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Thyroid and Selected metabolic panel among Multi- Drug Resistant tuberculosis patients attending Saint Peter Specialized Hospital, Addis Ababa, Ethiopia

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This is to certify that the dissertation prepared by Endalkachew Berhanu entitled: Thyroid and Selected metabolic panel among Multi- Drug Resistant tuberculosis patients attending Saint Peter's Specialized Hospital Addis Ababa, Ethiopia. Submitted in partial fulfillment of the requirements for the degree "Master of Science in Clinical Laboratory Sciences (clinical chemistry)" in the of Department of Medical Laboratory Sciences complies with regulations of the university and meets the accepted standards with respect to originality and quality.

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Abbreviations

BMI	Body mass index
BW	Body weight
CDC	Centers for Disease Control
DM	Diabetes mellitus
FMoH	The Federal Ministry of Health
HDL-C	High Density Lipoprotein-Cholesterol
IDF	International Diabetes Federation
IL	Interleukin
LDL-C	Low Density Lipoprotein-Cholesterol
LP	Lipid Profile
MDRTB	Multi-drug resistance Tuberculosis
PAS	Para-aminosalicylic acid
T3	Triiodothyronine
T4	Thyroxine
TB	Tuberculosis
TC	Total Cholesterol
TG	Triglyceride
TH	T-helper
TNF-α	Tumor necrosis factor
TSH	Thyroid stimulating hormones
WHO	World Health Organization

Abstract

Background: One of the challenges in multidrug resistance tuberculosis (MDR-TB) treatment is the side effect of drug reactions associated with its medications. Among this hypothyroidism and lipid profile are the adverse drug reactions (ADR) challenging in MDR-TB treatment. However, there is limited evidence on the level of hypothyroidism and lipid profile abnormalities and the risk factors associated with them among MDR TB patients in Ethiopia.

Objective: To determine thyroid profiles and associated factors among MDR-TB patients on treatment in Saint Peter's Specialized Hospital Addis Ababa, Ethiopia.

Methods and Materials: A cross-sectional study was conducted among patients on MDR-TB treatment at Saint Peter's Specialized Hospital in Addis Ababa, Ethiopia from January 2020 to November 2020. Consecutive sampling method was used to enroll a total of 162 participants. From each participant, after getting informed consent and interview on socio-demographic and anthropometric data, Five ml blood sample was collected in serum separated tubes (SST) and analyzed by Cobas Integra chemistry analyzer. Data were entered into Microsoft Excel window 2010, and analyzed by SPSS software version 23.Descriptive statistics were used to summarize socio-demographic and clinical characteristics of the participants. Logistic regression model was used to determine factors associated with hypothyroidism and lipid profile abnormality. P-values<0.05 were considered as statistically significant in all the analyses.

Results: Of 162 patients 99 (61.1%) were males. Mean age of the study participant was 35.9 ± 13.6 years. Of the total patients 26 (16%) were diabetic, and the prevalence of hypothyroidism was 32 (19.8%).The most frequently occurring lipid profile abnormalities were HDL-C and TG with 106(65.4%) and 23 (14.2%) respectively. On the other hand, among the study participants, the fasting blood sugar value was hypoglycemic and hyperglycemic in 24 (14.8% and 42 (25.9%) cases, respectively. The presence of co-morbidity ($p = 0.006$), Being underweight ($p = 0.050$) and Prothionamide drug use ($p < 0.001$) were significantly associated with hypothyroidism.

Conclusion: The proportion of hypothyroidism in MDR-TB patients was considerable. Presence of co-morbidity, being underweight and Prothionamide use were factors significantly associated with hypothyroidism. Lipid profile abnormalities also occurred among MDR-TB patients. Regular monitoring of thyroid function test and lipid profile in MDR-TB patients is required. Conducting large scale prospective cohort study is necessary to determine the risk factors associated with hypothyroidism and lipid profile abnormalities due to MDR-TB drugs.

Keywords: Tuberculosis**,** Drug resistant, Thyroid hormone, lipid profile

1. Introduction

1.1 Background

Tuberculosis (TB) is an infectious bacterial disease caused by *Mycobacterium* tuberculosis, most commonly affects the lungs. It is transmitted from person to person via droplets from the throat and lungs of people with the active TB disease. The bacteria become active due to reduced immunity which occurs as a result of HIV infection, advanced age, diabetes or other immunocompromising illnesses (1).TB bacteria have natural defenses against some drugs, and can acquire drug resistance through genetic mutations (2, 3). Spontaneous mutations in the TB genome can alter proteins which are the target of drugs that makes the bacteria drug resistant (2, 3).Multi-drug-resistant tuberculosis (MDR-TB) is caused by bacteria that resistant to isoniazid and rifampicin (4).

The regimen is recommended for pulmonary TB (PTB) patients with confirmed rifampicin resistant (RR)-/MDR-TB by rapid molecular first line drug susceptibility test (DST) with GeneXpert MTB/RIF assay or first line probe assay (FL-LPA). Shorter treatment regimen (STR) that includes injectable agents (SLI) Capreomycin (Cm) and Kanamycin (Km) and other core second-line agents including Moxifloxacillin, Clofazimine, and Prothionamide while Pyrazinamide, Ethambutol and High-dose isoniazid (INH) are considered as add on components of the STR (5). Intensive phase (IP) consists of Capreomycin (Cm)/Kanamycin (Km), Moxifloxacillin, Clofazimine, Pyrazinamide, Ethambutol, High-dose INH, and Prothionamide administered for 4 months (5). If the follow up sputum smears remain positive at the end of month 4, the treatment extended by one month (5). The IP can be extended up to a maximum duration of 6 months (5). The continuation phase (CP) consists of Moxifloxacillin, Clofazimine, Ethambutol, and Pyrazinamide for the fixed duration of 5 months (5).In the event of drug intolerance or severe adverse reactions (ADR) to kanamycin and cycloserine, para-aminosalicylic acid (PAS) may be used as a substitute drug (5).

Endocrine and metabolic abnormalities do not always reflect direct infection of the gland but could result from physiological response or as a consequence of therapy. The disease process of TB involves cellular immunity and phagocytosis of *Mycobacterium* tuberculosis by macrophages and releases of interferon, TNF-α and other cytotoxic molecules (6). Phagocytic activity of macrophages, neutrophils and monocytes also generates reactive

oxygen species (ROS) and free radicals that not only have destructive effects on serum lipids but also contribute to immune suppression (6).High serum levels of these free radicals and high concentration of lipid peroxidation products are characteristics of patients with advanced tuberculosis. The peroxidation could reduce concentration of serum lipids and tissue inflammation (7, 8).

Thyroid hormones induce the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is the first step in cholesterol biosynthesis (9-11). Moreover, triiodothyronine 3 (T3) up regulates low density lipoprotein (LDL) receptors by controlling the LDL receptor gene activation. This T_3-mediated gene activation is done by the direct binding of T3 to specific thyroid hormone responsive elements (TREs). Furthermore, T3 controls the sterol regulatory element-binding protein-2 (SREBP-2), which in turn regulates LDL receptor's gene expression. T3 has also been associated with protecting LDL from oxidation(9-11).Another effect of T3 is the up-regulation of apolipoprotein AV (ApoAV), which plays a major role in triglyceride (TG)regulation (12).

Thyroid hormones can influence high density lipoprotein (HDL) metabolism by increasing cholesteryl ester transfer protein (CETP) activity, which exchanges cholesteryl esters from HDL2 to very low density lipoproteins (VLDL) and TGs to the opposite direction (13).Thyroid hormones T3 and thyroxin 4 (T4) maintain a fine balance of glucose homeostasis by acting as insulin agonistic and antagonistic (14). Hypothyroidism can break this equilibrium and alter glucose metabolism, which can lead to insulin resistance that in turn to diabetic (14).

Hypothyroidism is a deficiency in thyroid hormones secretion and action. This disorder is associated with increased level of thyroid stimulating hormone (TSH) and decreased free T4 levels. Reduced concentration and availability of T3 and T4 lead to hypersecretion of pituitary, TSH and notable elevation in serum TSH concentration. Elevated in the concentration of TSH is an important laboratory finding, particularly in the early detection of hypothyroidism (15).

Lipids are important constituents that determine nutritional status and at the same time participate in immune function. Lipid profile abnormality could occur in TB patients on treatment which could challenge TB case management at clinical setup (16). If lipid profile

abnormality occurred as ADR in TB patients could interact with conditions such as atherosclerosis, coronary heart diseases (CHD) and increase morbidity and mortality among TB patients (17). Evidence indicates high level of HDL in TB patients which is important to increase lipid metabolism that prevent chronic illness in TB patients (18).

With this background the current study was conducted to determine thyroid profile TSH, T3 and T4 and lipid profile Cholesterol, Triglyceride, HDL-cholesterol, LDL-c and FBS among MDR –TB patients analyzed using fully automated chemistry analyzer.

1.2 Statement of the problem

The emergence of MDR-TB becomes the global public health problem. Globally in 2019, an estimated 10.0 million (range, 8.9–11.0 million) people fell ill with TB. In 2019, an estimated 3.3% of new TB cases and 18% of previously treated cases had MDR/RRTB. In absolute numbers, there were an estimated 465 000 (range, 400 000– 535 000) incident cases of rifampicin resistant TB; 78% had multidrug resistant TB. Based on a WHO report, Ethiopia is ranked as 10th among high MDRTB burden countries (19).

Severe ADR associated with MDR-TB treatment is the most challenging phenomenon which leads to treatment interruption (20).Treatment interruption is the most cause of prolonged morbidity, drug-resistance development, treatment failure and longtime morbidity and high mortality (21). Second-line drugs (SLDs) such as paraaminosalicylicacid, ethionamide, and prothionamide are known to cause hypothyroidism in MDR-TB patients on treatment (22, 23). Up to 58% of MDR-TB patients on treatment could develop hypothyroidism (22, 20). Moreover, 54% – 69% of MDR-TB patients developed hypothyroidism during treatment (12, 13, 24).A recently published review study indicated 17.0% of pooled prevalence of hypothyroidism (25).A retrospective study reported from Ethiopia that conducted in two MDR-TB treatment initiative centers revealed 17.2% of MDR-TB patients on treatment developed hypothyroidism(26).

Most patients with PTB have hypercholesterolemia (27). In contrast lower level of cholesterol is strongly associated with mortality in PTB patients (18). Thyroid function significantly affects lipoprotein metabolism and causes cardiovascular disease (CVD) (28-30).

Available studies have been indicated high prevalence of hypothyroidism among MDR-TB patients on treatment, and have recommended screening of hypothyroidism during follow-up period (25). However, previous studies based on representative data which could be severe from data missing on risk factors that associated with hypothyroidism. Moreover, there is limited evidence on the lipid profile abnormality among MDR-TB patients on treatment. Thus, the outcome of the current study was expected to generate empirical data on thyroid profile, lipid profiles and associated risk factors in MDR-TB patients on treatment in Addis Ababa, Ethiopia.

1.3. Significance of the study

Monitoring thyroid profile levels in MDR-TB patients is important for managing ADR that are associated with SLDs used for the treatment of MDR-TB. Through monitoring thyroid profile morbidity and mortality related to ADR could be reduced. The results of this study could generate information on the level of thyroid profile abnormality which leads helps as a reference to screen the abnormalities. Moreover, the results of this study could be used as a reference for the future studies. In addition, this study could generate evidence on the sociodemographic and clinical factors that are associated with hypothyroidism. Further, the current study revealed the lipid profile among MDR-TB patients which helps in improving the quality of care, and support TB program implementation.

2. Literature review

2.1 Thyroid hormone, metabolite and tuberculosis

The association between tuberculosis and thyroid diseases is known. In the study, the highest percentage of thyroid disease 95/350 (22.3%) was thought to be the result of the direct effects of a chronic disorder, like tuberculosis, on the thyroid and/or the suppressive effect of Ethionamide on thyroid function (12, 31).Research reports also indicate that patients with MDR-TB and a co-morbidity of DM have a poor treatment response compared with non-diabetic MDR-TB controls (32).Hypothyroidism is a common cause of secondary dyslipidemia (33, 34).

2.2 Thyroid profile

A systematic review and meta-analysis on studies reported around the world on the prevalence of hypothyroidism in MDR-TB patients on treatment was Conducted by Tola HH et al .The study included 30 studies and pooled data on a total of 6,241 MDR-TB patients. The crude prevalence of hypothyroidism was extremely heterogeneous. The pooled prevalence of hypothyroidism in MDR-TB patients on treatment was 17.0%. The pooled prevalence of hypothyroidism in Africa was 25.0% which was significantly higher than Asian prevalence of 13.0%, and European prevalence of 9.0% .Ethionamide and para-aminosalicylic acid (PAS) were the most frequently reported drugs that associated with the occurrence of hypothyroidism (25).

An observational study was done by Matveyeva SL et al. Among MDR-TB patients in Ukraine, The data obtained indicates a significant weakening of thyroid function in MDR-TB patients under the influence of individual chemotherapy with the inclusion of ethionamide and PAS. Of the 30 patients (18 men and 12 women), 10% developed clinical hypothyroidism, which was diagnosed by increasing the level of TSH and lowering the level of free T4. Two patients (6.6%) developed goiter, one of which was euthyroid and one hypothyroid with the decreasing the level of free T4 and increasing the level of TSH. The mean level of TSH increased at the end of the intensive care phase with an even greater increase towards the end of treatment (35).

A prospective, observational cohort study using routine clinical and laboratory data was done by Andries A et al., in Mumbai, India between October 2006 and March 2013, 116

patients were enrolled, and 69 of whom were included. There was a significant association between the use of both ethionamide and PAS in the regimen and developing hypothyroidism (p = 0.014). Co-administration of these two agents increased the risk of hypothyroidism by two-fold. Age, sex, category of TB (pulmonary or extra-pulmonary) and stavudine were not associated with the occurrence of hypothyroidism. No factors were found to be associated with the occurrence of hypothyroidism in multivariate models (13).

Prospective study conducted by Somashekar Munivenkatappa et al., among MDR-TB patients The study followed 188 euthyroidic persons undergoing treatment for multidrug resistant tuberculosis (MDR-TB) in the state of Karnataka, India to determine the incidence of hypothyroidism during anti-tuberculosis treatment. The result of this study was among MDR-TB patients with valid TSH values, about 23% developed hypothyroidism during antituberculosis treatment, with the majority (74%) occurring after 3 months of treatment (36).

Another prospective study was done by Bares R et al. 72 patients in Rawalpindi Leprosy Hospital, Rawalpindi, Pakistan, for treatment for MDR-TB, were enrolled into the study. The finding of this results showed Hypothyroidism during second-line treatment during the first 6 months, TSH levels increased significantly (P= 0.001). Overt hypothyroidism was diagnosed in 19 patients (38%), and subclinical hypothyroidism in 20 additional patients (40%). In 11 patients, TSH remained normal, slightly increasing in five of these. Between 3 and 6 months after the start of treatment, hypothyroidism was newly diagnosed in 13 patients (37).

Observational and cross-sectional study was conducted all the newly admitted patients in MDR-TB Referral Centre, University College Hospital, Ibadan, Nigeria.by Ige OM et al. Between July 2010 and December 2014.This study was carried out to determine if HIV co-infection alters serum levels of thyroid hormones (T3, T4) and thyroid stimulating hormone (TSH) in patients with MDR-TB patients and to find out the frequency of subclinical thyroid dysfunction before the commencement of MDR-TB therapy. Hundred and fifteen patients with MDR-TB were enrolled in the study, out of which 22 (19.13%) had MDR-TB/HIV co-infection. Sick euthyroid syndrome (SES), subclinical hypothyroidism and subclinical hyperthyroidism were observed in 5 (4.35%), 9 (7.83%) and 2 (1.74%) patients respectively.

The median level of TSH was insignificantly higher while the median levels of T3 and T4 were insignificantly lower in patients with MDR-TB/HIV co-infection compared with patients with MDR-TB only (38).

A retrospective study of 212 patients who initiated treatment for MDR-TB in Lesotho was performed by Satti H et al. Among the 186 patients screened for hypothyroidism, 129 (69%) had at least one documented TSH > 10.0 mIU/l; 100 (54%) had a maximum TSH >20.0 mIU/l, and 22 (12%) had a maximum TSH of 100.0 mIU/l, which was the upper limit of the laboratory test. Median time to hypothyroidism was 65 days. Treatment regimens were largely uniform. Nearly all patients received a combination of PAS (96.2%), cycloserine (96.2%), pyrazinamide (93.6%), ETH/ PTH (97.3%), a fluoroquinolone (95.7%), and a second-line injectable (95.2%). In total, 179 (96.2%) patients received both PAS and ETH/PTH (12).

A retrospective cohort study conducted by Meressa D et al., in Ethiopia.1044 patients were initiated on standardized second-line drug (SLD). 612 patients with confirmed or presumed MDR TB had ≥24 months of follow-up. Hypothyroidism occurred in105 (17.2%) of patients from February 2009 to December 2014 (26).

2.3 Lipid profile

A systematic review and a meta-analysis research done by Tegegne BS et al., 24 observational studies from 15 different countries revealed that DM has a significant association with MDR-TB (P= 0.031) (39).

A cross-sectional pilot study was done in Indian drug resistant TB patients by Nawaz A., Sixty‑six age‑ and gender‑matched controls were included. The result showed that DR‑TB patients had serum lipid levels (all fractions) significantly lower than the controls (*P* < 0.001).TC (p= <0.001, TG p= <0.001, HDL‑C p=<0.001, LDL‑C p= <0.001, VLDL‑**C** p=<0.001(40).

A pilot study done in India by Taparia P et al .The study aimed was to determine the level of Lipid fractions in Newly Diagnosed and Relapse Pulmonary Tuberculosis Patients and also to find correlation between serum lipid level with inflammation and disease severity. It included32 newly diagnosed and 26 relapsed cases to PTB were recruited for the study. 25 age and gender matched healthy subjects that were non-family members of patients were

taken as controls for comparison. The result showed All lipid parameters were significantly ($p<0.05$) 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 (18).

The study conducted by Akpovid et al., as a pre -posttest design study with a control group at Centre National Hospitalier de Pneumo-Phtyisiologie Cotonou (CNHPP) which is a reference center for TB diagnosis and treatment in West Africa. From October 2011 to June 2012, a total of 187 patients suffering from TB were admitted to CNHPP. Aimed of this study was to determine whether tuberculosis (TB) treatment normalizes the lipid profile strongly affected by pulmonary TB. Lipid profile were determined in 83 patients with pulmonary TB before and after treatment, and compared to results obtained from 100 control subjects without TB. Before treatment, levels of TC ($p<0.005$), HDL-C ($p<0.005$) and LDL-C ($p<0.005$) were significantly lower in pulmonary TB patients than normal subjects. Unlike TC and LDL-C, HDL-C decrease was correlated ($r = 0.96$, $p<0.05$) with smear positivity extent. At the end of TB treatment, which lasted six months, TC ($p<0.01$) and HDL-C ($p<0.005$) levels were significantly increased than before treatment while LDL-C stayed relatively unchanged. The results showed that tuberculosis treatment increases TC levels and normalizes HDL while reducing atherogenic indices to below levels of controls (28).

A Descriptive cross-sectional study was carried out to determine the relationship between Drug resistant tuberculosis (DR-TB), lipid parameters, albumin and zinc among Human Immunodeficiency Virus (HIV) infected patients in Nigeria Ojo Lagos state done by Odekunle Bola Odegbemi *et al.* The study showed that lipid profile decreases significantly in tuberculosis subject and even more among subjects with drug resistant-tuberculosis. It was evident from this study that One hundred and sixteen (116) subjects were enrolled for this study consisting of twelve (12) HIV and drug resistant-tuberculosis co-infected subjects (six females and six males), eighteen (18) drug resistant-tuberculosis subjects without HIV infection (six females and 12 males), ten (10) subjects with HIV and ordinary tuberculosis co-infection (three females and seven males) and thirty three (33) subjects with ordinary tuberculosis infection only (13 females and 20 males). Others were twenty five (25) HIV-infected subjects without tuberculosis (10 females and 15 females) and eighteen (18)

subjects with neither HIV nor tuberculosis infection (nine females and nine males). The results indicated that the levels of TC, TG, LDL-C and Albumin were significantly lowered in subjects with HIV and Drug Resistance- Tuberculosis co-infection when compared with those with HIV and ordinary TB co-infection. The levels of TC, TG, HDL-C, LDL-C, Albumin and Zinc were lowered in subjects with HIV and Tuberculosis co-infection when compared with the controls. Furthermore, the levels of TC, TG, LDL-C and Albumin were significantly lowered in subjects with HIV and Drug Resistance-Tuberculosis co-infection when compared with those with HIV and ordinary TB co-infection. There was an overall statistically significant difference in the means of all the lipid parameters analyzed including Albumin and Zinc P-value (0.000) (41).

An observational study was conducted by Gebremedhin Gebremicael G et al. At St. Peter Specialized TB Hospital, Akaki and Kality Health Centers, Addis Ababa, Ethiopia, between April 2007 and January 2011.*The study include a* total of 93 TB patients [49 HIV−TB+ and 44 HIV+TB+], 41 latent TB cases [17 HIV+TST+ and 24 HIV−TST+], and 25 HIV−TST− were included in the study. The result of this study showed that the concentrations of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in HIV−TB+ patients were significantly lower compared to HIV−TST+ and to HIV−TST− individuals. Similarly, the concentrations of the TC, LDL-C, and HDL-C in HIV+TB+ were significantly lower compared to HIV−TB+ patients. After the 6 months of anti-TB treatment (ATT), the concentration levels of TC, LDL-C, and HDL-C in HIV−TB+ patients were higher compared to the baseline concentration levels, while they were not significantly different compared to that of HIV−TST+ concentration (42).

Another Observational retrospective cohort study was conducted in Ethiopia by Yemane 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- (43).

3. Objectives

3.1 General Objective

To determine thyroid profile, lipid profile and fasting blood glucose abnormalities, and their factors associated among MDR-TB patients on treatment in St.peter's Specialized Hospital Addis Ababa, Ethiopia.

3.2 Specific objectives

To determine TSH, T3, T4, Cholesterol, Triglyceride, HDL-cholesterol, LDL-c and FBS levels in MDR-TB-patients on treatment in St.peter's Specialized Hospital Addis Ababa, Ethiopia.

To identify factors associated with hypothyroidism and lipid profile abnormalities in MDR-TB patients on treatment in St.peter's Specialized Hospital Addis Ababa, Ethiopia.

4. Materials and Methods

4.1 Study area

This study was conducted from January to November 2020 at St. Peter's specialized hospital in Addis Ababa, Ethiopia. St. Peter's hospital was established in 1953. It located in the Gullele sub-city of Addis Ababa. It administered under the Federal Ministry of Health of Ethiopia (FMoH). It has more than 1,000 medical and non-medical staff members. It is the first national hospital that stated MDR-TB treatment in Ethiopia in April 2009 (26). It was a center of excellence and training during the scaling up of MDR-TB treatment initiation centers in the country. This hospital is a referral hospital that receives patients from all parts of the country. It has a capacity to treat many patients as outpatient and has 32-40 bed to accommodate the inpatients. During the study period there were 197 MDR-TB patients on treatment in the hospital. Of these 30 were in patients.

4.2 Study design and period

A cross-sectional study was conducted in St.Peter's Specialized Hospital in Addis Ababa, Ethiopia from January to November 2020.

4.3 Population

4.3.1 Source population

All MDR-TB patients were the source of population.

4.3.2 Study population

All MDR- TB patients who visited St.Peter's specialized hospital for the treated were study population that fulfills the inclusion criteria.

4.4 Inclusion and exclusion criteria

4.4.1Inclusion criteria

The inclusion criteria of this study were:

- Age older than 18
- Bacteriologically confirmed MDR –TB patient
- Patients who has full data on HIV status, other co-morbidities and types of treatment
- Patients volunteer to participate in the study

4.4.2 Exclusion criteria

Patients who were critically sick, not initiated the treatment and had previous thyroid disease were excluded.

4.5 Study variables

4.5.1 Dependent

Serum level of total cholesterol (TC), triglyceride (TG), High density lipoprotein (HDL) cholesterol, Low density lipoprotein (LDL) cholesterol, thyroid stimulating hormone (TSH), free triiodothyronine (T3), free thyroxine (T4) and fasting Blood glucose (FBS) levels.

4.5.2 Independent

Sex, age, socio-economic status, body mass index (BMI), anti -MDR TB drugs type and duration on anti- MDR TB drug.

4.6 Measurement and data collection

4.6.1 Sample size calculation

Sample size was determined for each specific objective by single population proportion using Epi info sample size calculator. The largest sample size was determined based on the prevalence of hypothyroidism (17.2%) in Ethiopia which reported by previous observational cohort study (26). Thus, the determined sample size was 151 by considering 95% confidence level and 0.06 desired precision. After 10% contingency sample was considered, the total sample size was 166. However, 162 patients were included into this study after 4 patients refused to participate in the study.

4.6.2 Sampling method

Consecutive sampling method was used until the required sample sizes attained in the study period time.

4.6.3 Data collection procedure

All the professionals who participated in data or specimen collection were trained for two days on the objectives of the study and data management. Data on socio-demographic and clinical variables were collected on pre-designed check list. The data collection process was supervised by the assigned research directorate and principal investigator.

4.6.3.1. Sample collection

After an overnight fasting (9-12 hrs.), 5ml of peripheral venous blood was drawn from the median cubital vein of each subject using aseptic technique in a plain gel vacationer tube. The specimens were centrifuged at 3000rpm to separate the serum within one hour blood collection and stored at −20˚C until analyzed for TC, TG, HDL, LDL, FBS, T3, T4, and TSH. If tests were not done within 24 hours, the serum was stored at -80°C until the analysis. The samples were collected, handled and transported to the laboratory according to the guide lines given by clinical and laboratory standards institute/NCCLS (National Clinical Chemistry Laboratory Standards) (44, 45)

4.6.3.2. Thyroid profiles test

Thyroid function test includes TSH, Free T3 and Free T4.These tests analyzed within 24 hours by using the Cobas 6000 analyzer at Ethiopian public health institute (EPHI) which is national referral hospital. It is a fully automated discrete immunoassay worked with the principle of Electrochemiluminescence. Electrochemiluminescence (ECL) is a competitive principle that used for low molecular weight analyte, such as Free T3 and Free T4, whereas sandwich principle is used for large molecular weight analyte, such as TSH (45).

4.6.3.3. Lipid profiles testing

TC, TG, HDL, LDL, and FBS were measured using the Cobas Integra C311 at St.peter's specialized hospital laboratory. It is a fully automated and computerized analyzer which used for the analysis of a wide range of body fluids. It is optimized for high workloads in the professional environment using a combination of ion selective electrodes (ISE) and a photometric analysis unit. It can use serum, plasma, urine, cerebrospinal fluid (CSF), and supernatant sample types.

TC was analyzed by cholesterol oxidase phenol 4-aminoantipyrine peroxidase, HDL-C by direct enzymatic method, LDL-C by direct determination, VLDL-C by calculation, TG by glycerine phosphate oxidase peroxidase. FBS by Hexokinase catalyzes the phosphorylation of glucose to glucose‑6‑phosphate by ATP (46).

4.7 Data quality assurance

Pre-analytical stage: Pre-analytical step includes subject preparation, sample collection, transportation and instrument maintenance. These factors should follow the recommended quality procedure to come out with quality result. For this study the specimen were collected and transported based on the recommended procedure and the instrument was stablished based on manufacturer recommended.

Analytical stage: The reliability of the data generated is critical, because both the imprecision and inaccuracy of the method was determined its diagnostic utility. Therefore,

for this study, commercial quality control materials were used in the same manner as patient specimen test process.

Post-analytical stage: For the thyroid profile and lipid profile results were being performed for further investigation of any abnormality. The results were recorded and handled appropriately and stored in secured place.

The data collection and laboratory test process were supervised by principal investigator. The collected data was checked regularly for any error, and the necessary actions were taken in case of errors occurred. The data was double entered into different databases (SPSS version 23) by different persons to assure data quality.

4.8 Data analysis

Statistical Package for Social Sciences (SPSS) version 23 software was used for data analysis. Baseline characteristics and metabolic abnormalities of the study participants were summarized by descriptive statistics (mean ± SD and percentage). Tables and figure were used to present the results. Bivariate and multivariate logistic regression model were used to determine the factors that were associated with hypothyroidism and lipid profile abnormality. Variable scored 0.2 and above p-value during bivariate analysis were included into multivariate analysis. The P value of <0.05 was considered statistically significant.

4.9 Operational definitions

MDR-TB: refer to TB patient resistance to first line drug at least INH and Rifampicin.

Thyroid Profiles**:** refers to measurable or quantifiable characters which includeT3, T4 and TSH. Based on thyroid dysfunction, patients are classified as euthyroid (Normal TSH, free T3 and free T4), Primary hypothyroidism (High TSH with low free T4 and free T3), Subclinical hypothyroidism (High TSH and normal free T4 and free T3) and sick euthyroid (low free T3 with normal TSH and free T4 or low free T4 and free T3 with normal TSH) (47).

Lipid Profile: is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as TC, LDL-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. LDL-c termed as ‘bad cholesterol’ and is considered as major risks for cardiovascular diseases its normal range <100-130 mg/dL and Triglyceride normal range is 100-150mg/dl (48).

Normal: FBG levels below 100 mg/dl, without a history of diabetic medication.

Prediabetes: refers to a level of blood glucose between 100 and 125 mg/dl with no diabetic medication.

Diabetes: manifests when the FBG level equals or greater than 126 mg/dl (49).

4.10 Ethical Consideration

This study was approved by the Department of Research and Ethics Committee (DREC) of Medical Laboratory Sciences, College of Health Sciences Addis Ababa University.(Approval number-DRERC/479/19/MLS) and St.Peter’s Specialized Hospital (approval number-V143/28/01/2020). We have received from each review board a waiver of informed consent. In this study, sensitive information that could identify participants was not disclosed to protect confidentiality.

4.11 Dissemination of results

The results of this study will be submitted to School of Medical Laboratory Sciences in two hardcopies and a softcopy by compact disk (CD). Moreover, the hard copy of the results will be given to the St. Peter Specialized Hospital and other stakeholders. The results of this study will be submitted to peer-review journals for publication. The findings will also being communicated through presenting at scientific conferences.

Patient recruitment and laboratory work

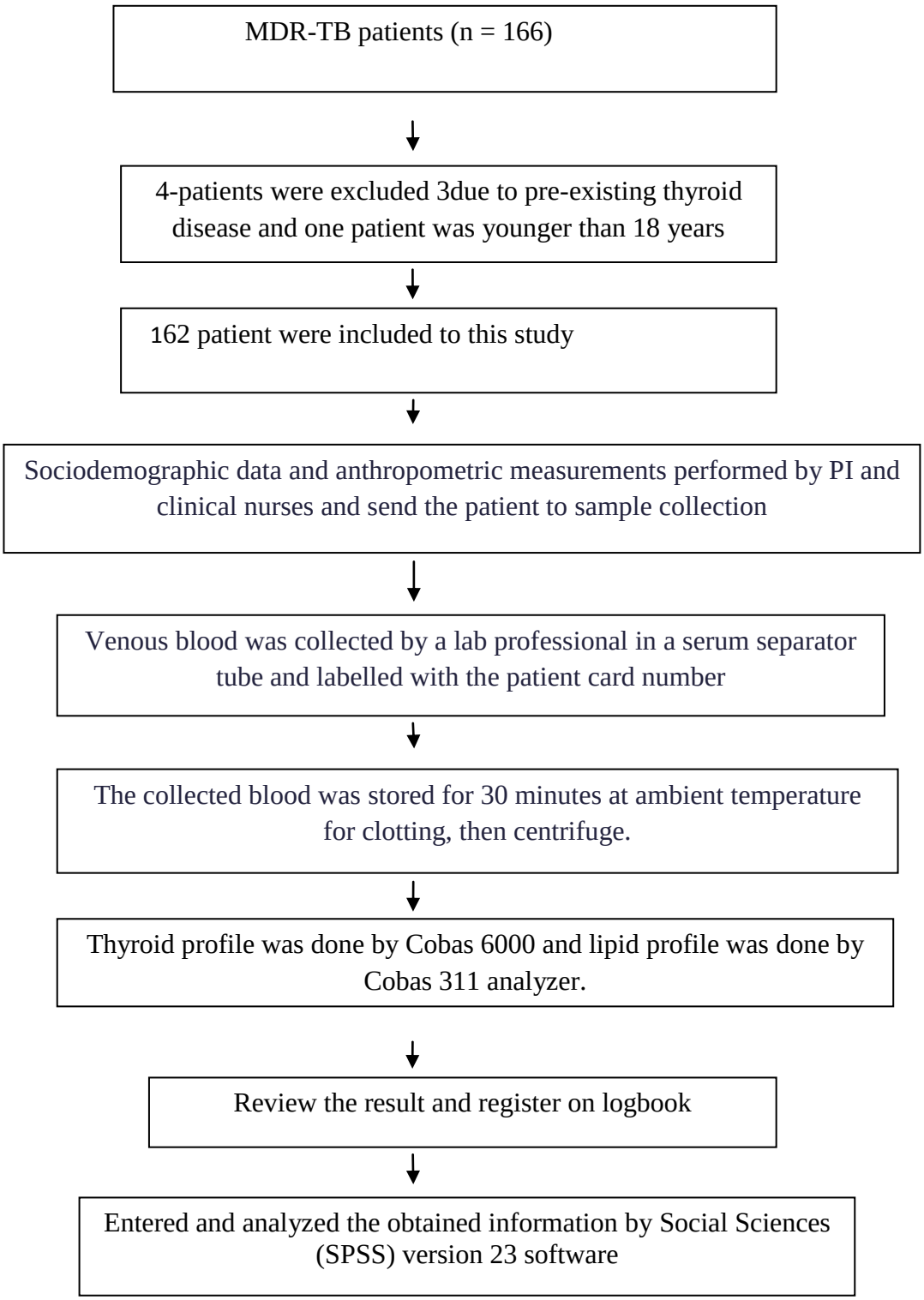

Figure 1 : *Flow chart of patient recruitment and the laboratory work for the study*

5. Results

5.1 Participants' background characteristics

A total of 166 MDR-TB patients study participants were included in this study. Of these, 3 patients were excluded from the analysis due to thyroid disease history and one was younger than 18 years and make participant rate 162 (97.6%). Of the total MDR-TB patients, 99 (61.1%) were males. The mean age (±SD) was 35.9 ± 13.6 years with age range 18 to79 years.

Table 1 depicts the socio-demographic and clinical characteristics of the patients. Of the total 162 patients, 40 (24.7%) had co-morbidities. From this 26(16%) had HIV, 10(6.2%) diabetes mellitus and 4(2.5%) other diseases. Of the total patients 16 (9.9%) had history of smoking, whereas 21 (13%) had alcohol consumption history. One hundred fifty one (92.6%) of patients had pulmonary TB, while 8 (4.9%) had extra pulmonary TB and 3(1.8%) both. Twenty three (14.2%) of the patients had previous history of MDR-TB treatment. Of the total patients included into this study, 57 (35.2%) were married, and 76(46.9%) patients were unemployed. Majority of the patients (85.8%) were urban dwellers, and 54 (33.3%) of the patients were earned less than 1000 birr. Majority of the patients (75.3%) were on anti-MDR-TB drug for ≥ three months and [**Table1**].The mean body mass index (BMI) of the patients was19.26m^2/Kg (±3.2).

Table 1: Sociodemographic characteristics of MDR-TB patients at St.peter's specialized hospital in Addis Ababa, Ethiopia, 2020 (n = 162)

Variable		Number	Percent
Sex	Male	99	61.1
	Female	63	38.9
Age category	18 – 25	43	26.5
	26 – 35	50	30.9
	36 – 45	37	22.8
	≥45	32	19.8
Residence	Urban	139	85.8
	Rural	23	14.2
Marital status	Married	57	35.2
	Single	105	64.8
Occupation status	Employed	69	42.6
	Laborer	17	10.5
	Unemployed	76	46.9
	Illiterate	22	13.5
	Primary school	45	27.6

Education status	Secondary school	59	36.2
	Certificate and above	36	22.1
Income (ETB)	<1000	54	33.3
	1001-2000	47	16.7
	>2000	81	50.0
Family size	1-3	72	44.2
	4-6	64	39.3
	7 and above	26	16.0
House room number	1	57	35.2
	2-3	84	51.9
	4 and above	21	44.4
Pervious TB history	No	23	14.2
	Yes	139	85.8
House hold currently treated for TB	YES	12	7.4
	NO	150	92.6
Co-morbidity	Yes	40	24.7
	No	122	75.3
Types of other disease	NO	122	75.3
	DM	10	6.2
	HIV	26	16.0
	Other	4	2.5
Taking drug	Yes	38	23.5
	No	124	76.5
Smoking history	Yes	16	9.9
	No	146	90.1
Taking alcohol history	Yes	21	13.0
	No	141	87.0
Duration of anti-MDR TB DRUG	<3month	40	24.7
	≥3month	122	75.3
Types of TB	EPTB	8	4.9
	PTB	151	92.6
	BOTH	3	1.8

ETB-Ethiopian Birr,DM-Diabetes mellitus, HIV -human immune deficiency virus, EPTB-Extra pulmonary tuberculosis, PTB-pulmonary tuberculosis, MDR-TB-Multidrug resistance tuberculosis.

Table 2 shows MDR-TB drug given to the patients. Cycloserine, Clofazimine, Linezoide and Bedaquiline were the drugs commonly used among the patients included into this study. However, Paraaminosalicylatesodium and Capreomycin were the drugs less likely used in the patients included into this study.

Table 2 : TB drugs in use MDR-TB patients at St.peter's specialized hospital in Addis Ababa, Ethiopia, 2020 (n = 162)

Drug type	Yes, N (%)	No, N (%)
Prothionamide	36(22.2)	126(77.8)
Paraaminosalicylatesodium	1(0.6)	161(99.4)
Capreomycin	6(3.7)	156(96.3)
Levofloxacin	151(93.2)	11(6.8)
Moxifloxacillin	24(14.8)	138(85.2)
Cycloserine	154(95.1)	8(4.9)
Clofazimine	156(96.3)	6(3.7)
Linezoide	153(94.4)	9(5.6)
Delamaid	43(26.5)	119(73.5)
Bedaquiline	154(95.1)	8(4.9)

N-number %-percent

5.2 Thyroid profile

Table 3 depicts the mean distribution of thyroid profile (TSH, T3 and T4).The mean level of TSH was 3.0uU/ml (±2.9), while the mean of T4 7.8ug/dl (±2.1) and the mean of T3 was 1.3ng/ml (±.36) .Thirty two (19.2%) patients had high level of TSH, and these patients considered as hypothesized patient. About 10% of the patients had low T4 and 6 (3.7%) low T3.

Table 3: Mean distribution of thyroid profile of MDR-TB patients at St.peter's specialized hospital in Addis Ababa, Ethiopia, 2020 (n = 162)

Variables	Minimum	Maximum	Mean (±SD)	Median (IQR)
Thyroid stimulating hormone (uU/ml)	0.01	26.49	3.0(2.9)	2.5 (2.2)
Thyroxine(µg/dl)	2.55	15.50	7.8 (2.1)	7.8(2.7)
Triiodothyronine(ng/dl)	0.47	3.19	1.3(.36)	1.3(0.4)
Height (m)	1.4	1.8	1.7 (8.2)	1.7(0.1)
Weight(kg)	29	79	53.4(10.3)	51(12)
BMI(kg/m^2)	12.8	27.3	19.26(3.2)	18.7(3.7)

SD-standard deviation, IQR-inter quartile range, ng/dl-nomogram per deciliter, µg/dl-microgram per deciliter, µU/ml-micro unit per milliliter, BMI-body mass index, m-meter, kg-kilogram, kg/m^2-kilogram per meter square

5.2.1Clinical classification based on thyroid profile

Majority (77.2%) of the patients had normal thyroid profile (euthyroid). The prevalence of hypothyroidism in MDR-TB patients was 32 (19.8%), whereas 28 (17.3%) had subclinical hypothyroidism and 4(2.5%) had overt hypothyroidism.

5.2.2 Thyroid profile Distribution by sex and age group

Figure 2 below showed that Thyroid profile Distribution by sex. There is no significant difference between male and female on TSH-Thyroid stimulating hormone (p=0.08832, T4-Thyroxine (p=0.121), and T3- Triiodothyronine (p=0.1012) levels among MDR-TB patients at St.peter's specialized hospital.

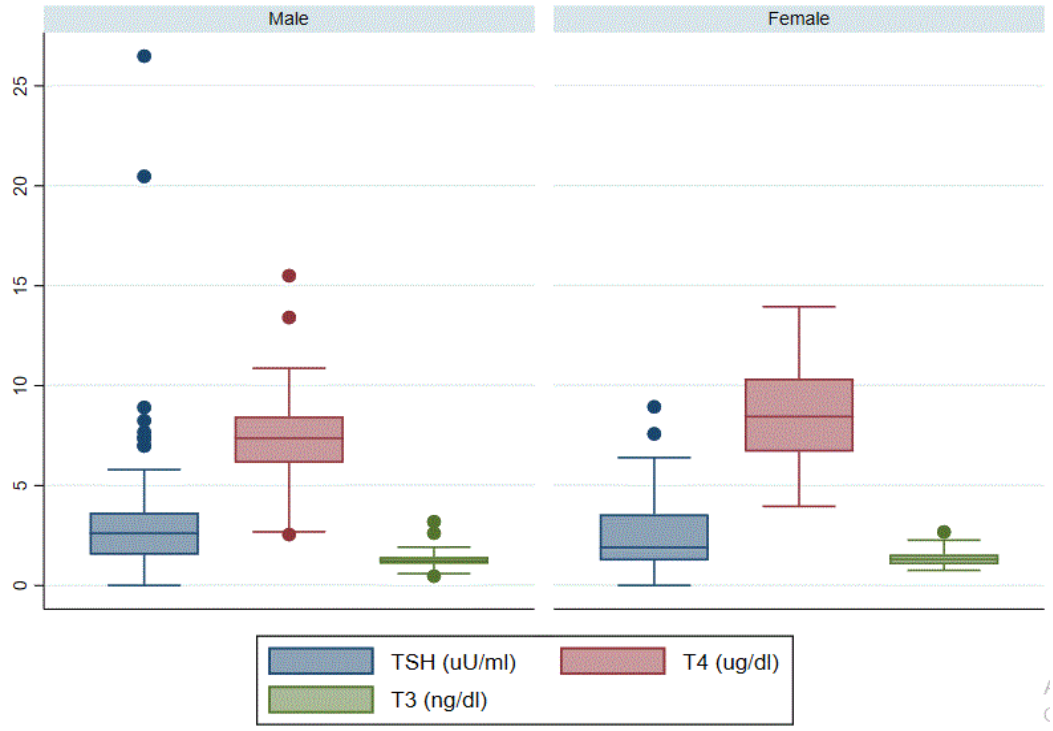

Figure 2 : Thyroid profile Distribution by sex TSH-Thyroid stimulating hormone, T4-Thyroxine, and T3- Triiodothyronine levels among MDR-TB patients at St.peter's specialized hospital.

Figure 3 below showed that Thyroid profile Distribution by age group. There is no significant difference between age group <36 and ≥36 on TSH-Thyroid stimulating hormone (p=0.2197), T4- Thyroxine (p=0.0.9472)), and T3- Triiodothyronine (p=0.5136) levels among MDR-TB patients at St.peter's specialized hospital.

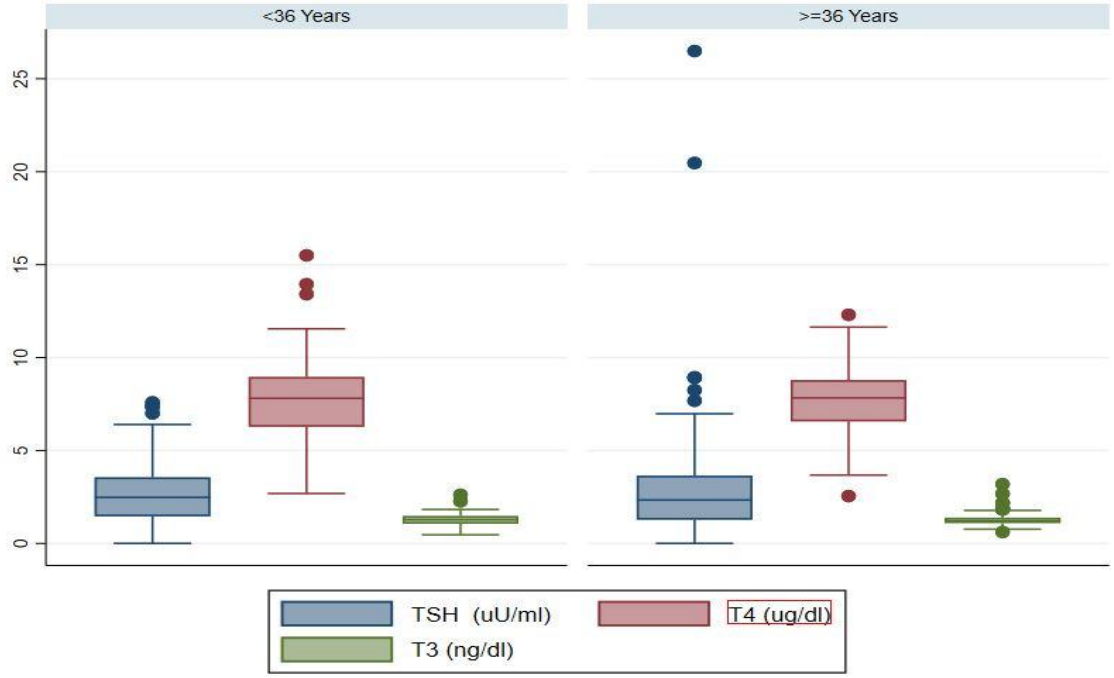

Figure 3 Thyroid profile Distribution by age TSH-Thyroid stimulating hormone, T4-Thyroxine, and T3- Triiodothyronine levels among MDR-TB patients at St.peter's specialized hospital.

5.2.3 Factors associated with hypothyroidism

5.2.4 Risk factors of hypothyroidism based on bivariate analysis

On bivariate analysis was indicated that, presence of any co-morbidity (COR = 2.6, 95%CI (1.1-5.9); p=0.022),being HIV infected (COR = 3.5, 95%CI (1.4-8.9);p =0.008), non MDR-TB drugs (COR = 3.4, 95% CI (1.5-7.9);p =0.003),duration on MDR-TB treatment ≥ 3months (COR= 3.8, 95%CI 1.1-5.7;p =0.034),Underweight (COR 2.5, 95%CI (1.1-5.7), p =0.034), and on Prothionamide drug (COR = 4.6, 95%CI (2.0-10.6);p =0.001) were significantly associated with hypothyroidism. Other risk factors were not significantly associated with hypothyroidism during bivariate analysis (p >0.05) [**Table 4**].

Table 4 Risk factors of hypothyroidism based on bivariate analysis in MDR-TB patients at St.peter's specialized hospital , Addis Ababa, Ethiopia, 2020 (n = 162)

Variable		COR (95% CI)	P-value
Sex	Male	1.0	
	Female	0.93 (0.42 – 2.1)	0.857
Age (in year)	Age(in year)	1.0 (0.97 – 1.0)	0.951
Marital status	Married	1.0	
	Single	1.6 (0.72–3.5)	0.260
Employment status	Employed	1.0	
	Unemployed	1.0 (0.47 – 2.3)	0.910
	Laborer	0.52 (0.11 – 2.6)	0.425
Income (in ETB)	≥2000.0	1.0	
	<2000.0	1.0 (0.46–2.2)	1.000
Family size (in number)	1-3	1.0	
	4-6	1.2 (0.43 – 3.3)	0.737
	7 and above	0.46(0.18 – 1.2)	0.100
Previous TB history	No	1.0	
	Yes	0.86 (0.30 – 2.5)	0.782
Type of TB	EPTB	1.00	
	PTB	1.7 (0.21 – 14.6)	0.612
	Both	3.5 (0.15 – 84.7)	0.441
Presence of any co-morbidity	No	1.0	
	Yes	2.6(1.1 – 5.9)	**0.022***
Type of co-morbidity	No co-morbidity	1.0	
	Diabetes mellitus	2.4(0.57 – 10.2)	0.230
	HIV Sero-reactive	3.5(1.4 – 8.9)	**0.008***
Anti-HIV Dugs	No	1.0	
	Yes	3.4 (1.5 – 7.9)	**0.003***
Cigarette smoking history	No	1.0	
	Yes	0.55 (0.12 – 2.6)	0.449

Alcohol use history	No	1.0	
	Yes	0.64 (0.16 – 2.3)	0.503
Duration on MDR-TB treatment	<3months	1.0	
	≥3months	3.8 (1.1 – 13.4)	**0.034***
BMI(Kg/m^2)	Normal	1.0	
	Underweight	2.5 (1.1 – 5.7)	**0.034***
	Overweight	1.5 (0.28 – 7.9)	0.640
Prothionamide	No	1.0	
	Yes	4.6 (2.0 – 10.6)	<**0.001****
Levofloxacin	No	1.0	
	Yes	0.79 (0.73 – 0.86)	0.810
Moxifloxacillin	No	1.0	
	Yes	0.79 (0.25 – 2.5)	0.681
Cycloserine	No	1.0	
	Yes	0.79 (0.73 - 0.86)	0.214
Clofazimine	No	1.0	
	Yes	0.80(0.73 – 0.86)	0.353
Linezoide	No	1.0	
	Yes	0.85 (0.17 – 4.3)	0.848
Delamaid	No	1.0	
	Yes	0.91 (0.37 – 2.2)	0.825
Bedaquiline	No	1.0	
	Yes	1.8 (0.21 – 14.9)	0.602

*COR -Crud Odds Ratio, BMI-body mass index, kg/m2-kilogram/meter square, ETB-Ethiopian birr, EPTB-Extra pulmonary tuberculosis, PTB- pulmonary tuberculosis *significant p<0.005*

5.2.5 Multivariate analysis

Multivariate logistic regression model contains five variables revealed that presence of co-morbidity (AOR = 3.8; 95% CI (1.5 – 9.9); p = 0.006), being underweight (AOR = 2.6; 95% CI (1.0 – 6.6); p = 0.050) and Prothionamide use (AOR = 5.4; 95% CI (2.0 – 14.4); p < 0.001) were significantly associated with hypothyroidism in MDR-TB patients on treatment **[Table 5].**

Table 5: Risk factors of hypothyroidism based on multivariable analysis of MDR-TB patients at St.peter's specialized hospital, Addis Ababa, Ethiopia, 2020 (n = 162)

Variable		(N)	AOR (95% CI)	P-value
Presence of any co-morbidity	No	122	1.0	
	Yes	40	3.8(1.5–9.9)	**0.006***
	No co-morbidity	122	1.0	
Types of co-morbidity	Diabetics mellitus	10	1.2 (0.37–0.37)	0.785
	HIV Sero-reactive	26	0.47 (0.17–1.3)	0.146
Duration on MDR-TB treatment	< 3 months	40	1.0	0.176
	≥3 months	122	2.5(0.66–9.7)	
BMI (Kg/m^2)	Normal	77	1.0	
	Underweight	74	2.6(1.0 – 6.6)	**0.050***
	Overweight	11	2.7(0.43 – 17.5)	0.287
Prothionamide	No	126	1.0	
	Yes	36	5.4(2.0 – 14.4)	<**0.001****

*AOR - Adjusted Odds Ratio, CI-confidence of interval, kg/m2-kilogram/meter square, BMI-body mass index *significant p<0.05*

5.3 Lipid profile

Lipid profile alteration has been described in association with tuberculosis. Serum lipid parameters (TC, LDL-c, HDL-c, and TG) diagnosed MDR-TB patients.

5.3.1 Descriptive Mean (± SD), proportion

The mean level of Total cholesterol (TC) was 134mg/dL (±34.6), Triglycerides (TG) was 138.6mg/dL (±64.2), High density lipoprotein cholesterol was 37.9mg/dL (±13.4) and Low density lipoprotein cholesterol was 69.8 mg/dL(±29.4), Very low density lipoprotein cholesterol was 27.6mg/dL (±12.9), Fasting blood glucose was 102.2mg/dL (±46.7).Body mass index was 19.26 kg/m2(±3.21.),Height was1.7m(±8.2), and weight was 53.4kg(± 10.3) [**Table 6**].

Table 6: Mean distribution of lipid profiles of MDR-TB patients at St.peter's specialized hospital in Addis Ababa, Ethiopia, 2020 (n = 162)

	Minimum	Maximum	Mean (±SD)	Median (IQR)
Total cholesterol(mg/dl)	51.10	278.00	134.0 (34.6)	132.9(42.1)
Triglycerides(mg/dl)	45.40	372.10	138.6(64.2)	123.4(80.3)
High density lipoprotein cholesterol(mg/dl)	10.40	94.70	37.9(13.4)	36.4(15.2)
Low density lipoprotein cholesterol(mg/dl)	9.7	193.00	69.8(29.4)	65.4(37.4)
Very low density lipoprotein cholesterol(mg/dl)	9.1	74.40	27.6(12.9)	24.7(16.1)
Fasting blood glucose(mg/dl)	38.4	502.4	102.2(46.7)	94.8(3.8)
Height (m)	1.4	1.8	1.7 (8.2)	1.7(0.1)
Weight(kg)	29	79	53.4(10.3)	51(12)
BMI(kg/m^2)	12.8	27.3	19.26(3.2)	18.7(3.7)

SD-standard deviation, IQR-inter quartile range, mg/dl-milligram per deciliters m-meter, BMI-body mass index, m-meter, kg-kilogram, kg/m^2-kilogram per meter square

5.3.2 Lipid profile Distribution by sex and age group

Figure 4 below Showed that lipid profile Distribution by sex group. There is no significant difference between male and female on TC-Total cholesterol (P=0.4418), TG-Triglyceride (P=0.2633), HDL-High density lipoprotein (p=0.294), LDL-Low density lipoprotein cholesterol (p=0.960), and VLDL-Very low density lipoprotein cholesterol (P=0.276) among MDR-TB patients at St.peter's.

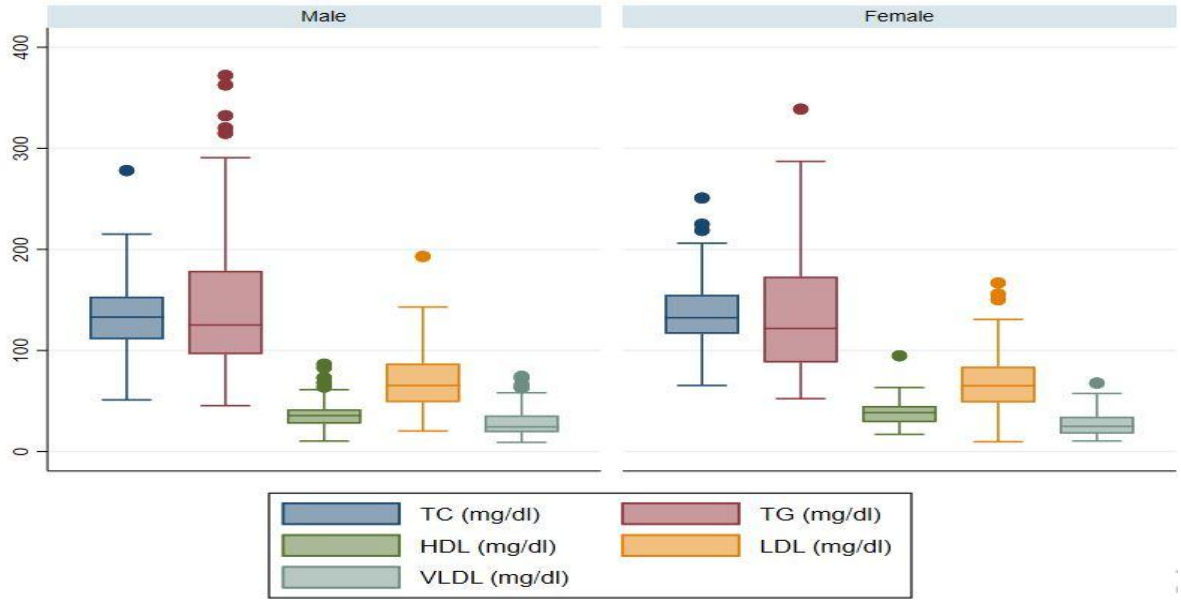

Figure 4 The box plot showing the mean distribution of lipid profile level according to sex group among MDR-TB patients at St.peter's specialized hospital

Figure 5 Showed that lipid profile Distribution by age group. There is no significant difference between There is no significant difference between age <36 and ≥36 on TC-Total cholesterol (P=0.260), TG-Triglyceride (P=0.154), HDL-High density lipoprotein (p=0.864), LDL-Low density lipoprotein cholesterol (p=0.135), and VLDL-Very low density lipoprotein cholesterol (P=0.140) among MDR-TB patients at St.peter's.

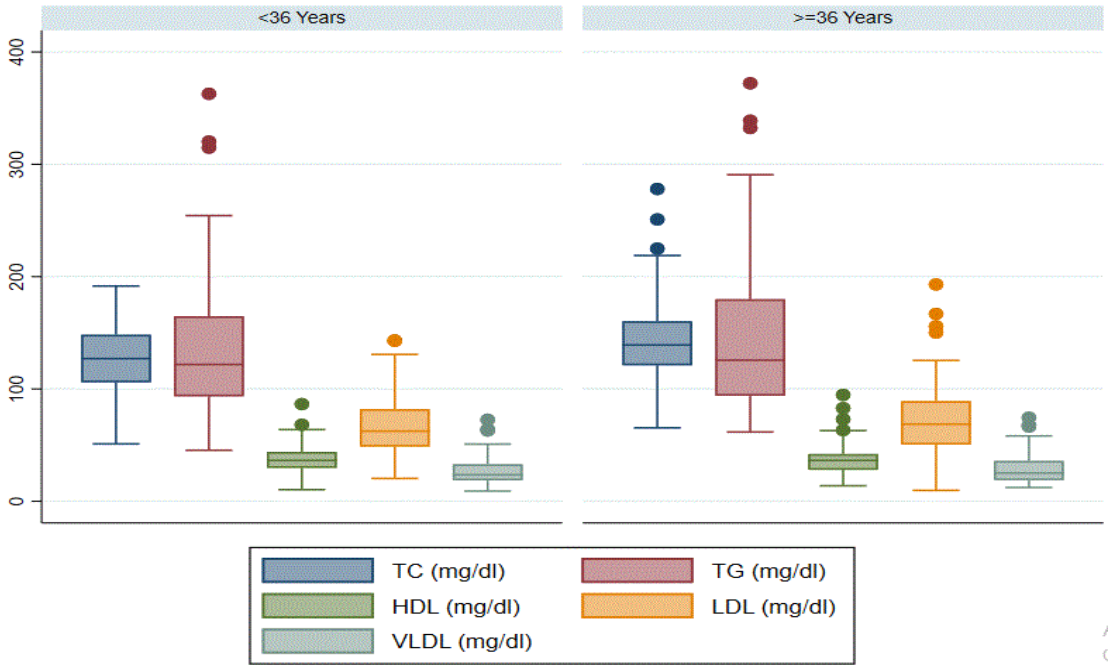

Figure 5 The box plots showing the mean distribution of lipid profile level according to age group between age <36 and ≥ 36 among MDR-TB patients at St.peter's specialized hospital.

5.3.3 Lipids profile (proportion)

Table 7 shows the distribution of lipid profile among the study participant based on the reference value. About (4.3%) of the patients had border-line TC level, but there was no high TC level in the current study [**Table7**].Similarly, 35(21.6%) of the patients had border-line TG concentration, while 23 (14.2%) of the patients had high TG (Table 7). Of the total patients included into this study 44(27.2%) had border-line HDL level, while 106(65.4%) were at high risk HDL. Majority 141(87.0%) of the patients had normal LDL and only 7(4.3%) had border line LDL. Moreover, 140(86.4%) of the patients had border line and 22(13.6%) had high very low density lipoprotein (VLDL). Forty two (25.9%) patients had high level of fasting blood sugar (FBS) [**Table 7**].

Table 7: Distributions of lipid abnormality among MDR –TB patients at St.peter's specialized hospital in Addis Ababa, Ethiopia, 2020 (n = 162)

Variable		N (%)
TC	Normal	155(95.7)
	Border-line	7(4.3%)
TG	Normal	104(64.2%)
	Border-line	35(21.6%)
	High	23(14.2%)
HDL-C	Normal	12(7.4%)
	Border-line	44(27.2%)
	High risk	106(65.4%)
LDL-C	Normal	141(87.0%)
	Good	14(8.6%)
	Border-line	7(4.3%)
VLDL-C	Border-line	140(86.4%)
	High	22(13.6%)
FBG	Normal	96(59.3%)
	Hypoglycemic(L)	24(14.8%)
	Hyperglycemic(H)	42(25.9%)

N-number, %- percent, TC-Total cholesterol, TG-Triglyceride, HDL-C-High density lipoprotein, LDL-C-Low density lipoprotein cholesterol, VLDL-C- Very low density lipoprotein cholesterol FBG- Fasting blood sugar

5.3.4 Factors associated with total cholesterol

Age above 35 years was significantly associated with abnormal TC (p = 0.02). However, age was not included in the multivariate logistic regression model because the number of abnormalities was very small.

Table 8: Factors associated with total cholesterol during anti-MDR –TB treatments at St.Peter's specialized hospital in Addis Ababa, Ethiopia, 2020 (n = 162)

Variables		Total cholesterol		
		Normal N (%)	Abnormal N (%)	P-value
Sex	Female	96(97.0)	3(3.0)	0.432
	Male	59(93.7)	4(6.3)	
Age (in year)	<36	93(100)	0(0)	**0.02***
	≥36	62(89.9)	7(10.1)	
Marital status	Married	54(94.7)	3(5.3)	0.698
	Single	101(96.2)	4(3.8)	
Residence	Urban	133(95.7)	6(4.3)	1.00
	Rural	22(97.5)	1(4.3)	
	Government employ	26(89.7)	3(10.3)	
	Laborer	17(100)	0(0)	

Occupation	Merchant	39(97.5)	1(2.5)	
	Other	13(81.3)	3(8.8)	0.113
	Student	22(100)	0(0)	
	No work	38(100)	0(0)	
Education level	Illiterate	20(90.9)	2(9.1)	
	Primary school	42(93.3)	3(6.7)	
	Secondary school	57(96.6)	2(3.4)	0.284
	Certificate and above	36(1000	0(0)	
Monthly income	<1000	53(96.7)	1(3.3)	
	1001-2000	25(92.6)	2(7.4)	0.694
	≥2000	77(95.1)	4(4.9)	
Types of MDR-TB	EPTB	8(100)	0(0)	
	PTB	144(95.4)	7(4.6)	1.00
	Both	3(100)	0(0)	
Comorbidity	Yes	39(97.5)	1(2.5)	1.00
	No	116(95.1)	6(4.9)	
Types of disease	HIV	26(100)	0(0)	
	DM	9(90.0)	1(10.0)	0.424
	OTHER	4(100)	0(0)	
	NO	116(95.1)	6(4.9)	
Anti HIV drug	Yes	36(94.7)	2(5.3)	0.667
	No	119(96.0)	5(4.0)	
Smoking history	Yes	14(87.5)	2(12.5)	0.143
	No	141(96.6)	5(3.4)	
Alcohol history	Yes	21(100)	2(12.5)	0.596
	No	134(96.6)	5(3.4)	
Duration of anti MDR-TB drugs	≥3months	118(96.7)	4(3.3)	0.365
	<3 months	37(92.5)	3(7.5)	
TSH	No hypothyroidism	123(94.6)	7(5.4)	0.347
	Hypothyroidism	32(100)	0(0)	
T4	Normal	141(95.3)	7(4.7)	
	Low	13(100)	0(0)	1.00
	High	1(0)	0(0)	
T3	Normal	142(95.9)	6(4.1)	
	High	6(100)	0(0)	0.475
	Low	7(87.5)	1(12.5)	
BMI	Normal	75(97.4)	2(2.6)	
	Underweight	70(94.6)	4(5.4)	0.284
	Over weight	10(90.9)	1(9.1)	

*DM-Diabetes mellitus, HIV--human immune deficiency virus, EPTB-Extra pulmonary tuberculosis, PTB-pulmonary tuberculosis, MDR-TB-Multidrug resistance tuberculosis, TSH-Thyroid stimulating hormone, T4-Thyroxine, T3- Triiodothyronine, kg/m2-kilogram per meter square, BMI-body mass index *significant p<0.005*

5.3.5 Factors associated with triglyceride

From the variables were assessed for the association with TG, none of them was not significantly associated with TG abnormality at the p-value less than 0.005 [**Table 9**].

Table 9: Factors associated with triglyceride during anti-MDR –TB treatments at St.Peter's specialized hospital in Addis Ababa, Ethiopia, 2020 (n = 162)

Variable		Triglyceride			P-value
		Normal	**Abnormal**	**High**	
Sex	Male	62(62.6)	19(19.2)	18(18.2)	0.16
	Female	42(66.7)	16(25.4)	5(7.9)	
Age (in year)	<36	60(64.5)	23(24.7)	10(10.8)	0.235
	≥36	44(63.8)	12(17.4)	13(18.8)	
Residence	Urban	87(62.6)	31(22.3)	21(15.1)	0.674
	Rural	17(73.9)	4(17.4)	2(8.7)	
Marital status	Married	37(64.9)	11(19.3)	9(15.8)	0.655
	Single	67(63.81)	24(22.86)	14(13.3)	
Occupation	Government employed	16(55.2)	8(27.6)	5(17.2)	0.377
	Laborer	9(52.9)	5(29.4)	3(17.6)	
	Merchant	23(57.5)	9(22.5)	8(20.0)	
	Other	11(68.8)	2(12.5)	3(18.8)	
	Student	15(68.2)	4(18.2)	3(13.6)	
	No work	30(78.9)	7(18.4)	1(2.6)	
Education level	illiterate	16(72.7)	5(22.7)	1(4.5)	0.427
	Primary school	26(57.8)	11(24.4)	8(17.8)	
	Secondary school	39(66.1)	9(15.3)	11(18.6)	
	Certificate and above	23(63.9)	10(27.8)	3(8.3)	
Monthly income	<1000	33(61)	14(25.93)	7(12.96)	0.517
	1001-2000	14(51.9)	7(25.90	6(22.2)	
	≥2000	57(70.4)	14(17.3)	10(42.3)	
Types of MDT-TB	EPTB	3(37.5)	3(37.5)	2(25.0)	0.374
	PTB	98(64.9)	32(21.2)	21(13.9)	
	Both	3(100)	0(0)	0(0)	
Comorbidity	Yes	23(57.5)	13(32.5)	4(10.0)	0.148
	No	81(66.4)	22(18.0)	19(15.6)	
Types of disease	HIV	17(65.4)	7(26.9)	2(7.7)	0.465
	DM	4(40.0)	4(40.0)	2(20.0)	
	OTHER	3(75.0)	1(25.0)	0(0)	
	NO	80(65.6)	23(18.9)	19(15.6)	
Anti HIV	Yes	23(60.5)	10(26.3)	5(12.5)	0.736

drug	No	81(65.3)	25(20.2)	18(14.5)	
Smoking history	Yes	9(56.3)	9(31.3)	2(12.5)	0.541
	No	95(65.1)	30(20.5)	21(14.4)	
Alcohol history	Yes	13(61.9)	5(23.8)	3(14.3)	0.942
	No	9(64.5)	30(21.3)	20(14.2	
Duration of anti MDR-TB treatment	≥3months	75(61.5)	29(23.8)	18(14.8)	0.411
	<3 months	29(72.5)	6(15.0)	5(12.5)	
TSH	NO hypothyroidism	81(62.3)	27(20.8)	22(16.9)	0.145
	Hypothyroidism	23(71.9)	8(25.0)	1(3.1)	
T4	Normal	94(63.5)	32(21.6)	22(14.9)	0.145
	Low	10(76.9)	3(23.1)	0(0)	
	High	0(0)	0(0)	1(100)	
T3	Normal	95(64.2)	32(21.6)	21(14.2)	0.561
	High	5(83.3)	0(0)	1(16.7)	
	Low	4(50.0)	3(37.5)	1(12.5)	
BMI	Normal	49(63.6)	17(22.1)	11(14.3)	0.312
	Underweight	49(66.2)	17(23.0)	8(10.8)	
	Overweight	6(54.5)	1(9.1)	4(36.4)	

DM-Diabetes mellitus, HIV-human immune deficiency virus, EPTB-Extra pulmonary tuberculosis, PTB-pulmonary tuberculosis, MDR-TB-Multidrug resistance tuberculosis, TSH-Thyroid stimulating hormone, T4-Thyroxine, T3-Triiodothyronine, kg/m2-kilogram per meter square, BMI-body mass index.

5.4 Fasting blood glucose level Distribution by sex and age group

Figure 6 below Showed that fasting blood glucose level Distribution by sex group. There is a significant difference between male and female on fasting blood glucose level (**p=0.0016**) among MDR-TB patients at St.peter's specialized hospital.

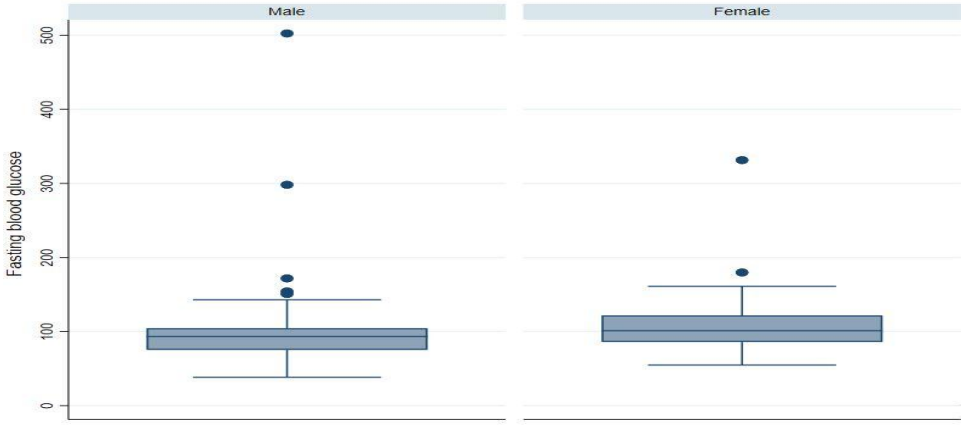

Figure 6 The box plot showing the mean distribution of fasting blood glucose level according to sex among MDR-TB patients at St.peter's specialized hospital

Figure 7 below Showed that fasting blood glucose level Distribution by age group. There is no significant difference between age group <36 and ≥36 group on fasting blood glucose level (p=0.2281) among MDR-TB patients at St.peter's specialized hospital.

Figure 7 The box plot showing the mean distribution of fasting blood glucose level according to age group between <36 and ≥36 among MDR-TB patients at St.peter's specialized hospital.

5.4.1 Prevalence of diabetes and pre-diabetes among study participants

Among the 162 MDR-TB patients who participated in this study 26 (16%) had diabetes mellitus and 40 (24.7%) were pre-diabetes (**Fig 8**). The mean value of two consecutive fasting blood glucose level of diabetes was 165.12mg/dl.

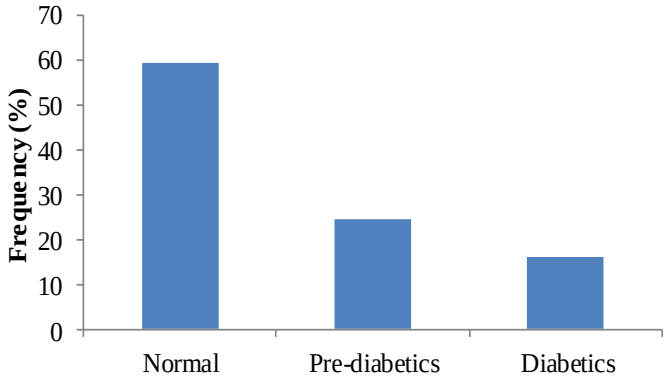

Figure 8 Distribution of prevalence of diabetes and pre-diabetes among study participants among multidrug resistance TB patients on treatment

5.4.2 Factors associated with fasting blood glucose

Female sex (p = 0.004), Non MDR-TB drugs (p =0.012), presence of other comorbidity (p =0.055), and hypothyroidism (p =0.002) were significantly associated with high fasting blood glucose level. However, these variables were not included in the final multiple logistic regression model since, the number of the abnormalities of FBS in each category were small [**Table 10**].

Table 10 : Factors associated with fasting blood glucose during anti-MDR –TB treatments at St.Peter's specialized hospital in Addis Ababa, Ethiopia, 2020 (n = 162)

Variable		Fasting blood glucose level			p-value
		Normal	Hypoglycemia	Hyperglycemia	
Sex	Male	61(61.6)	20(20.2)	18(18.2)	
	Female	35(55.6)	4(6.3)	24(25.9)	**0.004***
Age	<36	59(63.4)	16(17.2)	18(19.4)	0.076
	≥36	37(53.6)	8(11.6)	24(34.8)	
Residence	Urban	81(58.3)	20(14.4)	38(27.3)	0.659
	Rural	15(65.2)	4(17.4)	4(17.4)	
Occupation	Government employed	14(48.3)	3(10.3)	12(41.4)	NA
	Laborer	10(58.8)	4(23.5)	3(17.6)	
	Merchant	26(65.0)	4(10.0)	10(25.00	

	Other	5(31.3)	2(12.5)	9(56.3)	
	Student	16(72.7)	6(27.3)	0(0)	
	No work	25(65.8)	24(14.8)	8(21.1)	
Education level	illiterate	10(45.4)	3(13.6)	9(40.9)	0.529
	Primary school	30(66.70	4(8.9)	11(24.4)	
	Secondary school	35(59.3)	11(18.6)	13(22.0)	
	Certificate and above	21(58.3)	6(16.7)	9(25.0)	
Monthly income	<1000	34(62.96)	12(22.2)	8(14.8)	0.119
	1001-2000	15(55.6)	3(11.1)	9(33.3)	
	≥2000	47(58.0)	9(11.1)	25(30.9)	
Marital status	Marrieds	32(56.1)	8(14.0)	17(29.8)	0.694
	Single	64(61.0)	16(25.2)	25(23.8)	
Types of MDR-TB	EPTB	5(62.5)	1(12.5)	2(25.0)	0.965
	PTB	88(58.3)	23(15.2)	40(26.5)	
	Both	3(100)	0(0)	0(0)	
Anti HIV drug	Yes	15(39.5)	7(18.4)	16(42.1)	**0.012***
	No	81(65.3)	17(13.7)	26(21.0)	
Comorbidity	Yes	18(45.0)	6(15.0)	16(40.0)	**0.055***
	No	78((63.9)	18(15.2)	26(21.3)	
Smoking history	Yes	11(68.8)	2(12.5)	3(18.8)	0.811
	No	85(58.2)	22(15.1)	39(26.7)	
Alcohol history	Yes	15(71.4)	3(14.3)	3(14.3)	0.435
	No	8(57.4)	21(14.9)	39(27.7)	
Duration of anti MDR-TB treatment	≥3 months	69(56.6)	21(17.2)	32(26.2)	0.319
	<3 months	27(67.5)	3(7.5)	10(25.0)	
TSH	No hypothyroidism	85(65.4)	14(10.80	31(23.8)	**0.002***
	hypothyroidism	11(34.4)	10(31.3)	11(34.4)	
T4	Normal	88(59.5)	21(14.2)	39(26.4)	0.794
	Low	7(53.8)	3(23.1)	3(23.1)	
	High	1(100)	0(0)	0(0)	
T3	Normal	88(59.5)	23(15.5)	37(25.0)	0.554
	High	4(66.7)	1(16.7)	1(16.7)	
	Low	4(50.0)	0(0)	4(50.0)	
BMI	Normal	52(67.5)	7(9.1)	18(23.4)	0.152
	Underweight	37(50)	16(21.6)	21(28.4)	
	Overweight	7(63.6)	1(9.1)	3(27.3)	

*DM-Diabetes mellitus, HIV-human immune deficiency virus, EPTB-Extra pulmonary tuberculosis, PTB-pulmonary tuberculosis, MDR-TB-Multidrug resistance tuberculosis, TSH-Thyroid stimulating hormone, T4-Thyroxine, T3- Triiodothyronine, kg/m2-kilogram per meter square, BMI-body mass index *significant p<0.005*

6. Discussion

The current study was aimed to determine the prevalence of hypothyroidism, lipid profile and fasting blood glucose level abnormality and their associated factors among MDR-TB in Addis Ababa, Ethiopia. In the present study the prevalence of hypothyroidism was 19.8%.presence of co-morbidity, being underweight and Prothionamide use were significantly associated with hypothyroidism in MDR-TB patients on treatment .The prevalence of diabetes was 16% and the level of lipid profile was not considerable out the normal reference. Although the results were not adjusted for the potential confounders by multiple logistic regression, age were significantly associated with TG and Female sex, Non MDR-TB drugs, presence of other comorbidity, and hypothyroidism were significantly associated with high fasting blood glucose level in each category during bivariate analysis.

6.1 Thyroid profile

The most difficult thing in MDR-TB treatment is the severe adverse drug reactions associated with its medications. Hypothyroidism is one of them. Among Second-line anti-tuberculosis (TB) drugs prothionamide are known to cause hypothyroidism. These drugs inhibit thyroid hormone synthesis by preventing iodine organification thyroid hormone by reduce iodine transport due to inhibition of thyroid peroxidase (22, 50).

The need for detection of thyroid disease in patients with MDR-TB cannot be under-estimated since reports have shown that subclinical hypothyroidism increases the risk of depression and reduces adherence to MDR-TB and HIV treatment (51, 17).

This prospective cross-sectional study was conducted inpatients and outpatient MDR-TB patients in St.peter's specialized hospital Addis Ababa, Ethiopia. During the study period we included 162 patients that fulfill inclusion and exclusion criteria out of which 99 (61.1%) patients were male. The mean ±SD of study participants age were 35.9 ± 13.6 years (range 18to79), Co-morbidities in patients under study were 40 (24.7%) from this 26(16%) were HIV infected and 10(6.2%) known diabetes mellitus were observed.

The prevalence of hypothyroidism detected in this study was 19.8%.lower prevalence rate previously detected in a study done by Meressa D et al., 2009(17.2%) in Ethiopia (26).However, the rate was similar with a study done by Zhao Ben-nan et al., 2020 (19.78%)

in China (52).The highest prevalence rate was detected in a study done by Satti H et al., 2012 (69%) in Lesotho (12).

In *this* study 125 (77.8%) MDR-TB patients were having normal (euthyroid) status, 28 (17.3%) subclinical hypothyroidism was observed and also 4 (2.5%) patients had overt hypothyroidism. The study done by Ige Om et al., 2016(7.8%) in Nigeria observed lower subclinical hypothyroidism than this study(38).Bare R et al., 2016(40%) in Germany observed higher subclinical hypothyroidism and 38% higher overt hypothyroidism observed than this study (37).In the study done by *Dash M et al., 2020*(2.4%) in India observed comparable overt hypothyroidism, lower euthyroid(58.21%)and lower subclinical hypothyroidism (0%)status than the current study (53).

HIV Sero-reactive was observed in (16%) of patients in the current study. These findings lower than reports made by Meressa D et al., 2009(21.7%) in Ethiopia who reported patients had HIV Sero-reactive (26).The study done by Kirenga J et al., 2015 in Uganda observed HIV (41.9%) higher than this study finding (16%) (54).The study done by Fisher H et al., 2010 in USA among MDR-TB patients had lower HIV co-infection (1.54%) observed compare with this study finding (16%) (55).

On bivariate analysis HIV Sero-reactive and Drug used other than MDR-TB significantly associated with hypothyroidism. There was statistical significance (p=0.008) between the presence of comorbid illnesses HIV positive and development of hypothyroidism. This finding disagrees with the study done by Ige Om et al., 2016 (p=0.823) in Nigeria. There was no significant difference in the thyroid stimulating hormone in patients with MDR-TB and those with MDR-TB/HIV coinfection (38). Reports have shown that HIV and TB infections cause alteration in thyroid function (56, 57).

Duration on MDR-TB treatment ≥3 months was (p =0.034) significantly associated with development of hypothyroidism. This finding agrees with the study done by Somashekar M et al., (2016) in India and Bares R et al. (2016) in Pakistan the majority developing hypothyroidism after 3 months of treatment (36, 37).

Drug used other than MDR-TB drugs was significantly associated with hypothyroidism (p =0.003), in this study the association observed in HIV co-morbidity. Type of the drug

related with Anti-HIV drugs Stavudine associated with the development of subclinical hypothyroidism observed on a research done by Grappin M et al., 2000(p=0.01) in France.(58). However, the result of study was in contrast with a research done by Andries A et al., (2013 p=0.541) in India Stavudine were not associated with the occurrence of hypothyroidism in patients develop hypothyroidism and patients did not develop hypothyroidism among MDR-TB patients (13).

On multivariate logistic regression model presence of co-morbidity (p = 0.006), significantly associated with hypothyroidism in MDR-TB patients. Being underweight (p = 0.050) were significantly associated with hypothyroidism in MDR-TB patients. In this study Patients received prothionamide was significantly associated with hypothyroidism in MDR-TB patients (p < 0.001). This association also observed on a research done by Zhao Ben-nan, et al., 2020 (71.4%) in china reports hypothyroidism associated with prothionamide and para-aminosalicylic acid are the most caustic agent for hypothyroidism development. This also observed on the study done by Cheung YM, et al., (2019) in Australia (50, 51,).

6.2 Lipid profile

The percentage distribution of the lipid parameters into normal, low, borderline and high level category was carried out among MDR-TB patient's participants in St.peter's specialized hospital Addis Ababa Ethiopia.

The outcome indicated that the majority patients had normal value .About 95.7%TC, 87% LDL-C, 86.4% VLDL-C, 64.2% TG and 59.3% patients FBS had normal level.

The most frequently occurring serum lipid profile abnormalities among MDR-TB patients were HDL-C 92.6% had above borderline value, 35.8% had above borderline TG value and 25.9% had above the normal value (Hyperglycemic). During inflammation, catabolism of HDL increases. In successfully treated TB patients, HDL level is supposed to increase after anti TB treatment (ATT) (18).

The study showed that the mean ± SD 134mg/dL (±34.6), 37.9mg/dL (±13.4), 69.8 mg/dl (±29.4), and 19.26kg/m2±3.21 had TC, HDL-C, LDL-C, and BMI respectively. The finding of the current study higher TC 130±34.5, Higher HDL-C 34.7±16.1, lower LDL-C

79.03±27.5 and comparable BMI 18.3±3.9 with the study done by Prajapati N. et al., 2020 in Indian (59).

The study done by Alam N.et al., 2020 had mean ± SD lower TC129.76±33.7; lower TG 76.09±22.8, comparable HDL-C 35.22±13.5, higher LDL-C 86.06±29.9, and lower VLDL-C 15.29±4.5 when compare to the current study finding of the mean ± SD 134mg/dL (±34.6), 138.6mg/dL (±64.2),37.9mg/dL (±13.4), and 69.8mg/dL (±29.4), had TC, TG, HDL-C, and LDL-C respectively (40).

The study done by Mohamed M.et al., 2012 in Egypt had mean ± SD higher TC 148.6±30 mg/dL, lower TG 80.8±23 mg/dL, higher HDL-C 53±16.5 mg/dL and higher LDL-C 79±28.6 mg/dL compare to the finding of the current study which was 134 mg/dL (±34.6), 138.6mg/dL (±64.2), 37.9mg/dL (±13.4), and 69.8mg/dL (±29.4), had TC, TG, HDL-C, and LDL-C, respectively (60).

The current study showed that the median interquartile range of this study was 132.9(42.1) mg/dL, 123.4(80.3) mg/dL, 65.4(37.4) mg/dL, and 36.4(15.2) mg/dL TC, TG, LDL-C, and HDL-C respectively. The study done by Gebremedin G et al., 2017 in Ethiopia the median interquartile range of HIV negative TB patients had greater TC137(44) mg/dL, lower TG 76(22) mg/dL, greater LDL-C 71.2(35.6) mg/dL and comparable HDL-C 35.9(12.7) mg/dL when compare with current study (43).

The study done by Sushilendu V.et al., 2019 in Indian the mean ±SD of post anti TB treatment had higher TC 150±40 mg/dL, lower TG 134±39 mg/dL, lower 34±7 mg/dL HDL-C, and comparable LDL-C 70±14 mg/dL compare with the finding of the current study which was 134(34.6) mg/dL, 138.6(64.2) mg/dL, 37.9(13.4) mg/dL and 69.8(29.4) mg/dL of TC, TG, HDL-C and LDL-C respectively (61).

The study done by Shvets O.et al., 2019 in Ukraine on MDR-TB patients had lower median level of TC 162.2 mg/dL, lower TG 119.5 mg/dL, grater HDL-C 41.3 mg/dL and higher LDL-C 109.3 mg/dL compare with the median level of current study which was 132.9, 123.4, 36.4 and 65.4 TC, TG, HDL-C and LDL-C respectively (62).

Lipids and its metabolites have beneficial effect on tuberculosis resistance through the immune system. Abnormalities in serum lipid profiles play a central role in endothelial functional abnormality which is important in the pathogenesis of atherosclerosis, insulin resistance, and hypertension. Lipoproteins rich in TG and LDL-C have been recognized to be toxic to endothelium, while HDL-C may have a protective role (34).There are no sufficient literature about lipid profile abnormalities and associated risk factors in MDR-TB patients from Ethiopian population.

6.2.1Risk factors of Total cholesterol, Triglyceride and Fasting blood glucose

The current study showed that there were not a significant association between the study category and triglyceride ($p>0.05$). Age (in year) was found to be associated risk factors of abnormality of total cholesterol among MDR-TB patients. ($p = 0.02$) .The risk factors associated with fasting blood glucose were sex ($p=0.004$), Non MDR-TB Drug ($p=0.012$), presence of other comorbidity (0.005), and hypothyroidism ($p=0.002$).

6.3 Prevalence of diabetes and Pre-diabetes among MDR-TB Patients

In this study the prevalence of diabetes was 16%.Out of those who had diabetes, (9.8%) were newly diagnosed through the current study and 6.2% already knew their diabetes status. The prevalence of pre-diabetes in the current study was (24.7%).The current study had higher (24.7%) prevalence of pre-diabetes (15.5%) and higher (16%) prevalence of diabetes (12.8%) compared with the study done by Sarker, M, et al in 2016 in Bangladesh among TB patients (63). The prevalence of diabetes (16%) of current study lower when compared with (25.3%) and comparable with the prevalence of prediabetes (24.5%) with the study done by Viswanathan V, et al, 2012 in India among TB patients (27.5%) (64).This suggests that there may be an increased risk of diabetes among TB patients in the future.

7. Strength and Limitation

7.1. Strength

The research will express its intensity by adding many demographic and clinical parameters that are claimed to be correlated with the study variables. It examined thyroid profile, lipid profile and fasting blood glucose level of MDR-TB patients with detailed information on clinical history during MDR-TB treatment, including types of co-morbidity, duration of ATT, reported pre diabetes cases and new diabetes cases. Our findings highlight the importance of linking MDR-TB risk factors of hypothyroidism and lipid profile abnormalities among MDR-TB patients. It also gives floors to further researches on MDR-TB patients with thyroid and lipid profile issues.

7.2. Limitation

The present study is limited by a number of factors. The study was conducted in one healthcare setting due to budget constraint Therefore, difficult to generalized. In addition, the study could not compare the effects of thyroid and lipid profile variations in dietary habits, physical exercises and age and sex variation. The study also targeted only MDR-TB patients taking medications and did not compare with normotensive subjects. This cross-sectional study, which cannot address the future impacts ATT on thyroid and lipid profiles among MDR-TB patients. Lack of previous study findings limited the association of developing hypothyroidism with being underweight and the presence of comorbidity. This study also not discussed the associated risk factors of lipid profiles abnormalities among MDR-TB patients due to luck of study**.** In study participants could not enable the determination of the relationship between types of ART regimen and hypothyroidism, and thus the role of HIV and diabetics mellitus on changes in thyroid and lipid profile.

8. Conclusion and Recommendation

8.1. Conclusion

The current study was aimed to determine the prevalence of hypothyroidism, lipid profile abnormality and their associated factors in MDR-TB in Addis Ababa, Ethiopia. Hypothyroidism was detected in 19.8% of patients. The multivariate logistic regression revealed that presence of co-morbidity; Being underweight and Prothionamide use were significantly associated with hypothyroidism in MDR-TB patients on treatment. Hypothyroidism most commonly observed above three months of treatment, and also non MDR- TB Drugs are associated with risk factors. The most frequently occurring serum lipid profile abnormalities were HDL-C 92.6% had above borderline value. 35.8% had above borderline TG value and 25.9% of MDR-TB patients had above the normal value of fasting blood glucose (Hyperglycemic).The prevalence of diabetes were 16%.Out of those who had diabetes, (9.8%) were newly diagnosed through the current study.The alarming increase of diabetes and pre-diabetes indicate a threat to TB control and demands a need of increasing awareness regarding lifestyle changes. In addition to medications, nutritional supplementation of the diet to increase BMI in patients diagnosed with MDR-TB Patients seems to be a necessary measure required to curb infectivity, and therefore, decrease the incidence of hypothyroidism and lipid abnormality. According to this study results, we found that patients with MDR-TB under treatment developed hypothyroidism that proved to be a consequence of Prothionamide drugs, co-morbidity, being underweight are a risk factor. HDL-C has been the most frequently occurring lipid profile abnormality. Further research is needed with larger number of patients and longer follow up periods in order to provide additional support to this assertion.

8.2. Recommendation

From this study, we could summarize that there were significant statistical correlation between hypothyroidism and the risk factors prothionamide drug use, being underweight and the presence of comorbidity. Also HDL-C lipid abnormality highly observed among MDR-TB patients and therefore requires monitoring of thyroid function and lipid profile test for its timely initiation of therapy. The association between lipid profile abnormalities and hypothyroidism among MDR-TB patient further investigation and studies are needed.

Large observational studies are required to establish a possible role of hypothyroidism among MDR-TB patients in thyroid and lipid profile alteration and its associated risk factors by using appropriate sample size. These parameters are not well used in Ethiopia. Therefore, our findings suggest the need to test thyroid profile and lipid profiles among MDR-TB patients at the baseline for better patient management and treatment outcomes.

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10. ANNEXES

10.1 Annex 1. Information sheet (English Version)

Research Project: determination of thyroid parameters and selected metabolic profiles among MDR-TB patients at Saint specialized hospital Addis Ababa, Ethiopia.

Sponsored organization**:** Addis Ababa University, College of Health Sciences

Principal Investigator**:** Endalkachew Berhanu (B.Sc. in medical laboratory science, MSc in clinical chemistry candidate)

Advisors: Mistire Wolde (PhD), and Abebe Edao (PhD fellow)

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 thyroid and selected metabolic profiles among MDR -TB 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 5 ml of blood samples will also be collected for only the laboratory examination of TSH,T3,T4, HDL, LDL-C, total cholesterol, triglycerides, FBS, 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 MDR-TB 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 Endalkachew Berhanu: with 0913186148

10.2. Annex 2: Informed consent (English version)

Department of medical Laboratory science, 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 determine and correlate serum thyroid profile and selected metabolic panel among MDR-TB 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_____

11.3. 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 MDR-TB before current episode of MDR-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

11.4. Annex 4: Information sheet (Amharic version)

የተሳታፊዎችየፈቃደኝነትመተማመኛናመረጃመስጫቅፅ

በአዲስአበባዩኒቨርሲቲየጤናሳይንስኮሌጅየሕክምናላቦራቶሪሳይንስየክሊኒካልኬሚስትሪትምሕርትክፍል

ጥናቱንስፖንሰርያደረገውተቋምየአዲስአበባዩኒቨርሲቲየሕክምናጤናሳይንስኮሌጅነው፡

መረጃመስጫቅፅ

በአዲስአበባዩኒቨርሲቲየሕክምናጤናሳይንስኮሌጅየሕክምናላቦራቶሪሳይንስየክሊኒካልኬሚስትሪት/ክፍልለሁለተኛ ዲግሪየተማሪየመመረቂያየጥናትጽሁፍላይእንዲሳተፉተጋብዘዋል፡፡

እባክዎበዚህጥናትለመሳተፍከመስማማትዎበፊትከዚህቀጥሎየሚገኘዉንምንባብበጥሞናያንብቡናግልፅያልሆነሎት ንማንኛዉንምሃሳብይጠይቁ፡፡*To* determine thyroid profiles and its associated risk factors and selected metabolic panel among multi- drug resistant TB patients during MDR therapy regimen in St.peter's specialized hospital Addis Ababa, Ethiopia from January to November 2020.

የጥናቱርእስሲሆንአላማውምመድኃኒቱንየተላመደየቲቢታከሚዎችበደማቸውውስጥያለውንየቅባትመጠን፤ የታይሮይድሆርሞንመጠንናበደምውስጥያለውንየስኳርመጠንእንዲሁምሌሎችየቲቢንመድኃኒትከተላመደበሽታጋር ያላቸውንግኙኝነትናመጠንመለካትነው፡፡

የጥናቱውጤትለታካሚውብሎምለሌላውማህበረሰብየሚጠቅምናየተሻለየጤናእንክብካቤእንዲኖርየሚያደርግነው፡ ፡ስለሆነምእርስዎበዚህጥናትእንዲሳተፉተመርጠዋል፡፡

የእርስዎበዚህጥናትላይየሚያደርጉትተሳትፎሙሉበሙሉበበጎፈቃደኝነትላይየተመሰረተነዉ፡፡

በጥናቱከተሳተፉለናሙናይሆንዘንድ

5ሚሊሊትርያህልደምበሆስፒታሉጤናባለሙያዎችየሚሰጡሲሆንየደምናሙናውንበሚሰጡበትምሰአትሁልጊዜለ ምርመራከሚሰጡበትየተለየህመምናአለመመቸትየለውምለምናልባትቢኖርተገቢውንየጤናእንክብካቤየሚያገኙይሆ ናል፡፡

በዚህጥናትዉስጥለመሳተፍወይምላለመሳተፍከወሰኑበኃላለማቋረጥየሚወስኑቢሆንምእንኩዋንበዚህሆስፒታልየሚ ሰጠዎማንኛዉምአገልግሎትላይተጽዕኖየለውም፡፡

በጥናቱለመሳተፍየሚስማሙከሆነየስምምነትቅጹላይበጹሁፍወይምበጣትፊርማማስቀመጥይጠበቅበዎታልግልፅያ ልሆነሎትጥያቄካሎት

ሞባሌ፡**0913186148** እንዳልካቸወዉብርሃኑብለዉበመደወልመጠየቅይችላሉ

11.5. Annex 5: Informed consent (Amharic version)

የተሳታፊዎችስምምነትማረጋገጫቅጽ

የሚስጥርቁጥር----------

የተሳታፊዉስም----------

እኔስሜ ……..

ከላይየተገለጸዉግለሰብበዚህ
ጥናትእንድሳተፍስሆንመድኃኒቱንየተላመደቲቢያለባቸውንታካሚዎችበደማቸውውስጥያለውንየቅባትመጠን፤
የታይሮይድሆርሞንመጠንናበደምውስጥያለውንየስኳርመጠንእንዲሁምሌሎችየቲቢንመድኃኒትከተላመደበሽታጋርያላቸ
ውንግኙኝነትናመጠንለመለካትበሚደረገውጥናትኣላማውናጥቅሙተገልጾልኛል፤
ስለዚህለዚህጥናትየስምምነትቃሌንየምሰጠውየጥናቱንአላማናጥቅምበመረዳትናበፈቃደኝነትነው፡፡
በመጠይቁየምሰጠውላይየእኔመረጃእንደማይባክንናምስጢራዊእንደሚደረግተገልፆልኛልበተጨማሪምጥናቱውስጥላለ
መሳተፍከወሰንኩመብቴየተጠበቀእንደሆነናበማንኛውምግዜከጥናቱበራሴውሳኔመውጣትጭምርእንደምችልመብቴመሆ
ኑናከጥናቱበመውጣቴምንምኣይነትችግርእንደማይደርስብኝበሚገባስለሆነምሁኔታውንበሚገባበማጤንበፍቃደኝነትበም
ርምሩላይለመሳተፍፈቃደኝነቴንሰጥቻለው፡

በተጨማሪምየምሰጠውየደምናሙናየcholesterol, Triglyceride, HDL-C, LDL-C, Glucose እናየthyroid hormone ምርመራዎችብቻእንደሚውልተነግሮኝተስማምቻለው፡፡
ማንኛውንምያልገባኝንነገርየመጠየቅእድልተሰጥቶኝበሚገባኝቋንቋመልስአግኝቻለው፡፡
በተጨማሪምየሁሉንየላቦራቶሪውጤርበግዜውለሐኪምእንደሚሰጥልኝናውጤቱንማወቅእንደምችልተነግሮኛልበአጠቃ
ላይከላይበመተማመኛቅፅየተጠቀሱትንሁሉበተረጋጋመንፈስአንብቤያለውስለዚህበዚህጥናትለመሳተፍፈቃደኛመሆኔንበ
ፉርማዬአረጋግጣለው፡፡

እኔ_____የተባልኩትግለሰብይህንሁሉበማገናዘብበምርምሩላይመረጃናየደምናሙ
ናለመስጠትተስማምቻለው፡፡

ፉርማ ቀን

የተሳታፊ _____ _____

11.6. Annex 6: Questionnaire (Amharic version)

መጠይቅ

ውድተሳታፊቀጥሎያለውንመጠይቅለመሙላትስለተባበሩንእናመሰግናለን፡፡

የመጠይቁመለያቁጥር_____

Part-I: Socio-Demographic Characteristics

1. ጾታወንድ□ሴት□

2. ዕድሜ_____

3. የጋብቻሁኔታያገባ□ ያላገባ□ የፈታ□ የሞተበት□

4. የመኖሪያቦታገጠር□ ከተማ□

5. የስራዓይነትየቀንሰራተኛ□ ነጋዴ□ ገበሬ□

የመንግስትሰራተኛ□ ተማሪ□ ሌላ□

6. የትምህርትደረጃማንበብናመጻፍየማይችል□ አንደኛደረጃ□

ሁለተኛደረጃ□ ኮሌጅ/ ዩኒቨርሲቲ□

7. የወርገቢመጠን

ከ500 ብርበታች□ ከ500 እስከ 1000 □

ከ 1001 እስከ 2000 □ ከ2000 ብርበላይ□

8. የቤተሰብብዛት_____

9. ሀይማኖትኦርቶዶክስክርስቲያን□ ሙስሊም□ ፕሮቴስታንት□ ሌላ□

ክፍል 2፡- ከህክምናናጋርየተያያዙባህርያቶች

10. ከዚህበፊትቲቢታክመዉያዉቃሉአዎ□ አላቅም□

11. በአሁኑሰአትየቲቢመድኃኒትየሚወስድየቢተሰብአባልአለ□ የለም□

12. ከዚህበፊትየቲቢመድኃኒትየሚወስድየቢተሰብአባልአለ□ የለም□

13. ከቲቢሌላተጨማሪበሽታአለብዎትአዎ□ የለብኝም□

14. መዳኒትስይወስዳሉአዎ□ አልወስድም□

15. ሲጋራየማጨስልምድአለብዎትአዎ□ የለብኝም□

16. አልኮልየመጠጣትልምድአለበዎትአዎ□ የለብኝም

17. ቁመት_____

18. ክብደት_____

19. B.M.I. _____

አመሰግናለሁ

11.7 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). For adult patient 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 drying. 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.

11.8Annex 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 20-30 min 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.

11.9 Annex 9: Standard operating procedure (SOP)/ test procedure:

The sample collected for the purpose of this research will have the following basic procedure for all analytes studied in this study.

After the participants have agreed and signed the informed consent and are voluntary to give blood the following procedures will be applied to all.

- 4 ml of venous blood sample will be collected and stored at room temperature for 10-15 minutes until coagulated
- Then serum will be centrifuged at centrifugation at 2500 rpm for 5 minutes to separate the serum from the red cells.
- Finally the test analysis will be performed from the serum sample.

1. Test principle of TSH

Sandwich principle

▪ 1st incubation: 50 µL of sample, a biotinylated monoclonal TSH‑specific antibody and a monoclonal TSH‑specific antibody labeled with a ruthenium complex react to form a sandwich complex.

▪ 2nd incubation: After addition of streptavidin-coated micro particles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

▪The reaction mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

▪ Results are determined via a calibration curve which is instrument specifically generated by 2‑point calibration and a master curve provided via the reagent barcode or e‑barcode.

2. Test principle of T4

Competition principle

▪ 1st incubation: 15 µL of sample and a T4‑specific antibody labeled with a ruthenium complex; bound T4 is released from binding proteins in the sample by ANS.

▪ 2nd incubation: After addition of streptavidin-coated micro particles and biotinylated T4, the still‑free binding sites of the labeled antibody become occupied, with formation of an

antibody‑hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.

▪The reaction mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

▪ Results are determined via a calibration curve which is instrument specifically generated by 2‑point calibration and a master curve provided via the reagent barcode or e‑barcode.

3. Test principle of T3

Competition principle

▪ 1st incubation: 30 µL of sample and a T3‑specific antibody labeled with a ruthenium complex; bound T3 is released from the binding proteins in the sample by ANS.

▪ 2nd incubation: After addition of streptavidin-coated micro particles and biotinylated T3, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.

▪The reaction mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

▪ Results are determined via a calibration curve which is instrument specifically generated by 2‑point calibration and a master curve provided via the reagent barcode or e‑barcode.

4. Test principle of glucose

Enzymatic reference method with hexokinase.4,5 Hexokinase catalyzes the phosphorylation of glucose to glucose‑6‑phosphate by ATP.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

Glucose‑6‑phosphate dehydrogenase oxidizes glucose‑6‑phosphate in the presence of NADP to gluconate‑6‑phosphate. No other carbohydrate is oxidized. The rate of NADPH

formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

$$\text{G-6-P + NADP+} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P + NADPH + H+}$$

5. Test principle cholesterol

Enzymatic colorimetric method

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminophenazone to form a red quinone-imine dye.

$$\text{Cholesterol esters + H2O} \xrightarrow{\text{CE}} \text{cholesterol + RCOOH}$$

$$\text{Cholesterol + O2} \xrightarrow{\text{CHOD}} \text{cholest-4-en-3-one + H2O2}$$

$$\text{2 H2O2 + 4-AAP + phenol} \xrightarrow{\text{POD}} \text{quinone-imine dye + 4 H2O}$$

The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance.

6. Test principle of HDL-cholesterol

Homogeneous enzymatic colorimetric test

In the presence of magnesium ions, dextran sulfate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons which are resistant to PEG-modified enzymes.

The cholesterol concentration of HDL-cholesterol is 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 by cholesterol esterase.

PEG-cholesterol esterase

HDL-cholesterol esters + H2O ⟶ HDL-cholesterol + RCOOH

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide.

PEG-cholesterol oxidase

HDL-cholesterol + O2 ⟶ Δ4-cholestenone + H2O2

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

Peroxidase

2 H2O2 + 4-amino-antipyrine + HSDA* + H+ + H2O ⟶ purple-blue pigment + 5 H2O

*HSDA = Sodium N- (2-hydroxy-3-sulfopropyl) -3, 5-dimethoxyaniline.

7. Test principle LDL-cholesterol

Homogeneous enzymatic colorimetric assay

Cholesterol esters and free cholesterol in LDL are measured on the basis of a cholesterol enzymatic method using cholesterol esterase and cholesterol oxidase in the presence of surfactants which selectively solubilize only LDL. The enzyme reactions to the lipoproteins other than LDL are inhibited by surfactants and a sugar compound. Cholesterol in HDL, VLDL and chylomicron is not determined.

Detergent

LDL-cholesterol esters + H2O ⟶ cholesterol + free fatty acids

Cholesterol esterase

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

Cholesterol oxidase

LDL-cholesterol + O2 ⟶ Δ4-cholestenone + H2O2

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4‑cholestenone and hydrogen peroxide.

$$2\ H_2O_2 + 4\text{‑aminoantipyrine} + EMSE\ a) + H_2O + H^+ \xrightarrow{\text{Peroxidase}} \text{red purple pigment} + 5\ H_2O$$

a) N‑ethyl‑N- (3‑methylphenyl)‑N‑succinylethylenediamine

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4‑aminoantipyrine and EMSE to form a red purple dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

8. Test principle of triglycerides

Enzymatic colorimetric test

$$\text{triglycerides} + 3\ H_2O \xrightarrow{\text{LPL}} \text{glycerol} + 3\ RCOOH$$

$$\text{glycerol} + ATP \xrightarrow[Mg^{2+}]{\text{GK}} \text{glycerol‑3‑phosphate} + ADP$$

$$\text{glycerol‑3‑phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + 4\text{‑aminophenazone} + 4\text{‑chlorophenol} \xrightarrow{\text{Peroxidase}} 4\text{‑‑phenazone} + 2\ H_2O +$$

አዲስ አበባ ዩኒቨርሲቲ
የጤና ሳይንስ ኮሌጅ

Addis Ababa UNIVERSITY
Collage of Health Sciences

ሕክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

Department of Medical Laboratory Sciences

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Date: 30/12 / 2019

Ref.No. MLS/ /19

Departmental Research and Ethics Review Committee (DRERC) decision

Meeting No: 009/2019

Protocol number: DRERC/479/19/ MLS

Protocol title: Thyroid and selected metabolic panel among Multi-drug resistant Tuberculosis patients attending St. Peter Specialized Hospital, Addis Ababa, Ethiopia

Principal investigators: Endalkachew Berhanu

Institute: **AAU-CHS CLS**

Elements reviewed ☑ Attached ☐ Not attached

Review of revised application ☐ Yes ☑ No

Date of previous review: _____

Decision of the meeting: ☑ **Approved** ☐ Approved with recommendation

☐ Approved on Condition (Major revision) ☐ Disapproved

Obligation of the PI-

1. Should comply with the standard international and national scientific and ethical guidelines
2. All the amendments and changes made in protocol and consent form needs DRERC approval
3. The PI should report DRERC within 10 days of the event.
4. End of the study, including manuscripts and thesis works should be reported to the DRERC

Departmental Research and Ethics Review Committee (DRERC) Approval period: from **December 31, 2019 to January 1, 2021.**

Follow up report expected in

3 months ✓ 6 months _____ 9 months _____ one year _____

Chairperson, DRERC: Kassu Desta

Signature: _____

Date: 01/01/2020

Chair Person for DMLS: Rahel Alemu

Signature: _____

Date: 1/3/2020

አዲስ አበባ ዩኒቨርሲቲ
ጤና ሳይንስ ኮሌጅ
ነርሲንግ እና ሚድዋይፈሪንግ ት/ቤት
የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል

Addis Ababa University
College of Health Sciences
School of Nursing and Midwafery
Department of Medical Laboratory Sciences

ቀን፡-4/05/ 2012 ዓም
ቁጥር፡-ሕ/ላ/ላ/ት/112/12/20

ለ፡ አዲስ አበባ ጤና ቢሮ
ለ፡ቅዱስ ጴጥሮስ ስሌሻላይዝድ ሆስፒታል

ጉዳዩ ፡- ትብብር ስለመጠየቅ ::

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ በህክምና ላቦራቶሪ ሳይንስ ት/ክፍል የ2ኛ ዓመት የማስተርስ ተማሪ የሆኑት እንዳልካቸው ብርሃኑ መለያ ቁጥር GSR/4365/10 ከዚህ በታች በተጠቀሰው ርዕስ Thyroid and selected metabolic panel among Multi-drug resistant Tuberculosis patients attending St.peter specialized Hospital, Addis Ababa, Ethiopia.ጥናታዊ ፅሁፍ ለማዘጋጀት ይችሉ ዘንድ ተማሪ መሆናቸው ተጠቅሶ የትብብር ደብዳቤ እንዲፃፍላቸው በቀን 4/05/2012 ዓ.ም በማመልከቻ ጠይቀውናል ::

ስለዚህ ተማሪ እንዳልካቸው ብርሃኑ መለያ ቁጥር GSR/4365/10 መመረቂያ ጥናታዊ ፅሁፋቸውን ለመስራት እንዲችሉ መስሪያ ቤታችሁ አስፈላጊውን ትብብር ያደርግላቸው ዘንድ በትህትና እንጠይቃለን ::

ከሰላምታ ጋር
ወ/ሮ ሪዘቤል አለሙ
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Date 28/01/2020

St. PETERS SPECIALIZED HOSPITAL (SPSH)

RESEARCH & EVIDENCE GENERATION DIRECTORATE; THE OFFICE OF INTITUTIONAL REVIEW BOARD; ETHICAL CLEARANCE

Title: THYROID AND SELECTED METABOLIC PANEL AMONG MULTI-DRUG RESISTANT TUBERCULOSIS PATIENTS ATTENDING IN S'T PETER SPECIALIZED HOSPITAL

Principal Investigator/Project Holder : Endalkachew Berhanu (BSc)

Protocol/Version no: V143/28/01/2020

Project Type: For Partial Fulfillment of Requirements for Master of Science degree in Laboratory Science Clinical Chemistry

The office of IRB of SPSH has reviewed the research proposal with the above title on the day of **January 24 2020** & passed the following decision.

- Approved
- **Approved with recommendation**
- Approved on condition
- Disapproved

This decision is valid for consecutive 12months taking this decision date as day one, & the proposal should be implemented as presented to the office with the incorporated comments. If there is any plan to make changes in any part of the approved protocol, it is obligatory to inform the office to have another review. It is the researcher duty to inform & submit a summary of each and every activity of the study every three months to the office & finally submit the final completed work of the research to the office.

Regards

Cc

Academic, Research & Training Directorate

Dr.Million Molla Sisay
Research & evidence Generation
Directorate director

Declaration

I declare that this research paper entitled: Thyroid and Selected metabolic panel among Multi- Drug Resistant tuberculosis patients attending Saint Peter Specialized Hospital Addis Ababa, Ethiopia, 2020 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.

MSc candidate: Endalkachew Berhanu

Signature_____

Date of submission_____

This thesis has submitted with our approval as advisors.

Advisors:

Mistire Wolde (MSc, Ph.D)

Signature _____

Date of Submission _____

Abebe Edao (MSc, Ph.D candidate)

Signature _____

Date of submission _____

Habeteyese Hilu (MSc, Ph.D)

Signature _____

Date of submission _____

Mililion Mola (MD, MPH)

Signature _____

Date of submission _____