ADDIS ABABA UNIVERSITY
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Evaluation of Hematological and Lipid Profiles in Pulmonary Tuberculosis Patients: A case control Study at Metema and Gondar Referral Hospitals, North West Ethiopia

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This is to certify that the dissertation prepared by Mohammed Yesuf, entitled: **Evaluation of Hematological and Lipid Profiles in Pulmonary Tuberculosis Patients: A case control Study at Metema and Gondar Referral Hospitals, North West Ethiopia,** 2017 and Submitted in partial fulfillment of the requirements for the degree "Master of Science in Biochemistry" in the department of biochemistry complies with regulations of the university and meets the accepted standards with respect to originality and quality.

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Declaration

I declare that this research paper entitled: Evaluation of Hematological and Lipid Profiles in Pulmonary Tuberculosis Patients: A Comparative Cross Sectional Study at Metema and Gondar Referral Hospitals, North West Ethiopia, 2017 is my original work and has not been presented for any degree in any other university, and that all sources of materials used for the research have duly been acknowledged.

Mohammed Yesuf

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Date_____

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ABBREVIATIONS AND ACRONYMS

AFB	Acid Fast Bacilli
BMI	Body Mass Index
CAT	Community acquired tuberculosis
CBC	Complete Blood Count
EDTA	Ethylene Diamine Tetra acetic Acid
HCT	Hematocrit
HDL-c	High Density Lipoprotein Cholesterol
HGB	Hemoglobin
IFN-γ	Interferon-gamma
IL	Interleukin
LDL-c	Low Density Lipoprotein Cholesterol
MBTC	Mycobacterium Tuberculosis Complex
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Corpuscular Volume
PCV	Packed Cell Volume
PTB	Pulmonary Tuberculosis
RBC	Red Blood Cell
SD	Standard Deviation
TB	Tuberculosis
TC	Total cholesterol
TGs	Triglycerides
TNF	Tumor Necrosis Factor
WBC	White Blood Cells

ABSTRACT

Background: Tuberculosis is one of the first most common causes of deaths in the world alongside HIV/AIDS, causing more than 9.6 million new cases and 1.5 million deaths globally in 2014 alone. This infection also accompanied by hematological and lipid profile alterations. The hematological and lipid alteration related to the disease is not yet well determined and has variation in different studies.

Objective: This study aimed at evaluating hematological and lipid profiles in pulmonary tuberculosis patients in Metema hospital and Gondar referral Hospital, North West Ethiopia.

Materials and methods: A case control study design was implemented from January to July 2017. One to one case to control ratio was used and a total of 88 blood samples were collected. There were 44 samples from tuberculosis patients and 44 samples from apparently healthy individuals. Collected bloods were tested using Hematology analyzer (sysmex) and Mindray chemistry analyzer for hematological and lipid profiles, respectively.

Results: The mean $\pm$ SD of hemoglobin, hematocrit and red blood cell count of the pulmonary tuberculosis patients were significantly lower than control groups ($P < 0.05$). However, platelet counts, total white blood cell counts, and erythrocyte sedimentation rate were significantly increased as compared with control groups ($p<0.05$).The mean serum levels of triglyceride, total cholesterol, high density lipoprotein and low density lipoprotein were significantly lower than their respective control groups ($p=0.001$). Body mass index had significant positive associations with RBC counts, HGB, and HCT and serum levels of TC and LDL ($P < 0.05$).

Conclusion: Pulmonary Tuberculosis patients in this study had hematological and lipid profile abnormalities. RBC count, HCT and HGB were significantly reduced. However, TWBC count, thrombocyte, and ESR of TB patients were significantly elevated than control group. Total Cholesterol, HDL, LDL and TG concentrations were significantly reduced as compared with control groups. The factors associated with hematological and Lipid profiles also due attention to prevent further complication.

Keywords: Case, Control, Lipid profile, Hematological profile, Tuberculosis.

1. INTRODUCTION

1.1. Overview of tuberculosis

Tuberculosis is a disease which is caused by bacteria called *Mycobacterium tuberculosis* Complex. The *Mycobacterium Tuberculosis* Complex (MTC) comprises closely related species responsible for strictly human and zoonotic tuberculosis (TB). The complex consists of seven species and subspecies including *M. tuberculosis*, *M. canetti*, *M. africanum*, *M. pinnipedii*, *M.microti*, *M. caprae* and *M. bovis*. Despite the different species tropisms, the MTC is characterized by 99.9% or greater similarity at the nucleotide level and possess identical 16S rRNA sequence (Dye *et al.*, 2005).

Tuberculosis is one of the first most common infectious diseases leading to death alongside HIV/AIDS. It is causing more than 9.6 new cases and 1.5 million deaths globally in 2014 alone (WHO, 2015). 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 (WHO, 2009).

Tuberculosis remains a major public health problem claiming the lives of thousands of Ethiopians every year. Ethiopia is among the 22 high TB burden and 27 high Multi-Drug Resistance (MDR-TB) TB burden countries in the world with incidence estimated at 379 persons per 100,000 persons for all forms of TB and 168 persons per 100,000 persons for smear positive TB. The annual risk of TB infection is also estimated at 2.2% (WHO, 2009).

Accurate and rapid diagnosis is most important to control the disease but, nowadays most frequently used diagnostic tests for TB are chest x-ray, culture, tuberculin skin test and acid fast staining. In low income and middle income countries the primary method for diagnosing pulmonary tuberculosis is direct sputum smear microscopy, which is fast and inexpensive (Karen *et al.*, 2006 and Al-Zamel, 2009).

Some hematological and biochemical abnormalities are commonly seen in pulmonary tuberculosis patient and they are valuable aids or indicators for better diagnosis and patient care. According to Abraham *et al*., an infection with TB can lead to hematological abnormalities by affecting hematological parameters from different perspectives. These are profound bone marrow and peripheral blood abnormalities which comes due to modulating normal hematopoiesis. The change in normal hematopoiesis becomes more severe when it occurs as co-infection with Human Immunodeficiency Virus or diabetes (Abraham *et al*., 2015).

Anemia is a cardinal feature in patients with bacterial infections, particularly infections lasting longer than a month, including pulmonary tuberculosis; however, the precise mechanism of existence of anemia in pulmonary tuberculosis is not clearly known. The occurrence of anemia among patients diagnosed as active pulmonary tuberculosis was very high and it contributed anemia to chronic disease (Eyshi *et al.*, 2009).

There is a well-recognized association between TB and malnutrition. Lipids are important constituents that determine nutritional status and at the same time participate in immune function. Lipid abnormality is a prognostic indicator of increased morbidity and mortality connected with various pathological conditions. Study showed hypocholesterolemia induce lower total T cells, fewer CD8 + and CD4 + and lower production of interleukin (IL)-2 which are very crucial to combat various diseases (Basil *et al.*, 2012, Muldoon *et al*., 2007).

With this background this study was conducted to determine hematological profile such as total leukocyte count(TLC), Platelet count, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) analyzed using fully automated hematology analyzer (Sysmex KX 21). The study was also meant to determine the concentration of lipid fraction (Cholesterol, Triglyceride, HDL-cholesterol, LDL-c) which were analyzed using Mindray chemistry analyzer. The study also aimed at finding out whether any difference in hematological and lipid levels existed between pulmonary tuberculosis patients and apparently healthy individuals.

1.2 Literature review

1.2.1 Tuberculosis (TB) Disease

Tuberculosis disease means tuberculosis infection plus 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 (Kaufmann, 2001).

Active TB is an acute inflammatory condition associated with tissue injury due to increased generation of free radicals and ROS (Reactive oxygen species). ROS and RNI (Reactive Nitrogen Intermediates) are produced as a consequence of phagocytic respiratory burst (Deepak *et al.*, 2011).

Furthermore, the development process of TB disease involves cellular immunity, the phagocytosis of MTB by macrophages, and the releases of interferon‟s, TNF-α and other cytokine molecules. Phagocytic activity of macrophages, neutrophils and monocytes also generate ROS and free radicals that not only have destructive effect on serum lipids (by lipid peroxidation) but also contribute to immune suppression (Caner *et al.*, 2007).

1.2.2 Hematological Profiles in pulmonary tuberculosis:

Hematological parameters which include RBC and WBC counts, hemoglobin and hematocrit determinations, platelet count, and RBC indices are the backbones of any laboratory evaluation and their abnormal values may be associated with various pathological conditions (Abaker *et al.*, 2016).

Red blood cell count, hematocrit, and hemoglobin determinations provide estimates of red blood cell number, red cell proportion, and hemoglobin concentration, respectively, in a volume of blood. Therefore, there is the need to know those parameters of the blood, which are abnormal in patients living with pulmonary tuberculosis (Abaker *et al.*, 2016).

The mild to moderate anemia that is often observed in patient with infection, inflammatory or neoplastic disease that persist for more than 1-2 months are called anemia of chronic disease. It has been reported that anemia exists in 16 % to 94 % of patient with pulmonary tuberculosis. All chronic infections including tuberculosis can cause anemia. Typically anemia develops during the first 1-2 months of illness and there after does not progress. The hematocrit usually maintained between 25% and 40% but significantly lower values observed in 20-30 % patients. This is particularly likely in syndrome associated with increased levels of interleukin 6. Interleukin 6 produces a delusional anemia: expansion of the plasma volume resulting in a reduced hematocrit or hemoglobin concentration without changing in the circulating red cell mass (Atkins *et al.*, 1995).

Various theories of pathogenesis have been suggested in TB-associated anemia, but most studies have shown the suppression of erythropoiesis by inflammatory mediators as a cause of anemia, which indicates that patients with TB - associated anemia display an absence of bone marrow iron. The erythrocytes usually are normocytic and normochromic, however, hypochromic and microcytosis may be observed. The initial MCV was found reduced in subjects with untreated tuberculosis, but the level was not as low as that found in iron deficiency. Microcytosis (mean corpuscular volume (MCV) less than 80fL) was observed in 2-8% of patient with anemia of chronic disorder (Robert *et al.*, 2005).

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 (William *et al.*, 1983).

Neutrophilia describes a high number of neutrophil granulocytes in blood. Neutrophils are the primary white blood cells that respond to a bacterial infection, so the most common cause of neutrophilia is a bacterial infection, especially pyogenic infections. Neutrophils are also increased in any acute inflammation, so will be raised after a heart attack other infarct or burn. Neutrophila usually accompanies the leukocytosis. A relative or absolute neutrophilia is

documented in 29-57% of patients with tuberculosis (Tozkoparan *et al.*, 2007 and Single *et al.*, 2003).

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 (Bayokarirt *et al.*, 1998). 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 (Single *et al.*, 2003). Reversible mild thrombocytosis is seen in about 52% of patients with severe pulmonary tuberculosis. The elevated mean platelet count of untreated patients decreases and normalizes with successful treatment of pulmonary tuberculosis (Tozkoparan *et al.*, 2007).

A prospective cohort study was conducted in Pakistan to investigate the change in hematological parameters with effective anti-tuberculosis therapy. Differential leukocyte count was done manually by microscopy of Leishman"s stained blood films. The result of the study showed that hemoglobin concentration, monocytes, lymphocyte count, and platelet counts were significantly decreased, whereas total leukocyte counts was not significant in cases and controls at time of diagnosis (Sumaira *et al.*, 2015).

Another study was conducted in the same country to investigate different peripheral blood parameters and risk factors in TB patients. 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% cases, 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 (Shafee *et al.*, 2014).

A study conducted in Iraq investigated the changes of some hematological parameters in patients affected with pulmonary tuberculosis. Patients have been classified into 3 groups: includes newly

diagnosed patients, patients after two months from starting treatment and patients after six months from starting treatment. This study found that values of HGB, HCT, platelets and ESR for both sexes were significantly changed in newly diagnosed patients in comparison with patients after two months from starting treatment. Values of HGB, HCT, platelets and ESR for both sexes were significantly changed in newly diagnosed patients in comparison with patients after six months from starting treatment. This study showed that the values of HGB, HCT, platelets and ESR values for both sexes were significantly changed in patients after two months of treatment in comparison with healthy controls (Muhammad *et al.*, 2011).

A study also attempted 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 (Sei *et al.*, 2006).

A prospective study carried out in Bangladesh was intended to observe the hematological status of tuberculosis patients and the change in patients‟ medical condition (AFB smear test) followed by diagnosis after one month. The gender distribution of patients illustrated that men (90.9%) are more likely to be affected by TB. 95.5% patients were found to have higher ESR value. 81.8% patients were diagnosed to have anemia. The Mean (± SD) ESR was 58.18 (± 25.73) and White Blood Count was 10081.81 (± 2747.05) respectively. Among the patients, 7 were observed again after one month among which 4 patients became sputum smear negative (SS-) from sputum smear positive (SS+) in the initial diagnosis (Rabita, 2012).

A Case control study in Saudi Arabia was done to investigate hematological changes and abnormalities associated to pulmonary tuberculosis patients. A total of fifty proven pulmonary tuberculosis patients (30 males and 20 female Saudis) were included, a mild anemia was observed in 18 out of 30 male PTB patients (60%) and 9 out of 20 female patients (45%). The MCV in male patients (83.28 fL) was also lower than in the normal males (86 fL). In the study, anemia occurred in 60% male PTB patients and 45% female patients. The blood cell morphology

showed normocytic normochromic in 80% of the patients, while only 20% PTB patients had microcytic and hypochromic RBC. Such patients had correspondingly lower MCV and MCH values. Platelets count was found higher both in male and female untreated PTB patients as compared with the normal values for Saudi population. However, the neutrophil count was relatively higher in the female patients as compared to the male PTB patients (Al-Omar *et al.,* 2009).

Another study was done in India 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 patients had a normal leukocyte count. Leukocytosis as a response to infection was observed in 26 patients. Three patients had leucopenia. Thrombocytosis was observed in 24 patients while thrombocytopenia was observed in 9 patients (Yaranal *et al.*, 2016).

Other research was carried out on hematological profile of patients with pulmonary tuberculosis in Nigeria. 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 lymphopaenia in 4.8% of the patients were occurred. Thrombocytosis was occurred in 12.9%, while 8% had thrombocytopenia. None of the patients had leucopenia and only 8.4% had lymphocytosis (Olaniyi *et al.*, 2003).

A study done in Sudan was also aimed at measuring some of hematological 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, leukocytosis especially neutrophilia with monocytosis and moderate thrombocytosis was found in TB patients compared to controls (Abaker *et al.,* 2016).

The hematological profiles determination that is done in University of Gondar on TB patients before and after intensive phase treatment showed statistical difference in hematocrit (38.5 % versus 35.7 %), hemoglobin (12.7 g/L versus11.8 g/L) and platelet (268 × 103/μL versus 239 × 103/μL) values of patients before treatment and after tuberculosis treatment, respectively ($P < 0.05$). There was no significant difference on total white blood cell count among TB patients before and after completion of the 2 month treatment (Eyuel *et al.,* 2016).

1.2.3 Lipid profile and Pulmonary Tuberculosis

Lipid is synthesized from diet and endogenously by the liver and tissue particularly from acetate. LDL and HDL are carriers of cholesterol. Plasma levels of LDL cholesterol are mainly determined by the production of apo B, the apolipoprotein of LDL cholesterol, by the conversion of VLDL to LDL, and by LDL-receptor mediated clearance. LDL molecules are often informally called bad cholesterol because they can transport their content of many fat molecules into artery walls, attract macrophages, and thus drive atherosclerosis, whereas HDL carries cholesterol from tissues back to liver (Song *et al.,* 2010).

Association between tuberculosis and malnutrition is well recognized. TB can lead to malnutrition and malnutrition may predispose to TB. Lipids are important constituents that determine nutritional status and at the same time participate in immune function (Basil *et al.,* 2012).

Lower levels of TC found in tuberculosis patients compared to healthy controls were reported in different research results in conditions leading to tuberculosis and other infectious/inflammatory situations (e.g. pneumonia). The correlation between low serum levels of TC and progression of tuberculosis indicated an inverse relation between inflammation and serum value of TC. Accordingly, numerous studies report significant reduction in serum level of HDL (Rodriguez *et al.*, 1996).

In a study from Mexico conducted to evaluate the hypothesis that low serum levels of cholesterol might be a risk factor in development of pulmonary tuberculosis, levels of TC, HDL, LDL and TG were found to be lower in tuberculosis patients than in persons with household contact (Perez-Guzman *et al.*, 2008).

Other research carried out in India to determine the level of lipid fractions in newly diagnosed and relapsed pulmonary tuberculosis patients and also to find correlation between serum lipid level with inflammation and disease severity. Fasting serum lipid profile (Total Cholesterol (TC), Triglyceride (TG), HDL-cholesterol (HDL-c), Low density Lipoprotein (LDL) and Very Low density Lipoprotein (VLDL) and CRP along with ADA were estimated**.** All lipid parameters were significantly low in both newly diagnosed and relapse cases of Pulmonary Tuberculosis (PTB) than controls. TC and LDL level were significantly higher in relapsed patients than new PTB cases (Taparia *et al.*, 2015).

A case control study was done in Turkey, in title "Distinctive biochemical changes in pulmonary tuberculosis and pneumonia". To investigate the relationship between radiological extent and serum biochemical changes and body mass index in patients with PTB. The result of the study showed that TC, HDL and BMI values were significantly lower in active lung tuberculosis and community acquired TB (CAT) than healthy control. And also TG and BMI were significantly lower in active TB than CAP. In active TB; BMI, HDL, and TG, were found to decrease. But no significant difference was found in levels of TC and LDL (Fusun *et al.*, 2013).

Other study was conducted in Egypt from May to November 2006, to detect any differences in lipid profile between Egyptian TB patients and controls and to test whether this difference changes after treatment or not. This study was aiming to prove if the difference is a risk factor for tuberculosis or a consequence of the disease itself. Regarding the whole studied group, only serum triglyceride was significantly lower before treatment than control group ($P<0.01$) while both serum cholesterol and HDL showed a significant increase after treatment than before it ($P<0.01$ for both). Regarding pulmonary tuberculosis patients, both serum cholesterol and triglycerides were significantly lower on diagnosis than healthy controls ($P<0.05$ for both) and only serum cholesterol increased significantly after treatment than before it ($P<0.01$) (Mohamed *et al*., 2012).

Observational retrospective cohort study was conducted in 2 public health centers and 1 public hospital in Addis Ababa, Ethiopia, which are located in different parts of the city, to determine whether lipids are indirect biomarkers of PTB in patients with or without HIV infection. The result showed that at baseline, mean level of TC, LDL-C and HDL-C were significantly lower in

HIV-TB+, HIV+TB+ and HIV+TST+ as compared to HIV-TST+ and HIV-TST-. TG was also significantly lower in HIV+/TB+, HIV-TB+ and HIV+/TST+ than in HIV-TST- (Yemane, 2014).

1.3 Statement of the Problem

Tuberculosis (TB) is one of the earliest a major health risk, and socioeconomic burden in both developing and industrialized countries that kill approximately 2 million people annually. World Health Organization global report of TB by 2011, estimated the incidence and prevalence rate of 125 and 170 cases per100,000 population respectively, with 1.5 million deaths. It is primarily a disease of low socioeconomic community including Ethiopia (WHO, 2011).

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 (MOH, 2009). TB makes malnutrition worse and malnutrition weakens immunity, thereby increasing the likelihood that latent TB will develop into active disease. In Ethiopia, the annual risk of developing TB in people living with HIV infection (PLWHIV) who are co-infected with *M.* tuberculosis ranges from 5 to 15% as compared to a 5 to 10% life time risk for HIV negative individuals (Aragie *et al*., 2012).

The mycobacterium activates the invaded macrophages resulting to release free radical. High serum levels of these free radicals and high concentration of lipid peroxidation products are characteristics of patients with advanced tuberculosis (Yamanaka *et al.*, 2001). The peroxidation could cause reduced concentration of serum lipids and tissue inflammation (Rothschild *et al.,* 2001). This resulted low cholesterol level which hinders macrophage function and accelerates the disease process. This observation has important therapeutic implication in tuberculosis control programme (Deshpande *et al*., 2012).

Erythrocyte sedimentation rate may be requested as an indicator for screening of tuberculosis patients for underlying HIV infection which is also used as non specific diagnostic value for TB especially in developing countries (Sarkar *et al*., 2011). Lipids are negatively affected by pulmonary tuberculosis (Deniz *et al.,* 2007).

Studies indicated that there were significant changes in hematological profiles among tuberculosis patients as compared to non- TB infected individuals Peripheral blood abnormalities are commonly associated with pulmonary tuberculosis, insight into the relationship between hematological abnormalities and mycobacterial infection has come from an understanding of the immunology of mycobacterial infection (Fatimah *et al*., 2014, Bala *et al*., 2015 and Kurup *et al.,* 2016).

Hematological derangements are seen including low hemoglobin, decreased lymphocyte count with its subsets, neutrophilia, monocytosis, monocytopenia, thrombocytopenia and thrombocytosis in few cases. Anemia is one of the commonest findings seen in TB patients and considered to be responsible for poor prognosis (Morris *et al.*, 1989).

The first line of defense against any foreign organism is innate immunity characterized by phagocytosis mediated by neutrophils and macrophages. Tuberculosis is characterized by granuloma formation hence neutrophils play a vital role in its formation. Release of tumor necrosis factor alpha (TNF-α) from polymorphs efficiently kills the mycobacterium. Platelet count also has a significant role in immune functions and thrombocytosis is generally seen in chronic inflammation stated as „reactive thrombocytosis" (Kisich *et al.*, 2002).

Despite different literatures from several countries showed that hematological and lipid profiles were important for diagnosis, treatment, prognosis, drug response, evaluation of tuberculosis severity and the differentiation of tuberculosis from pneumonia, there is no sufficient comprehensive research done in our country especially in the study area.

Therefore, this study was primarily focused to evaluate hematological profile, lipid contents and factors associated with hematological and lipid profiles among tuberculosis confirmed patients as well as apparently healthy individuals at Metema and Gondar Hospital, Northwest, Ethiopia. These might be creating awareness of health professionals to consider various types of profiles in the diagnosis and management of TB patients.

1.4 Significance of the study

The findings of this study will provide information on:

- The overall Hematological and lipid profiles among TB patients so as to improve the management of TB patients in the study site
- The finding also provided information on association of the various factors that contribute for development of anemia and dyslipidemia among PTB patients that in turn used by physicians, policy makers and program evaluators
- Moreover, identification of associated factors is essential in designing the appropriate public health response to epidemic tuberculosis.
- It also serves as a base line data for further study.

1.5. Hypothesis of the study

The null hypothesis (Ho) assumes that tuberculosis has no effect on hematological profile, lipid profiles and BMI of TB patients. The alternative hypothesis (HA) supposes that TB affect hematological profile, lipid profile as well as BMI of TB patients.

2. OBJECTIVES

2.1 General Objectives

The aims of this study were to evaluate hematological parameters, lipid profiles and associated risk factors among pulmonary tuberculosis patients as well as to compare with control groups.

2.2 Specific Objectives

- ☞ To determine and compare lipid profile of pulmonary tuberculosis patients and apparently healthy individuals.
- ☞ To measure and compare hematological profiles of pulmonary tuberculosis patients and apparently healthy individuals.
- ☞ To identify association of various factors that affect hematological and lipid profiles of study population.

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted at Metema and Gondar Hospitals which found in northwest Ethiopia. Metema is 896 and 158 Km away from Addis Ababa and Gondar, respectively. Gondar is 738 km away from Addis Ababa. Both hospitals (Metema and Gondar) are serving about 2 and 5 million people living in the town and surrounding area respectively.

Metema is one of the areas in Ethiopia endemic for Tuberculosis disease. Metma hospital has many services those are medical, pediatric, orthopedic, gynecology and obstetrics words with more than 44 beds. It give a service for more than 1500 patient per year. The hospital has a direct observed treatment short course (DOTS) clinic where TB patients are treated and monitored according to the national guide line. All patients information‟s and services given to TB patients visiting the clinical have been registered on a TB log book available in this clinic.

University of Gondar referral Hospital is a referral hospital with 500 beds, serving approximately 5 million people and plays an important role in teaching students within the medical field since more than decades. In 2010, a TB isolation and treatment ward, with 28 beds capacity, was built to improve the hospital TB infection control. The need is much greater than the present beds available, but capacity building activities are noted, including a new hospital construction with another 400 beds are finalizing and some of them are starting to give service.

3.2 Study Design and Period

A case control study design was used to evaluate the required hematological and lipid profiles as well as assess the associated risk factors at Metema and Gondar Hospitals, North-West Ethiopia from January to July 2017.

3.3 Source and Study Population

3.3.1 Source Population

The source population for this study was all TB patients who visited Metema hospital and Gondar teaching and referral hospital.

3.3.2 Study Population

Study subjects were 44 newly diagnosed tuberculosis patients as well as 44 apparently healthy individuals who were volunteer to participate in the study were used as a study populations.

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion criteria

- Newly diagnosed tuberculosis patients in Metema and Gondar Hospitals
- Apparently healthy individuals coming to both hospitals
- Ages of between 18-65 years old were included after their consents approved.

3.4.2 Exclusion criteria:

- Patient on lipid lowering or rising medications like contraceptive
- Patients with hematological derangement
- Diabetes mellitus
- Hypertension

3.5 Sample size determination

Sample size was determined based on double population formula with the following assumption. The specific mean $\pm$standard deviation for each sub groups in Ethiopia were not obtained, the mean $\pm$standard deviation of Lymphocyte for TB cases, 1.83 $\pm$0.8 and for healthy control, 2.23 $\pm$0.5 were taken from a study done in Sudan entitle "Some Hematological Parameters among Patients with Pulmonary Tuberculosis"(Abaker *et al.,* 2016). Assuming 95% confidence interval and 80% power, and 1:1 ratio, the minimal sample size in each group were calculated by using open Epi info software. The sample size computed based on the above assumption were 88 which comprises each 44 sample from each category.

3.6 Sampling technique

Consecutive sampling methods were used for the case groups until the required sample sizes were attained and convenient sampling techniques were used to select the control group.

3.6.1 Methods of sample and data collection

A structured questionnaire translated to local language, Amharic, were used for socio-demographic data collection. Anthropometric measurements, including weight and height, were measured with the subjects wearing light clothing and no shoes. Body mass index (BMI) was calculated as kg/m^2.

3.6.1.1 Blood collection and processing

After the study participants had been asked for their consent to be interviewed and to give sample blood, about 8 mL blood was withdrawn from the study participants, who had fasted overnight. The sample was collected by qualified health care professionals in both hospitals for the immediate laboratory analysis of the blood sample. In addition, the questionnaire was filled by face to face interview and some anthropometric indicators were also assessed and measured side by side as well.

About 4ml of the blood was collected in EDTA coated tubes (2ml for CBC and remaining for ESR) and hematological profiles were determined for all samples using a hematological analyzer (sysmex, 2000) for CBCs, Westergreen method for ESR.

Blood collected in tubes without anticoagulant was allowed to stand for 30 minutes at room temperature to allow complete clotting and clot retraction. Samples were then centrifuged by (MEGAFUGE ^{R} 1.0 HERAEUS) at 3500 rpm for 15 min to extract serum. The serum extracted was then used to determine the levels of TC, HDL-cholesterol and triglycerides and conducted using Bio system kits on a mindray BS200 Chemistry Analyzer (Shenzhen, China). LDL-cholesterol was calculated using the Friedwald formula (Friedewald *et al*., 1972).

3.7 Test principles of the laboratory analytes

3.7.1 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 (KX-21N) 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) absolute neutrophils count (ANC) and absolute lymphocytes count (ALC) were determined in an automated hematological counter and the reference value of hematological parameters (Sysmex, 2000).

A. Principle and procedure of Sysmex KX-21N

Measurement of blood cells (RBCs, WBCs, & Platelet) and hematological concentration were measured obtained by aspiration of small volume of well mixed EDTA blood by sample probe and mixed with isotonic diluents in nebulizer. Diluted mixture aspiration was delivered to RBCs aperture both for providing information about RBCs and Platelet based on the cell size. Particles of 2 to 20 fL counted as platelet and above 36 fL were counted as reamed cell. Some portion of aspiration mixture induced in to WBCs both in which hemolytic reagent (Stromatolyzer) were added automatically to measure hemoglobin concentration in build calorimeter, based on cyanomethemogolobin method. Blood cell were counted, size information were also generated in triplicate pulses according to electronic conductivity, and translated into digital number using in build calculator programmed and designed for that RBCs ,WBCs count. Three parameters can be directly measured and displayed on (LCD). Other values of red cell indices, platelet, and leukocyte differential and absolute count were calculated from given information and automated constructed histograms. The results were printed out according to the setting mode. Commercial close system reagents were provided by Sysmex KX- 21 operator and consist of cell pack, stromatolyser, detergent and cell cleaner and the reference value of hematological parameters (Sysmex, 2000).

B. Erythrocyte Sedimentation Rate

ESR stands for erythrocyte sedimentation rate and is also known as sedimentation rate or Westergreen ESR. It is the rate at which red blood cells sediment in a period of 1 hour. It is a common hematology test, and is a non-specific measure of inflammation. It is a test that is conducted to check the speed at which the red blood cells precipitate over a period of time. The results are measured as millimeters per hour. The results of the ESR test are useful to plan further testing and to commence treatment depending on the condition that is suspected. Once a diagnosis has been made, this test may be used to monitor whether the illness is becoming more active or flaring up. It is a screening test, which means it cannot be used to diagnose a specific disorder. However, it is useful for detecting and monitoring (Lewis et al., 2006).

Procedure and methods: A Westergreen tube: length- 300mm (open at both ends), diameter 2.5mm were used. About 2ml of the blood was collected in EDTA coated tubes. Anti-coagulated blood was drawn into a Westergreen tube up to the zero mark and the tube was set upright in a stand with a spring clip on the top and rubber at the bottom. The level of the top of the red cell column was read at the end of 1 hour and reported as mm/hr (Lewis et al., 2006).

3.7.2 Estimation of Serum Lipid Profiles

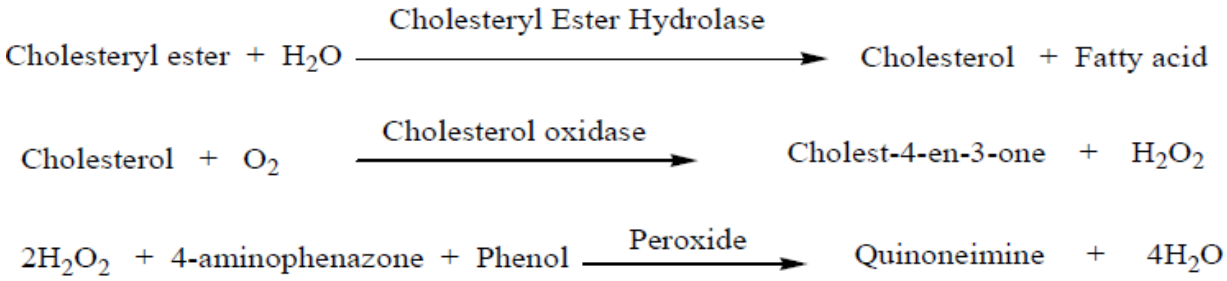
The remaining 4ml of the blood were used for measurement of biochemical parameters total cholesterol, triglyceride, HDL-cholesterols were estimated by enzymatic method by using Mindry automatic chemistry analyzer (Shenzhen, China). Low density Lipoprotein (LDL-c) was calculated by using Friedwald formula.

A. Determination of Total Cholesterol

Principles of the Method: Total cholesterol was measured enzymatically in serum in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3OH group of cholesterol.
Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase. The free cholesterol produced was oxidized by cholesterol oxidase to cholest-4-en-3-one with the simultaneous production of hydrogen peroxide (H_2O_2), which ox datively couples with 4-

aminoantipyrine and phenol in the presence of peroxidase to yield Quinoneimine dye with maximum absorption between 500-550 nm (Roschlay *et al.*, 1975).

The reaction sequence is as follows



Source:- (Roschlay *et al.*, 1975).

The test comes in the form of a commercial kit in which serum sample was incubated with enzymes and reagents from the kit and the change in absorption at 500nm was measured spectrophotometrically. This change in absorption is proportional to the concentration of total cholesterol in serum sample and can be calculated by comparison with absorption changes that occur with standard solutions containing known cholesterol concentrations (Roschlay *et al.*, 1975).

Procedure: Ten microliter (10µL) serum sample was added into the sample cups and put on the sample disk which rotates to bring the desire sample cup in to position next to the sample probe for specimen sampling. 1000µL reaction reagent (4-Aminophenazone, phenol, peroxidase, cholesterol esterase, cholesterol oxidase) were pipetted into reagent bottles leveled for TC and put on reagent disk and then on the screen menu of the machine TC were entered as a parameter to be tested. The sample probe was pipetted sample from the sample disk and transferred to the reaction disk which contains cuvettes. On the other side of the machine, the reagent probe were pipetted reagents from the reagent disk and transferred it into reaction disk which was a large rotatable disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly the sample and the reagents. The cuvettes were immersed into reaction water bath and incubated at 37^0C for 5 minutes. Next the reaction disk was rotated the cells to all reaction stations including

the photometer light path. Finally, the light was passed through the cuvettes and absorbance of the sample were measured at 500nm [Roschlay *et al*, 1975].

$$\text{TC concentration } (mg/dL) = \frac{\Delta A_{sample}}{\Delta A_{standard}} \text{ x } C_{standard}$$

B. Determination of Triglyceride

Principles of the Method: The method was based on the enzymatic hydrolysis of triglycerides to glycerol and frees fatty acids by lipoprotein lipase (LPL). Glycerol was converted to glycerol-3-phosphate and adenosine-5-phosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate was oxidized by glycerol phosphate oxidase to form dihydroxy acetone phosphate and H_2O_2. In the presence of peroxidase and H_2O_2, 4-aminoantipyrine couples with phenol to form a colored product (quinoneimine) that can be measured spectrophotometrically at a wavelength of 500nm.

The reaction sequence is as follows:

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerol phosphate oxidase}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 2H_2O$$

The triglyceride test comes in the form of a commercial kit containing the reagents, reactants and enzymes needed. Serum samples were incubated with the kit reagents and enzymes for 5 minutes at 37^0C and absorbance measured at 500 nm against the reagent blank and against known concentrations of standard triglyceride concentrations. The change in absorbance is proportional to the concentration of triglyceride in the serum sample.

Procedure: Ten micro liter (10µL) serum samples were added into the sample cups and put on the sample disk which rotates to bring the desire sample cup into position next to the sample probe for specimen sampling. 1000µL buffer and 1000µL substrate were pipetted into reagent bottles leveled for TG and put on the reagent disk. Then on the screen menu of the machine TG were entered as a parameter tope tested. The sample probe pipetted sample from the sample disk and transferred to the reaction disk which contains cuvettes. On the other side of the machine, the reagent probe pipetted reagents from the reagent disk and transferred it into rotatable reaction disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly the sample and the reagents. The cuvettes were immersed in to reaction water bath and incubated at 37^0C for 5 minutes. Next the reaction disk was rotated the cells to all reaction stations including the photometer light path. Finally, the light was passed through the cuvettes and absorbance of the sample measured at 500nm [Roschlay *et al*, 1975].

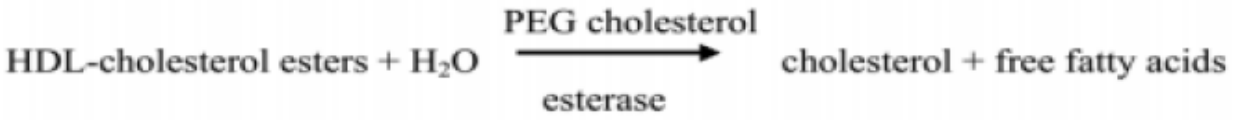
$$\text{TG level concentration } (mg/dL) = \frac{\Delta A_{sample}}{\Delta A_{standard}} \text{ x } C_{standard}$$

C. Determination of High Density Lipoprotein Cholesterol

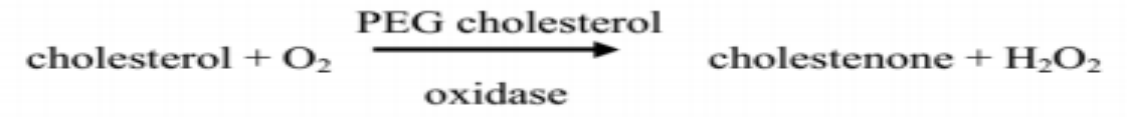
Principles of the Method: The basic principle of the method was as follows. The apoB containing lipoproteins in the specimen react with antibodies to apoB that renders them nonreactive with the enzymatic cholesterol reagent under conditions of the assay. The enzymes used were also pegylated, and this allows them to react only with HDL-c and not with antibodybound LDL-c, VLDL-cor chylomicrons. The apoBcontaining 39 lipoproteins were thus effectively excluded from the assay and only HDL-c was detected under the assay conditions (Jacobs *et al.*, 1990). The HDL-c test was a two reagent homogenous system for the selective measurement of serum or plasma HDL-c in the presence of other lipoprotein particles. The assay was comprised of two distinct phases.

In phase one, it was likely that in the presence of slightly alkaline buffer and magnesium sulfate and dextran sulfate selectively form water soluble complexes with LDL-c, LDL-cand chylomicrons, which were resistant to polyethylene glycol (PEG)modified enzymes. In phase two the cholesterol concentration of HDL-c cholesterol was determined enzymatically by

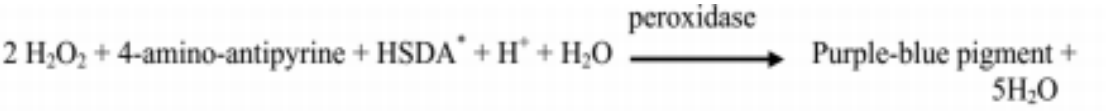
cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approx.40%).



Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids bycholesterol esterase.



In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to be converted in to cholestenone and hydrogen peroxide.



Where: HSDA= N-(2-hydroxy-3-sulfopropyl)-3, 5-dimethoxyaniline.

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrineand HSDA to form a purple blue dye. The color intensity of this dye is proportional to the cholesterol concentration and can be measured spectrophotometrically (Jacobs *et al.*, 1990).

Procedure: Ten micro liter (10µL) serum samples were added into the sample cups and put on the sample disk which rotates to bring the desire sample cup into position next to the sample probe for specimen sampling. 1000µL buffer and 1000µL substrate were pipetted into reagent bottles leveled for HDL-c and put on the reagent disk. Then on the screen menu of the machine. HDL-c was entered as a parameter to be tested. The sample probe pipetted sample from the sample disk and transferred to the reaction disk which contains cuvettes. On the other side of the machine, the reagent probe pipetted reagents from the reagent disk and transferred it into rotatable reaction disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly

the sample and the reagents. The cuvettes were immersed in to reaction water bath and incubated at 37^0C for 5 minutes. Next the reaction disk was rotated the cells to all reaction stations including the photometer light path. Finally, the light was through the cuvettes and absorbance of the sample will be measured at 500nm [Jacobs *et al*, 1990].

D. Determination of Low Density Lipoprotein Cholesterol

Most of the circulating cholesterol is found in three major lipoprotein fractions: VLDL, LDL and HDL. LDL-c is calculated from measured values of total cholesterol, triglycerides and HDL-c according to the Friedewald equation: LDL-c = TC [HDL-c + TG/5)]. Where [TG]/5 is an estimate of VLDL-c, all values are expressed in mg/dL. The equation is derived from another equation, [Total Cholesterol] = [VLDL-c] + [LDL-c] + [HDL-c], but TG is easier to estimate than VLDL and [TG/5] is a good estimate of VLDL, although the Friedewald equation is not valid for calculating LDL-c if the serum TG is above 400 mg/dL (Roschlay *et al.,* 1975, and Jacobs *et al.*, 1990).

3.7.3 Anthropometrical measurement procedure

The weight of the Pulmonary Tuberculosis patients was measured using a standard balance, and the height was measured by using a height measuring device attached to the balance. Body Mass Index (BMI) was then calculated from the body weight (kg) and height (meter) as follows: BMI = Weight (in kg) / (Height in m)^2 (Tambe *et al*., 2010). Using the WHO classification (WHO, 1997), four categories of BMI can be identified as follows: underweight, <18.5 kg/m^2; normal, >18.5–24.9 kg/m^2; overweight, >25.0–29.9 kg/m^2; and obesity, >30 kg/m^2. The participants‟ ages were also recorded.

3.8 Variables

3.8.1 Dependent variable

- Serum total cholesterol concentration (TC)
- Serum triglyceride concentration (TG)
- High density lipoprotein (HDL) cholesterol concentration
- Low density lipoprotein (LDL) cholesterol concentration
- Hemoglobin
- Hematocrit
- RBC
- WBC
- Platelets
- RBC indices

3.8.2 Independent variable

- Socio-demographic factors
- Family history
- Clinical and behavioral factors
- Anthropometric indicators

3.9 Operational definitions

Hematological profile in healthy individuals expected to be in range WBC (4-11x10^3/uL), RBC (4.3-5.9x10^6/uL), HGB(14-18g/dL), HCT(42-54%), and in RBC indices MCV(80-100fL), MCH(27-31pg), MCHC(33-37g/dL), PLT(150-400x10^3/uL) and part WBC differential (Neutrophils(2-7x10^3/uL), and Lymphocytes(1-3x10^3/uL) (Sysmex, 2000).

The definition of anemia used in this study was hemoglobin concentration less than 13 g/dL in men and 12 g/dL in women (WHO recommendation).

Lipid Profile: is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol, DDL-c, LDL-c and triglycerides. The normal range

of total cholesterol is from 150-200mg/dL, LDL-c refers as ‚good cholesterol. Normal range 40-60mg/dL (Carl A, 2006).

LDL-c termed as „bad cholesterol" and are considered as major risks for cardiovascular diseases its normal range <100-130 mg/dL and Triglyceride normal range is 100-150mg/dL (Silverton D, 2009).

Anthropometric indicators: parameters for the measurement of the human body and its individual parts thereby yielding a quantitative index of their variability. They include age, height, weight, body mass index, waist circumference and waist to hip ratio.

3.10 Data Quality Control and management

Standard daily quality control protocols were performed. All instruments were operated and quality controlled according to the manufacturer's instructions and normal and abnormal controls were run daily. No analysis was done if controls were out of range. Recommended values of quality control for low, normal and high level is WBC=7.1-7.8, 7.3-7.6 and 7.6- 8.1($x10^3$/uL) RBC=4.3-4.55, 4.50-4.52, 4.52-4.70 (10^6 /uL), HGB= 14.5-15.0, 14.8-15.0, 15.0-15.5 (g/dL).

Data collectors were received half day training about the objectives of the study and how to approach participants. The questionnaires were pretested at Aykel and Kolladiba hospital then modified accordingly. 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. The blood sample were collected and processed by trained laboratory professionals.

3.11 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 was performed by coding and entering the data into SPSS software version 20 package and the different variables were tested and analyzed. Simple descriptive statistics were used to present the socio-demographic and clinical characteristics of the study subjects. Continuous variables were presented as mean ± standard deviation and were compared using the student independent t-tests for groups. Other associations were performed with Pearson's correlation coefficient as well as linear regression

analysis. A p-value of <0.05 at 95% confidence level was considered to be statistically significant in all the analyses.

3.12 Result Dissemination

The results were reported and utilized for the purpose of patient care, and will be submitted to Addis Ababa University School of Medicine, Department of Biochemistry, Gondar University Hospital Tuberculosis center and published in reputable journal. It will also be presented at seminar and annual research conferences.

3.13 Ethical consideration

Before starting data collection and beginning study, ethical clearance letter with reference number SOM/DRERC/BCHM073/2009 was obtained from the Departmental Research and Ethics Review Committee, Department of Biochemistry, College of Health Sciences, Addis Ababa University. Collaboration letter for data collection was also obtained from Gondar university and Metema hospital. The objective of the study was briefly clarified and explained for each participant, before enrolling any of the eligible study participants. Samples and data were collected after informed consent had been obtained from the study participants. Confidentiality, anonymity, neutrality, accountability and academic honesty was maintained throughout the study, for example, by using codes. The findings of the study will be disseminated for health care professionals and other concerned bodies for better care of the tuberculosis patients than ever.

4. RESULTS

4.1 Socio-demographic characteristics of the study participants

The study included 88 study populations that comprise 44 patients already diagnosed as TB patients with mean ± SD of age 32.68 ± 13.44 years and 44 apparently healthy individual volunteers to participate with mean ± SD of age 32.25± 10.23 years. Thirty (68.2%) were male in case group and 27 (61.4%) were male in control group. The control groups were volunteers, apparently healthy and free from disease or medication therapy for three months before sample collection.

The study participants were Farmers (56.8% and 51.1%), married (50% and 45.5%) and income less than 500 ETB per month (47.7% and 47.7%) in cases and control groups, respectively.

Twenty five (56.8%) of the case group were under weight and 19 (43.2%) were normal weight, on the other hand 28 (63.6%), 7 (15.9%), 6(13.6%) and 3 (6.9%) of the control groups were normal weight, overweight, underweight and obese, respectively. While 23 (52.3%) of cases and 21 (46.7%) of control had history of alcohol dinking behavior, there were 10 (22.7%) of smoking behavior in both case and control groups. Nine (20.4%) of cases and 10 (22.7%) of control were both alcohol and tobacco users (**Table 1**).

Table 1. Socio demographic characteristics of the study participants at Metema and Gondar Hospitals, from January to July 2017 (n=88)

No	Variables	Case (44)		Control (44)	
		Frequency	%	Frequency	%
1	**Sex**				
	Male	30	68.2	27	61.4
	Female	14	31.8	17	38.6
2	**Age (Yrs)**				
	18-24	11	25	10	22.7
	25-34	19	43.2	20	45.5
	35-44	7	15.9	9	20.5
	$\geq$ 45	7	15.9	5	11.4
3	**Marital status**				
	Married	22	50	20	45.5
	Divorced	3	6.8	3	6.8
	Single	16	36.4	17	38.6
	Widowed	3	6.8	4	9.1
4	**Residence**				
	Rural	22	50	22	50
	Urban	22	50	22	50
5	**Occupation**				
	Daily laborer	5	14.4	3	8.9
	Students	5	14.4	6	13.3
	Civil servant	2	6.8	4	8.9
	Merchant	3	4.5	3	6.7
	Farmer	25	56.8	23	51.1
	Others	4	9.1	5	11.1
6	**Educational level**				
	Illiterate	14	31.8	17	38.6
	Elementary	21	47.7	11	25.0
	High school	3	6.8	11	25.0
	College	6	13.6	5	11.4

Continued Table: Socio-demographic characteristics					
No	**Variables**	**Case (44)**		**Control (44)**	
		Frequency	**%**	**Frequency**	**%**
7	**Income**				
	< 500	21	47.7	21	47.7
	500-1000	9	20.5	8	18.2
	1001-2000	8	18.2	12	27.3
	> 2000	6	13.6	3	6.8
8	**Family size**				
	<3	18	40.9	20	45.5
	3 – 5	14	31.8	16	36.4
	> 6	12	27.3	8	18.2
9	**BMI**				
	Under weight	25	56.8	6	13.6
	Normal	19	43.2	28	63.6
	Overweight	0	0	7	15.9
	Obese	0	0	3	6.9
10	**Smoking status**				
	Yes	10	22.7	10	22.7
	No	34	77.3	34	77.8
11	**Alcohol use**				
	Yes	23	52.3	21	46.7
	No	21	47.7	23	53.3
12	**Alcohol and smoke users**	9	20.4	10	22.7

4.2 Levels of hematological parameters in study participants

The mean ± SD of hemoglobin (11.93 ± 2.01 g/dL, 14.60 ± 2.15 g/dL), red blood cell (4.37 ± 0.85x 10^6/uL, 5.10 ± .82 x10^6 /uL), hematocrit (37.96± 6.36%, 44.042 ± 5.35 %) in case and control groups, respectively. Statistical significant difference was observed in hemoglobin (P. = 0.001), red blood cell (P. = 0.001), and hematocrit (P. = 0.001) of case and control groups.

The mean ± SD of mean cell volume was (87.95 ±10.45 fL, 87.97± 5.50 fL), in case and control groups respectively. But the mean reduction was not statistically significant in TB patients as compared with control groups (P=0.991). The mean± SD of mean cell hemoglobin (27.80 ± 4.66 pg, 29.57 ± 2.46 pg), mean cell hemoglobin concentration (31.50 ± 2.19 g/dL and 33.41± 1.88 g/dL) was in case and control groups, respectively. There were statistically significant difference in MCH (P. = 0.028), MCHC (P. = 0.001) of case and control groups as shown in **table 2**.

The mean ± SD of total white blood cell count (7.81 ±4.08 x10^3/µL, 6.03± 2.67 x10^3/µL), absolute neutrophil counts (4.86 ±3.11 x 10^3/µL, 3.66 ± 2.33x10^3/µL), platelet count (328.61±120.99 x10^3/µL, 272.77±69.23 x10^3/µL) and erythrocyte sedimentation rate (69.18 ± 22.86 mm/hr, 14.34 ± 4.38 mm/hr) for case and control groups, respectively. There were statistically significant different in total WBC (P. =0.018), A.N.C (P =0.044), platelet count (P = 0.009) and ESR (P =0.001) as compared with the control. The mean ± SD of absolute lymphocytes count was (1.81±0.85 x10^3/µL, 2.49±2.46x10^3/µL) did not show significant difference (P =0.086).

Table 2: Levels of hematological profiles in PTB patients and apparently healthy controls at Metema and Gondar Hospitals, Ethiopia, from January to July 2017

Variables	Cases (n=44)	Controls(n=44)	P-value
RBC(10^6/ uL)	4.37 ± .85	5.10 ± .82	**0.001***
HGB (g/dL)	11.93 ± 2.01	14.60 ± 2.15	**0.001***
HCT (%)	37.96± 6.36	44.02 ±5.35	**0.001***
MCV (fL)	87.95 ±10.45	87.97± 5.50	0.991
MCH (pg)	27.80 ± 4.66	29.57 ± 2.46	**0.028***
MCHC (g/dL)	31.50± 2.19	33.41 ± 1.83	**0.001***
ESR (mm/hr)	69.18±22.86	14.34 ±4.38	**0.001***
Platelet(10^3/µL)	328.61±120.99	272.77±69.23	**0.009***
TWBC(10^3/µL)	7.81 ±4.08	6.03 ± 2.67	**0.018***
Neutrophil (10^3/µl)	4.86 ± 3.11	3.66 ± 2.33	**0.044***
Lymphocyte(x10^3/µl)	1.81±0.85	2.49±2.46	0.086

* Values are expressed as mean ± standard deviation;* The mean difference is significant at $P \leq 0.05$
PTB (Pulmonary Tuberculosis Patient)

The mean ±SD of hematological profiles stratified by sex are depicted in **table 3** below. MCH, MCHC, TWBC and absolute neutrophil counts were not significant in females but significant in males as compared to their male and female counterparts. Only three were insignificant in male, such as absolute lymphocyte counts, mean cell volume, platelet counts while red blood cell counts, hemoglobin, hematocrit, erythrocyte sedimentation rate, and platelet counts were significant in females.

Table 3: Levels of hematological parameters among Male and female study participants at Metema and Gondar Hospitals, Ethiopia, from January to July 2017

Variable	Male (n=57)			Female (n=31)		
	Case (30)	Control(27)	P.value	Case (14)	Control(17)	P.value
RBC($x10^6$/uL)	4.55 (0 .70)	5.35(0.83)	**0.001***	3.98(1.03)	4.70(0.62)	**0.023***
HGB (g/dL)	12.28 (1.90)	15.37(2.07)	**0.001***	11.17(2.11)	13.38(1.70)	**0.003***
HCT (%)	39.36 (5.57)	46.04(5.27)	**0.001***	34.97(7.10)	40.80(3.74)	**0.007***
MCV (fL)	87.19 (9.77)	87.67(4.16)	0.625	89.59(12.0)	88.45(7.26)	0.748
MCH (Pg)	27.28 (4.38)	29.53(2.06)	**0.018***	28.90(5.19)	29.64(3.05)	0.625
MCHC (g/dL)	31.20 (2.02)	33.55(1.68)	**0.001***	32.15(2.48)	33.17(2.09)	0.222
ESR (mm/hr)	68.63(23.65)	15.48(4.81)	**0.001***	70.36(21.9)	12.53(2.89)	**0.001***
Platelet(10^3/µL)	311.2(108.5)	264.85(66.4)	0.060	365.93(141)	285.35(73.76)	**0.050***
TWBC(10^3/µL)	7.46(2.83)	5.71(2.25)	**0.013***	8.57(3.01)	6.55(3.23)	0.243
Neutrophil(10^3/µl)	4.61(2.16)	3.49(1.76)	**0.038***	5.40(4.60)	3.94(3.06)	0.301
Lymphocyt(10^3/µl)	1.76(0.75)	2.79(3.09)	0.082	1.92(1.06)	2.02(0.67)	0.750

Values are expressed as mean ± standard deviation;* The mean difference is significant at $P \leq 0.05$

4.2.1 Type of anemia in study participants

Based on MCV, anemia can be Normocytic anemia (80-100 fL) was the most common, and identified in 15 (50%) of patients. Microcytic anemia (MCV < 80 fL) was next common, and found in 8 (26.6%) of patients and Macrocytic anemia (> 100 fL) was identified in 7 (23.3%) of PTB patients. However, 42 (95.5%) of control groups were normal but only 2 individuals were shown abnormalities in MCV.

4.2.2 The severity of Anemia among study population

The severity of anemia was determined by the result of hemoglobin level. Anemia was identified in 30 (68.2%) patients at the time of diagnosis of tuberculosis. Moderate anemia (HGB from 8.1-9.9g/dL) was found in 8 (18.2 %) of total patients, the mild anemia (HGB, from 10-13g/dL) was found in 21 (47.7%) of total patients, and severe anemia (HGB, < 8g/dL) was found in 1 (2.3%) of total patients. The remaining 14 (31.8%) of total patients were having normal HGB levels. However, in control group mild anemia was seen in 13(29.5%) but the rest 31(70.5%) had normal HGB as shown below in **figure 1**.

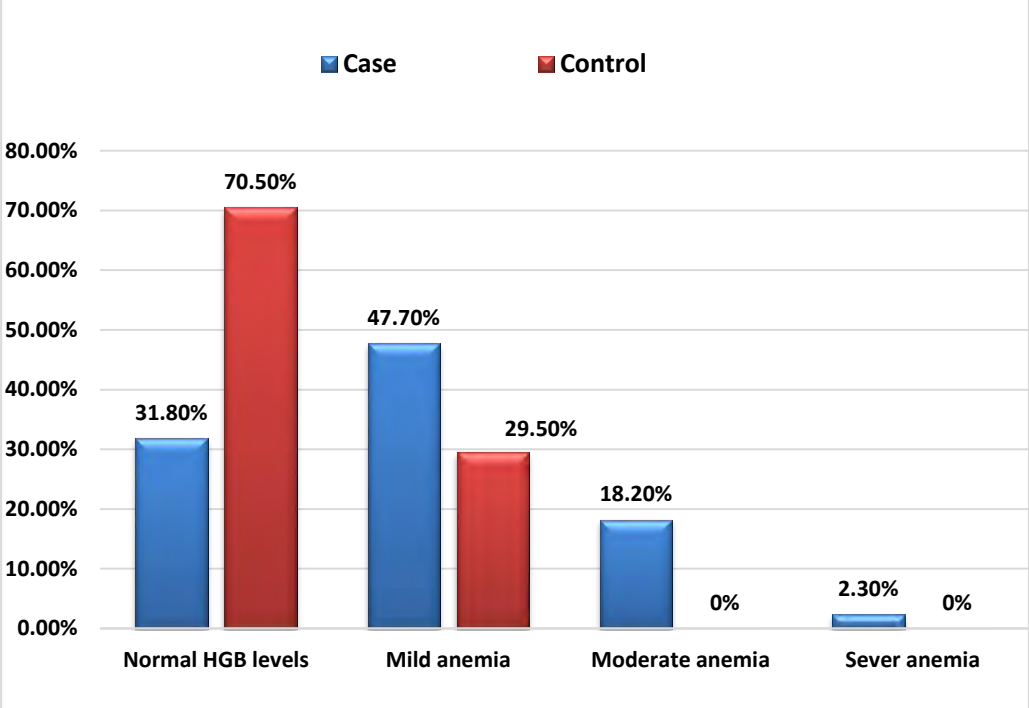


Figure 1: Severity of anemia stratified by hemoglobin concentration among study population.

4.2.3 Total leukocyte count in study population

Out of 44 patients, 9 (20.5%) had total leukocyte count below 4.0 x10^3/uL (leukocytopenia), 28 (63.6%) had normal total leukocyte count and 7 (15.9%) of TB patients had above 11.0 x10^3/uL (leukocytosis). However, out of 44 control groups 10(22.7%) of them were shown below normal range (leukocytopenia), 32(72.7%) in normal range, and 2(4.5%) were shown above range (leukocytosis).

4.2.4 Absolute Neutrophil Count (A.N.C) in study population

Out of 44 PTB patients, 6 (13.6 %) had A.N.C below 2 x10^3/uL (neutropenia), 30 (68.2%) had normal A.N.C and 8 (18.2%) had A.N.C above 7 x10^3/uL (neutrphilia) and out of 44 control groups 7(15.9%) were below normal (neutropenia), 32(75%) in normal range and remaining 4(9.1%) above normal range (neutropholia) as depicted in **figure 2**.

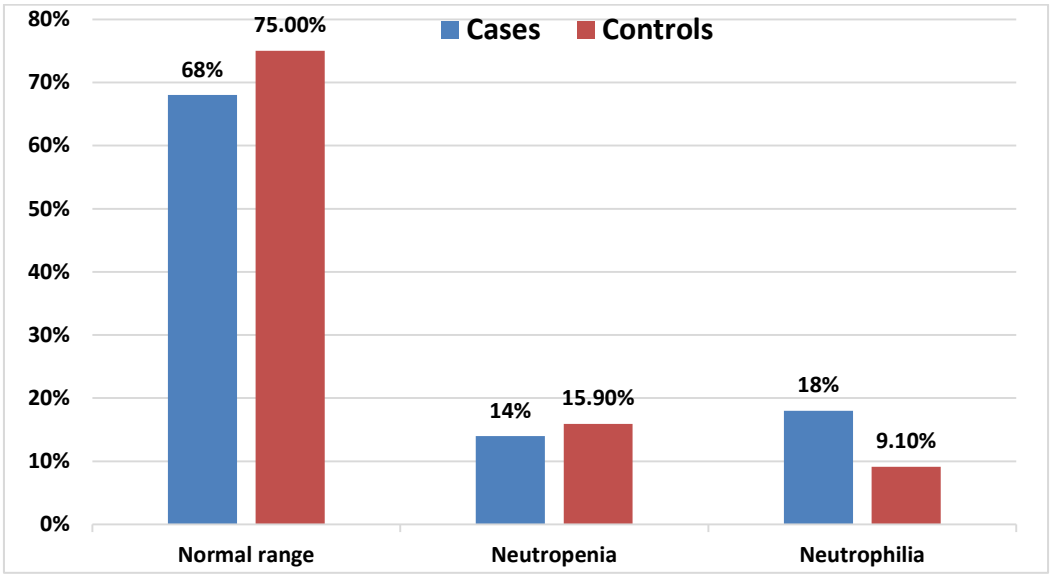


Figure 2: Absolute neutrophil count among study population.

4.2.5 Absolute Lymphocyte count in pulmonary TB patients

From 44 TB patients, 6(13.6%) had absolute lymphocyte count below1x10^3/uL (lymphopenia), 35 (79.5%) had normal absolute lymphocyte count and 3(6.8%) had absolute lymphocyte count above 3 x10^3/uL (lymphocytosis) and out of 44 control groups 2(4.5%) were below normal range (lymphopenia), 38(86.4%) in normal and 4(9.1%) above normal lymphocyte range (lymphocytosis).

4.2.6 Platelet Count among study population

In this study out of the 44 patients, 3 (6.8%) had less than 150 x10^3/uL (thrombocytopenia), 30 (68.2%) had normal platelet count which were between 150-400 x 10^3/uL and 11 (25.0%) had platelet count above 400 x10^3/uL (thrombocytosis) and from 44 control group only 2(4.5%) were above normal range (thrombocytosis) but the rest 42(95.5%) in normal range of thrombocyte as sown in **figure 3**.

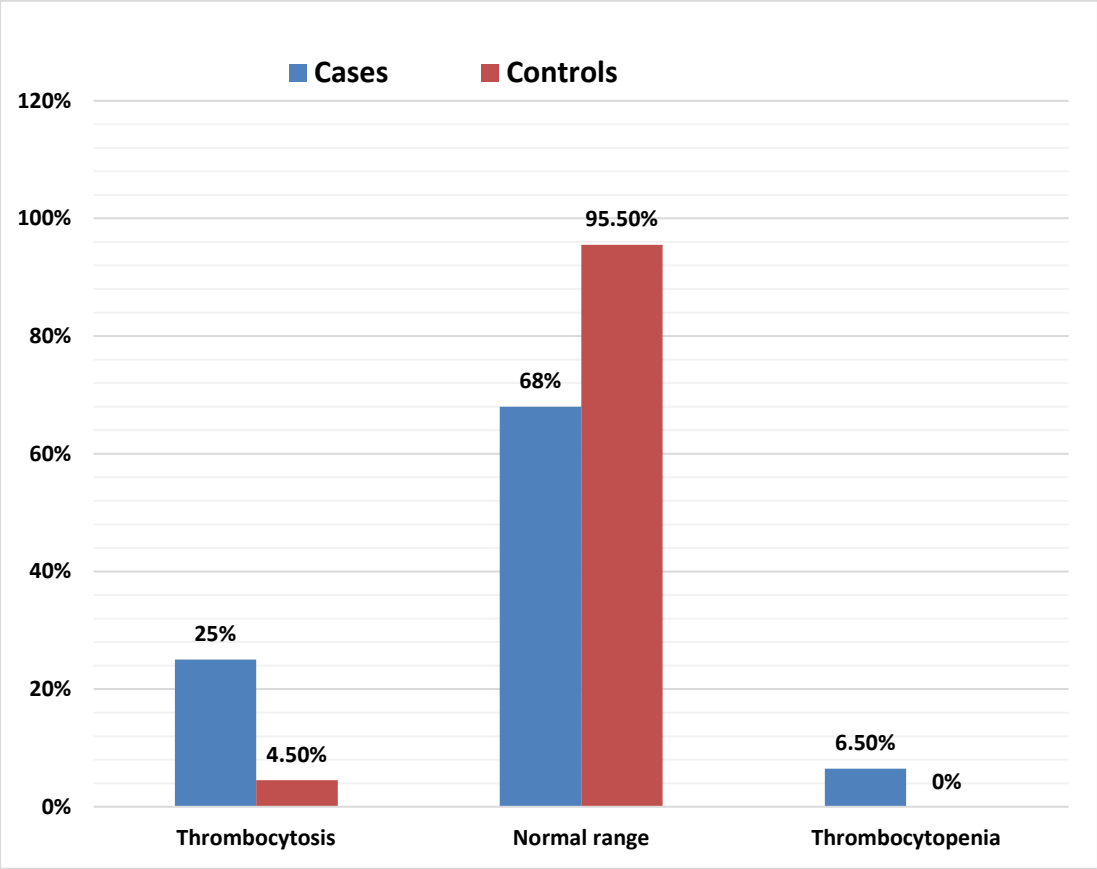


Figure 3: Platelet count among study population.

4.3 Levels of lipid panels in study participants

The mean ± SD of total cholesterol (101.72 ± 18.52 mg/dL, 139.77 ± 29.03 mg/dL), high density lipoprotein (29.38 ± 9.36 mg/dL, 41.75 ± 11.33 mg/dL), low density lipoprotein (58.25 ± 14.35 mg/dL, 75.98 ± 24.94 mg/dL) and triglyceride (77.59 ± 23.58 mg/dL, 102.29 ± 33.23 mg/dL) in TB patients and control group, respectively. Statistically significant mean reduction were observed in TC ($P < 0.001$), HDL-C (($P. < 0.001$), LDL-C (($P. < 0.001$) and PG (($P. < 0.001$) in case and control groups.

The mean ± SD of Body mass index was (18.28 ± 2.21kg/m^2, 22.08 kg/m^2) in case and control groups respectively. There was statistically significant mean reduction in TB patients as compared with control groups ($P. = 0.001$) as shown in table 4.

Table 4: Levels of lipid profiles in TB patients and apparently healthy controls at Metema and Gondar Hospitals, Ethiopia, from January to July 2017

Variables	Cases(44)	Controls(44)	P-value
TC (mg/dL)	101.72 ± 18.52	139.77± 29.03	**0.001***
HDL-c (mg/dL)	29.38 ± 9.36	41.75± 11.33	**0.001***
LDL-c (mg/dL)	58.25 ± 14.35	75.98± 24.94	**0.001***
TG (mg/dL)	77.59 ± 23.58	102.29 ± 33.23	**0.001***
B.M.I. (kg/m^2)	18.28 ± 2.21	22.08± 3.69	**0.001***

Values are mean ± SD; *supper scribe is significant at the mean difference P. value ≤ 0.05

PTB (Pulmonary Tuberculosis Patient)

Out of all study population, 9(20.5%) of cases as well as 23(52.5%) of control groups of triglyceride ranges were resulted in normal (100 – 150mg/dL). However, 35(79.5%) of cases and 17(38.6%) of control groups were shown below normal range (< 150mg/dL). There were no cases but 4(9.1%) 0f control groups shown above normal range (> 150mg/dL) of triglyceride.

Total cholesterol of all PTB patients were below normal range (<150mg/dL). However, 29 (65.9%), 14(31.8%) and 1 (2.3%) of control groups were shown in normal (150 – 200mg/dL), below normal and above normal ranges (> 200mg/dL), respectively.

All 44 (100%) of cases and 35(79.5%) of control groups had optimal level of LDL-C which is below 100 mg /dl, the cut-off value for the metabolite. The rest 9(20.5 %) of control groups had near optimal/above optimal level of LDL-C i.e. greater than 100 mg /dl.

On the other hand 3 (6.8%) of cases and 24(54.5%) of control groups were resulted in normal ranges (40 -60mg/dL) and 40 (90.9%) of cases as well as 17(38.7%) of control groups below normal range. The rest 1(2.3%) of cases and 3(6.8%) of control groups were shown above normal ranges of high density lipoprotein.

The results of lipid profiles in current study were also stratified by sex of participants. The TC, HDL-c, LDL-c and TG were significantly decreased in males. However, only TG was not significant in female as compared with control groups as shown in table 5.

Table 5: Levels of lipid profile among Male and female study participants at Metema and Gondar University Referral Hospital, Ethiopia, from January to July 2017

Variables	Male (n=57)			Female (n=31)		
	Case (n=30)	Control(n=27)	P.value	Case(n=14)	Control(n=17)	P.value
TC (mg/dL)	102.30(17.3)	142.48(27.05)	**0.001**	100.50(21.54)	135.47(32.30)	**0.002**
HDL(mg/dL)	30.47(10.67)	40.04(10.05)	**0.001**	27.07(5.22)	42.29(13.42)	**0.001**
LDL(mg/dL)	58.16(13.19)	76.72(25.21)	**0.001**	58.44(17.42)	74.80(25.21)	**0.048**
TG (mg/dL)	76.50(23.82)	105.70(36.55)	**0.001**	79.93(23.78)	96.88(27.33)	0.079
BMI(kg/m^2)	18.40(2.22)	23.27(3.48)	**0.001**	18.04(2.24)	20.21(3.30)	**0.046**

Values are expressed as mean ± standard deviation* The mean difference is significant at P. ≤ 0.05.
PTB (Pulmonary Tuberculosis Patient)

4.4 Factors associated with hematological and Lipid profiles

Bivariate, Pearson correlation, analyses showed that, status of study participant at the time of diagnosis were statistically significant positive correlation between being case and total WBC count (r=0.25, p< 0.05), Platelet count (r=0.27, p<0.05), and ESR (r=0.86, p< 0.05) but negatively correlated with RBC count (r= - 0.40, p<0.05), HGb (r= - 0.54, p <0.05) and HCT (r= -0.54, p <0.05); as well. All lipid profiles determined in this study like TC, HDL-c, LDL-c, and TG were negatively correlated with being case. Linear regression analysis also showed that 6.4%, 16.3%, 29.5%, 27.3%, 7.6%, and 73.9% of the variation in TWBC, RBC, HGB, HCT, Platelets, and ESR were respectively explained by being case or control.

In this study, age at the time of diagnosis was negatively correlated with total WBC (r= -0.23, p < 0.05), RBC (r = -0.141, p>0.05;); HGB (r = -0.079, p > 0.05); HCT (r = -0.103, p > 0.05) and platelet (r = -0.023, p > 0.05) but they are not statistically significant correlation with age except total WBC count. However, ESR (r= 0.156, p > 0.05) was positively correlated with age in the PTB patients. Linear regression analysis also showed that 5.4 %, 2.3 %, 0.6 %, 1.1%, 0.1% and 2.4% of the variations of TWBC, RBC, HGB, HCT, platelet and ESR were respectively explained by age. Age was positively correlated with serum Tc (r=0.162 p.> 0.05); HDL-c (r= 0.221, p <0.05; significant); LDL-c (r=0.089, p>0.05); TG (r=0.051, p>0.05); in the PTB patients and control groups. Linear regression also showed that 2.6%, 4.9%, 0.8% and 0.3% of the variation of Tc, HDL-c, LDL-c and TG were respectively explained by age.

The body mass index was statistically significant negative correlation with TWBC counts platelet count and ESR. However, RBC counts, HGB, and HCT were positively correlated. Also, lipid profiles such as TC, HDL-c and LDL-c were statistically significant and positively correlated but PG was not statistically significant correlation with BMI. Linear regression analysis also showed that 0.1 %, 1.1 %, 0.1 %, and 5.7%, of the variations of TWBC, RBC, HCT, and platelet were respectively explained by body mass index. On the other hand, lipid profile 11.1%, 0.7%, 12.5% and 0.3% showed variation in TC, HDL-c, LDL-c and TG respectively as explained by the body mass index.

Participants who were being case had lower level of TC, HDL-c, LDL-c and TG reduced by 38.04, 11.41, 14.05 and 22.66 units respectively compared to non-case (control). A unit increase

in age from one grade to the next showed an elevation of serum TC, HDL-c, LDL-c and TG by 0.42, 0.18, 0.18 and 0.05 units respectively.

BMI also showed significant association to all serum lipid profile except for TG. It was positively associated to TC, HDL-c, LDL-c and TG. An increase in BMI by 1 unit was associated with an increase in TC, HDL-c and LDL-c by 4.47, 1.24 and 1.48 units respectively.

Table 6: Linear regression analysis of factors associated with lipid level of study participants at Metema and Gondar Hospitals, January to July 2017 (n=88)

Variables	Tc		HDL-c		LDL-c		TG	
	β (95% CI)	**PV**	**β (95% CI)**	**PV**	**β (95% CI)**	**PV**	**β (95% CI)**	**PV**
Status(case)	-38.04(-48.36--27.72)	**.001***	-11.41 (-16.9 - -5.9)	**.000***	-14.05 (-24.63 - -3.47)	**.010***	-22.66 (-38.29 - -7.03)	**.005***
Age (per year)	0.42 (-0.13 - 0.97)	.133	0.18 (-0.30 – 0.40)	**.059***	0.18 (-.23 - .59)	.386	0.05 (-0.56 - 0.66)	.873
B.M.I	4.47(2.67 – 6.13)	**.001***	1.24(0.50 – 1.94)	**.001***	1.48(-0.03 – 2.98)	**.054***	0.17(-2.06 – 2.39)	.879
Resid(Rural)	-6.10 (-20.33 -8.09)	.394	-5.66 (-11.66 - 0.19)	**.046***	-1.01 (-11.12 - 9.09)	.842	-12.14 (-27.07 - 2.78)	.109
Income (<500)	-7.66 (-15.58 – 0.241)	**.057***	-1.36 (-4.39 - 1.66)	.373	-2.44 (-8.06 - 3.18)	.392	-4.86 (-13.16 - 3.43)	.247

β = Un Standardized coefficient, * = statistically significant at P<0.05, MS = Marital Status, BMI = Body mass index, Tc = Total cholesterol, HDL-c = High density lipoprotein cholesterol, LDL-c = Low density lipoprotein cholesterol, TG = Triglyceride, PV = P-value, CI = Confidence interval.

5. DISCUSSION

Tuberculosis is one of the most important communicable diseases in the world and is a major public health problem in Ethiopia (MOH, 2009). In fact, WHO has declared tuberculosis as a global emergency in 1993 (WHO, 2015). Various hematological and lipid profile alteration have been described in association with tuberculosis. There is no sufficient literature about the hematological and lipid profile abnormalities in pulmonary tuberculosis patients from Ethiopian population.

A total of 88 study population, 44 newly diagnosed TB patients and 44 control groups were involved in the study at Metema and Gondar hospitals, Ethiopia. The mean $\pm$ SD of study participants age were (32.68 $\pm$ 13.44 years, 32.25 $\pm$ 10.23 years), in case and control groups, respectively. Above two third of the study participant 30 (68.2%) were male in case group and more than half of the study subject 27 (61.4%) were male in control group. Higher percentages of study participants were Farmers (56.8% and 51.1%), married (50% and 45.5%) and income less than 500 ETB per month (47.7% and 47.7%) in cases and control category respectively.

The present study evaluated the hematological parameters (RBC count, WBC count, hematocrit, hemoglobin, Platelets, MCV, MCH, MCHC, and ESR) as well as serum lipid parameters (TC, LDL-c, HDL-c, and TG) in newly diagnosed TB patients and apparently healthy individuals. Significantly larger proportions of the patients were found to have decreased levels of hematological parameters such as hemoglobin, hematocrit, RBC counts, as well as RBC indices such as MCH, MCHC and the level of lipid profiles like TC, TG, HDL-c, LDL-C and anthropometric indicators like body mass index were also decreased in patients compared with control groups. Platelet counts, ESR and total WBC counts were also higher in TB patients than control groups. The present study discussed the findings of the hematological parameters and lipid abnormalities as well as body mass index of the TB patients with respect to control groups and factors associated with hematological and lipid profiles.

The present study found that hemoglobin, hematocrit, red blood cell count, and red blood cell indices like mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration were

showed statistically significant difference among TB infected patients ($P \leq 0.05$) compared to the control groups. On the contrary, pulmonary tuberculosis patients had statistically significant higher level of circulating platelets, erythrocyte sedimentation rate and white blood cell counts ($P < 0.05$) as compared to the control group.

These results are in agreement with the results of other related studies, which are conducted in different parts of the world including Ethiopia where statistically significant mean reduction of hemoglobin, hematocrit, and RBC indices of TB patients were reported (Shafee *et al.*, 2014, Abaker *et al.*, 2016, Muhammad, 2011 and Mohammed et al , 2016 thesis).

In our study the mean $\pm$SD of red blood cell in TB patients were ($4.37 \pm .85 \times 10^6$ /uL) and in control group were ($5.10 \pm .82 \times 10^6$ /uL) also showed significant decrease when compared with controls ($P < 0.05$). These finding were also supported with the results of other related studies which are conducted in different parts of the world (Kuppamuthu *et al*., 2016, Muhammad *et al*., 2011). The possible explanation for this RBC reduction might be due to suppression of erythropoiesis by inflammatory mediators‟ displays an absence of bone marrow iron (Robert *et al.*, 2005).

In this study 26.8% of patients had decreased MCV, 50% of patients had normal and remaining 23.3% of patients had increased but results were not statistically significant. These finding concede with other studies (Chakarbarti *et al*., 1995, Lee *et al*., 2006, Muhammad *et al*., 2011, Abaker *et al*., 2016).

There was statistically significant ($p < 0.05$) mean reduction in the value of mean cell hemoglobin and mean cell hemoglobin concentration in newly diagnosed pulmonary tuberculosis patients in comparison with healthy controls as shown in table 2 and this is in agreement with similar studies (Lee *et al*., 2006, and Abaker *et al*., 2016).

Anemia was occurred in 30 (68.7%) of the study populations. Normocytic anemia was the most common and identified in 15(50% of patients). These findings agree with reports made by Yaranal *et al.*, who reported in 74(68.9%) of patients had anemia. Normocytic anemia was the most common type and was found in 49 (66.2%) patients (Yaranal *et al.,* 2016).

The severity of anemia was determined as the result of hemoglobin level in which (18.2%) of total patients have moderate anemia and (47.7%) have mild anemia and only (2,3 %) of patients have severe anemia. These finding agree with similar study carry out by Al-Omar et al. (Al-Omar *et al.,* 2009).

There were highly significant ($p < 0.05$) decrease in the hemoglobin and hematocrit values in the newly diagnosed pulmonary tuberculosis patients in comparison with healthy controls as shown in table and this is in agreement with studies done by Eyshi *et al.*, who states that occurrence of anemia among patients that were diagnosed as active pulmonary tuberculosis is very high and it was contributed to anemia of chronic disease (Eyshi *et a*., 2009). This reduction may be associated with increased level of interlukin-6 (IL-6). Interlukin-6 produces delusion anemia and expansion of plasma volume resulting in reducing hematocrit and hemoglobin concentration in the circulating red blood cells (Atkins *et al*., 1995).

According to different scholars explained that the mechanism behind the occurrence of anemia in pulmonary tuberculosis patients might be when the cells or organs invaded by bacteria leads to activation of T-lymphocytes and macrophages, which induce the production of the cytokines like tumor necrosis factor-alpha (TNF-alpha), interferon gamma (IFN-gamma),interleukin-1 (IL-1) and interleukin-6(IL-6) which with their products often will cause diversion of iron into iron stores in the reticulo-endothelial system resulting in decreased iron concentration in the plasma thus limiting iron availability to red cells for hemoglobin synthesis, inhibition of erythroid progenitor cell proliferation and in appropriate production and activity of erythropoietin, the first leads to anemia and the latter two results in suboptimal response of the bone marrow to the anemia. Cytokines also impair red cell production in the marrow. IL-1 and TNF-alpha inhibit the production of erythropoietin, and together with IFN-gamma impair responsiveness of progenitor cells to erythropoietin. An inhibitory effect of TFN-alpha on red cell production in the bone marrow has been demonstrated by both in vitro and in vivo studies. IFN-gamma directly suppresses the proliferation of erythroid progenitor cells, in this way cytokines impair the physiological erythropoietin response to the anemia. In addition, TNF-alpha directly damages erythrocytes and decreases red cell life span. So the anemia of infection is therefore basically an underproduction anemia due to iron restriction, combined with inability of erythropoiesis to compensate adequately for the anemia (Weiss, 2002, Means, 2003, Nemeth *et al.,* 2004).

The result of leukocyte counts were shown in 20.5% 0f PTB patients decreased leukocyte count (Leukocytopenia), 63.6% of patients in normal and 15.9% patients increased leukocyte count (leukocytosis). These finding agree with similar study in Sudan by Abaker *et al*.,(Abaker *et al.*, 2016). However, disagree with Mohammed *et al*., 2016, his result was no statistically significant difference in the occurrence of leukopenia or leukocytosis between PTB positives and negatives patients (p=0.005). However, among the 15 leukopenic patients, 12(80.0%) were PTB patients as diagnosed by GeneXpert which is remarkable (Mohammed, 2016). Absolute neutrophil count was significantly increased when compared with control groups, these result agree with similar study (Abaker *et al.,* 2016).

Therefore, this study found a significant increase in total leukocyte count that may be resulted from immune reaction taking place in response to foreign antigen *Mycobacterium tuberculosis* that also resulted in increased cytokines levels. These cytokines are *IFN-γ, IL-1β*, and *IL-18* which in turn causes further proliferation of white blood cells (Abraham *et al*., 2015).

From this study resulting data showed that values of platelets count of pulmonary tuberculosis patients were highly significantly increased (p = 0.009) in comparison with values of healthy controls. These results may be similar with Abaker *et al*., to the reactive thrombocytosis which was found in a number of clinical situations including infectious diseases such as pulmonary tuberculosis (Abaker *et al*., 2016). And also agree with Al- Omar *et al*, the increase in platelet count was noticed in Saudi pulmonary TB patients as compared with the normal Saudi persons (Al- Omar *et al.,* 2009). However, the current results have been in contrast with similar research done in Pakistan and Nigeria. They found that there were significantly lower platelet counts in pulmonary tuberculosis patients (Sumaira *et al., 2015,* Awodu *et al*., 2007).

The possible explanation for these research results might be returns to elevated levels of IL-6 and IL-1 in patients with pulmonary tuberculosis suffering from anemia has been founded in a study done in Indonesia (Karyadi *et al.*, 2007). This interlukin-6 (IL-6) induced thrombocytosis is accompanied by enhanced hepatic thrombopoietin production and elevated thrombopoietin plasma levels (Kaser *et al*., 2001)

In this work it has been found that the values of erythrocyte sedimentation rate for pulmonary tuberculosis patients were highly significantly increased ($p < 0.001$) in comparison with healthy controls. These findings are fully supported by different reports and studies specifying high ESR levels in newly diagnosed and normal levels at the end of treatment of pulmonary tuberculosis patients (Al-Omar *et al*., 2009) who stated that erythrocyte sedimentation rate were elevated in pulmonary TB which is well influenced and corrected by using different combinations of anti-tuberculosis drugs. Other study stated that, erythrocyte sedimentation rate was significantly higher in pulmonary tuberculosis patients than controls; there was a significant reduction in the erythrocyte sedimentation rate from the 4^{th}week of therapy (Awodu *et al*., 2007). And other one says: statistically significant hematologic abnormalities like high erythrocyte sedimentation rate founded in pulmonary tuberculosis patients in Ibadan, Nigeria (Olaniyi *et al*., 2003).

The possible cause of an increased erythrocyte sedimentation rate of TB patient might be when an inflammatory process is present, the high proportion of fibrinogen, other acute-phase proteins (haptoglobin, ceruloplasmin and CRP) and immunoglobulins in the blood causes red blood cells to stick to each other, these jammed red cells forms what is called rouleaux, which will settle and sediment faster (Lewis *et al*., 2006).

The present study found that the average levels of serum TC, TG, HDL-c and LDL-c` were significantly decreased among pulmonary tuberculosis infected patients ($P< 0.001$) compared with the controls group. These lower mean levels of TC, TG, HDL and LDL-cholesterol in TB patients are in agreement with the results of other related studies which are conducted in different parts of the world including Ethiopia that reported hypocholesterolaemia, low level of HDL-c and LDL-c and hyportriglyceridemia in a patient with tuberculosis (Oyedeji *et al*., 2012, Mohamed *et al*., 2012, Füsun *et al*., 2013, Yemane, 2014, thesis, Taparia *et al*., 2015).

The decreased in TC levels in TB infections are related with concentrations of antioxidant were significantly lower ($P < 0.001$) in TB patients when compared with that of the control subjects (Oyedeji *et al*., 2012). Therefore, lipid peroxidation becomes increased resulting in reduction of lipids such as Tc, TG, HDL-c, and LDL-c and also several factors might be contributed in the reduction of lipid, such as low food intake, nutrient malabsorption, inadequate nutrient release from the liver, acute phase response to infection, inadequate availability of carrier protein may influence circulating antioxidants concentrations. The increased concentration of lipid

peroxidation products associated with TB infection may be a contributing factor to the significantly low level of lipid profile in TB patients compared with the control subjects (Oyedeji *et al*., 2012).

Triglyceride and LDL–cholesterol are major constituents of cell membrane while HDL–cholesterol protects arterial walls of the blood circulatory system (Oyedeji *et al*., 2012).The significantly low concentrations of triglyceride, total cholesterol and LDL–cholesterol observed among tuberculosis patients in this study correlates with the findings of previous studies, have reported increased lipid peroxidation in all categories of TB patients (Reddy *et al*., 2009).

The lower levels of the lipid fractions observed in TB patients when compared with the control subjects could also be as a result of impaired rate of lipid production and enhanced rate of lipid catabolic rate associated with pulmonary TB infection (Oyedeji *et al*., 2012).

In the present study, being case has a significant association with most of hematological and all serum lipid level among study participants. It affect in most of hematological and in all of lipid profile level negatively except for white blood cell, platelets, and erythrocyte sedimentation rate. The inverse association of PTB disease to hematological profiles like RBC, HGB, HCT, RBC indices and lipid profile like total cholesterol, high density lipoprotein, low density lipoprotein and triglyceride were supported by different studies carried out by various researcher which demonstrated statistically significant mean reduction in PTB patients compared to healthy control groups (Fusun *et* al., 2012, Muhammad *et al*., 2011, and Taparia *et al*., 2015).

Body mass index showed statistically significant positive correlation to TC, HDL and LDL but not significant with TG. The findings were supported by various studies which revealed a significant association between body mass index and lipid profile (Zamani *et al*., 2012, and Lior *et al*., 2011).

The association is related to adiponectin secreted from adipocytes, which is a potent modulator of glucose and lipid metabolism and an indicator of metabolic disorders. The increase in BMI decrease the concentration of adiponectin has metabolic effect on serum lipid profile (Martin *et al*., 2005).

6. CONCLUSIONS

In the present study many hematological and lipid profiles abnormalities have been demonstrated in pulmonary tuberculosis patients. According to our results, mild and normocytic anemia was found in the majority of pulmonary tuberculosis patients. This result may be due elevated levels of IL-6 and IL-1 in patients with pulmonary tuberculosis. Total WBC counts and absolute neutrophil counts were significantly increased in pulmonary tuberculosis patients compared to controls group. This increment may be resulted from immune reaction takes place in response to foreign antigen *Mycobacterium tuberculosis*. Erythrocyte sedimentation rate and platelet counts of almost all TB patients were significantly increased as compared with control groups.

Pulmonary tuberculosis disease was also associated with marked reductions in Tc, HDL-c, LDL-c and TG concentration when compared to healthy control group. This reduction of lipid may be resulted from impaired rate of lipid production and enhanced rate of lipid catabolic rate associated with pulmonary TB infection. The study provides reliable and current information regarding the relation of PTB with lipid and hematological alteration and calling up for an urgent attention for those aberrations.

The marked change in hematological and serum lipid concentration seems to be an additional feature of PTB associated disorders of lipid metabolism. The result helps to design an alternative ways of patients follow up and prediction of the disease prognosis.

7. RECOMMENDATIONS

Based on the above research finding the following recommendation are forwarded

- ☞ Hematological and lipid profile abnormalities in pulmonary tuberculosis are common and may be used as valuable aids in patients clinical management.
- ☞ Patients should be monitored for their marked change of lipid profile, hematological profiles and other risk factors at the time of diagnosis, treatment and follow up.
- ☞ The mechanism of lipid metabolism in PTB disease should be further studied for better understanding patho-physiology of the disease and consequently to novel treatment approaches.
- ☞ Large observational studies are required to establish a possible role of PTB in lipid and hematological alteration and its effect on the rest of biochemical values by using appropriate sample size.
- ☞ Furthermore studies are needed on tuberculosis applying technology advancement in molecular biology and bacterial genome to identify the genes and enzymes involved in the disease progression and lipid as well as hematological alterations should be considered in further investigation.

8. STRENGTHS AND LIMITATIONS OF THE STUDY

The study can express its strength that it includes several demographic, clinical and anthropometric parameters claimed to be associated with the variables under study. It also gives floors to further researches on PTB with lipid and hematological profile issues. In addition, the anthropometric indicators are measured directly than by self-report.

Despite the above mentioned strengths, this study has several weaknesses. Considering relatively smaller sample size and selecting those study sites purposefully makes difficulty to represent the whole tuberculosis patients in the population and therefore, failed to generalize. In addition, the study could not compare the effects of lipid and hematological profiles variations in dietary habits and physical exercises. The study could not test Malaria and HIV/AIDS at the time of sample collection but patient ID cards up to date history were chalked. Lack of ample previous study findings limited the comparison of these study findings with other findings in similar hospitals in Ethiopia. Finally, being a cross-sectional study by design it cannot observe prospectively and thus cannot associate causal relationships between the factors under study.

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10. ANNEXES

10.1 Annex 1. Information sheet (English Version)

Research Project: Evaluation of lipid profiles and hematological parameters in tuberculosis patients at Metema and Gondar University Referral Hospital.

Sponsoring organization: Department of medical Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa University

Principal Investigator: Mohammed Yesuf (B. Vet. Science, MSc in biochemistry candidate)

Advisors: Daniel Seifu (PhD), and Araya Mengistu (PhD)

Introduction

Dear participants you are kindly requested to take part in this research project as a study participant voluntarily. Read the information provided in this sheet carefully and then respond freely and voluntarily to what the investigator interviews you.

Objective of the research project

This information sheet is prepared by the investigator and the advisors at AAU for a project with the objective of evaluation of lipid profiles and hematological parameters among tuberculosis participants.

Procedure

If you agree to take part in the study, the investigator or a health worker will give you verbal and/or written information about the study and you will be given the consent form to sign, the physician or health professional will ask you some questions about your general health and perform a complete medical examination and assess whether you qualify to participate in the study. If you are fit for the study about 8 ml of blood samples will also be collected for only the laboratory examination of complete blood count, HDL, LDL-C, total cholesterol, triglycerides and face to face interview for additional questions.

Discomforts and risks and benefits from participation

The degree of discomfort you may encounter in giving the sample is no more than when one does in his/her routine examination. But, there could be cases in which minor pain and change in color of your skin following the blood drawing occur transiently. The blood will be withdrawn by licensed health care professionals in the hospital and appropriate care will also be taken. You will not be provided with any direct incentives for your participation in the research. But the cost for general medical examination will be covered by the project. In addition, based on the results obtained from the research you will be cared accordingly or the results may serve you as a baseline data. In addition, the result of the study will be beneficial for the better prevention and care of tuberculosis patients than before. Hence, you are indirectly benefiting other patients and the society in this aspect.

Confidentiality

All pieces of information about the patients will be kept confidential. Log books used in the laboratory will have no names but codes. The information sheet that links the coded number to patient name will be locked inside a box and it will not be revealed to anyone except your physician and the principal investigator. You have full right to withdraw from participating in this study at any time before and after consent even without explaining the reason. Your decision will not affect your right to get health service you are supposed to get otherwise.

Contact information: if you have any questions contact Mohammed Yesuf : with 0918784413

10.2. Annex 2: Informed consent (English version)

Department of medical Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa University, Consent form for the participation of the study participants in the research project

Name of the study participant ……

Code number…..……

I have clearly been informed about the research project that it aims to evaluate and correlate serum lipid panels and hematological parameters among tuberculosis patients. The objectives of the research project have clearly been explained to me and I have been told that the results obtained from me will help me as well as the community for better management of the disease. I had been also informed about the confidentiality of this research project. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and make myself withdraw from the study. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and blood sample as the doctors find best for me.

Signature: _____ Date_____

10.3. Annex 3: Questionnaire (English version)

Dear respondents, you are kindly requested to give correct information accordingly. Thank you for your time and participation.

Questionnaire code number_____

Part-I: Socio-Demographic Characteristics

1. Sex Male □ Female □
2. Age _____
3. Marital status Married □ Single □
   Divorced □ Widowed □
4. Residence Rural □ Urban □
5. Occupation Laborer □ Merchant □
   Gov't employee □ other _____
6. Educational status Illiterate □ Primary School □
   Secondary school □ College and University □
7. Monthly income Less than 500 □ 500 to 1000 □
   1001 to 2000 □ Greater than 2000 □
8. Family sise _____
9. Religious belief? Orthodox □ Muslim □ Protestant □ Other □

PART 2:- Clinical and anthropometric characteristics

10. Was the patient diagnosed with tuberculosis before current episode of TB?
    Yes □ No □
11. Household member currently taking treatment for TB? Yes □ No □
    If yes, who is on anti-TB treatment?_____
12. Household member previously treated for TB? Yes □ No □
13. Is there any other disease? Did you taking any drug? _____
14. Does the client smoke? Yes □ No □

15. Is the client taking alcohol? Yes □ No □

16. Height _____

17. Weight _____

18. BMI _____

I Thank

10.4. Annex 4: Information sheet (Amharic version)

የተሳታፊዎች የፈቃደኝነት መተማመኛና መረጃ መስጫ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ትምሕርት ክፍል፡
ጥናቱን ስፖንሰርያደረገዉ ተቋም አዲስ አበባ ዩኒቨርሲቲ ጤና ሣይንስ ኮሌጅ ነው፡፡

መረጃ መስጫ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምናባዮኬሚስትሪት/ክፍል ሁለተኛ ዲግሪ ተማሪ የመመረቂያ ጥናት ጽሁፍ ላይ እዲሳተፉተጋብዘዋል፡፡ እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነ ልዎትን ማንኛዉንም ሃሳብ ይጠይቁ፡፡

Evaluation of lipid profiles and hematological parameters in tuberculosis patients at Gondar University Referral Hospital የጥናቱ ርእስ ሲሆን አላማውም የትቢ ታካሚዎች በደማቸው ውስጥ ያለውን የቅባት መጠንና የደም ህዋሶች እንዲሁም ሌሎች ከትቢ በሲታ ጋር ግንኙነት ያላቸውን ነገሮች መጠንና ሁኔታ መለካት ነው፡፡የጥናቱ ውጤት ለታካሚው ብሎም ለሌላው ማህበረሰብ የሚጠቅምና የተሻለ የጤና እንክብካቤ እንዲኖር የሚያደርግ ነው፡፡እናም እርስዎ በዚህ ጥናት ለመሳተፍ ጠቃሚና ምቹ ሆነው ተመርጠዋል፡፡ የእርስዎ በዚህ ጥናት ላይ የሚያደርጉት ተሳትፎ ሙሉ በሙሉ በበነ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡

በጥናቱ ከተሳተፉ ለናሙና ይሆን ዘንድ 5ሚሊሊትር ያህል ደም በሆስፒታሉ ጤና ባለሙያዎች የሚሰጡ ሲሆን የደም ናሙናውን በሚሰጡበትም ሰአት ሁል ጊዜ ለምርመራ ከሚሰጡበት የተለየ ህመምና አለመመቸት የለውም ለምናልባት ቢኖር ተገቢውን የጤና እንክብካቤ የሚያገኙ ይሆናል፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋን በዚህ ሆስፒታል የሚሰጠዎ ማንኛዉም አገልግሎት ላይ ተጽዕኖ የለውም፡፡ በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅበዎታል፡

ግልጽ ያልሆነልዎ ጥያቄ ካለ
ሞባል፡ 0918784413 *መህመድ የሱፍ ብለዉ በመደወል መጠየቅ ይችላሉ*

10.5. Annex 5: Informed consent (Amharic version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚስጥር ቁጥር----------

የተሳታፊዉ ስም----------

እኔ ስሜ ከላይ የተገለጸዉ ግለሰብ የተፈለኩት በዚህ ጥናት እንድሳተፍ ሲሆን ትቢ ያለባቸው ታካሚዎች በደማቸው ውስጥ ያለውን የቅባት መጠንና የደም ህዋሶች እንዲሁም ሌሎች ከደም ግፊት ጋር ግንኙነት ያላቸውን ነገሮች መጠንና ሁኔታ መለካት የሚለው ጥናት አላማና ጥቅም ተገልፆልኛል፡፡ ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የምሰጠዉ በአጠቃላይ የጥናቱን አላማና ጥቅም በመረዳትና በፍጹም ፈቃደኝነት ነው፡፡ በመጠይቁ ላይ የምሰጠዉ የእኔ መረጃ እንደማይባክን እንደሚያዝም ተነግሮኛል፡፡ በተጨማሪም ጥናቱ ዉስጥ ላለመሳተፍ ከፈለኩኝ መብቴ የተጠበቀ እንደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት ጭምር መብቴ መሆኑንና ከጥናቱ በመዉጣቴ ምንም አይነት ችግር እንደማይደርስብኝ በሚገባ ተገልጾልኛል፡፡

ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ፈቃደኝነቴን ሰጥቻለሁ፡፡ በተጨማሪም የምሰጠዉ የደም ናሙና ለCholesterol.Triglycerides,HDL-C፣ LDL-C እና complete blood count ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛዉንም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡

በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች በጊዜዉ ለህኪሜ እንደሚሰጥልኝ እና ዉጤቱን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡ በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤ አለሁ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

እኔ_____የተባልኩት ግለሰብ ይህን ሁሉ በማገናዘብ በምርምሩ ላይ ስለ እኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ ቀን

የተሳታፊ _____ _____

10.6. Annex 6: Questionnaire (Amharic version)

መጠይቅ
ውድ ተሳታፊ ቀጥሎ ያለውን መጠይቅ ለመሙላት ስለ ተባበሩን እናመሰግናለን፡፡

የመጠይቁመለያቁጥር_____

Part-I: Socio-Demographic Characteristics

1. ጾታ ወንድ □ ሴት□
2. ዕድሜ_____
3. የጋብቻ ሁኔታ ያገባ □ ያላገባ □
   የፈታ □ የሞተበት □
4. የመኖሪያ ቦታ ገጠር □ ከተማ □
5. የስራ ዓይነት የቀን ሰራተኛ □ ነጋዴ □ ገበሪ □
   ተማሪ □ የመንግስት ሰራተኛ □ ሌላ□
6. የትምህርት ደረጃ ማንበብና መጻፍ የማይችል □ አንደኛ ደረጃ □
   ሁለተኛ ደረጃ □ ኮሌጅ/ ዩኒቨርሲቲ □
7. የወር ገቢ መጠን ከ500 ብር በታች □ ከ500 እስከ 1000□
   ከ1001 እስከ 2000 □ ከ 2000 ብር በላይ□
8. የቤተሰብ ብዛት_____
9. ሀይማኖት ኦርቶዶክስ ክርስቲያን □ ሙስሊም□ ፕሮቴስታንት□ ሊላ □

ክፍል 2፡- ከህክምናና ከልኬታ ጋር የተያያዙ ባህርያቶች

10. ከዘህ በፊት ትቢ ታክመዉ ያዉቃሉ አዎ □ አላቅም □
11. በአሁኑ ሰአት የትቢ መዳኒት የሚወስድ የቢተሰብ አባል አለ አለ □ የለም □
12. ከዚህ በፊት የትቢ መዳኒት የሚወስድ የቢተሰብ አባል አለ አለ □ የለም □
13. ከ ትቢ ሊላ ተጨማሪ በስታ አለበዎ መዳኒትስ ይወስዳሉ አዎ □ የለብኝም □ አልወስድም □
14. ሲጋራ የማጨስ ልምድ አለብዎት አዎ□ የለብኝም □
15. አልኮል የመጠጣት ልምድ አለበዎት አዎ□ የለብኝም □
16. ቁመት _____
17. ክብደት _____
18. B.M.I. _____

አመሰግናለሁ

10.7 Annex 7: Standard Operating Procedure (SOP) for blood sample collection

Blood must be collected with care and adequate safety precautions to ensure test results are reliable, contamination of the sample is avoided and infection from blood transmissible pathogens is prevented. Protective gloves should be worn when collecting and handling blood samples. Lancets, needles, and syringes must be sterile, and dry, and blood collecting materials must be discarded safely to avoid injury from needles and lancets.

Technique for collecting venous blood

Laboratory staff must not collect venous blood unless they have been adequately trained in the procedure. Newly qualified staff must be supervised until they have gained sufficient experience. When venous blood is required from infants, this should be collected by a medical officer. Do not collect blood for hematological tests from intravenous lines.

1. Select a sterile, dry, preferably plastic syringe of the capacity required, e.g. 2.5 ml, 5 ml, or 10 ml. Attach to it a 19 or 20 SWG needle (preferably a disposable one). If the patient is a child or adult with small veins, use a 23 SWG needle.

Note: When not using a disposable syringe or needle, check the syringe for good suction and the needle for any blockage, directing the syringe and needle *safely away from the patient*. Ensure all air is expelled from the syringe. Whenever possible use a disposable needle and syringe.

2. Apply a soft tubing tourniquet or Velcro fastening arm band to the upper arm of the patient to enable the veins to be seen *and felt*. Do not apply the tourniquet too tightly or for longer than 2 minutes. Ask the patient to make a tight fist which will make the veins more prominent.
3. Using the index finger, feel for a suitable vein, selecting a sufficiently large straight vein that does not roll and with a direction that can be felt.
4. Cleanse the puncture site with 70% ethanol and allow to dry. Do not re-touch the cleansed area.
5. With the thumb of the left hand holding down the skin below the puncture site, make the vein puncture with the bevel of the needle directed upwards in the line of the vein.

Steadily withdraw the plunger of the syringe at the speed it is taking the vein to fill*. Avoid moving the needle in the vein.

*If the plunger is withdrawn too quickly this can cause hemolysis of the blood and the collapse of a small vein.

6. When sufficient blood has been collected, release the tourniquet and instruct the patient to open his or her fist. Remove the needle and immediately press on the puncture site with a piece of dry cotton wool. Remove the tourniquet completely. Instruct the patient to continue pressing on the puncture site until the bleeding has stopped.

Remove the needle from the syringe and carefully fill the container(s) with the required volume of blood. Discard the needle safely. *Do not* attempt to re-sheath it because this can result in needle-stick injury.

Important: Do not fill a container with the needle attached to the syringe. Forcing the blood through the needle can cause hemolysis.

7. Mix immediately the blood in an EDTA or citrate anti-coagulated container. When required, make a thick blood film from the blood remaining in the syringe. Immediately label carefully all the blood samples.
8. Check that bleeding from the vein puncture site has stopped. Cover the area with a small dressing.

Avoiding hematoma: when collecting venous blood bleeding from a vein into the surrounding tissue (hematoma) can cause unnecessary distress to a patient and result in subsequent bruising. Hematoma can be avoided by ensuring an appropriate vein is selected and the needle is well positioned in it and not accidentally pulled out of the vein when withdrawing the plunger of the syringe. When removing the needle, always release the tourniquet *first* and apply pressure immediately to the puncture site, maintaining it until the bleeding has stopped completely (*always* check).

Avoiding hemolysis of blood samples

The hemolysis (rupture) of red cells can be a serious source of unreliable test results. If red cells are hemolyzed, substances from the cells are released into the serum or plasma leading to a false increase in the concentration of analytes, e.g. potassium. Hemolysis also interferes with many chemical reactions.

Hemolysis can be avoided by:

- Checking that the syringe and needle are dry and that the barrel and plunger of the syringe fit well.
- Not using a needle with too fine a bore.
- Not withdrawing the blood too rapidly or moving the needle once it is in the vein.
- Removing the needle from the syringe before dispensing the blood into the specimen container and allowing the blood to run gently down the inside wall of the container.
- Adding the correct amount of blood to anticoagulant. Do not shake the blood but gently mix it with the anticoagulant.
- Using clean dry glass tubes or bottles for blood from which serum is required. Allow sufficient time for the blood to clot *and* clot retraction to take place. Red cells are very easily hemolyzed by the rough use of an applicator stick to dislodge a clot.
- Centrifuging blood samples for a minimum period of time. Centrifuging for 5 minutes at about 1000 g is adequate to obtain serum or plasma. Not storing whole blood samples in, or next to, the freezing compartment of a refrigerator.

10.8 Annex 8: Standard operating procedure (SOP) for serum preparation:

Aim: Effective Separation of blood products

Purpose: To standardize separating procedures so that research samples will be uniform in quality

1. Select test tube with no anticoagulant, serum separator tube (SST)
2. Draw enough amount of blood (4ml) from the patient
3. Allow to stand for 20-30min for clot formation at room temperature before spinning and separating. A delay in centrifugation may have a detrimental effect on the sample quality and may result inaccurate results. Avoid hemolysis
4. Centrifuge the sample to speed separation and affect a greater packing of cells. Clot and cells will separate from clean serum and settle to the bottom of the vessel.

The supernatant is the serum which can be now collected by dropper or pipette for testing purposes or stored (-20C to -80C) for subsequent analysis or use.