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DETERMINANT OF HEPATOTOXICITY USING FIRST LINE ANTI-TUBERCULOSIS DRUGS AMONG TUBERCULOSIS INFECTED PATIENTS IN CENTRAL REGION, GHANA

BY

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DECLARATION

I, EMMANUEL N.Y.E. BOTCHWAY, hereby declare that with the exception of specific references which have been duly acknowledged, this submission is exclusively the outcome of my own study towards my Master of Philosophy In Chemical Pathology dissertation and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degrees of the University or elsewhere.

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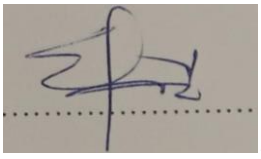

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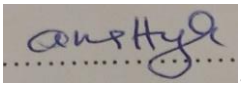

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DEDICATION

This piece of work is dedicated to, first of all, the Almighty father for granting me the strength, blessings, favor, and grace to go through this course; to Him I say, am forever grateful. This work, I also dedicate to my beloved mother, Miss Mercy Naa Otobia Tetteh, my two lovely children, Bethel Naa Otobia Botchway and Immanuel Nana Kwabena Botchway, for inspiring and being my source of strength for the entire period of this MPhil Programme.

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LIST OF ABBREVIATIONS

TB	Tuberculosis	CYP450	Cytochrome P450
MDR	Multi-drug risistance	NAT2	N-acetlytransferase -2
XDR	Extensive drug-resistant	NAPQI	N-acetyl-p-benzoquino
H	Isoniazid	SH-	Thiol
R	Rifampicin	M	Molar concentration
Z	Pyrazinamide	ATP	Adenosine triphosphate
E	Ethambutol	ATPase	Adenosine triphosphata
Anti-TB	Anti-tuberculosis	NADPH	Nicotinamide adenine d
Anti-TB DIH	Anti-tuberculosis drug induced hepat	PTB	Pulmonary tuberculosis
CCTH	Cape Coast Teaching Hospital	EPTB	Extra pulmonary tuberc
LFT	Liver function test	FBC	Full blood count
HIV	Human Immunodeficiency Virus	HB	Hemoglobin
AST	Aspirate aminotransferase	RBC	Red blood cells
ALT	Alanine aminotransferase	WBC	White blood cells
ALKP	Alkaline phosphatase	PLT	Platelet count
TBB	Total bilirubin	EDTA	Ethylenediamine tetrac
DOTS	Direct observation treatment short-co	Rpm	Rotation per minute
ULN	Upper limit of normal	SOP	Standard Operation Pro
IQR	Interquartile range	NAD^+	Nicotinamide adenine d
NTP	National tuberculosis Programme	Sd	Standard deviation
MPT	Mitochondrial permeability	GGT	Gamma glutamly pepti

ABSTRACT

Background: Tuberculosis (TB), remains a public health problem worldwide, especially with the emergence of multidrug-resistant (MDR), and the extensive drug-resistant (XDR). The recommended combination of a standard treatment regimen for new susceptible cases of TB, anti-TB drugs: Isoniazid [H], Rifampicin [R], Ethambutol [E], and Pyrazinamide [Z], have been of a greater benefit. Despite the efficacy of these drugs (particularly H, Z, and R), they are known to possess hepatotoxic effects causing a variety of adverse reactions. Anti-TB drug induced hepatotoxicity (anti-TB-DIH) accounts for significant morbidity and seems to be many in the third world nations such as Ghana where multidrug resistance is on the increase. The reason behind this higher incidence in developing countries remains uncertain. Ghana lacks evidential document on this adverse effects, hence the need for this present study which seeks to determine hepatotoxicity induced by anti-tuberculosis drugs and to find out risk factors among TB infected patients receiving first-line anti-tuberculosis drugs for TB treatment at the Cape Coast Teaching Hospital (CCTH).

Methods: A prospective cohort study on 40 newly diagnosed TB infected patients was conducted from May 2018 to October 2019 at the CCTH, Central Region of Ghana. These patients were of age eighteen and above and due for first-line TB drugs. Patients with normal baseline liver function tests (LFT) , with no viral hepatitis, HIV negative status, no chronic liver disease or renal insufficiency, and receiving the TB therapeutic regimen, were recruited for this study after obtaining informed consent and were followed up through the intensive phase (first two months) of the TB treatment. Participant's socio-demographic data and anthropometric measurements were collated. Six millimeters of venous blood was taken from each participant, before and after initiation of the anti-TB drugs. The liver enzymes levels (ALT, AST, ALP and TBB)

and hemoglobin, total white blood cell count, red blood cell count and platelet count were analyzed two weeks interval, during treatment. Patients with high levels of liver enzymes after repeated testing were categorized as having hepatotoxicity and then evaluated for various factors such as age, gender, alcohol intake and the concomitant use of other drugs. Statistical Package for the Social Sciences version 20 (SPSS Inc., Chicago, IL, USA), a statistical software for Windows was used to analyse data.

Results: An incidence of 15% (6 patients out of 40) anti-TB-DIH was observed. A significant rise in median concentrations of serum transaminases as well as aneamia and hypoalbumineamia, were seen among the hepatotoxic group. The onset of anti-TB-DIH was 4.3 weeks (range, 2 - 8) after the initiation of therapy. Of the several risk factors analyzed, age alone was found, related to the development of anti-TB-DIH ($p=0.008$).

Conclusion: The anti-TB-DIH incidence in the Central Region of Ghana was 15 %. The onset of the development of anti-TB-DIH was 4.3 weeks. Age emerged as a predictor for developing anti-TB-DIH.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Despite the tremendous advances in the control of tuberculosis (TB), TB remains a global health problem especially with the emergence of multidrug-resistant (MDR), and the extensive drug-resistant (XDR) events. It is estimated that Mycobacterium tuberculosis affects millions of people annually and ranked as a leading cause of death from infectious diseases (World Health Organization, 2015). Approximately nine million new cases of the disease occur annually worldwide and 1.5 million deaths can be attributed to this disease (Raviglione and Sulis, 2016). The majority of tuberculosis cases worldwide are concentrated in developing countries, mostly, affecting the economically active population (Cusack *et al.*, 2017). In Ghana, TB (seen as one of the commonest communicable diseases) prevalence has been reported to be 286 cases per 100,000 people by the recent 2013 prevalence survey by the National Tuberculosis Control Programme (Bonsu *et al.*, 2020). This is higher than World Health Organization (WHO) estimates of about 92 per 100,000 people (Boakye-Yiadom *et al.*, 2020). TB mortality rate is considered high at 7.5 per 1,000 infected and the prevalence is 264 per 100,000 in the general population (Frimpong-Mansoh *et al.*, 2018). In 2017, the Global TB Report estimated Ghana's TB prevalence rate stood at 290/100,000 population and incident rate at 156/100,000 population with a death rate of 36 cases per 100,000 (Sullivan, 2019).

The introduction of the Direct Observation Treatment Short-course (DOTS) Programme, by the WHO since 1993, has been suggested to be an effective control tool for the management of Tuberculosis worldwide (Tweed *et al.*, 2018; Shakya, S and Shrestha, 2006). The DOTS Programme involves the use of a recommended

combination of a standard treatment regimen (anti-tuberculosis drugs) for new susceptible cases of TB. These drugs include Isoniazid [H], Rifampicin [R], Ethambutol [E], and Pyrazinamide [Z], over a 2-month intensive phase, followed by a continuation phase with Rifampicin and Isoniazid for 4 months. Despite the efficacy of these drugs (particularly H, Z, and R), they are known to possess hepatotoxic effects causing a variety of adverse reactions (El Bouazzi *et al.*, 2016) sometimes potentiated by multiple drug regimens and the long duration of therapy (Shakya and Shrestha, 2006). Therefore, anti-TB-Drug induced hepatotoxicity (anti-TB-DIH), the commonest and most serious adverse effect, is regarded as the most important clinical consideration among patients receiving TB treatment (Chang *et al.*, 2019).

Anti-TB-DIH may be defined as the treatment-emergent increase in serum transaminases (ALT and AST) levels, five or three times the upper limit of normal (ULN), without symptoms of hepatitis or with the presence of symptoms of hyperbilirubinaemia (two time ULN [bilirubin]), respectively (Ramappa and Aithal, 2013). This condition may develop due to its metabolite, an immunologically mediated response, or the direct toxicity of the principal compound, affecting biliary epithelial cells, liver cells, and/or the vasculature of the liver (Kaplowitz, 2002). Metabolic idiosyncrasy is the most form of anti-TB-DIH. These result from accumulated metabolites or metabolites released throughout the metastasis. Largely the hypersensitivity or metabolic reactions arise independent of the dosage (Abera *et al.*, 2016).

Anti-TB-DIH ranges from the asymptomatic elevation in transaminase to acute liver failure since the main functions of the liver (protein, fat metabolism, carbohydrate, storage of vitamin , secretion of bile and detoxification that are crucial factors for the maintenance of a healthy liver and the overall health and well-being) may be altered,

inducing hepatitis and even be fatal. Thus must be a great priority during TB treatment (Senousy *et al.,* 2010).

Studies have implicated female gender, poor nutritional status, old age, pre-existing liver disease, high prevalence of viral hepatitis (especially in developing countries), hepatitis A, B and C carriage, advanced tuberculosis and hypoalbuminaemia, inappropriate use of drugs and acetylator status, chronic alcohol intake as risk factors that contribute to the development of drug-induced hepatotoxicity (Ramappa and Aithal, 2013). Jussi *et al.,* reports a history of chronic alcohol intake and old age as potential predictors for anti-TB-DIH (Jussi *et al.,* 2006). Female gender has been implicated in some studies as a risk factor for developing anti-tuberculosis hepatotoxicity (Kassa *et al.,* 2016),whilst, males have been observed to have a higher risk of developing anti-tuberculosis drug-induced hepatotoxicity (Devarbhavi *et al.,* 2010), Another study however reports that the extent of TB disease was not related to the development of anti-tuberculosis drug-induced hepatotoxicity (Wang *et al*. 2011). Malnourishment as a risk factor was documented to be related to the development of anti-TB-DIH, among researches studies in Spain (Abera *et al.,* 2016). Unlike in the Western part of the world, just a few studies have been done on drug-induced hepatotoxicity in Africans with TB.

In Ghana, where tuberculosis is considered a public health problem, there is a paucity of published research on anti-TB medications and their impact on liver function especially, for the duration of treatment. This study, therefore, aims to determine the hepatotoxic effect induced by the first-line TB drugs, among TB infected patients receiving treatment in the Central Region, Ghana.

1.2 Problem Statement

Although Ghana is not one of the high TB burdened ranked countries, it has been reported that the incidence of TB is of the increase (WHO, 2017). The annual incidence rate for TB alone was estimated at 156/100000 population and mortality rate of 36/100000 population as at 2017 (Sullivan, 2019), with the Central region being one of the high TB burdened regions. The drugs essential for the treatment of TB disease are anti-tuberculosis drugs. Although these drugs have significantly helped reduce morbidity and mortality rates (Sullivan, 2019), they are also known to possess hepatotoxic properties (especially Rifampicin, Isoniazid, and Pyrazinamide) causing adverse effects, with the risk enhanced when used in combination.

Anti-TB drug induced hepatotoxicity (anti-TB-DIH) accounting for significant morbidity, is considered the well-known reason for poor drug adherence and possibly causes drug resistance, suggesting that patients may remain infectious for a longer period and are more likely to relapse or succumb to the disease leading to reduced effectiveness of therapy (El Bouazzi *et al.*, 2016). This may result in selective compliance and treatment failure and may also promote the emergent of drug-resistance TB causing socioeconomic burden on both the patient and the society as a whole.

Anti-TB drugs may be responsible for jaundice in hospitals and even fulminant liver failure (Abera *et al.*, 2016). In most of the Western countries, all TB patients are routinely assessed, biochemically for drug-induced hepatotoxicity, since the liver is responsible for concentrating and metabolizing a majority of medications and also a prime target for drug-induced damage. In Ghana, however, although TB treatment in Ghana is free, per the treatment guideline used for TB by the National Tuberculosis Programme (NTP), only multi-drug resistance TB (MDR-TB) and TB/HIV co-infected patients are routinely assessed for their hepatic status) due to limited resources and

monetary challenge in running liver function tests (LFTs). Newly diagnosed smear-positive pulmonary TB patients are usually not assessed.

Despite the fact that similar regimens for the treatment of TB infection are used throughout the world, variations in the occurrence of liver injury during treatment has been observed. It appears to be much higher in the African and Asian countries (up to 39%) than in the Western countries (3-4%) (Kassa *et al.,* 2016). The increased incidence of hepatotoxicity caused by drugs in the third world countries is as at now unknown. Considering the known risk factors such as poor socioeconomic nature, malnutrition and alcoholism tendencies of people in the Central region of Ghana, TB patients may be at risk of anti-TB-DIH. Most of these TB patients also present late when TB has progressed, further aggravating their condition.

Again, the different definitions of hepatotoxicity, and differences in national guidelines proposed to treat TB and monitoring practices in hepatotoxicity, makes the underlying mechanisms of anti-TB-DIH and the factors predisposing its development unclear and controversial.

1.3 Justification

In Ghana, biochemical assessment of the liver during treatment of newly susceptible TB patients is limited. It is expensive for the National Tuberculosis Programme to have each patient tested. These assessments may inform the clinician of whether to proceed, modify, or stop TB drug treatment. During anti-TB treatment, elevated transaminases with no symptomatic are common. Significant elevation of three to five folds the upper limit of the normal range for transaminases should be detected early, since drug-induced hepatotoxicity could result in acute liver failure.

Studies have showed differences in the frequencies of drug-induced hepatotoxicity. There are also human variations that arise due to differences in genetic, cultural, and environmental factors among individuals, ethnic origins, and race.

There is a paucity published research on anti-tuberculosis drugs and their effects on liver function during the treatment period among the Ghanaian population. This study, therefore, aimed to determine the hepatotoxic effect induced by the first-line TB drugs, its incidence, and also determine associations between socio-demographic characteristics and the development of hepatotoxicity among TB infected patients receiving treatment in the Central Region, Ghana. The findings of this study may provide evident-based data on the prevalence of hepatotoxicity from first-line anti-tuberculosis drugs and factors associated with hepatotoxicity among the study population. The study will also help identify patients with increased hazard, which will help detect hepatotoxicity early and cause a reduction in the mortality and morbidity of hepatotoxicity. The study findings can impact National TB treatment by identifying patients at risk of hepatotoxicity and help regularize biochemical testing of TB patients in Ghana.

1.4 Null Hypothesis

Anti-TB drugs have no hepatotoxic effect on TB patients receiving first-line tuberculosis drugs for TB treatment.

1.5 Research Objectives

1.5.1 Main Objective

To evaluate hepatotoxic effect induced by the anti-tuberculosis drugs among TB infected patients receiving first-line anti-tuberculosis drugs for TB treatment in the Cape Coast Teaching Hospital.

1.5.2 Specific Objectives

To determine the hepatotoxic effect induced by first-line anti-tuberculosis drugs by measuring markers for liver damage.

To determine the incidence of anti-TB-DIH among TB patients in the Cape Coast Teaching Hospital.

To determine associations between selected characteristics (age, gender, poor nutritional status, chronic alcohol intake, and the concomitant use of drugs) and hepatotoxicity, among study participants.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Liver

The liver, the biggest inward organ in the human body, is the fundamental organ liable for the metabolism and detoxification of drugs and environmental chemical (Lima *et al*, 2011). Its different capacities incorporate glucose storage and synthesis, decomposition of red blood cells, plasma protein synthesis, hormone production, and bile formation (Kaur, 2019).

Anatomically, the liver lies marginally underneath the diaphragm and anterior to the stomach, a position that makes easier the maintenance of metabolic homeostasis of the body. The liver is fed by two distinct blood supplies: the portal vein and the hepatic artery. Blood containing digested nutrients from the gastrointestinal tract, spleen, and pancreas is supplied to the liver by the portal vein, while the hepatic artery conveys oxygenated blood from the lungs to the liver (Gu and Manautou, 2012). The human liver comprises of four lobes, and each lobe is comprised of numerous lobules, which is defined at the microscopic scale. The classical lobule is a hexagonal shaped unit revolved around a central vein. In each functional unit, blood enters the lobules from the portal vein and hepatic artery and then flows down past the cords of hepatocytes. The lobule is partitioned into three regions: the periportal (Zone 1), centrilobular (Zone 3) and the mid-zonal (Zone 2). Zone 1 is the nearest to the entering blood supply with the highest oxygen tension and whilst Zone 3 touches the central vein and has the poorest oxygenation, Zone 2 is intermediate (Kaur, 2019; Lima, 2011).

Because of the blood flows from the stomach and intestine, the liver is the internal organ, principally, to experience several injuries from ingested metals, drugs, and environmental toxicants (Kaur, 2019). Thus, liver cells are presented to critical degrees

of these exogenous materials like drugs, alcohol, and their metabolites, and may cause liver capacities be antagonistically influenced by acute or chronic exposure.

2.2 Hepatotoxicity due to drug induction

Diagnosis of hepatotoxicity due to drug induction (mostly seen as a clinical diagnosis of exclusion) is strongly confirmed by the appearance of an elevation of serum alanine aminotransferase (ALT) more than two folds, upon re-challenging with the suspected offending agent and a fall in ALT on discontinuation (Jussi *et al.*, 2006). Normally, the period for the start of an acute injury falls within months of starting a drug. Note that re-challenge, sooner or later of time, may put the patient in danger and consequently, is ordinarily restricted to fundamental medications or utilized when more than one conceivably hepatotoxic medications have been regulated simultaneously (Baghaei *et al.*, 2010).

2.3 Hepatic Drug Metabolism

Ingested drugs are carried directly into the liver by the splanchnic circulation, a phenomenon called the “first pass” through the liver (Pappano, 2012; Sharma, 2014). Metabolic enzymes convert these drugs to substances freely removed from the body via bile and urine. The conversion processes are through phase I pathways (oxidation reaction, reduction reaction, or hydrolysis) that are primarily processed by the phase I class of enzymes (cytochrome P450 enzymes), and phase II pathways that comprise glutathione conjugation, glucuronidation and sulfation, acetylation (Pappano, 2012). Other subsequent steps may incorporate deamination and deacetylation. Numerous medications might through alternative pathways be metabolized, and their comparative influences may clarify a few variations in toxicity among persons. In phase III pathways, protein transporters in cells help the secretion of these substances into bile or the systemic circulation (Pappano, 2012; Chan *et al.*, 2004). Factors like circadian

rhythms, cytokines, genetic factors, hormones, age, gender, malnutrition, disease state, ethnicity, as well as exogenous chemical substances may affect the activities of transporters and enzyme ((Saukkonen *et al.*, 2006). Bile is the main substance for excreting hepatic metabolites. Substances expelled into bile may experience enterohepatic circulation (Pappano, 2012; Sharma, 2014).

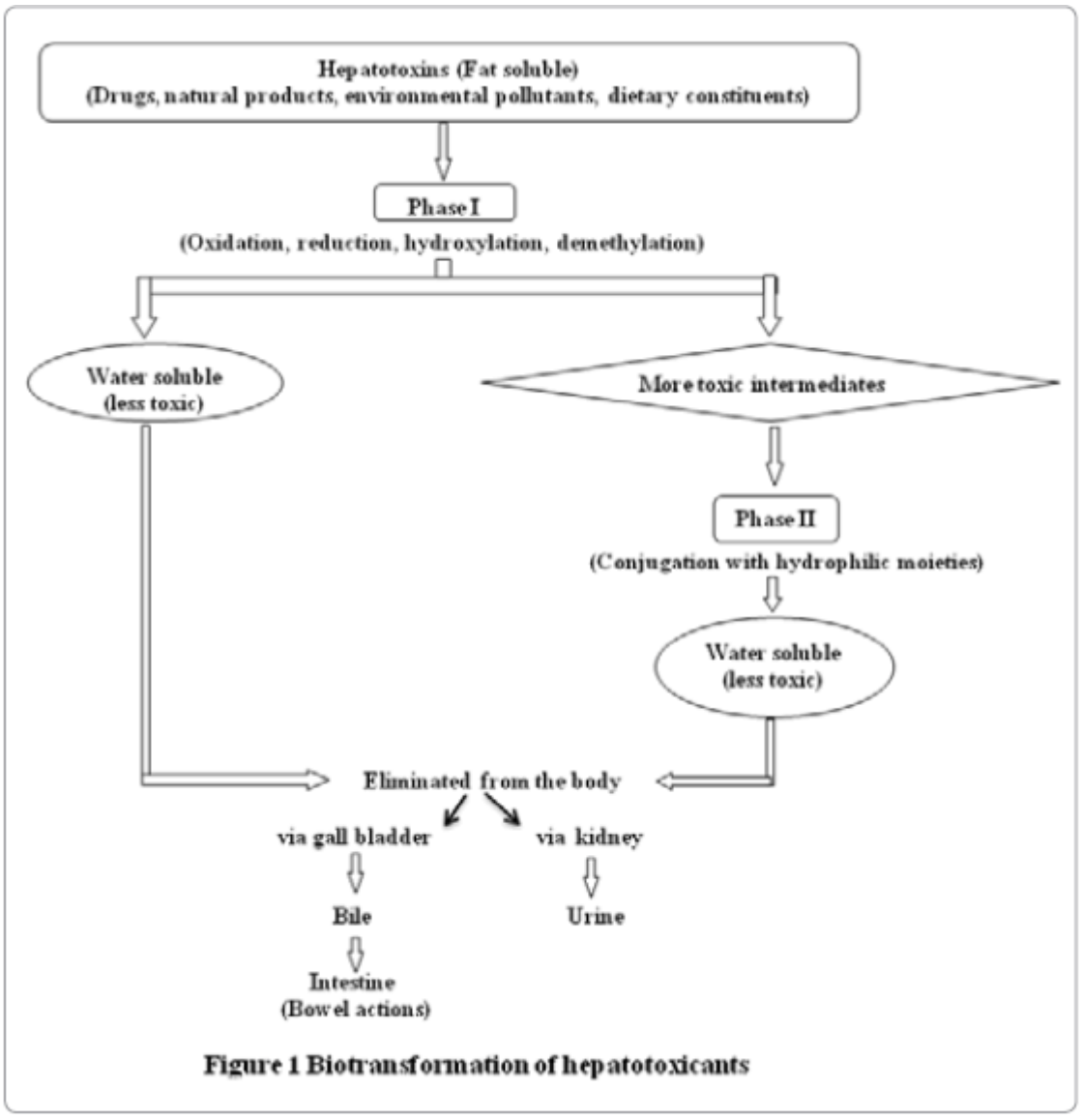


Figure 1 Biotransformation of hepatotoxicants

Source: Sharma OP, (2011) Clinical Biochemistry of Hepatotoxicity.

2.4 Mechanism of Hepatotoxicity

According to Mukesh K, (2015), there may be either drug-induced hepatocellular injury, which usually occurs in-between two to three months of initiating therapy, or hypersensitivity reaction to drugs, which is usually associated with eosinophilic infiltration in the liver. It occurs in between a few weeks from initiation of therapy.

The role of mitochondrial permeability transition (MPT) in the mechanism of H and R-induced liver injury in rats has been reported. Oxidative stress in the mitochondria and inappropriate MPT were discovered to be significant in the pathogenesis of apoptotic hepatotoxicity (Lingala and Ghany, 2016). Gene polymorphism predicted an increased risk for the development of I-induced hepatotoxicity in TB. However, no increased risk has been reported with N acetyltransferase -2 (NAT2) genotypes (Santos *et al.,* 2019). Metabolic idiosyncratic reactions may be due to acquired or genetic differences in the biotransformation pathways of medications, with synthesis or abnormally slow detoxification of a metabolite which are hepatotoxic. Metabolic idiosyncratic reactions may also have a broadly mutable latent period but reappear within days to weeks after re-introduction of drugs (Brown, 2017).

2.5 Biochemical Mechanism of Hepatotoxicity

Chemicals may have effect on liver injury. This effect may involve diverse mechanisms of cytolethality. These mechanisms may affect organelles such as cellular organelles, mitochondria, nucleus, the cytoskeleton, microtubules, and or endoplasmic reticulum thrugh the activation and hindering of signaling kinases, transcription factors, and gene-expression profiles, indirectly (Sharma, 2014; Sauer *et al.,* 1997). The resultant intracellular stress may result in cell death, caused by either cell shrinkage and nuclear disassembly [apoptosis] or swelling and lysis [necrosis] (Zhang *et al.,* 2018). Below are the main mechanisms involved:

2.5.1 Formation of reactive metabolites

Drugs such as Isoniazid (Lee, 2003), acetaminophen (Wallace, 2004) allyl alcohol and bromobenzene are some of the hepatotoxicants that are activated metabolically to poisonous metabolites that are chemically reactive and may form a covalent bond with vital macromolecules of cells consequently deactivating crucial cell capacities (Sauer *et al.,* 1997). Glutathione offers an operative pathway for detoxifying many reactive metabolites that are electrophilic. Nevertheless, excess substrates for conjugation, several alkylating agents, and oxidative stress can cause depletion of glutathione, therefore making cells more vulnerable to the chemical-induced toxic effects. The reactive metabolites may also alter liver-proteins causing an immune response and immune-mediated injury.

2.5.2 The impact of toxicant on crucial cellular systems

Crucial targets of the cell, such as lysosomes, nucleus, endoplasmic reticulum, mitochondria and plasma membrane can be attacked directly by hepatotoxicants, consequently disturbing their functions. Many substances that include drugs and metallic ions may fuse to membranes of mitochondria and enzymes, and disrupt energy metabolism and cellular respiration (Rines and Ardehali, 2013). Several liver toxicants function directly as uncouplers and inhibitors of mitochondrial electron transport (Kamalian *et al.,* 2015). Drugs that are covalently bounded to protiens in cells causes a fall in the levels of ATP resulting in the disturbance of actin and membrane rupture. Phalloidin, a mushroom toxin, bring about a rise in the permeability of plasma membrane through the banding to actin, disturbing the cell cytoskeleton (Ariani *et al.*, 2013). Some toxicants such as chenodeoxycholate and erythromycin salts possess surfactant effects on the liver cell plasma membrane directly (Sharma, 2014). Proteins of the mitochondria which have plasma membrane proteins and thiol groups associated

with calcium homeostasis forms adduct with *N*-acetyl-*p*-benzoquinone imine (NAPQI) covalently.

2.5.3 Lipid peroxidation and redox cycling

Redox cycling and Lipid peroxidation are associated with liver injury hepatotoxicity resulting in apoptosis because of oxidative stress that is due to a change in intracellular pro-oxidant to the antioxidant ratio in favor of pro-oxidants (Kurutas, 2016; Sies, 1985). Lipid peroxy radicals cause an elevated cell membrane permeability, diminished cell membrane fluidity, inactivation of membrane proteins, and loss of polarity of mitochondrial membranes. Some ions such as iron and copper participate in redox cycling while cycling of oxidized and reduced forms of a poisonous substances result in the generation of free reactive oxygen species that are capable of diminishing glutathione via oxidation or oxidize crucial protein sulfhydryl groups associated with enzymatic or cellular standardization, or capable of starting peroxidation of lipids. Excessive intake of liquor resulting in unbounded radical production, glutathione declination, and peroxidation of lipids (Mostafa Abd El-Aal, 2012). peroxidative process also contributes to severe hepatotoxicity due to α-amanitin (Zheleva *et al.*, 2007). Hydroperoxides, acrylonitrile, cadmium, halogenated hydrocarbons, iodoacetamide, chloroacetamide are also testified to show liver injury as a result of lipid peroxidation.

2.5.4 Calcium balance disturbance

Calcium is associated with a many differences in the body's crucial physiological tasks. Calcium homeostasis is specifically controlled in cells. The unbounded calcium in the cytoplasm kept comparatively low. The concentration gradient for calcium between the extracellular fluid [10^{-3}] and the intracellular cell [10^{-7}] is retained through an active membrane which is related to magnesium and calcium effluxion of ATPase enzymes

structure that is vital potential sitting duck for poisonous substances (Nicotera *et al.*, 1990). Chemically induced liver injury may result in calcium balance disturbance (Cullen, 2005). Unspecific elevations in the plasma membrane permeability, membranes of smooth endoplasmic reticulum and mitochondrial membrane resulted in calcium balance disturbance elevation in calcium levels in cells. The fall in unengaged cofactor (NADPH) required by calcium pump can as well disturb calcium balance. Disturbance of calcium balance may result in the activation of many membrane-damaging enzymes such as ATPases, phospholipases, proteases and endonucleases, disruption of mitochondrial metabolism and ATP synthesis and damage of microfilaments that are used to support cell structure.

2.6 Some Types of Hepatotoxicity

2.6.1 Drug-induced acute hepatitis

The clinicopathological portal for transaminase, substantial drug-related hepatitis has not been systematically established for many drugs. Persons on phenytoin usually experience increased AST/ALT up to 3×ULN, yet liver biopsies do not show substantial pathology (Amacher *et. al.*, 1998). Conversely, in patients on methotrexate for rheumatoid joint inflammation treatment, microscopic proof of hepatotoxicity has been established for any increase in AST/ALT ≥ ULN (Mori *et al.*, 2018). Acute liver cell damaged persons may be non-diagnostic or account for a prodromal of eligible symptoms, accompanied with lethargy, vomiting, nausea and anorexia. Histopathologically, focal hepatic necrosis may be revealed, with bridging in grievous cases (Butler *et al.*, 2018). Coagulopathy may happen 24 - 36 hours after beginning, in spite of the fact that this can along these lines resolve. Coagulopathy proceeding past 48 hours is a helpless prognostic sign in paracetamol-induced liver injury (Fontana, 2008).

2.6.2 Hepatic adaptation

Prologue to some medications may induce physiologic adaptive responses (Williams, & Iatropoulos, 2002). The stimulation of endurance genes, comprising those that control anti-inflammatory, anti-apoptotic pathways, and antioxidant may decrease poison-related adverse reactions. Such injury may also excite liver cell multiplication and protective adaptation. A brief increases of ALT may reflect mild, non-progressive injury to mitochondria of the liver cells, cell membranes, or other structures. Injury like this, hardly cause inflammation, apoptosis, or significant cell structure changes. Some toxins might probably compete through these adaptive formative reactions. Extreme determination of an adaptive reaction may, at certain point of time, cause hepatocytes more weak when exposed to additional new injuries (Dielh, 2000). The initiation of hepatic microsomal enzymes, capable of metabolizing the inducing medication (Chitturi, & Farrell, 2002; Williams, & Iatropoulos, 2002), is alternative type of adaptation in liver.

2.6.3 Cholestasis

Benign cholestasis, usually reported with estrogen therapy, comprises of asymptomatic, normally mutable, increased serum ALP and bilirubin level, brought about by a failure in bilirubin transport. There exist no inflammation in hepatic tissue (Chitturi, & Farrell, 2002).

2.7 Treatment for Tuberculosis

Mainly five drugs, Rifampicin (R), Isoniazid (H), Pyrazinamide (Z), Streptomycin (S), and Ethambutol (E) are used in the treatment of tuberculosis (El Bouazzi *et al.*, 2016). These drugs are often used in combination. The combined formulae are Isoniazid with Rifampicin (HR), Isoniazid combined with Rifampicin and Pyrazinamide (HRZ), and finally, Isoniazid combined with Rifampicin, Pyrazinamide, and Ethambutol (HRZE).

TB treatment involves two phases; intensive and continuation phases (Tang *et al.*, 2018). The first two months of treatment is referred to as intensive phase and the drugs are often taken, using DOT strategy. At this point, patients are required to take the drugs in the presence of a health provider. Patients living distance away from hospital facility or treatment center are given the medications and then referred to a treatment center nearer to their house for daily DOT. Meanwhile, patients nearer the health facility, attend the clinic for DOT every morning (Tang *et al.*, 2018).

Generally, almost all TB infected patients go by this daily ambulatory basis for the whole 2 month intensive phase in Ghana. However, severely ill patients or those with other clinical impediments are hospitalized for treatment. Depending on the outcome of the sputum test at the end of the intensive phase, or the type of treatment, patients are placed on either 4 or 7 month continuous phase. Most of the TB patients are placed on the 4 month continuous phase, while the 7-month continuous phase is endorsed for, (1) PTB resulted from drug-susceptible organism and those with month-2 sputum culture results is still positive; (2) patients who did not receive Pyrazinamide during the intensive phase; and (3) patients with sputum culture test results after the intensive phase is positive and took Isoniazid and Rifampicin once-weekly during the intensive phase. (Curry, 2006).

During the continuation phase, the drugs are given for a period to take home and are encouraged to take them in the presence of treatment supporters since no daily supervision of drug intake by a health provider is required. However, patients are expected to report at scheduled appointment times to receive their drugs and for medical assessment. The treatment regimens as stated in the WHO TB Treatment guide (WHO, 2010) comprises; category I, II and III.

All new TB cases of sputum smear positive or negative, both PTB and EPTB. This category of patients are placed on a 6-month therapy involving 2-month daily supervised R, H, Z and E f Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol accompanied by a 4-month H and R. However, EPTB patients may be put on treatment for up to nine months depending on the location of the infection.

Category II applies to TB patients who have been previously treated (such as treatment failure, treatment after default, and relapse cases). These patients often receive Isoniazid, Pyrazinamide, Ethambutol, and Rifampicin, supplemented under supervision during the intensive phase. This is then followed by five months of daily Rifampicin, Isoniazid, and Ethambutol.

This category refers to children under 12 years. They are put on a six-month treatment, comprising of two months of Isoniazid, Rifampicin, and Ethambutol. This is then followed by four months of Isoniazid and Rifampicin.

2.8 Pathogenesis of Drug-induced Hepatotoxicity

Drug-induced hepatotoxicity may occur due to the toxic metabolite formed, the direct toxicity of the drug itself, or an immunologically mediated response, affecting biliary epithelial cells, hepatocytes, and liver vasculature (Gu and Manautou, 2012). Mostly, the precise mechanism and factors contributing to drug-induced hepatitis continue to be ill understood. Predictable drug-induced liver injury has an increase developing rate, and is likely to happen speedily. Harmful free radicals result in liver cell necrosis in zones furthest from the arterioles in the liver, wherever metabolism is maximum and minimal antioxidant detoxifying ability (Ahmed and Mobasher, 2013).

Unpredictable or idiosyncratic reactions include almost all types of liver injury induced by drugs. These metabolic reactions or hypersensitivity which arise are mostly not dose dependent, but somewhat seldom for each drug, and can lead to liver cell injury and/or

cholestasis. Liver cell necrosis is usually dispersed all over hepatic lobules rather than being zonal, as usually observed with predictable drug-induced liver injury. In hypersensitivity reactions, immunogenic drug or its metabolites can be free or covalently bonded to hepatic proteins, forming haptens or "neoantigens." Antibody-dependent cytotoxic T-cell and irregularly eosinophilic hypersensitivity responses may be induced. Released tumor necrosis factor-α, interleukin (IL)-12, and IFN-γ stimulate hepatocellular programmed apoptosis, an effect opposed by IL-4, IL-10, IL-13, and monocyte chemotactic protein-1 (Feghali and Wright, 1997; Lovren *et al.,* 2010; Jayme *et al.,* 2020). Metabolic idiosyncratic reactions come about from inherited or self-developed differences in drug biotransformation pathways, with production or aberrantly slow detoxification of a liver toxic metabolite. Metabolic idiosyncratic reactions may have a broad adjustable latent time but reappear for days to weeks after re-introduction (Gu and Manautou, 2012).

2.9 Anti-TB drugs and Hepatotoxicity

The first-line anti-tubercular drugs are possibly hepatotoxic drugs (El Bouazzi *et al.,* 2016) and are metabolized by the liver. The adverse effects of anti-TB drugs might be potentiated by multiple drug regimens. Consequently, though each drug is possibly hepatotoxic, when administrated in combination, the toxicity is heightened. Grounded on the benchmarks for diagnosing hepatotoxicity and study participants, the incidence of anti-TB related liver injury is revealed to be in the range 5% -28% (Makhlouf *et al.,* 2008).

2.9.1 Isoniazid

Isoniazid is the recommended drug for essential prophylaxis of TB infection and remedy of latent infection to prevent active TB. Isoniazid induced hepatotoxicity that commonly develops in between 4–8 weeks after initiation of therapy, has been a

frequent problem of anti-TB treatment, and varies in harshness from the non-diagnostic rise in transferases to hepatic catastrophe necessitating hepatic transplanting (Abbas *et al.*, 2012). Bridging and multilobular necrosis are established by means of liver biopsy of patients. This, however, appears to characterize the idiosyncratic response. It is therefore not caused by high levels of plasma Isoniazid. Isoniazid is metabolized to monoacetyl hydrazine, which is then metabolized to a noxious product, hydrazine, via cytochrome P450 resulting to hepatotoxicity. Human genetic research have shown that cytochrome P4502E1 (CYP2E1) is involved in anti-TB-DIH (Tostmann *et al.*, 2008). The CYP2E1 c1/c1 genotype is linked to a rise in CYP2E1 activity and can additionally cause a higher creation of toxins in the liver. In rats, Isoniazid and Hydrazine induce CYP2E1 action (Hassan *et al.*, 2018). Isoniazid possesses a constraint impact on CYP1A2, 2C19, and 2A6 action. CYP1A2 proposed to be implicated in detoxification of hydrazine. Isoniazid may bring about its noxious toxicity by probably by way of the initiation or constraint of these enzymes (Abera *et al.*, 2016). Simultaneous use of Rifampicin and Isoniazid increases the risk of hepatic injury. (Dickinson, *et al.*, 1977; Sarma *et. al.*, 1986).

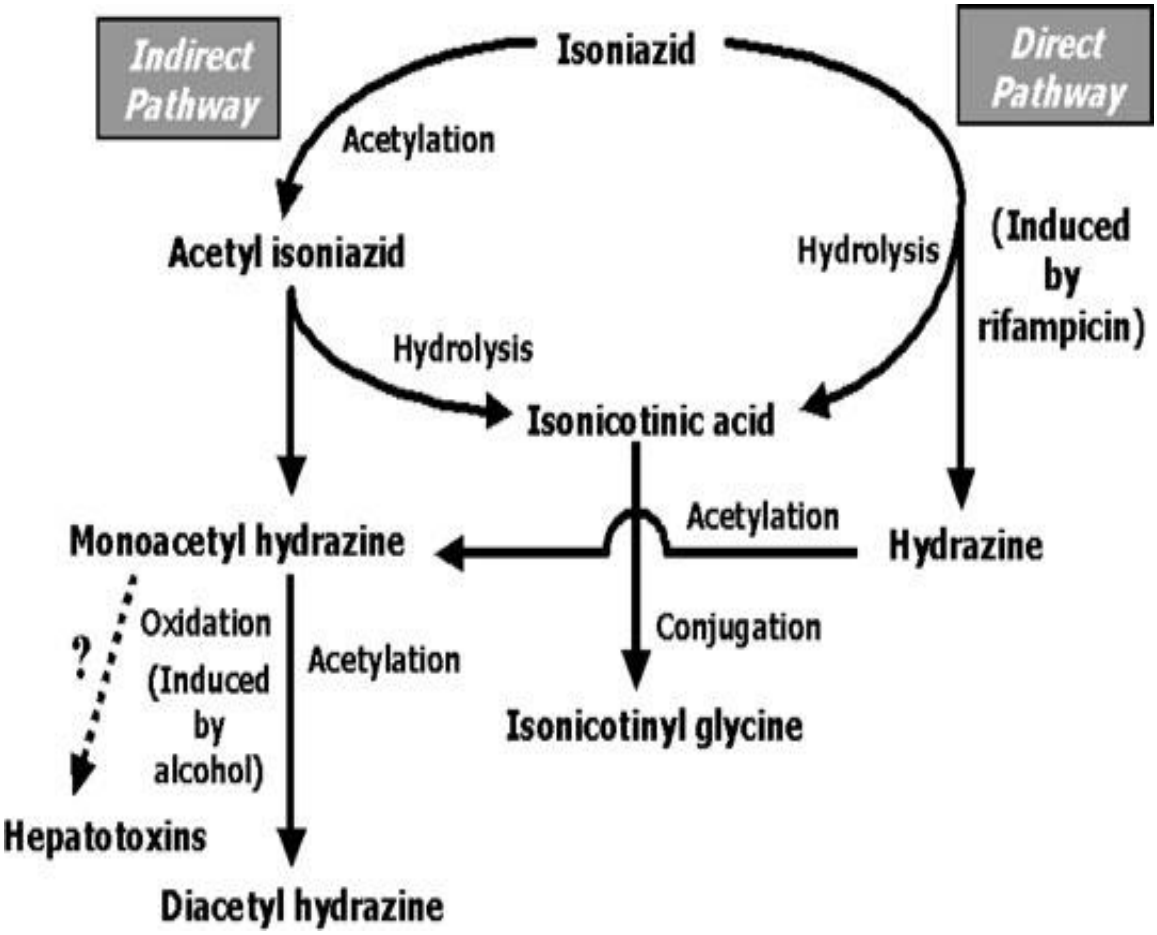


Figure 2 Influence of isoniazid metabolism by rifampicin and alcohol.

2.9.1.1 Isoniazid-induced injury mechanism

Monoacetyl hydrazine has its reactive metabolites to be poisonous to tissues thru the generation of free radical (Mitchell *et al*, 1975). The free radical, scavenger glutathione-related thiols and antioxidant glutathione peroxidase and catalase actions, are reduced by isoniazid, although glutathione reductase action is raised, in rats (Attri, *et. al.*, 2001; Sodhi, *et. al*., 1996). A substrate for glutathione synthesis, the antioxidant N-acetyl cysteine inhibits isoniazid-induced liver damage in pretreated rats (Attri *et. al.*, 2001), with anonymous significance in mankind. Covalently acetyl-hydrazine binds to macromolecules of the liver, a procedure facilitated via cytochrome CYP450 enzymes (Mitchell *et. al*., 1975). In a study, participants with unvaried cytochrome P450 2E1 c1/c1 host gene cytochrome P450 2E1 c1/c1 host gene polymorphic, and heightened

CYP2E1 activity, were identified to be at more serious danger liver injury, especially in slow acetylators like females (Huang *et. al*., 2003).

2.9.1.2 Clinical Presentation of Hepatotoxicity for Isoniazid

Some patients might appear with no symptomatic, while other patients might have symptomatic hepatotoxicity at different serum transaminase levels. In the early stages, constitutional symptoms might be observed in extreme cases and this may last for days to weeks. Among 50 – 75% of severely ill patients, nausea, vomiting, and abdominal pain may be seen, 10% may be seen with fever, and 5% with a rash. Overt jaundice, darkish urine, and clay-coloured stools are other symptoms at the worsening stage. Coagulopathy, hypoalbuminaemia, and hypoglycemia indicate serious hepatic dysfunction. The reversion of this condition normally last for weeks. Complete restoration in most cases happen after the withdrawal of the drug (Mitchell *et. al.*, 1976).

2.9.2 Rifampicin

Being on concomitant Rifampicin treatment has greater risk incidence of hepototoxicity (Chung-Delgado *et al.,* 2011). This effect could be as a result of Rifampicin-induced cytochrome P450 enzyme-induction, causing a rise in the generation of the noxious metabolites from acetyl hydrazine (AcHz) (Mahmood *et al.,* 2007). Rifampicin again raise the metabolism of Isoniazid to isonicotinic acid and hydrazine, both of which are toxic to the liver. The plasma half-life of AcHz, a metabolite of Isoniazid, is dropped by Rifampicin, and then rapidly changed to its active metabolites by raising the oxidative elimination rate of AcHz, which is associated with the higher incidence of liver necrosis caused by Isoniazid and Rifampicin in combination (particularly in slow acetylators). Factors such as alcoholism, old age and chronic liver disease raise the risk of severe hepatitis as rifampin is given alone or concurrently with Isoniazid. Rifampicin

may cause transient hyperbilirubinaemia, due to interference with bilirubin excretion that is rifampin hindering the main bile salt exporter pump (Byrne *et. al.,* 2002), and an increase in gamma-glutamyl transferase levels. Asymptomatic elevated bilirubin may also occur due to the interference (depending on dosage) in the clearance of bilirubin at the membrane of sinusoidal or obstructed discharge at the canalicular level (Grosset *et. al.,* 1983; Capelle *et. al.,* 1972). The plasma levels of Rifampicin is affected when the drug interacts with antiretroviral drugs and thus, the risk of liver injury (Mukonzo *et al.*, 2019; Tostmann *et al.,* 2008).

2.9.2.1 Mechanism of Hepatotoxicity for Rifampicin

Conjugated hyperbilirubinaemia perhaps is induced through rifampin hindering the main bile salt exporter pump. Asymptomatic rise in bilirubin may be due to the interference with bilirubin for clearance at the sinusoidal membrane or obstructed secretion at the canalicular level (Tátrai and Krajcsi, 2020; Čvorović and Passamonti, 2017). Rare hepatotoxicity seems to be a hypersensitivity reaction, and it may also be more frequent with more, discontinuous drug (Gauckler *et al.,* 2018). Hypersensitivity reactions have been accounted for in relation to kidney dysfunction, hemolytic aneamia, or “flulike syndrome” (Gauckler *et al.,* 2018; Lee and Boyer, 2000; Čvorović and Passamonti, 2017).

2.9.2.2 Clinical characteristics of hepatotoxicity for rifampicin

Cholestasis might be bewildering however unsafe. Idiosyncratic hypersensitivity reaction to rifampin may be uncovered as queasiness, heaving, fever, anorexia, discomfort, somewhat increased transaminaes, and increasd bilirubin, regularly appear during the principal month of therapy inception (Yilmaz *et al.,* 2018).

2.9.3 Pyrazinamide

The hepatotoxicity is the most serious side effect of Pyrazinamide therapy and is either dose-dependent and /or idiosyncratic. The conversion of Pyrazinamide results in pyrazinoic acid and then converted to 5-hydroxypyrazioic acid through oxidation reaction by xanthine oxidase. The $t_{1/2}$ of serum Pyrazinamide is no way associated with time therapy last, indicative of the inability of Pyrazinamide to induce the responsible enzymes for its metabolism (Pasipanodya and Gumbo, 2010). The enzymes employed in toxicity induced by Pyrazinamide is unknown and also it is uncertain whether Pyrazinamide toxicity is as a result of itself or its metabolites. A research in rat shown that the constrain the action of many CYP450 isoenzymes (3A, 2E1, 2C, ,2B), however, another research in microsomes of the liver of humans established that there was no repressive impact on the CYP450 isoenzymes by Pyrazinamide (Tostmann *et al.,* 2008; Cao *et al.,* 2017). In a cohort study conducted on 3,007 patients with active tuberculosis to compare continuation phase drugs including Pyrazinamide, Isoniazid, and/or rifampin with those containing Isoniazid and rifampin to assess Pyrazinamide-induced hepatotoxicity, it was observed that the addition of (Hassen Ali *et al.,* 2013a; Ramappa and Aithal, 2013; Abera *et al.,* 2016). Younossian *et al,* reported that the combination of Z and E for in treating latent tuberculosis infection after exposure to MDR-TB was associated with a remarkably high rate of drug-induced hepatitis (50%; occurring after a median of 4 months of treatment).

Aminotransferases (AST and ALT) levels returned to normal after Z and E were discontinued. In literature, a case report of severe hepatotoxicity associated with rifampin-Pyrazinamide preventative therapy, which required liver transplantation, has been published (Kunimoto *et. al.,* 2003). In a nonrandomized, observational study by Cook *et al.* (Cook *et al.,* 2006), patients received short-course therapy with 2 months

Pyrazinamide and rifampin or 4–6 months rifampin against conventional therapy with Isoniazid for 9 months for latent TB. The rate of completion was significantly higher with short-course regimens and although the general risk of hepatotoxicity in patients place on Pyrazinamide and rifampin was higher, liver function returned to normal after the medications were discontinued.

2.9.3.1 Pyrazinamide-induced injury Mechanism

Pyrazinamide may exhibit both dose-dependent and idiosyncratic hepatotoxicity. Pyrazinamide changes nicotinamide acetyl dehydrogenase levels in rodent liver (Shibata *et al.*, 2001), that may cause the production of free radical species. There may be similar mechanisms of injury for pyrazinamide and isoniazid on the grounds that there is some likeness in molecular structure. Patients with previous hepatotoxic reactions from isoniazid, had more stark reactions with pyrazinamide and rifampin given for Latent TB-infection. Pyrazinamide may induce hypersensitivity reactions with eosinophilia and liver injury (Centers for Disease Control and Prevention, 2001) or granulomatous hepatitis (Knobel *et. al.*, 1997).

2.9.4 Ethambutol

The mechanism of action of ethambutol is not completely known. There is evidence that the drug exerts its bacteriostatic activity by inhibition of arabinosyl transferase, an enzyme that polymerizes arabinose into arabinan and then arabinogalactan, a mycobacterial cell wall constituent.

2.10 Risk factors for Anti-TB-DIH

Many risk factors for anti-TB-DIH have been implicated. Clinical risk factors for drug-induced hepatotoxicity during tuberculosis treatment include advancing age, malnutrition, alcoholism, chronic viral hepatitis B and C infections, and HIV infection. A prospective cohort study from Spain (Fernandez-Villar *et. al.*, 2004) has shown the

incidence of anti-TB-DIH to be significantly higher in the group with risk factors (18.2%) than in the group without (5.8%). Severe hepatotoxicity occurred in 6.9% of the risk factor group and 0.4% of the group without risk factors. Detection of high risk persons is helpful in allowing early identification of liver toxicity and thus reduction of mortality and morbidity of hepatotoxic damages. Below are some risk factors that have been identified;

2.10.1 Advanced age

Because of clearance of substances like drugs mechanism by the cytochrome P450 and alterations in liver size, blood flow, drug distribution, or binding with growth, aged persons may be more exposed to liver toxin reactions. Advanced age has been observed not statistically significance as a risk factor for anti-TB-DIH in many studies (Dossing *et. al.*, 1996; Huang *et. al*., 2002; Hwang *et. al*., 1997; Ormerod & Horsfield, 1996; Schaberg *et al.*, 1996; Teleman, *et. al*., 2002; Yee *et. al.*, 2003). A prospective study by Hwang et al reported an anti-TB-DIH rate ranging from 2-8% as age advanced above 35years, with an average of 5 % (Hwang *et. al.*, 1997). Huang and Dufour in their studies uncovered that hepatotoxicity may change from 22-33% in patients over 35 years interestingly with 8 - 17% in patients under 35 years (Dufour *et. al.*, 2000; Huang *et. al.*, 2002).

2.10.2 Gender/Sex

Female gender is associated with higher cytochrome P3A enzyme activity compared to males and this may explain females being more vulnerable to anti-TB-DIH or drug-induced hepatotoxicity in general. Some studies have demonstrated an increased risk of hepatotoxicity in women (Shakya *et al.*, 2004; Teleman *et. al*., 2002). Shakya and colleges exhibited a fourfold more serious danger of hepatotoxicity (treatment-restriction) in females however with a general low frequency of 8% (Shakya *et. al.*,

2004) while Makhlouf *et al.*, (2014) and Assob *et al.*, (2008) uncovered no expanded danger in females.

2.10.3 Alcoholism and use of other drugs

Liquor addiction is related with a higher danger a higher risk of anti-TB-DIH on account of enzyme induction. Liquor initiates cytochrome P450 2E1, that invigorates the digestion of ethanol, paracetamol, and others (Tanaka *et al*., 2000). The digestion of liquor will create acetaldehyde that adds to glutathione exhaustion, free radical production, protein conjugation, and lipid peroxidation. Ongoing alcohol abuse initiates hepatic collagen-producing sinusoidal cells, possibly adding to fibrosis (Lieber, 1994). Some drugs, like calcium-channel blockers, can stimulus hepatic microsomes mechanism of conceivably hepatoxic drugs that may result in drug-induced hepatotoxicity (Eno and Cameron, 2015; Uebelacker, 2017). Patients with liquor misuse and associative utilization of other hepatotoxic medications have a higher risk of drug-induced hepatotoxicity (Hwang *et. al*., 1997). Mugusi *et al.*, (2012) detailed that there was no association between alcohol use and hepatotoxicity.

2.10.4 Malnutrition

Malnutrition (defined by a low BMI of less than 18kg/m^2 and additionally Hypoalbuminaemia under 35g/dl) brings about diminished xenobiotic clearance and higher plasma drug concentrations (Walter-Sack & Klotz, 1996). Glutathione stores decreased in malnourished persons than predisposes them to oxidative injuries from drug metabolites (Assob *et. al.,* 2014). In malnutrition, oxidative stress is heightened and poor nutritional status is associated with immune dysfunction (Younossian *et. al.*, 2005; Papastavros *et. al.*, 2002). Hepatic steatosis has also been found in malnourished animals and humans, with likely links to oxidative stress and mitochondrial dysfunction (Ridzon *et. al.*, 1997; Huang *et. al.*, 2002). It is possible that malnutrition would merit

attention in the elderly population with tuberculosis. Malnutrition as a predisposing factor for drug-induced hepatotoxicity has also been demonstrated by some researchers (Assob *et. al.*, 2014; Shakya *et. al.*, 2004; Sharma, *et. al.*, 2002).

2.11 Hepatic Enzyme Measurement

Increment in serum alanine aminotransferase suggest more of liver injury compared to aspartate aminotransferase that may also suggest anomalies in heart, muscle, kidney or brain (Sharma, 2014; Cuperus *et al.*, 2017). Serum enzyme levels are determined via functional catalytic analyses with standard values set up from "healthy" study participants. The standard value always falls within two standard deviations of the average of the distribution, with two and half percent of the healthy participants with levels above and below the normal limits on a single analysis (American Gastroenterologic Association Clinical Practice Committee., 2002). Participants used to establish the normal ranges in the previous days probably comprised of occult hepatic disease patients, whose rejection led to reductions in the upper limit of normal (ULN) (Prati *et. al.*, 2002). Inter-laboratory difference in analytic results can be significant. Thus, in comparing multiples of the ULN has come to be routine (Dufour *et al.*, 2000). Transaminases, in a person, may differ as far as 45% on the same day, with the peak levels happening at midday, or 10 to 30% on every other day. Factors such as exercise, hemolysis, or muscle injury may cause ALT and AST elevation. A review of healthy participants in a drug trial, who were put on a placebo revealed that 20% of them had at least one of their alanine transaminase result was higher than the upper limit of normal, and 7% had one value at least 2×ULN (Rosenzweig *et. al.*, 1999). Serum transaminase level increased in male gender and those with higher BMI. The transaminase concentrations tend to be lower in children and older adults. It has been suggested by the National Academy of Clinical Biochemistry that individual labs set

up their own normal ranges for enzymes acclimated for gender, the young and the old (Dufour *et. al.,* 2000). Elevations in ALP and/or bilirubin with slightly or no rise in ALT suggests cholestasis. ALP levels may also rise due to in bone, placenta, or intestine. Elevated serum GGT is helpful in differentiating liver-related from other related ALP increases other than the liver (Larrey *et al*., 2002). As bilirubin levels exceed 3.0 mg/dL, jaundice can be detected on physical examination.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design

A prospective cohort study was used in this study. Samples were taken for biochemical and hematological tests prior to the initiation and administration of the anti-TB drugs.

3.2 Study site

The study was carried out at the direct observation treatment short-course (DOTS) center of Cape Coast Teaching Hospital (CCTH) in the Cape Coast Metropolis, the capital of the Central Region of Ghana. CCTH serves as the referral hospital with a bed capacity of about nine hundred. It is equipped with a well-structured and well-attended DOTS center and see patients from various parts of the Central region.

3.3 Sampling Technique, Socio-demographic and Clinical characteristics

This study was based on a purposive sampling technique at the CCTH, from February 2019 to July 2020. Thus, all smear-positive pulmonary TB patients on first-line drugs who had been admitted to the hospital and/or referred to the DOTS center, and receiving the TB therapeutic regimen, who gave their consent were selected for this study and followed up through the intensive phase of the TB treatment.

Using a structured questionnaire, a complete history comprising of age, sex, concomitant use of other drugs, the extent of the disease for each patient, and alcohol consumption (48g per day continuously for a month) status were collected by a medical doctor and a trained DOTS nurse at the TB clinic. The height and weight of the patients were also taken for the calculation of body mass index (BMI) (BMI of 18.5kgs/m^2 or less was considered malnourished). Clinical information such as anorexia, vomiting, dark urine, jaundice, abdominal, rapid weight gain, fever and medical history related to TB, by a nurses who is trained (DOTS nurses) at the DOTS center, were also taken.

3.4 Study Participants

Newly diagnosed smear-positive pulmonary TB patients attending the DOTS center of the CCTH who were due for first-line anti-TB therapy and consented for the study by signing informed written consent. The participants were all residents of the central region. The patients were selected consecutively until the sample size was achieved. Participants exited the study at the end of the first 2 month of therapy but were also allowed to exit the study at any time and this did not affect the treatment they were receiving. Although all patients were receiving treatment for 6-9 months, this study focused on the intensive phase treatment period (the first 2 months) and evaluate the impact of the drugs used on liver function biomarkers and hematological parameters before and after the initiation of the TB drugs.

3.5 Eligibility

3.5.1 Inclusion Criteria

1. Newly susceptible TB-infected patients.
2. Patients of age eighteen and above due for first-line tuberculosis drugs
3. Patient having normal baseline Liver function enzymes concentrations
4. Patients who consented to the study were included in this study.

3.5.2 Exclusion Criteria

1. Patients diagnosed to have acute viral hepatitis A, B, C, or E or carrier for HBV & HCV
2. Patients with HIV positive status
3. Patients with their basal transaminases (ALT and AST) concentrations greater than twice the Upper Limit Normal (ULN).
4. Patients with chronic liver disease or renal insufficiency

5. Patients with concomitant administration of other potentially hepatotoxic drugs (methotrexate, phenytoin, valproate)
6. Patients who did not consent to participate in the study
7. Retreatment patients or patients with previous TB treatment for more than four weeks.

3.6 Sample size determination

The total regional population, **N**, for Cape Coast Municipal was 169894. 0.69% out of the 24,658,823 population for the whole country according to the 2010 Population & Housing Census Report (Ghana Statistical Service (GSS), 2013), and a total smear-positive TB case diagnosed in 2017, was, 1465 (NTP, 2018). A level of confidence of 95%, which corresponds to a **z** score of 1.96, and a 3% margin of error, **e**, was used in this study.

The proportion, **p**, possible TB positive case, therefore was calculated as,

$$p = a/N = 1465 / 169894 = 0.0086$$

And the minimum sample size, n, was calculated, using the Cochrane sample size formula,

$$n = Z^2 \times [p\,(1 - p)] / e^2$$

$$n = (1.96)^2 \times [0.0086\,(1 - 0.0086)] / (0.03)^2$$

$$n = 3.8416 \times [0.0086 \times 0.9914] / 0.0009$$

$$= 0.0330 \times 0.9914 / 0.0009$$

$$= 0.0327 / 0.0009$$

$$n = 36.333$$

$n \sim 36$ smear positive TB patients.

Forty newly diagnosed susceptible TB patients were selected for the study. The sample size was raised to 40 to enable me to make a good and meaningful inferences.

3.7 Drug regimen

The drug regimen was such that, the intensive phase comprised the following; H 50/mg/kg/day; maximum 300mg daily, R 10mg/kg/day; maximum 600mg/day, E 15mg/kg/day and Z 25mg/ kg/day.

Although all TB infected patients were receiving treatment for 6-9 months, this study focused on the first 2 months of the treatment period (intensive phase) and evaluated the impact of the drugs used on liver function biomarkers and hematological parameters before and after the administration of the TB drugs.

3.8 Diagnosis of drug-induced hepatotoxicity

A patient was considered to have developed hepatotoxicity when any of the following criteria were met; i) patient presented with signs and symptoms of hepatotoxicity following initiation of anti-TB drugs with the elevation of total Bilirubin and transaminases (ALT and/ or AST) to more than twice the ULN. (Normal reference range for ALT: 7- 41 U/L, AST: 12-38 U/L and Bil: 3.40 – 25.70µmol/L) ii) patient developed no clinical symptoms of hepatotoxicity but following the initiation of anti-TB drugs, ALT and/ or AST concentrations were observed to have elevated to more than five times the ULN and iii) patient developed anorexia, nausea, vomiting, abdominal pain, jaundice, or unexplained fatigue together with an increase in AST and /or ALT above pre-treatment levels and iv) if there was normalization or at least a 50% improvement in abnormal hepatic enzymes concentrations and when symptoms & signs of liver toxicity after the discontinued of all anti-TB drugs resolved (Sarda *et al.*, 2009). The reason to use clinical features of drug-induced hepatotoxicity as my definition for anti-TB-DIH was based on the fact that all major guidelines for TB treatment consider the presence of symptoms of hepatotoxicity as the absolute indication for stopping anti-TB therapy. This is because asymptomatic transaminase elevations occur in 20% of

patients started on standard anti-TB regimens before, or immediately after the start of treatment. Usually, hepatic adaptation occurs, that is, these elevations resolve spontaneously. This does not necessitate treatment interruption.

3.9 Sputum collection

Each participant was provided with two separate sterile, labeled, screw-cupped sputum containers to collect two separate samples at an hour interval. This was to confirm the presence of TB. Also, an X-ray and the medical history of the patients were reviewed to help diagnose the type of TB disease.

3.9.1 Laboratory Diagnosis of Tuberculosis

The Zeihl Neelson Staining Technique (Cheesbrough, 2006) was used in the detection of Mycobacterium tuberculosis among the patients. Briefly, smears were prepared on a microscope slide, allowed to air dry, gently heat-fixed and smear covered with carbol fuchsin solution. On each prepared slide, was flamed to apply heat until steaming and then let on to stand for 5mins. With a low pressured flowing tap water to wash off the stain. A 20% sulphric acid was then poured on the slide, allowed to stand for another 5mins for decolorization. After washing again with water, methylene blue solution was then applied for 30s to 1min to counterstain. The slide was then washed and allowed to air-dry. Under a ×100 oil immersion objective lens of a microscope, the slides were inspected. Results from the microscopy were reported as negative, scanty, 1+, 2+ and 3 + (Cheesbrough, 2006).

3.10 Blood Sample collection

Five milliliters venous blood samples were collected into gel separator vacutainer tubes and ethylene diethyltetramide (EDTA) tubes (to prevent clotting) separately, before initiation of anti-TB drugs (baseline), and two-week interval for the first two months after the administration of the anti-TB drugs. Blood samples for liver markers (LFT)

and full blood count (FBC) estimation were collected before and after the anti-tuberculosis medicines have been administrated, and transported to the laboratory for analysis, by a phlebotomist. Samples in the gel separator were allowed to clot for 10mins, centrifuged at 3000rpm for 5mins to obtain serum, aliquot into cryovalves and stored under -21^{o}C temperature for further analysis. The Full blood count (FBC) and liver function test were analyzed every two weeks during treatment with the final sample taking on the eighth week of treatment. Prior-therapy samples (baseline) were taken as controls. Patients with high levels of liver enzymes levels (ALT, AST, ALP, ALB and TBB) on repeated tests were categorized as having hepatotoxicity and were then evaluated for various factors such as age, sex, alcohol intake and the concomitant use of other drugs.

3.10.1 Liver Function Enzymes Measurements

The serum concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (alb), total bilirubin (TBil), gamma-glutamyltransferase (GGT), and alkaline phosphatase (ALP), were measured in blood serum, using a fully automated biochemistry analyzer (Pro S Selectra, EliTech Group Clinical System), following the manufacturer's instructions and the Standard Operational Procedures (SOP) of the Biochemistry Department of the CCTH laboratory.

3.10.2 Test Principles for Liver enzyme markers

3.10.2.1 Alanine Transaminase (ALT)

Alanine transaminase (ALT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate. The pyruvate is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH).

The reaction is monitored kinetically at 340nm by the rate of decrease in absorbance, resulting from the oxidation of NADH to NAD^+, proportional to the activity of ALT present in the sample. (Normal reference range: 7-41 U/L)

L-Alanine + 2-Oxoglutarate $\xrightarrow{\text{ALT // GPT}}$ L - Glutamate + Pyruvate

Pyruvate + NADH + H^+ $\xrightarrow{\text{LDH}}$ Lactate + NAD^+

3.10.2.2 Aspartate transaminase (AST)

Aspartate transaminase catalyzes the transfer of the amino group from aspartate to oxoglutarate with the formation of glutamate and oxaloacetate. The oxaloacetate is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH).

The reaction is monitored kinetically at 340nm 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. (Normal reference range: 12-38 U/L).

L-Asparatate + 2-Oxoglutarate $\xrightarrow{\text{AST / GOT}}$ L - Glutamate + Oxolacetate

Oxolacetate + NADH + H^+ $\xrightarrow{\text{MDH}}$ L - Malate + NAD^+

3.10.2.3 Gamma glutamyltransferase (γ-GT)

Gamma-glutamyltransferase catalyzes the transfer of a γ-glutamyl group from γ-glutamyl-3-carboxyl-4-nitroanilide to form glycylglycine with the formation of L-γ-glutamyl-glycylglycine and 5-amino-2-nitro-benzoate. An enzyme activity equivalent to the enzyme action present in the sample is then monitored at 405nm in a kinetic reaction. (Normal reference range: 9.0-36.0 U/L).

(L-γ-Glutamyl)-3-carboxyl-4-nitroanilide $\xrightarrow[\text{Glycylglycine}]{\gamma\text{GT}}$

(L-γ-Glutamyl)-glycylglycine + 5-amino-2-nitro-benzoate

3.10.2.4 Serum Albumin

The method is based on the specific binding of bromocresol green (BCG), an anionic dye, and the protein at acidic pH with the resulting shift in the absorption wavelength of the complex. The intensity of the colour formed is proportional to the albumin in the sample. (Normal reference range: 34.0-50.0 g/L).

BCG + Albumin $\xrightarrow{\text{pH 4.3}}$ BCG-albumin complex

3.10.2.5 Serum Bilirubin

The auto-analyzer uses a timed-endpoint Diazo method to measure the concentration of total bilirubin in serum or plasma. In the reaction, bilirubin reacts with the diazo reagent in the presence of caffeine, benzoate, and acetate as accelerators to form azobilirubin. The system monitors the change in absorbance at 520 nm at a fixed-time interval. This change in absorbance is directly proportional to the concentration of total bilirubin in the sample. (Normal reference range: 3.40-25.70 µmol/L).

3.10.2.6 Total Protein

In the biuret reaction, a chelate is formed between the Cu^{2+} ion and the peptide bonds of the proteins in alkaline solutions to form a violet coloured complex whose absorbance is measured photometrically. The intensity of the coloured produced is proportional to the concentration of the sample. (Normal reference range for serum total protein, TPro: 62.0-85.0 g/L).

Cu^{2+} + Serum protein $\xrightarrow{\text{pH 12 (25-37oC)}}$ Copper- protein complex

3.11 Hematological Parameters Measurements

The hematological parameters were determined on whole blood using a fully automatic hematology analyzer, Sysmex X500i; Sysmex Corporation, Kobe, Japan, in the Hematology Department of the Cape Coast Teaching Hospital Laboratory. This

analyzer performing full blood count (FBC) on EDTA anticoagulated blood, for counting the cellular blood components, concentrating more on the impact on with more emphases regarding the effects on hemoglobin level (HBs), white blood cells (WBCs) count, red blood cells (RBCs) count, and the platelet count.

3.11.1 Operational Procedure for Sysmex 500i (Hematology Analyser)

EDTA blood sample (10µl) was placed in the aspirator of the instrument. And the blood sample was aspirated. The results were provided within 1 minute on the LCD and documented.

3.11.2 Principle for Hematological Parameters Measurements

The Sysmex 500i analyzer performs blood cell count by DC detection method. In this technique, the aspirated blood sample is measured at a fixed volume, diluted at the specified ratio, and the fed into each transducer. A minute hole called the aperture is in the transducer chamber. The electrode between which the direct current flows are on both sides of the aperture. In the diluted sample, blood cells suspended pass through the aperture causing direct current resistance between the electrodes to shift. The size of the blood cell is detected as electricity as direct current resistance changes.

3.12 Viral Testing

Hepatitis A, B, C and HIV testing were performed on the blood serum, using their respective test strips.

3.12.1 HBsAg Test Principle

The surface antigen of the hepatitis B virus (HBsAg) Testing was grounded on the principle of a chromatographic immunoassay for the qualitative detection of the hepatitis B surface antigen in whole blood, plasma and serum samples. In particularly, Polyclonal and monoclonal antibodies are actively employed to identify hepatitis B

surface antigen. The test technique is highly sensitive and without employing any equipment results can be read visually.

A chromatographic lateral flow device system employs this test technique. On a nicotrocellulous membrane strip, colloidal gold conjugated monoclonal antibodies reacting to HBsAg (sAb-Au) are dryly immobilized. It migrates through capillary diffusion through thestip that rehydrates the gold conjugate when the sample is inserted. HBsAg can bind with the particle-forming gold-conjugated antibodies if present. These particles will continue to migrate along the strip until they are detected and visible in the Test Zone (T) by anti-HB antibodies immobilized there.

3.12.2 HCV Test Principle

Recombinant HCV antigen and mouse anti-human IgG antibody conjugated to colloidal gold are embedded in the sample pad. If the specimen is positive, the HCV antibody in whole blood, serum or plasma specimen combined with the colloidal gold conjugated recombinant HCV antigen and generate a complex. As the mixture moves along the test strip, the complex was captured by the recombinant HCV antigen immobilized on the membrane, forming a purplish red test band in the test region. A negative specimen did not form any test band due to the absence of a colloidal gold conjugate/HCV antibody complex. Regardless of whether HCV antibodies exist in a specimen, the unbound gold marked protein did bind to the sheep anti-mouse IgG in the control band region and form a purplish red band.

3.13 Data Management and Analysis

Using a statistical software (EpiData, version 3.1), all data were coded, entered, and cleaned, and afterwards exported into and analyzed with SPSS, version 20 for Windows (SPSS Inc., Chicago, IL, USA). After checking for skewness, the mean±standard deviation (SD) otherwise median and interquartile range and frequency of variables

were determined. T-test was used to determine statistical significant difference in the concentration of liver enzymes among levels of hepatotoxicity. A chi-square test or fisher exact test was used to assess associations between categorical variables. A lees than or equals to 0.05 p -value was statistically considered significant.

3.14 Ethical Considerations

The ethical approval was received from the Ethical and Protocol Review Committee of the School of Biomedical and Allied Health Sciences which was mandated by the College of Health Sciences, University of Ghana. An official introductory letter was also sent to the CCTH and the Central Regional Health Directorate, seeking permission to carry out the study. For participating voluntarily, an informed consent grounded on the unambiguous information of any potential hazard, harm, or even displeasures as a result of data or sample collection techniques, as well as any benefits was signed by all study patients. Additionally, issues in relation to intervention particularly in the case of hepatotoxicity development were thoroughly discussed. Oral and written informed consents were obtained from the patients prior to enrolment. Counseling for all the tests especially, HIV tests were done and those who give their consent were tested for. Tuberculosis treatments are given free of charge according to policy for tuberculosis management by National Tuberculosis Programme (NTP).

CHAPTER FOUR

4.0 RESULTS

4.1 Characteristic of Participants

A total of 40 newly diagnosed TB patients who were placed on anti-TB drugs were recruited and were monitored for 2 months. During this period, 2 participants died and 8 were also lost to follow-up by week 8. Participants' mean age was 39.7years and standard deviation of 14.2years with age ranging from 18 years to 67 years. Most (75%) of the patients were adults of age 25 – 64 years. Twenty-six were male, comprising two-thirds (65%) of the participants.

4.2 Serum Biochemical Analytes

Levels of liver markers and haematological parameters were determine for 8 weeks. The median and interquartile ranges (IQR) of these liver marker concentrations: ALT, AST, ALP, GGT, ALB, TPro, TBil are presented Table 4.2.

Table 4.1: Characteristics and clinical presentation of TB patients

Variable	Category	No. of patient	Percentage (%)
Age	Mean ±SD (range)	39.7±14.2 (18-67)	
.	15 – 24 years	7	17.5
	25 – 64 years	30	75.0
	> 64 years	3	7.5
Gender	Male	26	65.0
	Female	14	35.0
BMI, kg/m^2	Mean ±SD (range)	21.2±8.3 (5.8-49.1)	
	Under weight	15	37.5
	Normal weight	19	47.5
	Obese	6	15.0
Alcoholism	Yes	2	5.0
	No	38	95.0
Recreational drugs	Yes	2	5.0
	No	38	95.0
Aneamia	Yes	3	7.5
	No	37	92.5
Hypoalbumineamia	Yes	8	20.0
	No	32	80.0

Data presented as mean ±SD and as frequencies and percentages. SD = standard deviation

Table 4.2: Anti-tuberculosis drug effects on liver functions among TB patients

Liver function	Reference range	Pretreatment (median, IQR)	Post-treatment (median, IQR)			
			Week 2	Week 4	Week 6	Week 8
ALT, IU/L	10.0 – 50.0	22.7 (16.1-33.6)	15.9 (11.0-23.3)	27.6 (20.9-35.8)	16.9 (10.8-27.8)	14.4 (10.5-24.3)
AST, IU/L	5.0 – 34.0	48.6 (28.6-65.9)	24.1 (17.3-37.0)	59.3 (39.3-74.9)	39.6 (17.5-60.4)	33.5 (18.6-41.3)
ALP, IU/L	42.0 – 270.0	257.5 (209.5-315.6)	256.8 (138.0-346.7)	208.0 (142.0-307.4)	158.6 (104.8-256.1)	177.4 (126.8-307.4)
GGT, I U/L	9.0 – 36.0	50.5 (30.8-72.2)	46.1 (25.4-70.9)	41.8 (28.1-65.6)	39.2 (28.4-62.4)	28.9 (19.7-43.3)
ALB, g/L	34.0 – 50.0	37.3 (32.6-41.7)	38.2 (32.4-41.3)	40.7 (37.3-44.3)	40.9 (37.6-45.6)	41.0 (38.0-45.0)
TPT, g/L	62.0 – 85.0	79.3 (72.1-86.2)	88.9 (82.2-93.7)	85.2 (79.5-88.2)	84.7 (77.3-92.7)	84.4 (77.7-90.5)
TBB, µmol/L	3.4 – 25.7	9.9 (8.2-13.3)	10.6 (8.5-14.2)	9.9 (7.6-12.2)	10.1 (8.2-12.3)	9.3 (7.6-11.1)

Table 4.2 shows the anti-tuberculosis drugs effects on liver functions among TB patients IQR-interquartile range, AST-serum aspartate aminotransferase, ALT-alanine aminotransferase, ALP-alkaline phosphatase, GGT- Gamma-Glutamyl Transpeptidase, ALB- Serum albumin, TPT-Serum total Protein, TBB- Serum total bilirubin

The pattern of median concentration of liver function tests of TB patients within 8 weeks were illustrated in Figure 4.1. Generally, the median concentration of liver markers were not different between weeks and across time (p> 0.05)

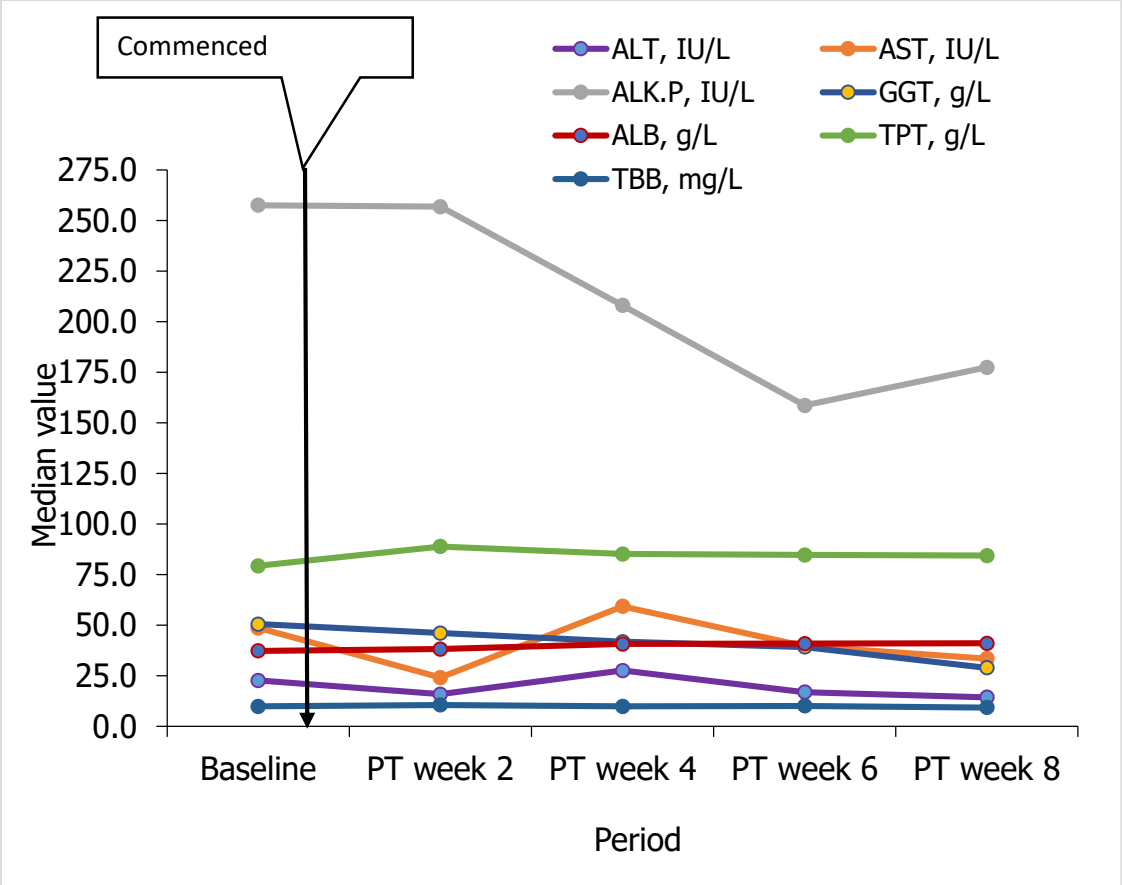


Figure 4.1: Median concentrations of liver function tests of TB patients within 8 weeks

AST-serum aspartate aminotransferase; ALT- alanine aminotransferase; ALK.P-alkaline phosphatase; GGT- Gamma-Glutamyl Transpeptidase; ALB- Serum albumin; TPT-Serum total Protein; TBB- Serum total bilirubin; and PT- post-treatment

Concentrations of liver markers between the hepatotoxicity induced by the drug (DIH) and non-hepatotoxicity induced by the drug (non-DIH) subjects at pretreatment and post treatment are shown in the Table 4.3. Generally, the median concentration of liver enzymes were much higher (p< 0.05) in DIH group than the non-DIH group at baseline and post-treatment. .

There were significant differences between the DIH group and non-DIH group for ALT, AST and GGT at baseline (P<0.05) and at post treatment (P<0.05) respectively. ALP,

ALB, TPT and TBB concentrations did not significantly (p>0.05) differ at baseline and at post treatment between DIH and Non-DIH groups.

Table 4.3: Pretreatment and post-treatment liver function tests in 6 DIH and 34 non-DIH

Liver function	Pretreatment (median, IQR)		P-value	Post-treatment (median, IQR)		P-value
	DIH(n=6)	Non-DIH(n=34)		DIH(n=6)	Non-DIH(n=34)	
ALT, U/L	45.5 (17.0-76.7)	21.5 (16.0-29.0)	<0.001	51.2 (39.9-79.5)	26.6 (16.6-33.1)	<0.001
AST, U/L	94.9 (67.7-124.2)	48.1 (27.5-60.0)	0.003	106.4 (81.5-170.3)	48.4 (36.6-67.1)	<0.001
ALP, U/L	499.2 (267.6-682.1)	253.8 (203.6-300.6)	0.6176	403.1 (155.7-570.1)	206.7 (135.9-303.0)	0.888
GGT, g/L	64.6 (36.2-100.0)	50.0 (25.7-70.0)	0.047	60.2 (36.3-78.6)	41.4 (27.0-57.3)	0.021
ALB, g/L	34.5 (32.3-39.4)	37.4 (32.9-42.0)	0.491	36.9 (35.5-44.6)	40.9 (37.6-44.3)	0.481
TPT, g/L	83.1 (79.1-86.2)	79.1 (71.7-86	0.405	86.1 (83.5-94.7)	85.1 (79.5-88.2)	0.889
TBB, mg/L	9.9 (8.9-16.9)	9.9 (7.7-13.3)	0.714	7.2 (6.4-10.2)	9.9 (8.1-12.3)	0.809

DIH-Drug-induced hepatotoxicity group; non-DIH: non drug-induced hepatotoxicity group; IQR-interquartile range; AST-serum aspartate aminotransferase, ALT- alanine aminotransferase, ALP-alkaline phosphatase, GGT- Gamma-Glutamyl Transpeptidase, ALB- Serum albumin, TPT-Serum total Protein, TBB- Serum total bilirubin. p< 0.05 is statistically significant.

Repeated measures ANOVA with a Greenhouse-Geisser correction showed that the median concentrations of the liver enzymes decreased between time points (F (1.208, 7.251) = 1.332, P = 0.208), but was however not statistically significant (p>0.05) (Figure 4.2).

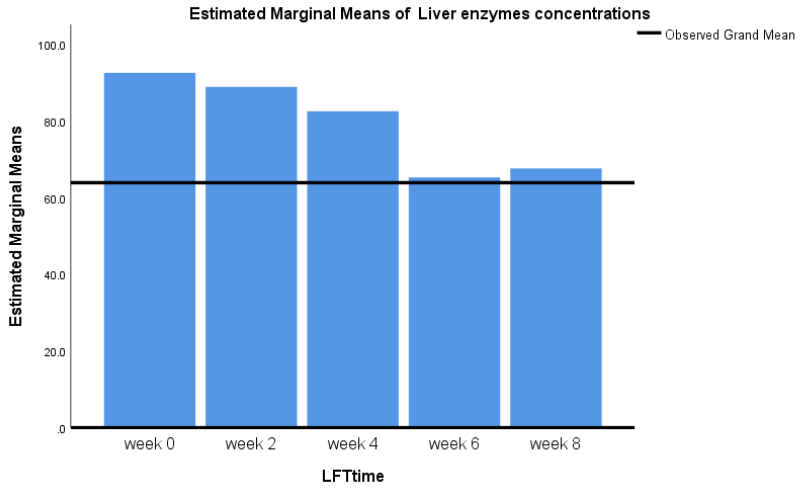


Figure 4.2: Estimated marginal medians for the liver enzymes concentrations among TB patients

The mean concentrations of hematological parametes**:** Haemoglobin (HB), red blood cells (RBC), platelets (PLT) and white blood cells (WBC) are presented Table 4.4. The HB was lower than the lower limit normal, LLN, (11.5 – 16.0 g/dL) at baseline (9.6 g/dL), abut returned within normal limits by week 8 (11 g/dL). RBC was slightly lower than the LLN (3.9 – 5.2 10^6/µL) at baseline (3.7 10^6/µL) but increased significantly after 8 weeks post medication. The mean concentration of WBC, and PLT did not differ significantly at baseline and during anti-TB medication and were within their acceptable reference ranges and with exception of. Generally, the anti-TB medication increased the mean concentrations of HB and RBC, but declined WBC and PLT.

Table 4.4: Anti-tuberculosis drug effects on hematological parameters among TB patients

Hematology	Reference range	Pretreatment (mean± SD)	Post-treatment (mean±SD)			
			Week 2	Week 4	Week 6	Week 8
HB, g/dL	11.5 – 16.0	9.6±2.0	9.8 ±2.0	10.5 ±1.7	11.1 ±1.8	11.0 ±1.8*
WBC, 10^3/µL	4.0 – 10.0	6.6 ±5.1	6.3 ±2.9	5.3 ±2.4	4.8± 2.9	5.7 ±5.6
RBC, 10^6/µL	3.9 – 5.2	3.7 ±0.8	3.8 ±0.8	4.1± 0.7	4.4 ±0.7	4.5 ±0.8*
PLT, 10^6/µL	140.0 – 440.0	253.9 ±129.6	249.8±110.5	226.6±118.7	257.9±103.9	243.0±104.7

Table 4.4 shows the anti-tuberculosis drug effects on hematological parameters among TB patients. SD-standard deviation, HB- Hemoglobin, RBC- Red Blood Cells, WBC- White Blood Cells, and PLT- Platelets. * means significant (p-value < 0.05) compared with baseline.

4.3 The anti-TB-DIH incidence among TB patients

The Proportion of anti-TB induce hepatotoxicity among TB patients are shown in Figure 4.3. The anti-TB induced hepatotoxicity among 6 TB patients representing 15% of the total sample.

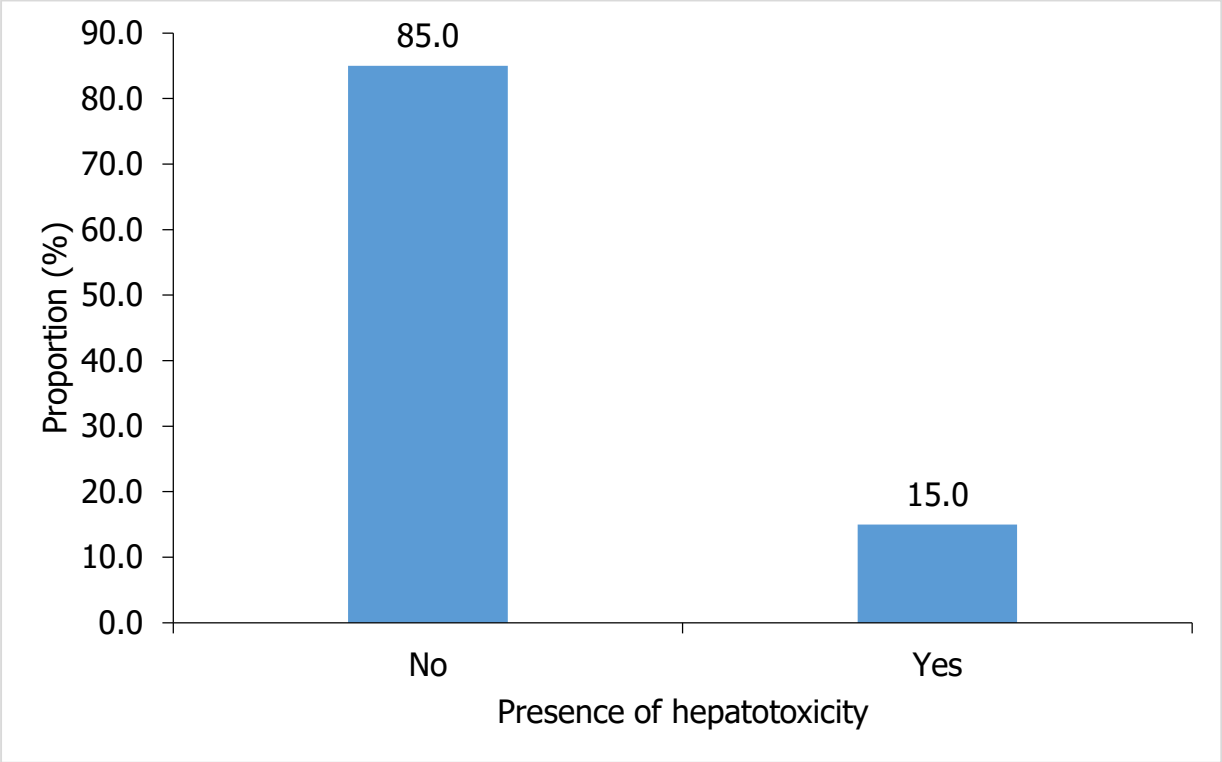


Figure 4.3: Proportion of anti-TB induced hepatotoxicity among TB patients

4.3.1 Severity of anti-TB drug induced hepatotoxicity

Table 4.5 summarized the severity of anti-TB drug induced hepatotoxicity**.** The intensity of liver toxicity was grounded on the level of bio-chemical imbalance according to the World Health Organisation side effect of medicine reaction scaling system. Among the DIH patients, moderate hepatotoxicity was observed in five (83.3%), severe hepatotoxicity in one patient (16.7%) with none suffering very severe hepatotoxicity.

Table 4.5: Extent of intensity of anti-TB drug induced hepatotoxicity

Severity level	Level of enzymes	No. of cases (%)
Moderate	<5 times ULN (121–200 IU/L)	5 (83.3)
Severe	5–10 times ULN (201–400 IU/L)	1 (16.7)
Very severe	>10 times ULN (.400 IU/L)	0 (0.0)
Total	**>3 times ULN (>120IU/L)**	**6 (100.0)**

Table 4.5 shows degree of severity of anti-TB drug induced hepatotoxicity

4.4 Factors associated with anti-TB drug induced hepatotoxicity

On the associations between socio-demographic characteristics (age, gender, alcohol intake, and use of recreational drugs), anti-TB combination, clinical presentation and the development of hepatotoxicity among study participants, Chi-square/Fisher exact test and Logistic regression were performed and are presented in Table 4.6.

The proportion of hepatotoxicity was much higher in younger (15 – 24years) TB patients (57.1%) and was followed by the adult group (6.7%). The proportion of hepatotoxicity was slightly higher among obese patients (33.3%) than normal patients (10.5%), non-alcoholics (16.2%), and taking recreational drugs (50%). No statistical association was found for gender, alcohol intake, use of recreational drugs, anti-TB combination, clinical presentation and anti-TB drug induce hepatotoxicity (at $p<0.05$).

Table 4.6: Demographics and clinical presentations associated with anti-TB induce hepatotoxicity, Cape Coast Teaching Hospital, 2020

Variable	Hepatotoxicity n (%)	Non-hepatotoxicity n (%)	X^2 Value	p-value	OR	95%CI
Age, years			9.208	0.002	0.026	2.53-598.608
15 – 24	4 (57.1)	3 (42.9)				
25 – 64	2 (6.7)	28 (93.3)				
> 64	0 (0.0)	3 (100.0)				
Gender			1.017	0.080	0.917	0.146-5.757
Male	4 (15.4)	22 (84.6)				
Female	2 (14.3)	12 (85.70				
BMI, kg/m^2			0.734	0.822	1.250	-
Mean (range)	26.1(14.1-49.1)	20.3 (5.8-46.1)				
Under weight	2 (13.3)	13 (86.7)				
Normal weight	2 (10.5)	17(89.5)				
Obese	2 (33.3)	4 (66.7)				
Alcoholism			1.017	0.313	0.912	0.821-1.012
Yes	0 (0.0)	3 (100.0)				
No	6 (16.2)	31 (83.8)				
Recreational drugs			1.451	0.228	0.152	0.01-2.823
Yes	1 (50.0)	1 (50.0)				
No	5 (13.2)	33 (86.8)				
Aneamia			1.017	0.313	0.912	0.821-1.012
Yes	0 (0.0)	3 (100.0)				
No	6 (16.22)	31 (83.8)				
Hypoalbumineamia			0.051	0.822	1.290	0.130-12.960
Yes	1 (12.5)	7 (87.5)				
No	5 (15.6)	27 (84.4)				

Table 4.6 shows the demographics and clinical presentations associated with anti-TB induced hepatotoxicity. n- Frequency; %- percentage; X^2- chi square value; p – value – level of statistical significance; kg/m^2 – kilogram per meter square; OR - Likehood ratio

CHAPTER FIVE

DISCUSSION

Tuberculosis remains a significant public health problem among developing countries, as it contributes the largest causes of death among world infectious diseases (Mirlohi *et al.*, 2016). The standard recommended combination therapy with anti-TB drugs has been proven to be a very effective treatment modality for new susceptible TB infection cases. However, these drugs possess hepatotoxic properties, thus may cause serious adverse effects. Anti-TB drugs induced hepatotoxicity (anti-TB DIH) is a critical predicament and major reason for interruption of treatment during TB treatment course (Ambreen, 2016) thus, increased length of hospitalization and economic burden to both patients and clinics. Son, 2016, reported that hepatic injury is the common adverse effect of first line anti-tubercular drugs. This present study aimed to determine the hepatotoxic effect induced by anti-tuberculosis drugs, incidence of DIH and identify TB patients at greater risk, who are receiving first-line anti-tuberculosis drugs for TB treatment in the Cape Coast Teaching Hospital.

In the present research work, 15% of the participants developed anti-TB drug-induced hepatotoxicity. This finding was in agreement with a previous study by Vijayalakshmi (2016), who also reported a 15% incidence rate. The present study finding was however lower compared to a 19.8% incidence reported from Karachi (Mahmood *et al.*, 2007) and 24.6% from a retrospective study in Morocco (El Bouazzi *et al.*, 2016). Other higher prevalence of 31.37% and 36.7% had also been reported in Iran (Khalili, 2009), and Brazil (Lima and Melo, 2012) respectively. Compared to the present study, a much lower prevalence,11.5 % amongst HIV/TB coinfected participants in Ethiopia (Hassen Ali *et al.*, 2013a), 9.48% in South India (Saha *et al.*, 2016) and 8.7% in Korea (Jeong *et al.*, 2015) has been reported. The discrepancy with these prevalence observed

in this study could probably have resulted from the differences in the study design, study participants, geographical areas, the diagnostic criteria used to define hepatotoxicity, characteristics, the risk factors of the population studied, laboratory analysis carried out during follow ups and the monitoring technique employed (Ramappa and Aithal, 2013).

The development of anti-TB DIH among study participants of this study was within an average of 4.3 weeks from start of therapy. This agreed with a similar study in Nepal which reported 12 – 60 days with a median of 28days (Shakya *et al.*, 2005). Makhlouf *et al.,* 2008, similarly documented the beginning of anti-tuberculosis induced hepatotoxicity ranging from 15 - 60 days (median, 30 days), correlating to the present study. In contrast, Mahmood *et al.*, reported that the onset of anti-TB-DIH in almost two-thirds of their patients was within 2 weeks from the start of therapy (Mahmood *et al.*, 2007).

Two patients each, developed anti-TB DIH in the second and fourth week while one patient each, had the condition in the six and eight week, respectively was observed in the present study. This affirmed the general perception that the development of anti-TB DIH is conventionally within the intensive phase of treatment (Nair *et al.*, 2020).

Anti-TB drugs are said to be toxic to the liver, particularly when pyrazinamide and rifampicin are administered concurrently (Devarbhavi *et al.,* 2010). Isoniazid-induced damage to the liver is as a result of either by hypersensitivity induced by acetyl hydrazine, acetyl hydrazine (AcHz) or by the toxicity of its metabolite. A cytochrome P450 enzyme-induction by Rifampicin, causes an acceleration in the generation of the toxic metabolites from AcHz (Abbas *et al.*, 2012). The rate of conversion of isoniazid to isoniazid acid and hydrazine (both hepatotoxic) is increased by rifampicin (Neha *et al.*, 2014). The plasma half-life of AcHz is reduced by Rifampicin and quickly

converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to a reported higher incidence of liver necrosis caused by the combined therapy of isoniazid and Rifampicin (Senousy *et al*., 2010). According to Semvua *et al.*, Rifampicin interaction with other hepatotoxic medicines impact the plasma concentrations, including the danger of toxicity of the liver (Semvua *et al.*, 2015). Pyrazinamide-induced hepatotoxicity is dependent on dose but rarely hepatic damage is not caused by the dose generally administer (Pasipanodya and Gumbo, 2010). Enzymes responsible for drug metabolism in hepatocyte microsomes may have an inborn defect, malformation, low activity, or be inhibited by drugs, making drug metabolites very toxic to hepatocytes (Nebert *et al.*, 2013). Hypersensitivity to drugs may be another reason. The drugs can behave like haptens producing allergic reaction by immune mechanism causing increment in liver enzymes (Meyer, 1996). Often anti-TB drugs cause disruption in hepatic function analysis and may lead to disturbance to liver function tests and may cause severe hepatic dysfunction (Hepatitis and Chemotherapy, 2011). I agree with the study by Ambreen, 2016, who suggested that laboratory confirmation is necessary to establish the presence of anti-TB drug-induced hepatotoxicity. This may help relieved the hepatotoxic effects of anti-TB-drugs, by interrupting the treatment at an early stage, since early detection can decrease the severity of hepatotoxicity if the drug is discontinued.

The present study revealed a significant higher median concentration of GGT, AST and ALT levels among TB patients at baseline and post treatment (Table 4.3). Note that, though GGT levels were high, it declined gradually, falling within the normal range. This may be attributed to the ability of transaminases and the GGT to increase as a direct involvement of the TB disease. It is of importance to note that the asymptomatic increase in the baseline median concentrations of transaminases and GGT were not

enough to disqualify study patients. Mokondjimobe *et al.*, (Mokondjimobe *et al.*, 2012) in their study reported an increase in serum transaminases and a decrease in the serum GGT during the initial two-month phase of DOTS, and this is consistent with my present study findings. The observed trend of the serum transaminases and GGT reflect the direct effect that the TB drugs had on the hepatocellular cells during the intensive phase of treatment.

Haematological findings from the present study, showed that at baseline, the mean HB level and mean RBC level were lower than the lower limit of normal (Table 4.2). This may be due to the fact that TB-infected participants have greater tendency of developing problems of absorption in the gastrointestinal tract, thus causing aneamia (defined as HB and/or RBC level below the lower reference range) perhaps, as a secondary adverse effect. Studies have reported aneamia as a scientific expression of TB and as a therefore of either iron deficiency or by chronic diseases including TB disease (Rupert and Katie, 2012; Chung-Delgado *et al.*, 2011). Aneamia in TB is usually as a result of suppression of the bone marrow, , failure of iron utilization, malabsorption syndromes and, deficiencies in nutrition (Oliveira *et al.*, 2014). Thus lower basal HB and RBC levels may be markers related to TB disease. However, in the course of treatment, the mean HBs and RBC levels improved to normal values (Table 4.2). This finding was in disagreement with the study by Mirlohi *et al.*, who decumented a decrease in both the mean HB and RBC during anti-TB therapy (Mirlohi *et al.*, 2016). Anaemia resulting from hemolysis can undeviatingly, be as a result of Rifampicin and Isoniazid, while sideroblastic aneamia is as a result of pyrazinamide (Nandennavar *et al.*, 2011). Chung-Delgado *et al.*, have suggested that aneamia may not be a danger for the presence of detrimental results, instead rather the detrimental results itself (Chung-Delgado *et al.*, 2011).

Neutropenia has been reported as the most frequent adverse effect of Isoniazid and to some extend related to rifampicin, ethambutol and streptomycin, during TB therapy (Belloumi *et al.,* 2018). This study did not observe possible neutropenia or thrombocytopenia among TB patients. Platelet count have been reported to decrease by many drugs; however, recover upon withdrawal of suspected drug **(**Hadida *et al.*, 2013**)**. Das, 2013 reported that generally, improvement in the haematological status of patient, depends solely on the improvement of the disease process, and this agrees with the observations of the present study.

Glutathione (a key antioxidant) stores deplete in malnourished persons and may predispose them to oxidative injuries from drug metabolites (Ramappa and Aithal, 2013). Mahmood *et al.,* had reported malnutrition as predator among 91% of TB-patients with anti-TB DIH (Mahmood *et al.,* 2007). The reliability of liver metabolism and detoxification of TB drugs depend on availability of adequate nutrient and weight balance of TB-patients, since the cytochrome P450 enzyme system (mainly responsible for drug metabolism and detoxification) is affected by nutrient intake, fasting and underweight states of the patients (Ramappa and Aithal, 2013). Another study by Hassen Ali *et al.,* found BMI less than 18.5 kg/m^2 as a significant predictor of anti-TB DIH with 3.6 folds odds compared to TB-patients with normal weight and obese patients (Hassen Ali *et al.,* 2013b). Mahmood *et al.,* 2007, documented BMI < 18.5 kg/m2 and hypoalbumineamia (a low albumin < 35 g/L and suggestive of lean body mass) as predictors of anti-TB DIH (Mahmood *et al.,* 2007). Contrary, the present study found BMI and hypoalbumineamia having relationship with the development of anti-TB-DIH. However, the mean BMI was observed to be in a higher proportion with the obese TB-patients who later developed anti-TB DIH. Statistically, anti-TB DIH could not be linked with hypoalbumineamia in this my present study. Yet, findings showed

that among the TB-patients with anti-TB DIH, 12.5% tended to developed hypoalbuminaemia (Table 4.6). A prior study found a much higher prevalence of hypoalbuminemia in 27% among TB-patients who developed anti-TB DIH (Mahmood *et al.*, 2007). The inconsistency observed in this present study may probably due to the fact that patients with other comorbidities were strictly excluded to minimize the influence on other factors of DIH in the present study, the extensive nutritional education given by health providers at the treatment center, it has been demonstrated that oxidative stress is heightened in malnourished person and poor nutritional status is associated with immune dysfunction (Bourke *et al*., 2016), and also the relatively small sample size used in the study.

Basing on the level of biochemical derangement according to WHO adverse drug reaction grading system, Hassen Ali *et al.*, 2013a, reported a 21.2 % incidence of severe hepatotoxicity and 24.4% incidence of very severe hepatotoxicity cases in TB/HIV co-infected patient on anti-TB drugs. The findings of the present study revealed, moderate hepatotoxicity, and severe hepatotoxicity in patient who developed anti-TB DIH (Table 4.3). These patients were mostly asymptomatic, therefore continued with their regimen whilst under strict monitoring.

In the present study no relationship was found between gender and anti-TB-DIH. This strengthens the studies of Makhlouf *et al.,* and Hassen Ali *et al.,* who reported no association between female gender and anti-TB-DIH (Hassen Ali *et al.,* 2013a; Makhlouf *et al.,* 2008). Contrary, other studies reported that being a woman makes a person stand at risk for developing anti-tuberculosis drug induced hepatotoxicity (Shakya, Rao and Shrestha, 2005; Devarbhavi *et al.,* 2010). This may be due to the fact that female gender is associated with higher cytochrome P3A enzyme activity compared to males and this may explain females being more vulnerable to drug-

induced hepatotoxicity in general (Ramappa and Aithal, 2013). However, the present study found the proportion of anti-TB- DIH slightly higher among male (15%) than females (Table 4.6). This discrepancy might be due to the fact that the present study included a high proportion (65 %) of male patients compared to females.

Several studies (Saukkonen *et al.,* 2006; Mahmood *et al*., 2007; Khalili, 2009; Pandit, Sachdeva and Bafna, 2012; Hassen Ali *et al.,* 2013; Jeong *et al.,* 2015; Ambreen, 2016; Saha *et al.,* 2016; P *et al.,* 2020) have documented that age is not dependent predictor of anti-TB DIH.

This findings correlates with the findings of the present study which found age significantly related to the development of anti-tuberculosis drug induced hepatotoxicity. Age as a risk factor for anti-TB-DIH may be as a result of reduced rate of clearing medicines metabolized by cytochrome P450 enzymes, and alterations in liver size, liver blood flow, drug binding. Contrary, Lima and Melo, 2012, had reported no significant relationship between age and the occurrence of hepatotoxicity. However, the present study observed much prevalent (57.1%) of anti-TB-DIH among the younger patients (Table 4.6).This agreed with a similar studies by Mahmood et al., 2007, and Wong, Wu, et al., 2000, who documented that hepatotoxicity was more frequent among younger patients. A direct contrast of what was reported from a systematic review that age over 60 years was associated with the risk of anti-TB DIH (Ramappa and Aithal, 2013). Makhlouf *et al.,* in a similar study reported an anti-TB-DIH rate ranging from 2-8% as age advanced > 35years, with a mean of 5 % (Makhlouf *et al.,* 2008). Naqvi *et al.,* also in their studies reported that hepatotoxicity was associated with patients older than 35 years (Naqvi *et al.,* 2015).The inconsistencies in the age pattern of anti-TB DIH in the different studies could be attributed to the differences in age distribution and sample size among studies.

In agreement with previous studies (Shakya *et al*., 2006; LUSWETI, 2015; Faiz, 2015) that have shown no association of high intake of alcohol as a risk factor with anti-TB-DIH during TB treatment, the present study found similarly. However, Mahmood *et al.*, in their study, reported high intake of alcohol and the use of potential toxic drugs to the liver, concomitantly, as a danger of hepatotoxicity, especially those receiving isoniazid therapy (Mahmood *et al.,* 2007). Other studies reported a significant association between high intake of alcohol and anti-TB DIH (Abera *et al.*, 2016; Khalili, 2009). This effect of alcohol intake might be attributable to the fact that alcohol metabolism yields acetaldehyde, that play a role in glutathione depletion, conjugation of protein, lipid peroxidation, and generation of free radical. Prolonged alcohol abuse induce liver collagen-producing sinusoidal cells, possibly resulting in fibrosis (Mello *et al.,* 2008). Certain drugs like calcium channel blockers, can stimulus cytochrome P450 metabolism of potentially hepatotoxic drugs, which then can initiate drug-induced hepatotoxicity (Gazzerro *et al.,* 2012). Thus, patients with alcohol abuse and concomitant use of other hepatotoxic drugs have an increased risk of drug-induced hepatotoxicity. This present study found the development of hepatotoxicity slightly higher in proportion (16.2%) among the non-alcoholic TB-patients. This might be explained by the fact that most of the alcoholics had normal baseline liver functions and therefore did not have alcohol related liver injury that could have predisposed them to drug adverse hepatotoxicity.

Studies have found the concomitant use of potentially hepatotoxic drug as a predictor for the incidence of anti-tuberculosis drug induced hepatotoxicity (Abera *et al*., 2016;Chang *et al.,* 2007). However, the concomitantly use of other drugs in this study was found to have no link with anti-tuberculosis drug induced hepatotoxicity. This

inconsistency can also be because of the variations in sample size among different studies.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

This study showed 15% of the TB-patients developed anti-TB drug-induced hepatotoxicity. The average period for the development of anti-TB DIH was 4.3 weeks. The liver enzymes, ALT, AST and GGT increase significantly among patients who developed hepatotoxicity. Only age (younger adults; 15-24 years) was found to be statistically significant association with the development of anti-TB-DIH.

6.2 Recommendations

In order to decrease the risk of liver damage of these drugs, a baseline liver enzymes assessment should be performed on all TB patients before prescribing anti-TB drugs. Furthermore, the polymorphisms of the cytochrome P450 and N-acetyltransferase 2 gene/enzyme on the metabolism of drugs, should be researched into determine their potential roles on susceptibility to DIH.

6.3 Limitations

The limitation of this study was the small sample size which was due to prospective nature of the study, making it costly and time-consuming. This study also failed to evaluate the impact of anti-tuberculosis drugs at the end of the 6-month treatment and thus, describe the continuation phase.

All the study participants who were recruited onto the present study were all placed on a four-combined anti-TB regimen. Hence, it was challenging to conclude on the drug accountable for causing the hepatotoxicity.

Additionally, the results obtained are clearly not representative of all tuberculosis patients, since patients with other comorbidities such as hepatitis A, B and C were excluded from the study.

References

Abbas, M. T., Mustafa, M. and Zayni, H. (2012) 'Effect of Morin on Isoniazid and Rifampicin Induced Hepatotoxicity in Rats', (4).

Abera, W., Cheneke, W. and Abebe, G. (2016) 'Incidence of antituberculosis-drug-induced hepatotoxicity and associated risk factors among tuberculosis patients in Dawro Zone, South Ethiopia: A cohort study', *International Journal of Mycobacteriology*. Asian African Society for Mycobacteriology, 5(1), pp. 14–20. doi: 10.1016/j.ijmyco.2015.10.002.

Ahmed, M. and Mobasher, A. B. D. E. (2013) 'Protection against Paracetamol induced adverse effects in the liver by the inhibition of the protein Tyrosine phosphatase 1B'. Available at: http://hdl.handle.net/10261/126062%0A.

Ambreen, K. (2016) 'Anti-Tuberculosis Drug-Induced Hepatotoxicity : A Review', *International Journal of Advanced Biotechnology and Research(IJBR)*, 5(3), pp. 423–437.

Ariani, H. H., Polkowska-nowakowska, A. and Bal, W. (2013) 'E ff ect of', (Ii), pp. 5811–5815.

Baghaei, P. *et al.* (2010) 'Incidence, clinical and epidemiological risk factors, and outcome of drug-induced hepatitis due to antituberculous agents in new tuberculosis cases', *American Journal of Therapeutics*, 17(1), pp. 17–22. doi: 10.1097/MJT.0b013e31818f9eae.

Belloumi, N. *et al.* (2018) 'Leukopenia Induced by Anti-Tuberculosis Treatment', *International Journal of Respiratory and Pulmonary Medicine*, 5(2), pp. 3–5. doi: 10.23937/2378-3516/1410093.

Boakye-Yiadom, A. *et al.* (2020) 'Tuberculosis surveillance system evaluation: case of Ga West municipality, Ghana, 2011 to 2016', *Ghana Medical Journal*, 54(2), pp. 3–10.

doi: 10.4314/gmj.v54i2s.2.

Bonsu, F. *et al.* (2020) 'National population-based tuberculosis prevalence survey in Ghana, 2013', *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease*, 24(3), pp. 321–328. doi: 10.5588/ijtld.19.0163.

El Bouazzi, O. *et al.* (2016) 'First line anti-tuberculosis induced hepatotoxicity: Incidence and risk factors', *Pan African Medical Journal*, 25, pp. 1–10. doi: 10.11604/pamj.2016.25.167.10060.

Bourke, C. D., Berkley, J. A. and Prendergast, A. J. (2016) 'Immune Dysfunction as a Cause and Consequence of Malnutrition', *Trends in Immunology*. Elsevier Ltd, 37(6), pp. 386–398. doi: 10.1016/j.it.2016.04.003.

Brown, A. C. (2017) 'Liver toxicity related to herbs and dietary supplements: Online table of case reports. Part 2 of 5 series', *Food and Chemical Toxicology*. Elsevier Ltd, 107(2016), pp. 472–501. doi: 10.1016/j.fct.2016.07.001.

Butler, D. C., Lewin, D. N. and Batalis, N. I. (2018) 'Differential Diagnosis of Hepatic Necrosis Encountered at Autopsy', *Academic Forensic Pathology*, 8(2), pp. 256–295. doi: 10.1177/1925362118782056.

Cao, L., Greenblatt, D. J. and Kwara, A. (2017) 'Inhibitory effects of selected antituberculosis drugs on common human hepatic cytochrome P450 and UDP-glucuronosyltransferase enzymes', *Drug Metabolism and Disposition*, 45(9), pp. 1035–1043. doi: 10.1124/dmd.117.076034.

Chan, L. M. S., Lowes, S. and Hirst, B. H. (2004) 'The ABCs of drug transport in intestine and liver: Efflux proteins limiting drug absorption and bioavailability', *European Journal of Pharmaceutical Sciences*, 21(1), pp. 25–51. doi: 10.1016/j.ejps.2003.07.003.

Chang, K. C. *et al.* (2007) 'Standard anti-tuberculosis treatment and hepatotoxicity: Do dosing schedules matter?', *European Respiratory Journal*, 29(2), pp. 347–351. doi: 10.1183/09031936.00090306.

Chang, T. E. *et al.* (2019) 'The role of regular liver function monitoring in antituberculosis drug-induced liver injury', *Journal of the Chinese Medical Association*, 82(7), pp. 535–540. doi: 10.1097/JCMA.0000000000000119.

Chung-Delgado, K. *et al.* (2011) 'Factors associated with anti-tuberculosis medication adverse effects: A case-control study in Lima, Peru', *PLoS ONE*, 6(11), pp. 1–5. doi: 10.1371/journal.pone.0027610.

Cullen, J. M. (2005) 'Mechanistic Classification of Liver Injury', *Toxicologic Pathology*, 33(1), pp. 6–8. doi: 10.1080/01926230590522428.

Cuperus, F. J., Drenth, J. P. and Tjwa, E. T. (2017) 'Mistakes in liver function test abnormalities and how to avoid them.', *Ueg Education*, 17(1), pp. 1–5.

Cusack, R. P. *et al.* (2017) 'Predictors of hepatotoxicity among patients treated with antituberculous medication', *Qjm*, 110(4), pp. 219–225. doi: 10.1093/qjmed/hcw160.

Čvorović, J. and Passamonti, S. (2017) 'Membrane transporters for bilirubin and its conjugates: A systematic review', *Frontiers in Pharmacology*, 8(DEC). doi: 10.3389/fphar.2017.00887.

Das, S. (2013) *Practical Textbook of Biochemistry for Medical Students*, *Practical Textbook of Biochemistry for Medical Students*. doi: 10.5005/jp/books/12054.

Devarbhavi, H. *et al.* (2010) 'Single-center experience with drug-induced liver injury from india: Causes, outcome, prognosis, and predictors of mortality', *American Journal of Gastroenterology*. Nature Publishing Group, 105(11), pp. 2396–2404. doi: 10.1038/ajg.2010.287.

Dots, T. (no date) 'Unit 5 , part 1 : The DOTS strategy for controlling TB', *International*

Journal, pp. 49–96.

Eno, M. and Cameron, M. (2015) 'Gauging Reactive Metabolites in Drug-Induced Toxicity', *Current Medicinal Chemistry*, 22(4), pp. 465–489. doi: 10.2174/0929867321666140826113520.

Faiz, S. N. (2015) 'Evaluation of Risk Factors for Hepatotoxicity in Patients Receiving Antitubercular Four Drugs Regimen in Initial Two Months', 14(1), pp. 15–18.

Feghali, C. A. and Wright, T. M. (1997) 'Introduction 3. Discussion 3.1 Cytokines involved in acute inflammation 3.1.1 Interleukin-1 3.1.2 Tumor necrosis factor 3.1.3 Interleukin-6 3.1.4 Interleukin-11 3.1.5 Interleukin-8/chemokines 3.1.6 Eotaxin 3.1.7 Interleukin-16 3.1.8 Interleukin-17 3.1.9 C', *Frontiers in Bioscience*, 2, pp. 12–26. Available at: https://www.bioscience.org/1997/v2/d/feghali1/htmls/feghali.pdf.

Fontana, R. J. (2008) 'Acute Liver Failure Including Acetaminophen Overdose', *Medical Clinics of North America*, 92(4), pp. 761–794. doi: 10.1016/j.mcna.2008.03.005.

Frimpong-Mansoh, R. P. *et al.* (2018) 'Evaluation of the tuberculosis surveillance system in the Ashaiman municipality, in Ghana', *Pan African Medical Journal*, 31, pp. 1–10. doi: 10.11604/pamj.2018.31.126.14993.

Gauckler, P. *et al.* (2018) 'Eosinophilia and Kidney Disease: More than Just an Incidental Finding?', *Journal of Clinical Medicine*, 7(12), p. 529. doi: 10.3390/jcm7120529.

Gazzerro, P. *et al.* (2012) 'Pharmacological actions of statins: A critical appraisal in the management of cancer', *Pharmacological Reviews*, 64(1), pp. 102–146. doi: 10.1124/pr.111.004994.

Ghana Statistical Service (GSS) (2013) '2010 Population & Housing Census National Analytical Report', *Ghana Statistical Service*, pp. 1–91.

Gu, X. and Manautou, J. E. (2012) 'Molecular mechanisms underlying chemical liver injury', *Expert Reviews in Molecular Medicine*, 14(February), pp. 1–21. doi: 10.1017/S1462399411002110.

Hadida, M., Abo-Smhadana, A. and Draid, M. (2013) 'Effects of Anti-Tuberculous Drugs on Liver Function Profile in Libyan Patients with Tuberculosis', *The Egyptian Journal of Hospital Medicine*, 52(52), pp. 740–751. doi: 10.12816/0000611.

Hassan, H. M. *et al.* (2018) 'Investigating the CYP2E1 potential role in the mechanisms behind INH/LPS-induced hepatotoxicity', *Frontiers in Pharmacology*, 9(MAR), pp. 1–14. doi: 10.3389/fphar.2018.00198.

Hassen Ali, A. *et al.* (2013a) 'Anti-Tuberculosis Drug Induced Hepatotoxicity among TB/HIV Co-Infected Patients at Jimma University Hospital, Ethiopia: Nested Case-Control Study', *PLoS ONE*, 8(5). doi: 10.1371/journal.pone.0064622.

Hassen Ali, A. *et al.* (2013b) 'Anti-Tuberculosis Drug Induced Hepatotoxicity among TB/HIV Co-Infected Patients at Jimma University Hospital, Ethiopia: Nested Case-Control Study', *PLoS ONE*, 8(5). doi: 10.1371/journal.pone.0064622.

Hepatitis, D. and Chemotherapy, A. (2011) 'Liv line', 5(4).

Hepatotoxicity, D. and Lee, W. M. (2003) 'Drug-Induced Hepatotoxicity', pp. 474–485.

Id, R. (no date) 'Reference ID: 4204409', pp. 1–22.

Ii, S. P. (2011) '" Pathology of Liver & Biliary Tract "', (Vpm 222).

J. Curry, F. (2006) 'Tuberculosis Care TREATMENT Endorsements ':, *Tuberculosis Coalition for Technical Assistance*.

Jayme, T. S. *et al.* (2020) 'Human interleukin-4-treated regulatory macrophages promote epithelial wound healing and reduce colitis in a mouse model', *Science Advances*, 6(23), pp. 1–13. doi: 10.1126/sciadv.aba4376.

Jeong, I. *et al.* (2015) 'Drug-induced Hepatotoxicity of Anti-tuberculosis Drugs and Their Serum Levels', *J Korean Med Sci*, 30, pp. 167–172.

Kamalian, L. *et al.* (2015) 'The utility of HepG2 cells to identify direct mitochondrial dysfunction in the absence of cell death', *Toxicology in Vitro*, 29(4), pp. 732–740. doi: 10.1016/j.tiv.2015.02.011.

Kaplowitz, N. (2002) 'Biochemical and cellular mechanisms of toxic liver injury', *Seminars in Liver Disease*, 22(2), pp. 137–144. doi: 10.1055/s-2002-30100.

Kassa, E. *et al.* (2016) 'Effect of anti-tuberculosis drugs on hematological profiles of tuberculosis patients attending at University of Gondar Hospital, Northwest Ethiopia', *BMC Hematology*. BMC Hematology, 16(1), pp. 1–11. doi: 10.1186/s12878-015-0037-1.

Kaur, G. (2019) 'Hepatic Toxicity Biomarkers', *Biomarkers in Toxicology*, pp. 251–266. doi: 10.1016/b978-0-12-814655-2.00014-1.

Khalili, H. (2009) 'Anti-tuberculosis drugs related hepatotoxicity; incidence, risk factors, pattern of changes in liver enzymes and outcome', *DARU*, 17(3), pp. 163–167.

Kurutas, E. B. (2016) 'The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state', *Nutrition Journal*. Nutrition Journal, 15(1), pp. 1–22. doi: 10.1186/s12937-016-0186-5.

Lee, J. and Boyer, J. L. (2000) 'Molecular alterations in hepatocyte transport mechanisms in acquired cholestatic liver disorders', *Seminars in Liver Disease*, 20(3), pp. 373–384. doi: 10.1055/s-2000-9390.

Lima, M. de F. S. de and Melo, H. R. L. de (2012) 'Hepatotoxicity induced by antituberculosis drugs among patients coinfected with HIV and tuberculosis Hepatotoxicidade das drogas antituberculose entre pacientes coinfectados HIV / tuberculose', *Cad. Saúde Pública, Rio de Janeiro*, 28(4), pp. 698–708.

Lingala, S. M. and Ghany, M. G. M. Mhs. (2016) 'HHS Public Access', 25(3), pp. 289–313. doi: 110.1016/j.bbi.2017.04.008.

Lovren, F. *et al.* (2010) 'Adiponectin primes human monocytes into alternative anti-inflammatory M2 macrophages', *American Journal of Physiology - Heart and Circulatory Physiology*, 299(3), pp. 656–663. doi: 10.1152/ajpheart.00115.2010.

LUSWETI, C. K. (2015) 'INCIDENCE AND RISK FACTORS OF ANTI-TUBERCULOSIS DRUG ASSOCIATED HEPATITIS AMONG PATIENTS AT THE MOI TEACHING AND REFERRAL HOSPITAL, KENYA', *thesis*, (September).

Mahmood, K. *et al.* (2007) 'Hepatotoxicity with antituberculosis drugs: The risk factors', *Pakistan Journal of Medical Sciences*, 23(1), pp. 33–38.

Mahmood, K., Hussain, A. and Jairamani, K. L. (2007) 'Hepatotoxicity with antituberculosis drugs : The risk factors', *Pak J Med Sci*, 23(1), pp. 33–38.

Makhlouf, H. A. *et al.* (2008) 'A prospective study of antituberculous drug-induced hepatotoxicity in an area endemic for liver diseases', *Hepatology International*, 2(3), pp. 353–360. doi: 10.1007/s12072-008-9085-y.

Mello, T. *et al.* (2008) 'Alcohol induced hepatic fibrosis: Role of acetaldehyde', *Molecular Aspects of Medicine*, 29(1–2), pp. 17–21. doi: 10.1016/j.mam.2007.10.001.

Meyer, U. A. (1996) 'Overview of enzymes of drug metabolism', *Journal of Pharmacokinetics and Biopharmaceutics*, 24(5), pp. 449–459. doi: 10.1007/BF02353473.

Mirlohi, M. *et al.* (2016) 'Hematological and liver toxicity of anti-tuberculosis drugs', *Electronic Physician*, (September), pp. 3005–3010.

Mokondjimobe, E. *et al.* (2012) 'Biomarkers of oxidative stress and personalized treatment of pulmonary tuberculosis: Emerging role of gamma-glutamyltransferase',

Advances in Pharmacological Sciences, 2012(May). doi: 10.1155/2012/465634.

Mori, S. *et al.* (2018) 'Non-alcoholic steatohepatitis-like pattern in liver biopsy of rheumatoid arthritis patients with persistent transaminitis during low-dose methotrexate treatment', *PLoS ONE*, 13(8), pp. 1–16. doi: 10.1371/journal.pone.0203084.

Mostafa Abd El-Aal, H. A. H. (2012) 'Lipid Peroxidation End-Products as a Key of Oxidative Stress: Effect of Antioxidant on Their Production and Transfer of Free Radicals', *Lipid Peroxidation*. doi: 10.5772/45944.

Mukesh K, S. (2015) 'Hepatotoxicity: A Major Complication with Critical Treatment', *MOJ Toxicology*, 1(3), pp. 114–120. doi: 10.15406/mojt.2015.01.00016.

Mukonzo, J. *et al.* (2019) 'Potential drug–drug interactions between antiretroviral therapy and treatment regimens for multi-drug resistant tuberculosis: Implications for HIV care of MDR-TB co-infected individuals', *International Journal of Infectious Diseases*. International Society for Infectious Diseases, 83, pp. 98–101. doi: 10.1016/j.ijid.2019.04.009.

Nair, R. S., Akhilesh, K. and James, E. (2020) 'INTERNATIONAL JOURNAL OF RESEARCH IN Anti-tubercular drugs , predisposing factors and management of drug-induced hepatotoxicity : A concise and up-to-date review', 11(3), pp. 3096–3104.

Nandennavar, M. *et al.* (2011) 'Immune hemolytic anemia in a patient with tuberculous lymphadenitis', *Journal of Global Infectious Diseases*, 3(1), pp. 89–91. doi: 10.4103/0974-777X.77303.

Naqvi, I. H. *et al.* (2015) 'Antituberculosis Drug-Induced Liver Injury: An Ignored Fact, Assessment of Frequency, Patterns, Severity and Risk Factors', *Open Journal of Gastroenterology*, 05(12), pp. 173–184. doi: 10.4236/ojgas.2015.512027.

Nebert, D. W., Wikvall, K. and Miller, W. L. (2013) 'Human cytochromes P450 in health and disease', *Philosophical Transactions of the Royal Society B: Biological*

Sciences, 368(1612). doi: 10.1098/rstb.2012.0431.

Neha, M., Deepshikha, K. P. and Vidhu, A. (2014) 'Cichorium intybus Linn: Its role in hepatoprotection', *International Journal of Pharmacognosy and Phytochemical Research*, 6(3), pp. 499–505.

Oliveira, M. G. *et al.* (2014) 'Anemia in hospitalized patients with pulmonary tuberculosis', *Jornal Brasileiro de Pneumologia*, 40(4), pp. 403–410. doi: 10.1590/s1806-37132014000400008.

P, H. B. *et al.* (2020) 'Antitubercular Drugs Induced Liver Injury : A Case Series & Review', *International Journal of Research and Review*, 7(3), pp. 458–462.

P Sharma, O. (2014) 'Clinical Biochemistry of Hepatotoxicity', *Journal of Clinical Toxicology*, 04(01), pp. 1–19. doi: 10.4172/2161-0495.s4-001.

Pandit, A., Sachdeva, T. and Bafna, P. (2012) 'Drug-induced hepatotoxicity: A review', *Journal of Applied Pharmaceutical Science*, 2(5), pp. 233–243. doi: 10.7324/JAPS.2012.2541.

Pappano, A. J. (2012) *Cholinoceptor-Activating & Cholinesterase-Inhibiting Drugs, Katzung Basic & Clinical Pharmacology 12th Ed2*.

Pasipanodya, J. G. and Gumbo, T. (2010) 'Clinical and toxicodynamic evidence that high-dose pyrazinamide is not more hepatotoxic than the low doses currently used', *Antimicrobial Agents and Chemotherapy*, 54(7), pp. 2847–2854. doi: 10.1128/AAC.01567-09.

Ramappa, V. and Aithal, G. P. (2013) 'Hepatotoxicity Related to Anti-tuberculosis Drugs: Mechanisms and Management', *Journal of Clinical and Experimental Hepatology*. Elsevier Ltd, 3(1), pp. 37–49. doi: 10.1016/j.jceh.2012.12.001.

Raviglione, M. and Sulis, G. (2016) 'Tuberculosis 2015: Burden, challenges and strategy for control and elimination', *Infectious Disease Reports*, 8(2), pp. 33–37. doi:

10.4081/idr.2016.6570.

Rines, A. K. and Ardehali, H. (2013) 'Transition metals and mitochondrial metabolism in the heart', *Journal of Molecular and Cellular Cardiology*. Elsevier Ltd, 55(1), pp. 50–57. doi: 10.1016/j.yjmcc.2012.05.014.

Rupert, M. and Katie, R. (2012) 'Reproduced with permission of the copyright owner . Further reproduction prohibited without', *Journal of Allergy and Clinical Immunology*, 130(2), p. 556. doi: 10.1016/j.jaci.2012.05.050.

Saha, A. *et al.* (2016) 'Prevalence of Hepatotoxicity From Antituberculosis Therapy : A Five-Year Experience From South India', *Journal of Primary Care & Community Health*, 7(3), pp. 11–14. doi: 10.1177/2150131916642431.

Santos, E. A. *et al.* (2019) 'Relationship of anti-tuberculosis drug-induced liver injury and genetic polymorphisms in CYP2E1 and GST', *Brazilian Journal of Infectious Diseases*, 23(6), pp. 381–387. doi: 10.1016/j.bjid.2019.09.003.

Sarda, P. *et al.* (2009) 'Role of acute viral hepatitis as a confounding factor in antituberculosis treatment induced hepatotoxicity', *Indian Journal of Medical Research*, 129(1), pp. 64–67.

Sauer, J. M. *et al.* (1997) 'The Liver as a Target for Chemical-Chemical Interactions', *Advances in Pharmacology*, 43(C), pp. 37–63. doi: 10.1016/S1054-3589(08)60201-X.

Saukkonen, Jussi J *et al.* (2006) 'American Thoracic Society Documents An Official ATS Statement : Hepatotoxicity of Antituberculosis Therapy', *American Journal of Respiratory and Critical Care Medicine*, 174, pp. 935–952. doi: 10.1164/rccm.200510-1666ST.

Saukkonen, Jussi J. *et al.* (2006) 'An official ATS statement: Hepatotoxicity of antituberculosis therapy', *American Journal of Respiratory and Critical Care Medicine*, 174(8), pp. 935–952. doi: 10.1164/rccm.200510-1666ST.

Semvua, H. H. *et al.* (2015) 'Pharmacological Interactions Between Rifampicin and Antiretroviral Drugs', *Therapeutic Drug Monitoring*, 37(1), pp. 22–32. doi: 10.1097/ftd.0000000000000108.

Senousy, B. E., Belal, S. I. and Draganov, P. V. (2010) 'Hepatotoxic effects of therapies for tuberculosis', *Nature Reviews Gastroenterology and Hepatology*. Nature Publishing Group, 7(10), pp. 543–556. doi: 10.1038/nrgastro.2010.134.

Shakya, R., Rao, B. S. and Shrestha, B. (2005) 'Management of antitubercular drugs-induced hepatotoxicity and therapy reintroduction strategy in a TB clinic of Nepal', *Kathmandu University Medical Journal*, 3 no. 1(9), pp. 45–49.

Shakya, R., S, R. B. and Shrestha, B. (2006) 'Evaluation of Risk Factors for Antituberculosis Drugs-Induced Hepatotoxicity in Nepalese Population', *Kathmandu University Journal of Science, Engineering, and Technology*, 2(1), pp. 1–8.

Shibata, K., Fukuwatari, T. and Sugimoto, E. (2001) 'Effects of dietary pyrazinamide, an antituberculosis agent, on the metabolism of tryptophan to niacin and of tryptophan to serotonin in rats', *Bioscience, Biotechnology and Biochemistry*, 65(6), pp. 1339–1346. doi: 10.1271/bbb.65.1339.

Son, C. (2016) 'A Severe Hepatotoxicity by Antituberculosis Drug , and its Recovery in Oriental Hospital', *J Korean Med.*, 37(2), pp. 119–124.

Sullivan, S. S. (2019) 'S t r a t e g i c f o c u s', (July), p. 2019.

Tátrai, P. and Krajcsi, P. (2020) 'Prediction of drug-induced hyperbilirubinemia by in vitro testing', *Pharmaceutics*, 12(8), pp. 1–16. doi: 10.3390/pharmaceutics12080755.

Tostmann, A. *et al.* (2008) 'Antituberculosis drug-induced hepatotoxicity: Concise up-to-date review', *Journal of Gastroenterology and Hepatology (Australia)*, 23(2), pp. 192–202. doi: 10.1111/j.1440-1746.2007.05207.x.

Tweed, C. D. *et al.* (2018) 'Liver toxicity associated with tuberculosis chemotherapy

in the REMoxTB study', *BMC Medicine*. BMC Medicine, 16(1), pp. 1–10. doi: 10.1186/s12916-018-1033-7.

Uebelacker, L. A. (2017) 'HHS Public Access', *Physiology & behavior*, 176(1), pp. 139–148. doi: 10.1016/j.bbadis.2018.08.037.ANIMAL.

Vijayalakshmi, A., Thanmayi, G. and Jayakumari, S. (2016) 'A prospective study on abnormal liver function test patterns in patients receiving anti-tuberculosis therapy', *Asian Journal of Pharmaceutical and Clinical Research*, 9(5), pp. 136–139. doi: 10.22159/ajpcr.2016.v9i5.12756.

Wallace, J. L. (2004) 'Acetaminophen hepatotoxicity: NO to the rescue', *British Journal of Pharmacology*, 143(1), pp. 1–2. doi: 10.1038/sj.bjp.0705781.

WHO (2017) *Global Tuberculosis Report 2017: Leave no one behind - Unite to end TB, WHO - Technical Report Series;727*. doi: 10.1001/jama.2014.11450.

Woldu, M. A. *et al.* (no date) 'The Risk of Hepatotoxicity Associated with HAART / Anti-Tb Co-Treatment : A Case Control Study in a Central Ethiopian Referral Hospital', *Family Medicine & Medical Science Research*, 3(3), pp. 1–6. doi: 10.4172/2327-4972.1000129.

World Health Organization (2015) 'Implementing the End TB Strategy: The Essentials', *Who*, pp. 1–130. doi: 10.1017/CBO9781107415324.004.

Yilmaz, M. *et al.* (2018) 'Rifampicin-Induced Fever in a Patient with Brucellosis: A Case Report', *Drug Safety - Case Reports*. Springer International Publishing, 5(1), pp. 18–21. doi: 10.1007/s40800-018-0074-3.

Zhang, Y. *et al.* (2018) 'Plasma membrane changes during programmed cell deaths', *Cell Research*. Nature Publishing Group, 28(1), pp. 9–21. doi: 10.1038/cr.2017.133.

Zheleva, A. *et al.* (2007) 'Free radical reactions might contribute to severe alpha amanitin hepatotoxicity - A hypothesis', *Medical Hypotheses*, 69(2), pp. 361–367.

Appendix 1:

CONSENT FORM

TITLE: **DETERMINANT OF HEPATOTOXICITY USING FIRST LINE ANTI-TUBERCULOSIS DRUGS AMONG TUBERCULOSIS INFECTED PATIENTS IN CENTRAL REGION, GHANA**

PRINCIPAL INVESTIGATOR: Emmanuel Nana Yaw Effrim Botchway

ADDRESS: Department of Chemical Pathology, School of Biomedical Sciences and Allied Health, University of Ghana. Tel: 0249634307. Email: immanuelbotchway1@yahoo.com

General Information about research

Dear Sir/Madam, this consent form contains information about the above titled study. We are carrying out this study in order to ascertain the extent of hepatotoxicity due to anti-tuberculosis regimens among TB infected patients in Cape Coast Teaching Hospital. As part of the efforts towards monitoring of treatment outcomes in TB patients (on first line drugs), it important to consider the impact, these medications have on the liver. We will need blood and sputum samples from you. If your consent to participate in the research, your blood and sputum sample shall be collected and test in the laboratory for liver function. You will answer a few questions about your illness.

Possible Risk and Discomforts

Blood and sputum samples will be collected. This will be done by experienced laboratory personnel and will cause minimal or no harm to you.

Possible Benefits

You stand to benefit from this study as your liver function will be monitored for you (free of charge). Your participation may also help us to understand the effects the drugs you are taking on your liver. This information may benefit the community as it may

provide information to health workers on how to offer better treatment and also inform policy making.

Confidentiality

All information will be protected and kept confidential. You will not be named in any report or publication. Your results may be made available to you upon request.

Compensation

You will not be paid for participating in this study. Your results may be made available to your doctor if there is the need to be given special attention.

Voluntary Participation and Right to Leave the Research

Your participation in my research stands free cost and can freely choose not to continue in the research at any point in time, without any negative consequences on you.

Contacts for Additional Information

If you have any questions about the research study or study-related problems, you may contact Emmanuel Nana Yaw Effrim Botchway (0249634307) or Prof. Anane-Asare of the Chemical Pathology Department, University of Ghana.

Your rights as a Participant

This study has been gone through review and approved by the Ethical Review Committee, School of Biomedical and Allied Health Sciences and that of the Cape Coast Teaching Hospital. The above mentioned committees can contacted,.should you face any human-right problem as a study participant,

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research titled **DETERMINANT OF HEPATOTOXICITY USING FIRST LINE ANTI-TUBERCULOSIS DRUGS AMONG TUBERCULOSIS INFECTED PATIENTS IN CENTRAL REGION, GHANA,** has been read and explained to me. I have been

given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

_____ _____

Date Name and Signature

If volunteers cannot read the forms themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

_____ _____

Date Name and Signature of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research has been explained to the above individual.

_____ _____

Date Name and Signature of Person

Appendix 2

Questionnaire study form

QUESTIONNAIRE FOR THE RESEARCH TITLED, DETERMINANT OF HEPATOTOXICITY USING FIRST LINE ANTI-TUBERCULOSIS DRUGS AMONG TUBERCULOSIS INFECTED PATIENTS IN CENTRAL REGION, GHANA

(For Tuberculosis patients receiving DOTs at CCTH)

Tick **[√]** where appropriate

Name of participant _____

Age _____ **Sex M [ ] F [ ]** **Date of visit** _____

Traceable address _____

Tel. _____ **Occupation** _____ **Weight** ____**Kg** **Height** _____**cm**

1. Check the status and extent of TB of participant

a. Have you been diagnosed of TB and when? Yes [ ] No [ ]Date ……

b. If 'Yes' to Q1a, confirm with lab result and ask, "have been placed on medications"?
Yes [ ] No [ ] Lab result for Sputum AFBs _____

Date for starting drug administration _____

c. Check the drug combinations physically and tick which one were given (this should be done by the DOTS center nurse or the researcher's supervision).

(IR) – Isoniazid + Rifampicin

[ ]

(IRZ) – Isoniazid + Rifampicin + Pyrazinamide

[ ]

(IRZE) – Isoniazid + Rifampicin + Pyrazinamide + Ethambutol

[ ]

2. Check for Viral status.

a. Have you been tested for HIV? Yes [ ] No [ ] Don't know [ ]

b. If 'Yes' to Q2a, what is the result? Positive [ ] Negative [ ]

c. What is your Hepatitis B status? Positive [ ] Negative [ ] No idea [ ]

d. What is your Hepatitis C status? Positive [ ] Negative [ ] No idea [ ]

e. Can you be tested to confirm? Yes [ ] No [ ]

Lab result(after confirmation) Hepatitis B -- Positive [ ] Negative [ ]

Hepatitis C -- Positive [ ] Negative [ ]

The below questions will be answered at month two follow-up

3. Check for the use of other things/drugs that may cause hepatotoxicity.

a. Do you take alcohol? Occasionally [ ] Most at times [ ] Not at all [ ]

b. Are you on other drug part from the TB medications? Yes [ ] No [ ]

c. If 'Yes' to Q3b, what kind of drug?

Paracetamol	Yes **[ ]**	No [ ]
Tramadol	Yes **[ ]**	No [ ]
Herbal concoctions	Yes **[ ]**	No [ ]
Marijuana / weed	Yes **[ ]**	No [ ]
Antacid based syrup	Yes **[ ]**	No [ ]

Others (specify) ……

d. Indicate by ticking [√] below if you experiences any of the following reactions after taking the TB drugs.

Anorexia	Yes **[ ]**	No [ ]
Nausea	Yes **[ ]**	No [ ]
Vomiting	Yes **[ ]**	No [ ]
Jaundice	Yes **[ ]**	No [ ]
Skin rashes	Yes **[ ]**	No [ ]
Reddish colouration of urine or tears	Yes **[ ]**	No [ ]
Blurred vision	Yes **[ ]**	No [ ]

Joint pains	Yes [ ]	No [ ]
Light colored stool	Yes [ ]	No [ ]
Severe abdominal pain	Yes [ ]	No [ ]
Weakness	Yes [ ]	No [ ]
Severe fatigue	Yes [ ]	No [ ]
Continuous bleeding	Yes [ ]	No [ ]
Generalized itching	Yes [ ]	No [ ]
Edema of the feet and/or legs	Yes [ ]	No [ ]
Abdominal and rapid accumulation of body mass in a short period of time	Yes [ ]	No [ ]
Darkish urine	Yes [ ]	No [ ]

e. First symptom experienced (Date observed) …… ( )

f. At what time do you take the TB drugs?

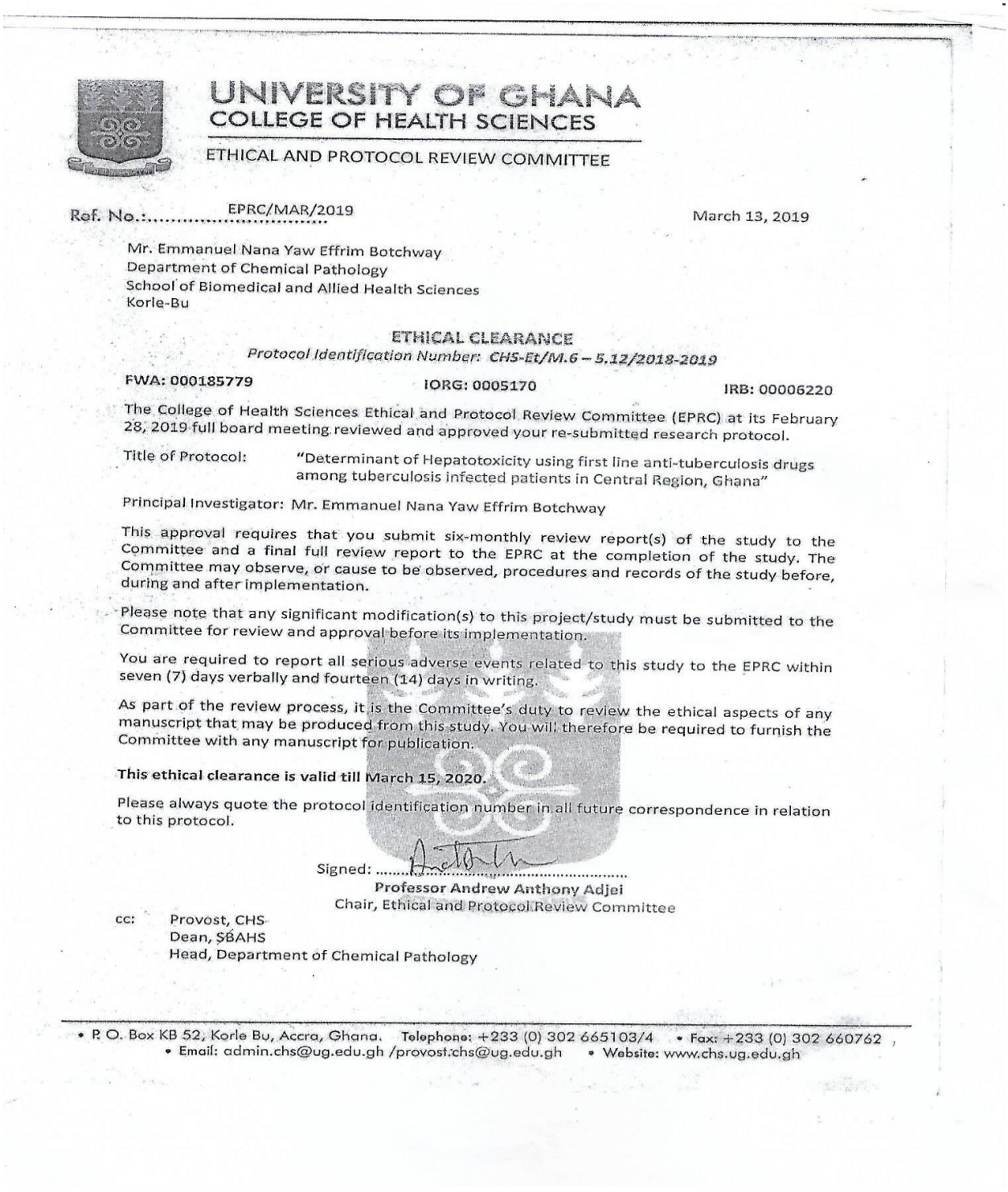
Just before eating	Yes [ ]	No [ ]
30 minutes before eating	Yes [ ]	No [ ]
1 hour before eating	Yes [ ]	No [ ]
Just after eating	Yes [ ]	No [ ]
30 minutes after eating	Yes [ ]	No [ ]
1 hour after eating	Yes [ ]	No [ ]

Thank you for participating.

Signature of DOTS nurse at the time of visit:_____

Signature of researcher:_____

Appendix 3: ETHICAL CLEARANCE



UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/MAR/2019 March 13, 2019

Mr. Emmanuel Nana Yaw Effrim Botchway
Department of Chemical Pathology
School of Biomedical and Allied Health Sciences
Korle-Bu

ETHICAL CLEARANCE

Protocol Identification Number: CHS-Et/M.6 – 5.12/2018-2019

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) at its February 28, 2019 full board meeting reviewed and approved your re-submitted research protocol.

Title of Protocol: "Determinant of Hepatotoxicity using first line anti-tuberculosis drugs among tuberculosis infected patients in Central Region, Ghana"

Principal Investigator: Mr. Emmanuel Nana Yaw Effrim Botchway

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till March 15, 2020.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, SBAHS
Head, Department of Chemical Pathology

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