ADDIS ABEBA UNIVERSITY
COLLEGE OF HEALTH SCIENCE
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DRUG INDUCED HEPATOTOXICITY AND ANEMIA AMONG TB INFECTED CHILDREN WITH AND WITHOUT HIV ATTENDING SOME RANDOMLY SELECTED GENERAL HOSPITALS IN TIGRAY, ETHIOPIA.

EFRIEM GEBREYOHANNES TESFAY

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE DEGREE OF MASTER OF SCIENCE IN MEDICAL BIOCHEMISTRY.

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This is to certify that the thesis prepared by Efriem Gebreyohannes titled: Drugs induced hepatotoxicity and anemia among TB infected children with and without HIV attending some randomly selected general hospitals; in Tigray, Ethiopia and Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Biochemistry complies with regulation of the university and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner _____ Sign_____ Date_____

Advisor:- Dr. Solomon Genet Sign_____ Date_____

Advisor:- Dr. Menakath Menon Sign_____Date_____

Dedication

TO
ARARSA BEKELLE

Efriem Gebreyohannes

Declaration

I declare that this research paper titled "Drug induced hepatotoxicity and anemia among TB infected children with and without HIV attending some randomly selected general hospitals in Tigray, Ethiopia is my original work and has not been submitted for a degree in any other university, and that all sources of materials used for the research have been properly and suitably acknowledged.

Efriem Gebreyohannes

Signature_____

Date_____

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ABBREVIATIONS

AFB	Acid Fast Bacilli
AIDS	Acquire immunodeficiency syndrome
ALT	Alanine aminotransferase
ART	Antiretroviral therapy
AST	Aspartate aminotransferase
ATLI	Anti-TB drug Liver Injury
ATT	Ant-Tuberculosis Treatment
DILI	Drug Induced Liver Injury
DOTS	Directly Observed Treatment Short Course
EPTB	Extra pulmonary TB
Hgb	Hemoglobin
HIV	Human immunodeficiency virus
INH	Isoniazid
LTBI	Latent Tuberculosis Infection
MDR	Multi-drug resistant TB
MOH	Ministry of Health Organization
MTB	*Mycobacterium tuberculosis*
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
NTP	National Tuberculosis Control Program
PCF	Passive Case Finding
PIs	Protease Inhibitors
PLWHA	People living with HIV/AIDS
PTB	Pulmonary TB
RIF	Rifampicin
TB	Tuberculosis
TRHB	Tigray Region Health bureau
WHO	World Health Organization

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ABSTRACT

Background: Drugs available to treat diseases are not without problems and the same is true for drugs of Tuberculosis and Human Immunodeficiency Virus infections. Drug induced hepatotoxicity is the major serious adverse drug reactions encountered during the treatment of these diseases. Anti tuberculosis drugs are very well known causes of drug-induced liver injury. Ethiopia is highly afflicted by the TB pandemic and is ranked 14th among the high Tuberculosis burden countries worldwide implying that large amount of drugs are used annually to treat these diseases which are capable of inducing liver injury. Anemia is also another aberration in such chronic diseases that may be induced by these drugs. Thus, this study focused on assessment of drug induced hepatotoxicity and anemia among Tuberculosis infected children.

Methodology:- Institutional based comparative cross-sectional study was conducted from May-November, 2019 at Maychew, Adigrat, Mekelle, and Axum general hospitals. A total of 188 participants (94 Tuberculosis infected children and 94 control groups) were recruited and blood samples were taken for Liver function and hemoglobin test to assess drug induced liver injury and anemia among the Tuberculosis infected children.

Result: Among the participants, 54.3% male and 45.7% female TB infected children and there was 58.5% male and 41.5% female in the control group. The mean age of Tuberculosis infected children was 9.46±(4.249), whereas the mean age of the control group was 8.35±(4.285) years. The mean serum enzymatic levels were elevated among portion of TB infected children (i.e. Aspartate aminotransferase 59/94(62.7%), Alanine aminotransferase 41/94(43.6%); and Total Bilirubin 37/94(39.4%)). Drug induced hepatotoxicity 8/94(8.5%) and anemia 29/94(30.9%) were observed in tuberculosis infected children. In this study only 11/94(11.7%) of the control group were anemic.

Conclusion: ALT, AST and Bilirubin in this study were significantly increased among Tuberculosis infected children and TB-HIV co-infected children when compared to control group and TB only infected children respectively. Whole blood hemoglobin concentration were significantly decreased in Tuberculosis infected children compared to control group. The Anti-Tuberculosis drugs seem to induce hepatotoxicity and anemia over a time of period.

Key Words: drugs induced Hepatotoxicity, Tuberculosis, Anemia, HIV/AIDS

1. INTRODUCTION

1.1. BACKGROUND

Tuberculosis (TB) is a respiratory disease which is a major public health problem across the world caused by different *Mycobacterium* species. Millions of people become sick every year and TB is ranked as the second most cause of death from an infectious disease worldwide (Seddon and Shingadia, 2014).

Globally, an estimated 10.0 million people fell ill with TB in 2018, a number that has been relatively stable in recent years. There were an estimated 1.2 million TB deaths among HIV-negative people in 2018 and an additional 251 000 deaths among HIV positive people. TB affects people of both sexes in all age groups but the highest burden is in men (aged ≥15 years), who accounted for 57% of all TB cases in 2018. By comparison, women accounted for 32% and children (aged <15 years) for 11%. Among all TB cases, 8.6% were people living with HIV (WHO, 2019).

There is difficulty in defining global epidemiology of childhood tuberculosis since there is ongoing transmission of TB and the difficulty was more in African countries. Ethiopia is one of the African counties with the same difficulty and according to the WHO estimate, TB case-detection rate was 60% with 40% case detection gap; which means that every year 80,000 TB cases remained un-diagnosed. However, the share of children of age less than 15 years old was expected to be big (Reves and Angelo, 2016).

According to WHO data, almost one million children had tuberculosis infection each year and 210,000 children lost their life during the course of illness (WHO, 2017).

About 233,000 children died from TB worldwide in the next year, which translates to 13% of total deaths in children. WHO report ranked Ethiopia 14th worldwide with regard to the global TB burden; 117,705 cases were reported in 2017, with an estimated incidence rate of 164 cases per 100,000 population per year. Of the reported cases in 2017, about 20,000 were in children under 15 years (WHO, 2018).

Management of childhood TB poses challenges to low- and middle-income countries. Morbidity and mortality are high enough to make this one of the three primary infectious diseases related to poverty (Seddon and Shingadia, 2014). A series of seminal TB treatment studies conducted by the British Medical Research Council defined the current 4-drug treatment regimen used as first-line drugs, which include a 2-month intensive

phase using isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E), and a 4-month continuation phase using isoniazid and rifampicin (Hong Kong Chest Service/British Medical Research Council, 1991). Where possible combination tablets are used to improve adherence and reduce bacterial resistance (Dheda *et al.*, 2016).

These treatments are not without any complication rather they are capable of inducing adverse drug reactions such as drug-induced liver injury (DILI) to first-line TB drugs which can be severe and may lead to death if not managed properly.

Drug Induced Liver Injury (DILI) is one component of the adverse effects of drugs. Its definition in this study is taken Hy's rule, that defines DILI as a serum ALT level greater than or equal to 3 times the UNL plus a serum bilirubin level greater than or equal to 2 times the UNL and this has also been advocated by the US Food and Drug Administration for use in the assessment of the hepatotoxicity of newly developed drugs (Yimer *et al.*, 2008; Bjornsson, 2010).

According to Shi *et al.*, report out of the estimated 10,000 documented human drugs, more than 1,000 have been associated with DILI, although causality has not always been established clearly (Shi *et al.*, 2010). In addition to TB disease, hepatotoxicity caused by anti-tuberculosis drugs cannot be ignored. Anti tuberculosis drugs are very well known drugs in causing drug-induced liver injury(Hartleb *et al.*, 2002; Meier *et al.*, 2005).

The incidence of impaired liver function tests (LFTs) varies from 10% to 25%, while symptomatic liver disease occurs in 0.5–3% of patients (Thompson *et al.*, 1995a).

Anemia is also another frequently encountered aberration in TB and/or HIV patients which may be clinically important. Many factors as causes of anemia may make it difficult to know its original cause and/or its suitable treatment. According to World Health Organization (WHO) definitions, anemia in children is defined as hemoglobin level less than 11 g/dL for children <5 years old, <11.5 g/dL for children 5–11.9 years old and <12 g/dL for children 12–14.9 years old after altitude adjustment. Anemia classification can be as mild degree, moderate, severe, and very severe with Hemoglobin range of (Hgb 10.0–10.9 g/dL), (Hgb 7.0–9.9 g/dL), (Hgb 4.0–7.0 g/dL), and Hgb less than 4.0 g/dL respectively (WHO, 2011). Over 2 billion people around the worldwide are anemic with more than 100 million of these anemic children living in Africa and in East Africa, the prevalence of anemia ranges from 15–93 % according to the WHO estimation (WHO, 2018). Significant association between TB and Anemia could be due to many

reasons. Some of the reasons mentioned are; involvement between tuberculosis and malnutrition consists of two relations, the effect of tuberculosis on the nutritional status and the effect of malnutrition on the clinical manifestations of tuberculosis, as a result of immunological impairment. A small study done in Sri Lanka revealed that more than 50% of TB cases were associated with underweight (Lanka, 2017). Anemia was observed in 32-94% of patients with TB (Agarwal, 2016). Among the anemia iron-deficiency anemia and anemia of chronic disease are the most common (Minchella *et al.*, 2015).

Among the anemia characterized by altered iron metabolism, iron-deficiency anemia and anemia of chronic disease are the most common. Iron-deficiency anemia is the most common nutritional deficiency worldwide, affecting primarily individuals residing in developing countries. It occurs as a result of chronic blood loss, urinary losses, poor iron intake/absorption, and increased blood volume. Individuals with iron-deficiency anemia, a decrease in plasma iron levels occurs, limiting erythropoiesis (Carvalho *et al.*, 2006).

Anemia of chronic disease, also known as anemia of inflammation, is a clinical syndrome characterized by the development of anemia in patients with (fungal, bacterial, or viral) infectious diseases, such as tuberculosis, inflammatory diseases, autoimmune diseases, and neoplastic diseases (Cançado and Chiattone, 2002). It is characterized by mild to moderate normocytic hypochromic anemia, and hypochromia and microcytosis, which can occur in 20-30% of cases. However, when microcytosis occurs, it is not as pronounced as it is in iron-deficiency anemia (Carvalho *et al.*, 2006). This type of anemia is associated with decreased serum iron levels and total iron binding capacity, as well as with increased ferritin levels (Cançado and Chiattone, 2002). In patients with active tuberculosis, few of the studies investigating the presence of anemia have determined whether anemia is associated with iron deficiency or chronic disease or have identified variables associated with its occurrence.

1.2. STATEMENT OF THE PROBLEM

According to the WHO 2014 report, there were an estimated 1.1 million cases of TB co-infected with HIV, and majority (90%) of the TB-HIV co-infected people were living in resource-limited settings, which includes Ethiopia (WHO, 2014; Zumla *et al.*, 2015)

Out of the 1.4 million TB-caused deaths reported in 2015, 0.4 million occurred in HIV-positive patients. Globally, it was estimated that there were 10.4 million TB cases, including 1.2 million among HIV-positive people (WHO, 2016).

Ethiopia is also one of the 22 high-burden countries for TB, and childhood TB accounts for 13% of the overall TB burden with case notification among children below 15 years of age. Even this could be an underestimate due to problems in confirmation of diagnosis of TB in children (WHO, 2015).

In Ethiopia, TB remains one of the leading causes of mortality and the third major cause of hospital admissions. In the last decade, 55 000 to 100 000 increment of new TB cases has been registered, and the reason behind is the rapid spread of HIV infection. According to the 2011 Ethiopian Demographic and Health Survey report, the average prevalence of HIV in Ethiopia was 1.5%. It was reported also that the prevalence of TB was 211 per 100 000 of the population, and the global TB report indicated that Ethiopia was among the 22 TB high burden countries, with a TB-HIV co-infection prevalence of 15% in 2012 (WHO, 2013; Zumla *et al.*, 2015).

TB-HIV co-infection, which constitutes a massive burden in the health system in a country, is associated with diagnostic and therapeutic challenges. TB epidemic has been draining resources and overburdening the limited workforce of health professionals (Pawlowski *et al.*, 2012).

Although there were progresses in the implementation of prevention of mother to child transmission (PMTCT), and provision of isoniazid preventive therapy (IPT) in our country, TB remains the major cause of death in HIV infected children and hospital admission (Ramos *et al.*, 2010).

The management of TB-HIV co-infection in children(less than 15yrs) is very difficult in resource-limited settings of developing countries such as Ethiopia because of the unavailability of proper formulations of drugs, pill burdens, drug to drug interactions, drug side effects and poor adherence (Marais *et al.*, 2007; Marais *et al.*, 2011; Pawlowski *et al.*, 2012;) which resulted in high death among TB-HIV co-infected children.

Anti-tuberculosis drug-induced liver injury (ATLI) is one of the most frequent and serious adverse reactions during TB treatment. Isoniazid, rifampicin and pyrazinamide are the most potentially hepatotoxic first-line anti-TB drugs, whereas ethambutol and streptomycin have no known hepatotoxicity reactions. ATLI has a significant role in diminishing treatment effectiveness by increasing patient morbidity and mortality and disrupting therapy adherence as an independent predictor of prolonged TB treatment. The high occurrence of ATLI results in discontinuation of therapy and potentially treatment

failure. ATLI can develop in children at any age or any dosage of anti-TB drugs, with signs and symptoms frequently ignored in many cases (Wares *et al.*, 2003; Yee *et al.*, 2003; Tostmann *et al.*, 2008; Donald, 2011; Devarbhavi *et al.*, 2013; Van't Boveneind-Vrubleuskaya N. *et al.*, 2016).

Moreover, almost all of the first-line anti-TB drugs i.e. isoniazid, rifampicin and pyrazinamide and all classes of antiretroviral drugs are reported to be associated with potential hepatotoxicity (Frieden *et al.*, 2003).

Many studies done in different areas have shown that anti-TB drugs are capable of inducing hepatotoxicity in children (Bhatia *et al.*, 2011; Donald, 2011; Antwi *et al.*, 2017; Sultan *et al.*, 2018).

Hepatotoxicity is one of those toxicities which lead to discontinuation of treatment, poor adherence, and consequently towards the development of resistance, and mortality if not handled properly. Anemia is also a common phenomenon in patients who are taking Anti-TB and/ or ART drugs separately or in combination. Among the 1st line anti-TB drugs: isoniazid, rifampicin and pyrazinamide are known to cause DILI, while all classes of ARV drugs are also incriminated as potential causes of anemic condition. DILI and Anemia secondary to anti-TB and ARV drugs are in general an area that has been less researched and the available few published data has limited emphasis on the incidence, risk factors, management and prognosis. Moreover, the available few studies has limitation on representing an African population, particularly when compared to other continents (Hoffmann *et al.*, 2007; Soriano *et al.*, 2008; Yimer *et al.*, 2008).

However, little is known about the effects of the above medications and the development of hepatotoxicity in patients in Ethiopia especially in children $\leq$15 years of age. Anemia is also another aberration in TB infected patients as well as in those who are co-infected with HIV and those taking the drugs.

Sadly, although the trend of anemia prevalence among Ethiopian children declined from 54 to 44% from 2005 to 2011, it increased to 57% in 2016 (EDHS, 2016). This high prevalence of anemia in 2016 may be due to iron deficiency which is a key component of hemoglobin, could be responsible for this high prevalence. Other attributes of anemia include malaria, hookworm and helminthes, nutritional deficiencies and chronic infections. In other words, although great advances have been made in the management of childhood HIV/AIDS, its burden continues to hamper progress in reducing childhood

mortality and morbidity in much of the developing world, particularly in this region (You *et al.*, 2010; EDHS, 2016).

Nonetheless, there is limited evidence on the prevalence of anemia among HIV infected children currently in Ethiopia. Similarly, the evidence based on the prevalence and effect of HAART on children with HIV/ AIDS and anemia comorbidity still remains inconsistent, uncertain and inconclusive. This may perhaps be due to paucity of data on the topic, particularly in HIV infected children. Thus, the aim of this study was to evaluate the association between the serum liver enzymes as indicators of hepatotoxicity and Hemoglobin concentration as indicator of anemia of children having the diseases. Early diagnosis of these conditions will help to decrease mortality from drug injuries and increase the life standard of children.

1.3. SIGNIFICANCE OF THE STUDY

TB is a common incurable chronic disease. During the past decades, TB has emerged as an important clinical and public health problem throughout the world. The prevalence of TB is reaching epidemic proportions, in large part low income countries in both adults and children. Likewise in Ethiopia, TB has become more common and it is a fast growing disease.

The prevalence of liver diseases is higher in patients who have suffered from this disease. Diagnostic and early therapeutic interventions are needed for treating liver disease in patients at risk for developing DILI and Anemia. On the other hand, prevention or early diagnosis of progressive liver disease and anemia are needed in TB patients. For that reason, the present study is attempting to provide a comparative explanation of liver function tests in TB infected children who are receiving anti- TB drugs with or without ART drugs by using lipid profile and enzyme studies in patients visiting selected general Hospital. Therefore, this study helps TB infected children receiving different anti-TB drugs with or without ARV drugs, to know the status of their liver status and anemic condition, and if there is any abnormality, and to treat them earlier. It may be useful as a base line for other researchers conducting study in related topics and also for organizations working with TB infected children. Besides, it may serve as a guide for prevention and early detection of liver problems in these children.

2. LITERATURE REVIEW

2.1. TUBERCULOSIS

TB is disease caused by the bacterium called *Mycobacterium tuberculosis* and it is also an airborne. *M. tuberculosis* and closely related mycobacterial species (*M. bovis, M. Africanum, M. microti,* and *M. mungi*) collectively comprise of what is known as the *M. tuberculosis* complex. Most of these species cause disease in humans. TB typically affects the lungs but it can also affect other sites as well (extra pulmonary TB). The disease is spread in the air when people who are sick with pulmonary TB expel bacteria, for example by coughing the organism and it can also spread by human to human contact through airborne droplets expelled when a person with active TB disease sneezes, and coughs, laughs or talks (Nelson and Wells, 2004; Pawlowski *et al.*, 2012; ; Dheda *et al.*, 2016).

M. tuberculosis is carried in the air on droplet nuclei, of 1 to 5 microns in diameter. Infectious droplet nuclei are generated during coughing, sneezing, shouting, or singing by persons who have pulmonary or laryngeal TB disease. Depending on the environment, these small particles can remain in the air for several hours. *M. tuberculosis* is not transmitted through surface contact; rather it is transmitted by air. Transmission of tuberculosis occurs when an individual with the disease exhales droplet nuclei containing *MTB,* other person inhales this droplet nuclei and pass through the mouth or nasal passages, Upper respiratory tract, and bronchi to reach the alveoli of the lungs (Dheda *et al.*, 2016). Tuberculosis (TB) is one of the major public health burden in developing countries (WHO, 2013). If untreated, the case fatality rate is estimated to be 70 percent and 20 percent for smear-positive and smear-negative TB patients, respectively (Tiemersma *et al.*, 2011). Untreated smear-positive TB patients are the main cause of infection. Thus, TB cases should be known and treated in an appropriate way. But, a large number of TB cases have not been identified in many sub-Saharan African countries. Internationally, about 3 million people who developed TB in 2012 were missed by national notification Systems(WHO, 2018).

Globally, TB is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS). A lot of people continue to fall sick with *M. tuberculosis* each year. According to WHO, TB disease caused an estimated 1.3 million deaths among HIV-negative people and additional 300 000 deaths from TB among HIV-

positive people, 5.8 million men, 3.2 million women developed TB disease. One million of them were children, 9% were people living with HIV (72% in Africa) and two thirds were in eight countries: South Africa (3%), Bangladesh (4%), Nigeria (4%), Pakistan (5%), the Philippines (6%), Indonesia (8%), China (9%), and India (27%). WHO's list of 30 high TB burden countries includes these and 22 other countries which accounted for 87% of the world's cases. Only 6% of global cases were in the WHO Region of the Americas (3%) and WHO European Region (3%) (WHO, 2018).

2.2. Tuberculosis in Children

Assessing the impact of TB in children less than 15 years of age is difficult and challenging since there's no universal diagnostic algorithm. The diagnosis of TB cases in children usually is combination of clinical criteria and a non-specific TB test. In 2016, an estimated 490,000 new cases occurred among children (around 6% of the all cases). Each year, 64,000 children die from TB, making it one of the top ten causes of childhood death (WHO, 2016).

In 2017, the World Health Organization (WHO) showed that smear-positive TB in children less than 14 years of age accounted for 0.6-3.6% of reported cases. The data underestimate the true burden of childhood TB since incidence is estimated using smear-positive cases. The majority of cases in children less than 12 years of age are smear-negative, and smears are seldom performed in high-burden countries. In low-burden countries, childhood TB constitutes about 5% of TB cases compared to the 20-40% in high-burden countries (WHO, 2017).

HIV infection has significantly impacted the epidemiology and severity of childhood TB since HIV-infected children have an increased risk of developing and disseminating TB. Initiating highly active antiretroviral therapy (HAART) reduces the risk for TB, but the risk still remains higher than that in HIV-uninfected children. Moreover, children living in a household with an HIV-infected adult also have a higher risk for TB exposure and infection (WHO, 2017).

TB cases prevention and control among children did not get adequate attention. The main reasons are limitation in generating quality epidemiologic data principally due to limited health workers, the inherent difficulties in diagnosing pediatric TB, and lack of the facilities or diagnostic tools (Nelson and Wells, 2004; WHO, 2006).

TB is one of the leading causes of morbidity and mortality in children. However, only about 5% of the estimated cases are detected due to the problems related to the health system and the community and 31% of globally estimated childhood TB is found in Africa, resource constrained high burden continent (WHO, 2016).

In Ethiopia, like other developing countries, the health facilities were not effectively organized to give full access to the majority of communities in the country and childhood tuberculosis was not given priority. TB diagnosis is mainly done by sputum smear microscopy, children often are unable to produce sputum and smear microscopy has a low detection rate in specimens, such as gastric aspirate, which is the best method recommended for sample collection in children (Marais, 2004).

To solve such problem recently Ethiopia stipulated Gene x-pert for TB case diagnosis and Mycobacterium/Rifampicin (*MTB*/RIF) as the initial test for children presumed to have TB, in order to increase bacteriological confirmed pediatric TB cases. Unfortunately, it is well known that testing only one specimen with Gene x-pert in children results in a small proportion (typically about 20%) of patients with a positive result and better ways to identify more children are needed (Graham *et al.*, 2004).

2.3. Therapy

The aim of effective TB treatment are patients to be cured from the disease and limiting bacterium transmission in the society. The current recommended treatment regimens for adults and children are essentially the same. Combination of drugs that eliminate the mycobacteria by different mechanisms to prevent the uprising of resistant organisms while having minimal toxicity. Isoniazid (INH) and rifampicin (RIF) are the most potent first-line bactericidal drugs. The drugs kill the bacteria and rapidly decrease their loads, leading to clinical improvements as well as restricting disease progression and transmission. Additionally, RIF and pyrazinamide (PZA) are sterilizing drugs that slow-replication and eradicate the organism. The combination of sterilizing with bactericidal drugs and the addition of a other drug, such as ethambutol (EMB) or streptomycin (S), protects against the appearance of drug-resistant *M. tuberculosis*. Because of the high risk of disseminated TB in infants and younger children, the treatment should be started as soon as a TB diagnosis is suspected (Graham, 2011; Abubakar *et al.*, 2012).

The principles of treating childhood TB largely parallel those for adults; short-course treatment consists of intensive therapy for 2 months with rifampicin (RIF), isoniazid

(INH), and pyrazinamide (PZA) with or without ethambutol (EMB), based on human immunodeficiency virus (HIV) status and extent of disease, followed by continuation therapy for 4 months with RIF and INH. Medication dosing recommendations for children historically were based on extrapolations from pharmacokinetics (PK) data among adults. Similarly, optimal drug-concentration targets for TB medications among children have been adapted from studies of their PK and pharmacodynamics (PD) in adults (WHO, 2017).

EMB is not used routinely in children less than 8 years of age because it can cause optic neuritis, which can lead to irreversible blindness if EMB is not discontinued when visual problems occur. However, since the toxicity is related to the dosage and duration of therapy, EMB can be safely used at the recommended dosage for the initial two months of treatment, even in children too young for routine eye examinations (American Thoracic Society, 2003; Graham, 2011; Abubakar *et al.*, 2012). (**Table 1**)

Table 1. ***Recommended first-line TB drugs for infants and children***

Drug	Daily dosage (max)
Isoniazid	10-15 mg/Kg (300 mg)
Rifampicin	10-20 mg/Kg (600 mg)
Pyrazinamide	15-30 mg/Kg (2.0 g)
Ethambutol	15-20 mg/Kg (1.0 g)

Adapted from the American Thoracic Society, CDC, and Infectious Diseases Society of America (American Thoracic Society, 2003).

Since anti-TB drug production has focused on adults, most drugs are in the form of tablets which have some advantages in poor settings because they can be more easily transported and stored than liquid drugs. However, infants and young children cannot swallow the tablets, and in these patients, galenic formulations must be prepared from the tablets (Ishiwada *et al.*, 2013; Nagarajana and Whitaker, 2018). There is no well known optimal treatment for pulmonary TB in children with HIV infection (American Thoracic Society, 2003).

2.3.1. Mechanism of action of first line Anti-Tuberculosis Treatment

2.3.1.1. Isoniazid (INH)

Isoniazid (isonicotnic acid hydrazide) is considered a pro-drug, which passively diffuses through the mycobacterial cell wall, and once inside the *M. tuberculosis*, is activated by

the KatG (catalase-peroxidase enzyme), a reaction supported by a range of oxidants, including superoxide, hydrogen peroxide and simple alkyl hydroperoxides. The subsequently generated isonicotinoyl radicals form covalent adducts with NAD+ (nicotinamide adenine dinucleotide), which in turn acts as a competitive inhibitor of InhA, a *M. tuberculosis* enoyl acyl carrier protein reductase involved in fatty acid oxidation via the FAS II (fatty acid synthesis pathway type 2) system, during mycolic acid synthesis. Since mycolic acids are the major structural components of the M. tuberculosis cell wall, INH-induced inhibition of mycolic acid synthesis will subsequently lead to a disruption in the cell wall structure, ultimately causing *M. tuberculosis* cell death (Unissa *et al.*, 2016).

Furthermore, the reactive species generated during the course of INH activation, are also accepted propose to result in DNA, carbohydrate and lipid damage, and the inhibition of the NAD+ metabolism (Du Preez and Loots, 2013; Unissa *et al.*, 2016).

2.3.1.2. Rifampicin

RIF freely diffuses over the *M. tuberculosis* cell wall, and once inside the cell, inhibits gene transcription by binding to the β-subunit of its DNA-dependent RNA polymerase (RNAP) (Pang *et al.*, 2013). Various studies have indicated that the RIF binding site is located within the DNA/RNA channel, but not at the active site, and that RIF bound RNAP sustains the capacity to catalyze the formation of the first phosphodiester bond in an emerging RNA transcript, suggesting that RIF does not inhibit catalysis. Alternatively, RIF physically obstructs the path of the growing RNA chain, which is two to three nucleotides in length. Considering this, once transcriptional elongation is at an advanced state, the RNAP is no longer susceptible to RIF inhibition. The precise mechanism by which this transcriptional inhibition leads to cell death, however, is still not completely understood (Nusrath and Hanna, 2017; Du Preez and Loots, 2018).

2.3.1.3. Pyrazinamide

Similar to INH, PZA is considered a pro-drug, which is converted to its active form, POA, via the bacterial enzyme, nicotinamidase/pyrazinamidase (PZase), after crossing the mycobacterial cell wall through a process of passive diffusion and possibly also an active transport mechanism. Although PZA has been used to treat TB for almost 70 years, its mechanism of action against *M. tuberculosis* is the least understood of all the first-line TB drugs, mostly owing to the drug's unusual, paradoxical properties (Sahu *et al.*, 2015).

In contrast to other TB drugs, which typically kill actively growing bacteria at a neutral pH, PZA is predominantly active against old, non-replicating *M.tuberculosis* at an acid pH. Additionally, *M. tuberculosis* is uniquely susceptible to PZA as a result of a deficient POA efflux mechanism. It is believed that, despite a defective efflux pump, POA can exit the *M. tuberculosis* cell via passive diffusion when extracellular pH is acidic. A small portion of the POA will take on an uncharged conjugated acid form, HPOA, which then re-enters the cell. As a proposed mechanism of action, it is further speculated that HPOA carries protons into the cell, eventually causing cytoplasmic acidification and the consequent inhibition of vital *M. tuberculosis* enzymes, which in theory explains the role of an acidic pH in PZA action; at an acidic pH, POA can de-energize the *M. tuberculosis* cell membrane by disrupting the *M. tuberculosis* membrane potential, thereby also disrupting membrane transport functionality. These results further explain the preferential activity of PZA against old, non-replicating bacilli, which are known to have a low membrane potential (Koch *et al.*, 2014). PZA and POA targets fatty acid synthase I (FAS I) as its mechanism of action, PZA acts as a competitive inhibitor of NADPH when binding to purified *M. tuberculosis* FAS I, and that POA also binds to FAS I, PZA and/or POA, as analogues of nicotinic acid, could affect NAD and NADP functionality as a means to kill *M. tuberculosis.* When considering the above, it is clear that the mechanism of action of PZA is complex, and that the antimicrobial activity of this drugs does not necessarily rely on one cellular target, but rather a multitude of targets (Koch *et al.*, 2014; Ramirez-Busby and Valafar, 2015; Sahu *et al.*, 2015; Du Preez and Loots, 2018).

2.3.1.4. Ethambutol

EMB invades the *M. tuberculosis* cells via porin channels and subsequently inhibits cell wall synthesis by interacting with the EmbCAB enzymes. The *embCAB* operon encodes a series of arabinofuranosyltransferases, which are involved in arabinofuranosyl (Araf) polymerization from DPA (ß-D-arabinofuronosyl-1-monophosphoryl decaprenol), in order to synthesize arabinogalactan (AG) and lipoarabinomannan (LAM). The EmbA and EmbB enzymes transfer Araf residues to a galactan chain after the initial Araf residues are linked to galactan by another priming arabinofuranosyltransferase, AftA (encoded by *Rv3792*). EmbC also uses DPA as the donor to transfer Araf residues to a mannan core after the Araf residues are linked to mannan by an uncharacterized arabinofuranosyltransferase. EMB-induced inhibition of EmbCAB, and the consequent

disruption of AG and LAM syntheses, ultimately disturbs the synthesis of the *M. tuberculosis* cell wall skeleton, which comprises of a mycolyl-arabinogalactan-peptidoglycan complex, and the important cell wall glycoconjugate, LAM, thereby interfering with normal bacterial growth, and eventually causing bacterial cell death (Sahu *et al.*, 2015; Du Preez and Loots, 2018)

2.4. Adverse Drug Reactions of first line Anti-Tuberculosis drugs

Most adverse reactions during treatment are predictable and related to the drug pharmacology or drug interactions (Kato *et al.*, 2018). For simple adverse events, such as gastrointestinal symptoms or mild itching, they will be self-limited or require supportive treatment. Around a third of all reactions are idiosyncratic with potential immunological mechanisms, they are outlined later (Usui *et al.*, 2016).

Reactions leading to discontinuation were hepatotoxicity, exanthema, and arthralgia. Pyrazinamide was the commonest culprit drug followed by isoniazid and rifampicin also it was identified as the most likely drug to cause major adverse effects in patients. In contrast, ethambutol was well tolerated with few adverse events. The rates of adverse reactions seen are even higher in patients with drug resistant tuberculosis receiving second-line drugs (Carroll *et al.*, 2012).(Table-2)

Table-2:- ***Adverse drug reactions to first line tuberculosis medication(Nagarajana and Whitaker, 2018)***

Drug	Adverse reaction	
	Common	Serious
Rifampicin	Discoloration of body fluids Gastrointestinal upset and indigestion Transient mild transaminitis Flu-like symptoms	Drug-induced liver injury Cutaneous hypersensitivity Drug-induced vasculitis and hemolytic anemia Nephrotoxicity Drug-induced liver injury
Isoniazid	Peripheral neuropathy Transient mild transaminitis	Cutaneous hypersensitivity Drug-induced lupus Seizures and encephalopathy Hemolytic anemia and thrombocytopenia
Pyrazinamide	Gout and arthralgia Nausea and reduced appetite Flushing Photosensitivity Transient mild transaminitis	Drug-induced liver injury Cutaneous hypersensitivity Sideroblastic anemia and thrombocytopenia
Ethambutol	Nausea Arthralgia and elevated uric acid	Optic neuritis Drug-induced liver injury (rare) Cutaneous hypersensitivity (rare)

Hepatotoxicity is the major drug-related adverse event to these drugs and is frequently associated with INH and RIF. In these cases, one of the two drugs should be interrupted. However, serious effects of these TB-drugs are rare in children. In situations where several first-line agents cannot be used because of intolerance, regimens based on principles described for treating MDR-TB should be used (Seddon *et al.*, 2012).

2.5. Drug Induced Liver Injury (DILI)

Drug induced liver injury is one of the most series adverse drug reactions that may result in death if it is not managed properly. It was defined by American Thoracic society which is further endorsed by international DILI Expert Working Group (Aithal *et al.*, 2011). For labeling DILI should fulfill any one of the following criteria:

- Elevation in alanine aminotransferase (ALT) more than or equal to fivefold Increase from upper limit of normal (ULN)
- Twofold or more than twofold rise in alkaline phosphatase (ALP) along with associated more than or equal to twofold elevations in concentrations of 5 nucleotidase or γ-glutamyl transpeptidase above the ULN without known bone pathology.
- More than or equal to threefold rises in ALT concentration and concurrent elevation of bilirubin concentration surpassing 2 times of the ULN.

2.5.1. DILI Severity Index

The severity of DILI was graded in accordance with *International DILI Expert Working Group*(Aithal *et al.*, 2011), where severity of DILI was graded as follow:

Mild: Elevated alanine aminotransferase or alkaline phosphatase concentration reaching criteria for DILI* but bilirubin concentration < 2× upper limit of normal (ULN).

Moderate: Elevated alanine aminotransferase or alkaline phosphatase concentration reaching criteria for DILI* and bilirubin concentration ≥2× ULN, or symptomatic hepatitis.

Severe: Elevated alanine aminotransferase or alkaline phosphatase concentration reaching criteria for DILI* and bilirubin concentration ≥2× ULN, and at least one of the following:

- International normalized ratio ≥1.5.

• Ascites and/or encephalopathy, disease duration <26 weeks, and absence of underlying cirrhosis.

• Other organ failure considered to be caused by DILI.

Fatal: Death or liver transplantation for drug induced liver injury.

*****(proposed by *International DILI Expert Working Group*).

Drug-induced liver injury (DILI; or *drug-induced hepatotoxicity*) is a hepatic injury caused by medications, herbal and dietary supplements (HDS), or another xenobiotics that ends with hepatic dysfunction or in elevations of liver function tests that cannot be explained by other causes (Suk and Kim, 2012). There are two types of DILI: intrinsic and idiosyncratic. *Intrinsic* DILI refers to hepatic injury induced by a given drug in a predictable and dose-related manner (e.g., acetaminophen [APAP]); whereas *idiosyncratic* DILI, which occurs rarely, is associated with a less consistent dose-toxicity relationship and a more varied presentation (Chalasani *et al.*, 2014).

The true prevalence of liver toxicity is hard to ascertain because premarketing clinical trials are underpowered to find the presence of idiosyncratic DILI. However, Drug induced Hepatotoxicity is estimated to have an annual incidence of 10 to 15 per 10,000 to 100,000 persons exposed to ordered medications. Therefore, about 44,000 people in the United States will experience DILI annually, making it costly in terms of not only its toll on humans, but also healthcare expenditures. Out of the 2,000 cases of acute liver failure (ALF) that occur in the U.S.A each year, medications account for >50%, and 13% attributable to idiosyncratic adverse drug reactions (Andrade *et al.*, 2007; Bell and Chalasani, 2009; Pandit and Sachdeva T., 2012; Larson, 2016).

2.6. Anti-tuberculosis drug-induced hepatotoxicity

Anti-tuberculosis drug-induced liver injury (ATLI) is one of the most frequent and serious adverse reactions during TB treatment. Isoniazid, rifampicin and pyrazinamide are the most potentially hepatotoxic first-line anti-TB drugs, whereas ethambutol and streptomycin have no known hepatotoxicity reactions. ATLI has a significant role in diminishing treatment effectiveness by increasing patient morbidity and mortality and disrupting therapy adherence. As an independent predictor of prolonged TB treatment, the high occurrence of ATLI results in discontinuation of therapy and potentially treatment failure. ATLI can develop in children at any age or any dosage of anti-TB

drugs, where signs and symptoms are frequently ignored in many cases (Wares *et al.*, 2003; Yee *et al.*, 2003; Tostmann *et al.*, 2008; Donald, 2011; Devarbhavi *et al.*, 2013; Van't Boveneind-Vrubleuskaya N. *et al.*, 2016).

2.6.1. Anti-tuberculosis drug-induced hepatotoxicity in children

Many studies done in different areas showed that anti-TB drugs are capable of inducing hepatotoxicity in children. For instance, study done in Indonesia reported that the rate of ATLI in children was about 7.4% (Akura *et al.*, 2009), according to Thompson et.al, study done in Southern India anti TB drugs induced 10-25% abnormal LFT and 3% hepatotoxicity due to ATT was seen in children (Thompson *et al.*, 1995b).

According to study done by Ohkawa et. al, severe TB DILI was diagnosed in 8% of pediatric patients, and was associated with age younger than 5 years, extra pulmonary TB, and use of pyrazinamide (Ohkawa *et al.*, 2002).

In a U.S. multicenter trial from 1993 through 1997, patients with TB-AIDS treated with regimens containing isoniazid, rifampicin, and pyrazinamide had an overall 4.4% rate of clinically significant or treatment-limiting hepatotoxicity (El-Sadr *et al.*, 1998).

According to Devarbhavi *et. al's* analysis of the causes, clinical and biochemical features, natural history, and outcomes of children with DILI, 39 children constituted 8.7% of 450 cases of DILI. There were 22 boys and 17 girls with Median age of 16 years (range, 2.6-17). Combination anti-tuberculosis drugs were the most common cause of drug induced Hepatotoxicity (Devarbhavi *et al.*, 2013)

Another study establishes that children form a small, but significant proportion (8.7%) of DILI with a high mortality rate (30.7%) and the excellent survival in patients with hypersensitivity features, such as skin rash, fever, lymphadenopathy, and/or eosinophilia. All deaths were caused by anti-tuberculosis drugs, except for 1 adolescent who died from overdose of some drugs (Harshad *et al.*, 2011).

Other study done in Spanish reported ≈4% from the age group 10-19 and the leading cause, anti-tuberculosis drugs (e.g., INH, RIF with or without PZA, and EMB) (Lucena *et al.*, 2009).

In a study in children done by Naqvi et. al. TB DILI was manifested in 34.17% (Naqvi *et al.*, 2015) which is greater than earlier studies where the frequency was 8% - 19.8% (Hno *et al.*, 2000; Mahmood *et al.*, 2007). A study done in Iranian has shown also frequency of

(31.37%) of TB DILI (Khalili *et al.*, 2009). TB DILI of 5% was reported in a meta-analysis (Steele *et al.*, 1991).

ATT DILI severity was noted from mild to severe in this study done by Naqvi et al. Majority of patients having TB DILI 41 (43.15%) had mild DILI, 29 (30.52%) had moderate DILI while 20 (20.01%) and 05 (5.26%) patients had severe and very severe DILI, respectively. A study done by Khalili *et al.*, (Khalili *et al.*, 2009) have also shown moderate (18.7%), severe (7.8%) and very severe (4.9%) hepatotoxicity with ATT.

Symptomatic liver disease was less commonly reported in 0.5–3% (Yew, 1998; Schreiber *et al.*, 1999; Devrim *et al.*, 2010; Bhatia *et al.*, 2011; Peter, 2011; Mugusi *et al.*, 2012).

Drug induced hepatotoxicity in children receiving ATT is between 3% and 10%; severe hepatotoxicity is documented below 1% in children (Devrim *et al.*, 2010; Bhatia *et al.*, 2011; Peter, 2011).

According to the study done by Mugusi *et.al,* Antiretroviral and anti-tuberculosis chemotherapy associated drug induced liver injury (DILI) is a common and challenging adverse event causing adherence problem leading to hospitalization and life-threatening events. DILI can be fatal if immediate action will not be taken such as therapy interruption on time, and this hepatotoxicity may also causes treatment failure and relapse or drug resistance. The prevalence of DILI in a study done by Mugusi *et al.* was 7.8%, it was also higher in patients receiving anti-TB and HAART (10.0%,) than those receiving ART drugs alone (5.9%) (Mugusi *et al.*, 2012).

Data on prevalence of DILI of different countries shown that this adverse event cannot be ignored.(Table-3)

Table-3:- Prevalence of DILI among different countries

Country	DILI%	Reference
Southern India	3%	(Akura *et al.*, 2009)
Spain	≈4%	(Lucena *et al.*, 2009)
Turkey	4%	Devrim *et al.*, 2010;
U.S.A	4.4%	(El-Sadr *et al.*, 1998)
Indonesia	7.4%	(Thompson *et al.*, 1995b)
Tanzania	7.8%	(Mugusi *et al.*, 2012).
Bangalore, India	8.7%	(Devarbhavi *et al.*, 2013)
Bangalore, India	8.7%	(Harshad *et al.*, 2011)
South Africa	8.8%	Peter, 2011
Pakistan	14%	Sultan et al., 2018

To our knowledge there is no data on DILI in children in Ethiopia but limited sources showed that the prevalence of DILI on adults is significant. Some of the data are shown as follow.(Table-4)

Table-4:- Prevalence of DILI in Ethiopia

Study Area	No. of Study Population	Occurrence of DILI N(%)	Reference
Ethiopia	197	34(17.2)	(Yimer *et al.*, 2008)
Southwest Ethiopia-Jimma	288	33(11.5)	(Hassen Ali *et al.*, 2013)
Ethiopia	1060	159(15)	(Yimer *et al.*, 2014)
South Ethiopia-Dawro Zone	124	10(8.1)	(Wondwossen *et al.*, 2016)

2.6.2. Mechanism of anti-tuberculosis drug-induced hepatotoxicity

It is not entirely clear the mechanism as well as pathogenesis of these drugs. The clinical relevance of therapeutic monitoring of ATT metabolite concentrations in managing anti-tuberculosis drug-associated toxicity is still being explored (Kato *et al.*, 2018; Nagarajana and Whitaker, 2018). Some of the mechanism understood till now are:-

By Isoniazid : Normally isoniazid is cleared mostly by the liver, primarily by acetylation by N-acetyl transferase2 (NAT-2). Acetyl-isoniazid is metabolized mainly to monoacetyl hydrazine (MAH) and to the no toxic diacetyl hydrazine, as well as other minor metabolites. The reactive metabolites of monoacetyl hydrazine (MAH) are probably toxic to tissues through free radical generation. The additional isoniazid metabolites acetyl hydrazine covalently binds to liver macromolecules, a process mediated by microsomal enzyme (Sheng *et al.*, 2014; Hassan *et al.*, 2015)

By Rifampicin : Rifampicin may occasionally cause dose dependent interference with bilirubin uptake resulting in subclinical, unconjugated hyperbilirubinemia or jaundice without hepatocellular damage. Conjugated hyperbilirubinemia probably is caused by rifampicin inhibiting the major bile salt exporter pump. Asymptomatic elevated bilirubin may also result from dose-depended competition with bilirubin for clearance at sinusoidal membrane or from impeded secretion at the canalicular level (Sharma *et al.*, 2014; Jeong *et al.*, 2015).

By Pyrazinamide : It may exhibit both dose dependent and idiosyncratic hepatotoxicity. Pyrazinamide alters nicotinamide acetyl dehydrogenase levels in liver which might result

in generation of free radical species. There may be shared mechanisms of injury for isoniazid and pyrazinamide, because there is some similarity in molecular structure. Patients who previously had hepatotoxic reactions with isoniazid have more severe reaction with RMP and PRZ (Pasipanodya and Gumbo, 2010; Jeong *et al.*, 2015)

By Ethambutol : This drug has less report in inducing liver toxicity with unclear circumstances (Jeong *et al.*, 2015).

The interactive hepatotoxicity of isoniazid and rifampicin, as previously alluded, may be due to additive or synergistic effects. According to Askgaard *et al.*, possible mechanism can be the enhancement of liver toxicity caused by monoacetyl hydrazine, hydrazine and related compounds resulting from hepatic metabolism through enzyme induction. (Askgaard *et al.*, 1995) This involves the hydrolase system (so-called direct pathway). The pathway is operationally more important in individuals with the slow acetylator phenotype. After some years of investigation, genotyping has revealed that the slow acetylator phenotype comprises a combination of mutant alleles of the N-acetyl transferase-2 (NAT2), as compared with the rapid acetylator phenotype (homozygous NAT2*4) and intermediate acetylator phenotype (heterozygous NAT2*4 and mutant alleles) (Fig-1)

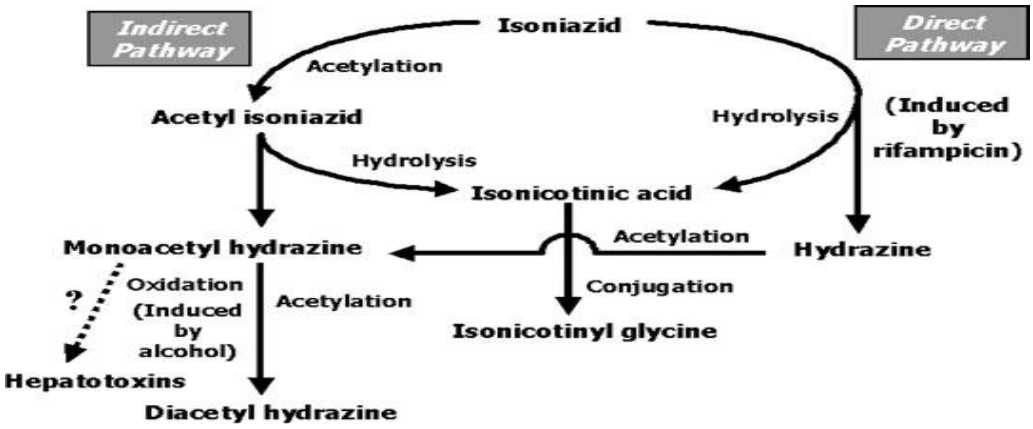


Figure-1:- Metabolism of isoniazid and its influence by alcohol and rifampicin.

Diagrammatic representation of Isoniazid and Rifampicin drug-drug interaction and common metabolite. (Ohno *et al.*, 2000).

2.7. Management of DILI

Lack of system for monitoring drug safety is a major problem contributing to poor health in Ethiopia. Monitoring the safety of drugs contributes to building evidence on the safety of medicines pertinent to the Ethiopian population (Federal Ministry of Health, 2015).

The incidence of DILI associated with first line anti-TB therapy was low but is still a serious problem in countries with high TB burden. Therefore, monitoring for possible ADRs, especially DILI in patients of TB, should be routinely conducted (Neuman, 2019); with over-the-counter and common prescription drugs. If they are mild, continuing the treatment regimen, with the help of ancillary drugs where necessary is the best option. Many ADRs disappear or diminish with time and patients should be encouraged to tolerate the effects until they subside. Psychosocial support is an important component of managing these events, (Bezu *et al.*, 2014; Federal Ministry of Health, 2015)

It has been repeatedly mentioned that DILI associated with first line anti-TB drugs become an obstacle in TB management (Nagarajana and Whitaker, 2018). It negatively affects therapy adherence, decrease treatment success rates and increase treatment failure, relapse or drug resistance (Reves and Angelo, 2016).

Regular laboratory screening is also important for occult the DILI using serum indicators such as ALT, AST, and Bilirubin. Otherwise, drugs may be stopped unnecessarily or treatment may be terminated prematurely by inexperienced health workers and patients may refuse to continue treatment. If DILI or other adverse events are not properly managed they can result in a high proportion of failure and further development of unnecessary resistance strain (Neuman, 2019).

2.8. Potential Risk Factor

2.8.1. ART/ATT concomitant use

More importantly, concomitant administration of NNRTIs and boosted PIs, with TB treatment has been shown to accelerate adverse drug reactions which are common among the dual infections, and predispose patients mainly to liver damage due to shared metabolic pathways (Kwara *et al.*, 2019). A high incidence of peripheral neuropathy (55%) has been documented in patients undergoing both d4T and INH treatment; concomitant use of both NVP and anti-TB drugs especially RMP subjects multiple overlapping toxicities including hypersensitivity skin rash and hepatitis; AZT administration has been discouraged in patients with low haemoglobin levels (<8 g/dL) due to likely hood of developing AZT associated anemia. Finally, gastrointestinal disturbances including malabsorption are reported with all first line anti-TB drugs and various antiretroviral regimen including NVP, that may possibly be attributed to presence

of gastrointestinal disease (Yimer *et al.*, 2008; Bhatt *et al.*, 2015; Kwara *et al.*, 2019). (Tables-5)

Table -5:-*Overlapping or additive adverse effect profiles due to antiretroviral and anti-tuberculosis agents (Karanja et al., 2016)*

Adverse effects	Antiretroviral drugs	Anti-tuberculosis drugs
Skin rash	NVP, EFV, APV, FPV	INH, RMP, pyrazinamide,
Peripheral neuropathy	d4T, ddl, ddC	INH, ethambutol
CNS toxicity	EFV	INH
Hepatotoxicity	EFV, NVP, all PIs and NRTIs	RMP, INH, pyrazinamide
Anemia, neutropenia	AZT	RMP, INH
Bone marrow suppression	AZT	RMP
Ocular effects	ddl	ethambutol
Nausea, vomiting	RTV, IDV, AZT	RMP, pyrazinamide
GIT side effects	All	All
Hepatitis	NVP, PIs	RMP, INH, pyrazinamide

Note: NVP: Nevirapine; EFV: Efavirenz: FPV: d4T: Stavudine; ddl: Didanosine; ddC: Zalcitabine; AZT: Zidovudine; INH: Isoniazid; RMP: Rifampicin;
PIs: Protease inhibitors; NRTIs: Nucleoside reverse transcriptase inhibitors;
GIT:Gastrointestinal tract

2.8.1.1. ART treatment regimen

Pediatric patients (≤15 years old) who need ART to determine the proportion of each metric that met the standard national guidelines. Ethiopian national guidelines included:

1) Weight must be recorded at every clinical visit
2) Bactrim prophylaxis must be given for every child.

   If Bactrim is not given, Dapsone is an alternative.
3) All patients must be screened for TB
   - ✓ If screened positive, there must be further documentation (options: treated for TB, TB infection not suspected, or follow-up)
   - ✓ If at the start of ART, the child had an active TB contact, then isoniazid prophylaxis must be given
4) May start ART initiation when they are positive for HIV test
5) ART Regimen
   - ✓ First line ART: 2 nucleoside reverse transcriptase inhibitors (NRTI) and 1 non-nucleoside reverse transcriptase (NNRTI)

- ✓ Different medication regimens for a child co-infected with TB (weight dependent) (FDRE MoH, 2018) (Table-6)

Table-6:- ***ARV treatment regimen of children with or without TB infection in Ethiopia***

TB Negative	
D4T+3TC+NVP	If Hemoglobin>8g/dL
	AZT+3TC+NVP
TB Positive	
<10Kg	>10Kg
AZT/D4T+3TC+ABC/NVP	2NRTI+EFV

Abbreviations
NRTI--> Nucleoside Reverse Transcriptase Inhibitors ,
3TC-->Lamivudine, ABC-->Abacavir
AZT-->Zidovudine, D4T -->Stavudine
NNRTI-->NON Nucleoside Reverse Transcriptase Inhibitors
EFV--> Efavirenz NVP--> Nevirapine

2.8.2. Mechanism of ART drugs liver toxicity

2.8.2.1. Mitochondrial injury

Mitochondrial products serve as the primary source of useful energy to the hepatocyte. As such, any process that impairs mitochondrial function can lead to hepatic injury. The primary mechanism by which mitochondrial injury occurs in patients with HIV is increased stress on the Endoplasmic Reticulum (ER). This increased ER stress may occur due to a variety of mechanisms, with a final common pathway of triggering increased production of inflammatory cytokines and a mitochondrial 'alarm response' that culminates in increased macrophage activation and beta-oxidation of accumulated fatty acids within the liver. This ER stress is initiated by activation of the IRE1/TRAF 2 (Inositol REquiring 1 / TNF receptor-associated factor 2) pathway (Kao *et al.*, 2012). In addition, antiretroviral drugs, in particular older NRTIs and protease inhibitors (PIs), can directly cause mitochondrial toxicity. In the case of the older NRTIs, this is primarily via a mechanism of increased lipid content of cell membranes leading to ER stress and subsequent mitochondrial dysfunction. In the case of the PIs (primarily indinavir and ritonavir), a PI-mediated decrease in sacroplasmic/ER calcium ATPase can lead to ER stress by decreasing levels of Ca^{2+} within the ER. This PI-mediated effect may be potentiated by concomitant alcohol use (Kao *et al.*, 2012; Sherman and Sterling, 2016)

2.8.2.2. Oxidative stress

Oxidative stress is a process by which free reactive oxygen species (ROS) cause increased activation of Kupffer cells in the liver. These activated immune cells promote

stellate cell activation via nuclear factor kappa-beta (NF-kB) and activator protein 1, leading to increased production of proinflammatory and profibrotic cytokines. Left unchecked, liver damage, fibrosis and cirrhosis may result (Mastroianni *et al.*, 2014). HIV and medications have been shown to cause liver damage via this mechanism. Accumulation of metabolites in the endoplasmic reticulum (ER) can lead to DNA damage, and the metabolite Negative Regulatory Factor (NEF) protein can affect metabolism of lipid droplets causing an increase in ROS. The HIV directly activates hepatic stellate cells (HSCs) via the gp120 receptor, activating metabolic pathways resulting in ROS. Finally, HIV medications, most notably nucleoside reverse transcriptase inhibitors (NRTIs) such as didanosine can cause mitochondrial toxicity and oxidative stress (Mastroianni *et al.*, 2014; Sherman and Sterling, 2016; Kaspar and Sterling, 2017).

2.8.2.3. Accumulation of toxic metabolites

Medications used with greater frequency in HIV-infected persons, including both HAART components and medications used to treat opportunistic infections, may lead to accumulation of toxic metabolites. For example, agents such as ketoconazole and erythromycin inhibit cytochrome P450 function in addition to having direct effects on liver chemistries, while drugs such as isoniazid are well known as a direct cause of hepatotoxicity and idiosyncratic liver injury (Sherman and Sterling, 2016). In addition, patients with HIV appear to have increased risk of hypersensitivity reactions when compared with the general population, possibly predisposing them to higher likelihood of Drug induced liver injury (DILI) from medications such as trimethoprim-sulfamethoxazole and acyclovir. Additionally, drug– drug interactions seen in these patients who are typically burdened by polypharmacy may potentiate hepatotoxic effects of other agents. For example, ribavirin (typically used as a component of treatment in HCV/HIV coinfected patients), if coadministered with didanosine, can decrease didanosine phosphorylation and increase cellular concentrations of didanosine, leading to mitochondrial toxicity. In the case of alcohol, PIs appear to cause down regulation of the P450 enzyme CYP2E1 (Sherman *et al.*, 2014; Sherman and Sterling, 2016; Kaspar and Sterling, 2017).

2.9. Tuberculosis related Anemia

2.9.1. Anemia related to Tuberculosis with and without HIV

TB is a curable disease although drug resistance is one of the major challenges in the treatment, prevention and control of the disease. However, the use of anti-TB drugs is not free from side effects. Allergic reactions, fever, rash, vasculitis, nausea, vomiting, hepatotoxicity, hepatocellular inflammation, peripheral neuropathy and others are some of the common side effects associated with TB treatment (Loulergue *et al.*, 2007). In addition, a wide range of hematological abnormalities has been reported as a result of administration of anti-TB drugs. The major hematological disorder due to anti-TB drug is aplastic anemia. Aplastic anemia is a hypo- regenerative bone marrow disorder characterized by a reduction in the amount of haemopoitic bone marrow and pancytopenia. It has been reported that pancytopenia due to aplastic anemia of moderate severity was observed in a patient while receiving antituberculosis therapy, probably caused by an idiosyncratic reactions (Agarwal, 2016).

Anemia is frequently encountered aberration in TB and/or HIV patients which may be clinically important. Many factors as causes of anemia may make it difficult to know its original cause and/or its suitable treatment.

2.9.2. Mechanism and Etiology of Anemia

Anemia arises through a variety of mechanisms and etiologies. Drug-induced hematological disorders can span almost the entire spectrum of hematology, affecting red cells, white cells, platelets, and the coagulation system. The wide spectrum of drug-induced hematologic syndromes is mediated by a variety of mechanisms, including immune effects, interactions with enzymatic pathways, and direct inhibition of hematopoiesis. Drug induced syndromes include hemolytic anemia, red cell aplasia, sideroblastic anemia, megaloblastic anemia, polycythemia, aplastic anemia, leukocytosis and others. There are four possible relationships of tuberculosis to hematologic disease (Agarwal, 2016). These are the hematologic disease predisposes to tuberculosis reactivation. Drugs may cause idiosyncratic reactions, malabsorption, interference with iron metabolism, and hemolysis in patients with red blood cell enzyme deficiencies. Idiosyncratic reactions manifested by depression of any or all of the three cellular blood elements (white cells, red cells and platelets) together with the coagulation system may be caused by any of the anti-tuberculosis drugs (Mintzer *et al.*, 2009).

Although the total leukocyte and platelet counts could be depressed after cessations of drugs, physicians rarely suspect anti-tuberculosis induced hematological complications which may have fatal consequences (Agarwal, 2016).

In addition to the direct effect of HIV, highly active antiretroviral therapy (HAART) also causes anemia. Although HAART has the capability of reducing the incidence of anemia (Enawgaw *et al.*, 2015) by suppressing viral replication and increases CD4 cell count, anemia remains a common problem even for patients treated with antiretroviral agents. Anemia due to drugs, such as cotrimoxazole, pentamidine, foscarnet, and zidovudine (AZT), often reflects reticuloendothelial iron block (Hermela *et al.*, 2017). There are several factors believed to contribute to the pathophysiology of anemia observed in HIV patients. First, many of the opportunistic infections or malignancies to which HIV patients are susceptible can lead to anemia (Camara-Lemarroy *et al.*, 2015).

As with anemia in the HIV population, micronutrient deficiencies can play a role in contributing to anemia in HIV patients (Muluken *et al.*, 2015). Finally, many of the most common ART drugs included in the standard also harm hematopoiesis and can thus contribute to anemia in HIV. The molecular mimicry between erythropoietin (EPO) and HIV-1 p17 protein can lead to circulating auto-antibodies against endogenous EPO in some HIV patients (Tsiakalos *et al.*, 2011; Meidani *et al.*, 2012).

Anemia is a common comorbid condition among HIV infected children and has a profound impact on disease progression and has been noted as a significant predictor of decreased survival time and death (Owiredu *et al.*, 2011). Drug induced syndromes includes hemolytic anemia, methemoglobinemia, red cell aplasia, sideroblastic anemia, megaloblastic anemia, polycythemia and aplastic anemia (Mintzer *et al.*, 2009). Especially, children are more prone to the consequences of anemia due to high iron requirements, low intake of iron from foods, and frequent episodes of infection (WHO, 2011). Anemia is one of the global public health problems that affect more than one third of the world population. It has been strongly associated with poor growth and development, limited psychomotor development, and poor long-term performance in cognitive, social, and emotional functioning in children. It is also associated with impaired mental, physical, and language development and poor coordination, scholastic achievement, and immune function (Owiredu *et al.*, 2011).

Anemia is a common childhood health problem in Africa, and its prevalence among children under the age of five years is estimated at 62%, which is above the cut-off points (40%). It is a leading cause of pediatric mortality and impaired development and is highly prevalent in young children in sub-Saharan Africa (Lumbiganon *et al.*, 2016).

2.9.3. Anemia related to Tuberculosis with and without HIV in Ethiopia

In Ethiopia, as many as six in ten under-five children are anemic. As per EDHS 2016, overall, more than half of the children 6–59 months (56 percent) suffered from some degree of anemia: 25 percent were mildly anemic, 28 percent were moderately anemic, and 3 percent were severely anemic (EDHS, 2016). Despite causing such high levels of burden, the public health community does not pay anemia the attention it deserves.

The government of Ethiopia has been working to reduce childhood anemia. Accordingly, it endorsed the national nutrition program and bimanual school deworming, developed micronutrient deficiency prevention and control guideline, and implemented micronutrient fortification (Hermela *et al.*, 2017). But studies from different corners of the country have shown that childhood anemia is still a major public health problem in this country (Muluken *et al.*, 2015).

Most of the studies on the HIV-anemia interplay were done in developed countries and mainly included adults. Determinants of anemia among HIV/AIDs positive children on HAART have not been very well understood in resource poor settings like Ethiopia, although there are few crosssectional studies conducted at different parts of the country (Bamlaku *et al.*, 2015).

However, knowing the determinants of anemia among HIV/ AIDS positive children on HAART through the case-control study will help to screen and manage anemia according to its cause. In the same vein, although HAART has been associated with a decrease in the incidence and severity of anemia, recent evidence shows that a decrease in hemoglobin level still occurs and is a predictors of poor clinical outcome. Moreover, according to EDHS 2016 reports, 57% of children age 6–59 months suffered from some degree of anemia (hemoglobin levels below 11 g/dl). Twenty-five percent of children are classified with mild anemia, 29% with moderate anemia, and 3% with severe anemia (EDHS, 2016).

2.9.4. Prevalence of Tuberculosis with and without HIV related Anemia

Anemia has been reported in 16% to 94% in patients with pulmonary TB, (Cameron and Horne NW., 1971; Baynes *et al.*, 1986b; Morris *et al.*, 1989; Olaniyi and Aken'Ova YA., 2003) although a different definition was applied.

Anemia occurred in 31.9% of TB patients according to the study done by Sei Won Lee *et.al,* but it had a benign course in most cases. TB associated anemia completely resolved with anti-TB treatment in 64.5% of patients (Sei *et al.*, 2006). In addition, the anemia improved considerably in the other patients, with rare exceptions. All chronic infections including TB can cause anemia (Weiss, 2002). Various pathogeneses have been suggested in TB-associated anemia, but most studies have shown suppression of erythropoiesis by inflammatory mediators (Baynes *et al.*, 1986a; Baynes *et al.*, 1986b; Morris *et al.*, 1989; Means, 2003; Weiss and Goodnough LT., 2005) as a cause of anemia. Nutritional deficiency (Schwenk and Macallan, 2000) and malabsorption syndrome (Ramadan and Abdul-Ghaffar, 1997) can deepen the severity of anemia. However, the observation that patients with TB-associated anemia display an absence of bone marrow iron (Isanaka *et al.*, 2012) and the same red blood cell distribution width as that observed with iron-deficiency anemia (Muluneh and Fessahaye, 2009), suggests that iron-deficiency is a possible cause of anemia in patients with TB. The prevalence of anemia usually increases with age (Choi *et al.*, 2004), according to some scholars (Ania *et al.*, 1994; Ania *et al.*, 1997; Smith, 2000; Balducci, 2003). The increasing prevalence of anemia with age has been explained by increased chronic disease, poor nutritional status, decreased marrow cellularity, and low serum vitamin B12 levels (Isanaka *et al.*, 2012)

According to Lidwiczek *et al.,* chronic infections such as Tuberculosis has disturbance of iron homeostasis developed with increased uptake and retention of iron within the reticuloendothelial system (Tilg *et al.*, 2002; Ludwiczek *et al.*, 2003; Weiss and Goodnough LT., 2005). Because iron is important growth factor of *Mycobacterium tuberculosis,* the iron retention to reticuloendothelial system is considered one of host defense mechanisms and many therapeutic trials are performed(Cronje and Bornman, 2005). The effect of iron-retention might be exaggerated in women with TB because women are more likely than men to be iron deficient (Muluneh and Fessahaye, 2009).

3. OBJECTIVE

3.1. General objectives

The general objective of this study is to assess drug induced Hepatotoxicity and Anemia among TB infected children with and without HIV in some randomly selected General Hospitals in Tigray, Ethiopia.

3.2. Specific Objectives

- To assess drug induced liver toxicity among TB infected children
- To measure anemia among TB infected children
- To evaluate the difference in selected serum liver enzymes and metabolites (ALT,AST and Bilirubin) by comparing TB infected children with or without HIV to control group
- To detect and compare anemic condition between TB infected children with or without HIV and apparently healthy control group.

4. METHODS AND MATERIALS

4.1. Study Area

The study was conducted in some randomly selected General Hospitals of Tigray, Ethiopia. These hospitals were Adigrat General Hospital, May chew (Lemlem Kharl) General Hospital, Mekelle General Hospital, and Axum General Hospital.

4.2. Study Design

A comparative cross-sectional study was conducted to assess drug induced hepatotoxicity and Anemia by evaluating serum enzymatic levels of transaminases and hemoglobin respectively among children who were taking anti-TB drugs with and without anti-retro viral treatments. (1^{st} line drugs:- *Efavirenz, Lamovidine, Nevirapine, Stavudine,* and *Zidovudine,* 2^{nd} line drugs:- *Abacabir, Didanocine, Liponavir* and *Retronavir*)

4.3. Study Period

The study was conducted from May, 2019 to November, 2019

4.4. Population

4.4.1. Source population

The Source population was all TB patients who visited the selected hospitals of Tigray, Ethiopia.

4.4.2. Study Population

The study population was children having TB with and without HIV infection and follow treatment in the selected hospitals of Tigray, Ethiopia, during the study period.

4.5. Eligibility Criteria

4.5.1. Inclusion Criteria:

Children of either gender having age less than 15 years and on Anti TB with and / or without HIV-AIDS treatment, to whom voluntariness and willingness to participate in the study was obtained from their families.

4.5.2. Exclusion Criteria:

- ✓ Hepatitis A,B, C, and E infection after testing the cases and controls
- ✓ Confirmed Biliary obstruction

- ✓ Congestive Hepatomegaly
- ✓ History of hepatotoxic drug intake
- ✓ Confirmed Liver abscesses or nay focal lesions
- ✓ Any other apparent cause for the elevation of liver chemistries and
- ✓ Previous history of anemia and Schistosomiasis infection
- ✓ Non-consenting children were excluded

4.6. Sampling Method

The general hospitals were randomly selected by lottery method and the study units were selected by non-probability purposive sampling technique to include all TB infected children during the study period (Adigrat 23/24, Mekelle 29/30, Michew 24/23 and Axum 18/17 TB infected children/control groups). All the study units were tested for HBS Ag and HCV tests and excluded when they positive. There was a well-structured questionnaire (annexure) which was used to select appropriate study participant; and record clinical and socio-demographic data.

4.7. Study variables

4.7.1. Dependent variables

- ✓ ALT, AST
- ✓ Bilirubin
- ✓ Hemoglobin
- ✓ Adverse Drug Reactions
- ✓ Anti TB Drug induced Hepatotoxicity
- ✓ TB related Anemia

4.7.2. Independent variables

- ✓ Age, Sex,
- ✓ Malnutrition and BMI,
- ✓ HIV/AIDS infection and ARV treatments
- ✓ TB infection history of family

4.8. Blood sample and Data Collection

4.8.1. Data collection procedure

After a brief explanation, the patient's family consent was asked for their willingness to enroll their children in the study and willing parents were asked to

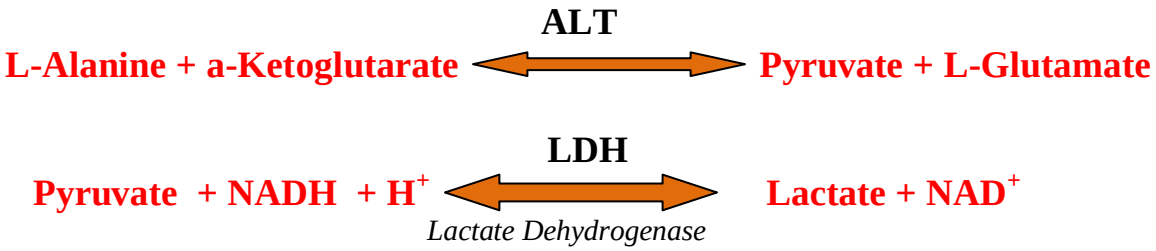
sign on the consent agreement form. Then, before blood sample drawing, responses of patients or their guardians were taken by professional nurses using structured questionnaire.

4.8.2. Blood Samples

Five mL venous blood samples were drawn aseptically from each volunteer participants using a disposable plastic syringe. The blood was poured in to standardized two types of test tubes (one with anticoagulant for hematological tests and the other with serum gel separator for liver function tests). The samples, which were negatively tested for HBs Ag and HCV, were tested for hemoglobin test within 8hours of collection by using CBC machine Mindray BC-5800(Germany) and the blood sample in a test tube with serum separator gel was centrifuged after it clotted. Serum was kept at -20°C in the refrigerator till analysis. AST, ALT, and Bilirubin were measured by using Jouri Lab reagents in AuthoChemistry Analyzer Mindray (Serial No. XR-28000394T, Document No. AHL/ALS/F5.3-007, Address:-servi@mindray.com Sales & technical support contact information:-afrogermen company, tel-0911888096, Germany) according to the manufacturer's procedures and standard operating procedures (Shenzhen Mindray Bio-medical Electronics, 2006).

Alanine Aminotransferase (ALT) assay method

ALT catalyzes the transfer of the amino group from alanine to α-ketoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD+ through the activity of ALT present in the sample. The method is linear up to 800 U/L (Hsiang-Li *et al.*, 2016).



Procedure

Samples and reagent (Jouri Lab ALT reagent) were brought to room temperature and vortexed prior to the analysis. After running normal and pathological high control, 37

samples at a time were placed in a fully automated clinical chemistry analyzer (Authochemistry Analyzer-Mindray). The analyzer was programmed for appropriate wavelength, temperature and volume of plasma sample (50 µL) and 1.0 mL of reagents. The analyzer takes appropriate volume of reagent and sample, mix and incubates at 37^{o}C for one minute. Based on the intensity of color compound formed, the automated analyzer reads the absorbance at 340 nm kinetically at one, two, three minutes and determines the concentration (activity) of ALT in the sample by calculating the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

Aspartate Aminotransferase (AST) assay method

AST catalyzes the transfer of the amino group from asparate to α-ketoglutarate with the formation of glutamate and oxaloacetate. The latter is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD+ proportional to the activity of AST present in the sample. The method is linear up to 800 U/L(Hsiang-Li *et al.*, 2016).

$$\text{Aspartae + a-Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate + L-Glutamate}$$

$$\text{Oxaloacetate + NADH + H}^{+} \xrightarrow[\textit{Malate Dehydrogenase}]{\text{MDH}} \text{Malate + NAD}^{+}$$

Procedure

Samples and reagent (Jouri Lab AST reagent) were brought to room temperature and vortexed prior to analysis. After running normal and pathological high control, 37 samples at a time were placed in a fully automated clinical chemistry analyzer. The analyzer was programmed for appropriate wavelength, temperature and volume of plasma sample (50 µL) and 1.0 mL of reagents. The analyzer takes appropriate volume of reagent and sample, mix and incubates at 37^{o}C for one minute. Based on the intensity of color compound formed, the automated analyzer reads the absorbance at 340 nm kinetically at one, two, three minutes and determines the concentration (activity) of AST in the sample by calculating the mean of the results to obtain the average change in absorbance per minute (ΔA/min) (Authochemistry Analyzer-Mindray).(Shenzhen Mindray Bio-medical Electronics, 2006)

Total Bilirubin determination method

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and its absorbance is measured spectrophotometrically at 540 nm. The intensity of color formed is directly proportional to the concentration of bilirubin in the specimen (Kwo *et al.*, 2016). Of the two bilirubin fractions in plasma–bilirubin-glucuronide and free bilirubin which is bound to albumin– only the former reacts directly, while free bilirubin reacts after being displaced from protein by an accelerator. The method is linear up to 20 mg/dL(Wu *et al.*, 1978).

Total bilirubin + Sodium Nitrite + Diazotized Sulfanilic ⟶ Acid Azobilirubin

Procedure

Samples and reagents (Jouri Lab Bilirubin reagents) were brought to room temperature and each plasma was vortexed. Into each of respective tubes the following reagents were pipetted. The reaction mixture was mixed and allowed to stand at room temperature for 2 minutes. The absorbance of sample and standard was read against reagent blank at 540 nm using Cobas integra automated clinical chemistry analyzer (Authochemistry Analyzer-Mindray).(Shenzhen Mindray Bio-medical Electronics, 2006)

Body Mass Index (BMI)

Body Mass Index is a useful clinical calculation to diagnose obesity because it is correlated with total body fat and is relatively unaffected by height. It is most often used to diagnose obesity, but it is equally applicable to defining those who are underweight (Margo and Wood., 2012).

$$BMI = \frac{\text{Weight (Kg)}}{\text{Height}^2\ (m^2)}$$

BMI(Kg/m^2)		
Under weight		<18.5
Normal		18.5-24.9
Overweight		25-29.9
Obesity class	Obesity I	30.0-34.9
	II	35.0-39. 9
	III (Extreme Obesity)	>40

4.9. Data Quality Assurance

- ✓ There was a well-prepared data collection questionnaire to assess participant's demographic and other information.

- ✓ The sample had been taken in aseptic techniques and collected considering proper procedure. The kit had been made free from contamination and kits were checked for consistency. Collected results were checked for completeness on daily basis by the immediate supervisor. Attention in data insertion to software on computer. The completed result was rechecked repeatedly to maintain the quality of data.
- ✓ There was quality control for the clinical chemistry and hematology analyzers which was run daily in the morning before the actual sample running.
- ✓ Standard working protocol was followed for sample analysis.

4.10. Data processing and software used in statistical analysis

Data was checked, cleared and fed into excel and then exported to SPSS (version 25.0, 2018, America) software for statistical analysis. Collected quantitative data was coded, entered to computer, processed, edited, and analyzed using SPSS (25^{th} version) and expressed at 95% confidence interval and the p-value were considered significant at $p < 0.05$. Then data were computed using appropriate statistical methods (mean, standard deviation, p-value) and the results were presented using tables. Clinical and laboratory data were expressed as the mean ± standard error of mean (SE). Differences in the means between the studies group and control group were evaluated by independent samples t-test. The data collected during the current study were recorded and analyzed statistically to determine the significance of different parameters by using SPSS package for windows version 25.0.

4.11. Ethical consideration

Ethical clearance was obtained from Research and Ethical Committee of the Department of Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University after full review was conducted meeting No. DRERC 01/19 attended by the research committee with approval with protocol number M.Sc. 03/19. Structured Questionnaire (attached as appendix) and consent form was prepared with detailed explanation of objectives, risks, and benefits to the study subject and the confidentiality of responses were given to participants. Data were collected after obtaining informed consent and agreement from the patients under study. Sample collection was performed by trained health professionals following ethical steps and procedures.

5. RESULT

5.1. Demographic and clinical characteristics

A total of 188 (94 children diagnosed with TB and 94 children who do not have TB infection and anti-TB medication as control group) were selected for this study. Among the TB infected children, 51(54.3%) were male and 43 (45.7%) were females where as 55 (58.5%) male and 39 (41.5%) females were included in control group. The average age of TB infected children was 9.46± 4.252 years, ranging between 1-15 years, and for the control group it was 8.35 ± 4.285 years, ranging from 1-15 years. Among the TB infected children on Anti-TB treatment 8(8.5%) were found to have Severe Acute Malnutrition(SAM) and 22(23.4%) had Moderately Acute Malnutrition (MAM), the remaining 64(68.1%) were normal when their nutritional status was assessed. It was also observed that 24(25.5%) of the TB infected children were in the intensive phase of treatment and the remaining were in the continuous phase; out of which 12(12.8%) were TB-HIV co-infected children treated with both anti-TB and ART drugs.(Table-7)

Table-7:- ***Socio-demographic data of TB infected children and Control groups in the selected General hospitals***

Characteristics	TB infected children N(%)	Control Group N(%)
Gender	**94(100)**	**94(100)**
Male	51(54.3)	55(58.5)
Female	43(45.7)	39(41.5)
Age	**9.46±4.249**	**8.35±4.285**
0-5	20(21.3)	23(24.5)
5-10	21(22.3)	30(31.9)
10-15	53(56.4)	41(43.6)
BMI	**18.964±1.9616**	**20.493±1.2543**
<18.5	30(31.9)	0(0)
18.5-24.5	64(68.1)	94(100)
>24.5	0(0)	0(0)
Nutritional status		
SAM	8(8.5)	0(0)
MAM	22(23.4)	0(0)
Normal	64(68.1)	94(100)
Family TB infection history		
Yes, there is.	30(31.9)	0(0)
No, there isn't.	23(24.5)	94(100)
We don't no.	41(43.6)	0(0)
Registration group		

New	94(100)	--
Retreatment	0(0)	--
Unknown	0(0)	--
ATT Duration		
Intensive Phase	24(25.5)	--
Continuous Phase	70(74.5)	--
TB-HIV con-infection	**12/94(12.8)**	**0/94(0)**
Male	6/12(50)	0(0)
Female	6/12(50)	0(0)

Hemoglobin and serum level of ALT, AST, and Bilirubin in blood samples of TB infected children and control groups were significantly different. Hemoglobin level of TB infected children and control groups of this study were significantly different ($P<0.05$) (95% CI (-0.9458--0.2585)). The average hemoglobin level of TB infected children was 12.78±1.35 g/dL whereas, those of the control group, was 13.38±1.02 g/dL. Similarly, serum ALT level in TB infected children and control groups was also significantly different ($P<0.05$) (95% CI (10.828-17.214)). ALT level in blood samples of TB infected children was 45.71±14.555IU/L, whereas in control groups, it was 31.69±5.8681IU/L. Total Bilirubin levels of TB infected children and control groups was also significantly different ($P<0.05$) (95% CI (0.1954-0.4514)). Total Bilirubin in blood samples of TB infected children was 1.163±.5931mg/dL whereas; in control groups, it was 0.839±0.2096mg/dL. (**Table-8**)

Table-8:- *Whole blood Hemoglobin concentration and Serum enzymatic levels (ALT,AST, Bilirubin) of TB infected children(N=94) and control groups (N=94) in the selected General hospitals.*

Parameters	TB infected Children Mean ± SD	Control Groups Mean ± SD	Mean Diff.	P-value	95% CI
Hemoglobin	12.781±1.3475	13.383±1.0183	-0.6021	0.001**	-.9458- -.2585
ALT	45.71±14.555	31.69±5.868	14.021	0.001**	10.828-17.214
AST	49.64±17.441	34.85±5.931	14.787	0.011*	11.039-18.536
Direct Bil	.276±.2652	.230±.0878	0.0463	0.110	-.0106- -.1031
Total Bil	1.163±.5931	.839±.2096	0.3234	0.001**	.1954-.4514

*The mean difference is significant at $p \leq 0.05$

**The mean difference is significant at $p \leq 0.01$

Table-9 also shows that there is significant difference on Hemoglobin and serum level of ALT, AST, and Bilirubin in blood samples of TB infected children without HIV and TB-HIV co-infected children. Hemoglobin level of TB infected children without HIV and

TB-HIV co-infected children were significantly different (P<0.05) (95% CI -2.58- -1.05)). Hemoglobin in blood samples of TB infected children without HIV was 12.98±1.28g/dL whereas, in the TB-HIV co-infected children, it was 11.16±0.94g/dL. Similarly, serum level of ALT in TB infected children without HIV and TB-HIV co-infected children were also significantly different (P<0.05) (95% CI (43.27-64.44)). ALT level in blood samples of TB infected children without HIV was 41.23±11.52IU/L whereas, in TB-HIV co-infected children, it was 96.08±38.85IU/L. Total Bilirubin level of TB infected children without HIV and TB-HIV co-infected children was also significantly different (P<0.05) (95% CI (0.7067-1.3083)). Total Bilirubin in blood samples of TB infected children without HIV was 1.034±0.4874mg/dL whereas, in TB-HIV co infected children, it was 2.042±0.5089mg/dL. (**Table-9**)

Table-9:- *Whole blood Hemoglobin concentration and Serum enzymatic levels (ALT,AST, Bilirubin) of TB-HIV co-infected children (N=12) and TB infected children without HIV(N=82) in the selected General hospitals.*

Parameters	TB infected Children without HIV Mean ± SD	TB- HIV co infected Children Mean ± SD	Mean Diff.	P-value	95% CI
Hemoglobin	12.978±1.2793	11.158±.9385	-1.8197	0.000**	-2.5830- -1.0564
ALT	41.23±11.523	96.08±38.85	53.852	0.000**	43.266-64.437
AST	46.12±11.934	80.50±18.218	35.378	0.000**	27.490-43.350
Total Bil	1.034±0.4874	2.042±0.5089	1.0075	0.000**	0.7067-1.3083
Direct Bil	0.221±0.1646	0.675±0.4413	0.4543	0.004**	03210-0.5876

**The mean difference is significant at p. value ≤0.01

Serum enzyme levels of ALT and total Bilirubin of TB infected children with or without HIV infection are shown in Table-10. ALT and Total Bilirubin were normal in 50(53.2%) and 2(2.1%) of TB infected children among without HIV and TB-HIV co-infected children respectively; whereas, 29(30.9%) of TB infected children without HIV and 5(5.3%) of the TB-HIV co-infected children show elevation but less than three times of the upper limit of the normal (reference) value and 3(3.2%) of TB infected children without HIV and 5(5.3%) of TB-HIV co-infected children show elevation of this enzyme more than three times of ULN. Total bilirubin were show elevation more than two times

of the ULN in 9(9.6%) of the TB infected children without HIV and 6(6.3%) of the TB-HIV co-infected children.**(Table-10)**.

Table-10:- *Serum enzymatic level of ALT and Total Bilirubin of TB infected children without HIV (N=82) and TB-HIV co-infected children (N=12) in the selected General hospitals*

Parameter		HIV condition	Total Bilirubin <1 N(%)	1-2 N(%)	≥2 N(%)	Total N(%)
ALT	<42	Yes	2(2.1)	0(0.0)	0(0.0)	2(2.1)
		No	48(51.1)	2(2.1)	0(0.0)	50(53.2)
		Total	**50(53.2)**	**2(2.1)**	**0(0.0)**	**52(55.3)**
	42-125.9	Yes	0(0.0)	4(4.3)	1(1.1)	5(5.3)
		No	6(6.3)	17(18.1)	6(6.3)	29(30.9)
		Total	**6(6.3)**	**21(22.3)**	**7(7.4)**	**34(36.1)**
	≥126	Yes	0(0.0)	0(0.0)	5(5.3)	5(5.3)
		No	0(0.0)	0(0.0)	3(3.2)	3(3.2)
		Total	**0(0.0)**	**0(0.0)**	**8(8.5)**	**8(8.5)**
	Total	Yes	2(2.1)	4(4.3)	6(6.3)	12(12.8)
		No	54(57.4)	19(20.2)	9(9.6)	82(87.2)
		Total	**56(59.5)**	**23(24.5)**	**15(15.9)**	**94(100.0)**

5.2. Laboratory DILI and Anemia evaluation test results

Of the 94 TB-infected children, 12 of which were TB-HIV co-infected, tested for different laboratory tests. These test evaluations were done according to the SOPs protocol of the Adigrat General hospital laboratory department and the guide manual of manufacturer of the clinical chemistry machine used. Every children in this study was checked whether if they had had abnormality of liver function and hemoglobin concentration using serum enzymatic level measurement and whole blood test. After ALT, AST, Direct Bilirubin, Total Bilirubin and Hemoglobin were done for TB infected without HIV and TB-HIV co-infected children the following results were found.**(Table-11& Table-12)**

Table-11:- *ALT, AST, Bilirubin(Direct and Total) and Hgb laboratory evaluation test results in TB infected children without HIV in the selected General hospitals.*

No.	Code	ALT(IU/L)	AST(IU/L)	Dir Bil(mg/dL)	Tot Bil(mg/dL	Hgb(mg/dL)
1	HACS-001	37	36	.1	.8	13.0
2	HACS-002	56	68	.2	.8	13.3
3	HACS-004	55	68	.3	1.3	11.0
4	HACS-005	44	48	.3	1.2	11.5
5	HACS-006	56	52	.1	.7	11.3
6	HACS-008	39	37	.1	.8	14.0
7	HACS-009	127	97	1.3	2.3	13.0
8	HACS-010	41	37	.1	.6	11.5
9	HACS-011	39	41	.2	.8	14.0
10	HACS-012	40	37	.1	.9	13.0
11	HACS-013	67	68	.3	1.4	12.3
12	HACS-014	128	94	1.6	2.3	11.0
13	HACS-016	41	40	.2	.9	13.0
14	HACS-017	40	50	.2	.7	12.7
15	HACS-018	39	34	.1	.8	13.0
16	HACS-019	53	55	.3	1.1	11.3
17	HACS-020	59	63	.5	2.3	11.3
18	HACS-021	40	47	.1	.8	13.0
19	HACS-022	55	77	.4	2.0	11.7
20	HACS-023	28	36	.1	.6	13.7
21	HACS-024	40	48	.2	.8	13.0
22	HACS-025	41	41	.2	.7	14.0
23	HACS-026	53	61	.3	1.3	11.0
24	HACS-027	126	76	1.2	2.1	10.0
25	HACS-028	64	65	.3	1.6	12.7
26	HACS-030	54	55	.4	2.3	11.0
27	HACS-031	42	43	.2	.9	13.0
28	HACS-032	41	35	.3	1.0	13.0
29	HACS-034	43	51	.5	2.2	10.7
30	HACS-035	38	36	.1	.8	13.0
31	HACS-036	56	68	.3	1.2	12.3
32	HACS-037	40	33	.1	.7	14.3
33	HACS-038	38	43	.2	1.0	11.0
34	HACS-039	39	36	.1	.9	13.3
35	HACS-040	51	54	.2	.9	12.7
36	HACS-042	58	68	.3	1.4	10.7
37	HACS-043	41	49	.2	.8	13.0
38	HACS-044	64	59	.2	1.0	14.3
39	HACS-045	39	41	.1	.8	14.7
40	HACS-046	54	60	.3	1.1	10.7
41	HACS-047	38	40	.2	.8	14.3
42	HACS-048	56	66	.2	.9	13.7
43	HACS-050	71	49	.4	1.9	14.7
44	HACS-051	62	77	.4	2.4	10.0
45	HACS-052	48	54	.3	1.4	14.3
46	HACS-053	40	37	.1	.6	14.3

47	HACS-054	40	46	.2	1.0	13.0
48	HACS-055	39	43	.1	.8	13.7
49	HACS-056	27	34	.2	.7	14.7
50	HACS-057	56	68	.3	1.2	12.7
51	HACS-059	38	41	.2	1.1	15.0
52	HACS-061	40	36	.2	.8	13.0
53	HACS-062	37	35	.1	.7	13.3
54	HACS-063	24	27	.2	.8	14.3
55	HACS-064	39	33	.1	.4	15.0
56	HACS-065	34	37	.1	.8	13.0
57	HACS-066	53	66	.4	1.5	11.0
58	HACS-067	39	35	.1	.5	14.7
59	HACS-068	53	58	.3	1.6	13.0
60	HACS-069	26	35	.1	.8	14.7
61	HACS-070	40	35	.1	.6	13.5
62	HACS-071	44	56	.5	2.3	14.0
63	HACS-072	49	56	.6	2.1	10.0
64	HACS-073	48	53	.4	1.4	14.0
65	HACS-074	40	34	.1	.6	13.0
66	HACS-076	41	35	.1	1.0	12.7
67	HACS-077	35	37	.1	.8	15.0
68	HACS-079	41	30	.1	.9	13.0
69	HACS-080	29	36	.2	.6	13.7
70	HACS-081	38	35	.1	.7	14.0
71	HACS-082	44	56	.4	1.4	13.7
72	HACS-083	41	44	.1	.4	13.3
73	HACS-084	41	31	.2	.9	12.7
74	HACS-086	42	36	.1	.7	13.3
75	HACS-087	42	32	.1	.7	12.3
76	HACS-088	42	36	.1	.4	13.0
77	HACS-089	39	36	.1	.5	14.0
78	HACS-090	41	48	.1	.6	13.3
79	HACS-091	42	36	.2	.8	13.7
80	HACS-092	42	37	.1	.7	14.0
81	HACS-093	38	38	.1	1.0	13.7
82	HACS-094	39	35	.1	.8	14.3

Table-12:- *ALT, AST, Bilirubin(Direct and Total) and Hgb laboratory evaluation test results in TB-HIV co-infected children in the selected General hospitals.*

No.	Code	ART	ALT(IU/L)	AST(IU/L)	Dir Bil(mg/dL)	Tot Bil(mg/dL	Hgb(mg/dL)
1	HACS-003	Yes	126	93	1.2	2.4	9.0
2	HACS-007	Yes	63	66	.4	1.3	11.1
3	HACS-015	Yes	40	32	.1	.9	11.3
4	HACS-029	Yes	126	89	.5	2.5	11.3
5	HACS-033	Yes	127	93	.6	2.5	11.0
6	HACS-041	Yes	126	94	.5	2.1	11.3
7	HACS-049	Yes	62	76	.4	2.0	13.3
8	HACS-058	Yes	130	92	.6	2.3	11.3
9	HACS-060	Yes	56	65	.5	2.2	10.7
10	HACS-075	Yes	66	68	.3	1.7	11.0
11	HACS-078	Yes	70	75	.4	1.4	11.3
12	HACS-085	Yes	39	40	.2	.8	11.4

5.3. Evaluation of ALT, AST, Bilirubin and Hemoglobin concentration differences

There was significant difference in laboratory test results among TB-infected children and the control group. AST 59/94(62.8%), ALT 41/94(43.6%), and Total Bilirubin 37/94(39.4%) were elevated in TB-infected children compared to control group (ALT 9/94(9.6%), AST 13/94(13.8%), and Total Bilirubin 9/94(9.6%)). The laboratory test result for hemoglobin concentration was significantly different among the two groups 29/94(30.9%) and 11/94(11.7%) respectively.**(Fig-2)**

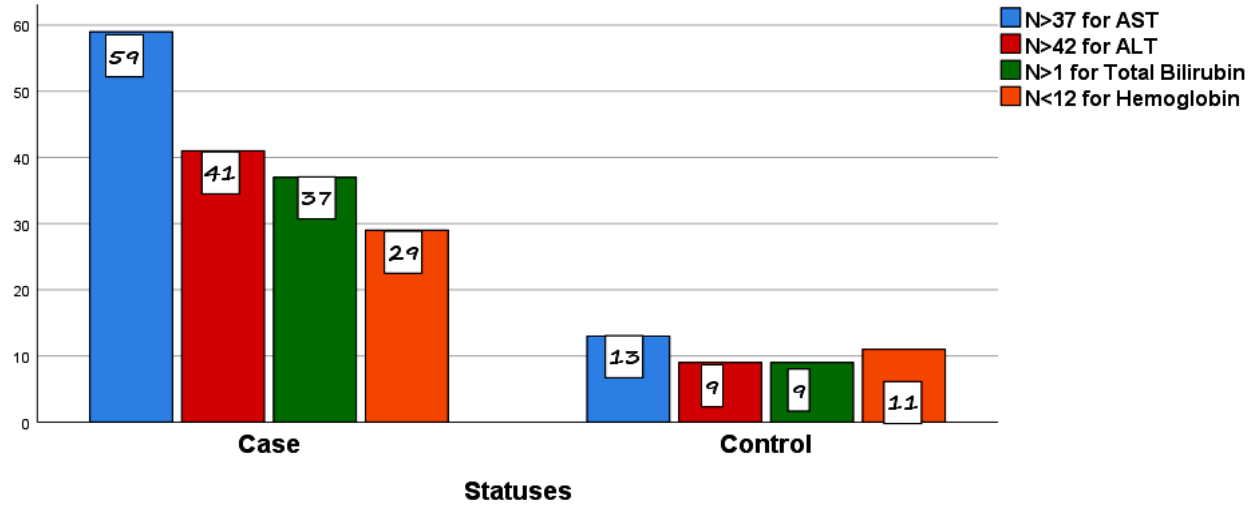


Fig:-2:- Evaluation of laboratory test results difference among cases and controls

Comparison of deviation from Normal limit value of Laboratory tests between TB infected children(Case) and Control groups(Control)in the selected General hospitals

Significant difference in Laboratory test result evaluation among Male and Female TB-infected children, was also obtained. In male TB-infected children elevation of AST and ALT enzymes was recorded as 29/51 (56.8%), and 19/51(37.3%) respectively, Total Bilirubin was increased in 18/51(35.3%) and Direct Bilirubin in 17/51(33.3%). Among females it was 30/43(69.8%) for AST, and 22/43(51.2%) for ALT. Elevated Total Bilirubin was obtained in 19/43(44.2%) and Direct Bilirubin in 20/43(46.5%) among female participants. This study also found that the decrement in hemoglobin concentration was significantly different between Males and Females 14/51(27.45%) and 15/43(34.88%) respectively.**(Fig-3)**

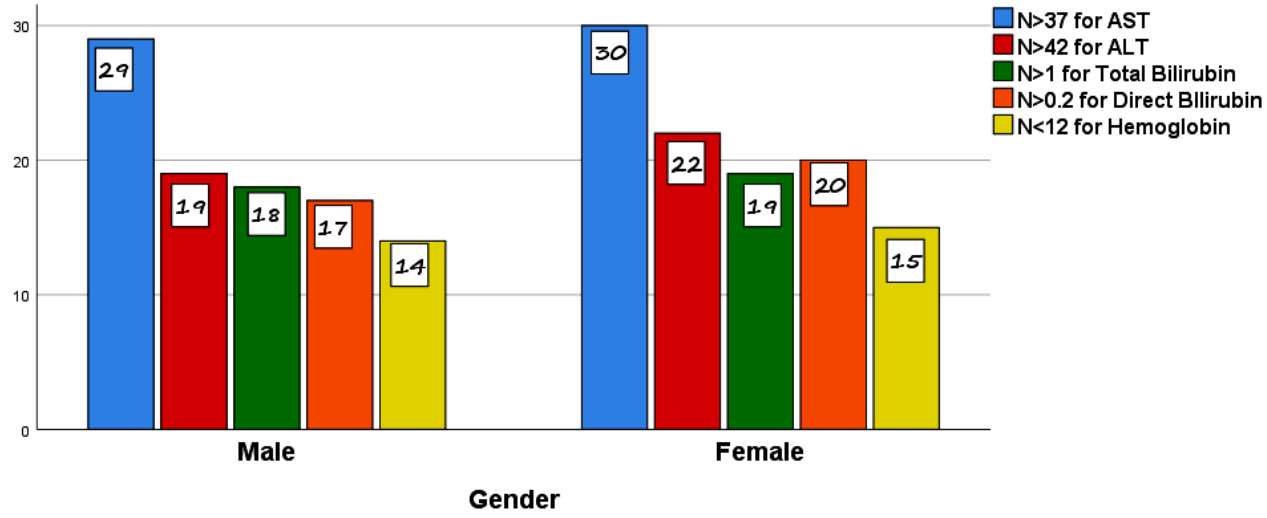


Fig-3:- Evaluation of laboratory test results difference by Gender

Comparison of deviation from Normal limit value of Laboratory tests between Males and Female TB infected children(N=94) in the selected General hospitals

5.4. Prevalence of Adverse Drug Reactions

Even thought the adverse drug reactions are more prominent in adults as it was mentioned by different scholars (Carroll *et al.*, 2012; Bezu *et al.*, 2014; Sahu *et al.*, 2015; Lee *et al.*, 2016; Nagarajana and Whitaker, 2018) but it is not also ignorable in children. Gastrointestinal (GI) related adverse events were the most common ADRs. Among the 94 TB infected children Anorexia was present in 18(19.1%), Nausea and vomiting in 11(11.7%), Headache in 9(9.6%), Sleeping disturbance 13(13.8%), Abdominal pain in (3.2%), and Seizure in1(1.1%).**(Table-13)**

Table-13:- *Prevalence of Adverse Drug reactions caused by ATT drugs in males and Female TB infected children in the selected General hospitals.*

Adverse Drug Reactions	Total Number N(%) N=94	Male N(%) N=51	Female N(%) N=43
Anorexia	18(19.1)	8(8.5)	10(10.6)
Nausea and vomiting	11(11.7)	4(4.3)	7(7.4)
Arthralgia	10(10.6)	4(4.3)	6(6.3)
Headache	9(9.6)	4(4.3)	5(5.3)
Pruritus	7(7.4)	3(3.2)	4(4.3)
Dizziness	4(4.3)	1(1.1)	3(3.2)
Sleeping disturbance	13(13.8)	5(5.3)	8(8.5)
Abdominal pain	3(3.2)	1(1.1)	2(2.1)
Dehydration	2(2.1)	--	2(2.1)
Seizure	1(1.1)	--	1(1.1)

5.5. Potential risk factors (Age, Gender and ATT/ART parallel use)

Comparison was made on the prevalence of DILI in age category <5years, 5-10years and 10-15years of age to observe effect of different age category in the abundance of the DILI events. The result of this study showed that the proportion of DILI found in 5-10years and 10-15years was 4.76% and 13.21% respectively. Prevalence of anemia was also compared in the same age category to observe the effect of different age categories in the abundance of the anemia events, our result showed that the proportion of anemia found was 10% in the age group of <5 years, 66.67% in the age group of 5-10years and 24.53% in the age group of 10-15years of age.(Table-14)

Table-14:- *Prevalence of DILI and Anemia by age category in the selected General hospitals*

Age	Number tested	Occurrence of DILI(%)	95% CI	Occurrence of Anemia(%)	95% CI
0-5	20	0(0)	-0.957-1.158	2(10)	1.581-13.22
5-10	21	1(4.76)	0.876-6.321	14(66.67)	0.897-14.53
10-15	53	7(13.21)	2.021-14.69	13(24.53)	0.48, 8.63
Total	94	8(8.5)	5.311-12.54	29(30.9)	1.098-13.25
X^2= 0.89 OR(1.12), P >0.05				X^2= 0.93 OR(1.01), P >0.05	

Gender assessment for the association of DILI was found to be 3.92% in Males and 13.95% in Females. Association of gender with anemia was also studied and the percentage was 27.45% in Males and 34.88% in Females (Table-15).

Table-15:- *Prevalence of DILI and Anemia by gender category in the selected General hospitals.*

Gender	Number tested	Occurrence of DILI (%)	95% CI	Occurrence of Anemia(%)	95% CI
Male	51	2(3.92)	0.021-0.581	14(27.45)	0.58-0.87
Female	43	6(13.95)	0.041-0.745	15(34.88)	0.39- 6.38
Total	94	8(8.5)	0.012-1.21	29(30.9)	0.30-1.11
	X^2= 1.68 OR(2.241), P >0.05			X^2= 1.81 OR(1.6), P >0.05	

The association of DILI and ART concomitant to Anti-Tuberculosis treatment was also found to be 41.67% (5/12) during their treatment follow up. The association of anemia and ART concomitant to anti-tuberculosis treatment was also investigated as 91.67%(11/12) during their treatment follow up (Table-16).

Table-16:- *DILI and Anemia associated with ATT/ART concurrent use among TB-HIV co-infected children in the selected General hospitals*

ART/ATT Status	Number tested	Occurrence of DILI(%)	95% CI	Occurrence of Anemia(%)	95% CI
ART/ATT drugs co-infected	12	5(41.67)	0.12-0.95	11(91.67)	0.271-11.35
Treated with ATT	82	3(3.66)	0.02-0.56	18(21.95)	0.47-0.921
Total	94	8(8.5)	0.01-0.41	29(30.9)	0.21-01.12
	X^2= 1.76 OR(2.4), P<0.05			X^2= 1.87 OR(3.624), P<0.05	

6. DISCUSSION

TB infection is the most common communicable disease worldwide and has a high prevalence in developing countries; it is common in Ethiopia. The patients have been reported to be associated with higher incidence of abnormal liver function tests (LFT) compared to the individuals without TB infection, and elevated ALT being the most common abnormality (EMoH, 2012).

The liver is vulnerable to injury from drugs and many other toxins because of its unique anatomy and physiology. The liver has many functions that includes: secretary, synthetic, metabolic and detoxifying roles. In its metabolic roles, it transforms some drugs into toxins that can cause injury to hepatocytes (Neuman, 2019).

In this institutional based cross sectional study with comparative nature, prevalence and associated factors of DILI among TB infected children with and without HIV that attend in some randomly selected general hospitals of Tigray, Ethiopia were studied. By using Hy's rule, prevalence of DILI was found to be 8.5 % of the TB infected participants. The prevalence of DILI was higher in children having TB-HIV co-infection.

In the present study, serum levels of ALT, AST, Bilirubin and whole blood test for hemoglobin measurement were assessed in searching the prevalence of DILI and anemia among TB infected children. Those serum and whole blood parameters were studied on 188 (94 TB infected children and 94 age-sex matched apparently healthy children as control group) participants.

The current study revealed that significantly higher serum levels of ALT, AST, and Bilirubin were observed in TB infected children compared to serum samples of the control group with no TB infection. This finding of elevation in serum enzymatic level agreed with studies done by other scholars such as Peter (2011); Aishatu *et al*., (2017); Sultan *et al.,* (2018); Gafar *et al.,* (2019).

ATT drugs can affect the liver, and although no patient in this study presented with jaundice, elevated liver enzymes at baseline was found in about one-third of the study participants. ALT, AST or Bilirubin singly or in combination were elevated in patients taking ATT. The range of ALT, AST and Bilirubin level recorded in the patients at 1 up to 6 months of therapy was different. No patient had jaundice at presentation or during the course of treatment. This is important as jaundice is used as a clinical sign for referral of patients on ATT from lower level facilities to higher levels of care. The present finding

supports the fact that drug induced hepatotoxicity in children receiving ATT is uncommon despite the frequent finding of elevated LFT at baseline, and it is consistent with the literature which shows that incidence of drug-induced hepatotoxicity in childhood is between 3% and 10%; severe hepatotoxicity is documented below 3% in children (Ohkawa *et al.*, 2002; Saukkonen *et al.*, 2006; Devrim *et al.*, 2010; Bhatia *et al.*, 2011; Peter, 2011).

This finding is important as it will provide a basis to counsel patients for ATT. In addition, this can allay the anxiety among those who care for children with TB. While this may not be enough to make recommendations on monitoring hepatotoxicity in children, it will provide data for systemic reviews for future reference on monitoring.

Even though routine monitoring of LFT will increase the cost of TB treatment and may create significant financial barrier to care, for safety reasons of liver injury may occur in children, it could be done when necessary. There are calls for early monitoring of LFT in patients to reduce cases of hepatotoxicity (Shu *et al.*, 2013; Lee *et al.*, 2016).

Generally, the result of this study indicates that drug induced hepatotoxicity on TB infected children was 8.5% and TB related anemia was also found to be 29/94 (30.9 %). This finding of serum enzymatic level elevation was supported by Parthasarathy *et al.*, (1986); Ohkawa *et al.*, (2002); Akura *et al*., (2009); Harshad *et al.,* (2011) studies done in different countries reported that children who were taking Anti-TB drugs developed drug induced liver injury. They found that Anti-TB drugs have positive and significant effect on developing DILI which is similar to findings by Lucena *et al.,* (2009) done in Spain. This scholar reported that ATT has positive and significant effect on DILI.

This result also supported by Mugasi *et al.,* (2009), which found that Anti-TB and/or ART drugs have significant and positive effect on developing drug induced hepatotoxicity.

The prevalence of Anemia found in this study was also supported by a study done in Korea by Sei *et al.,* (2006) that reported 31.9% of TB patients were anemic.

Associated risk factors

Age has been one of the risk factors affecting the development and frequency of DILI occurrence in TB patients during treatment. In the present study prevalence of DILI based on age was 0/20(0%) in 0-5 years, 1/21(4.76%) in 5-10 years, and 7/53(13.21) in 10-15 years. However, the difference is not statistically significant (P-value>0.05). This finding

is in agreement with the work done by (Vycke *et al.*, 2019). The slight difference in the prevalence of DILI in this study could be a significant reduction in clearance rate of metabolized drug agents by the cytochrome-P450 enzyme and/or changes in the hepatic blood flow distribution into liver, as age increases (Aka *et al.*, 2017).

In this study, prevalence of DILI based on gender was 13.95% in female and 3.92% in male. This finding is in agreement with studies done by Mansukhani and Shah, (2012) and Vycke *et al.*, (2019) and assures that gender has some association with the occurrence of DILI in TB infected patients under anti-tuberculosis treatment with risk factor of 1.5-1.7-fold greater risk of developing DILI for females compared to male patients (Mansukhani and Shah, 2012; Vycke *et al.*, 2019). The high prevalence of DILI found in female than male patients in this study could be explained due to anatomical difference; females are less muscular than males. Despite this variation, the tradition of ATT dosage usually is given without normalizing by body weight, rather simply put a bench mark above and below would receive the same dosage. Consequently, high dose, a dose beyond the recommended might be given to women in comparison to men and as a result the more DILI may occur in females (Mansukhani and Shah, 2012). In addition, the glomerular filtration rate in female is slower than male. Therefore, drugs eliminated through the urinary system might be delayed in female than male and as a result more insult will happen to the proximal tubules in the kidney (Chakkera *et al.*, 2018; Nawaz *et al.*, 2019)**.**

ATT/ART concurrent therapy for HIV-TB co infected children were reported as risk factor for poor outcomes of anti-TB treatments and this has great contribution in the development of adverse drug reactions such as hepatotoxicity (Belay and Wubneh, 2020). In this study the prevalence of DILI based on ATT/ART drugs treatment was 41.67% compared to 3.66% treated with ATT only. ART drugs are capable of inducing hepatotoxicity. Some of them (e.g. EFV) are also able to involve in drug-drug interactions with ATT drugs (Abongwa *et al.*, 2019). Therefore, the increased DILI prevalence observed in the current study may be due to contribution of ART drugs effect on liver. Anemia is also higher in females compared to males and in co-infected children when it was compared with the TB infected children without HIV and it was in agreement with findings of many scholars (Zelalem and Bamlaku, 2014; Bamlaku *et al.*, 2015; Muluken *et al.*, 2015; Eyuel *et al.*, 2016; Feven *et al.*, 2018)**.**

7. CONCLUSION

In conclusion, this study identified prevalence of DILI and Anemia (8.5 % and 30.9%, respectively) among TB infected children with and without HIV that attend some randomly selected general hospitals of Tigray, Ethiopia.

Serum liver enzymatic levels of ALT, AST, Bilirubin and Hemoglobin concentration among TB infected children with and without HIV were determined. In conclusion, ALT, AST, and Bilirubin tests were significantly increased in serum of TB infected children and whole blood Hemoglobin concentration among TB infected children with and without HIV were decreased. Furthermore, low nutritional status as determined by low BMI, TB infection history in family and having HIV infection had a higher risk for DILI among TB infected children than those who had normal nutritional status, no TB infection history in family and those negative for HIV.

ALT, AST, and Bilirubin play critical role in following up the progress and regulation of the adverse drug effects like DILI of TB infection in children.

This work confirms that anti-TB and/or ART drugs were found to have an effect in increasing serum liver enzyme levels (ALT, AST and Bilirubin) and inducing liver injury and lowering hemoglobin concentration that results in anemic condition. The effects were found to be prominent in children who were taking both anti-TB and ART drugs in comparison with the group who were not taking ART drugs. Similarly, serum liver levels (as indicators of DILI) were also elevated and hemoglobin concentration was lowed in female TB infected children when compared with male TB infected children.

8. RECOMMENDATION

Forwarded recommendations are:-

- Further study is needed with large sample size to investigate anti-TB and/ or ART drugs induced liver injury and anemia among TB infected children with and without HIV in our country which must include techniques such as histopathological, pathological, anatomical parameters, immunohistochemistry (to detect genetic expression) and sequencing of the gene among TB infected children with and without HIV in comparison to control groups to understand more specific effects of the drugs.
- Clinicians should follow TB infected children with and without HIV regularly, in order to understand the progressive biochemical and hemoglobin changes within the duration of treatments.
- Continuous and regular evaluation of serum enzymatic levels and hemoglobin concentrations of TB infected children with and without HIV should be performed.
- Chemoprophylaxis for those children who are HIV positive as most of them could harbor smear-negative TB should be routine.
- Drug compliance by the children taking anti-TB and/ or ART drugs should be highly monitored and interventions should be taken during treatment
- Pediatric formulation of these drugs is important to prevent breakup from follow up of the treatments.

9. Strength and Limitation of the study

Strength of the Study

Assessment of drug induced liver injury among TB infected children is attempted for the first time in Tigray region as well as in Ethiopia. It is hoped that assessment of serum enzymatic level of ALT, AST and Bilirubin should favorably used to follow up the progress of the disease and to monitor the treatments. Therefore, this study is expected to provide the baseline information for further studies of drug induced hepatotoxicity among TB infected children in Ethiopian hospitals, in relation to ART drugs.

Limitation of the study

The limitations in the present study include:-

- Small study population, lack of histopathological studies on the liver, and sequential pathological and anatomical studies on liver functions during the drug regimen could not be undertaken.
- Also, this short cross- sectional study could not follow up the patients, who were taking anti-TB and/ or ART drugs for long duration of biochemical and enzymatic progression due to limitation of money and time.
- Absences of tests for Hepatitis A& E to assess their comorbidity effect on liver of TB children
- The compliances of children in the study to the drugs and the regimen of treatment could not be ascertained.

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APPENDEX

A) Information sheet in English Version

Title of the Research Project: assessment of drug induced hepatotoxicity and anemia among TB infected children with and without HIV attending in some randomly selected general hospitals in Tigray, Ethiopia.

Principal Investigator: Efriem G/yohannes (BSc, MSc candidate)

Name of the Organization: Addis Ababa University College of Health Sciences School of Medicine Department of Biochemistry.

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, two advisors from Addis Ababa University biochemistry department. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to assess drugs induced Hepatotoxicity and Anemia among TB infected children at some randomly selected general hospitals of Tigray, Ethiopia so that we will suggest best strategy for treatment option of TB and HIV.

Procedures and what will be expected from you for participation

In order to perform the indicated study at the five hospitals of Tigray, Ethiopia, you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required clinical sample will be collected by a principal investigator and nurses. Then, you are requested to give your consent to the sample collector. After consent, 5mL blood specimen will be collected from you by specimen collector and face to face interview for additional questions.

Potential risks and Discomforts

There will be minor discomfort during blood specimen collection. During collection of specimen from you, appropriate precaution will be taken and all samples will be collected

by trained health professionals. If anything happened, appropriate medical care will be provided to you.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. But based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial for the detection and managing of the diseases. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is completely voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result for free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

Exclusion criteria*(Case- TB-infected children, Control- TB-non infected children)*

- ✓ Hepatitis B and/or C infection
- ✓ Confirmed Biliary obstruction & Congestive Hepatomegaly
- ✓ History of hepatotoxic drug intake
- ✓ Confirmed Liver abscesses or nay focal lesions
- ✓ Any other apparent cause for the elevation of liver chemistries

Efriem G/yohannes **Phone:** +251913520097 **E-mail:** *efriemg@gmail.com*

B) Information sheet in Amharic version

የተሳታፊዎች ፈቃድና ሙተማመኛ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡፡ እባክዎ በዚህ ጥናት ለማሳተፍ ከማመማሙዎ በፊት ከዚህ ቀጥሎየ ሚ ኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነ ልዎትን ማነኛዉምሃሳብ ይጠይቁ፡፡

መግቢያ

የጥናቱ ርዕስ assessment of drug induced hepatotoxicity and anemia among TB infected children with and without HIV attending in some randomly selected general hospitals in Tigray, Ethiopia.

የጥናቱ አላማህፃናት የቲቢ ህመምተኞችን ላይ በሽታውን ለማታተል(የ ሜዳን ሂደቱን ለመ ት) የ ሚከቅምወይምየ ሚያስችል አዲስ ጥናት ነ ው፡፡

እናምእርስዎ በዚህ ጥናት ለማሳተፍ ጠቀሜ ምቹ ሆነ ውተመ ጠዋል፡፡ የእርስዎ በዚህ ጥናት ላይ የ ሚኖርዎት ተሳትፎ ማሉ በማሉ በበጎ ፈቃደኝነ ት ላይ የተማኅረተ ነ ው፡፡ በዚህ ጥናት ዉስጥ ላለማሳተፍ ወይም ለማሳተፍ ከወሰኑ በኃላ ለ ማቋረጥ የ ሚወስኑ ቢሆን ምእንኩዋ በዚህ ሆስፒታል የ ሚሰጡዉማንኛዉምአገልግሎት አይቋረጥም ፡ በጥናቱ ለማሳተፍ የ ሚስማሙከሆነ የስምማ ት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡

የጥናቱ ተሳታፊ ለሚሆን የ ሚጠበቅበዎት ምንድን ነ ው?

በዚህ ጥናት ለማሳተፍ የ ሚስማሙከሆነ የደምናመኛ እንደሚወሰድና ለጥናቱ እንዲሚዉል ማስማሙት ይጠበቅብዎታል፡፡ ከተወሰደዉናመኛ ላይ የ ሚ ኙ ሚጃዎች ከዚህ ሆስፒታል ዉጭለ ሚ ኙና ለስራዉአግባብነ ት ላላቸዉሰዎች ቢነ ገር የ ሚይቃወሙሚሆኑን ማስማሙት ይጠበቅብዎታል፡፡ ይሁን እንጅ ይህ አይነቱ ሚጃ የርስዎን ማንነ ት የ ሚልጡ ሚጃዎችን ማለትም ስም፤ አድራሻና የስልክ ቁጥር የ ማሰሉትን ሚጃዎቸን አይጨምር ፡ ይልቁንምለዚህ አገልግሎት ብቻ የ ሚዉል አርስዎን ለ ማወቅ የ ሚያስችል ማለያ ቁጥር ጥቅምላይ እንዲዉል ይደረጋል፡፡ በተጨማሪ ምስለርስዎ አጠቃላይ የጤና ሁኔታ ለ ማቋርቡአንዳንድ ተጨማሪ ጥያቄዎች ሜልስ ማነጋት፡፡

በዚህ ጥናት ማሳተፍ የ ሚያስከትላቸዉችግሮች ምንድን ናቸዉ?

ናመኛ በ ሚሰበሰብበት ወቅት ምንምአይነ ት የከፋ ችግር አያጋጥምምት ፡ ነገር ግን ደም ሲወሰድ ማነ ኛ የህመም ስሜት ሊያስከትል ይችላል፡፡ ሆኖም ግን ናመኛዉን ለማነብሰብ ልምድ ያለዉ ባለ ሞያ ስለ ሚሞዉና አስፈላጊዉ የጥንቃቄ እርምጃ ስለ ሚወሰድየህመምስሜት አይኖርም ፡

የህክምና ሚጃ በ ሚስጥር ተጠብቆ ማቆየ ት የ ሚቻለዉእንዴት ነ ዉ?

ስለራስዎ የሰጡት ማንኛውምመረጃና ከተወሰደዉናማኛ ላይ የተገኘዉየላቦራቶሪ ዉጤት የሚመለዉለጥናቱአላማብቻነዉ፡ ይህን ማህደር ሊያገኙየሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸዉ፡ ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለውየኮምፒውተር የሚጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል ፡፡

በዚህ ጥናት ማሳተፍ የሚያስገኛቸውጥቅሞች ምንድን ናቸው?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ ማስን በዚህ ጥናት በማሳፈልዎ በገንዘብ የሚያገኙት ጥቅምባይኖርምከጥናቱ በሚገኘውውጤት ግን ተጠቃሚነዎት፡ ፡ የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎትን በሽታ ለማወቅና ለማከታተል ከፍተኛ ጥቅምይኖረዋል፡ ፡

በዚህ ጥናት ተሳታፊ የሚሆንዎ ማብቶች ምንድን ናቸው?

በዚህ ጥናት ማሳተፍ ማሉ በማሉ በእርስዎ ፈቃደኝነት የተማሳረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ማሉ ማብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማገለልዎ ምክንያት የማቋርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም ፡ ፡ ከዚህምበተጨማሪ ጥናቱን በተማላከተ ማንኛውንምአይነት ጥያቄ የማከየቅና ገለጻ የማገኝት ማብት አለብዎት፡ ፡ የላብራቶሪ ምርማራ ውጤቱንምበነጻ ማገኝት ይችላሉ፡ ፡ ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለማላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለማቋርብልዎት ጥያቄ ቀጥተኛ ሜልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን፡ ፡

ጥያቄ ካለኝ ወይምችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተማላከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይምጥያቄ ካለዎት በሚማላከተውአድራሻ ይጠቀሙ ፡

በዚህ ጥናት ማሳተፍ የሚያስችሉ ሁኔታዎች

- የጉበት B እና C ቫይረስ በሽታ
- የተረጋገጠየሃሞት ከረጢት ችግርና የጉበት እብጠት
- የጉበት በሽታ ማድሃኒት ማውሰድ
- የተረጋገጠየጉበት ፈሳሽ ማቋጠርና ቁስለት ማኖር
- ማንኛውምየጉበት ምርማራ ውጤት ሊያሳስት የሚያስችል ሁኔታ

ኤፍሬም ገ /ዮሃንስ ማባይል፡ +251-913-520-097 ኢሜል፡ efriemg@gmail.com

C) Informed consent(child or Guardian) form in English version

Code number…..……

I/I as a guardian had been informed that the objective of this study is to assess drug induced Hepatotoxicity and Anemia among TB infected children with and without HIV at some randomly selected general hospitals of Tigray, Ethiopia.

The results of this study have an importance to treat me/my child and other patients, and to be used as an input for the future development of strategies or guidelines for follow up and treatment of TB and HIV in Ethiopia. I had been also informed about the confidentiality of this study. The principal investigator requested me/my child to participate in the study that would require my willingness to provide the required data that include blood sample and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my/my child's benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent/on behalf of my child for providing the requested information and specimens as the doctors find best for me/my child.

Signature: _____ Date_____

D) Informed consent(child or Guardian) form in English version

የተሳታፊዎች ስምምነት ሚገልጫ

የሚስጥር ቁጥር ----------

የተሳታፊዉስም----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ/የልጅ ሞግዚት "assessment of drug induced hepatotoxicity and anemia among TB infected children with and without HIV attending in some randomly selected general hospitals in Tigray, Ethiopia." በሚል ጥናት ላይ በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም የደም ናሙና እንደሚያስፈልግ ተገልጾልኛል፡፡ የጥናቱንምአላማዎችምተረድቻለሁ፡፡

በማከይቁ ላይ የገለጽኳቸው ሚጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለማሳተፍና ማነኛውንም ሚጃ ያለማስጠት እንዲሁም በማነኛውምጊዜ ከጥናቱራሴን የማገለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡ ስለዚህ ለዚህ ጥናት ሚጃና የስምምነት ቃሌን የሰጠሁት በአጭቃላይ ሁኔታውን በሚዳትና በፍጹምፍቃደኝነት ነው፡ በተጨሜምጥያቄ ለማከየቅ ተፈቅዶልኝ ለሙከቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡፡ የዚህ ጥናት ተሳታፊ በሚሄ የማኘው ጥቅምየሁሉንምምር ሚራ ውጤት በነጻ ማገኘት እንደሆነ ተረድቻለሁ፡፡

በአጠቃላይ እኔ/ልጄ ከላይ በሙሙሙኛ ቅፅ የተጠቀሱትን ሁሉ በሚባና በተረጋጋ መነፈስ አንብቤዋለሁኝ፡፡ ስለዚህ በዚህ ጥናት ለማሳተፍ ፈቃደኛ ሚሄን በፊርሜ አረጋግጣለሁ፡፡

ፊርማ---------- ቀን ----/---/----------

(የስምምነት ቅጹን ማነበብለሚይችሉ ተሳታፊዎች)

የአማካሪ ነርስ ስም---------- ፊርማ----------

ቀን----------

E) QUESTIONNAIRE (please put ✗ mark/result for tests in the space given)

Biological characteristics					
Age (years)	< 5				
	5-10				
	10-15				
Gender	Male				
	Female				
Nutritional Status	SAM				
	MAM				
	Normal				
BMI	Low Body Weight				
	Normal Body Weight				
	Over Body Weight				
Family TB infection history	Yes				
	No				
	We don't know				
HIV/AIDS infection characteristics					
Do you have HIV/AIDS infection	Yes				
	No				
If you answer "Yes " for the above question, please answer the following four questions					
T CD4+ count (cells/mm³)(it can be taken from Card or Clinicians)	< 200				
	200-350				
	≥ 350				
Start of antiretroviral therapy (it can be taken from Card or Clinicians)	Previous to TB treatment				
	Simultaneous to TB treatment				
	Yes	1st line drugs	4a	d4T+3Tc+NVP	
			4b	d4T+3Tc+EFZ	
			4c	AZT+3Tc+NVP	

Antiretroviral therapy (it can be taken from Card or Clinicians)		2nd line drugs	5a	ABC+ddI+LPV/R	
			5b	ABC+3TC+LPV/R	
	No				
Other opportunistic infection (it can be taken from Card or Clinicians)	Yes				
	No				
TB characteristics					
TB disease presentation (it can be taken from Card or Clinicians)	Pulmonary				
	Extrapulmonary				
	Pulmonary and Extrapulmonary				
	Disseminated				
TB treatment characteristics					
TB disease treatment regimen (it can be taken from Card or Clinicians)	RHZ				
	ERHZ/RH				
	SRHZ				
	ESH				
How do you feel after you took the treatment? (Please write if you have any feeling)					
Laboratory Test Results	LFT	sGPT/ALT(IU/L)			
		sGOT/AST(IU/L)			
		Bil-Total(Mg/dL)			
		Bil-Direct (Mg/dL)			
	Hemoglobin(g/dL)				

NB:- d4T= Stavudine, 3Tc= Lamovudine, NVP= Nevirapine, EFZ= Efavirenz, AZT= Zidovudine, ABC= Abacabir, ddI= Didanocine, LPV= Liponavir, LPR=Retronavir, E=Ethambutol, H= Isoniazid, R= Rifampcin, S= Streptomycin, Z= Pyrazinamide

F) ማስታወቅ (እባክዎት ፈገግ ክት/ ውጤት በተሰጠውበታ ያስቀምጡ)

ማስታወቅ					
ዕድሜ(በዓመት)	< 5				
	5-10				
	10-15				
ፆታ	ተባዕታይ				
	አንስታይ				
ምግብ ሁኔታ	SAM				
	MAM				
	Normal				
ክብደት፤ ቁምት ንፅፅር	‹18.5				
	18.5-24.5				
	›24.5				
የ ቤተሰብ የ ቲቢ ህመምሁኔታ	አዎ አለ				
	አይየለም				
	አናውቅም				
የ HIV/AIDS ህመምበተመለከተ					
የ HIV/AIDS ህመምአለብዎት	አዎ				
	አይ				
አዎ ካሉ እባክዎ እሚቀጥሉ አራት ጥያቄዎችን ይሞልሱ (የ ህክምና ካርድ ሞት ወይምህኪምማስየቅ ይቻላል)					
የ CD_4+ ማስጎን (cells/mm^3)	< 200				
	200-350				
	≥ 350				
የ HIV/AIDS ህመምመድሀኒት የጀመሩት (የ ህክምና ካርድ ሞት ወይምህኪምማስየቅ ይቻላል)	የ ቲቢ ህመምመድሀኒት ከ ሞሰድዎ በፊት				
	የ ቲቢ ህመምመድሀኒት ከ ሞሰድዎ በኋላ				
	አዎ	1st line	4a	d4T+3Tc+NVP	

የ HIV/AIDS ህ ማ ም ሞ ድ ህ ኒ ት ዓ ይ ነ ት (የ ህ ክ ም ና ካ ር ድ ሞ ዞ ት ወ ይ ም ህ ኪ ም ማ ከ የ ቅ ይ ቻ ላ ል)		drugs	4b	d4T+3Tc+EFZ	
			4c	AZT+3Tc+NVP	
		2nd line drugs	5a	ABC+ddI+LPV/R	
			5b	ABC+3TC+LPV/R	
	አ ይ				
ተ ጓ ዳ ኝ በ ሽ ታ	አ ዎ አ ለ				
	አ ይ የ ለ ም				
የ ቲ ቢ ህ ማ ም በ ተ ጣ ላ ከ ተ ዓ ይ ነ ት					
የ ቲ ቢ ህ ማ ም ዓ ይ ነ ት ሞ ድ ህ ኒ ት በ ተ ጣ ላ ከ ተ (የ ህ ክ ም ና ካ ር ድ ሞ ዞ ት ወ ይ ም ህ ኪ ም ማ ከ የ ቅ ይ ቻ ላ ል)	Pulmonary				
	Extrapulmonary				
	Pulmonary and Extrapulmonary				
	Disseminated				
የ ቲ ቢ ህ ማ ም ሞ ድ ህ ኒ ት ዓ ይ ነ ት በ ተ ጣ ላ ከ ተ (የ ህ ክ ም ና ካ ር ድ ሞ ዞ ት ወ ይ ም ህ ኪ ም ማ ከ የ ቅ ይ ቻ ላ ል)					
የ ቲ ቢ ህ ማ ም ሞ ድ ህ ኒ ት ዓ ይ ነ ት (የ ህ ክ ም ና ካ ር ድ ሞ ዞ ት ወ ይ ም ህ ኪ ም ማ ከ የ ቅ ይ ቻ ላ ል)	RHZ				
	ERHZ/RH				
	SRHZ				
	ESH				
ሞ ድ ህ ኒ ቱ ን ከ ወ ሰ ዱ በ ኋ ላ ም ን ዓ ይ ነ ት ስ ሜ ት ይ ሰ ማ ዎ ታ ል ? (እ ባ ክ ዎ ስ ሜ ት ካ ል ዎ ት ይ ፃ ፉ)					
የ ላ ቦ ራ ቶ ሪ ም ር ሚ ራ ዉ ጤ ት (የ ህ ክ ም ና ካ ር ድ ሞ ዞ ት ወ ይ ም ህ ኪ ም ማ ከ የ ቅ ይ ቻ ላ ል)	LFT	sGPT/ALT(IU/L)			
		sGOT/AST(IU/L)			
		Bil-Total(Mg/dL)			
		Bil-Direct (Mg/dL)			
	Hemoglobin(g/dL)				

NB:- d4T= Stavudine, 3Tc= Lamovudine, NVP= Nevirapine, EFZ= Efavirenz, AZT= Zidovudine, ABC= Abacabir, ddI= Didanocine, LPV= Liponavir, LPR=Retronavir, E=Ethambutol, H= Isoniazid, R= Rifampcin, S= Streptomycin, Z= Pyrazinamide