ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCES.

ASSESSEMENT OF PLATELET PARAMETERS AMONG ADULT PTB PATIENTS AT TWO HCs, IN NSC AND LSC, ADDIS ABABA, ETHIOPIA.

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THIS 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. (HEMATOLOGY AND IMMUNOHEMATOLOGY SPECIALITY).

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This is to certify that the thesis paper prepared by Desalegn Alemu, entitled:
The assessment of platelet parameters among pulmonary tuberculosis patients at two health centers of Nefas Silk and Ledeta, Addis Ababa, Ethiopia, and the 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. (Hematology and immunohematology Specialty). Complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABBREVIATIONS

AAU	Addis Ababa University
CBC	Complete Blood Count
DOT	Direct Observation Therapy
EDTA	Ethylene Diamine Tetra Acetic acid,
ESR	Erythrocyte Sedimentation Rate
EPTB	Extra Pulmonary Tuberculosis
HC	Health Centre
IQR	Inter Quartile Range
LSC	Lideta Sub City
MCV	Mean Corpuscular Volume.
MCH	Mean Corpuscular Hemoglobin.
MCHC	Mean Corpuscular Hemoglobin Concentration.
MMP	Matrix MetalloProteinases
MPV	Mean Platelet Volume.
NC	Normal Control
NSC	Nefas silk Sub City
PDW	Platelet Distribution Width
PI	Principal Investigator
PTB	Pulmonary Tuberculosis
RBC	Red Blood Cells
RR	Reference Range
SD	Standard Deviation
SOP	Standard Operating Procedures
URTI	Upper Respiratory Tract Infections
WBC	White Blood Cell

ABSTRACTS

Background:

Pulmonary Tuberculosis (PTB) is one of the most burdensome infectious diseases in the world. By the year 2022, 10.6 million people developed symptomatic tuberculosis disease and 1.3 million people died. Studies have shown that not only the lungs, but the disease also affects bone marrow and hematological abnormalities were reported. However, little is known about platelet profile of pulmonary tuberculosis patients in Ethiopia.

OBJECTIVE*:* To assess Platelet parameters among adult Pulmonary Tuberculosis patients at two health centers in NSC and LSC, Addis Ababa, Ethiopia.

METHODS*:* Institution based Comparative cross–sectional study was conducted among pulmonary tuberculosis patients. A convenience sampling technique, to recruit 156 PTB patients were newly starting treatment immediately after the diagnosis, among 156 PTB patients were 100 from woreda 02 and the rest was 56 from woreda 04. Also 104 apparently healthy individuals, 52 from woreda 02 and 52 were from 04 HCs. Socio-demographic data was obtained from structured, closed ended and pre-tested questionnaires. Clinical data obtained from their medical records. Sysmex XP-300 and Weisman AC310 of woreda 02 and 04 HCs automated hematology analyzers that used for blood analysis. The data presented with words, tables, and figures and it analyzed by descriptive statistical tool, Epi-data V4.6.0.6 used for data entry, data transferred to SPSS version 20.0 for analysis, compared PTB versus control of platelet parameters with Pearson correlation and weight, independent t-test and Mann-Whitey U test compared the median value, P value <0.05 was considered as significant.

RESULTS: Out of 156 total PTB patients, 81(51.9%) were males and majority (37%) of them were in the age group of 28-37years and 18-27 years of control were female, 52(50%). The mean platelet count of PTB patients was higher than that of the control (317 ± 89 versus 230±49); however, there was not statistically significantly difference (P > 0.05). Regarding the magnitude of platelet abnormalities, 86(55%) had thrombocytosis and no thrombocytopenia was reported among PTB patients. The median of MPV and Platecrite values for PTB patients were slightly higher than the healthy control (10.00 ± 5.67 versus 9.3 ± 1.38 and 0.27 ± 0.07 versus 0.23 ± 0.07) respectively, then statistically significant difference (P < 0.05). In contrast, the median PDW of PTB patients were slightly less than the healthy control, however, it was not statistically significantly difference.

CONCLUSION: This study demonstrated platelet indices such as platelet count, MPV and PCT values of PTB patients were higher but PDW was lower compared with healthy control. Thus, the clinicians could assess parameters for routine PTB treatment follow-up. Further studies are required to understand the role of platelet parameters for follow up and progress.

Key words*:* Pulmonary Tuberculosis, Platelet parameter.

1. Introduction

1.1.Background

Tuberculosis (TB) is human diseases that affect any tissue and organs of human body. It caused by *Mycobacterium Tuberculosis* which is curable and preventable disease. It mainly affects the lungs that known as pulmonary tuberculosis. It also affects out of the lungs is called extra pulmonary tuberculosis, such as, intestine, bone, skin, liver, the reproductive system, and brain [1].

Pulmonary TB represents about 70% of all cases of TB and is the most contagious form TB and remains the main target for TB control [2].

The hematopoietic system is seriously affected by TB infection, there are myeloid, lymphoid cell lines and plasma components. Myeloid linage is derived from the common myeloid progenitor cells in the bone marrow, the linage includes monocytes, granulocytes, erythrocytes and platelet. They are a primary component of the innate immune system, and they serve as the first line of defense mechanism of infection [3].

Among the haematological values, thrombocytosis is common in pulmonary tuberculosis but in case of Miliary/disseminated/ tuberculosis, thrombocytopenia is observed. Rapid response of innate immunity is initially accelerated and detected by platelets during *Mycobacterium tuberculosis* infection [4].

Hematological abnormalities are associated with tuberculosis disease such as anemia, increased erythrocyte sedimentation rate, leukocytosis, and neutrophilia [5]. The production of platelets are stimulated by the secretion of IL-6 during pulmonary tuberculosis infection [6].

Inflammatory cytokine, tumor necrotic factor alpha (TNF- alpha) increase the risk of pulmonary tuberculosis infection while the monoclonal antibody produced [6].

Wound healing, thrombosis, and hemostasis are the main functions of platelets, there are significant roles in immune system, it has shortened survival. Tuberculosis disease is influenced by Megakaryocytes because the platelet is preprogrammed to defense the disease of tuberculosis. In the alteration of internal environment, the morphology and function of platelet to be changed [7].

Thrombocytopenia in tuberculosis patients could be occurred due to the defect of platelet production (marrow suppression) in case of TB treatment side effect, some viral infections, histiophagocytosis, cytokine –induced myelosuppression, tuberculosis complication due to thrombotic thrombocytopenic purpura (TTP) and disseminated intravascular coagulopathy (DIC). When compared with the platelet parameter count of Pulmonary Tuberculosis was higher than the control [7].

Tuberculosis immunopathology most likely derived by Platelet through the effect of immune cells

especially monocytes; it is used for an up regulation of activation markers. Platelet can sense the pathogens directly through Toll-like Receptors (TLR), such as Toll Like Reciptor2 and Toll Like Receptors4 [8].

MPV is increased during intestinal disease, cancer, diabetes, cardiovascular disease but decreased during tuberculosis, neoplasm, systemic lupus erythromates, and ulcerative collites [9].

Platelets are leading to the activation and release of reactive oxygen species and prion i n flammatory cytokines. Human tuberculosis granuloma has the presence of platelets, and it is associated with increased gene transcription ribonucleic acid (RNA) in relative to healthy individuals [10].

The soluble form of platelet activation marker is P-selectine, the level of P-selectine is higher in tuberculosis patients than healthy controls; it is used to activate platelet goes to aggregation [11].

A leukocyte is directly interacting with activated platelet to facilitate cellular recruitment towards the site of PTB infection. Immune response of TB may be involved by Platelet activity that depends on the concentrations of P-selectine. The positively correlate of a platelet activation marker with levels of well characterized marker of disease severity including IL-1β, MMP-1, -3, -7, -8 and -9 in TB patients, there are the final effectors for the host of innate inflammatory response (12).

Cluster Differentiation 42b(CD42b) is one of the Platelet specific markers that observed with epithelial cells and multinuclear giant cells in human TB lung granulomas [13].

MPV, PDW and PCT are Platelet indices which are changed in any phase of reaction. A Patient frequently attacked by pulmonary tuberculosis infections there is highly increased the number of platecrit values [14].

The normal range of MPV between 7.2 up to 11.7 fL was 95 % of individuals and the healthy individual value of PDW ranges between from 8.3% to 56.6% [15].

For Pct value from 0.22% to 0.24 % of PCT is in normal range [16]. Among tuberculosis patients of mean platelet volume was (8.03fl) and control groups (7.96 fL), so there was no difference between the groups, but the platelet count is highly increased in tuberculosis disease [17].

There are different diagnostic approaches for pulmonary tuberculosis infection detections, such as screening tests, tuberculin skin test and interferon gamma release assays, and Imaging like chest radiography. Laboratory tests such as, Zehiel Nelson techniques, culture, Gene xpert and Urine lame rapid tests [18].

As some studies have shown, the importance of hematological parameters abnormalities that indicating the risk of pulmonary tuberculosis development, identifying active tuberculosis infection, or monitoring the response of the anti-tuberculosis therapy and prognosis.

1.2.Statement of the Problem

Pulmonary Tuberculosis (PTB) is one of the most burdensome infectious diseases in the world. By the year 2022, 10.6 million people developed symptomatic tuberculosis disease and 1.3 million people died, in each year of the total number of people who develop TB. It accounted 5.8 million men, 3,5 million women and 1.3 million children and it affects all across the world and age group [19].

Mortality of TB occurred primarily in developing countries, it accounted for 60% and in Ethiopia was 73/100,000 population had died by tuberculosis infection [7].

The highest rate is observed in Africa, in Guinea-Bissau at 361 per 100,000 population, and the lowest observed in Togo at 36 per 100,000. In 2018, in Africa TB incidence was 275(range: 238-314) per 100,000 population compared, in Ethiopia, TB killed above 19000 people in the year 2022.

The incidence of tuberculosis infections status as indicated in the horn of Africa, it was an estimated range from 65 cases per 100,000 people per year in Eretria, 274 cases per 100,000 per year in Somalia, 378 cases per 100,000 per year in Djibouti and 192 cases per 100,000 per year in Ethiopia [20].

Pulmonary tuberculosis is affecting mainly hematopoietic stem cells like myeloid and lymphoid lineage that are caused anemia, leukocytosis, neutrophilia, lymphocytosis, monocytosis, and thrombocytosis; also, it affects the production and life span of hematologic cellular component [21,22].

The hematological change is given a clue to diagnosis, follow up, and prognosis to indicate the complication of underlying infections, as well as help to response of therapy [23]. One of the changes of platelet parameter's structure and function is described for tuberculosis infection [24].

In TB infections, platelet parameters abnormalities are one indication of disease severity and marker for pulmonary tuberculosis infection, it is known as reactive thrombocytosis [24].

Thrombocytosis is an indication for much chronic inflammatory disease including the tuberculosis infections. However, little attention is given attention to the use of platelet parameters during tuberculosis infection case management. Thus, aimed at the study to be assessed and recommended for the use of platelet parameters based on the findings. So that it provides additional and valuable information that support for clinicians' decisions during tuberculosis treatment monitoring and prognosis.

1.3.Significance of the study

The study finding will enabling physicians to consider the change of hematological parameters such as platelets indices, it is for assessing the disease progressing and treatment monitoring. There is an already established protocol for pulmonary tuberculosis patients to evaluate the disease prognosis. However, using easily available and relatively cheap tests like complete blood count provided additional and valuable data for the physician. In addition, the study will also provide reference data for further study as there were only a few studies with inconsistent findings.

2. Literature review

Tuberculosis is a chronic infectious disease and health concern in the world. In the study of 82 PTB patients and 87 were as controls done by the year 2007 in USA, they were equal age groups.The acute phase reaction for the values of MPV, PDW and PCT were significantly lower than in the tuberculosis group. However, the values of MPV, PDW and PCT were changes in tuberculosis, but the change may not reflect only acute phase reaction and disease activity [25].

In the study included 106 newly diagnosed Pulmonary TB patients were evaluated in the year 2021, in Romania, the participant was equal age groups, 63 pulmonary tuberculosis patients of hematological parameter were significantly lower than negative result after a treatment of positive pulmonary tuberculosis patients. But in pulmonary tuberculosis patients the value of hematological parameters were higher than apparently healthy individuals [26].

Of 265 pulmonary tuberculosis patients was involved by the year 1989, 40 % of leukocytosis and 52% of thrombocytosis was reported with hematological abnormality for pulmonary tuberculosis patients in East London [27].

The study in European respiratory Journal of 2011, about Platelet count and indices with 98 active tuberculosis patients (mean age 38.78±15.42) and 20 healthy controls (mean age 36.20±17.34) were evaluated. The result showed that the active tuberculosis group values were significantly higher PLT(381.683 ±125.046, MPV :8.57±1.39; PDW: 14.67±2.10; PCT : 0.31±0.09); ESR: result raised during pulmonary tuberculosis infection 75.03 ±4.01 than the healthy control group(PLT: 266.150±550.84; mean Platelet volume: 8.57±0.42, Platelet distribution width: 13.74±1.34 ; Platecrite : 0.24±0.03) and ESR: 5.04 ± 10.0 [28].

study was done in India 2015, 227 population were participated on hematological profile study on pulmonary tuberculosis patients. The result indicated that WBC abnormalities included leukocytosis in 65(28.63%) cases, leukopenia in 5(2.20%) cases, neutrophilia in 43(66.15%) cases, lymphocytosis in 14(21.53%) cases, platelet count was normal in majority 175(77.09%) cases 40(17.62%) cases showed that thrombocytosis, and 12(5.28%) cases showed thrombocytopenia [29].

A study was done in India, hematological parameters among pulmonary tuberculosis patients, the body weight correlated with platelet count of PTB patients and healthy controls, which means platelet count of PTB patients was increased while the body weight was decreased, and it was negative correlation [30].

Thirty- two Pulmonary tuberculosis patients involved in the study of India, the year 2001.The more common result of Pulmonary tuberculosis patients were thrombocytosis [31].

Active pulmonary tuberculosis patients, n=82 and 95 healthy subjects (control group) was studied in Istanbul,Turkey in 2014.The finding showed that the MPV was decreased than the control group [32].

A study conducted in South Africa was 15% leukopenia, 23% thrombocytopenia, and 87 % lymphopenia reported the effect of anti-tuberculosis drug treatment [33].

The study was performed on Nigeria by the year 1995 with comparable cross sectional study design, of 50 pulmonary TB patients and 50 of apparently healthy individuals matched with age and sex. The result of the study for thrombocytosis and thrombocytopenia was equal, it accounts 18% [34].

Study conducted in *Nigeria* the year 2003, of sixty-two pulmonary tuberculosis patients was participated. As the result indicated that the haematological parameters were anaemia accounted in 93.6%, 22.3% leucocytosis, 45.2% neutrophilia, 4.8% lymphopenia, 8% thrombocytopaenia while 12.9% occurred in thrombocytosis of the patients [35].

Out of the 70 PTB patients observed, the same age group of the participant, of 49(70%) patients had thrombocytosis, due to natural vascular aging, change in bone marrow tissue and altered blood plasma make up, the platelet count decreases when the age is increased. While the observed 3(5%) patients were thrombocytopenia, also the rest 18(25%) patients were in the normal range of platelet count [3].

In the study of 2007, 82 patients with TB and 87 non-TB as a control. The values of PDW and MPV were higher in the TB groups [36].

The hematological prognostic markers especially platelet indices and the other such as, hemoglobin, hematocrit, red blood cell count, white blood cell count and ESR are significantly important for the outcome monitoring of pulmonary tuberculosis treatment for the survival and quality of life [37].

One of the markers of platelet average size is MPV, that is present in various diseases such as diabetics mellitus, Hepatitis B, metabolic syndrome, and TB. But the role of MPV in TB study was disputed [38,39].

MPV is routinely tested platelet parameters in medical laboratory by using automated hematology analyzer, the management and diagnostic role has not been fully investigated yet [39].

Tuberculosis patients of Platelet count of the female is slightly increased than the male, the reason is not known clear physiological mechanism but, in some studies, showed that smoking in male could be the cause. The women do not accrue equal therapeutic benefit of antithrombotic therapy when compared with male because high platelet reactivity in women, so that it needs P2Y12 inhibitors to low rate of the platelet [11,40].

In Ethiopia, according to the year 2018 study showed that PTB patients of thrombocytosis, 11.6 %, thrombocytopenia, 9.8%, and majority were in normal range [41].

In the year 2018, University of Gonder Hospital was done research on hematological abnormality of pulmonary tuberculosis without HIV which was taken 50 pulmonary tuberculosis patients. There were provided different hematological abnormalities that accounts for 46% were anemic, 6% leucopenia, 22% neutropenic, 8% lymphopenia and 8% detected thrombocytopenic [42].

The study included a total of 50 pulmonary tuberculosis patients in University of Gonder, Ethiopia, in 2018. The study also compaired hematological parameters, before initiation of anti- tuberculosis treatment with after the completion of the intensive phase of TB treatment was significantly difference in statically. Those were hemoglobin (12.7g/dl versus 11.8g/dl), hematocrit (38.5% versus 35.7%), and platelet count ($268x10^3$/µl versus $239x10^3$/µl) [42].

Study participants of 168 were new TB patients, out of that 87(51.8%) of pulmonary TB patients, the rest was extra pulmonary tuberculosis, which was studied in University of Gondar Hospital, Ethiopia in the year 2016. The study finding of h e m a t o l o g i c a l Parameter was decreased as well as MPV and PCT values after the beginning of anti-tuberculosis treatment [43].

A total of 108 TB cases was studied in Jimma University Specialized Hospital, females TB patients the mean absolute WBC count ($8.62 \pm 2.89\ x10^3$cells/µl) was higher than the mean absolute WBC count for female's healthy controls ($6.67 \pm 1.5\ x10^3$cells/µl). The tuberculosis cases of the mean absolute platelet count were ($455.9\pm 329\ x10^3$cells/µl) higher than the mean absolute platelet counts of healthy controls ($315 \pm 124\ x10^3$cells/µl) [44].

A cross-sectional study the year 2018 involving forty pulmonary tuberculosis patients in St Paulose Millenium Medical College, Ethiopia, the findings of the study for thrombocytosis was detected in majority (65%) of the PTB patients, and the remaining patients had normal platelet count, the platelet count of Ethiopian is lower than the commonly utilized standard values [45].

3. OBJECTIVES

3.1.General objective

- To assess the platelet parameters among adult pulmonary tuberculosis patients at two health centers of Nefas Silk and Ledeta sub city, Addis Ababa, Ethiopia.

3.2.Specific objectives

- To assess platelet parameters among pulmonary tuberculosis patients.
- To compare platelet parameters abnormalities between pulmonary tuberculosis patients and apparently healthy individuals.

4. Materials and Methods

4.1.Study site.

The two health centers were found in the sub city for Nefas Silk lafto and Ledeta, there are Woreda 02 and 04 Health center, they were established in 2006 and 2010 respectivelly. Both health centers are in the Southwest Addis Ababa Region. Woreda 02 health center catchment population is 73,400, and their staff was 180 and Woreda 04 health Center served catchment population is 26,880 and their staff was 125. There are separate rooms from other clinical services, they provide TB treatment DOT service in the form of kit, and they have served in full day of the week including holiday. The health centres were found around the dry waste storage of an open dump site that is caused the disease of tuberculosis, Pneumonia, tetanus, and diarrhea [46].

4.2.Study design and period.

A facility-based comparative, prospective, cross-sectional study design was used, and the study period was from April to September 2023.

4.3.Population

4.3.1. Source population.

All TB patients and apparently healthy persons who were visited in the health centers during the study period.

4.3.2. Study population.

Adult Pulmonary TB patients and apparently healthy persons who were visited in health center during the study period and who full fill the eligible criteria of the study.

4.4.Eligibility criteria

4.4.1. Inclusion criteria

All volunteer adult pulmonary tuberculosis patients and apparently healthy persons who were given consent and the patients were newly starting the treatment immediately after the diagnosis.

4.4.2. Exclusion criteria

Pulmonary tuberculosis patients and apparently healthy person who have HIV in the blood, bleeding disorder, diabetics mellitus, myocardial infraction, EPTB, URTI, hematological malignancy, acute surgical conditions such as appendicitis, and pregnant women, they were excluded from the study.

4.5.Study variables.

4.5.1. Dependent variable

-Platelet parameters among PTB patients and apparently healthy person.

4.5.2. Independent variable

- ✓ Age
- ✓ Sex
- ✓ Residence
- ✓ Educational Status
- ✓ Occupational status
- ✓ Body weight

4.6.Sample size and sampling technique.

4.6.1. Sample size determination.

It was a comparable cross-sectional study, two (unpaired) means in two populations used to calculate sample size. The total sample size is determined using the following formula [47].

$n \geq (Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2 . (\sigma 2\ 1 + \sigma 2\ 2)/(M_1\text{-}M_2)^2$

Where,

n= number of samples for each group

$Z1-\alpha/2 = 1.96$ (For 5% significance level), $Z1-\beta = 0.84$ (For 80% power)

M1 =455.9 (mean of Platelet for Case group) σ1 = 329 (standard deviation for Case group)

M2 = 315 (mean of Platelet for Control group) σ2 = 124(Standard deviation for control group) [44].

n ≥ 7.84 x 123617 =49, by adding 5% non -response, total is equal to 52

19,853

Finally, by using 3:2 ratios for cases –control respectively, then a minimum sample size of 156 for case group and 104 for control group, among those (woreda 02, 100 cases and woreda 04, 56 cases) and (woreda 02, 52 control and woreda 04, 52 control) over all total sample size was 260.

4.6.2. Sampling method

A convenience sampling technique was used to recruit the study participants who fulfill the inclusion criteria. The control groups were selected from those who visited the health center for driving license health check-up, caretaker, and volunteer staff.

4.6.3. Laboratory Data collection

Laboratory data collected by two trained Laboratory technologists, who have done in hematology laboratory section in the health centers and by the principal investigator following Standard operating procedures. Patients were welcomed and informed about all the procedures involved in collecting laboratory data. The participants who fulfilled the inclusion criteria were asked to give sample of blood for complete blood count, CBC. Result interpretation, validation, verification, and reporting were performed by the principal investigator. The demographic data of the participant was collected in face-to-face method. However, they were prior to given informed consent. The questionaries were prepared for easily understandable language which is in English then translated to Amharic versions (Annex V and VI). The data was checked by principal investigator for completeness and consistency on daily basis. The collected data was kept in secured place for clients confidential.

4.6.4. Clinical Data Collection Procedures

Clinical data of the participants were collected thorough evaluations of patients' medical history, medical record reviews. Before medical record reviewing and screening tests, careful observation of participants for symptoms of any diseases was made. The clinicians extensively reviewed the participant's medical records along with history takings for the history HIV, bleeding disorder, diabetics mellitus, myocardial infraction, EPTB, URTI, hematological malignancy, and acute surgical conditions after getting permission from the participants unless known medical records and history were obtained.

4.7. Laboratory analysis and Data quality assurance

Automated Hematology analyzer, SYSMEX XP 300 of woreda 02 and Weisman AC 310 of woreda 04 health center, quality control performed by their operating manuals recommendations (annex VIII), both are used whole blood or pre-diluted mode

. About 4 ml venous blood was collected in K2/K3EDTA anticoagulant from each study participant by experienced phlebotomist. 30 µl blood mix with diluent in tube and it moves through a tube thin enough that cells pass by one at a time. Characteristics of the cell are measured using lasers (fluorescence flow cytometer) or electrical impedance. Whole blood is passed between two electrodes through an aperture so narrow that only one cell can pass through at a time.

To ensure the quality of the data, training was given to data collectors, and socio-demographic information was collected using a pretested questionnaire. Standard operating procedures were strictly followed during specimen collection and laboratory analysis procedures. The accuracy and completeness of the data were checked every day by the Principal Investigator. Data were cleaned, coded, and entered correctly.

4.7.1. Pre-analytical

To assure the quality of the data, enough information was given to data collectors, and socio-demographic information was collected using a self-administered questionnaire. Standard operating procedures (SOPs) were strictly followed that all reagents, test kits meet expiration date, quality control parameters as well as specimen collection and laboratory procedures.

4.7.2. Analytical

Commercially available low, normal, and high-quality control reagents were used to check the Reliability (accuracy and precision) of the data generated by SYSMEX XP 300 and Weisman AC 310 hematology analyzer. These hematology analyzers were run by the principal investigator and well-trained laboratory technologist in the health centers.

4.7.3. Post analytical.

The accuracy and completeness of the data was checked every day by the principal Investigator. Data was entered correctly by coded for confidentiality.

4.8. Statistical tool for Data analysis and interpretation:

Data entry and analysis was used in Epi-data version 4.6.0.6, and SPSS version 20, Data was checked as (Mean ± standard deviation) and the data was not normally distributed.

graph, it used (Median ± interquartile range), when reported continues variable and used number of subject(n) and percentage (%), when used for categorical variables. Testing for normality was checked by Kolmogorov-Smirnov (K-S) method.

According to the test results of the data, it was used an inferential statistical tool, the two groups were analyzed and compared the median for non-parametric test method, it was Mann-Whitney U test (two sample test) and the comparison of the mean is known as unpaired sample t -test (independent sample t- test). Also, the P value used two-sided < 0.05 was considered as statically significant. Finally, the result was presented in tables, figures, and graphs.

4.9. Ethical considerations

The protocol was reviewed, and ethical clearance permission obtained from the Research and Ethical Review Committee (DRERC) of the Department of Medical Laboratory Sciences College of Health Sciences of Addis Ababa University and then, the official letter was written to Nefas silk Lafto sub city Woreda 02 and Ledeta Sub city Woreda 04 health centers by asking the permission for performing the research. Strict confidentiality was ensured through coding of questionnaires, and it was used for the purpose of the study only. The purpose of the study and data collection was explained to the patients. Besides anyone who did not have interest to participate, they were excluded from the study. Privacy, culture, values, and traditions of the respondents were respected.

4.10. Dissemination of results

The findings of this study will be presented to Addis Ababa University on thesis defense and the result will be preserved at Addis Ababa University library and publication office and department of medical laboratory Sciences, College of health sciences. The findings were communicated to

the health centers higher officials through reports. The finding of this study will be published in peer reviewed on national or international journals.

4.11. Operational definitions

- **Thrombocytosis:** in this study, it was defined as the Platelet count higher than 300 $x10^3$cell/µl and 400 $x10^3$cell/µl.
- **Thrombocytopenia:** is defined as a platelet count less than < 100 $x10^3$cell/µl.
- **Platelet count:** is defined as the reference value between 100-300 $x10^3$cell/µl and 100-400 $x10^3$cell/µl respectively [48,49].
- **Progress:** is defined as pulmonary tuberculosis patients have seen improvement in terms of platelet parameter result come to reference range.

5. Conceptual framework

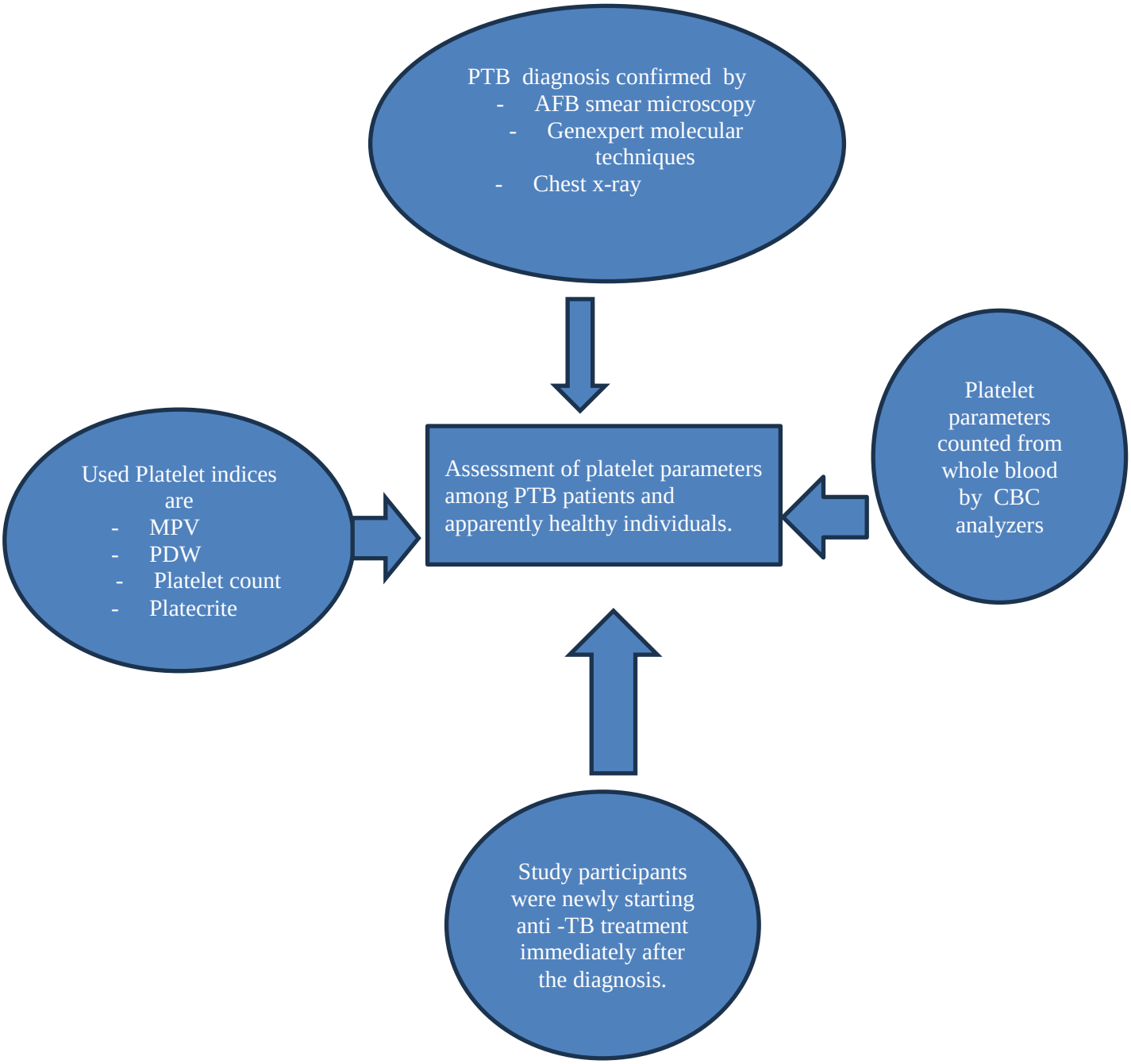

Fig. 1: conceptual framework for assessment of platelet parameters among PTB patients

6. Results

6.1.Socio-demographic characteristics of the study participants.

A total of 260 subjects, among those 156 pulmonary tuberculosis patients starting the treatment immediately after the diagnosis and 104 apparently healthy individuals were enrolled in this study. Among the patients 81(51.9%) were males while in healthy controls, the proportion of males and females were equal (50%). Most of the case, (93.6%) and control, (88.5%) individuals were resident in urban and similarly, most of the patient (37.8%) and apparently health group (28%) was daily labor (Table 1).

Table 1: Socio-demographic characteristics of pulmonary tuberculosis patients and apparently healthy individuals at Nefas Silk Sub city Woreda 02 and Ledeta sub city Woreda 04 health centers.

Socio-demographic Characteristics		Pulmonary tuberculosis		Healthy Controls	
		frequency	%	frequency	**%**
Sex	Male	81	51.9	52	50
	Female	75	48.1	52	50
	total	156	100	104	100
Age group in years	18-27	41	26	33	32
	28-37	58	37	32	31
	38-47	34	22	19	18
	48-57	21	14	17	16
	≥ 58	2	1	3	3
Residence	Urban	146	93.6	92	88.5
	Rural	10	6.4	12	11.5
	total	156	100	104	100
Occupational status	Governmental worker	22	14.1	22	21
	Daily labor	59	37.8	29	28
	Private worker	33	21.2	18	17
	Student	7	4.5	18	17
	Housewives	24	15.4	8	8
	Merchant	3	1.9	3	3
	Others	8	5.1	6	6
	total	156	100	104	100
Educational status	Can't read and write	29	18.6	4	3.8
	Read and write only	18	11.5	13	12.5
	elementary school	29	18.6	15	14.4
	High school	55	35.3	46	44.2
	Certificate	8	5.1	14	13.5
	Diploma and above	17	10.9	12	11.5
	Total	156	100	104	100

6.2. Platelet parameters

For normally distributed, mean and standard deviation are calculated while for not normally distributed data median and interquartile range is used. The mean value of platelet counts and the median of MPV for PTB were higher than the healthy individuals. Platelet count, MPV, PDW, and Pct has correlations with body weight of pulmonary tuberculosis. Platelet count, MPV, and Pct were significant in contrast, PDW were not significant difference (Table 2).

Table 2: Platelet parameters among pulmonary tuberculosis patients and apparently healthy individuals at Nefas Silk Sub city Woreda 02 and Ledeta sub city Woreda 04 health centers.

Platelet parametrs and body weight	PTB patients		Controls		P-value
	Mean±SD	Median±IQR	Mean±SD	Median±IQR	
Platelet count (cell/µl)	317 ± 89	-	230±49	-	0.08
MPV (fL)	-	10.00 ± 5.67	-	9.3 ± 1.38	0.00
PDW (%)	-	13.00 ± 6.00	-	13.85 ± 3.15	0.24
PCT (%)	-	0.27 ± 0.07	-	0.23 ± 0.07	0.00
Body weight in kgs	-	42.20 ± 2.12	-	54.23± 3.16.00	0.00

6.3 Magnitude of platelet parameters abnormality among tuberculosis patients versus control.

Thrombocytosis is platelet count above the expected values while compared to control. The frequency of abnormal platelet parameters among pulmonary tuberculosis patients versus control, out of 156 PTB patients were 57(37%) thrombocytosis, but no thrombocytopenia, in contrast, out of 104 healthy control, 3(2.9%) of thrombocytosis. Among the age group 18-27years of PTB was 17 (30%) versus control, most of the age group 28-37 years was 21(37%) versus control (Fig 1).

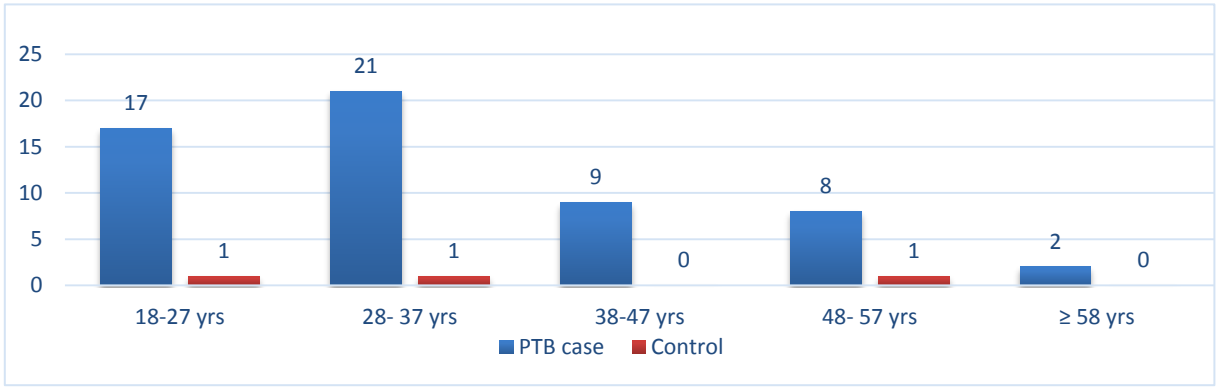

Figure 2: Thrombocytosis among pulmonary tuberculosis patients versus control at two HCs of NSC and LSC, Addis Ababa, Ethiop

7. Discussion

Platelet count is high in peripheral blood during active tuberculosis infections, because it regulates the human inflammatory response that goes to infection. However, the role of platelet parameters in tuberculosis infection is unclear. An increase MPV in patient with bacterial infection indicated that the infection has become invasive. A high PDW means platelet size variation so that platelet activation. As a result, higher MPV and PDW levels may be associated with inflammation. High platecrite may be a marker of increased platelet reactivity; thrombocytosis is an increase the level of platelet counts in pulmonary tuberculosis [2].

As a summary of the findings, among 156 pulmonary tuberculosis patients, the frequency distribution of male and female was 81(51.9%) and 75(48.1%) respectively. Majority of the age group of pulmonary tuberculosis infections was 28-37years, it accounted for 21(37%). But there was not statistically significance difference among case and control ($P > 0.05$).

The mean value of platelet counts of PTB, and control was 317 ± 89 and 230±49 respectively. Whereas the median value of mean platelet volume and platecrite for PTB was increased which accounted for 10.00 ± 5.67 and 0.27 ± 0.07 compare with control, 9.3 ± 1.38 and 0.23 ± 0.07, respectively. In contrast, the median value of platelet distribution width decreased, 13.00 ± 6.00 versus control, 13.85 ± 3.15.

Out of 227 study subjects the platelet count normal majority was 175(77.09%), thrombocytosis 40(17.62%), and thrombocytopenia 12(5.28%), in the same year in India studied showed, among 70 patients 49(70%) thrombocytosis, 3(5%) thrombocytopenia and 18(25%) platelets count in normal range [20].

Thrombocytosis is a cardinal feature of pulmonary tuberculosis. Some published studies have shown that an increase in platelet count, MPV, PDW and platecrite during repeated PTB infections [42].

Some study showed that in European respiratory Journal 2011, Jimma University 2014, and University of Gonder 2016, the mean values of active tuberculosis patients for platelet count, MPV, PDW and PCT higher in compare with the healthy control [40].

This study the median of PDW of PTB patients were slightly less than the healthy control, though, it was not statistically significant ($P > 0.05$). whereas the median of PCT value higher. there was statistically significant difference ($P< 0.05$).

Thrombocytosis is an increased platelet count during pulmonary tuberculosis infections that is used as a marker for tuberculosis. Raised concentrations of serum thrombopoiesis stimulating activity in patient with PTB suggest that the circulatory stimulating factor which increased thrombopoietin concentrations in tuberculosis patient, according to the study done in India by the year 2015 [29].

In this study thrombocytosis 57(37%), thrombocytopenia (0) and the normal range was 99(64%) when compared with the control group, it was out of 104 participants the thrombocytosis was very few numbers 1(0.96%) also majority of the participants were in normal range,103(99.40%). The mean platelet count was higher also the median values of MPV and PCT but less PDW when compared with the control, as a result, there was statistically significantly differences ($P < 0.05$).

Studies showed that, thrombocytosis was varied country to country among pulmonary tuberculosis patients, such as in India, out of 32 PTB patients that more of the patient had thrombocytosis. But in Nigeria the study indicated that thrombocytosis and thrombocytopenia on PTB patients and control were equal (18%) among 100 study subjects. According to a study from University of Gonder, on 50 PTB patients among these 8% was thrombocytopenic [22,35, 40].

In Ethiopia, according to the year 2018 study showed that PTB patients of thrombocytosis, 11.6 %, thrombocytopenia, 9.8%, and majority were in normal range [41].

According to University of Gonder study, abnormality of hematological test, 8% platelet count decreased among studied subjects and platelet count increased before PTB treatment started and decreased after the completion of anti-TB [2, 42].

As a summary of this study, high in platelet count, MPV and platecrite but low PDW vales in pulmonary tuberculosis patients when compared to control.

Tuberculosis could be occurred due to the defect of platelet production (marrow suppression) in case of anti-TB side effect, some viral infections, histiophagocytosis, and cytokine when compared with control. When Low values of MPV, PDW and PCT means bone marrow does not produce enough platelets and activation of clone B lymphocytes by Mycobacterium TB leading to anti-platelet antibody production.

In this study, the mean body weight of PTB was significantly lower when compared with healthy individuals. The platelet count, MPV, PDW, and PCT correlations with body weight of pulmonary tuberculosis. Therefore, Platelet count, PCT, MPV were significant, but PDW were not significant difference.

The body weight of the PTB patients was low, in contrast, the platelet count was elevated that had been negative correlation. The clinical Pearson negative correlation of body weight among platelet count of PTB and apparently healthy control was 0.4, it was medium. The correlation of body weight between MPV of PTB patients versus healthy controls was less than 0.1, that was no correlation of its. And the positive correlation of body weight among PDW of PTB patients versus healthy controls was 0.1, which was a very high correlation. Also, the negative correlation of body weight among PCT of PTB patients versus healthy controls was 0.4, there was medium correlation [19].

8. Strength and Limitations of the study

8.1.Strength of the study

The study was conducted with dedicated, patiency and honestly since data collected up to full fill sample size. Study was the first to be conducted in this area and thus can be used as base line data for future research on related topics.

8.2.Limitations of the study

Chronic disease status of the respondents was not known because as they have told us information about them during data collection which hinder the result to characterize the status of them. And shortage of CBC reagent and control due to that prolong the time of data collection. Finally, there was a limited number of studies on this topic.

9. Conclusion and Recommendation

9.1.Conclusion

This study demonstrated that platelet parameters, the mean value of platelet count was high which is a marker for inflammation. The median value of MPV and PCT was increased whereas PDW was decreased during pulmonary tuberculosis infections when compared with healthy individuals.The mean platelet count increase during pulmonary tuberculosis infection, due to the increased productions of platelets that stimulated by the secretion of IL-6 during pulmonary tuberculosis infection. Platelet count, MPV, PDW, and Pct has correlations with body weight of pulmonary tuberculosis. As the result, Platelet count, MPV, and pct were significant in contrast, PDW were not significant difference. In general, pulmonary tuberculosis patients in this study showed that significant alterations in platelet parameters.

9.2. Recommendation

The study showed that there was significant difference in platelet parameters between that starting anti-TB treatment immediately after the diagnosis and control participants. Therefore, we recommend that routine Platelet parameters at baseline level prior to treatment of PTB patients to follow up and prognosis.

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Annex

Annex: I Information sheet

Title of the research project: The assessment of platelet parameters among pulmonary tuberculosis patients at Nefas Silk Lafto Subcity woreda 02 and Lideta woreda 04 health centers TB clinic, Addis Ababa, Ethiopia.

Principal Investigator: Desalegn Alemu (BSC, MSC candidate)

Name of the organization: Nefas Silk Lafto Subcity woreda 02 and Lideta woreda 04 health `Centers TB clinic, Addis Ababa, Ethiopia.

Purpose of the research**:** I am asking you to take part in this study as a study subject of my endeavour to assess the MPV, PDW and PCT for pulmonary Tuberculosis. Finding of the research can be an input for helping the treatment or one indicator of tuberculosis management of pulmonary tuberculosis program. Therefore, at the end of the study based on the result found all the necessary recommendations were forwarded to all concerned bodies.

Procedure and the expected participation**:**

If you willing to participate, you need to sign consent form and respond to a short questionnaires interview. This implies that you give interview also blood samples were collected from you. The required blood sample collected by laboratory personnel from venous blood.

Potential risks

During the collection of specimens from you, appropriate precautions were taken, and all samples were collected by trained laboratory personnel. If anything happened appropriate medical care was provided to you.

Confidentiality:

All information you gave, and data obtained from laboratory analysis was kept confidential and it was communicated only to responsible figures. The format containing data was kept locked.

Benefits:

You were not receiving any payment for your participation in this research study as compensation. However, if there is any abnormality finding in the blood examination of Complete Blood Count the result was reported to your clinicians for appropriate treatment and management.

Participation and withdrawal from the study:

The participation was voluntary, and you have the right not to participate in this study. You may withdraw at any time and place without consequence of any kind. You may also reject to give any sample. You can ask any questions regarding this study, and you have a right to get a laboratory result. Contact information: if you have any questions about this study, you can contact the following principal investigator and advisor for further information.

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Annex II. የምርምር ጥናት ማበራሪያ/አማርኛ ቅጂ /

የምርምር ጥናቱ እርዕስ፡ በቲቢ በሽታየተያዟ ስዉበደምወስጥ ያለወን የፕላትሌት ቤተሰብን ሁኔታ ዳሰሳ ጥናት ሜድረግ፡ ፡

ተመራማሪ፡ ደሳለኝ አለመ፟ የ 2ኛ አመት የማስተርስ ዲግሪ ተማሪ

የ ድርጂቱ ስም፡ በን/ስ/ላ/ክ/ከ/ወ02/ እና በልደታክ/ከተማወረዳ 04 ጠፍጣቢያዎች ቲቢክሊኒክ የ ጥናቱአላማ፡

የ ቲቢህመምካፕላትሌትቤተሰብጋርያለወን ግንኙነ ት

ጥናትበምን ደርገ ወእርስዎምየ ዚህእድልተጠቃሚእን ዲሆኑበአክብሮትተጋብ዗ዋል፡ ፡

ይህምጥናትበህገ ራዊየ ቲቢህክምናአስጣኮላይአስተዋዖእን ዲያደርግነ ው፡ ፡

ተሳትፎ፡ በዚህጥናትለመሳተፍ ፍቃደኛ ከሆኑ የ ስምምነ ት ፎርመን አሜፎ ቃለ _ምልልስ ሊሰጡ፟ ይችላሉ፡ ፡

በተጨማሪ ምክሰልጠኑየ ላብራቶሪ ባለሙያዎችከክንድላይትንሽድምጢወስዳሉ፡ ፡

በዚህጥናትመሳተፍየ ሜደስከትላቸውችግሮች፡ ናመና በሚከበሰብበትወቅትምንምአይነ ትየ ከፋችግርአያጋጥመዎትም፡ ፡

ሆኖምግንናመናወንለመሳብሰብልምድያለወላለሙያስለሜሙደብናአስፈላጊወንየ ጥንቃቄእርምጃስለሚ

ወሰድየህመምኪሜ፞አይኖረወም፡ ፡

ሚስጥራዊነ ት:

ስለ ራስዎየ ሰጡትመንኛወምሙረጃናከተወሰደወናሜላይየ ተገኘወየ ላብራቶሪ ወጤት የ ሜው

ለወለጥናቱአላማብቻነው፡ ፡ይህንማህደርሊያገኙትየሚችሉትየተወሰኑየጥናቱተባባሪሰዎችብቻናቸው፡ ፡

ከዚያምበላይስለእርስዎያለውማንኛውምመረጃየተለየየይለፍቃልባለየኮምፒውተርየሚጃማህደርወስጥ

እንዲቀመጥይደረጋል፡ ፡

ጥቅም

በዚህጥናትለሚደርጉትቀጥተኛተሳትፎምንምአይነትክፍያአያገኝምሆኖምግንከምርመራውበሚገኘው

ወጤትመሰረትአስፈላጊውንየህክምናአገልግሎትከጠፍባለመያዎችያገኛሉ፡ ፡

ተሳትፎንስለማቅዋረጥ፡

በዚህጥናትመሳተፍመሉበመሉበእርሰዎፍቃደኝነትላይየተመሰረተሲሆንበማንኛውምሰአትናቦታየ ማቅዋረ

ጥመሉመበትየተጠበቀከመሆኑባላይእራስዎንከጥናቱበማለልዎምክንያትየሚቅርብዎትምንምአይነትየሆ

ስጢታልአገልግሎትየለም ፡

ከዚህበተጨማሪጥናቱንበተመለከተማንኛውንምአይነትጥያቄየማጠየቅናገለዓየማግኝትመበትአልዎት፡

የተመራማሪወአድራሻ፡ደሳለኝአለማኸልክቁጥር: 0913315489 ኢ-ሜል: desualex60@gmail.com ጀማል

አለሙ(ኤምኤስ ሲ)ስልክቁጥር፡ 0911429989 ኢ-ሜል፡ jemal.alemu@aau.edu.et

ሞገስ ኦርዶፋ (ኤምኤስ ሲ) ስልክቁጥር፡ 0984742173ኢ-ሜል፡ heranmakmow@gmail.com

የአዲስ አበባ ዩኒቨርሰቲ ጠፍ ሳይንስ ኮሌጅ ላብራቶሪ ዲፓርትመንት አድራሻ፡

መለሚያ ፡ሰፈረ ሰላም፡ ስልክ ቁጥር ፡ 0112755170.

Annex III. የአዋቂዎች የስምማ ት ፎርም(≥18 አመት)

እኔ ስሜከዚህ በታች የተጠቀሰውተሳታፊ ስለ ጥናቱ አላማ፤ ግብ ፤አካሄድና ሚስጥራዊነ ት ላይ በቂ ገለጣ ተደርጎልኛል፡ ፡በጥናቱ ላይ የመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ እራሴን የማለል መቡቱ የተጠበቀ እንዲሆን ተገልጦልኛል፡ ፡ ስለዚህ ስለ ጥናቱ አስፈላጊነት በማንጸብ በፍጡኡምፍቃደኝነ ት የጥናቱ ተሳታፊ ለመሆን እኔን በሚመለከት አስፈላጊውን መረጃ እና የላብራቶሪ የደም

ምርመራ ናሙና ለመስጠት ተስማምቻለሁ፡ ፡

የህመምተኛውፊርማ_____ቀን_____(ቀን/ወር/አም).

የ ተመራማሪው ስም፣ ቀን ፤ እና ፊርማ ፊርማ_____ቀን ____(ቀን/ወር/አ ም).

Annex IV. Adult Consent form (≥18 years)

I have been informed about the objectives, purpose, procedures, and confidentiality of this study. I understand that I have the right to withdraw from the study at any time. Therefore, with full understanding of the importance of the study, I agreed voluntarily to be participant and provide the required data that include blood sample for laboratory investigation and response for the interview.

I _____hereby give my consent for providing the requested information and specimen.

Signature _____date _____(dd/mm/yy).

Name of researcher,_____signature of the researcher _____date _____/___/___(dd/mm/yy)

Signature of the patients _____date_____(DD/MM/YY)

Annex V. Questionnaires/in English Version/

Addis Ababa University College of health science, department of medical laboratory science in the study of hematological parameters of platelet indices such as, platelet count, PDW, MPV and PCT. which is assessed during pulmonary tuberculosis infections that was coming to health centers to attend anti TB treatment at N/S/L SC W02 and Ledeta Woreda 04 HCs Tb clinic

from April to September 2023.I request kindly to give appropriate response for each question. Your response was kept confidential.

Name of interviewer_____ Date____

Please put a tick mark (√) for your response in the space provided. **Part-I. Socio demographic characteristics**

S/ no	Question		Answer	Comment
1	Identification number:		_____	
2	Age:		_____	
3	Sex:		Male ☐ Female ☐	
4	Educational status:	Can you read and write?	yes ☐ No ☐	

5	If the answer is yes,	✓ primary school ☐ ✓ secondary school ☐ ✓ Diploma,degree and above ☐ ✓ Other specify _____	
6	Marital status:	✓ married ☐ ✓ unmarried ☐ ✓ divorce ☐ ✓ widowed ☐ ✓ widwer ☐ ✓ Other Specify _____	
7	Residence:	✓ Urban ☐ ✓ Rural ☐	
8	Occupation:	✓ student ☐ ✓ daily labor ☐ ✓ housewife ☐ ✓ governmental worker ☐ ✓ Private worker ☐ ✓ merchant ☐ ✓ other specifies -------	
9	How much your income per month	_____	
10	Do you have children/children?	yes ☐ No ☐	
11	If yes,	how many children did you have? _____ no of other relatives _____	

Part II: Clinical condition/characteristics/

12				
13	Did you have common cold/URTI?		yes ☐ No ☐	
14	Did you have any chronic disease?		yes ☐ No ☐	
15	If the answer is yes		✓ RetroViral infection ☐ ✓ Cancer ☐ ✓ pregnant ☐ ✓ PTB On treatment ☐ ✓ Acute surgical condition such as appendicitis ☐ ✓ Heart problem ☐ ✓ Diabetic mellites ☐ ✓ Bleeding disorder ☐	
16	Did you feel/sense any of the following signs and symptoms?		Yes ☐ No ☐	
17	If yes,	Coughing more than two weeks:	Yes ☐ No ☐	
		Productive Sputum:	Yes ☐ No ☐	
		Night Sweating:	Yes ☐ No ☐	
		Head ach:	Yes ☐ No ☐	
		Loss of appetite:	Yes ☐ No ☐	
		Back pain	Yes ☐ No ☐	
		General body weakness	Yes ☐ No ☐	
18	Body weight		_____kgs (killograms)	
19	Disease condition	Severe	yes ☐ No ☐	

Annex VI. የማጠይቅ ፎርም/ አማርኛ ቨርሽን/

አዲስአበባዩኒቨርሲት የጠፍሳይንስኮሌጅ

የሕክምናየላቦራቶሪሳይንስት/ትክፍል

በአዲስ አበባ ዩኒቨርስቲ ጠፍ ሳይንስ ኮሌጅ የ ፐላትሌት ፓራሜቱር ምርመራ የሳንባ በሽታ ታመውመድሃኒት ለመወሰድ የሚማጡታማሚዎችላይ የሚደረግ ጥናት ሲህን ይህም ጥናት የሚደረገው በን/ስ/ላ/ክ/ከ/ ጠፍ ዕ/ቤት በወረዳ 02 እና በል/ክ /ከ/ ጠፍ ዕ/ቤት የወረዳ 04 ጠፍ ጣቢያ ቲቢ ክሊኒክ ከ ሚያዝያ እስከ ጥቅምት 2023 በዚህ ጊዜ ወስጥ የሳንባ ቲቢ በሽታ ለተገኘባቸዉህሙማን የተዘጋጀ ሲሆን ይህ ማጠይቅ ለመሚቂያ ፅሁፍ ጥናት በማሆኑ የሚጠውምላሽ ሚስጥሩ የተጠበቀ ይሆናል፡

የጠያቂውስም፡ ________________________________ ¨ቀን፡ ______________ በተሰጠው ባዶ ሳጥን ወስጥ ይህን ምልክት ተጠቀም (☐)

ክፍል አንድ፡ የማህበራዊ እና የዲሞግራፊ መረጃ

ተ/ቁ	ጥያቄዎች		መልስ	አስተያየት
1	ማለያ		__________	
2	እድሜ		__________	
3	ጾታ:		ወንድ ☐ ሴት ☐	
4	የትምህርት ሁኔታ:	ማንበብና መጻፍ ይችላሉ?	አዎ ☐ አይ ☐	
5	መልሱ አዎ ከሆነ		✓ የመጀመሪያ ደረጃ ☐ ✓ ሁለተኛ ደረጃ ☐ ✓ ዲፕሎማ ፡ ዲግሪ እና ከዚያ በላይ ☐ ✓ ሌላ ካለ ይጠቀስ ______	

6	የጋብቻ ሁኔታ:	✓ ያገባ/ች ✓ ያላገባ/ች ✓ የፈታ/ች	
		✓ ባልዋየ ሞተባትሴት ✓ ሚስቱ የሞተባት ወንድ ✓ ሌላ ካለ ይጠቁስ ____	
7	የመኖሪያ ቦታ ፡	✓ ከተማ ✓ ገጠር	
8	የስራ ሁኔታ፡	✓ ተማሪ ✓ የቀን ሰራተኛ ✓ የቤት እማቤት ✓ የመንግስት ሰራተኛ ✓ የግል ሰራተኛ ✓ ነጋዴ ✓ ሌላ ካለ ይጠቁስ ____	
9	የወር ገቢክ/ሽ ስንት ነዉ?	_____	
10	ልጅ/ልጆች አለዎት?	አዎ አይደለም	
11	ሚልሱ አዎ ከሆነ	ስንት ልጆች አለዎት ?_____ ሌላ ቤተሰብ ካለ _____	
ክፍል ሁለት ፡ የህክምና ሚጃ			
12	የቲቢ ህመምአይነ ት	የሳንባ ከሳንባ ዉጪ	
13	ጉንፋን /የ ትንፋሽ ህመምአለዎት	አዎ አይደለም	
14	የቆየ ህመምአለዎት ?	አዎ አይደለም	

15	መልስዎ አዎ ከሆነ	የኤች አይ ቪ ህመም ☐ ካንሰር ☐ የስኳር ህመም ☐ እርግዝና ☐ የሳንባ መድሃኒት ይወስዳሉ፡ ☐ ሰርጀሪ አለዎት፡ ለምሳሌ፡ የአፔንዴክስ ☐	

			የልብ ህመም ☐ ደም ከፈሰሰ ቶሎ የሚይቆም ☐	
16	ከዚህ በታች የተዘረዘሩትን የህመም ምልክቶች ይሰማዎታል?		አዎ ☐ አይደለም ☐	
17	መልስዎ አዎ ከሆነ	ከ2 ሳምንት ያለፈ ሳል ነበረዎት:	አዎ ☐ አይደለም ☐	
		ወፍራም አክታ ነበረዎት:	አዎ ☐ አይደለም ☐	
		ሚታ ሚታ ያልብዎት ነበር:	አዎ ☐ አይደለም ☐	
		ራስ ምታት ነበረዎት:	አዎ ☐ አይደለም ☐	
		የምግብ ፍላጎት አለዎት:	አዎ ☐ አይደለም ☐	
		ህመም ነበረዎት:	አዎ ☐ አይደለም ☐	
		የሰውነት ድካም ነበረዎት:	አዎ ☐ አይደለም ☐	
18	የሰውነት ክብደትዎ		በኪሎግራም _____	
19	የህመሙ ሁኔታ፡	አደገኛ ደረጃ ላይ ነው?	አዎ ☐ አይደለም ☐	

Annex VII. Venous blood Specimen collection

Material needed such as:

- Tourniquet and Disposable gloves
- 4ml EDTA tubes
- Alcohol (70%) and gauze or alcohol wipes
- Sterile needle with syringe Sterile Collection procedure

1. Patients will be welcomed and informed about the purpose of taking samples.
2. Materials needed for sample collection will be assembled.
3. Visual inspection of both arms will be performed by the phlebotomist assigned to do so.
4. Punctures site that is free of bruises, abrasions, and sites of infection will be chosen as site of sample collection.
5. Once the appropriate site has been chosen a tourniquet will be applied just above the area to be punctured.
6. The area chosen for puncturing will be cleaned inside out in a circular fashion with cotton soaked with 70% alcohol.
7. A needle with it bevel faced upward will be inserted gently into the selected site and the tourniquet will be removed as soon as blood begins flowing into the syringes. About 4ml blood will be pooled into the syringe.
8. Once the desired volume taken, and the needle and syringe will be removed from the patients' arm and patients will be informed to hold the punctured area with cotton ball for a minute to stop bleeding.
9. Blood withdrawn will be dispensed into a tube containing EDTA.
10. The tube will then be inverted many times in figure of eight to ensure proper mixing of sample to anticoagulant present in the tube or use blood Mixer machine.
11. The sample will be labeled with unique identification numbers assigned to match to the corresponding patients.
12. Materials used will be removed from the work area, and patients will be departed with thanks presented by the phlebotomist.

Annex VIII: SYSMEX XP 300 and Weisman AC310 Hematology Analyzers

❖ SOPs for SYSMEX XP 300 Hematology Analyzers

- ✓ Specimen Requirements
- ✓ Whole blood collected in an (K2E) EDTA tube.
- ✓ Minimum sample volume is 30μL.
- ✓ Samples are stable at room temperature for eight hours.
- ✓ Reagents and supplies for analyzer.
- ✓ Cell pack of 20 lit
- ✓ Stromatolyzer WH of 500 ml
- ✓ Cell cleans of 50 ml.
- ✓ Normal, high, and low control
- ✓ Thermal paper
  - Reagent stability
- ✓ The reagents are stored at room temperature, 15-25^{0}C.
- ➢ Daily Operating Procedures
  - Pre-operation checks are used for both machines.
- ✓ Verify the following before powering on the XP-300
- ✓ Check that the power cable is connected.
- ✓ Check that there is sufficient printer paper.
- ✓ Check pneumatic unit trap chamber of fluid. Empty if necessary.
- ✓ If needed, discard any waste fluid in the waste container.
- ❖ Power on/Self check
- ✓ Switch power on by pressing the power switch located on the right side of the analyzer.
- ✓ Three automatic rinse cycles are performed followed by a background check.
- ✓ Should any values fall outside of the acceptable limits, a maximum of two extra background checks will be performed automatically.

✓ Record background check on a daily checklist or keep a copy of the printout for documentation.

✓ Compare the results to the acceptable background limits.

☐ Acceptable background limits.

WBC	≤	0.3 x 10^3/µl
RBC	≤	0.02 x 10^6/µl
HGB	≤	0.1 g/dl
PLT	≤	10 x 10^3/µl

➢ Quality Control Analysis for Sysmex XP-300.

✓ Running QC

- Verify the XP -300 is ready.
- Press the QC button.
- Press the quality control file to be analyzed.
- Mix the control blood according to the package insert.
- Remove the cap and set control blood container to the sample probe.
- Press the **Start Switch**
- When analyzing is displayed and two beeps sound, remove the control blood.
- Use ( ) and ( ) buttons to scroll through pages.
- Press (**IP)** to print to internal printer. Press (**NG**) to reject.
- Touch (OK)

❖ **SOP for Weisman AC310 Hematology Analyzers Specimen Requirements**

- Whole blood collected in an (K2E) EDTA tube.
- The minimum sample volume is 9.7µL.
- For prediluted mode 20 µL sample with 200 µL diluent, aspirate blood sample volume 80 µL.
- Samples are stable at room temperature for eight hours.

- Reagents and supplies for analyzer.

- Cell pack of 20 lit
- Cell lyze of 500 ml
- Probe cleaner of 40 ml
- Normal, high, and low control
- Thermal paper

➢ Quality Control Analysis for AC310

- Running QC
- Verify the AC310 is ready.
- Press the QC button.
- Press the quality control file to be analyzed.
- Mix the control blood according to the package insert.
- Remove the cap and set control blood container to the sample probe.
- Press the **sample bar.**
- When analyzing is displayed and two beeps sound, remove the control blood.
- Use (⬅) and(➡) buttons to scroll through pages.
- Press **prints** to print out manually.

➢ QC Files set-up Barcoded Entry of Target and Limit Values

- Press (QC) on the main screen
- Select the correct file corresponding to the (QC) vial.
- Press **(**settings)
- Scan lot from the assay sheet. Lot number loads automatically.
- Manually enter the expiration date
- Using the (➡), scroll to the PARAMETER page beginning with WBC.
- Scan in WBC. TARGET and LIMIT Values load automatically.
- Continue to scan in all parameters.

  Note: scroll to the right to advance the screen and input data for all parameters

- Press (**Save**), then, Touch (**OK**)
- Repeat Steps from Press the QC button until save to input QC data for the remaining levels of QC.

Quality Controls Analysis for CBC analyzer

- ✓ Erasing a File for a new lot of controls
- ✓ Verify the XP -300 is ready.
- ✓ Press the (QC) button, the QC file list will be displayed.
- ✓ Press the**(**settings) button.
- ✓ Press the (Clear) button.
- ✓ Press (Enter)
- ✓ Press (OK) to erase selected file.
- ✓ Press (Cancel) to cancel file deletion.

Working principle of hematology analyzer

Blood is sampled and diluted and moves through a tube thin enough that cells pass by one at a time. Characteristics about the cell are measured using lasers (fluorescence flow cytometer) or **Electrical impedance.** Whole blood is passed between two electrodes through an aperture so narrow that only one cell can pass through a small aperture. The change in impedance is proportional to cell volume, resulting in a cell count and measure of volume.

Colorimetric method: A method of measuring HB based on chromogenic substances. When reagent lyses red blood cells, HB is released. The released HB is measured in WBC counting chamber.

When light interacts with medium containing HB, part of is absorbed based on the concentration of hemoglobin present. The amount of light absorbed is proportional to the amount of hemoglobin present.

Optical Light Scatter and Diffraction Method: The white cell differential counts are possible with this method of measurements. Focused leaser source illuminates' cells suspended in fluids, and light scatter happens depending on the nature of cells. A special detector detects light scattered by cells with characteristic intensity proportional to cell volume and internal complexity. Electronics these changes are then converted to electrical pulses.

The difference in dimensional and granular scattering properties of WBCs enables the analyzer to differentiate and group them into different cellular compartments.

Annex IX: Health centers standard operating procedures and manuals.

- Standared operating procedures for Sysmex XP-300
- Standared operating procedures for Wheisman AC-310
- Operating manuals for sysmex XP-300 equipment
- Operating manuals for Wheisman AC-310 equipmen

Declaration

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

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This thesis has been submitted with our approval as advisors.

Advisor: Jemal Alemu (MSc)

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Date: _____

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Advisor: Moges Wordofa (MSc)

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Place: Addis Ababa, Ethiopia.