HEMATOLOGICAL ABNORMALITY AMONG PULMONARY TUBERCULOSIS PATIENTS IN WOLKITE UNIVERSITY SPECIALIZED HOSPITAL GURAGE, SOUTH WEST, ETHIOPIA

BY:

1. EBRAHIM YIMER
2. HAILE DEJENE
3. KEYRAT KEDIR

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1. EBRAHIM YIMER
2. HAILE DEJENE
3. KEYRAT KEDIR

ADVISORS:-1. Mr. KASSAHUN HAILE (BSC, MSC)

2. Mr. REBIE KEDIR(BSC.MSC)

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ACRONYM

AFB	Acid Fast Bacilli
ESR	Erythrocyte sedimentation rate
HCT	Haematocrit
HGB	Haemoglobin
HIV	human immuno-deficiency
HSDP	Health Sector Development Program
MCH	Mean Corpuscular Haemoglobin
MCV	Mean Corpuscular Volume
MDR	Multi-Drug Resistance
PCV	Packed Cell Volume
PTB	Pulmonary Tuberculosis
RBC	Red Blood Cell
TB	Tuberculosis
WBC	White Blood Cells
PLT	Platelet
WHO	World Health Organization

Abstract

Background: - *Tuberculosis (TB) is highly prevalent chronic infections disease caused by Mycobacterium tuberculosis. Tuberculosis is a contagious disease that can affect almost any tissue and organs of the human body but mainly cause infection of the lungs. Various hematologic abnormalities such as anemia, leukocytosis, monocytosis, lymphopenia, leukopenia, thrombocytopenia, thrombocytosis, leukemoid reactions and pancytopenia can be seen among tuberculosis patients. Hematological abnormality in tuberculosis patients is multi-factorial.*

Objective: *The objective of this study was to assess hematological abnormality among tuberculosis patents in Wolkite University specialized hospital, southwest of Ethiopia.*

Methods*: A health institutional based cross-sectional study was conducted among 86 PTB positive patients from May to July 2023 at Wolkite University specialized hospital. All patients who have positive for AFB and fulfill inclusion criteria during the study period was included in the study by using convenient sampling technique. A structured questionnaire was used to collect data on socio-demographic and related characteristics. Five milliliters of venous blood was collected and hematological parameters were determined by Sysmex analyzer. Additionally, erythrocyte sedimentation rate (ESR) was measured by using the ESR analyzer. Data was analyzed using SPSS version 23 software and P-value less than 0.05 was considered as statistically significant. Data is presented as frequency and percentages for numerical and categorical variables respectively using tables and figure.*

Result: *Among 86 study participants, 38(44.2%) of patients had anemic out of this 20(64.52%) patients were female and 23(26.7%) patients develop Leukocytosis out of this 23(56.52%) patients was under the age of 45 year. Thrombocytosis was observed in 32(37.2%) patients while thrombocytopenia was observed in 6(6.97%) patients.*

Conclusion: *from our finding Hematological abnormality like anemia, leukocytosis and thrombocytosis were commonly detected in patients with pulmonary tuberculosis*. Therefore, i*t is conclude that patients with MTB should be assessed at diagnosis for various hematological abnormalities and this will help in preventing morbidity and mortality rates. A further large study must be undertaken to substantiate these findings.*

Keyword: - *Hematological abnormality, Tuberculosis, Wolkite, Ethiopia*

CHAPTER ONE

1. INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Tuberculosis (TB) is highly prevalent chronic infections disease caused by *Mycobacterium tuberculosis*, an aerobic intracellular binding bacterium, because of this characteristic it prefers tissues which always in contact with high oxygen levels, such as in the lung [1]. Inhalation of single viable organs has been shown to lead to infection, although close contact is usually necessary for acquisition of infection [1].

Tuberculosis remains one of the greatest publichealth problems in developing countries [2]. one third of the world‟s population are infected with *Mycobacterium tuberculosis*, this reservoir of infected individuals results in 8 million new cases of TB and 3 million deaths annually from Pulmonary tuberculosis (PTB) [3].

Hematological abnormalities, hyponatremia, and psychiatric issues are among the indications and symptoms of active PTB [4]. Anemia, iron deficiency, and elevations in peripheral blood leukocytes are the most typical hematological symptoms of PTB [5]. Researches have shown a strong relationship between the presence of acid-fast bacilli in sputum and hematological abnormalities [6]. TB cause profound bone marrow and peripheral blood abnormalities by modulating normal hematopoiesis [7].

The hematopoietic system is seriously affected during TB infection. Both myeloid and lymphoid cell lines and plasma components are affected [8].

Red blood cells are best known for their role in transporting oxygen between lungs and tissues, they also play two important roles in mycobacterial infections. They play a defensive role against infections by capturing pathogens and then transferring them to the macrophages in the liver and spleen, where they are then eliminated. This study reveals that red blood cells may also get targeted as host cells for mycobacteria. although anemia is the most common complication of TB infections, Anemia in tuberculosis is most often due to nutritional deficiency, malabsorption syndromes, failure of iron utilization, and bone marrow suppression [9].

Leukocytosis during infection due to the increased polymorphonuclear leukocytes and macrophages as part of the body‟s immune defense mechanism to combat the invading bacterial population. Mechanistic studies have shown that anti-tuberculosis drugs such as rifampicin can bind to plasma macromolecular proteins, promote antibody generation, and form antigen-antibody complexes. When these complexes are absorbed on leukocytes, they can cause leukocyte lysis and target cell damage, leading to leukopenia [10].

Platelets are important players in the immune response against Mycobacterium tuberculosis. They are present at the site of infection; they can down regulate the proliferation and cytokine production of T lymphocytes, and they may enhance the effector function of macrophages, decreasing mycobacterial replication. Various inflammatory cells, cytokines and mediators are involved in the formation of granulomatous lesions encountered in tuberculosis. Among the variety of cytokines, interleukin-6 (IL-6) is known to promote platelet production [11].
Thrombocytosis due to disease severity and a hypercoagulable profile, thrombocytes are present human TB granulomas and platelet-associated gene transcripts are increased in TB patients [11].

Reversible peripheral blood abnormalities are commonly associated with pulmonary tuberculosis and these hematological changes act as marker for the diagnosis, prognosis and response to therapy. TB cause profound bone marrow and peripheral blood abnormalities by modulating normal hematopoiesis and the disease become more severe when it is co infected with HIV because of weakened immunity[7].In pulmonary tuberculosis many hematological and biochemical abnormalities are common and they are valuable aids to diagnosis[12].

Routine diagnostic methods for MTB include acid-fast bacilli (AFB) microscopy, MTB culture, conventional polymerase chain reaction (PCR), and GeneXpert® MTB/RIF assay [13]. The delay of diagnosis in some cases was attributed to the PTB, because the PTB was presented in the form of multiple nodules through the lungs [14].

Hematological abnormalities associated with TB infection have not been completely investigated. To the best of our knowledge, there is no comprehensive study assessing the hematological abnormalities in this study area among patients suffering from pulmonary tuberculosis.

1.2 STATEMENT OF THE PROBLEM

Tuberculosis is a highly prevalent chronic infectious disease caused by mycobacterium.
One-third of the world population is infected and approximately 3 million people die [15].Geographically, the burden of TB is highest in Asia (the rate of TB among Asian person is 14.4 cases per 100,000 person) and Africa (in 2016, 2.5 million people fell ill with TB cases and 540,000 TB- related deaths occur) while, India and China together account for almost 40% of the world's TB cases [16]. According to data from Ethiopian Ministry of Health, TB is the leading cause of morbidity, the third cause of hospital admission and the second cause of death in Ethiopia(its incidence was estimated to be 164 per 100,000 people and the death rate was 16 cases per 100,000 people) [17,32].

In developing countries more people are dying of TB than any other infectious diseases. It comprises 25% of all avoidable deaths. Nearly 95% of all TB cases and 98% deaths due to TB are in developing countries and 75% TB cases are in productive age groups [18].

TB can cause diverse laboratory abnormalities such as anemia [19, 20], increased erythrocyte sedimentation rate [21, 22], low serum albumin level [6, 23], hyponatremia, abnormal liver function, leukocytosis and hypocalcaemia [23]. A number of studies have documented anemia in patients with TB [20, [23-25], however, these studies involved only small numbers of patients andthe results were not uniform.

In most cases, TB is treatable and curable; however, people with TB can die if they do not get proper treatment [26]. Sometimes drug-resistant TB occurs when bacteria become resistant to the drugs used to treat TB. This means that the drug can no longer kill the TB bacteria [26]. Tuberculosis (TB) treatment may present significant hematological disorder and some anti-TB drugs also have serious side effects [27]. Although many other diseases may be reflected by the blood and its constituents, the abnormalities of red cells, white cells, platelets, and clotting factors are considered to be primary hematologic disorder as a result of tuberculosis treatment [27], Leukocytosis during infection due to the increased polymorphonuclear leukocytes and macrophages as part of the body‟s immune defense mechanism to combat the invading bacterial population[9].Thrombocytosis due to disease severity and a hypercoagulable profile, thrombocytes are present human TB granulomas and platelet-associated gene transcripts are increased in TB patients[8,9].

A study in Sao Paulo State University, Brazil, on 80 PTB patients revealed that platelet count values were higher in those with less clinical disease duration. This was, because of the fact that, at the beginning of the TB process, there was strong pro-inflammatory cytokine activity (IFN-γ & TNF-α) which stimulates expression of acute-phase proteins and thrombocytosis [28]. The severity of tuberculosis epidemics varies widely among countries. Ethiopia ranked 10th among the thirty high burden tuberculosis countries worldwide, with an estimated 164 incidents of all forms of TB cases per 100 000 population [29, 32].

In response to prevailing and newly emerging health problems, the government has set up the Health Sector Development Program (HSDP), which incorporates a 20- year health development strategy, through a series of five-year rolling programs [30]. The country is implementing the third phase of this program, HSDP III. It focuses on poverty related health conditions, communicable diseases such as human immune-deficiency virus, tuberculosis (TB), malaria and diarrhea, and other health problems that affect mothers and children with particular attention to rural areas [31].

Ethiopia is one of the 30 high TB and MDR-TB burden countries with an estimated TB incidence of 164 per 100,000 populations [32]. The burden of tuberculosis in this study area was 27.3 % and the prevalence of TB was higher in males than females [33]. The economic impact of tuberculosis comes both from the size of the problem and from the fact that in developing countries the majority of disease and death occurs among the most economically active segment of the population: more than 75% among those 15 to 54 years of age [35]. TB is accounts for almost 20% of all deaths in this age group and 26% of preventable deaths [35]. Social discrimination was observed to have been affecting tuberculosis patients and their families to a great extent. Divorce rate due to tuberculosis among patients was high. Patients have reported loss or threat to lose their job. Dietary misconceptions were rampant [36].

To the best of our knowledge, few studies are available in Ethiopia. Studies done in Addis Ababa and Gondar, Ethiopia stated the occurrence of hematological changes in pulmonary tuberculosis patients. Even though their results are inconsistent in some of the hematological indices. As well, to the best of our knowledge, no study has been conducted in the study area. Therefore, this study planned to evaluate hematological abnormality among PTB patients at Wolkite University specialized hospital, Gurage Zone, south west, Ethiopia. This study will be creating awareness for health professionals to consider various types of hematological abnormality in the diagnosis and management of PTB patients.

1.3 SIGNIFICANT OF THE STUDY

Tuberculosis remains a major public health problem in developing countries, as it is the most common cause of death in the world from a single infectious disease. The hematopoietic system is seriously affected during TB infection. Both myeloid and lymphoid cell lines and plasma components are affected, because TB modulates normal hematopoiesis. Data regarding hematological profile abnormality among PTB patient in this study area is limited. Therefore, the findings of this study will provide information on the overall burden of hematological abnormality among TB patients so as to improve the management of TB patients in the study site and the community at large. The findings of this study will be also provided information about anemia, leukocytosis, thromobocytosis and thrombocytopenia among PTB patients that in turn used by physicians, policy makers and program evaluators to give effective management of hematological abnormality and to reduce mortality rate due to hematological abnormality among pulmonary tuberculosis patients. The findings of the study also will be serving as base line data for further study or serve as secondary data for other studies.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Tuberculosis (TB) Disease

Tuberculosis disease means tuberculosis infection plus the presence of signs and symptoms of TB. TB bacilli multiply in the lungs or other organs and produce the symptoms and signs. Around 10% of the people infected with TB bacilli may progress to TB disease in their lifetime. Around 5% of them develop TB disease within months or years and the remaining in their old age, which is known as reactivation of the disease [37].

Haematological parameters which include RBC and WBC counts, haemoglobin and haematocrit , platelet count, and RBC indices are the backbones of any laboratory evaluation and their abnormal values may be associated with various pathological conditions [38].

Platelets are considered to be pulmonary immune cells, because they possess many of the classical features of immune cells and participate in the pathogenesis of some pulmonary diseases. Platelets have a role in the inflammatory response; including defense against mycobacterium [39].

Several studies demonstrated that the hemoglobin level, white blood cell count, red blood parameters, erythrocyte sedimentation rate, high platelet count and body weight loss is useful indices of severity in tuberculosis, and the return of these indices to normal level is good indication of disease control in that they correlate with sputum conversion to acid-fast bacilli negative [40].

Other study conducted in Ethiopia demonstrated that Alteration of leukocyte parameters is one of the most frequently used indicators of infection. The changes can be in cellular morphology and number. Regarding the number of white blood cells, they keep normal except when the tuberculosis disease is advanced and active. Although changes occur in the relative number of lymphocyte, monocyte and neutrophil, this is not useful either as a clinical or prognostic index [41].

The study conducted in Pakistan on 600 participants to investigate different peripheral blood parameters and risk factors in TB patients revealed that Erythrocyte Sedimentation Rate (ESR), hemoglobin (Hgb) and lymphocytes were markedly changed in both sexes. Hemoglobin was recorded lower than normal value in 55% and 53% of male and female population respectively. Total leukocyte count was also lower than normal values in 8% and 6% of male and female respectively. Similarly, neutropenia was observed in 5% and 8% of male and female cases respectively, while neutrophilia was recorded as 60% and 64% in male and female patients respectively. In the same study, lymphocytopenia was also observed in 59% and 43% patients in male and female respectively. Illiteracy, smoking habits, overcrowding and living in shared houses were the main associated risk factors contributing in the enhancement of the disease [42].

A study also done in 2006 to show TB associated anemia by clarifying its prevalence characteristics, and evolution, involving large numbers of patients with TB. Among 880 patients with TB, 281 (31.9%) had anemia on diagnosis of TB, however, the hemoglobin concentration was less than 10 g/dL in only 45 patients (5.0%). Anemia was more frequently associated with the female and old age. The anemia found in 202 (71.9%) patients was normocytic and normochromic and 175 (64.6%) of the 271 patients cured from the anemia during or after anti TB treatment without iron intake [30].

Another study was done in India on 100 study participants in order to evaluate the hematological parameters in pulmonary tuberculosis patients who were positive for Mycobacterium tuberculosis bacilli in sputum. In this study anemia was seen in 74% of patients. In spite of the infection, 71% of patients had a normal leukocyte count. Leukocytosis as a response to infection was observed in 26% of patients. 3% of patients had leukopenia. Thrombocytosis was observed in 24% of patients while thrombocytopenia was observed in 9% of patients. 99% patients had increased erythrocyte sedimentation rate [42].

Other research was carried out on hematological profile of patients with pulmonary tuberculosis in Nigeria in 2003. It was found that the hematological indices of 62 pre-treatment, sputum-smear-AFB positive pulmonary tuberculosis patients were examined. Hematocrit, white cell count and differentials, and erythrocyte sedimentation rates (ESR) were estimated by manual methods. Statistically significant hematologic abnormalities including anemia in 93.6%, leukocytosis in 22.3%, neutrophilia in 45.2% and lymphopenia in

4.8% of the patients were occurred. Thrombocytosis was occurred in 12.9%, while 8% had thrombocytopenia. None of the patients had leukopenia and only 8.4% had lymphocytosis [22].

A study done in Sudan 0n 60 participants was also aimed at measuring some of haematological parameters among pulmonary tuberculosis patients. The results in pulmonary tuberculosis patients when compared with control showed that there were significant lower values in HGB, RBC count, HCT, MCV, MCH and MCHC. Total leukocyte count (TWBCs) and absolute neutrophil count showed significant increase in TB patients compared with control. The type of anemia found most was normocytic normochromic anemia. The significant increase in platelet count was showed in TB patients compared with controls group. Moderate normocytic normochromic Anemia (72.5%), leukocytosis (50%) especially neutrophilia with monocytosis and moderate thrombocytosis was found in TB patients compared to controls [38].

The study conducted in 2018 on 100 participants in hematological abnormality in pulmonary tuberculosis patients with HIV and without HIV at Gonder the PTB infected patients without HIV shows that 46% were anemic, 6% leukopenic, 22% neutropenia, 8% lymphopenic, and 8% thrombocytopenic [43].

To the best of our knowledge, few studies are available in Ethiopia. Studies done in Addis Ababa and Gondar, Ethiopia stated the occurrence of hematological changes in pulmonary tuberculosis patients. Even though their results are inconsistent in some of the hematological indices. As well, to the best of our knowledge, no study has been conducted in the study area. Therefore, this study planned to evaluate hematological abnormality among PTB patients at Wolkite University specialized hospital, Gurage Zone, south west, Ethiopia. This study will be creating awareness for health professionals to consider various types of hematological abnormality in the diagnosis and management of PTB patients.

CHAPTER THREE

3. OBJECTIVE OF THE STUDY

3.1 General objective

- To assess hematological abnormality among pulmonary tuberculosis patients at Wolkite University specialized hospital, Gurage Zone, south west, Ethiopia

3.2. Specific objectives

- To determine prevalence of anemia among pulmonary tuberculosis patients at Wolkite University specialized hospital
- To determine prevalence of leukocytosis pulmonarytuberculosis patients at Wolkite University specialized hospital
- To determine prevalence of thrombocytosis and thrombocytopenia among pulmonary tuberculosis patients at Wolkite University specialized hospital

CHAPTER FOUR

4. METHODS AND MATERIALS

4.1 Study area and study period

4.1.1 Study area

The study was conducted in Wolkite University Specialized Hospitals, which is found in the Gurage zone, Southern Nation Nationality and Peoples Regional State (SNNPR), Ethiopia. The hospital is located 170km far away from the capital city of Ethiopia, Addis Ababa and 14.7km from Wolkite town. It has an average annual temperature of 25 °C and an average rainfall of 1244 mm. The town has an elevation between 1910 and 1935 meters above sea level [44].The hospital is established in 2018 as a part of teaching hospital for health science students to produce qualified health professional by providing practical skill. The hospital delivers health service for medical, surgical, gynecology and pediatrics to 4 million catchment population living in Gurage zone.

4.1.2 Study Period

The study was carried out in Wolkite University specialized hospital from May 2023 to July 2023.

4.2 Study Design

Health institution based cross sectional study design was conducted

4.3 Population

4.3.1 Source population

All TB patients attending Wolkite University specialized hospital for direct observed treatment program.

4.3.2 study population

All TB patients attending Wolkite University specialized hospital during data collection period who fulfill the inclusion criteria.

4.3 Eligibility criteria

4.3.1 Inclusion criteria

- All TB patients greater than 18 years old and voluntary to participate will be included in the study.

4.3.2 Exclusion criteria

- TB patients who have been in treatment of any hematological abnormality including anemia, white blood cell abnormality, and platelet disorder were excluded.
- In addition, TB patients who had blood transfusion 4 months prior to data collection were not included in this study.

4.4 Sample size determination and sampling technique

The sample population was obtained by using single population formula.

According to study conducted in Gonder we were take the 8% thrombocytopenic for the „P‟of population [43]

$$n = \frac{z_{\alpha/2} * p(1-p)}{d^2} = \frac{(1.96)^2 * 0.08 * 0.92}{(0.05)^2} = 113.2$$

Where; p =Proportion of population and is the maximum expected prevalence rate 8% was used

d= the margin of error to be tolerated while sampling which is 5%

$z_{\alpha/2}$= critical value at 95% confidence of certainty (1.96)

n= sample size =113

For the non-respondents we added 10% of the sample sizes, there for the final sample size in our study were 113*10% +113=124.3=124

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4.5.1 Sampling Technique

The study subjects were selected by using convenient sampling technique to sequentially enroll patient's positive for AFB until the required sample size is reached.

4.5 Variables

4.5.1 Dependent variables

- Anemia
- Leukocytosis
- thrombocytosis
- Thrombocytopenia

4.5.2 Independent variables

Age

Gender

Marital status

Education

Occupation

Residence place

4.6 Data collection

Socio-demographic and related data were collected by using a structured questionnaire from the study participants. It has mainly close type of questions that are commonly used in cross-sectional studies. The questionnaire was prepared in English languages, and translated to Amharic language. So, the participants were interviewed with this method accordingly.

4.6.1 Sample collection: - about 5 ml of venous blood was collected with a K3EDTA anticoagulant tube from each study participants. After the blood was collected it was analyzed by Sysmex hematology analyzer for the determination of hematological parameters. Additionally, erythrocyte sedimentation rate (ESR) was measured by using the ESR analyzer. To ensure quality of data, hematological quality control materials were analyzed parallel to the patient‟s samples.

4.6.2 Determination of hematological parameters (CBC)

Principally Sysmex analyzer is based on the electronic resistance (impedance) detection method for counting and sizing recognition of the leukocytes, erythrocyte, and platelet using three hydraulic systems for, WBC, RBC, platelet and hemoglobin, and displays the results on the liquid crystal displayer (LCD) and printed out the results in thermal paper. The analyses were performed by using automated hematology analyzers Sysmex hematology analyzer (XP-300) using EDTA anti coagulated fresh venous blood sample. For each sample of blood the following hematometric variables: red blood cells (RBC), hematocrit (HCT), hemoglobin (HGB),mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH),total leucocyte(WBC) absolute neutrophils count (ANC) and absolute lymphocytes count (ALC) were determined in an automated hematological counter and the reference value of hematological parameters[45].

4.7 Data Analysis and Interpretation

After checking for completeness and cleaning, processing and analysis of the data obtained from laboratory analyses of the blood samples and questionnaires were performed by coding and entering the data into SPSS software version 23 package and the different variables were tested and analyzed and represented by using table and figure.

The result was compared with different studies done in different places. Principal investigator was responsible for coordinating all activities involved in laboratory, data collection management of the resource, and analysis of data supervised by the advisor.

4.8 Quality assurance and quality control

Data collectors were received half day training about the objectives of the study and how to approach participants. The principal investigator and supervisors were daily supervising during the whole period of data collection. Questionnaire were reviewed and checked for completeness, accuracy and consistency by supervisors and investigator and the questionnaires was translated from English to Amharic. We use Standard operating procedures during venous blood collection and we use standard daily quality control protocol during operating complete blood count. All instruments were operated and quality controlled according to the manufacture’s instructions and normal and abnormal controls were run daily. No analysis was done if controls out of range.

4.9 Ethical clearance

Ethical clearance was obtained from Wolkite University College of Medicine and Health Science, department of Medical Laboratory Science. The letter of support was taken from department of Medical Laboratory Science to Wolkite University specialized Hospital. Written informed consents were obtained from each study participant.

4.10 Dissemination plan of the study finding

The finding of the study will be presented and submitted to School of Medical Laboratory Science and College of Medicines and Health Science, Wolkite University

4.11 Operational definitions

- Hematological profile in healthy individuals expected to be in range WBC (4-11x103 /uL), RBC (4.3-5.9x106 /uL), HGB (13-18g/dL), HCT (42-54%), and in RBC indices MCV (80-100fL), MCH (27-31pg), MCHC (33-37g/dL), PLT (150-400x103 /uL) and part WBC Differential (Neutrophils in percent (40-75%), and Lymphocytes (20-40%) and monocyte (2-9%) [45].

- The definition of anemia used in this study was hemoglobin concentration less than 13 g/dL in men and 12 g/dL in women [46].

- Leucopenia, leukocytosis, neutropenia, neutrophilia, lymphocytosis, and thrombocytosis, were defined as WBC less than 4x 109 /L, WBC >11 x 109 /L, neutrophil <40 %, neutrophil > 70 %, lymphocyte >9% and PLT> 400x103 /Ul respectively [45].

- Normocytic normochromic anemia: anemia with MCV value of 80-100fl and MCHC value of 33-37 g/dL.

- Microcytic hypochromic anemia: anemia with MCV value of <80fl and MCHC value of <33 g/Dl.

- Macrocytic Normochromic anemia: anemia with MCV value of >100fl and MCHC value of 33-37g/dL.

CHAPTER FIVE

5. RESULT

5.1 Socio-demographic data of the study participants

A total of 86 PTB positive patients were included in this study with a response rate of 100%. Of the participants 55 (64%) were males. The mean (±SD) age of the study participants was 39 ± 14.14 years (age ranges from 18 to 70). The majority of the study participants were under the age of 45(67.3%) and rural residents 66 (76.7%). Out of 86 respondents, 40 (46.5%) were married. Regarding their educational status, 38(44.2%) had finished primary education. The majority, 33 (38.4%), of our participants were farmers and the mean monthly income was 2742.12 birr (table 1).

Table 1 Distribution of Socio-demographic of study participants at Wolkite University specialized Hospital from May to July

Variable	Category	Frequency	Percent (%)
Gender	Male	55	64
	Female	31	36
	Total	86	100
Age	>=18-45	57	67.3
	>45	29	33.7
	Total	86	100
Marital status	Married	40	46.5
	Unmarried	26	30.2
	Divorced	9	10.5
	Widowed	11	12.8
	Total	86	100
Residence	Urban	20	23.3
	Rural	66	76.7
	Total	86	100
Occupation	Laborer	8	9.3
	Merchant	21	24.4
	Farmer	33	38.4
	Government	14	16.3
	Student	10	11.6

	Total	86	100
Family Size(in number)	1-3	27	31.4
	3-5	25	29.1
	>5	34	39.5
	Total	86	100
Educational status	Illiterate	20	23.3
	Primary	38	44.2
	Secondary	10	11.6
	College/University	18	20.9
	Total	86	100
Monthly income(in birr)	<1500	26	30.2
	1501-3000	32	37.2
	3001-4500	12	14
	>4500	16	18.6
	Total	86	100

5.2 Hematological profile of the study participants

According to the analysis, the patients mean ± standard deviation for red blood cell counts were (4.28±0.81X106/uL), with 42 (48.8%) having less RBC count than normal range and 43 (50%) having normal RBC counts.

The Hgb level of PTB patients ranges from 5 to 18.20 g/dl, with a mean ± SD value of 12.05 ± 2.707 g/dl. The mean ± SD of hematocrit count was 35.11 ± 7.7 % with the range of 16 to 58.4% and the majority of patients 82.5% have lower hematocrit value (Table 2).

The mean ± SD of WBC count was 9.92 ± 5.03 x103cells/μL with a range of 3.28 to 28×103 cells/μL and Leukocytosis was observed in 23(26.7%) patients. Only 2 patients (2.3%) had leucopenia.thrity-eight (44.2%) patients had neutrophilia and 5(5.8%) patients have neutropenia.The majority of patients had lymphocytopenia (62.8%).twelve (14%) of patients have monocytosis and 17(19.85%) of patients had monocytopenia (table 4). The platelet count ranges from 57 to 961×103cells/μL, with a mean ± SD value of 359.05 ± 163.2 x103cells/μL(table 2).

Table 2 RBC,WBC and PLT count result of participants at Wolkite University specialized hospital May to July 2023

RBC PARAMETER	CATEGORY	FREQUENCY	PERCENT%
RBC(x106 /uL)	<4.3	42	48.8
	4.3-5.9	43	50.0
	>=6	1	1.2
Hemoglobin(g/dl)	<13	38	44.2
	13-18	47	54.7
	>18	1	1.2
Hematocrit (%)	<42	71	82.5
	42-54	14	16.3
	>54	1	1.2
MCV(fl)	<80	35	40.7
	80-100	49	57
	>100	2	2.3
MCH(pg)	<27	39	45.3
	27-31	41	47.7
	>31	6	7
MCHC(g/dl)	<33	35	40.7
	33-37	51	59.3
	>37	0	0

Total WBC count (x 103 cells/uL)	<4	2	2.3
	4-11	61	70.9
	>11	23	26.7
Neutrophil (%)	<40	5	5.8
	40-75	43	50
	>75	38	44.2
Lymphocyte (%)	<20	54	62.8
	20-40	26	30.2
	>40	6	7

Monocyte (%)	<2	17	19.8
	2-9	57	66.3
	>9	12	14
Platelet(x103cell/uL)	150-400	48	55.81
	<150	6	6.97
	>400	32	37.21

5.3 The magnitude of hematological abnormalities among PTB patients

The magnitude of anemia, its association with socio-demographic factor and types of anemia among PTB patients

Thirty-eight of the PTB patients were found to be anemic, resulting in an overall magnitude of 44.2% .Of them, 20 (64.52%) were female. Twenty-three (60.52%) patients were aged less than 45 years, 34(89.5%) patients were rural dweller and 21(55.26%) were farmers.

Table 3 **Anemia associated with socio-demographic factor**

Socio-demographic factor	Category	Anemic		
		No	Yes	p-value
Gender	Male	37(67.27%)	18(32.73%)	0.591
	Female	11(35.48%)	20(64.52%)	
Age	>=18-45	34(59.65%)	23(40.35%)	0.315
	>45	14(48.27%)	15(51.73%)	
Marital status	Married	23(57.5%)	17(42.5%)	0.524
	Unmarried	16(61.5%)	10(38.5%)	
	Divorced	3(33.3%)	6(66.7%)	
	Widowed	6(54.5%)	5(45.5%)	
Residence	Rural	32(48.5%)	34(51.5%)	0.013*
	Urban	16(80%)	4(20%)	
Occupation	Laborer	5(62.5%)	3(37.5%)	0.006*
	Merchant	18(85.7%)	3(14.3%)	
	Farmer	12(36.4%)	21(63.6%)	
	Student	7(70%)	3(30%)	

	Gov‟t worker	6(42.9%)	8(57.1%)	
Educational level	Illiterate	10(50%)	10(50%)	0.138
	Primary	17(44.7%)	21(55.3%)	
	Secondary	7(70%)	3(30%)	
	College/university	14(77.8%)	4(22.2%)	
Monthly income(birr)	<1500	16(61.5%)	10(38.5%)	0.178
	1501-3000	14(43.8%)	18(56.3%)	
	3001-4500	7(58.3%)	5(41.7%)	
	>4500	11(68.8%)	5(31.3%)	
Family size(in number)	1-3	16(59.3%)	11(40.7%)	0.789
	3-5	14(56%)	11(44%)	
	>5	18(52.9%)	16(47.1%)	

*refers to its significant ($p<0.05$)

Based on their MCV and MCHC, 47.37%, 39.47%, and 13.25% of the anemic patients had normocytic normochromic, microcytic hypochromic, and macrocytic normochromic type of anemia respectively (figure 1).

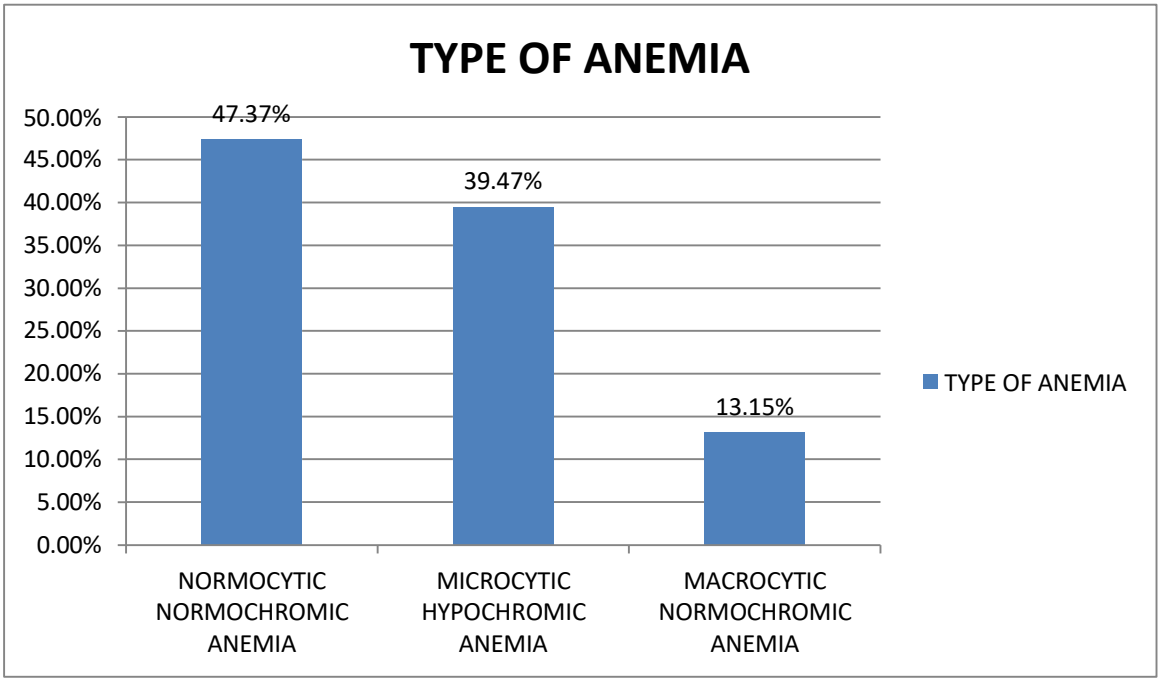

Figure 1 types of anemia observed on PTB patients at Wolkite University specialized hospital From May to July 2023

5.4 Erythrocyte sedimentation rate (ESR) of study participants

The mean ± SD of ESR of our study participants was 46.61±23.26 mm/hr and the maximum and minimum value was 100 and 6mm/hr.

In this study 77(89.5%) of patients with pulmonary tuberculosis had ESR greater than normal range. Only 9(10.5%) patient had a normal ESR value (figure 2)

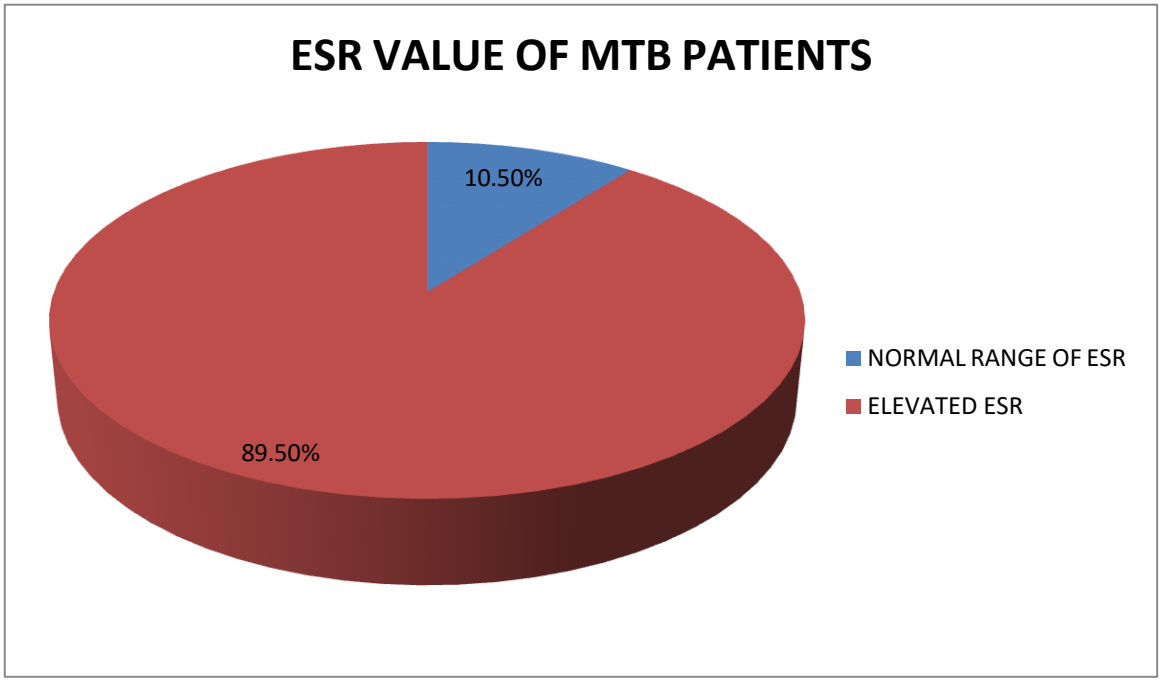

Figure 2 erythrocyte sedimentation rate determinations of participants at Wolkite University Specialized hospital May to July 2023.

The magnitude of leukocytosis, thrombocytosis and thrombocytopenia among PTB patients

The magnitude of leukocytosis in our study was 23(26.7%),of them 23(56.52%) patients was under aged of 45 year,17(73.91%) was rural dweller and 11(47.82%) was farmers. The magnitude of thrombocytosis in our study was 32(37.2%) of patients. Whereas thrombocytopenia was detected in 6(6.7%) of patients.

Table 4 Leukocytosis association with socio-demographic factor

Socio-demographic factor	Category	Leukocytosis		p-value
		No	Yes	
Gender	Male	43(78.2%)	12(21.8%)	0.169
	Female	20(64.5%)	11(35.5%)	

Age	>=18-45	44(77.2%)	13(22.8%)	0.247
	>45	19(65.5%)	10(34.5%)	
Marital status	Married	28(70%)	12(30%)	0.256
	Unmarried	21(80.8%)	5(19.2%)	
	Divorced	8(88.9%)	1(11.1%)	
	Widowed	6(54.5%)	5(45.5%)	
Residence	Rural	49(74.2%)	17(25.8%)	0.707
	Urban	14(70%)	6(30%)	
Occupation	Laborer	6(75%)	2(25%)	0.803
	Merchant	17(81%)	4(19%)	
	Farmer	22(66.7%)	11(33.3%)	
	Student	7(70%)	3(30%)	
	Gov‟t worker	11(78.6%)	3(21.4%)	
Educational status	Illiterate	12(60%)	8(40%)	0.282
	Primary	29(74.4%)	10(25.6%)	
	Secondary	7(70%)	3(30%)	
	College/university	15(88.2%)	2(11.8%)	
Monthly income(in birr)	<1500	22(71%)	9(29%)	0.839
	1501-3000	19(70.4%)	8(29.6%)	
	3001-4500	10(83.35%)	2(16.7%)	
	>4500	12(75%)	4(25%)	
Family size(in number)	<3	22(81.5%	5(13.5%)	0.149
	3-5	20(80%)	5(20%)	
	>5	21(61.8%)	13(38.2	

CHAPTER SIX
6. Discussion

Mycobacterium tuberculosis remains a major public health problem in developing countries, as it is the most common cause of death in the world from a single infectious disease and various hematological manifestations have been described in association with tuberculosis.Therefore , this study planned to evaluate hematological abnormality among PTB patients at Wolkite University specialized hospital, Gurage Zone, south west, Ethiopia.

In our study, 76.7% of patients were from rural areas and 23.3% were from urban areas.Various studies have reported similar findings [47-49],TB cases increased in rural areas, the most probable reason could be lack of awareness about the disease, bad social conditions, and living in shared house.

In this study 44.2% of the PTB patients had developed anemia which was similar with the study reported from Gonder,Ethiopia[43].Whereas, our finding was higher compared to a study from Korea (31.9%)[42] and Addis Ababa (25%)[50] .but lower than the prevalence reported from Iran (73%)[51] and India (74%) [52]. those differences could reflect the differences of the study participants in their nutritional status, malabsorption syndrome and stage of the disease during diagnosis. Indeed, the small sample size of the current study may have also an effect on the differences observed.

High prevalence of anemia is supported by several studies which showed that high prevalence of anemia among PTB patients. Various pathogeneses have been suggested in TB associated anemia, but most studies have showed infection of the hematopoietic progenitor cells, effect of treatment on erythropoiesis and folate activity, nutritional deficiencies and malabsorption, absence or depletion of bone marrow iron, and suppression of erythropoiesis by inflammatory mediators as a potential explanation for TB related anemia [24],[53-55]. For instance it has been reported that mild-to-moderate anemia is common during chronic inflammatory infections, including TB [54].

Erythrocyte sedimentation rate has been reported to be raised in infections and inflammations which could be linked to elevated synthesis of acute phase proteins usually seen in chronic infections and release of proteins by Mycobacterium tuberculosis into the circulation. In this study, we found high ESR values in 89.5% of TB patients. It is comparable with findings of other previous studies [50, 56]. ESR value usually increased in PTB [57]. This might be due

to alterations in the plasma proteins [58], which in turn affect ESR values. Elevated ESR to a different level is one of the indicators of the severity of disease and used as a prognostic tool [30, 59].

Based on our findings, thrombocytosis was observed in majority (37.2%) of the PTB patients, and most of the remaining patients had normal platelet count. One study conducted by Rathod.et.al reported as 75% of tuberculosis patients were with thrombocytosis [60].Thrombocytosis is assumed to be due to increased thrombopoietic factors as an inflammatory response. Lower thrombocytosis percentage (22%) also reported [42]. The variation could be stage of disease during diagnosis of patients in the current study which could have an effect on platelet count. Moreover, a study hypothesizes that increased platelet count in many cases is causally related to elevated interleukin-6 (IL-6) which is known to promote megakaryocytopoiesis during the acute phase of infection [61]. This study was limited to a small number of participants because we didn‟t have got enough patients during the time of our study. Therefore, this might have an effect in our conclusion.

The prevalence of thrombocytopenia in our study among PTB patients was 6.97% which is similar with reported from Gonder, Ethiopia [43], India [42] and Nigeria [22]. Different mechanisms such as immune mechanisms, bone marrow fibrosis, direct megakaryocyte infection, and hypersplenism had been implicated as possible causal factors for thrombocytopenia in PTB patients.

Leukocytosis in patients with pulmonary tuberculosis was increase in number count as response to infection, and leukopenia also was documented in study patients (2%), these results similar with study reported from Nigeria [22] and India [42]. Neutrophilia was documented in (44.2%) of PTB patients these result agree with study reported from Nigeria [22] and Khartoum state [38].this may due to acute inflammation and bacterial infection. The prevalence of monocytosis in our study was 14% which was somewhat close with the reported in other research‟s because monocyte plays an important part in the cellular reaction to the tubercle bacillus. The phospholipids of the organism are partly degraded within the monocyte and macrophage and cause the transformation of these cells to epitheliod cells. Monocyte where there is a high monocyte – macrophage turn over. This activity reflected in the blood, with monocytosis regarded as evidence of active extension of the tuberculosis infection [62].

CHAPTER SEVEN

7. CONCLUSION AND RECOMMENDATION

7.1 CONCLUSION

In this study, different hematological abnormalities in pulmonary tuberculosis were observed. There was high prevalence of anemia, leukocytosis and thrombocytosis. It is conclude that patients with MTB should be assessed at diagnosis for various hematological abnormalities. This will help in preventing morbidity and mortality.

7.2 RECOMMENDATION

We recommend that:

- Physicians treat patients suffering from pulmonary tuberculosis not only for pulmonary tuberculosis but also underlining hematological disorders as a result of pulmonary tuberculosis.
- Hematological investigation to be regularly part of the differential diagnosis modalities so as to improve the management of TB patients
- Further studies should also focus on finding the extent of damage caused by these abnormal changes as a co-infection with pulmonary tuberculosis. This will help establish whether these changes significantly affects the progress of pulmonary tuberculosis or the vice versa in pulmonary tuberculosis patients, it will help establish the relation between pulmonary tuberculosis and abnormal hematological profile in PTB patients.

7.3 Limitations and strength of the study

Limitation of the study

- This study was limited to a small number of participants because we didn‟t have got enough PTB patients during the study period .

Strength of the study

- Willingness of staffs of the hospital in providing necessary information and supporting participation during data collection and laboratory diagnosis.
- Good respond and willingness of the study subject to participate.

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Appendix

Annex I

Informed consent (English version)

Department of medical laboratory science, College of medicine and Health Sciences, Wolkite University, Consent form for the participation of the study participants in the research project
Name of the study participant ……
Code number…..……

We have clearly been informed about the research project that it aims to evaluate hematological abnormality among tuberculosis patients. The objectives of the research project have clearly been explained to us and we have been told that the results obtained from this research will help the hospital as well as the community for better management of the disease. We had been also informed about the confidentiality of this research project. Moreover, we have also been well informed of our right to keep hold of information, decline to cooperate and make our self withdraw from the study. Therefore, with full understanding of the importance of the study, we agreed voluntarily to provide the requested samples and our benefit was only from the free laboratory investigation result/s.

we_____hereby give my consent for providing the requested information and blood sample as the doctors find best for us.
Signature:_____Date_____

Annex II

QUESTIONARIES IN ENGLISH

ID number for the study participant. _____

PART-I**:** Socio-Demographic Characteristics

1. Sex 1 Male 2 Female

2. Age_____

3. Marital status 1 Married 2 Single 3 Divorced 4 Widowed

4. Residence 1 Rural 2 Urban

5. Occupation 1 Laborers 2 Merchant 3 Government employee

4 others _____

6. Educational status 1Illiterate 2Primary School 3 Secondary school

4 college or university

7. Monthly income_____

8. Family size _____

THANK YOU

Informed consent (Amharic version)

የወልቂጤ ዩኒቨርሲቲ የህክምና እና ጤና ሳይንስ ክፍል የህክምና ላብራቶሪ ክፍል፣የጥናቱ ተሳታፊዎች በምርምር ፕሮጀክቱ እንዲሳተፉ የፈቃድ ቅጽ

የጥናቱ ተሳታፊ ስም …….

የመለያ ቁጥር ……

በሳንባ ነቀርሳ በሽተኞች መካከል ያለውን የሂማቶሎጂ መዛባት ለመገምገም ያለመ ስለ የምርምር ፕሮጀክቱ በግልፅ ተነግሮኛል። የምርምር ፕሮጀክቱ ዓላማዎች በግልጽ ተብራርተውልኛል እና ከእኔ የተገኘው ውጤት ለእኔም ሆነ ለህብረተሰቡ በሽታውን በተሻለ ለመቆጣጠር እንደሚረዳ ተነግሮኛል. የዚህ የምርምር ፕሮጀክት ሚስጥራዊነትም ተነግሮኛል። በተጨማሪም ፣ መረጃን የመያዝ ፣ የመተባበር እና ከጥናቱ የመውጣት መብቴን በደንብ ተነግሮኛል ። ስለዚህ የጥናቱን አስፈላጊነት ሙሉ በሙሉ በመረዳት የተጠየቁትን ናሙናዎች ለማቅረብ በፈቃደኝነት ተስማምቻለሁ እና የእኔ ጥቅም ከነፃ የላብራቶሪ ምርመራ ውጤት / ውጤት ብቻ ይሆናል።

እኔ_____የተጠየቀውን መረጃ እና የደም ናሙና ለማቅረብ ፈቃኛ ነኝ።

ፊርማ፡-_____ቀን_____

QUESTIONARIES AMHARIC VERSION:

መጠይቅ

ውድ ተሳታፉ ቀጥሎ ያለውን መጠይቅ ለመሙላት ስለተባበሩን እናመሰግናለን።

የመጠይቁ መለያ ቁጥር_____

Part-I: Socio-Demographic Characteristics

1. ጾታ 1 ወንድ 2 ሴት

2. ዕድሜ_____

3. የጋብቻ ሁኔታ 1 ያገባ 2 ያላገባ 3 የፈታ 4 የሞተበት

4. የመኖሪያ ቦታ 1 ገጠር 2ከተማ

5. የስራ ዓይነት 1 የቀን ሰራተኛ 2 ነጋዴ 3 ገበሪ 4 ተማሪ 5 የመንግስት ሰራተኛ

6. የትምህርት ደረጃ 1 ማንበብና መጻፍ የማይችል 2 አንደኛ ደረጃ 3 ሁለተኛ ደረጃ 4 ኮለጂ/ ዩኒቨርሲቲ

7. የወር ገቢ መጠን_____

8. የቤተሰብ ብዛት_____

እናመሰግናለን

ANNEX III

Principle of Sysmex hematology analyzer (CBC machine)

The Sysmex Hematology Analyzer performs a complete Blood count (CBC), platelet, and a three part differential. Whole blood is aspirated, diluted, and then divided in to two samples. One sample is used to analyze the red blood cells and platelets while the second sample is used to analyze the white blood cell and hemoglobin. Electrical impedance used to count the white blood cells, red blood cells, and platelets as they pass through an aperture. As each cell is dawn through the aperture, a change in electrical resistance occurs generating a voltage pulse. The number pulses during a cycle corresponds to the number of cells counted. The amplitude of each pulse is directly proportional to the cell volume. Lyse reagent is added to the diluted sample and used to count the white blood cells after the white

Blood cells have been counted and sized; the remainder of the lysed dilution is transferred to the Hgb flow cell to measure Haemoglobin concentration. The cell dyne uses electronic to determine a three-part automated differential.

The percentage and absolute count are determined for lymphocytes,neutrophil,and mid-size population of monocyte, Basophils, eosinophils, blasts,and other immature cells. Specimen requirements

1. whole blood connected in an EDTA tube
2. Minimum sample volume is 0.5ml using the open sample mode.
3. sample are stable at room temperature for eight hours

Procedure for entering and running patient specimen in Sysmex hematology analyzer

Prior to running samples, perform daily start-up procedures. When the **READY** message is displayed on the run screen, the instrument is ready to run specimens.

Entering specimen ID

Manual entry

From **RUN** screen, **[SPECIMEN TYPE**] has been press

In the SPECIMEN TYPE screen, **[PATIENT SPECIMEN**] has been press

The cursor has been placed in the **<NEXT ID #>** entry field. Use the alphanumeric keys on the keyboard to enter a specimen ID of up to 16 characters.

Running patient specimen

To run patient specimens, proceed as follows:

- ✓ With the cap tightly secured on the specimen tube, it has been slowly inverted the tube 10 to 15 times.
- ✓ Cap from the pre-mixed specimen tube has been removed.
- ✓ The tube under the aspiration probe has been placed and raised so that the end of the probe is deeply immersed in the specimen.
- ✓ The touch plate has been pressed to aspirate the run.
- ✓ When the sample has been aspirated from the tube, the probe has been moved up through the wash block. Specimen tube has been removed and replaced the cap.
- ✓ After the cycle was completed, run results were displayed on screen and the aspiration probe has been moved into position to accept a new specimen. The current run data is saved to the data Log.

[PRINT REPORT] has been press to obtain a copy of the results.

NOTE: if a system has been idle for 15 minutes or more, a normal background should be run immediately prior to running patient specimens.

Annex IV

Laboratory registration form for CBC auto hematology analyzer result

Parameter and unit of measure	RBC PARAMETERS						WBC PARAMETERS				PLATLATES	ESR DETEMINATION
	RBC (Cells/L)	MCV (fl)	HCT (%)	HGB (mg/dl)	MCH (pg)	MCHC	TWBC (Cells/L)	NEU (cells/L)	LYMP (cells/L)	MON Cells/L	PLT (cells/L)	ESR value
1												

Annex V: Declaration form

The undersigned declares that this thesis complies with the university‟s regulations and meets the accepted standards concerning originality and quality. Principal investigators also agrees to take responsibility for the research project's scientific, ethical, and technical conduct and the provision of required progress reports.

BSc candidate students name:

1. EBRAHIM YIMAR Signature: _____Date of submission: 15/12/2015 E.C

2. HAILE DEGANE Signature: _____Date of submission: 15/12/2015 E.C

3. KEYRAT KEDIR Signature: _____Date of submission: 15/12/2015 E.C

APPROVAL OF THE ADVISORS

This research paper approved by University advisors:

1. Name of 1st advisor: Mr. KASSAHUN HAILE (BSc, MSc,)

Signature: _____Date of submission: _____/_____/_____

2. Name of 2nd advisor: Mr.REBIE KEDIR (BSc, MSc)

Signature: _____Date of submission: _____/_____/_____

Examiner

Name: _____Signature: _____Date_____/_____/_____

Name: _____Signature: _____Date_____/_____/_____

Name of School head: Mr.KASSAHUN HAILE (BSc, MSc,)

Signature: _____Date of submission: _____/_____/_____

AUGUST, 2023

WOLKITE, ETHIOPIA

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