THE INCIDENCE OF ANTI-TUBERCULOSIS DRUGS INDUCED HEPATOTOXICITY AND ASSOCIATED RISK FACTORS AMONG TUBERCULOSIS PATIENTS IN DAWRO ZONE, SOUTH ETHIOPIA: A COHORT STUDY

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COLLEGE OF HEALTH SCIENCES DEPARTMENT OF MEDICAL LABORATORY SCIENCE AND PATHOLOGY:

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Abstract

***Background**: Anti-tuberculosis drugs causes hepatotoxicity in some individuals leading to acute liver failure which results in death. Such phenomena limit the clinical use of drugs, contributing to treatment failure that possibly causes drug resistance. Furthermore, associated risk factors for the development of anti-tuberculosis-drug induced hepatotoxicity are found to be controversial among different study findings.*

***Objective**: To determine the incidence rate of anti-tuberculosis drug induced hepatotoxicity and associated risk factors among tuberculosis patients in Dawro zone, southern Ethiopia.*

***Method**: A prospective cohort study was conducted from 1 May to 30 October2014 in Dawro zone Tercha district hospital laboratory.124 new tuberculosis positive individuals available from Tercha hospital and 5 health centers during data collection were consecutively included. Socio demographic data and anthropometric measurement was obtained.5 ml of venous blood was drawn from each individuals and Alanine amino transferase, Aspartate amino transferase and total bilirubin was measured photometricallyat baseline and then continuously monitored by measuring these liver enzymes every two weeks for two months. Data was analyzed with statistical package for social science (version 20 Chicago inc.).Logistic regression, (odds ratios) with 95% confidence intervals was calculated to evaluate the possible association of all variables. P-value of less than 0.05 was considered as statistically significant.*

***Result:** The incidence of anti-tuberculosis induced hepatotoxicity was found to be 8% (10 patients out of 124). Raised serum transaminase and bilirubin level as well as sign and symptoms of hepatotoxicity(nausea,anorexia, vomiting,malaise, and jaundice) were observed in the cases. The onset of hepatotoxicity ranged from 13-58 days (median: 26 days) aftertreatment was initiated.Of the various risk factors analyzed, only high alcohol intake was associated with the incidence (OR=9.3, 95% CI (1.8-47),P<0.007). Age, sex, extent of tuberculosis and malnutrition were not significantly associated with anti-tuberculosis induced hepatotoxicity.*

Conclusion:*The incidence of anti-tuberculosis induced hepatotoxicity in Dawro zone was high.The drug responsible for the hepatotoxicity was not known.However chronic high alcohol intake was associated with the development of anti-tuberculosis induced hepatotoxicity.*

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List of Abbreviation and acronym

- ALF Acute Liver Failure
- ALT Alanine Amino Transferase
- AST Aspartate Amino Transferase
- ATS American Thoracic Society
- BMI Body Mass Index
- DOTS Directly Observed Treatment Short course
- DIH Drug induced hepatotoxicity
- EMB Ethambutol
- HBsAg Hepatitis B Surface Antigen
- HCV Hepatitis C Virus
- HIV Human immunodeficiency virus
- IFN Interferon
- IL Interleukin
- INH Isonicotinic acid hydrazide(Isoniazid)
- LFT Liver Function Test
- MAH Mono acetyl hydrazine
- NACB National Academy of Clinical Biochemistry
- NAT-2 N-Acetyl Transferase 2
- COR Crude Odds Ratio
- PZA Pyrazinamide
- RIF Rifampin
- SOP Standard Operating Procedure
- SPSS Statistical Package For Social Science
- TB Tuberculosis
- ULN Upper Limit Normal

Operational Definition

Anti-tuberculosis drug induced hepatotoxicity: increased concentration of transaminases (AST and/or ALT) up to 5 timesof the ULN in the absence of symptoms or up to 3 times of the ULN or/and 2 times the ULN ofbilirubin in the presence of symptoms like anorexia, nausea, vomiting, jaundice, hepatomegally, epigastric distension, right upper abdominal discomfort, malaise and weakness.

Upper limit of normal: The maximum value of reference range of AST, ALT and bilirubin concentration in plasma/serum of healthy population which is 37U/L, 42U/L & 1.2mg/dl respectively.

Healthy population: Population having AST, ALT and bilirubin level of up to 37IU/L,42 U/L and 1.2mg/dl respectively.

Alcoholism: Men who drink more than 35 melekia (175ml) of alcohol a week for 10 years or more and Women who drink more than 28 melekia (175ml) of alcohol a week for 1 year or more

Malnutrition: patients having BMI less than 18.5Kg/m^2

All form TB:TB includingsmear negative pulmonary,Smear positive pulmonary TB**,** and extra pulmonary TB

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CHAPTERONE: INTRODUCTION

1.1 Background

Tuberculosis is a chronic or acute bacterial infection caused by *Mycobacterium tuberculosis* that affects the lungs causing small round swellings called tubercles to form on the mucous membrane. Italso affects the kidneys, bone, lymph nodes and the brain. It is transmitted from person to person through infectious droplets from individuals with active respiratory disease [1]. It is endemic disease in developing countries, especially in immuno-compromised patients [2].

New cases of tuberculosis infected individual are treated by combination of four drugs: Isoniazid (INH), Rifampin (RIF), Pyrazinamide (PZA), and Ethambutol(EMB). Total treatment period is 6 months (2 months for intensive and 4 months for continuation phases). The intensive phase comprised INH (5 mg/kg day^{-1}; maximum 300 mg/day), RIF (10 mg/kg day^{-1}; maximum 600 mg/day), EMB (15–20 mg/kg day^{-1}), and PZA (20–25 mg/kg day^{-1}), or streptomycin. The continuation phase comprised daily similar doses of INH and RIF [1,2]. However a variety of adverse reactions of these drugs have been reported; one of the well-known toxic effects is hepatotoxicity [3,4].Among the drugs (isoniazid, rifampin, pyrazinamide and ethambutol), the first three have the potential for hepatotoxicity with pyrazinamide being the most hepatotoxic followed by isoniazid and rifampin[5].

Anti-TB drug induced hepatotoxicity (anti-TB-DIH) is defined as elevation of transaminases up to 5 times of the upper limit of normal in the absence of symptoms. Elevation of transaminases up to three times the upper limit of normal(ULN) or twice the ULN of bilirubin in the presence of symptoms like anorexia, nausea, vomiting, jaundice, hepatomegally, epigastric distension, right upper abdominal discomfort, malaise and weakness, provided competing causes such as acute viral hepatitis, autoimmune hepatitis and others liver diseases are ruled out[5, 6].

Anti-TB-DIH may result from direct toxicity of the primary compound, a metabolite, or from an immunologically mediated response, affecting hepatocytes, biliary epithelial cells, and/or liver vasculature. Predictable anti-TB-DIH is generally characterized by certain dose-related injury in experimental animal models, has a higher attack rate, and tends to occur rapidly. Injurious free radicals cause hepatocyte necrosis in zones farthest from the hepatic arterioles, where metabolism is greatest and antioxidant detoxifying capacity is the least[7,8].Recent evidence indicates that drugs taken in quantities of >50 grams/day[9]are more likely to produce hepatotoxicity, which results from the formation or reduced clearance of toxic metabolites[5,10].

Most type of anti-TB-DIH is due to metabolic idiosyncrasy due to the metabolites released or accumulated during the metabolic process. These hypersensitivity or metabolic reactions occur largely independent of dose. In hypersensitivity reactions, immunogenic drug or its metabolites may be free or covalently bound to hepatic proteins, forming haptens or "neoantigens." Antibody-dependent cytotoxic T-cell and occasionally eosinophilic hypersensitivity responses may be evoked and releases tumor necrosis factor-α, interleukin (IL)-12, and IFN-γ which promotes hepatocellular programmed cell death, apoptosis [8, 11]. Most of the time anti-TB-DIH curs after taking the drugs for few weeks to months especially, starting from the second week [5,6, 8].

1.1.1. Liver Enzymes

Liver metabolizes carbohydrate, protein, and lipids and synthesizes many proteins. It conjugates bilirubin, and detoxifies drugs and other substance. For this reason clinical laboratory uses several tests for the assessment of liver function [12]. Enzyme activity or concentration levels in body fluids can reflect leakage from cells due to cellular injury, changes in enzyme production rate, actual enzyme induction due to metabolic or geneticstates or proliferation of neoplasm. In the latter case, increased enzyme activity can be used as a tumor marker [13].

Enzymes like alkaline phosphatase (ALP), ALT, AST, Gama glutamyltransferase (GGT), lactate dehydrogenase (LDH) and 5′-nucleotidase and the metabolite bilirubin are helpful in the assessment of the proper functioning and inflammatory status of the liver[14].

ALP, ALT, AST and LDH are found in the cytoplasm of hepatocytes though; can originate from other organs and are hence released in to plasma with mild inflammation of the liver with increased permeability of the cell membrane.Since ALT and AST are also mitochondrial enzymes they are released with cell death or necrosis and their increased concentration can be indicator of severe injury of liver. Distributions of these enzymes within specific types of hepatic tissues vary. ALP and GGT are more concentrated in the biliary ducts or tissues of the small ducts (canaliculi), while AST, ALT, and LDH are found mainly in structural (parenchyma) hepatic cells. Multiple forms of enzymes, called isoenzymes, are distributed in several different tissue types [14, 15].

ALT and AST perhaps are the most commonly used indicators as they are used to generally measure the degree of acute liver (hepatocellular) injury. The ALT is felt to be more specific indicator of liver injury as AST is also found in other organs such as heart, skeletal muscle, kidneys,brain, pancreas, lungs, leucocytes, and red cells [16].

Bilirubin is derived from the breakdown heme of hemoglobin released when aged red blood cells are phagocytized by the reticuloendothelial system. The protoporphyrin ring of the heme is further broken down and forms biliverdin, which is then reduced to bilirubin. It is transported to the liver, from extra-hepatic sources as bilirubin-albumin complex. Within the hepatocyte, the liver enzyme uridyldiphosphate glucurony ltransferase (UDPG-transferase) transfers molecules of glucuronate, a sugar, to the bilirubin molecule. Conjugated bilirubin passes into the intestine through the bile duct, where intestinal bacteria reduce bilirubin to urobilinogen.Some urobilinogen may be reabsorbed through the intestinal mucosa and returned to the portal circulation and theliver. The remaining urobilinogen is excreted into urine or oxidized to form urobilin and excreted in the feces [17,18].

1.1.2. Measuring transaminases and bilirubin

Both ALT and AST are measured using absorbance spectrophotometer technique. ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD. The system monitors the rate of change in absorbance at 340 nm over a fixed-time interval. The rate of change in absorbance is directly proportional to the ALT activity in the sample. AST catalyzes the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with the concurrent oxidation of NADH to NAD. The system monitors the rate of change in absorbance at 340 nm over a fixed-time interval. The rate of change in absorbance is directly proportional to the AST activity in the sample [19].

Bilirubin is coupled with diazotized sulphanilic acid in acidic medium to form the pink colored azobilirubin. Using automated absorbance spectrophotometric technique, the intensity ofthe color produced is directly proportional to bilirubinconcentration present in the sample [17, 19].

1.1.3. Interpretation of liver enzyme measurement

Serum enzyme concentrations are measured by functional catalytic assays with normal values established from “healthy” populations. Transaminase concentration may vary as much as 45%on a single day on an individual with the highest levels occurring in the afternoon,or 10 to 30% on successive days. ALT and AST elevation may occur afterexercise, hemolysis, or muscle injury. A recent retrospectivereview of healthy volunteers participating in drug trials who received placebo found that 20% had at least one ALTvalue greater than the ULN, and 7% had one value at least two times the ULN [20]. Serum hepatic transaminase concentration is higher in men and in those with greater body massindex. Children and older adults tend to have lower transaminase concentrations [21].

There is a mild increase of transaminases level in approximately 20% of patients receiving anti tuberculosis drug which is usually asymptomatic [22]. There is a peculiar feature to anti-TB drugs to undergo a phenomenon called adaption, through which the liver undergoes tolerance to the drugs. Adaption is a process in which increased concentration of transaminases and bilirubin without symptom resolves with continuation of drugs [23]. But a mild elevation of transaminase and bilirubin in serum must be identified whether it is a sign of adaption or a sign of early development of liver toxicity. The current recommended diagnostic criteria of Anti-TB-DIH as laid down by the Drug induced liver injury network(DILIN) and other groups may assist in resolving this issue. Anti-TB-DIH is confirmed by elevated level of AST/ALT to 5 × ULN, in the absence of jaundice or other symptoms; or up to 3 × ULN in the presence of symptoms or hyperbilirubinemia (bilirubin 2 × ULN) whereas Competing etiologies particularly acute viral hepatitis may need to be excluded [3, 5, 20, 22].

1.2. Problem statement

Tuberculosis continues to remain a significant infectious disease across much of the world. It poses a formidable socioeconomic burden on the individual and society. There were 8.6 million newer TB cases and estimated 1.3 million deaths occurred worldwide in 2012. There are 22 high burden countries in the world, among which Ethiopia ranks seventh position. Tuberculosis is a major cause of morbidity and mortality in Ethiopia. On a survey conducted in 2010/11, the prevalence of TB that was confirmed bacteriologically was found to be 156/100,000 populations and the prevalence of all form of TB was estimated to be 240/100,000 populations [24].

The high TB burden countries that account 82% of all estimated cases worldwidehave been given the highest priority level since 2000. For this reason directly observed treatment short course (DOTS) program expansion have been the major concern to reduce the burden of TB on these countries [11, 24]. Drugs used in the DOTS strategy to treat TB are four combinations of drugs that consist of INH, RIF, PZA and EMB. From 10% to 20% of patients using the drugs present aslight increase in transaminase levels around 2–3 times above their normal values during the first 2 months and frequently it resolves in spite of going on with the treatment [25]. Although a vast majority of patients tolerate the drugs, some 3% to 25% develop anti-TB-DIH worldwide.Anti-TB-DIH accounts for 7% of reported drug adverse effects, 2% of jaundice in hospitals, and approximately 30% of fulminant liver failure [26, 27]. It has replaced viral hepatitis as the most apparent cause of acute liver failure [28]. In a study conducted at King's College Hospital in India the case mortality rate of anti-TB-DIH was 31.4% [30]. In Ethiopia according to a study conducted in Addis Ababa at Saint Peters hospital the incidence of anti-TB-DIH was found to be 8.9% [31].

The spectrum of Anti-TB-DIH is diverse, ranging from asymptomatic rise in transaminase (to fivefold) in 2.3% to 28% to acute liver failure (ALF) in approximately less than 0.01% of the individuals [29].The most serious consequence of Anti-TB-DIH is that it limits the clinical use of drugs, contributes to treatment failure and thereby leads to drug resistance [8,29].Furthermore,it is a leading cause of drug-induced liver injury and of drug-induced acute liver failure leading to

death or liver transplantation. Hepatitis, hepatic necrosis and death have been described with INH drug and the only treatment being given for fulminant hepatic failure (FHF) is liver transplantation[5, 8, 29].

There are many factors that contribute to the development of anti-TB-DIH. Age, gender, nutritional status, extent of TB disease, alcoholism and genetic polymorphism are most studied risk factors for anti TB drug induced hepatotoxicity. Young age, malnutrition, alcoholism and severe TB like multi bacillary TB and extra pulmonary organ involvement are found to be positive predictors of Anti-TB-DIH [5].

TB patients who resume anti-TB drugs need routine follow up of clinical presentation of hepatotoxic effect. If suspected, examination is done both biochemically and clinically. If serum transaminase concentrations are more than five times the ULN (with or without symptoms) or more than three times the ULN with jaundice and/or hepatitis symptoms, then potentially hepatotoxic medications should be stopped immediately and the patient evaluated promptly until transaminase level returns to normal or baseline [32].

The risk factors that contribute to the development of anti-TB-DIH are still obscure and controversial. Understanding Anti-TB-DIH is restricted by difference in study population, definition of hepatotoxicity and monitoring practices. There are no studies that determined the incidence of anti-TB-DIH and assessed risk factors for developing anti-TB-DIH among TB patients in the study area. Since the risk factors are found to be controversial, not all studies have identified the same risk factors. Therefore, this study is aimed at determining the incidence of anti-TB-DIH and identifying the possible risk factors.

1.3. Significance of the study

Depending on the definition of hepatotoxicity and methodologies used the incidence rate of anti-TB-DIH varies. The incidence rate, pattern, and predictors of anti-TB-DIH have not been prospectively studied in Dawro zone and the overall incidence of anti-TB-DIH in the population is unknown and is probably unrecognized.

This study determined the incidence of anti-TB-DIH and assessed the risk factors so that higher risk groups can be identified and early intervention will be taken to manage severe hepatotoxicity and prevent treatment failure. This study will also be used as valuable baseline data for stake holders and policy makers to intervene in drug formulation and recommend treatment monitoring.

CHAPTER TWO: LITERATURE REVIEW

2.1. Drugs that cause anti-TB drug induced hepatotoxicity

Anti-TB-DIH is a minor but significant cause of liver injury across the world. Anti-TB-DIH is a leading cause of drug induced hepatotoxicity (DIH) [5]. RIF, INH and PZA are the three key drugs that are used to treat TB and are potentially hepatotoxic.Hepatotoxicity level of each of the three drugs is different PZA being the first followed by INH and RIF [34]. When anti-TB-DIH occurs following the use of 4-drug combination regimen, it is difficult to quantify the contribution of each drug in the development of anti-TB-DIH. However as a result of meta-analysis, incidence rate of hepatotoxicity is shown to be 2.6% with INH and RIF co-administration, 1.6% with INH alone and 1.1% with RIF alone [35]. But RIF combined with PZA is more hepatotoxic than RIF combined with INH [36].

2.2. Mechanism of Hepatotoxicity

The exact mechanism of liver toxicity has not been understood well enough but anti-TB-DIH may result from direct toxicity from the primary compound, a metabolite or as a result of host immunologic response which damages liver cells, billiary epithelial cells and the liver vascular system [8].

2.2.1. Isoniazid

Liver clears INH from plasma through acetylation by the enzyme N-acetyl transferase 2 (NAT-2). The acetylated INH or acetyl-isoniazid is metabolized to mono acetyl hydrazine (MAH), to non-toxicdiacetyl hydrazine and other minor metabolites [37]. The metabolites of reactive MAH cause toxicity to liver tissues by generating free radicals [38]. On a study conducted in rats INH reduces glutathione-related thiols that scavenge free radicals thereby reducing the activity of the antioxidant glutathione peroxidase and catalase [39]. In INH toxicity of liver, metabolic idiosyncrasy is also observed in which the INH metabolite acetyl hydrazine covalently binds to

liver macromolecules mediated by microsomal enzymes which producesacylating agent that causes liver necrosis [38].

2.1.1. Pyrazinamide

Pyrazinamide is de-aminated to pyrazinoic acid in the liver and subsequently metabolized in to 5-hydroxy pyrazinoic acid by xanthine oxidase [40], aldehyde oxidase [41] and xanthine dehydrogenase [42]. A study conducted in rats showed that in idiosyncratic manner PZA alters nicitinamide acetyl dehydrogenase level in the liver. This process leads to generation of reactive free radicals. Since there is similarity of molecular structure between pyrazinamide and isoniazid there is a shared mechanism of injury to liver [43].

2.1.2. Rifampin

RIF causes conjugated hyperbilirubinemia or jaundice without causing hepatocellular damage by inhibiting bile exporter pump [44].

2.3. Follow Up

Patients receiving anti tuberculosis drugs are followed clinically and biochemically. Liver function parameters that consists ALT, AST and total bilirubinwere performed before the initiation of the treatment. After initiation ofthe treatment, biochemical tests were performed every two weeks during the first two month and then monthly until completion of treatment [32, 45, 46].

Hepatotoxicity of anti-tuberculosis drug often develops within two weeks to two months from the onset of therapy. On astudy conducted in Nepal the onset of hepatotoxicity was found to be within 12 – 60 days (median of 28days)[3], in India the onset ofhepatotoxicity was within 15-50 days (median of 25 days) [47], andin Egypt the onset of hepatotoxicity was within 15–60 days (median: 30 days)[2].

2.4. Incidence rate of Anti-TB-DIH

The overall incidence of anti-TB-DIH in the population is unknown and is probably unrecognized. Recent studies that utilized the ATS transaminase criteria for definition of

hepatotoxicity placed the incidence rate about 3 to 13%, but depending on the definition of hepatotoxicity and methodologies used, the incidence rate of anti-TB-DIH ranges from 3% to 25% [33]. On a prospective cohort study conducted in Nepalese population, the incidence of anti-TB-DIH was found to be 8% (4 patients out of 50) detected by clinical examination and laboratory confirmation of liver function tests [3]. Another studies conducted in Spain and India showed the incidence of anti-TB-DIH to be 18.2% (42 patients out of 231) and 14.3% (35 patients out of 244) respectively [46, 48]. A study that was conducted in Ethiopia, in Addis Ababa at Saint Peter's hospital on 516 admitted TB patients showed the incidence of anti-TB-DIH to be 8.9% [31].

2.5. Risk factors for Anti-TB-DIH

There are many risk factors that have been found to be associated with anti-TB-DIH. Advanced age, early age, female sex, poor nutritional status, extent of TB disease and high alcohol intake are found to be positive predictors of anti-TB-DIH. However, the exact risk factors associated with anti-TB-DIH are still controversial and obscure.

2.5.1. Age

Age is a controversial predictor of anti-TB-DIH. On a prospective cohort study conducted in India,univariate analysis indicated that the prevalence of anti-TB-DIH was significantly (P=0.03) higher in younger age group (≤35 years) as compared to older[33]. Another study conducted in Nepal showed that Patients belonging to younger age group were found to be at higher risk for anti-TB drugs induced hepatotoxicity (P=0.368) [3]. But a study conducted in India in another hospital shows no significant correlation of age with anti-TB DIH [49].

2.5.2. Gender

There is currently no clear evidence to point out an overall sex-related difference in the incidence of hepatotoxicity. But the female gender is felt to be at increased risk of developing anti TB-DIH. A prospective study showed 4-fold increase in the risk of severe hepatotoxicityin women, but with an overall incidence of only 2% [50]. Another study showed that

hepatotoxicity induced by anti-TB drugs was more frequent in females as compared to males(p=0.005) [33]. It has been suggested that slow acetylator are more prone to hepatotoxicity compared to rapid acetylator and females being a slow acetylator, are at higher risk for anti-TB-DIH[51]. However some studies showed no increased risk of anti-TB-DIH in women[52, 53].

2.5.3. Malnutrition

Malnourishment may be one of the risk factors of anti-TB drugs-induced hepatotoxicity.In malnutrition, glutathione stores are depleted which makes the patient vulnerable to oxidative injury [3]. According to recent reports hypoalbuminemia is continuously being used as a surrogate marker of malnutrition and a risk factor for anti-TB-DIH [5]. Body mass index (BMI) measurement is also used in assessing malnutrition in many researches. In a study done in India, incidence of hepatotoxicity was found to be three times higher in malnourished patients which is assessed by lower BMI(<20Kg/m^2) and hypoalbuminemia(<3.5 mg/dl)[54].

2.5.4. Extent of TB disease

The extents of tuberculosis including cavitory disease, multibacillary TB and extra pulmonary organ involvement have been considered aspositive predictors for anti-TB-DIH [55, 56]. In a study conducted in Nepal three patients out of four who developed hepatotoxicity had advanced TB infection, proven microbiologically as well as radiologically, which has shown that the extent of disease has a role in predisposing the patient toward hepatotoxicity[3]. A study conducted in India showedthat pulmonary patients had 68% lower risk for anti-TB-DIH than extra pulmonary TB patients. Of the total extra pulmonary TB (47%), cervical lymphadenitis comprises 39.1%, pleural effusion (24.3%), bone (7.8%), meningitis (7.0%), ocular (6.1%), genitourinary (8.7%) and gastrointestinal (7.0%). (Adjusted OR=0.32)[33]. According to another study conducted in India, disease extent was also a significant risk factor for the development of hepatitis, with14% of the cases having extensive disease but only 3-5% of the controls (OR=4-54, p<0001). Four of the cases (4.6%) and six of the controls (1-5%) had milliary shadows on their radiographs [54].

2.5.5. Alcoholism

Alcoholism is defined as consuming alcohol as much as >6 units (48 g ethanol) per day for more than one year [41]. The influence of alcohol as a risk factor for anti-TB DIH is equivocal. Yet it is felt to be predisposing factor for anti-TB-DIH [54, 57, 58].

A study conducted in India showed that the proportion of patients with a high alcoholintake was significantly higher amongst the cases than the controls (19. 8% versus 4.9%; OR=4.76, p<0-001) [41]. Yet a study conducted in Nepal showed that there was no correlation between hepatotoxicity and alcoholism [3].

In conclusion, different studies conducted indifferent areas of the world so far have shown that the anti- TB drugs induce hepatotoxicity with different rate of incidence. The exact mechanism of hepatotoxicty of the drugs is still not explicit. But two mechanisms of toxicity have been proposed: the unpredictable which is dose independent (idiosyncratic in nature) and dose dependent. The risk factors for development of anti-TB-DIH are controversial in that all studies did not identify the same factors. So risk factors for anti-TB-DIH have to be evaluated and incidence rate should be determined in areas of unknown prevalence.

CHAPTER THREE: OBJECTIVES

3.1. General objective

To determine the incidence of anti-TB-DIH and associated risk factors among TB patients in Dawro zone, southern Ethiopia.

3.2. Specific objectives

- To determine the incidence rate of anti-tuberculosis drug induced hepatotoxicity among TB patients in Dawro zone.
- To demarcate the time at which the anti-TB drugs induces anti-TB-DIH among TB patients at Dawro zone.
- To identify associated risk factors for the development of anti-tuberculosis drug induced hepatotoxicity among TB patients at Dawro zone.

CHAPTER FOUR: METHODS AND MATERIALS

4.1. Study area and period

This study was conducted from 1 May to 30 October 2014 E.C at Dawro zone. Dawro zone is found in southern nations and nationalities and peoples regional government (SNNPRG) Ethiopia. It is located 7^0 N longitude and37.16^0 E latitude. The zone covers 4,814.52 square

kilometer and has a total population of 489,577. Among these 249,263 are men and 240,314 women.The zone isdivided in to 5 woredas: Mareka, Tocha, Essera, Gena and Loma and 1 administrative town (Tercha). It has 5 health centers (Waka, Tocha, Bale,Gessa,Woldehani) and 1 district hospital. Catchment population of Tercha hospital is about 601,904 and it has annual utilization rate of 40,000(Dawro Zone Health Bureau).

4.2. Study design

A prospective cohort study was conducted. Cohorts were all newly diagnosed patients of TB of all forms.

4.3. Population

4.3.1. Source population

TB patients of Dawro zone who enrolled for therapy at 5 health centers and Tercha hospital TB clinic

4.3.2. Sample population

TB patients who were newly diagnosed for TB and had enrolled for DOTS program at 5 health centers and Tercha hospital TB clinic during the study period were the sample population of the study.

4.4. Sample size and Sampling technique

4.4.1. Sample size calculation

Sample size was calculated by using STATCALC calculator of the EpiInfo program to give a total of 124 participants.
Using single proportion
$N=z^2pq/w^2$Where,

N= the desired sample size

Z=the standard normal deviation

P=the proportion of the target population estimated to have particular characteristics

q=1-p

w=degree of accuracy desired (marginal error).

By using the incidence of Anti-TB-DIH, 0.089 (8.9%) [31]. Desired accuracy of 0.05 at 95% confidence interval (z-statistics=1.96), then the sampling size is:

$$n=\frac{1.96^2(0.089)(1-0.089)}{0.05^2}=124$$

4.4.2. Sampling technique

A convenient sampling technique was used to collect data. All newly diagnosed of all forms of TB cases available during the time of data collection were consecutively included in the sample population.

4.5. Eligibility criteria

4.5.1. Inclusion criteria

A total of 124 who were new cases of TB, negative forHBsAg, anti-HCV antibodies and HIV) were included in the study.

4.5.2. Exclusion criteria

Patients who hadALT and AST value greater than 2 times of ULN of reference rangei.e. ULN> 62 U/l, patient positive for HBsAg and patients positive for HIV and retreatment case of TB and patients takingany herbal medicine were excluded from the sample.

4.6. Study variables

Dependent variable

- Anti-TB-drug induced hepatotoxicity (Anti-TB-DIH)

Independent variable

- Age
- Sex
- BMI
- Alcoholism
- Extent of TB disease

4.7. Data collection

Nurses were hired and trained to collect socio-demographic, behavioral and anthropometric (weight, height to yield BMI and arm circumference in children) data using pre-tested structured questionnaire which requires face-to-face interview and reviewing patient's medical record, from

HIV negative patients that were newly diagnosed for tuberculosis and who had signed written informed consent.

I. Blood sample collection

Venous blood samples (5ml) were collected using test tubes that contain separator gels. Whole blood was allowed to Clot for 30 minutes at room temperature andwascentrifuged at 2000rpm for 10 minutes [59]and the serum was separated using nunc tubes for ALT, AST and bilirubin tests.

II. Sample Transportation and storage

Since ALT, AST and total bilirubin are stable in refrigerated sample for 4,4 and 3 days respectively [60,61]. The serum sample was transported from the 5 health centers to Tercha Hospital laboratory within 3 days of collection as the temperature (2 to 8^0c) was maintained using cold boxes.

III. Hepatitis B and C screening

All patients were screened for hepatitis B and C using Rapid Hepatitis B Surface Antigen Test and rapid anti-HCV test respectively. Anti-HCV rapid test is a lateral flow, immunochromatographic assay, screening serum or plasma using recombinant HCV proteins. Recombinant antigens of HCV labeled by gold conjugates are used in test lines as capture materials, and anti-rabbit HCV antibody is used in the control line. When a sample is added to a sample pad of the test strip, it migrates through the membrane strip. If the antibodies to HCV present in the specimen a complex of antibody-gold conjugate d recombinant antigens will be formed which is then captured by antigen immobilized in the test line region of the membrane, producing a visible pink color line of immunocomplex conjugate on the membrane.The hepatitis B surface antigen test uses the same lateral flow, immunochromatographic assay based on the principle of the double antibody-sandwich technique. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of test. During testing hepatitis B surface antigen in the serum or plasma specimen reacts with the particle coated with anti-HBsAg antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a colored line [62]. Patients who were found to be positive for hepatitis B and C test were excluded from the study.

IV. ALT, AST and total bilirubin

Baseline measurement of ALT, AST and total bilirubin was performed before initiation of anti-TB treatment by absorbance photometry using Mindray BS-200E chemistry analyzer machine (SHENZHEN MINDRAY BIO-MEDICAL ELECTRONICS CO., LTD.Mindray Building, Keji 12th Road South, Hi-tech Industrial Park, Nanshan, Shenzhen, P.R.China, 518057, **Phone:**+86 755 26582479 26582888, **Fax:** +86 755 26582500 26582501).Patients who had baseline ALT, AST and bilirubin level beyond normal limits (>2 ULN) were excluded from the study. But patients who had ALT, AST and bilirubin levels within normal limits were included in the study and followedwith biochemicaltests(ALT, AST and bilirubin measured 4 times for each patient) every two weeks for two months andphysical examination was done (only when symptoms of hepatotoxicity were observed).

4.8. Quality control

Questionnaire was translated to Dawro Donna language and data collectors were trained prior to data collection so as to maintain quality of data. Standard operating procedures and manufacturer instructions were strictly followed throughout the procedures. All reagents were prepared according to the manufacturer's instruction. Expiry date of the reagents was checked. Multi Control Sera normal (N) and pathologic (P) which were a lyophilized control based on human serum were used in routine quality control for monitoring accuracy and precision of Mindray BS 200-E measurement.

4.9. Data Analysis

Data was coded, entered and cleaned using statistical software (epidata, version 3.1). Statistical analysis wasperformed using statistical package for social science (SPSS version 20 Chicago Inc.). Mean, standard deviation and frequency of variables was calculated.Bivariate logistic regressionwas calculated to evaluate the possible association of all variables and P-value of less than 0.05 was considered as statistically significant.

4.10. Ethical Consideration

Ethical clearance was obtained from Jimma university ethical review committee and official letter was written to Dawro zone, health bureau. For voluntary participation of

research subjects' informed consent was signed based on explicit information of any possible risk, harm and even discomfort caused by data/sample collection procedures as well as any benefits. Minors were included according to their surrogate consenters. Moreover, issues concerning intervention especially in the case of induced hepatotoxicity were discussed with concerned bodies to continue or discontinue treatment.

4.11.

Dissemination of study results

The finding of this study will be presented to Jimma university department of medical laboratory science and pathology. The study finding will be sent to Dawro zone health bureau. The paper will also be presented on seminars and conferences. It will also be submitted for publication to reputable journals.

CHAPTER FIVE: RESULT

5.1. Demographic and Anthropometric data

During the study period 124 TB patients taking anti-TB drugs participated in the study and were followed for two months. Amongthem 66 (53.2%) were females. Age of the cases range from 10 to 80 years with the mean (±SD) age being 34.5(±15.2 years)but the highest number of participants were found in the age group of 20-49 years, which is 84 (67.7%). The BMI measurement of the participants ranges from 17.08 to 24.78 kg/m2, the mean value being 20.60 ± 1.77kg. The BMI measurement of the majority (119 (96%)) of the participants was within the normal range i.e. 18.5-24.00 kg/m2 [Table 1].

Table 1 Demographic and anthropometric data of study participants in Dawro zone, Tercha hospital and 5 health centers, Southern Ethiopia from 1 May to 30 October.

character		**No (%)**
	Male	58(46.8)
Sex	female	66(53.2)
	total	124(100)
	10-19	20(16.1)
Age(year)		
	20-49	84(67.8)
	>50	20(16.1)
	total	124(100)
	underweight	4(3.2)
BMI(Kg/m^2)		
	normal	119(96)
	Overweight	1(0.8)
	Total	124(100%)

5.2. Clinical and laboratory data of study participants

Out of 124 participants 13 of them were taking different antibiotics during the study period of which 9 (69%) were males and 4 (31%) were females. None of them were reported to be taking paracetamol or other potentially hepatotoxic drugs during the study or 1 month prior to the study period. Among 124 participants 8 (6.5%) were reported to be alcoholics accounting 6 (75%) females and 2 (25%) males. Smear positive pulmonary TB accounted for 99 (79.8%) of all cases and extra pulmonary TB accounted about 25(20.2%).

Table 2 laboratory data (mean+ SD) of patients at Dawro zone, Tercha hospital and 5 health centers, southern Ethiopia, from May 1 to October 30 2014

Laboratory test		**Patients with anti-TB-DIH N=10**	**Patients without anti-TB-DIH N=114**
ALT (U/l)	Baseline	22.70 ± 9.71	23.34 ± 3.67
	During treatment (Peak value)	304.80 ± 93.67	33.90 ± 5.21
AST (U/l)	Baseline	21.60 ± 6.67	27. 74 ± 4.54
	During treatment (peak value)	261.80 ± 66.07	30.13 ± 2.22
Tot. Bilirubin (mg/dl)	Baseline	0.34 ± 0.21	0.45 ± 0.33
	During treatment (peak value)	2.26 ± 0.91	0.65 ± 0.77

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Table 3Laboratory data (mean + SD) based on gender of patients at Dawro zone among Tercha hospital and 5 health centers, southern Ethiopia, from May 1 to October 30 2014.

Laboratory test		**Patients with anti-TB-DIH N= 10**		**Patients without anti-TB-DIH N=114**	
		maleN=4	**female N=6**	**Male N=54**	**female N =60**
ALT (U/l)	Baseline	29.70 ± 11.71	23.11±9.01	24.74 ± 10.67	21.90±9.34
	During treatment (Peak value)	349.80 ±63.67	309±57.45	48.41 ± 16 .81	49.33±18.10
AST (U/l)	Baseline	26.90 ± 12.67	21.32±9.34	23. 54 ± 12.11	24.08±8.13
	During treatment (peak value)	291.40 ±66.07	245.51±31.4	41.45 ± 14.13	43.05± 14.94
Tot. Bil. (mg/dl)	Baseline	0.28 ± 0.19	0.21±0.17	0.32 ± 0.46	0.45±0.28
	During treatment (peak value)	1.96 ± 0.81	1.87±0.67	0.48 ± 0.28	0.71±0.24

10 patients whowere hepatotoxic showed elevated serum concentration of ALT, AST and total bilirubin beyond 3 times of ULN accompanied with symptoms and 5 times of ULNwith or without symptom.Some of them showed total bilirubin concentration greater than 2.4 mg/dl.

Table 4 Cut-off points of liver function tests of ALT, AST and total bilirubin for anti-TB-DIH at Dawro zone Tercha hospital and 5 health centers ,southern Ethiopia, from May 1 to October 30 2014.

parameters	Cut-off value			Patients with DIH N=10
				No (%)
AST(U/l)	>3X ULN + symptoms	male	111	3(30)
		female	93	1(10)
	>5X ULN with/ without symptom	male	185	4(40)
		female	155	2(20)
ALT(U/l)	>3X ULN + symptoms	male	126	1(10)
		female	96	3(30)
	>5X ULN with/ without symptom	male	210	3(30)
		female	160	3(30)
Tot. bilirubin(mg/dl)	>2X ULN	male	2.4	2(20)
		female	2.4	3(30)

Patients were kept under close observation and instructed to report any unusual signs and symptoms(malaise, anorexia, vomiting, nausea, jaundice) immediately. Most of the patients who had developed ant-TB-DIH showed the same sign and symptoms of hepatotoxicity. Treatment was discontinued for some time untilserum concentration of the liver enzymes return to the baseline state or normal reference range.

Table 5 Clinical presentation of anti-TB-DIH among TB patientsat Dawro zone Tercha hospital and 5 health centers, southern Ethiopia, from May 1 to October 30 2014

Sign and symptoms	**Patients with anti-TB-DIH**	
	number	(%)
Vomiting	4	(40)
Jaundice	4	(40)
Anorexia	8	(80)
Nausea	9	(90)
Malaise	6	(60)

10 patients who had developed anti-TB-DIH were followed weekly for 3 weeks with liver function test (AST, ALT & tot. bilirubin) until their liver enzyme level returned to normal or baseline state. Liver toxicity resolved within 21 days (median of 20 days) and continued drug.

5.3. Hepatotoxicity in TB patients taking anti-TB drugs

During the study period, 10 patients taking anti-TB drugs developed hepatotoxicity which is confirmed by clinical examination and liver function test. The time interval from initiation of treatment to the onset of hepatotoxicity was 13 – 58days (median of 26days). Most of the patients 111 (89%) showed elevated level of liver transaminases (>2 x ULN but < 5 x ULN).

Table 6 baseline characteristics of patients with anti-TB-DIH and without anti-TB-DIH in Dawro zone Tercha hospital and 5 health centers, southern Ethiopia from May 1 to October 30 2014

Characteristics		No. of patients	Patients with DIH	Patients without DIH	COR(95%)CI
sex	Male(ref)	58 (46.8%)	4(3.2%)	54(43.5%)	1.3(0.36-5.04)
	Female	66 (53.2%)	6(4.8%)	60(48.3%)	0.74(0.12-2.34)
Extent of disease	Pulmonary(ref)	99 (79.8%)	7(5.6%)	92(74.2%)	1.79(0.42-7.49)
	Extra Pulmonary	25(20.2%)	3(2.4%)	22(17.7%)	0.76(0.11-1.54)
Alcohol status	Alcoholic	8(6.5%)	3(2.4%)	5(4%)	9.343(1.84-47.3)
	Non-alcoholic(ref)	116 (93.5%)	7(5.6%)	109(87.9%)	0.064(0.13-7.5)
age	10-19	20(16.1%)	2(1.6%)	18(14.5%)	0.11(0.4-3.76)
	20-49(ref)	84(67.8%)	7(5.6)	67(54%)	0.818(0.157-4.27)
	>50	20(16.1%)	1(0.8%)	19(15.3%)	0.474(0.039-5.688)
BMI	<18.5	4 (3.2%)	3 (2.4%)	1(0.8%)	0
	18.5-24.99(ref)	119(96%)	7(5.6%)	112(90.3%)	0

Table 7 follow up result of patients who had anti-TB-DIH (N=10) in Dawro zone Tercha hospital and 5 health centers, southern Ethiopia, from May 1 to October 30 2014

test	1st week	2nd week	3rd week
ALT(U/l)	324± 60.10	135.35 ± 24.12	47.34 ± 8.21
AST(U/l)	268± 48.42	107.08 ± 14.76	36.33 ± 7.98
Tot. bilirubin(mg/dl)	1.92± 0.710	1.25 ± 0.53	0.95 ± 0.34

5.4. Factors associatedwith hepatotoxicity

Alcoholism (which is defined as consuming >35 and > 28 units of alcohol per week for at least 10 years for men and women respectively) was found to be predisposing factor for the incidence anti-TB-DIH (COR= 9.343, 95% CI (1.8 - 47.3). according to this study BMI (kg/m2), extent of TB disease, sex and age had no significant association with incidence of anti-TB-DIH.

Table 8 Association of predictors with incidence of anti-TB-DIH in patients taking anti-TB drug at Dawro zone Tercha hospital and 5 health centers, southern Ethiopia from May 1 to October 30 2014.

variables		OR	95% CI	P-value
sex	male	1.3	0.36-5.04	0.65
	female	.0.74	0.12-2.34	0.56
Age (years)	10-19	0.11	0.4-3.76	0.838
	20-49	0.818	0.15-4.27	0.81
	>50	0.474	0.039-5.7	0.556
BMI (kg/m^2)	<18.5	161547162	0	0.999
	18.5-25	11643038	0	0.99
	>25	1454367	0	0.999
Extent Of disease	pulmonary	1.792	0.42-7.4	0.424
	Extra	0.76	0.11-1.54	0.89

	pulmonary				
Alcohol status	alcoholic	9.3	1.8 - 47.3		0.007
	Nonalcoholic	0.064	0.13-7.5		0.92

CHAPTER SIX:DISCUSSION

124 cohorts of newly diagnosed TB patients who were negative for HIV, hepatitis B and C and started taking anti-TB drugs were included in this study. The analysis in this study showed the incidence of anti- TB-DIH to be 8.1%. This incidence is almost similar to previous reports of study in Addis Ababa, St. Peter's TB specialized hospital (8.9%) [31]and reports from Asia (8.0–19.8%)[3,37]. However, this incidence is lower than that from Egypt 15% [2] and higher than that of western world 4.3% [35].The variation in the incidence of anti-TB-DIH worldwide may be attributed to differences in patients' characteristics, indiscriminate use of drugs, and the definition criteriaof hepatotoxicity[59].

According to this study the time interval for the onset of hepatotoxicity after initiation of treatment was 13-58 days (median: 26 days). This is similar to the result reported in Nepal(12–60 days (median: 28 days)) [3]. One study also reported that the onset of anti-TB-DIH to be 15-60 days (median: 30 days) [2]which is similar to the result of this study. But another study [60] reportedthe onset of anti-TB-DIH in almost two thirds of their patients (61.2%) was within 14 days from the start of therapy.

This study showed that history of high alcohol intake was a potential risk factor for anti-TB-DIH ($p<0.007$, OR= 9.3). Similarly, one study[54]reported that history of chronic alcohol intake was common among the cases. Other studies also reported that high alcohol intake as a predisposing factor for anti-TB-DIH[52, 58]. In the contrary, a study report from Dossing et al[62] and study done in Egypt [2]showed that high alcohol intake had no correlation with incidence of anti-TB-DIH. This difference can be explained by the fact that high alcohol intake as a predisposing factor for anti-TB-DIH has been considered as the most equivocal. However, according to some study report higher alcohol consumption as a risk factor was ascribed to malnutrition and glutathione store depletion [61].

Several studies reported that old age is a potential risk factor for anti-TB-DIH[3, 5,37]. A study done in Egypt [60] reported that older age group was affected more than the younger age group. In contrast, a study done in Nepal [3] reported that the incidence of anti-TB-DIH was higher in younger

patients. The current study showed that there was no correlation between age and anti-TB-DIH. In agreement to this, another study reported that age had no significant relation to anti-TB-DIH[49]. The discordance between our finding and the study done in Nepal and Egypt may be explained by the fact that the age categorization for young and old people is different.

Several studies suggested that female gender is independent predictor of anti-TB-DIH [3, 39].However, recent report suggested that male has higher risk of developing anti-TB-DIH[64].The reason for female susceptibility was believed to be variations in pharmacokinetics and slower acetylation status[63]. However, gender showed no correlation with anti-TB-DIH in this current study. This difference may be explained by the fact that females are slow acetylaters and INH is cleared by acetylation. So, females in our study might have developed anti-TB-DIH by anti-TB drug other than INH. Some other studies are in agreement with this current study finding[2, 53].

Extent of TB disease or involvement of extra pulmonary organ had no significant association with incidence of anti-TB-DIH according to the current study. In congruent to this, another study reported that there was no significant association between extent of TB disease and incidence of anti-TB-DIH[65]. However, extra pulmonary organ involvement was reported to be associated with incidence of anti-TB-DIH in studies from India [55, 56]. This difference may be attributed to the fact that extra-pulmonary TB may not necessarily indicate severity of the disease.

In this study malnutrition as assessed by BMI <18.5kg/m^2had no significant association with anti-TB-DIH. Despite, some studies from Nepal [3], Spain [48] and India [52,56] showed that malnourishment had significant association with incidence of anti-TB-DIH.This might be due to depletion of glutathione stores, which makes patients more vulnerable to oxidative injuries. The reason for the deviation of our finding may be explained by the fact that the majority of patients included in our study were not malnourished.

In this study, since there was no severe hepatotoxicity no death was recorded. During the study period 100% of patients who developed anti-TB-DIH had their transaminase level below 10X of ULN. Patients having signs and symptoms suggestive of hepatotoxicity were put under close follow up and had their liver function tests monitored and physical examination done regularly. For confirmed hepatotoxic cases anti-TB drugs were discontinued for some time until it was normalized. Fortunately, all of the cases recovered after few days and continued treatment.

Thoughas a result of meta- analysis, incidence rate of hepatotoxicity was shown to be high with INH followed by PZA and RIF [35], Tuberculosis patients who were included in the current study were taking combination of four anti-TB drugs: Isoniazid (INH), Rifampin (RIF), Pyrazinamide (PZA), and Ethambutol (EMB). Therefore, it was difficult to infer which drug was responsible for the cause of hepatotoxicity. The role of N-acetyltransferase 2 gene/enzyme polymorphisms on the metabolism of INH plays role on susceptibility to anti-TB-DIH [5].Butwe could not determine genetic polymorphism because of resource in this study. Most of the time anti-TB-DIH was expected to happen during initial phase (the first 2 months) of treatment though; it may develop during continuous phase. In this current study, liver function test for monitoring of TB patients was done only for initial phase of treatment. We couldn't get enough financial and time resource to follow the patients during their continuation phase and thus unable to describe the incidence during this phase.

CHAPTER SEVEN: CONCLUSIONAND RECOMMENDATION

7.1. Conclusion

This study determined the incidence (8.1%) of idiosyncratic type of anti-TB-DIH which was probably unknown in the area and assessed the possible risk factors using the concentration of ALT,AST and total bilirubin in patient's serum. Patients who had their serum ALT and or AST level > 5X upper limit of normal (ULN), bilirubin level > 3X ULN were considered to have developed anti-TB-DIH. Hepatotoxicity developed within the first two months after initiating treatment. Chronic high alcohol consumers had increased risk of developing anti-TB-DIH rates. However, according to this study there was no significant association between ages, sex, extent of TB disease, BMIof patients with the incidence of anti-TB-DIH.

7.2. Recommendation

As tuberculosis continues to remain a significant infectious disease across much of the developing world, determining the incidence of anti-TB-DIH and identifying higher risk groups should be important task to prevent the disease. Difference in population characteristics, type of regimen used and definition criteria of anti-TB-DIH posed challenge in predicting the incidence worldwide. However, making consensus on the definition criteria and using similar regimen should be done to precisely predict the incidence. Researchers should perform meta-analysis in order to identify the drug that exactly causes hepatotoxicity so that the agent could be withdrawn to avoid further injury.

Population characteristics that determine the susceptibility of patients to anti-TB-DIH should be identified and controlled. In this regard, this study had shown that hepatotoxicity of liver due to anti-TB drugs was higher among those who chronically consume alcohol. So, patients taking anti-TB drugs should quit drinking alcohol. Most of the time hepatotoxicity develops within the initial phase of the treatment. However, frequent and close follow-up of patients should be done both in intensive and continuation phase of treatment in our laboratory so that early diagnosis and immediate withdrawal of the causative agent could be ensured to prevent progression.

In general the incidence of anti-TB-DIH varies across different regions and most of the risk factors identified were not similar. However, researchers should continue determining the incidence and assess the risk factors until they get steadfast. Furthermore, the finding of this study can provide baseline information for researchers of the same interest and government bodies, stakeholders and policy makers to intervene in drug formulation and recommend treatment monitoring.

Annexes

Annex I: References

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Annex II:Standard Operating Procedures

Venous blood collection

1. All the equipment which are needed for the procedure were collected and placed within safe and easy reach on a tray or trolley.
2. Patients were identified and prepared.
3. Antecubital fossa of the forearm was selected locating the vein that is visible, straight and clear and tourniquet was applied about 4–5 finger widths above the veni-puncture site.
4. The entry site was disinfected using 70% alcohol.
5. Take blood. By inserting the needle in the vein swiftly at a 30 degree angle or less, and draw sufficient and withdraw the needle.
6. The laboratory sample tubes were filled with blood.
7. Needles and syringe were disposed in a safety box container prepared for sharp objects.

Serum preparation

1. Venous blood specimen was obtained in a testtube containing separator gel.

2. The specimen was placedvertically in a test tube rack as this will speed up the clotting action.

3. When the clotting had completed, the specimen was centrifuged according to centrifuge manufacturer's recommendations at 2000 rpm's for 10 minutes.

4. Serum was separated from the red cells to avoid test interference and transferred to nunc tubes.

ALT (Alanine Aminotransferase)

Clinical significance

Alanine aminotransferase (ALT), formerly called Glutamic Pyruvic Transaminase (GPT), is one of liver-specific enzymes. It can catalyze the interconversion of amino acids and a-ketoacids by transfer of amino groups. Elevated ALT levels can indicate myocardial infarction, muscular dystrophy, especially in hepatobiliary diseases. Measurement of ALT is often used in diagnosis

and monitoring treatment of liver diseases and heart diseases. The AST/ALT ratio is often used for differential diagnosis in liver diseases: if the AST/ALT ratio < 1, it indicates mild liver damage; otherwise it is associated with severe, often chronic liver diseases.

Method

UV-assay according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) without pyridoxal phosphate activation, Mindray BS 200E chemistry analyzer.

Reaction Principle

Alanine aminotransferase catalyzes the reversible transamination of L-alanine and a-oxoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of reduced ß-nicotinamide adenine dinucleotide (NADH) to ß-nicotinamide adenine dinucleotide (NAD). This change in absorbance is directly proportional to the activity of ALT in the sample.

ALT

a-oxoglutarate + L-alanine ----------→ L-glutamate + pyruvate

←----------

LDH

pyruvate + NADH + H------ ----------→ L-lactate + NAD

←----------

Reagent Components and Concentrations

R1

- TRIS buffer (150 mmol/L)
- L-Alanine (750 mmol/L)
- LDH (1200 U/L)

R2

- a-oxoglutarate (90 mmol/L)
- NADH (0.9 mmol/L)

Storage and Stability

Stable up to expiry date indicated on the label, when stored unopened at 2^0C-8^0C and protected from light. Once opened, the reagent is stable for 28 days when refrigerated on the analyzer or refrigerator.

Assay procedure

1. Prepare
   Reagent 1 (1000 µL) blank (1000 µL) sample
Dist water (100 µL)
   Sample (100 µL)
2. Mix, incubate for 5 min, then add:
   Reagent 2 (250 µL) (250 µL)
3. Mix thoroughly, read the absorbance after 1 min and monitor time. Read the absorbance again for additional 3 min.

Reference Intervals

Male (0-42 U/L)
Female (0-32 U/L)

AST (Aspartate Aminotransferase)

Clinical significance

Aspartate aminotransferase (AST),formerly called Glutamic Oxalacetic Transaminase (GOT), is present in both cytoplasm and mitochondria of cells,belonging to the transaminase family, which catalyze the conversion of amino acids and a-oxoglutarate by transfer of amino groups. AST is commonly found in various human tissues. The heart muscle is found to have the most activity of the enzyme, secondly in the brain, liver, gastric mucosa, skeletal muscle and kidneys. The serum AST present low activity in the healthy human body, but when these tissues injury or damage, AST is released into blood and results in high blood AST activity. Measurement of AST in serum and plasma is mainly used for the diagnosis of heart muscle damages, liver damages and skeletal muscle diseases as well as for monitoring the treatment. The AST/ALT ratio is often used for differential diagnosis in liver diseases. While the ratio < 1, it indicates mild liver damage, otherwise it is associated with severe, often chronic liver diseases.

Method

UV-assay according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) without pyridoxal phosphate activation, Mindray BS 200E chemistry analyzer.

Reaction Principle

In the assay reaction, the AST catalyzes the reversible transamination of L-aspartate and a-oxoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with NADH being oxidized to NAD The rate of the photometrically determined NADH decrease is directly proportional to the rate of formation of oxaloacetate and thus the AST activity.

a-oxoglutarate + L-aspartate -------AST---------→ L-glutamate + oxaloacetate

←----------

MDH

oxaloacetate + NADH + H------ ----------→ L-malate + NAD+

←----------

Reagent Components and Concentrations

R1

- TRIS buffer (100mmol/L)
- L-Aspartate (750 mmol/L)
- MDH

R2

- a-oxoglutarate (60 mmol/L)
- NADH (0.9 mmol/L)

Storage and Stability

Stable up to expiry date indicated on the label, when stored unopened at 2^0C-8^0C and protected from light. Once opened, the reagent is stable for 28 days when refrigerated on the analyzer or refrigerator.

Assay procedure

1. Prepare
   Reagent 1 (1000 µL) blank (1000 µL) sample

Dist water (100 µL)

Sample (100 µL)

2. Mix, incubate for 5 min, then add:
   Reagent 2 (250 µL) (250 µL)
3. Mix thoroughly, read the absorbance after 1 min and monitor time. Read the absorbance again for additional 3 min.

Reference Intervals

Male 0-37 U/L)

Female (0-31 U/L)

Bil-T (Bilirubin Total)

Clinical significance

80-85% bilirubin is breakdown product of hemoglobin, and 15-20% bilirubin roots in proteins containing hemoglobin. Measuring the bilirubin in plasma is used for diagnosing and discriminating the reason of jaundice. Hyperbilirubinemia is due to the excess increase of bilirubin and may cause by prehepatic jaundice (e.g. hemolysis), intrahepatic jaundice (e.g. virus

hepatitis) or post-hepatic jaundice (e.g. gall-stone). Some chronic and congenital diseases can also result in Hyperbilirubinemia.

Method

Diazotized Sulfanilic Acid (DSA) Method

Reaction Principle

By using a special surfactant to accelerate the solubility of conglutinated bilirubin, total bilirubin with diazo salt at an acid condition to form a red product of azobilirubin. The absorbency increase is directly proportional to the concentration of bilibrubin.

Bilirubin + Diazo salt----------→Azobilirubin
←----------

H
+
Surfactant

Reagent Components and Concentrations
R1

- HCl (100mmol/L)
- Sulfanilic acid (5 mmol/L)
- surfactant (1% m/v)

R2

- sodium nitrite(72 mmol/L)

Storage and stability
Up to expiration date indicated on the label, when stored unopened at 18-25^{0}c and protected from light. Once opened, the reagents are stable for 28 days at 18-25^{0}c. And the working solution is stable for 14 days when refrigerated on the analyzer or refrigerator.

Assay procedure

To prepare the working solution, mix 4 parts R1 with 1 part R2, e.g. 4 mL R1 + 1 mL R2

1. Prepare
   Reagent 1 (1000 µL) blank (1000 µL) sample

Dist water (100 µL)

Sample (100 µL)

2. Mix thoroughly at 37^0c then read the absorbance after 10 min later.

Reference Intervals

Serum / Plasma - Adult (0.1-1.2 mg/dL)

Anti-HCV Rapid Test

Test principle

Anti-HCV rapid test is a lateral flow, immunochromatographic assay, screening serum or plasma using recombinant HCV proteins. Recombinant antigens of HCV labeled by gold conjugates are used in test lines as capture materials, and anti-rabbit HCV antibody is used in the control line. When a sample is added to a sample pad of the test strip, it migrates through the membrane strip. If the antibodies to HCV present in the specimen a complex of antibody-gold conjugate d recombinant antigens will be formed which is then captured by antigen immobilized in the test line region of the membrane, producing a visible pink color line of immunocomplex conjugate on the membrane. The color intensity depends on the concentration of the anti-HCV present in the sample. Absence of the test line suggests a negative result. The test contains an internal control which should exhibits a pink colored line of the immunocomplex conjugate regardless of color development on the test line. Otherwise the test result is invalid and the specimen must be retested with another test strip.

Reagent and material provided

50X Tests. Each test strip is sealed in a foil pouch with a package of desiccant.

50X 25micro liter specimen dropper

2X Assay buffer

1X Instruction for use

Clean container

Timer

Storage and stability

Store the kit in cool and dry places at a temperature between 2-30^{0}c. Do not freez. The shelf life of the kit under this condition is 24 months.

Assay procedure

Test device, patient's sample and controls should be brought to room temperature (15-30c) prior to testing. Do not open pouches until ready to perform the assay.

1. The test strip was removed from its protective pouch and labeled the strip with patient or specimen number.
2. Using the dropper/ pipette 1 drop (about 25 micro liters) was added on to the sample pad. Then 2 drops (about 100micro liter) assay buffer were added as well.
3. Start the timer and wait for the red lines to appear. The result should be read at 10-20 minutes. Result read after 20 minutes is invalid.

Interpretation of result

Negative result: only control line appears red

Positive result: both control and test line appears red

Invalid result: both line do not appear

Rapid Hepatitis B Surface Antigen Test (HBsAg)

Principle

The hepatitis B surface antigen test is a lateral flow, immunochromatographic assay based on the principle of the double antibody-sandwich technique. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of test. During testing hepatitis B surface antigen in the serum or plasma specimen reacts with the particle coated with anti- HBsAg antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result.

Reagent and material provided

Package insert

Test cassette

Desiccant

Pipette dropper

Instruction for use

Clean container

Timer

Storage and stability

Store the kit in cool and dry places at a temperature between 2-30c. Do not freeze. The shelf life of the kit under this condition is 24 months.

Test procedure

1. The test strip was removed from its protective pouch and labeled the strip with patient/specimen number.
2. The test cassette was placed on a clean and level surface. Holding the dropper vertically, 2-3 full drops of serum were transferred.
3. Wait the colored line to appear. Read results in 15 minutes. Do not interpret the result after 15 minutes.

Interpretation of result

Negative result: only control line appears red

Positive result: both control and test line appears red

Invalid result: both line do not appear

Annex III: Information sheet (English version)

This information sheet is prepared for newly diagnosed TB positive subjects. The detailed explanation about what will be undertaken in the study is presented as follows and it is after reading the description that informed consent will be obtained.

Title of the project: the incidence of anti-tuberculosisdrugs-induced hepatotoxicity and risk factors among TB patients in Dawro zone.

Name of Principal Investigator: WondwossenAbera

Organization: Jimma University (Medical laboratory science and pathology department)

Name of sponsor: Jimma University

Description and Purpose of the study

Tuberculosis continues to remain a significant infectious disease across much of the world. The drugs that are used to treat TB found to be potentially hepatotoxic in some individuals causing a disease condition known as anti-TB drug induced hepatotoxicity. And there are some risk factors that are associated with Anti-TB-DIH. The aim of this study is to determine incidence of anti-TB-DIH and evaluate risk factors.

Procedures

Following your willingness you are asked to sign a consent form and the following procedures will be undertaken

- ✓ Your medical history will be reviewed
- ✓ You will provide us 10 minutes interview
- ✓ Blood sample will be collected(5ml) every two weeks for at least two months.
- ✓ The sample will be analyzed for LFTs

Risks and discomforts

During sample collection we will follow Standard operational procedures. The blood drawing may cause minor pain, at the place where blood is taken. However, this pain will disappear in few hours.

Benefits

There is no direct financial benefit you get by participating in this study but the test result will be delivered timely and appropriate intervention will be pointed.

Confidentiality

Any information obtained during this study will be kept confidential. This is assured by avoiding use of any identifier and information will be recorded with code number.

Voluntary participation

Participation on this study is voluntary and you have the right to refuse participation at any time.Your decision will not result in any penalty or loss of benefits to which you are entitled. Your decision will not put at risk any present or future medical care or other benefits to which you otherwise entitled. You may ask questions now and in the future if you do not understand something that is being done. For the success of our study, we will be asking you to give the correct answer for the respective questions. Thank you for your assistance. Continue answering the questions.Here are addresses of individuals who you can contact:

WondwossenAbera, phone no- +251916867428, Email address- aberawondwossen@yahoo.com

Dr. GemedaAbebe, phone no- +251911991285, Email address- Gemeda.abebe@ju.et

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Annex IV:<u>የጥናቱተሳታፊዎችመረጃቅፅ (የአማርኛግልባጭ)</u>

የጥናቱርዕስ፡የጥናቱተሳታፊዎችመረጃቅፅ(የአማርኛግልባጭ)
የጥናቱርዕስ፡ በፀረ፡ቲቢመድሃኒትአማካኝነትየሚከሰተዉንየጉበትመመረዝአደጋሊያስከትሉየሚችሉስጋቶችመመዘን
የዋናተመራማሪስም:ወንድወሰንአበራ
የድርጅቱስም፡-ጂማዩኒቨርሲቲ
ድጋፍሰጭተቋም:
ይህየመረጃቅፅየተዘጋጀውከላይበተጠቀሰው ጥናት ለሚሳተፉየቲቢህሙማንሲሆንበአጠቃላይበጥናቱውስጥልናካሂዳቸውስለፈለግናቸውጉዳዮችእናስለጥናቱጠቅላላማብራርያይሰጣል::በመሆኑበጥናቱየሚሳተፉትበራስዎፍላጐትብቻመሆኑንበትህትናእንገልፃለን፡፡

<u>የጥናቱአላማ</u>

የቲቢበሽታበዓለምላይዕጅግብዙሰዎችንየሚያጠቃሲሆንበሽታውንለመከላከልየሚሰጡመድሀኒቶችበአንዳንድምክንያትየጉበት መመረዝዐደጋሊያመጡይችላሉ።የዚህጥናትዐላማእነዚህንምክንያቶችለይቶዐቅማቸውንመመዘንነው።

<u>የጥናቱሂደትዝርዝር</u>

በጥናቱለመሳተፍከተስማሙየሚከተሉትንመረጃዎችናናሙናእንወስዳለን፡

- የህክምና :: እንዲሁምከራስዎአንደበት የ5 ደቂቃቃለ-መጠየቅይደረግሎታል፡:
- 5 ሚሊሊትርያክልየደምናሙናይወስዳል፡:
- የተወሰደዉናሙናአስፈላጊዉምርመራይደረግበታል።

<u>ስጋትናጉዳት</u>

ህክምናውየሚያስገድደውንየአሰራርሂደትስለምንከተልሊያጋጥሙየሚችሉየህመምስሜትበጣምአነስተኛነዉ ::
ቢሆንምየደምናሙናበሚወስድበትጊዜትንሽየህመምስሜትሊያጋጥምይችላል፡፡ነገርግንይህህመምበአጭርጊዜይጠፋል፡፡

ሊያስገኛቸውየሚችሉትጥቅሞችእናየካሳክፍያ

በዚህጥናትውስጥበመሳተፍዎበጥሬገንዘብየሚደረግየካሳክፍያአይኖርም::ነገርግንየምርመራዉዉጤትበወቅቱየሚሰጥሲሆንበም ርመራዉዉጤትመሰረትላስፈላጊዉየህክምናእርዳታይጠቆማል።

የጥናቱምስጢራዊነት

ማንኛውምበጥናቱ የሚገኙመረጃዎችበምስጢርይጠበቃሉ። የጥናቱመረጃዎችበሙሉየሚቀመጡትከእርሶስምጋርሳይሆንለጥናቱተብሎበሚሰጠውስውርቁጥርሲሆንጥናቱንከሚያካሂዱትባ ለሙያዎችበስተቀርማንምሊያውቅአይችልም፡; የእርስዎንማንነትበሚገልጥመልኩየተዘጋጀውንመረጃበፌርማዎ የተረጋገጠፍቃድሳናገኝይፋአናደርግም:፡

ይህጥናትሳይንሳዊመረጃእንደመሆኑመጠንበወረቀትታትሞቢወጣወይንምበሚድያቢነገርየእርስዎ ስም በምንምመልኩአይጠቀስም፡:

ያለመቀበልወይምጥናቱንየማቋረጥመበት

በዚህጥናትውስጥየሚኖርዎትተሳትፎሙሉበሙሉፈቃደኝነትላይየተመሰረተይሆናል:፡

በማንኛውምጊዜይህንንጥናትየማቋረጥመብትዎሙሉበሙሉየተጠበቀነው::

በጥናቱባለመሳተፍዎወይምከጥናትበመገለልዎምክንያትበአሁኑወይምየወደፊትየህክምናእርዳታላይተፅዕኖአይኖረውም፡

ከዚህበፊትሲያገኙከነበሩትጥቅሞችአንዳችነገርአይጐሎቦትም፡

ጥናቱንየሚያከናውነውአካልወይምድጋፍሰጭአካልከእራስዎጥቅምሲባልበጥናቱእንዳይሳተፉሊከለከልይችላል::

ጥያቄካለወት

ስለጥናቱማንኛውንምጥያቄወይምእርስዎበዚህጥናትውስጥለሚኖርዎትድርሻ፣

አሳሳቢጉዳትወይምቅሬታካለዋትየሚከተሉትንስልኮችወይምኢሜልአድራሰመጠቀምየጥናቱንባለቤቶችማነጋገርይችላሉ::

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ለጥናታችንስኬትትክክለኛዉንመረጃእንዲሰጡበኣክብሮትእንጠይቃለን።

Annex V: Patient consent form (English Version)

Participant Code Number_____

Participant full name _____

I am informed fully in the language I understand about the aim of above mentioned research. I understood the purpose of the study entitled with" Determining the incidence rate of anti-tuberculosis drugs induced hepatotoxicity and assessing the risk factors in Dawro zone at Tercha district hospital"I have been informed that medical history, blood samples will be taken and there will be minimal risk during sample collection. In addition I have been told all the information collected throughout the research process will be kept confidential. I understood my current and future medical services will not be affected if I refused to participate or with draw from the study.

_____Agree Not agree_____

Therefore I give my consent freely for my participation in this study.

Patient Name _____ signature _____ Date_____

Investigator name _____signature _____Date_____

Witness

1. Name _____signature _____date _____
2. Name _____signature _____date_____

Annex VI: የስምምነትቅፅ (የአማርኛግልባጭ)

የተሳታፊውልዩመለያቁጥር _____

የተሳታፊውስም _____

እኔስሜከላይየተጠቀሰውግለሰብበፀረ፡ቲቢመድሃኒትኣማካኝነትየሚከሰተዉንየጉበትመመረዝኣደጋሊያስከትሉየሚችሉስጋቶች መመዘንበሚልርዕስበታሰበውምርምርላይበሚገባኝቋንቋበቂመረጃአግኝቻለሁ፡፡የህክምናመረጃናየደምናሙናምንምኣይነትጉዳ ትበማያደርስመልኩእንደሚወሰድተረድቻለሁ፡፡በተጨማሪምየሚወሰዱማናቸውምመረጃዎችበሚስጥርእንደሚያዙተነግሮኛል

::እንድሁምየምጠየቀውንመረጃያለመስጠትናለጥናቱያለመሳተፍከጥናቱበማናቸውምወቅትራሴንማግለልእንደምችልየተገለፀልኝሲሆንይህንንምበማድረጌወደፊትምሆነአሁንየማገኛቸውየህክምናግልጋሎቶችእንደማይጓደሉብኝበመረዳት

_____እስማማለሁ _____አልስማማም

በመሆኑምለዚህምርምርለመሳተፍወስኛለሁ::

የታካሚ/ የተሳታፊስም _____ፊርማ ----------ቀን ----------

የተመራማሪውስም ----------ፊርማ ----------ቀን ----------

ምስክሮች

1.ስም ----------ፊርማ ----------ቀን ----------

2.ስም ----------ፊርማ----------ቀን----------

Annex VII: Questionnaire

For TB "positive"

- ✓ HIV positive Individuals and retreatment cases are not included.

Date___/___/__

Card No_____ Code no _____

Information from the participant

1. Sex ☐ Male ☐ Female
2. Age_____
3. Weight ----------
4. Height ----------
5. Do you drink alcohol Yes☐ no ☐
6. If yes how long have you been drinking? ----------How much unit per day?--------
7. How often do you drink alcohol 7/week ☐ 3/week?☐1/week?☐

8. Have you done physical exercise today yes ☐ no ☐

9. Had you been treated for TB previously yes ☐ no ☐

Information from patient record

i. Extent of TB disease ☐ PTB ☐ Extra-pulmonary TB

ii. Pre-existing liver disease yes ☐ no ☐

iii. Muscle injury yes ☐ no ☐

Follow up information

10. Any sign or symptom

- Nausea ☐
- Anorexia ☐
- Jaundice ☐
- Abdominal discomfort ☐
- Malaise ☐
- Hepatomegally ☐
- Vomiting ☐
- Others ☐

11. Baseline and follow up assessment value

Lab. Test	**baseline**	**2nd week**	**1st month**	**6th week**	**2nd month**
AST (IU)					
ALT (IU)					
Tot. bilirubin (mg/dl)					

12. Any drug or cultural drug taken other than anti-TB drugs ----------

13. When (if yes) ----------

Annex VIII: Ooyyiishsha

Echchaybiyyanadee'yaaassaoyiisheetenna

Qaaniyya___/___/__

Karddiyyaqutturiyya_____ kooddiyyaqutturia _____

Haarggaanchaoddua

1. Maacca☐attumma☐

2. Yeellettoolaayiita_____

3. Deetstsuwwa----------

4. Geessaa ----------

5. Harraqqiyaushaay? A ushaay☐Ushikke☐

6. Ushaaygoopeappunwoodeegiddeedde? ----------gaalaassanappunmaalakkiyaushaay?----

---

7. Saamminttaanappuntarraaushaay? 7/saamintta?☐ 3/saamintta ?☐1/saamintta?☐

8. Haachchiisspoorttiyaaottaadi ?Aottas☐ottabaykke☐

9. Haawwaapekoyyirro TB haarrggiyaasaakkettaerraay? A saakettaddi☐saakettabayke☐

Kaardiyaabollandee'yaahaarrgganchchamarrajjaa

i. TB haarrggiyaaayyinaattiyaaGooffinniyaa TB ☐boollaa TB ☐

ii. HawwappeeKooyyirrogubbaattahaarrggiyaade'ee☐baawwaa☐

iii. Bollaaanmaassuunttayde'ee? De'ee☐bawwaa☐

TB xaalliyyaadommowodeepppede'eeyyasaakkuwwaa

10. Siiyyettiyyaasaakkuwwa/maalatta

- Biiccoyii ☐
- Quummaadiggii ☐
- Ayyffiiyyaabiiccaatti ☐
- Ulluwwaasaakkii ☐
- Laabbaantstsi ☐

- Ulluuggaaffii ☐
- Ccooshshii ☐
- Hharraasakkuwwaa ☐

11. Ddoomettaanneeguuyyeende'eeyyaalaabbrraattorriyyaawuuxxeettiyyaa

Lab. Test	**ddoometta**	**2tosaamintta**	**1roagginnaa**	**6tosaminttaa**	**2tosamintta**
AST (IU)					
ALT (IU)					
Tot. bilirubin (mg/dl)					

12. Tiibbiyyaapehaarraaxaalliyyaaekkaaddii?

13. Awwuddee? ----------

Annex IX: Declaration sheet

We, the undersigned, agree to accept responsibility for the scientific ethics and technical conduct of the biomedical research and for the provision of required progress reports as conditions of your institution in effect of grant provision. Moreover, all investigators will assure to guarantee the safety and proper care of the study participants.

Principal investigator

WondwossenAbera (BSc, MSc candidate; Jimma University)

Signature _____ Date_____

First Advisor

This thesis has been submitted with my approval as university advisor

Dr. GemedaAbebe (PhD)

Signature _____ Date_____

Second advisor

This thesis has been submitted with my approval as university advisor

Mr. WaqtolaCheneke (BSc, MSc)

Signature_____Date_____

Examiner

This thesis has been submitted with my approval as internal examiner

Mr. WondimagegnAddisu(BSc, MSc)

Signature _____Date_____