites. Increased activities of liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are indicators of hepatocellular injury. Increased activities of these bio markers are associated with insulin resistance, metabolic syndrome and type 2 diabetes [24].

The excess amount of free fatty acids found in the insulin-resistant state is known to be exhausted mitochondrial oxidation in the hepatic cells, that is toxic to the liver. Mechanisms include cell membrane disruption, toxin formation, activation and inhibition of key steps in the regulation of metabolism [22, 25]. Other potential causative effect for elevated transaminases in insulin-resistant states include oxidant stress from reactive lipid peroxidation, peroxisomal beta-oxidation, and recruited inflammatory cells [20, 25]. The insulin-resistant state is also characterized by an increase in pro inflammatory cytokines such as tumor necrosis factor-α, which may also contribute to hepatocellular injury. In numerous studies, an increased frequency of specific TNF-α–promoter was found to cause nonalcoholic steatohepatitis (NASH) patients, which suggesting a possible genetic link or predisposition to fatty liver found in insulin-resistant states. The above theories all attribute elevated transaminases to direct hepatocyte injury [26]. And also hypothesized that elevation in ALT, the gluconeogenic enzyme whose gene transcription is suppressed by insulin, could indicates an impairment in insulin signaling rather than purely hepatocyte injury [27].

1.2. Statement of the Problem

Globally, the number of people suffered with diabetes mellitus has quadrupled in the past three decades and it is the ninth major cause of death. According to International Diabetes Federation 2017 report, globally around 426 million people are living with diabetes and the number is expected to rise by 629 million by 2045 [1]. Worldwide now, among the 11 adults one person have diabetes mellitus, of them 90% have type 2 diabetes mellitus. Asia is the major area of the rapidly emerging T2DM global epidemic, with China and India are the top two epicenters. Although genetic predisposition partly plays important role in individual susceptibility to T2DM, an unhealthy diet, sedentary lifestyle and stress are important drivers of the current global epidemic of the disease [2].

Diabetes mellitus is affecting a majority of the world population and reaching to epidemic proportions in some countries [3]. In Saudi Arabia, diabetic patients are increasing daily and these may be due to shifts in way of life, increase consumption of sweet foods and beverages containing high levels of sugars, lack of exercise, unhealthy diet, obesity, and socioeconomic changes over the past decades [11].

An estimated 15.5 million adults aged between 20 and 79 years have diabetes in Africa. The highest prevalence of diabetes in Africa is between ages 55 and 64. Africa has the highest proportion of undiagnosed diabetes; over two-thirds (69.2%) of people with diabetes are unaware they have the disease. More than half (55.3%) of people with diabetes live in cities, even though the population in the region is predominantly (60.3%) lives in rural. The highest prevalence of diabetes in Africa in adults from age 20-79 years is found in the island of Reunion (13.8%), followed by Comoros (11.9%) and the Seychelles (10.6%) [1].

Some of Africa's most populous countries have the highest numbers of people with diabetes, including Ethiopia 2.6 million, South Africa 1.8 million, Democratic Republic of Congo 1.7million, and Nigeria 1.7 million. About 45.1% of all adults aged 20-79 years with diabetes in the region live in these four countries. As urbanization expanded and populations age increased, type 2 diabetes mellites will pose an ever-growing threat. It is expected that by 2045 there will be 40.7 million adults aged 20-79 years in this region living with diabetes, more than double the number in 2017 [1]. The projected national diabetes prevalence in Ethiopia as estimated by IDF Atlas was 5.2 % [28].

Type two diabetes mellitus is most complicated disease. Studies conducted in our country are not adequate as well as specific to various physiological abnormalities that may cause by T2DM. So, our physicians have no enough updated information and recent findings related to the hematological and biochemical alterations that caused by diabetes mellitus, so they may not use as their main diagnosis and management means in their day-to-day activities.

In our study area Aksum, the problem is far worse, to the best of our knowledge there is no study conducted related to diabetes mellitus and its relation with the hematological as well as biochemical parameters. Therefore, this study was intended to assess the hematological and biochemical alterations among T2DM patients in the study setting.

1.3 Significance of the study

Diabetes mellitus is a very common endocrinal disorder affecting a majority of the world population and reaching to epidemic proportions in some countries, including Ethiopia. In our country there is no adequate studies conducted on the alteration of hematological and biochemical parameters on T2DM patients. The main purpose of this study is to assess the alteration of hematological and biochemical parameters of type two diabetes mellitus patients in Aksum Tigray, North Ethiopia. This study will have more significance to the physicians that will help them to consider their management of patients, that may provide them an alternative way to monitor their status. This study will help the policy makers, health institutions and administrators to improve their diabetic patients management and follow up system. This study will also be used as reference for further studies.

2. Literature Review

Diabetes is becoming the world's headache as the incidence of both diabetes mellitus type one and diabetes mellitus type two become large and larger. It is the most complicated disease and needs complex studies worldwide. There are so many studies conducted to assess the hematological and biochemical changes [11,23].

2.1 Review of both hematological and biochemical parameters

A comparative cross-sectional study was conducted in Saudi Arabia from November 2016 to April 2017 to assess the biochemical and hematological difference among 205 diabetic and 200 non-diabetic participants. The study results showed that an increase in the mean values of SGPT, alkaline phosphatase, urea, serum creatinine, total cholesterol, triglyceride, and LDL in the T2DM group relative to the control group. Meanwhile, the mean value of HDL was significantly decreased in the T2DM group compared to the control group. The mean values of hemoglobin, RBC, MCV, MCHC and MCH were significantly decreased in the T2DM group compared to the control group. In contrast, the red cell distribution width, MPV, total WBC significantly increased in the T2DM group versus control group [11].

2.2 Review of hematological parameters

Cross sectional study which was conducted at the Korle-bu Teaching Hospital, Accra, Ghana between January and December 2017, the total number of individuals recruited into the study was 304 comprising of 171 (56%) males and 133 (44%) females. The outcome of the study indicates that, there was significant difference in almost all of the hematological parameters. There was also, both strong positive and negative correlations between the hematological parameters [5].

Hospital based case control study was conducted in a rural tertiary care center for a period of May 2016 to April 2017 among type 2 diabetic patients. 70 diabetics and non-diabetics as controlled were enrolled for the study. In diabetics mean hemoglobin, RBCs, PCV, and MCV was significantly lower than in non-diabetics. Whereas mean MCHC, WBCs and lymphocytes were significantly higher in diabetics compared to non-diabetics. No difference was observed for MCH, neutrophils and platelets between two groups. This shows that diabetics are prone for anemia, leukocytosis and lymphocytosis [13].

Cross-sectional study was carried out in Al-Sadder general hospital Iraq, 2015. From 100 patients (55 male and 45 female) having age group (28-70), year and same number of non-diabetic subjects having age group (20-62) year. The values of hematological parameter in diabetic and non-diabetic; Hb concentration and PCV percentage were found significantly unchanged in both diabetic patients and non-diabetic, while the WBC count were found significantly increased in diabetic patients than non-diabetic [14].

A study conducted in western Algeria done in 2017 on a sample of 1852 subjects, 1059 with type 2 Diabetes and 793 witnesses, were evaluated for peripheral Blood parameters using hematology. The logistic model retained the mean corpuscular hemoglobin concentration reveals that subjects with concentration over the normal ratio (>36g/dl) have an exposure risk six and half times higher than subjects with normal concentration. As regards to the platelets blood ratio, subjects with a ratio lower are five times more exposed to type2 diabetes compared to subjects with a normal ratio. The logistic model also retained basophils ratio and sedimentation rate at one hour [9].

Study was conducted at El-Beida Hospital, El-Beida City, Libya, 2017. This study consists of 103 Libyan type 2 diabetic patients (79 males + 24 females) and 39 healthy non-diabetic subjects (29 males and 10 females) acted as controls. Hematological studies in the diabetic patients showed significantly lower HCT values, hemoglobin content, RBCs count and MCV concentration than in the controls. Greater total WBC counts, MCHC concentrations, MCH, lymphocytes and neutrophils counts were observed in the patients than in the controls. No differences were found between platelets counts in the diabetic patients and the control [6].

A cross-sectional study from Gondar University Hospital that was done from February to April 2015. A total of 296 participants which enrolled 148 cases and 148 healthy controls, deference was observed in result of red blood cell distribution width, total WBC count, neutrophil count, lymphocyte count, mean platelet volume and platelet distribution width were significantly increased in T2DM patients compared with controls [5]

2.3 Review of biochemical parameters

Prospective study that performed on 200 T2DM patients in the Department of Medicine, Gandhi Medical College from January 2015 to June 2016, out of 200 patients, there were 82.45% male and 17.55% female in patients in Group A whereas in group B there were 78.76% male 22.24% female, the result show Abnormal liver function results were common among diabetes mellitus patients. The result from this study shows, liver function test parameters such as GGT, SGOT and SGPT were higher in patients with T2DM but comparable with patients with hypertensive diabetes mellitus [20].

Cross-sectional study was done in Sudanese type 2 diabetic patients with long period of suffering from diabetes. From the study conducted the liver function tests in patients with type 2 diabetes mellitus were significantly higher than that of control. i.e., 22% of the patients had at least one or more elevated liver enzyme levels in the serum of patients. Because of high liver enzymes level in serum of patients with type 2 diabetes mellitus, performance of liver function tests is highly recommended for diabetic patients [21].

A cross-sectional study was conducted at North West Ethiopia from May to August 2017, on a total of 384 selected participants. They nominated an equal number of cases (diabetes) and control (apparently healthy) study participants. From the study, 93 (48.4%) of diabetes participant and 8 (4.2%) of control had elevated serum AST levels. Of all the diabetes case, 77 (40.1%) had elevated serum ALT, and 192 (100%) of control group had normal values of ALT. In addition, 3 (1.6%) of diabetes case and 1 (0.5%) of the controls had elevated serum GGT levels. The mean values of ALT and AST were significantly higher in type 2 diabetes participants than the control group, the result of this study showed that the elevation of the liver enzyme test (ALT and AST) was significantly associated with type 2 diabetes mellitus compared with the control group [19].

Case control Study conducted in Rajasthan, among 25 newly diagnosed cases of type 2 diabetes mellitus and 25 healthy controls. The study showed as the mean serum urea levels were 25.80±6.75 mg/dL in controls and 34.08±9.62 in cases, which was statistically significant. Serum creatinine and uric acid values were also statistically significant (p=0.0002). Also, significant positive correlation was found between FBS and renal function tests in both cases and controls [26].

Conceptual Frame Work

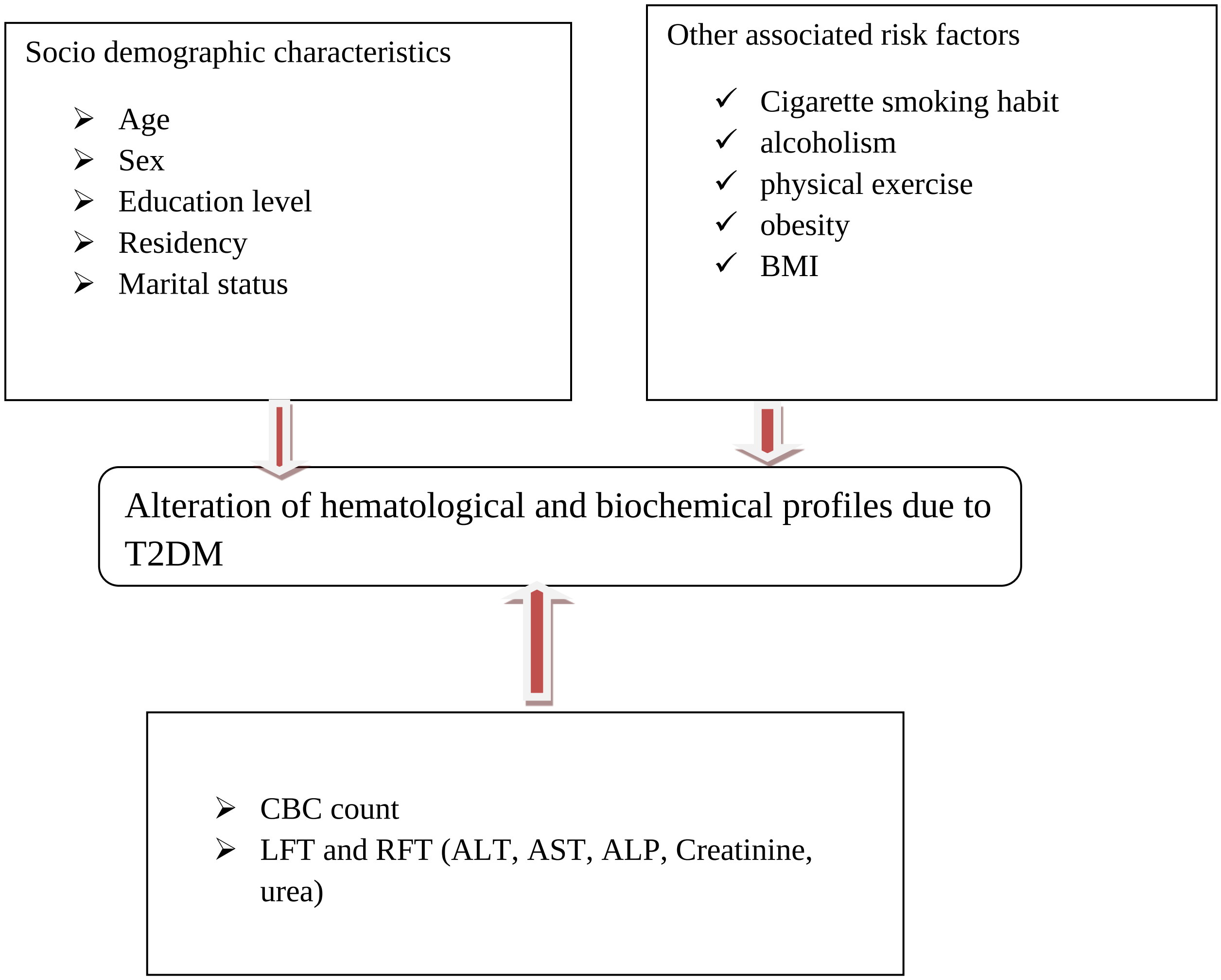

Figure 1.Conceptual frame work to assess hematological and biochemical parameters at Aksum St. Marry Hospital

3. Objectives

3.1. General objective

To assess hematological and biochemical alterations among T2DM patients attending at St. marry hospital Aksum, Northern Ethiopia in Comparison to control groups from December 2019 to Aril 2020

3.2. Specific objectives

- To compare the hematological parameters of T2DM patients at St. marry hospital with apparently healthy controls
- To compare the biochemical parameters of T2DM patients attending at St. marry hospital with apparently healthy controls

4. Hypothesis

H_o -There is no significant difference of hematological and biochemical parameters among T2DM patients and control groups.

5. Materials and methods

5.1. Study area

Theis study was conducted in Aksum St. Marry Hospital, Aksum town, Tigray, Ethiopia. Aksum is a historic town located in Tigray Region, northern Ethiopia which is around 1020 km far from the capital city Addis Ababa. It is located in the central Tigray with elevation of 7000ft (2100 meters) above the sea level [52]. Aksum St. marry Hospital is a general hospital which serves to Aksum town and surrounding weredas with an estimated 250,000-catchment population. It has different wards and OPDs (medical, surgical gynecological pediatrics and special TB ward, chronic disease clinic). Among these services the chronic disease clinic is the main one that serves the diabetic patients. The chronic disease clinic's service is run and followed by two internists.

5.2. Study design and period

Comparative cross-sectional study was conducted among T2DM patients from December 2019 to April, 2020 at Aksum St. marry Hospital.

5.3. Population

5.3.1. Source population

All DM patients who attended Aksum St. marry Hospital during the study period.

5.3.2. Study population

5.3.2.1. Case group

All T2DM -patients, who attended at St. Marry Hospital during the study period

5.3.3. Sample population

T2DM patients who full filled the required criteria during the study period at Aksum St. marry Hospital

5.3.4. Control Group

Any voluntary apparently healthy person who have similar age and sex to study participant without confirmed diabetes mellites.

5.4. Inclusion and exclusion criteria

5.4.1. Inclusion criteria

5.4.1.1. Case group

All voluntary T2DM patients with age of 18years and above who attended at St. marry hospital during the study period were included in this study.

5.4.1.2. Control Group

- Any voluntary apparently healthy person who had the same age, sex and status to study participants.

5.4.2. Exclusion criteria

5.4.2.1. Case group

- Patient of T1DM
- Patients who have history of chronic diseases (HIV, hypertension, chronic liver disease, chronic kidney disease, chronic heart failure, malignancy.
- Pregnant mothers

5.4.2.2. Control group

- Patients who have history of malignancies, HIV, hypertension, chronic liver disease, chronic kidney disease

5.5. Study variables

5.5.1. Dependent variables

- Hematological (CBC) parameters
- Biochemical parameters

5.5.2. Independent variables

- Socio-demographic variables (age, sex, marital status, educational level, residence, occupation)
- Smoking habit
- Habit of drinking alcohol
- Physical exercise
- BMI
- Blood pressure

5.6. Sample size calculation and Sampling method

5.6.1. Sample size calculation

The sample size for this study was calculated using sample size calculation for comparison of two independent means at 95% confidence level and 80% power. From research done in Gondar to assess the hematological parameter of type 2 diabetic patients and equal proportion of controls without diabetes. Mean platelet volume and total WBCs count used to calculate sample size. Because those result have significant association with T2DM [10].

Table 1 Mean and standard deviation of the T2DM patients and control subjects in Gondar

Mean and standard deviation of the T2DM patients and control subjects in Gondar				
no	CBC count result	Mean and SD of the T2DM patients	Mean and SD of control subjects	Calculated sample size
1	MPV (fl)	10.4±1.1	9.9±1.1	76
2	Total WBC (*10^3/µl)	6.59±1.42	5.56±1.38	30

$$n = \frac{(s_1^2+s_2^2)*(Z\alpha+Z\beta)^2}{d^2}$$

Where, **n**= desired sample size, **S**= Standard deviation (s1=study group i.e., 1.1, s2= control group i.e., 1.1) from previous study, $Z\alpha$=1.96, $Z\beta$ = 0.8, **d**= effective size /difference between two means = |u1-u2|

= |10.4-9.9| =0.5

$$n = \frac{(s_1^2+s_2^2)*(Z\alpha+Z\beta)^2}{d^2} = \frac{(1.1^2+1.1^2)*(1.96+0.84)^2}{(0.5)^2} = 75.8 \text{ round to } 76$$

Non response rate (10%) = 76 *0.1= 7.6 round to 8

Minimum sample size =76+8

= 84

Therefore, the minimum sample size calculated for this study is 168 which is (84 T2DM cases and 84 control group).

5.6.2. Sampling Method

A total number of 84 T2DM patients and 84 volunteer controls participants from Aksum St. marry Hospital were included in this study by convenient sampling method.

5.7. Measurement and Data collection

5.7.1. Data collection procedure

Before the main data collection procedure, pilot study was conducted to assess the main gaps and to run the study effectively. All T2DM patients who attending St. marry hospital during the data collection period were interviewed for socio demographic data by using pre tested structured questionnaire. The questionnaire mainly consists of closed and open-ended questions focusing on socio-demographic data, age, gender, occupation, and health status, smoking status and getting any medications.

The anthropometric measurements conducted for every study person. This measurement was performed by the help of the nurses. The measurements performed by a calibrated instrument for every measurement as follows.

Waist circumference: As measure of central obesity; measured at the midpoint between the bottom of the ribs and the top of the pelvis by using well calibrated and have good standard meter measurement. Central obesity is most easily measured by waist circumference with cut-points that are gender and ethnic group specific. For example, the waist-circumference cut point for Europe males is ≥ 94 cm and for Europe females is ≥ 80 cm

Body mass Index: It is measured by the ratio of weight to height^2. The measurement used well standardized and calibrated balance. The height measurement used calibrated and have good standard meter measurement. BMI will be calculated by the WHO recommended formula; BMI =weight/height^2 with cut point for BMI to be at risk is >24kg/m$^{2.}$

Blood pressure: blood pressure was measured by instrument called sphygmomanometer cuff. The cuff we use was well standardized, i.e., fulfill the AAMI/ESH/ISO standard protocol, and large adult cuff used had (width 12.5-13 cm, length 35 cm).

Blood was collected from the participant who complete the questionnaire and fulfill the criteria, also who are agreed to give blood. After collection, blood analysis was done for the hematological parameters using sysmex KX-21N differential hematology analyzer and the biochemical parameters done by BE 200.

5.7.2. Laboratory analysis

5.7.2.1. Specimen Collection and Processing

About 10 ml of Venous blood sample was collected by using syringe method. The collection procedure followed standard venous-puncture method from the T2DM patients and controls hands after cleaning with 70% ethanol. Then, 4ml of the blood poured on vacutainer tube containing, Ethylene Die amine Tetra Acetic Acid (K_2EDTA).to determine clinical chemistry and hematological profile of T2DM patients. As soon as the sample is collected and labeled, it was transported to the hematology and biochemical working areas to be analyzed.

For hematological parameters analysis 4ml of the blood sample poured on vacutainer tube containing, Ethylene Die amine Tetra Acetic Acid (K2EDTA) from both T2DM patients and healthy controls. To determine biochemical parameters about 5 ml venous blood sample poured to serum separator tube from both T2DM patients and healthy controls. The sample transported to required work area for clinical chemistry analysis, serum sample was separated after centrifugation at 4000 RPM for 5 minute and analyzed.

5.7.2.2. Complete blood count (CBC)

The complete blood count (CBC) is one of the most common blood tests used. It analyzes: red blood cells, white blood cells, and platelets. The CBC counts these cells, measures hemoglobin, estimates the red cells' volume, and sorts the white blood cells into five subtypes or three types depends of the type of hematology analyzer. It is a very common and important test that is frequently ordered by a physician to check a patient's general health status.

5.7.2.2.1. Sysmex KX-21N hematology analyzer

The sysmex KX-21N is a quantitative automated hematology analyzer for in vitro diagnostic use. Examination of the numerical and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemia, allergic reactions, malignancies, viral infection, bacterial infection, and parasitic infections. The sysmex KX-21N processes approximately 60 samples an hour and displays on the screen the particle distribution curves of WBC, RBC, and platelets, along with data of 17 parameters, as the analysis results. Sysmex KX-21N analyzer directly measures the WBC, RBC, HGB, HCT, PLT, LYM count, BASO count, Eosinophil count, Monocyte count, and NEUT count. The remaining parameters are calculated or derived, MCV, MCH, MCHC, MPV, RDW-CV and RDW-SD, and differential percentages LYM%, Monocyte%, BASO%, EOS%, NEUT% [47].

5.7.2.2.1.1. Detection Principle

The sysmex KX-21N counts and detects the sizes of red blood cells, WBC and platelets by using electronic resistance detection method. Hematocrit is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin is converted to methemoglobin, and read photo metrically at 555 nm. White blood cells are analyzed using direct current and discriminated into a three-part differential using Particle Distribution Analysis. The resulting WBC histogram is discriminated into lymphocyte, neutrophil, mid cells [47].

5.7. 2.3 BS- 200 Chemistry analyzer

A discrete, random access and automated chemistry analyzer that can run up to 200 testes per hour. It has reversed optic system with 8wavelengths 340-670nm, Built-in ISE module, internal sample/reagent and On-board reagent refrigeration. It has automatic probe cleaning, liquid level detection, depth adjustment, disposable cuvettes ensure minimal carryover, Low-cost instrument, low-cost reagents and minimum reaction volume: <=180µl. Perform tests on serum, plasma, or urine samples to determine concentrations of analytes (e.g., cholesterol, electrolytes, glucose, calcium) [48].

Principle

After the tray is loaded with samples, a pipette aspirates a precisely measured aliquot of sample and discharges it into the reaction vessel; a measured volume of diluent rinses the pipette then reagents are dispensed into the reaction vessel. After the solution is mixed incubate (if necessary), then measures its absorbance and calculates the analyte’s chemical concentrations [50].

5.7. 2.3.1 Alanine amino transaminase

Principle of the test

Colorimetric assay is based on the oxidization of NADH to NAD in the presence of pyruvate and lactate dehydrogenase. The ALT activity is determined by assaying the rate of NADH oxidation, which is proportional to the reduction in absorbance at 340nm over time (ΔOD340nm/min) [50].

L-Alanine + 2-Oxoglutarate - ALT→ L-Glutamate + Pyruvate

Pyruvate + NADH + H -----LDH --→ Lactate + NAD+

5.7.2.3.2 Aspartate amino transaminase

Principle of test

Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to -ketoglutarate to yield oxaloacetate and L-glutamate. Malate dehydrogenase (MDH) catalyzes the reduction of oxaloacetate with simultaneous oxidation of NADH+ to NAD [50].

L-Aspartate + 2-Oxoglutarate AST L-Glutamate + Oxalacetate

Oxalacetate + NADH + H+ MDH L-Malate + NAD

5.7.2.3.3 Alkaline phosphatase

Principle of the test

It is a colorimetric assay in accordance with a standardized method. In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol, the p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance at 409 nm. [50].

4-Nitrophenylphosphate + H2O ALP 4-Nitrophenol + Pi

PH>9

5.7.2.3.4 Creatinine

Principle

The method is based on the Jaffe reaction. Creatinine reacts with picrate ion formed in alkaline medium to develop a red-orange color. The color produced from the sample is then compared in a colorimeter at wavelength of 505 nm with that produced by a known amount of creatinine under the same condition [50].

5.7.2.3.5 Urea

Principle

Urea is hydrolyzed by urease to ammonia and carbon dioxide. The ammonia formed then reacts with αketoglutarate and NADH in the presence of glutamate dehydrogenase (GLDH) to yield glutamate and NAD+. The decrease in absorbance due to consumption of NADH is measured kinetically [50].

5.7.2.4 glucometer

A glucose meter is a medical device for determining the approximate concentration of glucose in the blood. It is a key element of home blood glucose monitoring. A small drop of blood, obtained by pricking the skin with a lancet, is placed on a disposable test strip that the meter reads and uses to calculate the blood glucose level. The glucometer apparatus then displays the level in mg/dl or mmol/l [49].

Principle of test

Glucose in the blood samples mixes with chemical in the test strip and produces a small electronic current. The amount of the current produced changed with the amount of glucose in the blood. The glucose meter measures the strength of the current and displays the results as a blood glucose level [49].

5.8. Data Quality Assurance

The quality of any research depends on the quality of data that are used as input for that research. Therefore, the quality of the blood and the participant information was ensured by collecting and processing using a standard operating procedure.

5.8.1. Pre analytical

To collect the socio-demographic data, age, gender, job, and health status, smoking status and getting any medications the questioner was translated to local languages Tigrigna and Amharic to make certain that the participants can understand the questions at the time of interview.

Concerning sample collection, transportation and processing the principal investigator together with senior laboratory technologist assembled blood sample collection materials. Principal investigator strictly followed the SOP to assure that sample collected on EDTA tube, labeling with participant identification number, and check for hemolysis, clot, correct volume etc... Before participants sample analyzed the status and integrity of reagents were checked. For those other biochemical parameters, the collected venous sample was waited for 20-25 minute to clot, then centrifuged the clotted specimen at 4000rpm for 5 minutes to separate serum for analysis. To ensure the quality of the result, the quality of the instrument, reagent expiration, reagent volume, and room temperature were checked by daily, weekly and monthly maintenance.

5.8.2. Analytical

The reliability of the study finding especially the analytical part was guaranteed by running three levels of commercially prepared hematology cell controls (Normal, Low and High) before analyzing participants specimen. For the chemistry analysis the quality controls normal (N) and pathologic condition (P) were run. Sample was analyzed by using standard operating procedure (SOP) after running quality controls and the control result was passing.

5.8.3. Post analytic

For avoiding any clerical error, printout results that generated by the analyzer used. No result from the screen of the analyzers was recorded by hand. Data entered using double entry method to trace data entry errors which has strong negative effect on study results and conclusions.

5.9. Data analysis and interpretation

The completed questionnaire was checked for completeness, consistency and coded by the principal investigator. Data was entered and analyzed by using SPSS Version 23.

Tables were used for descriptive data. Mean used as measurement of location. Independent t-test used to evaluate the compression between T2DM patients and controls subjects regarding of the hematological and biochemical parameters. Also, P-value used to check the presence or absence significant association between T2DM and hematological or biochemical changes. The P value <0.05 considered as significant association between independent variables and outcome. Percentage and frequency used to evaluate the age, gender distribution between T2DM and control subjects. Pearson correlation also used to determine correlation of hematology or biochemical changes.

5.10. Operational definitions

- **Hyperglycemia** – A condition when there is excess amount of glucose (130mg/dl) in the blood circulation.
- **Type 2 diabetes mellitus patients-** the diabetic patients that at least two months period from their diagnosis
- **Hematological parameters** - are parameters such as RBC count, HB, HCT, MCV, MCH MCHC and RDW; WBC count, lymphocyte count, neutrophil count, monocyte count, eosinophil count, basophil count, platelet count and MPV.
- **Anemia-** is a hemoglobin level <10.5 mg/dl in the blood
- **Thrombocytosis** -A condition in which platelets count >450*10^3/µl
- **Leukocytosis**– the number of white blood cells (leukocytes count) >14.5*10^3 µl
- **Biochemical parameters:** ALT, AST, ALP, urea and creatinine
- **Physical exercise:** scheduled and regular activity practice by participants, minimum 3 days a week.

5.11. Ethical considerations

Ethical clearance obtained/approved from the ethical review committee of the department of Medical Laboratory Science Addis Ababa University, letter of cooperation was written to St. Marry Hospital by the department. Every participant permitted us their consent to be included in this stud by his/her willingness. Confidentiality of the information collected maintained by using code numbers for participants. Data captured on the questionnaire with a code and transferred to the database anonymously. In addition, the clinical specimens collect during the study period was used for the stated objectives. For those participants who had haematological abnormality associated with type 2 diabetes mellitus, farther diagnosis was done and appropriate treatment prescribed by physicians and health education was given to create awareness.

5.12. Dissemination of the result

The final result of the study will be submitted to department of medical laboratory science, College of Health Sciences, Addis Ababa University. In addition, a copy of this material will be given to study site Aksum St. Marry Hospital. The result will also be submitted to peer reviewed local and international journals for publication.

6. Result

Socio demographic characteristics of study groups

In this study a total of 168 participants, 84 Type II diabetes mellitus patients(T2DM) and 84 apparently healthy controls having age range from 18 to 70 years were enrolled. Of the study participants majority were females, among cases (59.5%) as well controls (51.2%). Most of the T2DM patients were at primary level (32.2%) of education whereas majority of the controls were diploma and above (40.5%). Regarding marital status, majority of the study participants were married (82.1%) T2DM and (88.1%) controls. Based on occupation majority of T2DM patients (33.3%) and controls (36.9%) were privet business owners. More over 91.7% of T2DM patients and 89.3% of the controls live in urban. About (69%) of T2DM and (52.4%) of the controls were shown not to have do regular physical exercise practices. Similarly, 31% and 9.5% of the cases and controls do not have the habit of smoking respectively [table 2].

Table 2. Socio demographic characteristics of study participants at Aksum St. Marry Hospitals Tigray, Northern Ethiopia from December 2019- April 2020, N=168

Study variables		T2DM patients		Control cases	
		Frequency	Percent	Frequency	Percent
Sex	Male	34	40.5	41	48.8
	Female	50	59.5	43	51.2
Age in years	18-24	1	1.2	0	0.0
	25-45	21	25.0	26	30.9
	46-70	62	73.8	58	69.1
Marital status:	Not married	0	0	0	0
	Married	69	82.1	74	88.1
	Divorced	8	9.5	7	8.3
	Widowed	7	8.3	3	3.6
Education:	Illiterate	18	21.4	7	8.3
	Primary (1-8)	27	32.1	13	15.5

	Secondary (9-12)	23	27.4	30	35.7
	Diploma and above	16	19	34	40.5
Residence:	Urban	77	91.7	75	89.3
	Rural	7	8.3	9	10.7
Occupation	Government or private employee	14	16.7	7	8.3
	Private business owner	30	35.7	31	36.9
	Daily worker	7	8.3	6	7.1
	House maid	19	22.6	19	22.6
physical exercise	Yes	21	25	40	47.6
	No	63	75	44	52.4
Smoking habit	Yes	26	31	8	9.5
	No	58	69	76	90.5
Alcohol drinking habit	No	41	48.8	51	60.7
	Yes	43	51.2	33	39.3

Anthropometric measurements

The results of this study showed anthropometric measurements of T2DM patients and controls, mean measure of diastolic blood pressure significantly higher in T2DM patients (82±10.3 mmHg) compared to controls (72 ±3.6 mmHg) ($p<0.001$). Also, the mean measure of systolic blood pressure was significantly higher in T2DM patients compare to controls that had ($p<0.001$). The mean level body mass index of T2DM patients was significantly higher than the controls ($p<0.001$). And the mean measure of whist circumference was significantly higher in T2DM patients in compare to controls ($p<0.05$) [table 3].

Table 3. anthropometric measurements of the study participants at Aksum St. Marry Hospitals Tigray, Northern Ethiopia from December 2019- April 2020, N=168

Variable	Study group	N	Mean	Std. deviation	P-value
Body mass index	T2DM patients	84	23.84	1.722	<0.001
	Control group	84	21.72	1.296	
Blood pressure(diastolic)	T2DM patients	84	81.54	10.29	<0.001
	Control group	84	72.26	3.60	
blood pressure (systolic)	T2DM patients	84	135.65	22.43	<0.001
	Control group	84	110.60	9.08	
Waist Circumference	T2DM patients	84	83.83	5.48	<0.001
	Control group	84	80.61	2.0	

. Comparison of hematological and biochemical parameters

In this study of the 84 T2DM patients and 84 controls, the mean of total WBC, neutrophil, lymphocyte, eosinophils, basophils, platelet count and MPV were significantly higher in the case group than the control group ($P<0.05$). On the other hand, the means of, RBC count ($p=0.014$)

and hemoglobin level ($p<0.001$) were significantly decreased compare to controls. The mean of platelet count and MPV was significantly increased ($p=0.025$& $p<0.001$) respectively compared to control group [table 4].

Regarding to the biochemical parameters the means level of ALT, AST, ALP, urea and creatinine were significantly higher in compare to controls ($p<0.05$). Additionally, the mean level of fasting blood sugar was significantly higher than that of controls ($p<0.05$) [table 5]

Table 4. Hematological parameters in T2DM patients and Control Subjects at Aksum St. Marry Hospital Tigray, Northern Ethiopia from December 2019- April 2020, N=168

Variables	Study group	N	Mean	Standard deviation	P-value
Total WBC count	T2DM patients	84	8.156	2.295	<0.001
	Controls	84	6.315	1.631	
Neutrophil count	T2DM patients	84	3.865	1.517	0.011
	Controls	84	3.364	0.936	
Lymphocyte count	T2DM patients	84	3.254	0.943	<0.001
	Controls	84	2.056	0.738	
Monocyte count	T2DM patients	84	0.613	0.197	0.132
	Controls	84	0.568	0.180	
Eosinophil count	T2DM patients	84	.352	.211	0.019
	Controls	84	.287	.138	
Basophil count	T2DM patients	84	0.070	0.036	<0.001
	Controls	84	0.041	0.019	
RBC count	T2DM patients	84	4.587	.458	0.014
	Controls	84	4.746	.364	

Hemoglobin	T2DM patients	84	13.690	1.063	<0.001
	Controls	84	14.500	.892	
Hematocrit	T2DM patients	84	41.513	3.203	0.030
	Controls	84	43.042	3.326	
MCV	T2DM patients	84	87.350	3.088	0.542
	Controls	84	87.617	2.561	
MCH	T2DM patients	84	28.695	.947	0.936
	Controls	84	28.683	.984	
MCHC	T2DM patients	84	34.2131	.760	0.235
	Controls	84	34.082	.651	
RDW	T2DM patients	84	13.466	.544	0.094
	Controls	84	13.309	.661	
RDW.SD	T2DM patients	84	41.571	2.187	0.943
	Controls	84	41.550	1.523	
PLT count	T2DM patients	84	256.119	54.795	0.025
	Controls	84	232.274	42.723	
MPV	T2DM patients	84	9.423	.903	<0.001
	Controls	84	8.666	.690	

Table 5. Biochemical parameters of study participants at Aksum St. Marry Hospital, Tigray, Northern Ethiopia from December 2019- April 2020, N=168

Variables	Study group	N	Mean	Std. deviation	P-value
ALT	T2DM patients	84	36.235	6.015	<0.001
	control groups	84	20.313	3.475	
AST	T2DM patients	84	37.509	5.966	<0.001
	control groups	84	21.816	3.725	
ALP	T2DM patients	84	228.559	58.192	<0.001
	control groups	84	141.047	26.533	
Urea	T2DM patients	84	45.071	9.205	<0.001
	control groups	84	22.929	3.728	
Creatinine	T2DM patients	84	1.063	.237	<0.001
	control groups	84	.812	.101	
Fast blood sugar	T2DM patients	84	140.916	35.235	<0.001
	control groups	84	85.190	8.931	

7. Discussion

Several research outcomes suggest that hematological indices and biochemical markers were altered in T2DM patients when compared to controls [10, 14, 30]. In type two diabetes mellites patients, persistent hyperglycemia condition exposes RBCs to elevated glucose concentrations, thus resulting in glycation of hemoglobin, prothrombin, fibrinogen, and other proteins. Uncontrolled glucose level makes pressure on the vital organs that results in alteration of the biochemical parameters. In this study a total of 168 study participants (84 T2DM patients and 84 controls) were included to assess hematological and biochemical profiles.

In this study total WBC count was significantly increased in T2DM patients compared to the control groups. Similar findings were reported from the studies conducted in Gondar, North West Ethiopia and El-Beyda, Libya [6, 10]. Also, Lymphocytosis was significantly observed in T2DM patients in comparation to control group. This finding was consistent with studies conducted in Nigeria and karnatak, India [12, 13]. On contrary, study conducted in Pakistan reported that lymphocyte count was decreased in diabetic patients compared to control groups [8]. Additionally, Basophil and Eosinophil count were significantly higher in T2DM than control groups, these results were in agreement with the study conducted in Gondar [10]. On the other hand, our study revealed increased monocyte count among T2DM patients than the control group, but it was not statistically significant. The possible cause for the increasing of WBC count might be due to Oxidative stress and inflammation that caused by chronic hyperglycemia [16]. It is also possible that immune system activation caused by inflammation, and cytokine production such as cytokines (e.g., IL-6) a potent white blood cell differentiation factor, that is produced mostly in adipose tissue by activated white blood cells [15]. Activated leukocytes can also liberate superoxide radicals and proteases that promote oxidative stress. It is expected that low grade chronic inflammatory responses, together with other risk factors can lead to extensive vascular damage, endothelial dysfunction, and increased oxidative stress, ultimately participating in the pathogenesis of diabetic microvascular and macrovascular complications [42].

Our study also showed that mean RBC count was significantly decreased (lowered) in T2DM patients than that of the control groups. Similarly, a study conducted in Uttar Pradesh, India reported that the mean RBC count was decreased in T2DM patients compared to the controls [13]. Additionally, the mean hemoglobin and hematocrit (PCV) level were significantly lower in

T2DM patients than the controls. On the other hand, the study conducted in Karachi, Pakistan revealed significantly higher RBC count, hemoglobin level and PCV level in T2DM patients than the control group [8]. Elevation of glucose concentration in blood is one of the major factors that effects the erythrocyte morphology. The possible mechanism of effect may be that chronic hyperglycemic condition causes to non-enzymatic glycosylation of RBCs protein membrane that leading to accelerated aging of RBCs and decrease in count [17]. Uncontrolled glucose may increase the production of lipid peroxides that causing hemolysis of RBCs or it may cause anemia as secondary disorder [43]. Hyperglycemia can also contribute to the formation of advanced glycation end products that elevates oxidative stress by stimulating increased production of free oxygen radicals, that damage the cell DNA and cause apoptosis of cells. And also, chronic hyperglycemia makes consistent pressure on the vital organs especially on kidney, as a result affects the erythropoietin production and leads to low RBC production. Hyperglycemia affects RBC indices by changing their properties through alteration and deformation of the RBC membrane and aggregation [13].

Hemoglobin level was also the other parameter that had significantly lowered in cases than the control group. This was in agreement with a study conducted in University of Gondar [43]. The reduction Hgb level in DM patients may be due to the heme part of Hgb is subjected to glycation, the excess glucose present in blood reacts with Hgb to form glycosylated Hgb, as a result Hgb level is decreased [43]. The other RBC indices like MCV, MCH, MCHC, and RDW-SD did not show statistically significant variation between the T2DM patients and the controls.

The current study also revealed that the mean platelet count and MPV of T2DM patients were significantly higher than the controls. study conducted in Karachi, Pakistan reveled findings in agreement with this study [8]. The increment of platelet count in T2DM patients may be because of the response of body to complications of the vascular systems caused by diabetes mellitus [11]. There might be small vascular bleeds due the rupture of atherothrombotic plagues leading to bone marrow stimulation to tackle bleeding as result produce larger hyperactive platelets [42]. Moreover, chronic hyperglycemia and the platelet granule secretions, may result in variation of platelet size and increase in MPV among T2DM patients [41].

According to this study, the mean levels of ALP, ALT and AST were significantly higher in T2DM compared with control group. Our finding is supported by a, study conducted in Northwest Ethiopia which reported as the levels of ALT and AST are significantly higher in cases than the controls [19]. Similarly, a study conducted in Madhya Pradesh, India reported results of ALT and AST significantly increased which are similar to this study finding [20]. Another study conducted in Missan University, Iraq also showed the levels of ALT, AST and ALP were significantly elevated compared to controls, which were in agreement with this study findings [14]. The elevation of these biochemicals may be due to increases in glycogen/insulin effect on liver cells. The increase of glycogen lysis (breakdown of stored glycogen) and gluconeogenesis (glucose production from non-carbohydrate precursors) to compensate and maintain the glucose balance in the body. So, the accumulation of fat on liver cells leads to hepatic cell injury and this leads to leak out of the liver enzymes (ALT, AST, ALP). These bio markers serve as indicators of hepatocytes injury [24, 25]. The insulin-resistant state is also characterized by an increase in pro-inflammatory cytokines such as tumor necrosis factor (TNF), which may also contribute to hepatocellular injury [40].

In this study, the mean levels of creatinine and urea were significantly elevated in T2DM patients than the control groups. This study was in agreement with a study conducted in Rajasthan India [26]. Diabetes is the most common cause of kidney failure. Hyperuricemia has been found to be associated with obesity and insulin resistance and consequently with type 2 diabetes mellitus. So, hyper uremia induces endothelial dysfunction which results in nephropathy in type 2 DM patients [27].

8. Conclusion

Hematological and biochemical parameters in diabetes patients had altered compared with control group. Poor control of blood glucose causes alteration in various biochemical parameters as well as hematological indices. Total WBC count, neutrophil count, lymphocyte count, eosinophil count, basophil count, platelet count, MPV, ALT, AST ALP, Urea and creatinine were significantly increased in T2DM patients than in controls. RBC count, hemoglobin and PCV were significantly decreased in T2DM in contrast to control group.

9. Strength and limitation of the study

9.1 Strength of the study

- This study used primary data as the source of data so it had good quality of data
- The study includes both hematological and biochemical profiles

9.2 Limitation of the study

- The study is difficult to generalize for the population as it is institutional based study
- The study was cross-sectional therefore it doesn't show cause and effect
- Single test evaluation may not show exactly alteration of test values in the patients due to potential variability
- Other important tests were not included due to resource limitation e.g., lipid profiles

10. Recommendation

- In parallel to fast blood sugar checking hematological profiles and biochemical markers assessment should be performed (at least ones in two appointments to evaluate prognosis.
- Health care providers especially Physicians should consider the alteration in hematological and bio chemical parameters to prevent sever complications
- Future research should be directed to conducting a multicenter study of similar objectives to provide data set for evaluating and monitoring this important intervention, and provide information for health services provision.

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Annex I. Participant Information Sheet

1. English Version

Principal investigator: Kidane Yhdego

Institution: Addis Ababa University, College of health Science, Department of Medical Laboratory Science

Title of the project: Alteration of some Hematological biochemical parameters of type two diabetes mellitus Patients follow at Aksum St. Marry, Aksum, Tigray, North Ethiopia.

Introduction: Dear study participants I am MSc student of Addis Ababa University, School of Medical laboratory Sciences. You are invited to participate on the study of Alteration of some Hematological & biochemical parameters of type two diabetes mellitus Patients follow at Aksum St. Marry, Aksum, Tigray, Northern Ethiopia.

Purpose of the study: The purpose of the study is Assessment of some Hematological biochemical parameters of type two diabetes mellitus Patients follow at Aksum St. Marry, Aksum, Tigray, North Ethiopia.

Duration: the duration of this study depends on the availability of reagent supply and study subjects that it can take about 5 months.

Procedures to be carried on: dear study participant if you agree to participate in the study, you will provide 10 ml of blood sample to assess your hematological & biochemical profile tests.

Risks and Discomfort: then only risk if you participate is there will be minor discomfort or feel pain during blood samples collection. During sample collection from your hand appropriate precaution will be taken and all samples will be collected by trained health professionals.

Expected Benefit: There will not be any cash payment or direct benefit for participating, but you will get free laboratory examination for the testes. If you want the result, it will be given to you. If your result is clinically significant, we will inform you for further diagnosis and treatment.

Confidentiality: Any information that that I am going to collect about you during this research will be kept confidential. Your name and identity on the request paper will be changed to confidentiality code for the purpose of this study.

Withdrawal from the study: Your participation in this study is by your voluntary, and you may drop the participation at any time or you may refuse to answer some of the questions if you feel uncomfortable. You may withdraw at any time without giving reason if you are uncomfortable. You can ask any questions regarding to this study and you have a right to get a laboratory result for free.

If you have any question regarding to this study, please contact by the following address:

Principal Investigator: Kidane Yhdego

Mobile no: +251932050732

Email: bykidane13@gmail.com

Advisors: Zemenu Tamir (BSc, MSc, PhD candidate, Assistant Professor of Hematology and Immunohematology)

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2. Amharic Version

ለተሳታፊዎች መረጃ መስጫ ሰነድ

የጥናቱ ርእስ፡ ኣክሱም ቅ/ማርያም ሆስፒታል የሚገኙ የስኳር ሕሙማን ላይ ሙሉ የደም ምርመራ እና ሌሎች ባዮኬሚካል ሚታየው ለውጥ ምርመራ ና ጥናት

የአጥኚው ስም: ኪዳነ ይህደጎ ባህታ

የተቋሙ ስም: አዲስ አበባ ዩኒቨርሲቲ የህክምና ና ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ትምህርት ክፍል

መግቢያ:የተከበሩ የጥናቱ ተሳታፊ በአክሱም ቅ/ማርያም ሆስፒታል የሚገኙ የስኳር ሕሙማን ላይ የሚደረገዉ የሄማቶሎጂ እና ባዮኬሚካል ምርመራ ዓይነቶች ሚታየው ለውጥ ምርመራ ጥናት ለመሳተፍ

ተጋብዛል ።ይህ ጥናት በ አዲስ አበባ ዩኒቨርሲቲ ላቦራቶሪ ትምህርት ክፍል የጥናትና የሰነምግባር ኬሚቴ የጸደቀዉ ጥናተ መሆንን መግለፅ እንወዳለን ። በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፍቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰአትና ቦታ የማቋረጥ ሙሉ መብትዎን ኣሎት።

የጥናቱ ዋና አላማ፡- በአክሱም ቅ/ማርያም ሆስፒታል የሚገኙ ሰስኳር ሕሙማን ላይ ምርመራ ማድረግ ና ማጥናት ።

የጥናቱ ጊዜ፥ የጥናቱ ግዜ በምናገኘዉ የስኳር በሽተኞች ብዛት የሚወሰን ሲሆን እስከ 5 ወር ለወስድ ይችላል፡፡

ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት: የደም ናሙና በሚሰጥበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም ። ነገር ግን ደም ሲወስድ መጠነኛ የህመም ስሜት ልያስከትል ይችላል ። ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ባላቸዉ ባለሞያ ስለሚመደብና አስፈላጊውን ጥንቃቄ እርምጃ ስለሚወስድ የህመም ስሜት የቀነሰ ነው።

ከጥናቱ የሚያገኙት ጥቅም :ያለ ምንም ክፍያ የሙሉ ደም ምርመራ የስኳር መጠን እና ሌሎች ምርመራ ያደርጋሉ።ችግር ካለ በፍጥነት ህክምና ይሰጣችዋል።

ከርስዎ የምናገኘዉ መረጃ እና ሚስጥራዊነቱ: የእርሶ ስም በዚህ መጠይቅ ላይ አይጠቀስም። በተጨማሪም የሚሰጡት መረጃ እና በሰጡትም ደም ላይ የሚደረገው የምርመራ ውጤት ከተባለለት ጉዳይ ውጪ እንደማይውል እና ሚስጥራዊነቱ የተጠበቀ እንደሚሆን አረጋግጣለሁኝ፡፡

በዚህ ጥናት ላይ ያለዎትን ጥያቄ በሚከተሉት ኣድራሻ በማንኛውም መጠቀም ይችላሉ።

የኣጥኚው ስም: ኪዳነ ይሕደጎ ባህታ ስልክ: +251932050732 Email: bykidane13@gmail.com

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Email: rahelalemu2014 @gmail.com

3. Tigrigna Version

ናይ መፅናዓይ ሽም: ኪዳነ ይሕደጎ ባህታ

ናይ ትካል ሽም: ዩኒቨርሲቲ ኣዲስ አበባ ኮሌጅ ጥዕናን ሕክምና ሳይንስን ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ

ናይቲ መፅናዕቲ ርእሲ፡- ኣብ አክሱም ቅ/ማርያም ሆስፒታል ዝርከቡ ናይ ስኳር ሕሙማት ሙሉእ ደም ምርመራ ምግባርን ዘሎ ለውጢ ምጽናዕን

መእተዊ: ዝተከበሩ(ራ) ናይዚ ጽንዓት ተሳታፊ/ት ኣነ ናይ ዩኒቨርስቲ አድስ አበባ ጥዕናን ሕክምናን ሳይንስ ኮሌጅ ናይ ሕክምና ላቦራቶሪ ክፍሊ ትምህርቲ ብማስተርስ ድግሪ ተምሃራይ እየ። ስለዚ ንሰም ወይ ንሰን ኣብ ሆስፒታል ቅ/ማርየም ዝርከቡ ናይ ስኳር ሕሙማን ናይ ሙሉእ ደም ምርመራ ምግባርን ዝረአ ለውጢ ምጽናዕን ኣብ ዝብል ናይ መመረቂ መፅናዕታዊ ፅሑፍ ተሳታፊ ንክኾኑ ይዕድሞም አለኩ።

ናይቲ መፅናዕቲ ዋና ዓላማ፡- ኣብ አክሱም ቅ/ማርያም ሆስፒታል ዝርከቡ ናይ ስኳር ሕሙማን ናይ ሙሉእ ደም ምርመራ ምግባርን ምፍላጥን።

እቲ ጽንዓት ዝካየደሉ እዋን ፡ ናይቲ ጽንዓት እዋን ዝዉሰን በቶም ንረክቦም ናይ ስኳር ሕሙማት እንትከዉን ክሳብ 5 ወርሒ ክወስድ ይክእል እዩ።

ቐደም ሳዓብ እቲ ጽንዓት፡ ዝተከበሩ(ራ) ናይዚ ጽንዓት ተሳታፊ/ት እዞም ዝስዕቡ ናይዚ ጽንኣት ምስ ተረድኡ እና ፍቃደኛ እንድሕር ኮይኖም ነዚ ጽንዓት ዝከዉን ክሳብ 10 ሚ.ሊ ዝከዉን ደም ክንወስደሎም ኢና።

ምሰቲ መፅናዕቲ ተታሒዙ ዝመፅእ ሳዕቤን፡- ንምርመራ ዝከዉን ደም አብ ዝህብሉ እዋን ምንም ዓይነት ዝኸፍአ ፀገም አየጋጥሞምን። ነገር ግን ደም አብ ዝውሰደሉ እዋን ዝተወሰነ ናይ ምሕማም ስሚዕት ክህሉይ ኽእል እዩ። ይኹን ዳአምበር ደም ንምስብሳብ ልምዲ ብዘለዎም ባዓል ሞያታት ስለ ዝምደቡን አድላዪ ዝኾነ ጥንቃቑን ስለዝውሰድ ናይ ምሕማም ስምዕት አይህሉን።

ካብቲ መፅናዕቲ ዝረክብዎ ጥቕሚ፡ እዚ መፅናዕቲ ናይ ማስተርስ ድግሪ መመረቕ ፅሕፍ ከም ምኻኑ መጠን አብዚ መፅናዕቲ ብምስታፎም ዝረኽብዎ ናይ ገንዘብ ጥቕሚ ዋላኳ እንተዘይሃለዎ ካብቲ መፅናዕቲ ብዝርከብ ውፅኢት ግን ተጠቃሚ እዮም። ነቲ መፅናዕቲ ካብ ዝተወሰደ ደም ዝርከብ ውፅኢት

ብነፃ ይረኽቡ እዮም። ብተወሳኺ አብቲ ናይ ደም ውፅኢት ለውጢ እንተሃልይዎ ምስ ሓኻይም ንክራኽቡን ንኽምርመሩን ይግበር እዩ።

መረዳእታ ብምስጥር ምሕላዉ ዝምልከት ፡ ኣብዚ ጽንዓት ስለ ናቶም ወይ ናተን ንእክቦ ዝኮነ ዓይነት መረዳእታ ብሚስጥር ከም ንሕዘለኩም ነፍልጥ። ነዚ መጽናዕቲ ኢልና ዘሎ ናቶም/ተን መንነት ዝገልጽ ኩሉ መረዳእታ ናብ ሚሽጥር ክንቕይሮ ኢና።ብተወሳኪ እቲ ትህቡና ደም ኮነ መረዳእታ ካብቲ ጽንዓት ወጻኢ ኣይንጥቀመሉን።

ካብቲ መፅናዕቲ ስለምቁራፅ:- አብቲ መፅናዕቲ ምስታፍ ብናቶም/ተን ፍቓደኝነት ዝተመስረተ ኮይኑ ኣብ ማእከል ምቑራፅን ዘይደለይዎ ሕቶ ዘይምምላስ ይኽእሉ/ላ እዮም/የን። ኣብዚ መፅናዕቲ ዘለዎም/ወን ሕቶን/ ርኢቶን አብ ዝኾነ ይኩን ግዜ ክሓቱ/ታ ይክእሉ/ላ።

ንተወሳኺ ሓበሬታ ነዞም ዝስዕቡ ኣድራሻ ይጠቀሙ።

ናይ መፅናዓይ ሽም: ኪዳነ ይሕደጎ ባህታ ሞባይል፡ 0932050732 ኢሜል ፡ bykidane13@gmail.com

ኣማከርቲ 1) ዘሙኑ ታምር ስልኪ፡ +251-911699710

2) ራሐል አለሙ ስልኪ፡ +251929045451

Annex II: Consent Form (study participants)

A. English version

Principal investigator: Kidane Yhdego

Research title: Alteration of some Hematological biochemical parameters of type two diabetes mellitus Patients follow at Aksum St. Marry, Aksum, Tigray, Northern Ethiopia.

I have read, or have had this document read to me in a language that I understand, and I understand the purposes, procedures and risks of this research project as described within it. I understand that at any time I may withdraw from this study without giving a reason. I have had an opportunity to ask questions and I am satisfied with the answers I have received. I know that no special payment for being participating in the study. I freely agree to participate in this study, as described. I understand that I was given a signed copy of this document to keep.

Name of participant____________ Age___ Address__________ Signature_____ Date________

Interviewer's name______________________________________Signature_____________________

Principal investigator Name________________________ Signature________________________

B. Amharic version

የፍቃደኝነት ማረጋገጫ ቅጽ

የጥናቱ ርእስ፡ አክሱም ቅ/ማርያም ሆስፒታል የሚገኙ የስኳር ሕሙማን ላይ ሙሉ የደም ምርመራ ና ጥናት

የአጥኚው ስም: ኪዳነ ይሕደጎ ባህታ

የተቋሙ ስም: አዲስ አበባ ዩኒቨርሲቲ የህክምና ና ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ትምህርት ክፍል

እኔ ከዚህ በታች የተገለጸዉ በዚህ ጥናት ተሳታፊ ልመሆን ስወሰን የጥናቱ ዓላማዎች ኣሰራሮችና ቅድመ ሁኔታዎች በግልጽ በመረዳትና እንዲሁም ከጥናቱ ተሳታፋይነት ፈቓደነኝነቴን በማነኛዉም ግዜ የማስወገድ መብቴን በመራጋገጥ ነዉ።

ሰለዚህ በጥናቱ ተሳታፊ መሆኔን በፈርማየ እየራጋገጥኩ ይህንን ስውስን በጥናቱ ሊከሰቱ የሚችሉ ስጋቶችን በሚገባ የተረዳሁና ከጥናቱ በማነኛዉም ግዜ ራሴን ለምግለል ብወስን ተገቢ የሆኑ ህክምናዎችና እገዛዎች ሁሉ እንደማትነፈጉኝ በማመን ነው። እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጸልኝ መሆኑን በፈርማየ ኣጋግጣለሁ።

የተሳታፊው ስም ....

ፊርማ.............................. ቀን ....

የአጥኚው ስም .... ፊርማ .... ቀን ....

ስለ ትብብርዎ አመሰግናለሁ!

C.Tigrigna version

ናይ ወለንታዊነት መረጋገፂ ቅጥዒ

ናይ መፅናዓይ ሽም: ኪዳነ ይሕደጎ ባህታ

ናይ ትካል ሽም: ዩኒቨርሲቲ አዲስ አበባ ኮሌጅ ጥዕናን ሕክምና ሳይንስን ናይ ሕክምና ላበራቶሪ ትምህርቲ ክፍሊ

ናይቲ መፅናዕቲ ርእሲ፡- ኣብ አክሱም ቅ/ማርያም ሆስፒታል ዝርከቡ ናይ ሰስኳር ሕሙማት ናይ ሙሉእ ደም ምርመራ ምግባርን ምጽናዕን

ኣነ ኣብዚ ታሕቲ ተገሊጹ ዝኒሆ መጽናዕቲ ተሳታፋይ ንምኳን እንትዉስን እንተለኩ ናይዚ ጵንዓት ዘድልዩ ነገራት ብምርዳእ እና ካብዚ ጵንዓት እዚ ምስታፍ ኣብ ዝኮነ ይኩን እዋን ከም ዘግልል ብምርግጋጸይ እዩ። ስለዚ ኣብዚ ጾንዓት ተሳታፋይ ምኳነይ በፈረማይ የራጋግጽ።እዞም ኩሎም መረደእታታት ብዝግባእ ብዝሩድኡኒ ቋንቋ ዝተገለጹለይ ምኳኖም በፈረማይ የራጋግጽ።

ናይ ተሳታፊ/ፊት መለለዪ ቑፅሪ ------------------------- ፊርማ ----------------- ዕለት-----------------

ሓበሬታ ዝኣክበ በዓል ሞያ ሽም ----------------------------- ፊርማ ----------------ዕለት----------------

ስለ ዝተሓባበሩኒ የቐንየለይ!

Annex III: Structured Questionnaire for T2DM patients

Research Title: Assessment of some Hematological biochemical parameters of type two diabetes mellitus Patients follow at Aksum St. Marry, Aksum, Tigray, Northern Ethiopia.

A. English Version

Region ___________ Zone ___________ Woreda __________ Tel: -----------------

Note: Please Encircle or Write the appropriate answer on the provided space.

No.	Variable	Response
1	Sex	1. Male 2. Female
2	Age	
3	Marital Status	1. Single 2. Married 3. Divorced 4. Widowed
4	Education level	1. Illiterate 2. Primary 3.Secondary 4.Diploma and above
5	Residence	1. Rural 2. Urban
6	Occupation	1. Employee 2. Business owner 2. Daily worker 4. House maid
7	Do you do daily physical exercise?	1. Yes 2. No
8	Do you have smoking habit	1. Yes 2. No
9	Do you have habit of drinking alcohol?	1. Yes 2. No
10	If your answer for Q#12 is yes, for how much?	1. 500 ml/day 2. Above 500ml

11	Do you have history of cancer disease?	1.Yes 2. No
12	do you have history of liver disease	1. yes 2. No
13	If your answer to Q#14 is yes, do you take medication	1. Yes 2. No
14	Do you have history of kidney disease?	1. Yes 2. No
15	If your answer to Q #17 is yes, do you take any medication?	1. Yes 2. No

I thank you for your cooperation

B. Amharic version

መለያ: የተቋሙ ስም ___________________

ክልል___________ ወረዳ___________ ቀበሌ__________

ማሳሰብያ: እባክዎ ትክክለኛውን መልስ ያክብቡ ወይም ይፃፉ።

ተ.ቁ	ጥያቄዎች	መልስ
1	ጾታ	1. ወንድ 2. ሴት
2	እድሜ	
3	የጋብቻ ሁኔታ	1. ያላገባ/ች 2. በትዳር ላይ 3. የፈታ/ች 4. በሞት ምክንያት የተለያዩ
4	የትምህርት ደረጃ	1. ያልተማረ 2. ኣንደኛ ደረጃ(1-8) 3. ሁለተኛ ደረጃ (9-12) 4.ዲፕሎማ/ድግሪና ከዛ በላይ
5	መነርያ	1.ገጠር 2.ከተማ
6	ስራ	
7	የኣካል እንቅስቃሰ ያዘወትራሉ?	1. አዎ 2. . ኣላዘወትርም
8	ሲጋራ የመጨሰ ልምድ ኣሎት	1. አዎ 2. የለኝም
9	አልኮል የመውሰድ ልምድ አሎት?	1. አዎ 2. የለም
10	ለተራ ቁጥር 12 መልስዎ አዎ ከሆነ	1.500ሚሊ 2. ከዛ በላይ

	በቀን ስንት?	
11	በካንሰር በሽታ ተይዞ ያዉቃሉ	1. አዎ 2. የለም
12	የጉበት በሽታ ተይዞ ያዉቃሉ	1. አዎ 2. የለም
13	ለተራ ቁጥር 12 መልስዎ አዎ ከሆነ መድሃኒት ወስድዋል	1. አዎ 2. የለም
14	የከላሊት በሽታ ተይዞ ያዉቃሉ	1. አዎ 2. የለም
15	ለተራ ቁጥር 16 መልስዎ አዎ ከሆነ መድሃኒት ወስድዋል	1. አዎ 2. የለም

ስለ ትብብርዎ አመሰግናለሁ!

C. Tigrigna version

መለለዪ: ናይቲ ተቋም ሽም:__________________ ናይቲ ተቋም ኮድ:__________________

ክፍለ ከተማ__________ ወረዳ__________ ጣበያ_________ስልኪ: ----------------

መተሓሳሰቢ: ትኽክለኛ ዝኾነ መልሲ የክብቡ ወይ ከዓ ይፅሓፉ።

ተ.ቑ	ሕቶታት	መልሲ
1	ፆታ	1. ተባዕታይ 2. አንስታይ
2	ዕድመ	
3	ኹነታት ሓዳር	1. ዘይተመርዓወ/ወት 2. በዓለ ሓዳር 3. ዝፈትሐ//ሐት 4. ብሞት ምኽንያት ዝተፈላለዩ
4	ናይትምህርቲ ደረጃ	1. ዘይተምሃረ 2.ቀዳማይ ደረጃ (1-8) 3. ካልኣይ ደረጃ (9-12) 4. ዲፕሎማ/ድግሪንልዕሉኡን
5	መንበሪ	1.ገጠር 2.ከተማ
6	ስራሕ	
7	አካላዊ ምንቅስቃስ የዘውትራ ዶ?	1. እወ 2. አይፋል
8	ሽጋራ ናይ ምትካክ ልምዲ ኣለዎም /ን ዶ?	1. እወ 2. ኣይፋል
9	መስተ ናይምስታይ ልምዲ አለዎም/ን	1.እወ 2. አይፋል
10	ንተራ ቑፅሪ 10 መልሶም/ሰን እወ እንተኾይኑ,ክንደይ ዝኣክል ግዚ	1. 500ሚሊ 2. ልዕሊኡ

11	ሕማም መንስሮ ተታሒዞም/ን ይፈልጡ/ጣ ዶ?	1.እወ 2. ኣይፋል
12	ሕማም ጸላም ከብዲ ተታሒዞም/ን ይፈልጡ/ጣ ዶ?	1.እወ 2. ኣይፋል
13	ንሕቶ ተራ ቑፅሪ 12 መልሶም/ሰን እወ እንተኾይኑ, መድሃኒት ከ ወሲዶም ዶ?	1.እወ 2. ኣይፋል
14	ሕማምከላሊት ተታሒዞም/ን ይፈልጡ/ጣ ዶ?	1.አወ 2. ኣይፋል
15	ንሕቶ ተራ ቑፅሪ 16 መልሶም/ሰን እወ እንተኾይኑ,መድሃኒት ከ ወሲዶም ዶ?	1.አወ 2. ኣይፋል

ስለዝተሓባበሩኒ ካብ ልቢ ኣመስግን

Annex-IV: standard operating procedures for blood collection

Equipment

- 21-gauge needle for each participant
- Blood collection tubes for each participant
- Tourniquet
- Box of nitrile /vinyl gloves
- 70% Alcohol wipes
- Cotton balls/swabs
- Bandages
- Pillow/pad for raising arm to comfortable elevation
- Apple/orange juice and snacks for fasting participants
- Disposable, single use materials or equipment are to be used whenever possible
- Any reusable materials or equipment must be cleaned and disinfected with

  Alcohol-based sanitizers before use with another participant

Safeguards /safety procedures

• A new pair of disposable latex/vinyl gloves is used with each participant. Gloves are for single-procedure use only. Gloves should always be removed using a glove-to-glove or skin-to-skin technique which will prevent contaminating the hands.

• The use of gloves does not replace the need for hand hygiene. Hands should be properly washed before the gloves are put on and after the gloves are removed. Hand hygiene is also needed before and after the replacement of gloves during a procedure or in between tasks.

• Participants are reminded to do no heavy lifting for 24 hours.

Procedure for drawing blood

Steps 1; Assemble equipment

Collect all the equipment needed for the procedure and place it within safe pack which is simple for transport to collection site and place easy reach on a flat surface table ensuring that all the items are clearly visible.

Step 2; Identify and prepare the participants and allow to sit comfortably preferably be stretching his/her arm

Step 3; Perform hand hygiene and put on gloves

Step 4; Select the site of injection

Step 5; apply the tourniquet

Step 6; Prepare the arm by swabbing the antecubital fossa with a gauze pad or cotton moistened with 70% alcohol.

Step 7; insert the needle properly into the vein

Step 8; draw the required amount of blood

Step 9; Fill the laboratory sample tubes and mix properly

- When obtaining multiple tubes of blood, use evacuated tubes with a needle and tube holder. This system allows the tubes to be filled directly. If this system is not available, use a syringe or winged needle set instead.
- If a syringe or winged needle set is used, best practice is to place the tube into a rack before filling the tube. To prevent needle-sticks, use one hand to fill the tube or use a needle shield between the needle and the hand holding the tube.
- Pierce the stopper on the tube with the needle directly above the tube using slow, steady pressure. Do not press the syringe plunger because additional pressure increases the risk of hemolytic.
- Where possible, keep the tubes in a rack and move the rack towards you. Inject downwards into the appropriate colored stopper. DO NOT remove the stopper because it will release the vacuum.

- If the sample tube does not have a rubber stopper, inject extremely slowly into the tube as minimizing the pressure and velocity used to transfer the specimen reduces the risk of hemolysis. DO NOT recap and remove the needle.
- Before dispatch, invert the tubes containing additives for the required number of times (as specified by the local laboratory).

Step 10; Draw samples in the correct order and label the sample using unique code of participants

Step 11; Clean contaminated surfaces and complete patient procedure

Step 12; Prepare samples for transportation

Step 13; Clean up spills of blood or body fluids

Annex V: SOP for Sysmex KX-21N hematology analyzer

The Sysmex KX-21N is a quantitative automated hematology analyzer for in vitro diagnostic use. Examination of the numerical and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemias, leukemias, allergic reactions, viral, bacterial, and parasitic infections. The KX-21 processes approximately 60 samples an hour and displays on the LCD screen the particle distribution curves of WBC, RBC, and platelets, along with data of 17 parameters, as the analysis results. The Sysmex KX-21N analyzer directly measures the WBC, RBC, HGB, HCT, PLT, LYM#, MIXED# and NEUT#. The remaining parameters are calculated or derived, MCV, MCH, MCHC, MPV, RDW-CV and RDW-SD, and differential percentages LYM%, MIXED%, NEUT%.

DETECTION PRINCIPLE

The KX-21N counts and sizes red blood cells (RBC) and platelets (PLT) using electronic resistance detection. Hematocrit (HCT) is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin (HGB) is converted to methemoglobin, and read photometrically at 555 nm. White blood cells (WBC) are analyzed by direct current and discriminated into a three-part differential using Particle Distribution Analysis

(PDA). The resulting WBC histogram is discriminated into lymphocyte, neutrophil and mixed cell populations. The mixed cell population contains monocytes, basophils and eosinophils**.**

DC Detection Method

Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both side of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data.

Non-Cyanide Hemoglobin Analysis Method

To analyze hemoglobin by automated methods, the Cyanmethemoglobin method or Oxyhemoglobin method have so far been the main stream. Cyanmethemoglobin method was recommended as the international standard method in 1966 by ICSH (International Committee for Standardization in Hematology). This method, however, is so low in hemoglobin conversion rate that it cannot be said an appropriate method in the automated process in which multi-sample processing is the pre-condition. In addition, this method uses the reagent of cyanide compound which is a poisonous substance and requires waste processing; thus, it can hardly be called an environmentally favorable method. At present, this method cannot be said suitable for a fully-automated instrument which is required to handle a large amount of waste. The Oxyhemoglobin method, on the other hand, is faster in hemoglobin conversion rate; in fact, blood hemoglobin is converted instantaneously into oxyhemoglobin. Also, it does not contain poisonous substance as cyanmethemoglobin method, making the method suitable for automation. This method, however, is unable to convert methemoglobin into oxyhemoglobin. Consequently, when a great amount of methemoglobin is contained as in control blood, lower-than-real values result, although usual human blood poses no problems.

Non-cyanide hemoglobin analysis method utilizes the advantages of both of the above methods. Non-cyanide hemoglobin analysis method rapidly converts blood hemoglobin as the Oxyhemoglobin method and contains no poisonous substance, making it suitable for automated method. Being capable of analyzing methemoglobin, this method can accurately analyze control blood, etc. which contain methemoglobin.

Reagents

Cell Pack: - is ready to use for impedance and photoelectrical analysis of whole blood and its ingredients are: sodium chloride, boric acid, sodium tetra borate, and K2EDTA.

Stromatolyzer WH: - is ready to use lysing reagent to analyze the leucocytes by lysing the RBC and left the WBC free and easy to count; whole blood sample by resistance measurement and photometric measurement and its ingredients are: nonionic surfactant, organic quaternary ammonium salt.

Cell Clean: - is a strong alkaline detergent to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of Sysmex analyzer.

Reagent preparation: Reagents are commercially prepared.

Reagent stability and storage: All reagents are stable at room temperature up to their expiry date.

Supplies

- Disposable glove
- BD Vacutainer tube (EDTA anticoagulant tube)
- Dry gauze
- Cotton Swab
- Vacutainer
- Needle with holder
- 70% Ethanol alcohol
- Tourniquet68

Equipment:

- Sysmex KX-21N

- Electrical blood mixer
- Electrical power stabilizer (500 or 1000 w)

Sample

- Whole blood specimen collected in K2EDTA anticoagulant tube.
- Serum collected on yellow tope serum separator tube

Amount required

¾ of the lavender collection tube (3 ml)

Enough amount in yellow top

Transport and Storage: 2-8 0C

Aspiration

- Whole blood mode- Approximately 50µl
- Pre-diluted mode-Approximately 20µl

Dilution

In pre-dilution mode a sample is diluted in to 1:26 before analysis.

Instrument dilution

In whole blood mode

- The dilution for HGB and WBC 1:500
- The dilution for RBC 1:25000

In pre-dilution mode

- The dilution for HGB and WBC is 1:1000
- The dilution for RBC is 1:25000

CBC Analysis using sysmex Sysmex KX-21N

WBC/HGB Analysis procedure

In WBC and HGB analysis, the volume of WBC and hemoglobin in the blood are measured. The flow of WBC/HGB analysis is described below:

Whole Blood Mode

1. Blood is aspirated from the sample probe into the sample rotor valve.
2. 6 ul of blood measured by the sample rotor valve is transferred to the WBC transducer chamber along with 1.994 mL of diluent. At the same time, 1.0 mL of WBC/HGB lyse is added to prepare 1:500 dilution sample. When the solution is made to react in this status for approximately 10 seconds, RBC is hemolyzed and platelets shrink, with WBC membrane held as they are. At the same time, hemoglobin is converted into red colored methemoglobin.
3. Of the diluted/hemolyzed sample in the WBC transducer chamber, approximately 1 mL is transferred to the HGB flow cell.
4. 500 ul of sample in the WBC transducer is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection method.
5. In the HGB flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) is applied to the sample in the HGB flow cell. Concentration of this sample is measured as absorbance. This absorbance is compared with that of the diluent alone that was measured before addition of the sample, thereby calculating HGB (hemoglobin value).

Pre-diluted Mode

1. Blood sample that was diluted beforehand to 1:26 dilution using CELLPACK. This sample is aspirated from the sample probe into the sample rotor valve.
2. 78 ul of diluted blood measured by the sample rotor valve is transferred to the WBC transducer chamber along with 1.922 mL of diluent. At this time, 1.0 mL of WBC/HGB lyse is added to prepare 1:1000 dilution sample. When the solution is made to react in

this status for approximately 10 seconds, RBC is hemolyzed and platelets shrink, with WBC membrane held as they are. At the same time, hemoglobin is converted into red colored methemoglobin.

3. Of the diluted/hemolyzed sample in the WBC transducer chamber, approximately 1 mL is transferred to the HGB flow cell.
4. 500 ul of sample in the WBC transducer chamber is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection method.
5. In the HGB flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) is applied to the sample in the HGB flow cell. Concentration of this sample is measured as absorbance. This absorbance is compared with that of the diluent alone that was measured before addition of the sample, thereby calculating HGB (hemoglobin value).

RBC/PLT Analysis procedure

In RBC/PLT analysis, RBC and platelet count in the blood are measured. The flow of RBC/PLT analysis is described below:

Whole Blood Mode

1. Blood is aspirated from the sample probe into the sample rotor valve.
2. 4.0 ul of blood measured by the sample rotor valve is diluted into 1:500 with 1.996 mL of diluent and brought to the mixing chamber as diluted sample. (1st step dilution)
3. Out of the 1:500 dilution sample, 40 μL is measured by the sample rotor valve, diluted into 1:25000 with 1.960 mL of diluent, then transferred to the RBC/PLT transducer chamber. (2nd step dilution)
4. 250 ul of the sample in the RBC/PLT transducer chamber is aspirated through the aperture. At this time, RBC and PLT are counted by the DC detection method. At the same time, HCT (hematocrit value) is calculated by RBC pulse height detection method.

Pre-diluted Mode

1. Blood sample that was diluted beforehand to 1:26 dilution using CELLPACK. This sample is aspirated from the sample probe into the sample rotor valve.
2. 2.08 ul of diluted blood measured by the sample rotor valve is transferred in 1.99792 mL of diluent to the RBC/PLT transducer chamber, and is made into 1:25000 dilution sample.
3. Of the sample in the RBC/PLT transducer chamber, 250 µL is aspirated through the aperture. At this time, RBC and PLT are calculated by the DC detection method. At the same time, HCT (hematocrit value) is calculated by RBC pulse height detection method.

Calculation of RBC Constant

RBC constant (mean RBC volume, mean RBC hemoglobin, mean RBC hemoglobin concentration) is calculated from RBC, HGB, and HCT.

1. Mean RBC Volume (MCV)

   Calculation is made from RBC and HCT by the formula below:

   $$MCV\ (fL) = \frac{HCT\ (\%)}{RBC\ (x10^6/ul)} x10$$

2. Mean RBC Hemoglobin (MCH)

   Calculation is made from RBC and HGB by the formula below:

   $$MCH\ (pg) = \frac{HGB\ (g/dL)}{RBC\ (x10^6/ul)} x10$$

4. Mean RBC Hemoglobin Concentration (MCHC)

   Calculation is made from HCT and HGB by the formula below:

   $$MCHC\ (g/dL) = \frac{HGB\ (g/dL)}{HCT\ (\%)} x100$$

General Analytical procedure

1. Mix the sample sufficiently

2. Remove the plug while taking care not to allow blood scatter

3. Set the tube to the sample probe and in that condition, press the start switch

4. The buzzer sounds two times - "beep, beep" - and when the LCD screen displays "Analyzing,"remove the tube. After that, the unit executes automatic analysis and displays the result on the LCD screen. Then the unit turns to the Ready status, becoming ready for analysis of the next samples.

5. When the LCD screen displays "Ready," prepare the next samples and repeat the above procedures.To analyze the sample in Pre diluted Mode, first switch the analyzer to Pre diluted mode and follow the procedure as Whole Blood analysis.

Alanine amino transaminase

Principle of the test

Alanine aminotransferase (ALT/GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD+, proportional to the activity of ALT present in the sample.

L-Alanine + 2-Oxoglutarate - ALT→ L-Glutamate + Pyruvate

Pyruvate + NADH + H -----LDH --→ Lactate + NAD+

Procedure

1. Pre incubate working reagent, samples and controls to reaction temperature.

2. Set the photometer to 0 absorbance with distilled water.

3. Pipette into a cuvette:

Reaction temperature	37ºC	30ºC
Working reagent Sample	1.0 mL 50 µL	1.0 mL 100 µL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.

5. Incubate for 1 minute and record initial absorbance reading.

6. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.

7. Calculate the difference between absorbance.

8. Calculate the mean of the results to obtain the average change in absorbance per minute ($\Delta A/min$).

Calculations

$$U/L = \Delta A/min \times 3333\ (37^{\circ}C)$$

$$U/L = \Delta A/min \times 1746\ (30^{\circ}C)$$

Samples with $\Delta A/min$ exceeding 0.160 at 340 nm should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

Result interpretation

The liver is especially rich in ALT, being the enzyme measurement used primarily as a test for infectious and toxic hepatitis, although high levels of both ALT and AST may also be found in cases of liver cell damage and acute pancreatitis, suggesting that the obstruction of the biliary tree by the edematous pancreas and the presence of associate hepatic disease may contribute to elevated AST levels in these patients. Slight or moderate elevations of AST and ALT activities may be observed after intake of alcohol and after administration of various drugs, such as salicylates, opiates and ampicillin. [50]

Aspartate amino transaminase

Principle

Aspartate aminotransferase (AST/GOT) catalyzes the transfer of the amino group from aspartate to oxoglutarate with the formation of glutamate and oxalacetate. The latter is reduced to malate-by-malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide

(NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD+, proportional to the activity of AST present in the sample1.

L-Aspartate + 2-Oxoglutarate $\xrightarrow{AST}$ L-Glutamate + Oxalacetate

Oxalacetate + NADH + H+ $\xrightarrow{MDH}$ L-Malate + NAD

Procedure

1. Preincubate working reagent, samples and controls to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette

Reaction temperature	37ºC	30ºC
Working reagent Sample or control	1.0 mL 50 µL	1.0 mL 100 µL

4Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.

4. Incubate for 1 minute and record initial absorbance reading.
5. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.
6. Calculate the difference between absorbance.
7. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

Calculations

U/L = ΔA/min x 3333 (37ºC)

U/L = ΔA/min x 1746 (30ºC)

Samples with ΔA/min exceeding 0.160 at 340 nm should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

Result interpretation

The liver is specially rich in ALT, being this enzyme measurement used primarily as a test for infectious and toxic hepatitis, although high levels of both ALT and AST may also be found in cases of liver cell damage and acute pancreatitis, suggesting that the obstruction of the biliary tree by the edematous pancreas and the presence of associate hepatic disease may contribute to elevated AST levels in these patients. Slight or moderate elevations of AST and ALT activities may be observed after intake of alcohol and after administration of various drugs, such as salicylates, opiates and ampicillin [50].

Alkaline phosphatase

Principle of the test

Alkaline phosphatase (ALP) catalyze the hydrolysis of 4- nitrophenylphosphate (4-NPP) with the formation of free 4- nitrophenol and inorganic phosphate, acting the alkaline buffer as a phosphate-group acceptor. The reaction is monitored kinetically at 405 nm by the rate of formation of 4-nitrophenol, proportional to the activity of ALP present in the sample.

$$\text{4-Nitrophenylphosphate} + H2O \xrightarrow[\text{PH>9}]{\text{ALP}} \text{4-Nitrophenol} + Pi$$

Procedure of the test

1. Preincubate working reagent, samples and controls to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette**:**

Working reagent Sample or control	1.0 mL 20 µL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
5. Incubate for 1 minute and record initial absorbance reading.
6. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.
7. Calculate the difference between absorbances.
8. Calculate the mean of the results to obtain the average change in absorbance per minute ($\Delta A/min$)

Calculations

$U/L = \Delta A/min \times 2764$

Samples with $\Delta A/min$ exceeding 0.250 at 405 nm should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

Interpretation of result

Serum ALP measurements are of particular interest in the investigation of two groups of conditions: bone disease and hepato biliary disease. Physiological bone growth elevates ALP in serum of growing children and a transient elevation may be found during healing of bone fractures. The response to the liver to any form of biliary tree obstruction is to synthesize more ALP. Intrahepatic obstruction of the bile flow by invading cancer or drugs raises serum ALP. Any drug that is hepatotoxic or induces cholestasis will greatly increase serum ALP [50].

Sysmex Hematology Analyzer Reference Range

Parameter	Range for Females	Range for Males	Unit
WBC	3.17 – 8. 4	3.58-8.15	$\times10^3$/ml
RBC	3.72-5.06	4.29- 5.7	$\times10^6$/ml
Hgb	11.0-14.7	13.3-16.6	Mg/dl
Hct	35.2-46.7	41.3-52.1	%
MCV	87.1. - 102.2	86.3 - 101.4	fL
MCH	26.8 - 32.4	267.5- 32.4	Pg
MCHC	30.2 - 35.4	30.8 - 36.3	g/L
Plt	178 - 408	134 – 377	$\times10^3$/ml
Neutrophil	39.7 – 71.2	38.3 – 69	%
lymphocyte	21.9-50.5	24-48.4	%
Monocyte	4.2-9.6	4.8-10.1	%
Eosinophil	0.6-4.8	0.8 – 5.8	%
Basophil	0.2 - 1.4	0.2 - 1.4	%
RDW-CV	12.2-15	12.2 - 14.9	%
RDW-SD	42 - 53.6	39.9 - 52.2	fL
MPV	9.2 - 12.4	9.1 - 12.4	fL

Biochemical normal reference range

S.no	Parameter	Reference		unit
		Men	Women	
1	ALT	≤ 45 (37°C)	≤ 34 (37°C)	U/L
2	AST	< 35 (37°C)	< 31 (37°C)	U/L
3	ALP	80-380	80-306	U/L
4	Urea	12-43	10-38.7	Mg/dl
5	creatinine	0.48-1.13	0.47-1.09	Mg/dl
6	FBS	65-110	65-110	mg/dl

Normal Population Reference Range

Quality Control

The reliability of this instrument and reagents is monitored by quality control. By use of control blood or control materials the stability of the measured value is monitored over a certain period of time, and problems can be detected early or prevented.

Control material

The control materials, EIGHTCHECK-3WP-N (Normal), EIGHTCHECK-3WP-L (Low level) and EIGHTCHECK-3WPH (High level) are used. These are equivalent to Low, Normal and High level. For other biochemical the normal and pathogenic condition materials are used

For chemistry analysis we use P and N controls these are run simultaneously with sample.

Annex VII: Declaration

The undersigned declares that this proposal complies with the regulations of the University and meets the accepted standards with respect to originality and quality. I also agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

Principal Investigator: Kidane Yhdego (BSc, MSc candidate)

Signature: ___________________

Date of submission: ___________________

This proposal has been submitted with our approval as advisors.

Advisors:

Zemenu Tamir (BSc, MSc, PhD candidate)

Signature: _________

Date: _____ _____ __________

Rahel Alemu (BSc, MSc)

Signature: _________

Date: _____ _____ __________

Place: Addis Ababa, Ethiopia.

Thank you!!