INCIDENCE AND RISK FACTORS OF ANTI-TUBERCULOSIS DRUG ASSOCIATED HEPATITIS AMONG PATIENTS AT THE MOI TEACHING AND REFERRAL HOSPITAL, KENYA

BY

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A Thesis submitted to the Moi University School of Medicine in partial fulfillment for the award of the degree of Masters of Medicine in Internal Medicine.

SEPTEMBER 2015

DECLARATION

I hereby declare that this thesis is my original work and has not been presented for the award of any academic credit in any Research institution or University.

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DEDICATION

I would like to dedicate this thesis to my husband Joseph and children Andrew and Angel for the sacrifices they made and their unending love.

ABSTRACT

Background: Tuberculosis (TB) is highly prevalent in Sub-Saharan Africa and hepatotoxicity is one of the serious adverse events complicating its treatment. It remains one of the main causes of treatment interruption during tuberculosis treatment that leads to increased morbidity and mortality. However, there is paucity of data on anti-TB drug associated hepatitis (anti-TB DAH).

Objectives: The aim of this study is to determine the incidence of anti-tuberculosis drug associated hepatitis and to identify associated risk factors.

Methods: A prospective study was carried out at the Moi Teaching and Referral Hospital medical wards, TB and chest clinics from October 2013 to March 2014. All newly diagnosed TB patients above 18 years, who gave an informed consent, were enrolled consecutively until the desired sample size was obtained. Baseline Liver function tests (LFTs), Human Immunodeficiency Virus (HIV), Hepatitis B and C status were determined. They were then reviewed at 2 week intervals clinically by use of signs and symptoms during the 6 month course of treatment to determine those who developed anti-TB DAH. DAH was diagnosed when a participant developed symptoms and signs of hepatotoxicity with elevation of transaminases and bilirubin twice the upper limit of normal following commencement of TB therapy, followed by resolution of symptoms and normalisation of LFTs when the drugs were stopped. Participants who had DAH had serial LFTs done until recovery.

Data was collected using a specialized data collection form, entered into a computerized database using Epidata software and analyzed using STATA version 13 special edition. Associations between continuous and categorical variables were assessed using Wilcoxon rank sum test. Risk factors for DAH were evaluated by multivariate logistic regression and odds ratios and the corresponding 95% confidence limit (95%CL) were reported.

Results: A total of 161 participants were enrolled and 158 completed the study, of whom 82 (52 %) were females. The median age was 34 (MAD: 8) years. Majority 89 (56%) had secondary level of education. Thirty (19%) had a history of alcohol consumption. Pulmonary TB was the commonest form of TB 112 (71 %) and 95 (68%) were smear negative. TB/HIV co-infection rate was 70%. There were 13 (8.2%) mortalities 10 cases among patients with DAH and 3 in patients without DAH. The incidence of anti-TB DAH was 35 (22%). Gender, age, alcohol use and presence of other comorbid illnesses did not have a significant association with DAH. Development of anti-TB DAH was significantly associated with HIV infection (P=0.002), Co-trimoxazole use (P=0.005), Anti-retroviral therapy (P=0.019) and serum albumin levels below 35g/dl (P <0.001). On multivariate logistic regression, only HIV infection and low serum albumin levels were independently associated with DAH. HIV infected participants had 4 times increased risk for developing anti-TB DAH Adjusted Odds Ratio (AOR):3.90 (95% CL: 1.08, 14.13). Similarly, participants with serum albumin bellow 35g/dl had 11% increased risk of developing anti-TB DAH AOR:1.11 (95% CL:1.03, 1.19).

Conclusion: The incidence of anti-TB DAH in MTRH is 22%.The findings suggest that patients with HIV infection and serum albumin<35g/dl have an increased risk for development of anti-TB DAH.

Recommendation: TB patients presenting with HIV infection and serum albumin< 35g/dl should be closely monitored by serial Liver function tests during TB therapy for early detection of anti-TB DAH.

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ACKNOWLEDGEMENT

I acknowledge all those whose efforts resulted to the development and writing of this thesis.

I am very grateful to my supervisors Dr. Evangeline Njiru and Dr. David Lagat for their guidance, valuable advice and time spent in the entire challenging process.

I would also like to acknowledge all the other faculty members of the Department of Medicine for their positive criticism and contribution.

I thank all the patients for participating in this study, the staff of MTRH chest and TB clinics for their cooperation. I am also very thankful to the biostatisticians Mr. Keter and Dr. Ann Mwangi and laboratory technicians for their technical support.

Special thanks go to the Government of Kenya which supported this study by a research grant from National Commission for Science Technology and Innovation (NACOSTI).

Finally, am grateful to my family and colleagues for their support and encouragement during the entire process.

LIST OF ABBREVIATIONS

ALT	Alanine transaminase
AMPATH	Academic Model Providing Access To Healthcare
Anti-TB-DAH	Anti-tuberculosis drug associated Hepatitis
ART	Anti-retroviral Therapy
AST	Aspartate transaminase
CDC	Centre for Disease Control and Prevention
CI	Confidence interval
DAH	Drug Associated Hepatitis
DIH	Drug induced hepatotoxicity
EMB	Ethambutol
HBV	Hepatitis B virus
HbsAg	Hepatitis B surface Antigen
HbeAg	Hepatitis B envelope antigen
HCV	Hepatitis C virus
HIV	Human Immune-deficiency Virus
INH	Isoniazid
IREC	Institutional Research and Ethics Committee
LFT	Liver function test
MTRH	Moi Teaching and Referral Hospital
NACOSTI	National Commission for Science, Technology and Innovation
PZA	Pyrazinamide
RIF	Rifampicin
TB	Tuberculosis
WHO	World Health Organization

OPERATIONAL DEFINITION OF TERMS

Drug Associated Hepatitis

A patient was considered to have DAH if

1. They developed signs and symptoms of hepatotoxicity following initiation of anti-TBs with elevation of Bilirubin, ALT and/ or AST to more than twice the upper limit of normal.

2. They presented with anorexia, nausea, vomiting and jaundice together with had any increase in AST and /or ALT above pre-treatment levels.

3. There was normalization of liver enzymes and resolution of signs & symptoms of hepatotoxicity after withdrawal of all anti-TB drugs.

Incidence

For the purpose of this study, incidence will be reported as cumulative incidence.

This is defined as the proportion of a candidate population that gets a disease/condition over a specified period of time

It will be expressed as a percentage

$$\textbf{Cumulative incidence} = \frac{\textbf{Number of new DAH cases}}{\textbf{Number of persons at risk}} \times 100\%$$

Newly diagnosed TB patient

This was a patient who had been diagnosed with any form of TB and was due for anti-tuberculosis therapy.

Primary care provider

This was the clinician directly involved in managing the patient with TB. The primary care providers included the following cadre of health care practitioners; medical specialists, registrars, medical officers, medical officer interns and clinical officers.

Diagnosis of TB

TB was diagnosed by different methods:

Ziel Neilsen staining and microscopy for acid fast bacilli in specimens such as sputum, pus, Cerebro-spinal fluid, pleural fluid and ascitic fluid.

Culture was also done for the above specimens and urine whenever genito-urinary TB is suspected.

Histology was used for biopsied specimens.

Other methods of supporting diagnosis were imaging especially Chest X-rays, Vertebral X-rays, Computerized Tomography scans and Magnetic Resonance Imaging

Alcohol consumption

Regular consumption of beverages containing ethyl alcohol as reported by the participant

Liver Function Tests

This is a panel of tests that measures the different physiologic functions of the liver. This panel includes

- Alanine Aminotransferase (ALT) upper limit of normal of 41u/l
- Aspartate Aminotransferase (AST) upper limit of normal of 32u/l
- Alkaline Phosphatase (ALP) upper limit of normal 129u/l
- Total bilirubin level will be 17.1umol/l
- Direct bilirubin 4.0umol/l
- Serum albumin level 35-50g/l
- Total serum protein 63-84g/l

Hypoalbuminaemia

Serum albumin level below 35g/l

Malnutrition

Body mass index (BMI) less than $18.5Kg/M^2$

Transfer out

A patient diagnosed with TB at MTRH but opted to be followed up at a different TB care facility due to logistical reasons.

Transfer in

Patient who was diagnosed with TB at a facility other than MTRH and then opted to be followed up at MTRH

CHAPTER ONE : INTRODUCTION

1.1 Background

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It remains a major global health problem causing ill health among millions of people each year. It is also the second leading cause of death from an infectious disease worldwide after HIV.

According to WHO reports, there were an estimated 5.7 million incident cases (13% co-infected with HIV) and 1.5 million deaths (including deaths from TB among HIV-positive people) in 2013 (WHO., 2014).

Tuberculosis (TB) remains a major cause of morbidity and mortality in Kenya. It affects all age groups but has its greatest toll in the most productive age group of 15 to 45 years (MOPHS, 2010). The major factor responsible for the large TB disease burden is the current HIV epidemic. Other factors that have contributed to this large TB disease burden include poverty and social deprivation that has led to malnutrition and mushrooming of peri-urban slums and congestion. Although TB cases notified in Kenya has stagnated recently annual incidence of rate of 268/100000 persons(WHO., 2014), a new challenge of resistant strains of TB is gradually but surely increasing and in particular multidrug resistant (MDR) TB (Ogaro, 2012). The cases of drug resistant TB are partly attributed to poor compliance due to drug adverse effects such as drug-induced hepatitis (Muture et al., 2011).

Standard short-course chemotherapy regimen, which comprises of a combination of Isoniazid, Rifampicin, Pyrazinamide and Ethambutol for at least 6 months is recommended by WHO and currently used in most high TB burden countries including Kenya (WHO, 2002). Due to the long duration of therapy and concurrent use of multiple drugs, adverse effects are regarded as the most important clinical consideration in patients undergoing TB treatment (Yee et al., 2003). Hepatotoxicity is the most serious one, which not only leads to high morbidity and mortality, but also diminishes anti-TB treatment effectiveness owing to non-adherence (Kaona, Tuba, Siziya, & Sikaona, 2004).

Clinical risk factors for drug-induced hepatotoxicity during treatment of tuberculosis include advancing age above 35 years, extensive tuberculosis disease, malnutrition, alcoholism, chronic viral hepatitis B and C infections, and HIV infection. One prospective cohort study from Spain has shown the incidence of mild Anti-tuberculosis drug-induced hepatotoxicity 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 in 0.4% of the group without risk factors (Fernandez-Villar et al., 2004). Patients with chronic viral hepatitis infections or HIV infection are subject to 3 to 5 times increased risk of drug associated hepatitis. Chronic hepatitis B and C are of particular relevance in many developing countries, and HIV infection is also soaring in these countries.

The incidence of anti-TB drug induced hepatitis varies from one region to another and it ranges from 2-30% (A. Tostmann et al., 2008; Yimer et al., 2008). There are very few studies done in Africa, a study done in Egypt showed an incidence of 15%, in Ethiopia an incidence of 30%, 11.5% and 8.9%, Rwanda 9%, Cameroon 13.6%, Tanzania10% and 0.9%, while in Malawi 2%, has been reported (Ali, Belachew, Yami, & Ayen, 2013; Assob, Nde, Nsagha, Njunda, & Ngum, 2014; Lorent, Sebatunzi, Mukeshimana, Van den Ende, & Clerinx, 2011; Makhlouf, Helmy, Fawzy, El-Attar, & Rashed, 2008; Mekonnen, 2002; Mugusi et al., 2012; A Tostmann et al., 2007; Alma Tostmann et al., 2010; Yimer et al., 2008).

1.2 Problem Statement

Kenya is one of the countries with high incident rates of TB and it annual incident rate was estimated to be 268/ 100,000 in 2013 (WHO., 2014). Kenya also has a high HIV prevalence of 6.0% (De Cock, Rutherford, & Akhwale, 2014) and the co-infection rate of TB and HIV is high approximately 48% (WHO., 2014).

Treatment of TB requires a combination of four drugs; Rifampicin, isoniazid, pyrazinamide and ethambutol during the intensive phase. Three of these four first line anti-TB drugs;

rifampicin, isoniazid and pyrazinamide have hepatotoxic potential and therefore patient are at risk for drug induced hepatitis.

There are no studies that have been done in Kenya to determine the rate of drug induced hepatitis and factors that influence its occurrence.

In Kenya, there is a high prevalence of risk factors for DAH such as HIV infection and malnutrition which have been reported in previous studies yet no study has been done to assess their role in the development of DAH.

HIV tends to co-exists with hepatitis viruses then TB patients with these comorbidities are at a high risk for drug induced hepatitis. There is also a high degree of malnutrition in Kenya (Ngare & Muttunga, 1999) due to poverty and therefore our patients are at risk of anti-TB drug induced hepatitis, since malnutrition is a well-recognized predisposing factor for drug induced hepatitis. Most of our patients present late when TB has progressed, hence have extensive disease that further predisposes them to drug induced hepatitis.

Adverse drug effects, such as hepatotoxicity are a well-known reason for poor drug adherence (Muture et al., 2011). Poor drug adherence to anti-TB therapy means that patients remain infectious for longer, and are more likely to relapse or even succumb to TB (Salla, 2007). Erratic or selective compliance to treatment and default can result in treatment failure and foster emergence of drug resistant TB leading to an increased cost of treatmen t(Pablos-Mendez, Knirsch, Barr, Lerner, & Frieden, 1997). Kenya has a high TB defaulter rate of 9% (MOH, 2007) and this could be the driving force for the growing MDR-TB problem. Ten percent of default rate is attributed to side effects of anti-TB drugs.

1.3 Justification

This study was done to determine the magnitude of drug induced hepatitis in our set-up, where no similar studies have been published.

Determining the risk factors for anti-TB drug induced hepatitis unique in our set-up sensitizes care givers and makes them more cautious when prescribing anti-TBs to patients at risk.

Education to care providers to be able to have closer follow-up for patients at risk leading to early detection and treatment of drug induced hepatitis and therefore reduce associated morbidity, mortality and treatment interruption.

Education of patients on symptoms of drug associated hepatitis leads to early detection of DAH and may contribute to a reduction in associated morbidity, mortality and non-compliance.

Findings from this study will facilitate stratification of patients in the treatment of TB as per their risk factors and hence development of protocols that improve care.

This study hoped to fill the gap in knowledge of the magnitude and risk factors and of anti-TB drug associated hepatitis in our set-up.

1.4 Research Questions

1) What is the incidence proportion of anti-TB drug associated hepatitis among TB patients in MTRH?
2) What are the risk factors for development of anti-TB drug associated hepatitis among TB patients in MTRH?

1.5 Study Objectives

1.5.1 Broad objective

To determine the incidence and identify risk factors for anti-TB drug associated hepatitis among TB patients in MTRH.

1.5.2 Specific objectives

1) To determine the incidence of anti-TB drug associated hepatitis among TB patients on follow-up at the MTRH.
2) To determine the risk factors for anti-TB drug associated hepatitis among patients on follow-up at the MTRH

CHAPTER TWO : LITERATURE REVIEW

2.1 Burden of Tuberculosis.

TB is the most common infectious disease worldwide. According to WHO report, global TB burden remains enormous accounting for an estimated 5.7 million new cases in 2013 (13% infected with HIV) and 1.5 million deaths in 2013. TB is also one of the top killers of women with 300,000 deaths among HIV negative women and 200,000 deaths in HIV positive women in 2011 (WHO., 2014).It is one of the diseases that is curable and preventable and in millennium development goal, 6, the target is to half and reverse the TB epidemic by 2015. Major steps have been achieved towards halving TB mortality by 2015, though Africa and Europe regions are still lagging behind (WHO, 2012).

Mortality and incidence rates of TB have been falling in all the six WHO regions and in most of the 22 high burden countries which account for 80% of the world's TB cases. There has also been a 45% decrease in TB prevalence worldwide since 2002. This is attributed to the implementation of the directly observed therapy short-course (DOTS) therapy (WHO, 2012).

The emergency of multidrug resistant TB (MDR-TB) is a new challenge in control of TB. There has been an increase in the number of MDR-TB cases in the 27 high MDR-TB burden countries reaching almost 60,000 worldwide in 2011. This is considered to be only a fifth of all MDR- TB and it has been estimated that 3.5% of all new cases and 20.5% of previously treated cases have MDR-TB (WHO, 2012).

Africa accounts for 24% of all TB cases in the world and has the highest rates of cases and deaths per capita. Almost 80% of TB cases among people living with HIV reside in Africa (WHO, 2012).

Kenya has been in the group of the 22 high burden countries that collectively contribute 80% of the global burden of TB disease for a long time. In 2011, the country was ranked 8th among these 22 high TB burden countries. The high TB burden in Kenya is attributed the HIV epidemic with a 48% co-infection rate in 2013 (WHO., 2014).

There has been a decreasing incidence of TB in Kenya with a 2013 incidence of 268/100,000 persons having dropped from 288/100,000 in 2011 (WHO, 2012). It was estimated that 130,000 people had TB in Kenya in 2013 (WHO., 2014). Mortality from TB in 2013 was 9.1% (WHO., 2014).

There are many reasons for the continued large burden of TB despite the long presence of a strong national TB control programme. These factors include high poverty levels, delays in TB diagnosis from both patients and health care system related factors which facilitate TB transmission and the concurrent HIV epidemic (Disease, 2010).

2.2 Treatment of TB

2.2.1 Anti TB regimen.

Treatment for new cases of susceptible TB consists of a 6 month regimen of 4 first line drugs; Isoniazid, Rifampicin, Ethambutol and pyrazinamide. The treatment for MDR-TB (defined by resistance to Rifampicin and isoniazid) is longer, up to 18 to 24 months and requires more expensive and toxic drugs.

A standard short course chemotherapy consisting of a 2- month intensive therapy combining 4 drugs (Rifampicin, isoniazid Ethambutol and Pyrazinamide) and a 4- month continuation phase combining Rifampicin and isoniazid can cure up to 90% of all cases. In 2010, TB treatment success rate was 85% and 87% among new smear positive and smear negative pulmonary TB respectively (WHO, 2011).

Due to long duration of therapy and concurrent use of multiple drugs, adverse effects are regarded as the most important clinical consideration in patients undergoing anti-tuberculosis therapy (Yee et al., 2003).

2.2.2 Hepatotoxicity of anti-TB drugs.

Active TB is usually treated with multiple drugs and therefore there is limited data on toxicity rates of anti-TB drugs individually except for isoniazid, which has been widely used

as prophylactic monotherapy for latent TB infection (Jussi, 2006). Multidrug therapy has complicated attribution of a reaction to a specific medicine and therefore only temporal relationships can provide evidence that a given drug is responsible for adverse effects. This temporal relationship is made when symptoms appear with start of a new drug, resolve with its withdrawal and/or reappear with re-challenge of the same drug. Anti-TB drug induced hepatotoxicity can be fatal if treatment is not interrupted in time (Sharifzadeh, Rasoulinejad, Valipour, Nouraie, & Vaziri, 2005; Tost et al., 2005).

Hepatotoxicity of specific drugs is described below;

Isoniazid

Approximately 10-20% of patients during the first 4 to 6 months of therapy have a mild hepatic dysfunction shown by mild and transient increase in serum AST, ALT and bilirubin concentration. But in some patients the hepatic damage may be progressive and cause fatal hepatitis. Acetyl hydrazine, a metabolite of INH is responsible for liver damage when there is slow acetylation by N-acetyltransferases (Santos et al., 2013). INH should be discontinued if the AST increases to over 5 times the normal value. In retrospective study with 111 cases of DAH in Taiwan, the incidence rate of isoniazid induced hepatotoxicity was 0.59 per 100 person months. Twenty one of the 111 DAH cases were attributed to isoniazid (Shu et al., 2013)

Rifampicin

Transient abnormalities in liver function are common in the initial stages of therapy. But in some cases it may cause severe hepatotoxicity, more so in those with pre-existing liver disease. Rifampicin causes transient elevations in hepatic enzymes usually within the first 8 weeks of therapy in up to 20% of patients, with less than 1% of the patients demonstrating overt rifampicin-induced hepatotoxicity. The occurrence of Rifampicin induced hepatotoxicity was estimated to be 0.66 per 100 person months in a study by Shu and colleagues (Shu et al., 2013). A higher incidence of hepatotoxicity has been reported in patients receiving rifampicin with other anti TB agents, and is estimated to be fewer than 4%

(Kishore, 2007). A higher incidence of hepatotoxicity has also been reported in patients receiving rifampicin in combination with pyrazinamide for the treatment of latent TB (Jasmer et al., 2002; McNeill, Allen, Estrada, & Cook, 2003). This data has led to the recommendation that this regimen should generally not be offered for the treatment of latent tuberculosis (CDC, 2003).

Pyrazinamide

The most common adverse effect of this drug is hepatotoxicity. Hepatotoxicity is dose related and may occur any time during therapy. Shu et al reported the incidence rate of pyrazinamide induced hepatotoxicity to be 3.21 events per 100 person months. Seventy of the 111 cases of DAH were attributed to Pyrazinamide (Shu et al., 2013). In the Centre for Diseases Control and Prevention (CDC) update, 48 cases of hepatotoxicity were reported in association with a 2-month regimen of Rifampin-pyrazinamide for the treatment of latent tuberculosis between October 2000 and June 2003. Thirty-seven patients recovered and 11 died of liver failure. Of the 48 reported cases, 33 (69%) occurred in the second month of therapy (CDC, 2003). Pyrazinamide is therefore considered the most hepatotoxic anti-TB drug.

Ethambutol

There are very few reports of hepatotoxicity with Ethambutol in the treatment of TB. Abnormal liver function tests have been reported in some patients taking Ethambutol; however, these patients were also taking other anti-TB drugs known to cause liver dysfunction (Tahaoglu et al., 2001).

Streptomycin

No hepatotoxicity has been reported.

2.3 Prevalence of drug induced hepatotoxicity in TB patients

The incidence of anti-TB DAH with standard multidrug TB therapy has been variably reported as between 2-30% (A. Tostmann et al., 2008; Yimer et al., 2008). This variability is partly due to different investigators definition of hepatotoxicity and differences in the populations studied. This difference in incidence can also be explained by variations in risk factor profiles among different regions in the world.

The rate of hepatotoxicity in sub-Saharan Africa is not known since the few studies done have mentioned hepatotoxicity but did not report on its incidence. This is probably due to the fact that LFTs are not carried out routinely in monitoring TB patients. One study done in Egypt reported an incidence of 15% in an area endemic for liver disease (Makhlouf et al., 2008) while a study done in Cameroon reported an incidence of 13.6% (Assob et al., 2014) other reports from Africa include 11.5% (Ali et al., 2013) and 8.9% (Mekonnen, 2002) in Ethiopia, 10% in Tanzania (Mugusi et al., 2012), 9% in Rwanda (Lorent et al., 2011) and 2% in Malawi (A Tostmann et al., 2007). There are no published reports on the rate of DAH in Kenya.

2.4 Risk factors for Anti-TB DAH

Many risk factors for anti-TB DAH have been reported. Clinical risk factors for drug-associated hepatotoxicity during treatment of tuberculosis include advancing age above 35 years, 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-tuberculosis drug-associated hepatotoxicity 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 in 0.4% of the group without risk factors. The identification of high risk patients is useful in allowing early detection of hepatotoxicity and reduction of morbidity and mortality of this condition.

The following risk factors have been identified;

2.4.1 Age over 35 years

Older patients may be more vulnerable to hepatotoxic reactions due to a decreased clearance of drugs metabolized by cytochrome p 450 (CYP450) enzymes and changes in liver blood flow, size, drug binding or distribution with aging.

Several studies suggest increasing age is a risk factor for anti-TB DAH, but often statistical significance was not achieved, or hepatotoxicity was not treatment-limiting(Dossing, Wilcke, Askgaard, & Nybo, 1996; Huang et al., 2002; Hwang et al., 1997; Ormerod & Horsfield, 1996; Schaberg, Rebhan, & Lode, 1996; Teleman, Chee, Earnest, & Wang, 2002; Yee et al., 2003). In one prospective study, Hwang et al reported anti-TB DAH rate ranging from 2-8% as age increased above 35years, with an average of 5%(Hwang et al., 1997). Huang and Dufour in their studies reported that hepatotoxicity ranges from 22-33% in those older than 35 years compared with 8-17% in those younger than 35 years (Dufour et al., 2000; Huang et al., 2002).

2.4.2 Female sex

Female sex is associated with higher cytochrome P3A enzyme activity compared to males which may explain females being more vulnerable to anti-TB DAH.

Some studies have reported increased risk of hepatotoxicity in women (Shakya, Rao, & Shrestha, 2004; Teleman et al., 2002). Shakya and colleagues showed a fourfold higher risk of treatment limiting hepatotoxicity in women but with an overall low incidence of 8% (Shakya et al., 2004) while Makhlouf et al and Assob et al showed no increased risk in women (Assob et al., 2014; Makhlouf et al., 2008).

2.4.3 Alcohol use

Alcoholism is associated with a higher risk of anti-TB DAH because of enzyme induction. Patients with alcohol abuse and concomitant use of other hepatotoxic drugs have an increased risk of DAH (Hwang et al., 1997). Mugusi et al and Lorent et al in their studies in

Tanzania and Rwanda respectively found no association between alcohol use and hepatotoxicity (Lorent et al., 2011; Mugusi et al., 2012).

2.4.4 Malnutrition

Malnutrition considered to be defined by a low BMI less than $18kg/M^2$ and/or Hypoalbuminaemia less than 35g/dl, results in decreased xenobiotic clearance and higher plasma drug levels (Walter-Sack & Klotz, 1996). Malnourished persons have decreased glutathione stores than predisposes them to oxidative injuries from drug metabolites (Assob et al., 2014). Malnutrition as a predisposing factor for DAH has also been demonstrated in other studies (Assob et al., 2014; Shakya et al., 2004; Sharma, Balamurugan, Saha, Pandey, & Mehra, 2002).

2.4.5 HIV infection

HIV infection increases the risk of anti-TB DAH. The exact mechanism of increased risk for anti TB DAH (Breen et al., 2006; Ungo et al., 1998; Yee et al., 2003; Yimer et al., 2008) is not known, though it's been postulated that acute HIV infection alters oxidative pathways. Severe HIV immunosuppression as measured by low CD4 counts has also been demonstrated to increase the risk of DAH indicating a possible immunologic mechanism of DAH (Yimer et al., 2008). Also concurrent therapy of HIV/TB is complicated by overlapping toxicities and drug to drug interactions. Nevirapine is the most hepatotoxic NNRTI. Majority of NRTIS are potentially hepatotoxic e.g. didanosine and stavudine while protease inhibitors with hepatotoxicity include ritonavir, indinavir and sequinavir. Generally hepatotoxicity due to HAART is estimated at 2-18% (Nunez, 2006). Mugusi in Tanzania reported an increased risk of DAH in patients on anti-TBs and ART (Mugusi et al., 2012). In a study of patients with HIV and hepatitis C on TB treatment, HIV independently increased the risk of anti TB DAH fourfold; approximately 27% of HIV infected individuals developed DAH compared with 12% of the HIV uninfected individuals (Ungo et al., 1998). Yimer in Ethiopia found a 3.6 increased odds of developing DAH among HIV infected persons (Yimer et al., 2008).

2.4.6 Hepatitis B Carriage

Studies by Wong et al and Lee et al suggest that hepatitis B carriers get more severe hepatic disease due to TB therapy (Lee et al., 2005; Wong et al., 2000). Assob and colleagues in Cameroon collaborated this finding (Assob et al., 2014). Some studies did not support the fact that hepatitis B is a risk factor for anti TB DAH (Assob et al., 2014; Hwang et al., 1997). It should be noted that these studies did not stratify patients according to evidence of active infection.

2.4.7 Hepatitis C infection

A study done in Florida by Ungo and colleagues evaluated the impact of HCV infection on anti TB DAH during treatment of TB disease (Ungo et al., 1998). Approximately 30% of HCV infected individuals developed DAH compared to 11% among Hepatitis C uninfected individuals. Hepatitis C was an independent risk factor for development of anti-TB DAH, elevating the risk fivefold of transaminase elevation of at least 12u/l or serum bilirubin at least 1.5 mg/dl Co-infection with HIV and HCV elevated risk of DAH by fourteen fold. A study from Tanzania by Mugusi et al corroborated this finding (Mugusi et al., 2012).

CHAPTER THREE: METHODOLOGY

3.1 Study design

This was a prospective study.

3.2 Study setting

This study was carried out at the Moi Teaching and Referral Hospital (MTRH) which is the second largest public referral hospital in Kenya and the teaching hospital for Moi University, College of Health Sciences. MTRH is also classified as one of the districts under the National Leprosy and Tuberculosis Programme (NLTP). Patients were received from the medical wards, chest and TB clinics.

3.3 Study population

The study population was newly diagnosed patients with tuberculosis due for first line anti-TB therapy. The subjects were patients residing within Eldoret and its vicinity who were willing to be followed up at MTRH chest clinic and AMPATH TB clinic throughout the course of TB treatment.

3.3.1 Sampling technique

Sampling was done by convenient sampling, where every patient aged eighteen years and above and diagnosed with TB seen in the Medical wards, AMPATH TB and MTRH Chest clinics was eligible for recruitment into the study.

Patients who agreed to participate in the study were recruited and followed at MTRH chest clinic and AMPATH TB clinic throughout the course of TB treatment.

3.3.2 Sample Size

The sample size was estimated at 150 based on the following formula;

The sample size formula required for a logistic regression by Agreisti(Agresti, 2002), given by;-

$$n = \left(\frac{\left[z_{\alpha} + z_{\beta} \exp\left(-\lambda^2 / 4 \right) \right]^2 \left(1 + 2\bar{\pi}\delta \right)}{\bar{\pi}\lambda^2} \right)$$

Where n = Sample size for one predictor (risk factor)

Zα = Chance of a type I error

Zβ = Chance of a type II error

π = Proportion of DAH from a study by Makhlouf et al 15%

λ = Odds ratio of developing DAH at one standard deviation above the predictor which is derived when there is a change in prevalence by 15%

δ = estimator of the sample size at one standard deviation of the predictor

This sample size would be sufficient to model the relationship between the quantitative predictor (risk factor) and the outcome, drug associated hepatitis (DAH). The predictor in this case is taken as the most important predictor such as age, serum albumin level or BMI. The incidence of DAH from a prospective study conducted in Egypt was 15%(Makhlouf et al., 2008). For computing the sample size it is taken that these prevalence values were values at an average age, average serum albumin and average BMI. Using the incidence of 15% from the study conducted in Egypt, we estimated a sample size that allowed the test to be sensitive to at least 15% difference in the prevalence of DAH that is to increase by an equivalent proportion of 15% to 30% from the population prevalence, at one standard deviation increase in the predictor.

The odds of DAH at mean value of any predictor is

= (15/100) ÷ (1-15/100)

=0.15/0.85=0.18

The odds of DAH at a unit standard deviation above the mean is

= (30/100) ÷ (1-30/100)

= 0.3/0.7=0.43.

This means that the odds ratio $\theta = 0.43/0.18 = 2.4.$

Hence $\lambda = \log(\theta) = \log(2.4) = 0.88$,

$\lambda^2 = 0.77$

For $\beta = 0.2$ chance of type II error in an $\alpha = .05$ level test, $z_\alpha = 1.645$, $z_\beta = 0.842$.

$$\delta = \left(\frac{1 + \left(1 + \lambda^2\right) \exp\left(5\lambda^2 / 4 \right)}{1 + \exp\left(-\lambda^2 / 4 \right)} \right)$$

$$= \left(\frac{1 + (1 + 0.77) \times \exp\left(5 \times 0.77 / 4 \right)}{1 + \exp\left(-0.77 / 4 \right)} \right)$$

$$= \left(\frac{1 + (1.77) \times \exp(0.9625)}{1 + \exp(-0.1925)} \right)$$

$$= \left(\frac{1 + 1.77 \times 2.6182}{1 + 0.8245} \right)$$

$$= \left(\frac{1 + 4.6343}{1.8249} \right)$$

$$= \left(\frac{5.6343}{1.8249} \right)$$

$$= 3.0875$$

The sample size required for one predictor n will be calculated as follows;

$$n = \left(\frac{\left[z_{\alpha} + z_{\beta} \exp\left(-\lambda^2 / 4 \right) \right]^2 \left(1 + 2\bar{\pi}\bar{\delta} \right)}{\bar{\pi}\bar{\lambda}^2} \right)$$

$$= \left(\frac{\left[1.645 + 0.842 \times \exp\left(-0.77 / 4 \right) \right]^2 \left(1 + 2 \times 0.15 \times 3.088 \right)}{0.15 \times 0.77} \right)$$

$$= \left(\frac{\left[1.645 + 0.842 \times 0.825 \right]^2 \left(1 + 2 \times 0.15 \times 3.088 \right)}{0.15 \times 0.77} \right)$$

$$= \left(\frac{\left[1.645 + 0.695 \right]^2 \left(1 + 2 \times 0.15 \times 3.088 \right)}{0.15 \times 0.77} \right)$$

$$= \left(\frac{2.34 \times 2.34 \times 1.926}{0.116} \right)$$

$$= 90$$

However, for a multivariate logistic regression model where we have other covariates to adjust for in the model we needed to calculate a sample size N that will be powered to detect the effect in predictors with low prevalence.

This is determined by the formula $N = n \div (1-R^2)$

Where R^2 is the coefficient that determines the correlation between the predictors with a value of 0.4

Therefore N= 90 ÷ (1 - 0.4)

N= 150

3.4 Eligibility

3.4.1 Inclusion criteria

- Newly diagnosed patients with TB
- 18 years and above

3.4.2 Exclusion criteria

- Patients who did not give consent to participate in the study
- Transfer outs

3.5 Study Methods

3.5.1 Participants

Participants were recruited once a diagnosis of TB had been made by their primary care provider. These were patients due for first-line TB therapy who agreed to participate in the study by signing an informed written consent. The patients were recruited consecutively until the sample size was achieved (Appendices IV and V)

3.5.2 Drug regimen

Total treatment period for most patients was 6 months, including intensive and continuation phases of 2 months and 4 months respectively. The continuation phase was prolonged to 10 months in patients with TB meningitis and TB spine.

The intensive phase comprised the following;

- INH 5mg/kg/day; maximum300mg daily
- RIF 10mg/kg/day; maximum 600mg/day
- EMB 15-25mg/kg/day
- PZA 20-25mg/ kg/day

The continuation phase comprised of either INH and RIF or INH and EMB at similar doses as intensive phase.

3.5.3 Diagnosis of drug-induced hepatitis

A patient was considered to have DAH if

1) They developed signs and symptoms of hepatotoxicity following initiation of anti-TBs with elevation of Bilirubin, ALT and/ or AST to more than twice the upper limit of normal.

2) They had anorexia, nausea, vomiting and jaundice together with any increase in AST and /or ALT above pre-treatment levels.
3) There was normalisation of liver enzymes and resolution of signs & symptoms of hepatotoxicity after withdrawal of all anti-TB drugs.

The decision to use clinical features of DAH as our definition for DAH was based on the fact that all major guidelines consider presence of symptoms of hepatotoxicity as the absolute indication for stopping anti-TB meds. This is because Asymptomatic transaminase elevations occur in 20% of patients started on standard anti-TB regimens prior to, or immediately after the start of treatment. Usually these elevations resolve spontaneously. This is called hepatic adaptation and does not necessitate treatment interruption.

3.5.4 Laboratory values

- Normal liver enzymes: - ALT upper limit of normal of 41iu/l, AST upper limit of normal of 32iu/l, Normal total bilirubin level was be 17mmol/l.
- Hypoalbuminaemia: - Serum albumin level below 35g/l.

3.5.5 Baseline assessment

Pretreatment evaluation included;

- Clinical history
- Physical examination
- BMI (body weight in kg / height in M^2)
- Liver function test
- Hepatitis B & C markers
- HIV test

3.6.6 Follow up

Patients were followed up closely in the MTRH Chest Clinic and AMPATH TB Clinic by clinicians as per standard of care. They were seen fortnightly for the first 2 months then monthly till the end of the six month period.

In each visit, the patients were assessed clinically for response to therapy, any adverse effects and nutritional status. Biochemical assessments by LFTs were done whenever symptoms suggestive of hepatotoxicity occurred. These symptoms and signs included nausea, anorexia, malaise, vomiting, hepatomegaly or jaundice.

3.5.7 Exclusion of other causes of liver disease

Before attributing hepatotoxicity to anti-TB drugs, other causes of liver disease were excluded. The tests that were done to exclude the other causes included HBsAg, anti-HCV antibody and an abdominal ultrasound to exclude biliary obstruction in patients with features of cholestasis.

3.5.8 Management of DAH

Whenever a patient developed signs and symptoms of anti-TB DAH and LFTs were suggestive of anti-TB DAH, all anti-TB meds were stopped immediately. The patients were then started on ethambutol and streptomycin which are not hepatotoxic until the cause of hepatotoxicity was identified. Isoniazid and rifampicin were restarted once the transaminase levels were back to less than 2 times the ULN. In patients who had deranged enzymes at baseline, these drugs were re-introduced once their liver enzymes were back to pre-treatment levels. These patients were treated by the primary clinician as per the AMPATH TB hepatitis complication protocol as show in Appendix IV.

3.6 Data collection

3.6.1 Consent

Patients who qualified for the study were enrolled into the study after signing an informed consent form (Appendices IV and V).

Consent was sought by offering the patient the written consent form and the investigator explained the contents of the consent form. Consent forms were written in English and Kiswahili. For patients who could not read in either English or Kiswahili, the consent form was read out and the contents explained in either language by the investigator or research assistant.

Where participants were not able to communicate in either language, a translator to the language that patient could understand was sought.

Where such a translator was not available or where patient reported they could not understand, such a patient was excluded from the study.

Those patients who did not know how to write were requested to put a thumb print in the space provided for signature.

Patients who were unable to give consent for any other reason were excluded from the study.

3.6.2 Collection of demographic data and clinical characteristics of the patients;

The demographic and clinical characteristics of the participants were collected by the investigator by use of a special data collection form. This information included age; gender; level of education; employment status, alcohol use and any other prescribed medication.

Whenever this information could not be acquired through the interview, reference was made to the patients' records.

Weight and height were measured and BMI calculated by the investigator or research assistant.

The participants were then requested to allow the investigator to draw blood for LFTs, HBsAg and anti-HCV tests. The samples were taken to the laboratory by the investigator and results followed up and recorded. HIV status was determined through Provider Initiated Testing and Counselling (PITC) which is the standard of care.

The findings were recorded in the data collection sheet using participant's hospital registration numbers and study number. Unauthorized persons were denied access to collected information which was kept under the custody of the investigator.

3.7 Data Management and Analysis

3.7.1 Study Variables

The variables in this study were;

- Age – Participants were divided into two groups, those 35 years and above and those bellow 35 years of age
- Sex – two categories; male and female
- BMI – A BMI of 18.5kgs/M^2 and bellow was considered low
- ALT upper limit of normal of 41iu/l
- AST upper limit of normal of 32iu/l
- Normal bilirubin level was 17mmol/l
- Serum albumin level below 35g/l and 35g/dl and above
- HCV status – Positive versus Negative
- HBsAg – Positive versus Negative
- HIV status – Positive versus Negative
- Current alcohol use (as reported by participant) – Yes versus No

3.7.2 Data Cleaning, Data Entry and data analysis

At the end of data collection, data collection forms were reviewed for completeness and coded. Data was then entered into a computerized database using Epidata data entry software. Data analysis was done using STATA version 13 special edition. Categorical variables were summarized as frequencies and the corresponding percentages. Continuous variables violated the Gaussian assumption therefore they were summarized as median and the corresponding median absolute deviation (MAD). Normality assumption was assessed

using Shapiro Wilks test. Association between categorical variables was assessed using Pearson's Chi Square test while the association between continuous and categorical variables was assessed using Wilcoxon rank sum test. Association between the continuous variables was assessed using spearman rank correlation coefficient. Factors associated with the outcome were investigated using logistic regression model. We reported the odds rations (OR) and the corresponding 95% confidence limits (95% CL).

The statistically significant variables were then included in a logistic regression model in order to estimate magnitude of their effect. Assessment for possible correlation or association was done to avoid the effect of multi-collinearity. We established that HIV status, ARV use and cotrimoxazole use were all associated. As a result of that we modelled three separate models in order to be in a position to estimate the true impact of HIV status, ARV use, and cotrimoxazole use on DAH.

The results are presented as tables and graphs.

3.8 Study Limitations

This study diagnosed DAH on the basis of clinical symptoms and it is possible that we under estimated the incidence of DAH by not capturing assymptomatic cases

This study was carried out in a tertiary hospital and AMPATH, a HIV care center and it could have suffered referral bias with high rates of TB/HIV co-infection. Our results may not be transposed to other centers where TB/HIV co-infection rate is low.

We did not have an objective tool to assess alcohol consumption and therefore we cannot rule alcohol as a risk factor for DAH.

History of use of herbal medicine was not taken, yet they have hepatotoxic effects that may then be attributed to anti-TB drugs.

3.9 Ethical consideration

Voluntary participation- Patients were not coerced into participating in the study and they were free to withdraw at any time during the study.

Informed consent – The patients were informed of the purpose of the study and were only interviewed if they gave consent.

Confidentiality – All raw data/ information was strictly handled by the researcher and research assistants. There was no identifying information included in data collection instrument. Data was stored in a password-protected computer.

Approval - this was sought and obtained from Institutional Research & Ethics Committee (IREC). Permission was sought and obtained from hospital management. (See Appendices IX and X)

CHAPTER FOUR : RESULTS

4.1 Enrollment into the study

During the study period, 303 patients were diagnosed with TB (Figure 1).

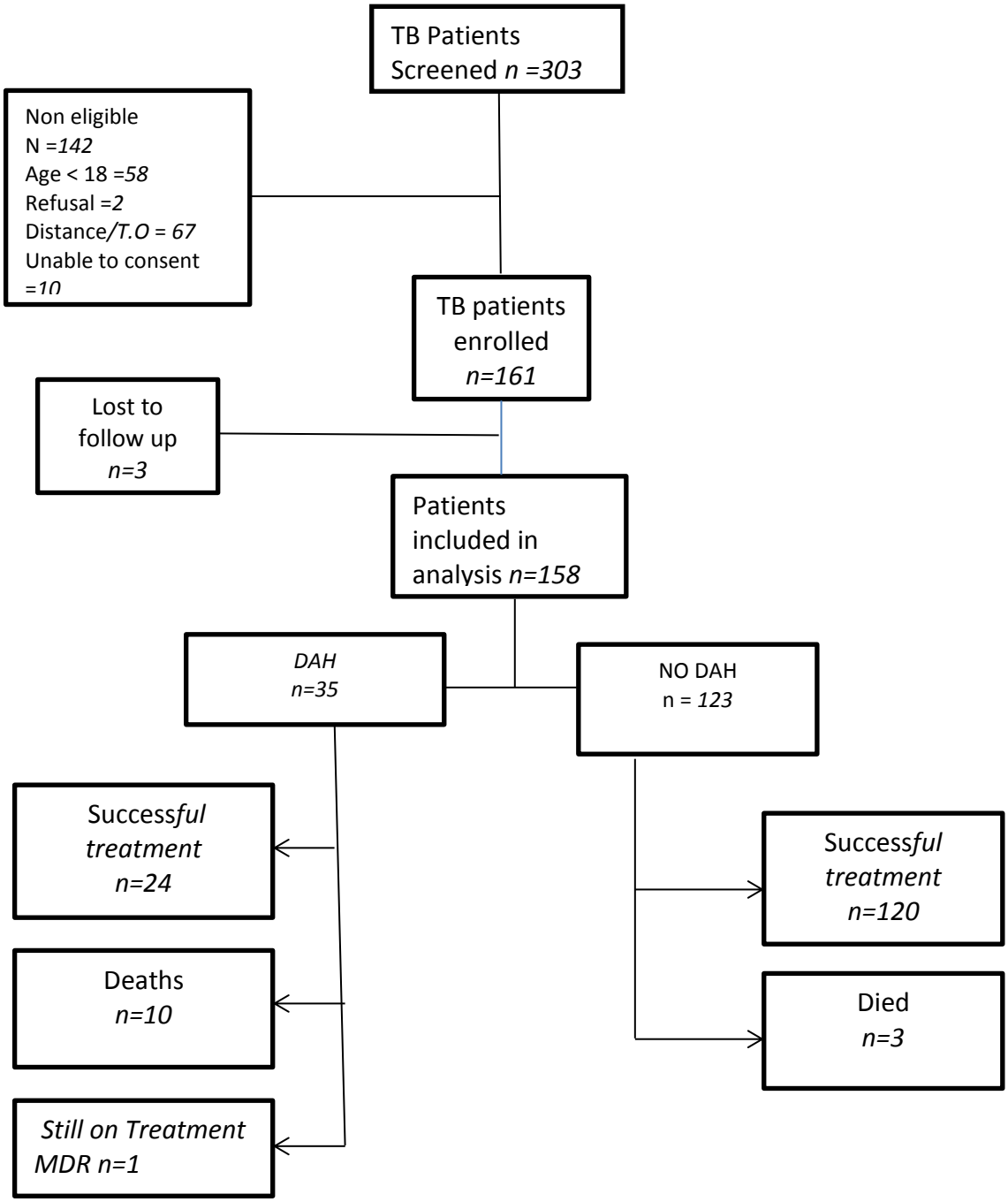

Figure 1: Flowchart showing screening and recruitment of participants

4.2 Socio-demographic characteristics

4.2.1 Age and gender

A total of 158 participants were included for analysis.

The median age was 34 (MAD: 8) years with a minimum of 18 years and a maximum of 61 years. Female participants were 82 (52%) as shown in Figure 2.

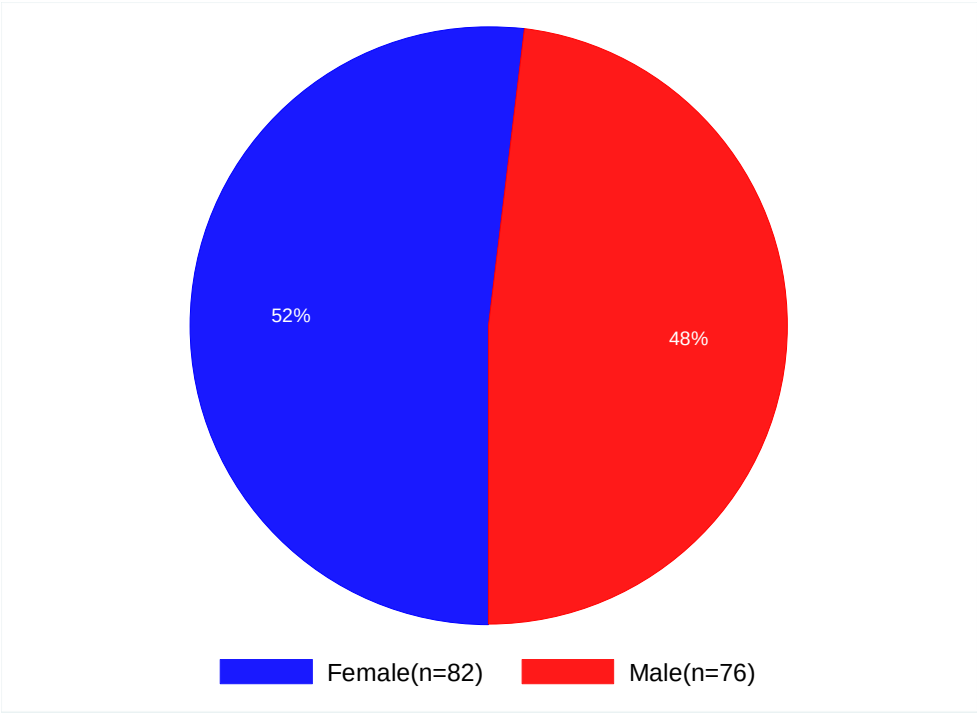

Figure 2: Distribution of participants by gender

4.2.2 Education level

More than half of the participants had a secondary level of education as shown in Figure 3.

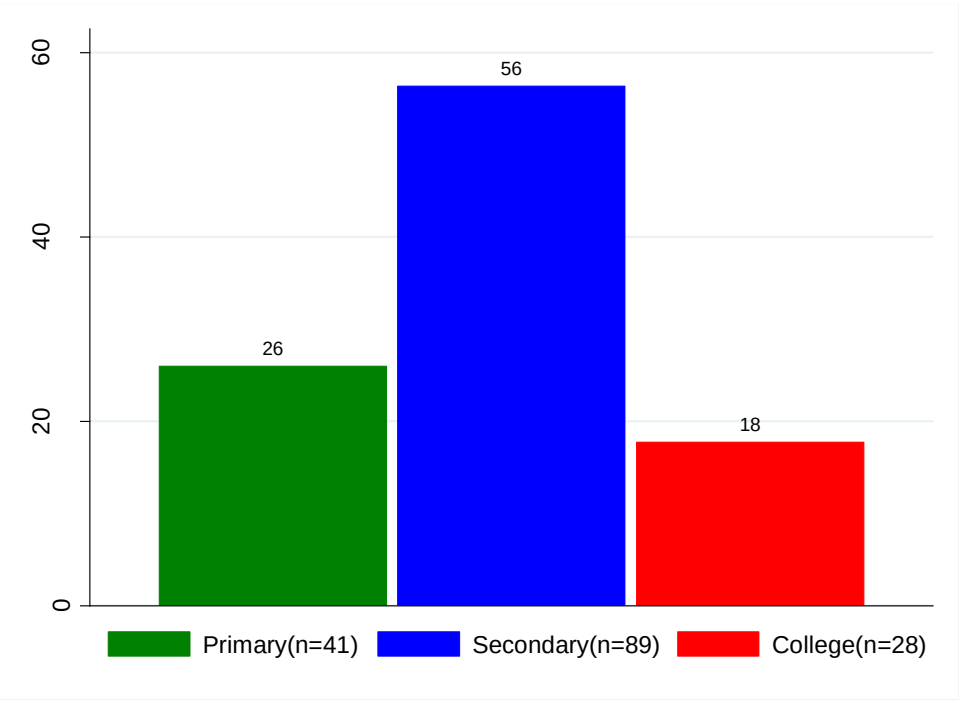

Figure 3: Distribution of participants by education level

4.2.3 Employment status

Majority of the participants, 76 (48%), were unemployed.

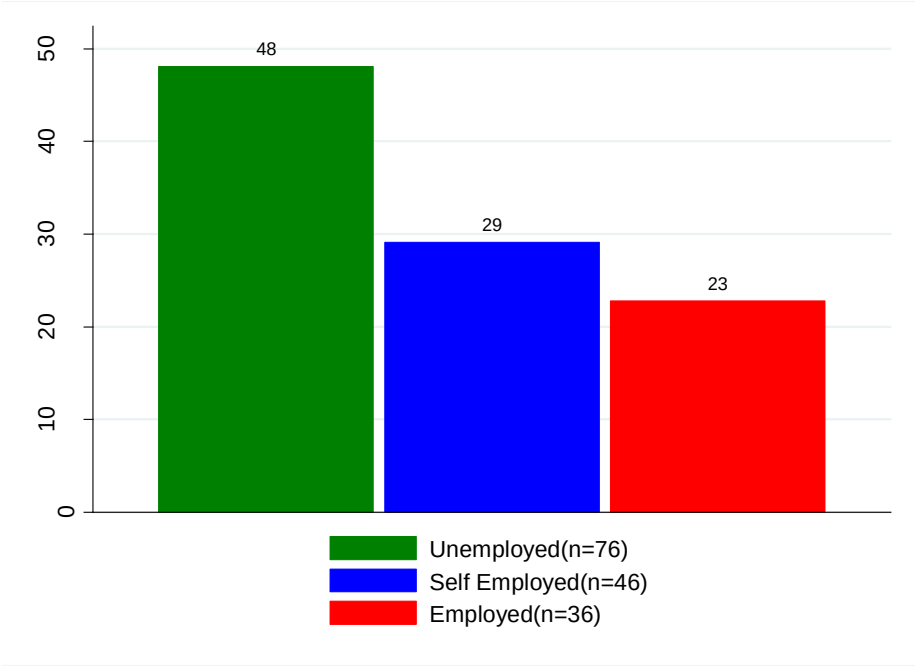

Figure 4: distribution of participants by employment status

4.2.4 Alcohol consumption

Almost one fifth of the participants, 30 (19%), had a history of alcohol consumption.

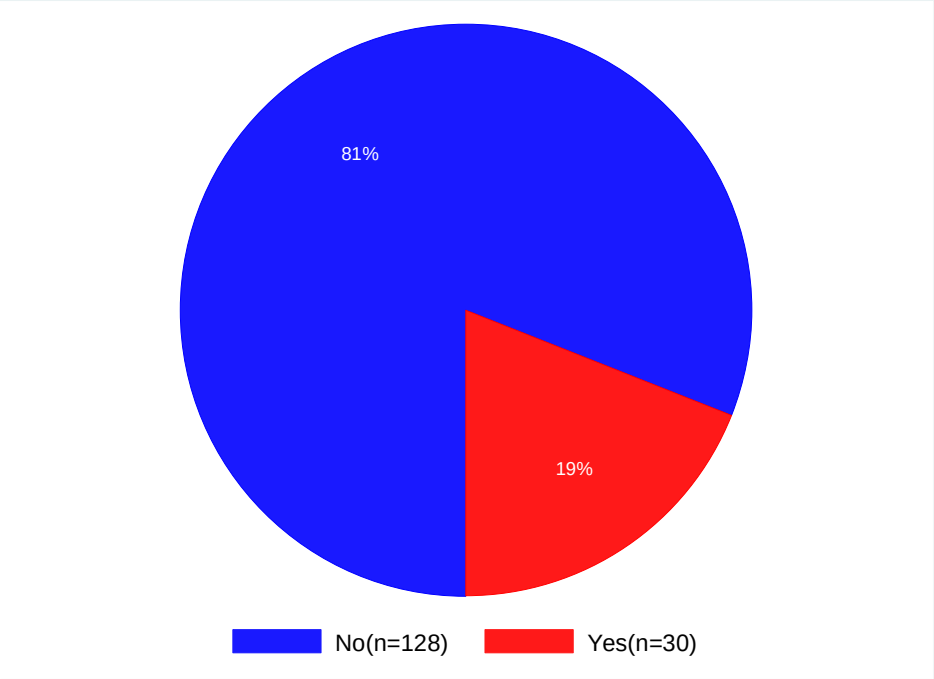

Figure 5: Distribution of the participants by the history of alcohol consumption

4.3 Clinical characteristics

4.3.1 Form of TB

Table 1: Form of TB

Variable		n (%)
TB Form	PTB	112 (71%)
	TBM	10 (6%)
	Disseminated	8 (5%)
	Pleural	8 (5%)
	Milliary	6 (4%)
	Pericarditis	4 (3%)
	Adenitis	3 (2%)
	Peritonitis	3 (2%)
	Spine	3 (2%)
	Genito-urinary	1 (1%)

Majority of the participants, 112 (71%), had pulmonary TB. The extra-pulmonary forms represented below 10% each.

The distribution of the participants based on the form of TB they were suffering from is as shown in Table 1 above.

4.3.2 Sputum smear results

Of the 139 participants who had sputum subjected to staining for acid fast bacilli (AAFBs), 95 (65%) were smear negative for AAFBs.

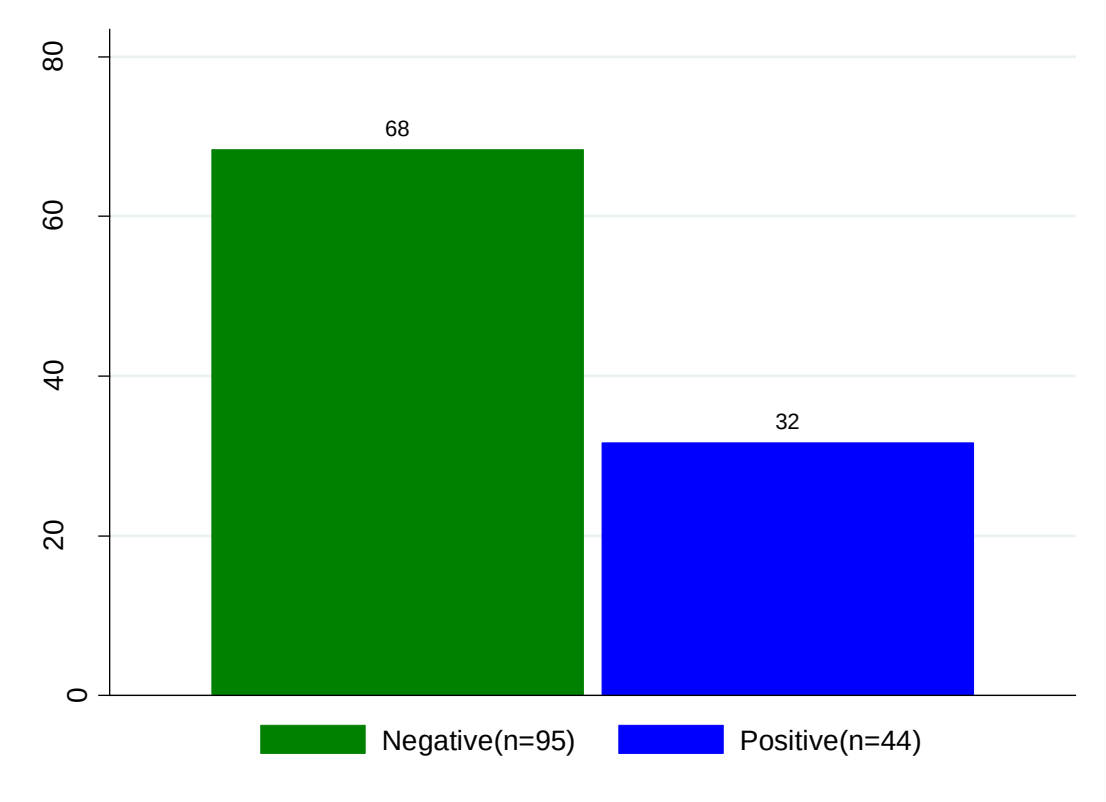

Figure 6: Distribution of the participants by sputum results

4.3.3 Nutritional status

The median BMI of the participants was 18.4 (MAD: 2.5). More than half of the participants 82 (52%) had a BMI<18.5 kg/m^2 and 71 (45%) had BMI between 18.5 and 25 kg/m^2 as shown in Table 1. Most of the participants had a low pretreatment serum albumin level with a median of 25.7g/dl (MAD: 5.9)

4.3.4 Liver function tests

A large proportion of the participants, 145 (92%) had normal baseline liver function test parameters, the median AST, ALT, ALP, direct bilirubin, and total bilirubin for the participants were, 29.1 (MAD: 21.9), 20.8 (MAD: 15.6) 109 (68.8), 2.6 (MAD: 5.6), and 5.2 (8.1) respectively. Only 13 (8%) of the participants had abnormal liver function tests characterized elevation of either AST,ALT and/or elevated bilirubin level above the upper limit of normal at the induction of therapy.

4.3.5 Hepatitis B and C infection

There were 2 (1.2%) participants who were tested positive for hepatitis B. One of the patients developed anti-TB DAH and the other one did not. None of the participants tested positive for hepatitis C.

4.3.6 HIV status

A total of 111 (70%) (95% CL: 62%, 77%) participants were HIV infected. Of these, 90 (81%) were on ARVs while 109 (98%) were on co-trimoxazole prophylaxis. The most commonly used ARV combination was Tenofovir/ Lamivudine/ Efavirence by 72% of our HIV infected participant. The remaining 28% were on Zidovudine/Lamivudine/Efavirence.

4.3.7 Other illnesses and medications

Ten participants, representing 6%, had other comorbid illnesses. These illnesses included Acute Renal Failure, candidiasis, cor-pulmonale, cryptoccoccal meningitis, Diabetes mellitus, End stage renal disease, herpes simplex encephalitis, Hodgkins lymphoma, Non-hodgkins lymphoma, and Peptic ulcer disease.

Fourteen participants, representing 9%, were on other medications. These medications included Acyclovir, spironolactone, dapsone, dexamethasone, chlopropamide, enalapril, fluconazole, omeprazole and Adriamycin/Bleomycin/Vinblastin/Darcarbazine (ABVD).

Table 2: Clinical features

Continous variable		**Median (MAD) or n(%)**
Age (years)		34 (8)
BMI (Kg/m^2)		18.4 (2.5)
Albumin (g/l)		25.7 (5.9)
BWT(Kg)		52 (8.3)
Height (meters)		166 (5.4)
AST (u/l)		29.1 (21.9)
ALT (u/l)		20.8 (15.6)
ALP (u/l)		109 (68.8)
Direct bilirubin (umol/l)		2.6 (5.6)
Total bilirubin (umol/l)		5.2 (8.1)
Categorical variables		
BMI (Kg/m^2)	<18.5	82 (52%)
	18.5-25	71 (45%)
	25-30	2 (1%)
	>30	3 (2%)
HBV	Positive	2 (1.2%)
HCV	Positive	0
HIV	Positive	111 (70%)
ARV use[$]	Yes	90 (81%)
Co-trimoxazole use[$]	Yes	109 (98%)
Presence of other illness	Yes	10 (6%)
Other medication	Yes	14 (9%)
LFTs	Deranged	13 (8%)
DAH	Positive	35 (22%)
DAH type[*]	Hepatotoxic	16 (46%)
	Cholestatic	19 (54%)
DAH severity[*]	Mild	7 (20%)
	Moderate	11 (31.5%)
	Severe	17 (48.5%)

[*] - n=35, [$] - n=11

4.4 Drug associated hepatitis

4.4.1 Incidence of DAH and forms of DAH

There were 35 (22%), (95% CI: 16%, 29%) participants who developed anti-tuberculosis drug associated hepatitis. Of this number, 16 (46%) had hepatotoxic form while the rest had cholestatic form. DAH mainly occurred during the first 60 days of TB therapy. The median time lapse to the development of DAH was 14 (MAD: 18) days. However two patients developed DAH after 5 months of therapy and one in the last week of treatment and ended up being having rifampicin resistant TB. The degree of severity of DAH was guided by the WHO toxicity classification standards as mild, moderate and severe. About half, 48.5% of patients had severe DAH (Table 3).

Table 3: Frequency and severity of DAH

Severity of anti-TB DAH	Number of participants	Percentage
Mild	7	20
Moderate	11	31.5
Severe	17	48.5

4.4.2 Factors associated with DAH

Table 4 presents the rates of hepatotoxicity stratified by the variables of interest. There was no significant difference in age, BMI and sputum smear status between the participants who developed DAH and those who did not have DAH. The other variables that showed no association with DAH were alcohol use, form of TB, presence of other comorbid illnesses, being on other concomitant medication, and abnormal LFTs at the start of therapy.

The factors that showed statistically significant association to development of DAH included gender (P-value 0.009), low serum albumin (P-value <0.001), HIV status (P-value0.002), ARV use (P-value 0.019) and cotrimoxazole use (P-value 0.005).

The results show that male participants had significantly lower rate of hepatotoxicity compared to the female participants. That is, male had 65% reduced risk of hepatotoxicity compared to the male participants, OR: 0.35(95% CL: 0.14, 0.83).

Table 4: Rate of hepatotoxicity stratified by covariates of interest

Variables		**Sample size**	**Hepatotoxicity n(Rate)**	**OR (95% CL)**	**P**
Sex	Male	76	10 (13%)	0.35(0.14, 0.83)	0.009
	Female	82	25 (30%)		
Age	≤35 years	85	21 (25%)	0.72(0.31, 1.65)	0.404
	>35 years	73	14 (19%)		
BMI	<18.5 kg/m^2	80	22 (28%)	0.53(0.22, 1.21)	0.101
	≥18.5 kg/m^2	78	13 (17%)		
Albumin	≥35g/dl	79	27 (34%)	4.61(1.83, 12.60)	<.0001
	<35g/dl	79	8 (10%)		
Alcohol use	No	128	28 (22%)	1.09(0.36, 2.97)	0.863
	Yes	30	7 (23%)		
PTB	No	46	12 (26%)	0.73(0.31, 1.81)	0.445
	Yes	112	23 (21%)		
Sputum	Negative	114	27 (24%)	0.72(0.26, 1.82)	0.455
	Positive	44	8 (18%)		
HIV	Negative	47	3 (6%)	5.94(1.69, 31.76)	0.002
	Positive	111	32 (29%)		
ARV use	No	68	9 (13%)	2.66(1.09, 6.97)	0.019
	Yes	90	26 (29%)		
Cotrimoxazole	No	49	4 (8%)	4.47(1.43, 18.41)	0.005
	Yes	109	31(28%)		
Other illnesses	No	10	4 (40%)	0.40(0.09, 2.05)	0.160
	Yes	148	31(21%)		
Other meds	No	14	3 (21%)	1.05(0.26, 6.19)	0.946
	Yes	144	32 (22%)		
LFTs	Normal	145	30 (21%)	2.40(0.57, 8.97)	0.139
	Abnormal	13	5 (38%)		

Participants who had a low serum albumin level <35.0 had more than four times increased risk of hepatotoxicity compared to those who had albumin level ≥35, OR: 4.61(95% CI: 1.83, 12.60).

HIV positive participants, participants who were on ART, and those who were using co-trimoxazole has increased risk of hepatotoxicity, OR: 5.94(95% CI: 1.69, 31.76), 2.66(95% CI: 1.09, 6.97), and 4.47(1.43, 18.41), respectively.

Table 5 below presents the results of Odds ratios after three logistic regression models that were fitted. The common variables in the three models were Gender, serum albumin, HIV status, co-trimoxazole use and ARV use. Only variables that were significant in bivariate logistic regression model were included in the multivariate logistic regression model.

Table 5: Showing Odds Ratios after adjusting for the joint effect of the variables

Variable		**UOR**	**AOR**
Gender	Male Vs Female	0.35 (0.15,0.78)	0.43 (0.18,1.03)
Serum albumin g/dl	<35 vs ≥35	4.61 (1.83, 12.60)	1.11 (1.03, 1.20)
HIV status	Positive vs Negative	5.94 (1.72,20.52)	3.9 (1.08, 12.24)
Cotrimoxazole use	Yes Vs No	2.66 (1.15,6.15)	1.86 (0.76, 4.59)
ARV use	Yes vs No	4.47 (1.48,13.49)	2.52 (0.78, 8.20)

UOR: Unadjusted Odds Ratio, AOR: Adjusted Odds Ratio.

CHAPTER FIVE : DISCUSSION

5.1 Incidence of anti-TB DAH

This study found the incidence of anti-TB DAH in MTRH to be 22%.This is incidence is much higher than reports from African countries Egypt 15% (Makhlouf et al., 2008), Cameroon 13.6% Assob et al (Assob et al., 2014) Ethiopia 8.9% Mekonnen et al (Mekonnen, 2002) and 11.5% Ali et a l(Ali et al., 2013) Rwanda 9% Lorent et al (Lorent et al., 2011), Tanzania 10% Mugusi et al (Mugusi et al., 2012) and 0.9% Tostmann et al (Alma Tostmann et al., 2010) and 2% in Malawi Tostmann et al (A Tostmann et al., 2007). The variation in the incidence can be attributed to differences in patient characteristics, populations studied, regimens used, type of monitoring, study designs and diagnostic criteria defining anti-TB DAH (Fernandez-Villar et al., 2004; A. Tostmann et al., 2008)**.** The difference in our study and that by Makhlouf (Makhlouf et al., 2008) could be due to difference in HIV prevalence which is higher in our set-up and had a significant association with anti-TB DAH in our study. The lower prevalence of 8.9% reported by Mekonnen et al (Mekonnen, 2002) and 11.5% Ali et al (Ali et al., 2013) in their studies, could be attributed to the retrospective nature of these studies that could have resulted in low prevalence rates due to poor documentation and record keeping and also a different definition criteria for anti-TB DAH .The very low incidence rates reported in Tanzania 0.9% (Alma Tostmann et al., 2010) and Malawi 2% (A Tostmann et al., 2007) could be explained by the fact that these studies were not specifically designed to detect anti-TB DAH, but reported observations made in TB treatment clinical trials. The higher incidence in our study could also be attributed to our study setting being a referral hospital and AMPATH being an HIV clinic leading to high HIV positivity rate which had a significant association with DAH in our study. However our study findings are consistent with other studies including Yimer (Yimer et al., 2008) 21.4%. Despite these variations, DAH remains very important especially, in countries that are endemic for TB, HIV and malnutrition as is the case in Sub- Saharan Africa and parts of Asia.

5.2 Demographic Factors

A predominant number 82 (52%) of the study participants were females. This is in keeping with the national statistics that indicate there are more females than males.

The study population consisted of mostly younger age group. The median age of our participants was 34 (MAD 8). This is in keeping with the KAIS 2012 report which shows the median age of people leaving with HIV/ AIDS have a median age of 35-39years (De Cock et al., 2014).This is also consistent with study by Muture et al n =1978 in Nairobi where 71% of persons with TB were aged between 20 and 40 years (Muture et al., 2011). Makhlouf and colleagues in Egypt also had a similar age group mean 33.6 years (Makhlouf et al., 2008).

Nineteen percent of our study participants reported use of alcohol. This is an alcohol abstinence rate of 81% among our participants which is consistent with reports from Bloomfield et al in a study done in Western Kenya that reported rates of abstinence rate for men and women of 62% and 88% respectively (Bloomfield et al., 2013).

5.3 Clinical Characteristics of the participants

Majority of our study participants 112 (71%) had pulmonary TB. This is consistent with results from WHO Kenya's TB profile 2013 (WHO., 2014), 82% and other studies from Makhlouf et al (63%) and Muture et al (78.9%) who had similar findings (Makhlouf et al., 2008; Muture et al., 2011). This could be explained by the fact that TB transmission is air borne and the lungs are the port of entry for Mycobacteria tuberculosis and therefore this is the most likely manifestation of TB. It is also the site where TB is easily diagnosed both by sputum smear and radiographs.

A large proportion of the participants 65% had smear negative TB. This is higher than the smear negative rate of 51% in the country (WHO., 2014). High numbers of smear negative TB in our study can be explained by the fact that most of the participants had HIV infection which is known to result in smear negative TB due to immunosuppression that results in less inflammation and cavitation in the lung parenchyma hence no site for the bacteria occupy.

This could also be due to use of Gene-X-pert technique to diagnose TB in smear negative samples hence more cases were diagnosed.

Majority of our patients had a low BMI less than 18.5 Kg/M^2.This is consistent with reports from Karyadi et al in Indonesia which indicated that TB patients are 11 times more likely to have BMI less than 18.5Kg/M^2 (Karyadi et al., 2000). This can be explained by the fact that TB leads to reduction in appetite, nutrient malabsorption and altered metabolism leading to wasting. TB patients also have a failure to channel food protein into endogenous protein synthesis, a process called anabolic block. Overexpression of increased inflammatory cytokines especially tumor necrosis factor alpha also results in wasting (Gupta, Gupta, Atreja, Verma, & Vishvkarma, 2009). Most of our participants also had co-infection with HIV that also could have contributed to malnutrition. Malnutrition is also a risk factor for TB as it leads to secondary immunodeficiency that may have led to hosts' susceptibility to TB.

Eight percent our study participants had deranged pretreatment transaminases and bilirubin levels. This could be explained by sub-clinical liver disease such as TB infection affecting the liver, TB Immune reconstitution syndrome in the HIV infected participants, use of concomitant hepatotoxic drugs such as co-trimoxazole, ARVs, Fluconazole, and chemical cofactors such as ethanol use.

There were very few participants with HBV in our study and there was none with HCV. This result is consistent with results by Karoney et al (unpublished) in which there was low prevalence's of 1.09% and 0.26% of HBV and HCV infection respectively among blood donors from Western Kenya (Karoney, 2014). This means our study population was drawn from a population of western Kenya which has low prevalence of HBV and HCV. However, our study findings had very low HBV/HIV co-infection rates compared to a study by Biko et al (unpublished) in the same setting among HIV infected individuals in which he found a HBV/HBV co-infection rate of 9.7% (Biko, 2010). This disparity could be because not all our participants were HIV infected, hence the low rate of HBV. Our findings are also low

compared to the WHO reports which regards Kenya as an intermediate HBV endemicity region of 8%.

There was a high TB/HIV co-infection rate of 70% among our study participants. This is inconsistent with the estimated Kenya TB/HIV co-infection rate of 48% (WHO., 2014). This can be explained by the fact that our study setting included the AMPATH TB clinic which caters for only HIV infected persons. The high TB/HIV co-infection rate can also be explained by the fact that HIV immunosuppression is the main risk factor for TB disease development in our country and therefore most TB patients have HIV infection.

Most of our HIV infected participants 98% were on co-trimoxazole prophylaxis. This is consistent with WHO rate of co-trimoxazole prophylaxis therapy in Kenya of 98% (WHO., 2014). The remaining participants were on dapsone due to allergy to co-trimoxazole.

Eighty one percent of our HIV infected participants were on ART, a rate similar to the Kenya national rate of 84% (WHO., 2014). However, this did not meet the TB/HIV care policy which requires that all TB/HIV co-infected patients should be on ART (Organization, 2010) this could be because some of our patients had the HIV diagnosis made at the time of TB diagnosis and therefore had not yet been started on ART.

5.4 Clinical Presentation of participants with DAH

Anti-TB DAH in our patients manifested as anorexia, nausea, vomiting, and jaundice and right upper quadrant abdominal pain. This is in agreement with reports by Makhlouf and Shakya (Makhlouf et al., 2008; Shakya et al., 2004).

In this study most cases of anti-TB DAH (87%) occurred during the first 60 days of treatment. The median time before development of hepatotoxicity in our participants was 14 days range (6-60 days). This is comparable to other studies conducted in Ethiopia, Egypt and Malawi which showed that hepatotoxicity occurred within the first 6 weeks of treatment (Ali et al., 2013; Makhlouf et al., 2008; A Tostmann et al., 2007). The current finding is also in agreement with results from Shakya et al (Shakya et al., 2004) who reported an interval of

12-60 days (median 28 days).It is also similar to the results from Mahmood et al who reported that onset of anti-TB DAH in almost two thirds of their patients (61%) was within 14 days from start of therapy (Mahmood K & S., 2007). This emphasizes the importance of close and more frequent monitoring in the first 2 months of therapy. The higher occurrence in the first 2 months of therapy can also be attributed to the combination of four drugs in the intensive phase as compared to two drugs in the continuation phase.

5.5 Risk factors for DAH

5.5.1 Age

Age was divided into two groups, thirty five years and bellow and above 35 years for the purpose of analysis based on previous 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). Although several studies suggested that increasing age especially above 35years is a potential risk factor for ant-TB DAH (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), but often statistical significance was not achieved or hepatotoxicity was not treatment limiting. Hwang reported that the rate of anti-TB DAH ranges from 2-8% as age increases with an average of 5% but this was not the case in our study (Hwang et al., 1997). Other studies reported that hepatotoxicity ranges from 22-33% in patients older than 35years compared with a range of 8-17% in those younger than 35years (Hwang et al., 1997; Sharma et al., 2002)**.** Mahmood and colleagues also reported that the older age group was affected more than the younger age group (25.8% and 14.4%) respectively but in our study the two groups were equally affected (Mahmood K & S., 2007). In contrast, Shakya and colleagues reported that the incidence of anti-TB DAH is higher in the younger patients (Shakya et al., 2004). This can be explained by the fact that the majority of patients in his study were young. Our study has shown that age is not a risk factor for the development of anti-TB DAH.This can be attributed to the fact that most of our study participants were younger than 35 years.

5.5.2 Gender

Female gender has been reported to be a risk factor for anti-TB DAH in some studies (Shakya et al., 2004; Shu et al., 2013). Shakya in his study of 50 patients undergoing TB treatment showed that female gender was an in dependent risk factor for the development of hepatotoxicity (Shakya et al., 2004). The relationship between gender and hepatotoxicity was not found to be significant in the present study. Other studies that have demonstrated an absence of correlation between gender and occurrence of hepatotoxicity include (Assob et al., 2014; Lorent et al., 2011; Makhlouf et al., 2008; Mugusi et al., 2012).

5.5.3 Malnutrition

Malnutrition may be a risk factor for anti-TB DAH when defined by a BMI less than 18.5kg/m2 and/or serum albumin level below 35g/dl. The association between malnutrition and hepatotoxicity has been linked to the derangement and disruption of drug metabolism pathways during protein energy malnutrition. This may also be due to depletion of glutathione stores which makes patients more vulnerable to oxidative injuries and the slower pace at which the liver metabolizes drugs. Indeed low albumin level was associated with higher rates of anti-TB DAH in our study as it is in other studies (Assob et al., 2014; Shakya et al., 2004; Sharma et al., 2002).

5.5.4 Alcohol

Although alcohol consumption has been reported as a risk factor for anti-TB DAH (Hwang et al., 1997; Pande, Singh, Khilnani, Khilnani, & Tandon, 1996; Tost et al., 2005) no relationship was observed in the present study. This could be explained by the fact that most of the patients who reported alcohol consumption had normal baseline liver functions and therefore did not have alcohol related liver injury that could have predisposed them to DAH. Other studies that are in agreement with our study include Lorent et al and Mugusi et al (Lorent et al., 2011) (Mugusi et al., 2012).

5.5.5 HIV infection

This study did demonstrate that HIV positive participants were at risk for anti-TB DAH (P-value 0.002). HIV infection independent of Anti-retroviral therapy and cotrimoxazole use was associated with anti-TB DAH. This compares with other studies (Breen et al., 2006; Lorent et al., 2011; Ungo et al., 1998; Yimer et al., 2008). Ungo and his colleagues in 1998 reported that HIV positive patients had a fourfold increased risk of developing DAH (Ungo et al., 1998). Yimer et al in Ethiopia also reported 3.6 increased odds of developing DAH in HIV infected persons and this is in keeping with our results (Yimer et al., 2008). Our study is also in agreement with the findings of Lorent et al who found that HIV/TB co-infection increased the risk of DAH and all other adverse events (Lorent et al., 2011). In this study Lorent and colleagues reported that 13% of HIV positive compared with 2% of HIV negative had DAH. Breen and colleagues 2006 found that treatment interruption due to hepatotoxicity occurred in HIV positive and negative patients at similar frequency, 85% and 87% respectively (Breen et al., 2006).

5.5.6 Hepatitis B infection

With regards to HBV infection, some studies Assob et al**,** Wong et al and Lee et al have shown that it increases the risk for anti-TB DAH (Assob et al., 2014; Lee et al., 2005; Wong et al., 2000). This study had very few cases of HBV that we were not able to assess the impact of hepatitis B. Only two patients were HBsAg positive, one developed DAH and the other one did not.

5.5.7 Hepatitis C infection

Ungo and his colleagues in 1998 reported that chronic HCV infection increased the risk for anti-TB DAH (Ungo et al., 1998). Mugusi corroborated this finding in his study (Mugusi et al., 2012). We were not able to assess the impact of HCV since none of our participants had HCV infection.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Hepatotoxicity is a common finding in patients on treatment for TB. In this study, the incidence of anti-TB DAH was 22% .This is a significantly high proportion of patients who had treatment interruption.

HIV infection and low pretreatment serum albumin levels were identified as independent predictors of occurrence of anti-TB DAH.

Patients with anti-TB DAH had a higher all cause mortality compared to those without DAH

6.2 Recommendations

1) Patients commencing TB treatment should have pretreatment serum albumin levels checked.
2) High risk patients with HIV infection and those with low serum albumin levels should be identified and closely monitored for symptom and signs of anti-TB DAH and serial LFTs through the course of their therapy.
3) Patients commencing TB treatment should be educated on side effects and symptoms of hepatotoxicity to detect drug induced hepatotoxicity early.

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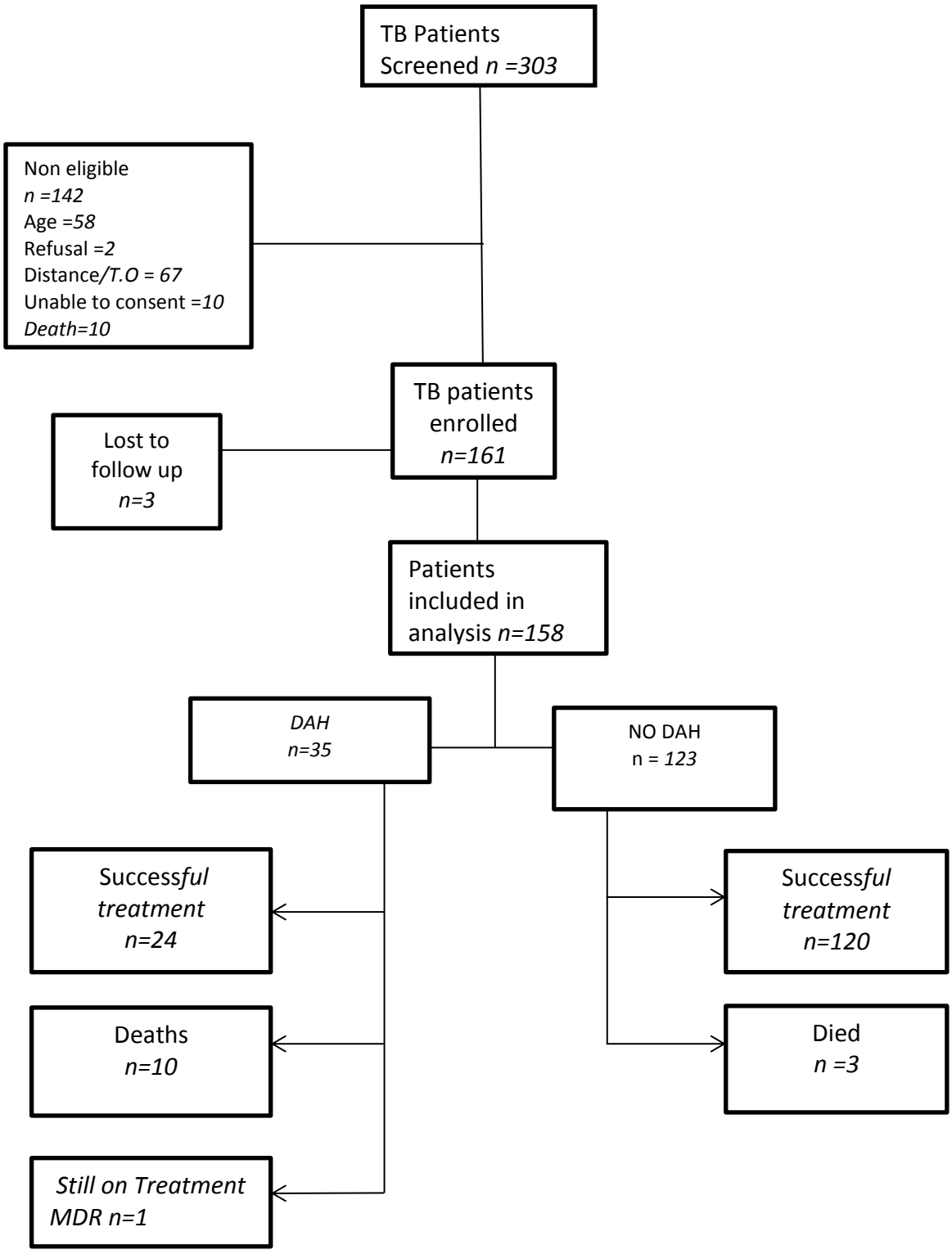
APPENDICES

APPENDIX I: FLOW CHART SHOWING PARTICIPANT RECRUITMENT



APPENDIX II: INITIAL ENCOUNTER DATA COLLECTION SHEET

Hospital number _____ Study number _____

1. Demographic characteristics of the patients

i) Age_____

ii) Gender male ☐ female ☐

iii) Occupation; ☐ employed ☐ self-employed ☐ unemployed

iv) Education level; no formal education ☐ primary ☐

Secondary ☐ college ☐

2 Clinical characteristics

i) Form of TB; Pulmonary_____ Extra-pulmonary_____

ii) Weight_____

iii) Height _____

iv) BMI_____

v) Current Alcohol use YES ☐ NO ☐

Amount of alcohol consumed per day

vi) HIV status _____ Positive ☐ Negative ☐ Not Tested ☐

vii) If HIV positive, are you on HAART YES ☐ NO ☐

ART regimen _____

Cotrimoxazole prophylaxis YES ☐ NO ☐

Are you on any other medicines YES ☐ NO ☐

If yes list the medicines_____

ix) Do you suffer from any other chronic illnesses? YES ☐ NO ☐

If yes in (ix) above, list the disease(s) and medicines that you are taking

_____

_____

_____

_____

x) Are you on any other medications at the moment? YES ☐ NO ☐

Indicate name of drug(s) _____

xi). Laboratory investigation results;

AST	
ALT	
ALP	
Direct bilirubin	
Total bilirubin	
Albumin	
HIV	
HBsAg	
HCV antibodies	

APPENDIX III: FOLLOW-UP DATA COLLECTION SHEET

Hospital number _____ Study number _____

i)Date _____

ii) Time period of anti-TB intake in weeks _____

iii) Do you have any complaints? YES NO

iv) Are you experiencing any of the following symptoms?

Symptom	YES	NO
Nausea	☐	☐
Vomiting	☐	☐
Right upper quadrant pain	☐	☐
Yellowness of eyes	☐	☐
Anorexia	☐	☐
Any complaints	☐	☐

v) Weight _____

vi) Height _____

vii) BMI _____

viii) Jaundice _____

ix) Right upper quadrant tenderness _____

x) Hepatomegaly _____

xi) Are you taking any other medications at the moment other than anti-TBs? Yes ☐ o ☐

If yes above, list the medications _____

xii) Are you taking alcohol at the moment? Yes ☐ No ☐

If yes, how much? _____

xiii). Laboratory investigation results;

AST	
ALT	
ALP	
Direct bilirubin	
Total bilirubin	
Albumin	
HIV	
HbsAg	
HCV antibodies	

xiv) Abdominal ultrasound results

xv) Conclusion

-Continue anti-TBs, to be seen in the next review date

-Possible anti-TB DIH; Repeat LFTs

APPENDIX IV: CONSENT FORM

I am Dr. Lusweti Carolyne Khisa, a post graduate student in the Moi University school of Medicine. I am undertaking a master's degree in the field of internal medicine.

Please read through this document and append your signature in either of the two provided blank spaces as appropriate.

I intend to undertake a study on the prevalence of anti-TB drug induced hepatotoxicity and associated risk factors in MTRH.

The aim of this study is to assess the prevalence of Anti-tuberculosis medicines liver injury and associated risk factors in MTRH. No harm is anticipated in the procedures that will be undertaken, that is; Phlebotomy to get blood for measurement of liver function, HBsAg, Anti-HCV antibodies and you will be kindly requested to take a HIV test. What you are being requested to do is to voluntarily give me permission to include you in this study.

There are no hidden intentions.

In the event that you consent to participate, every bit of information you provide will be held confidential and no part of that information will be printed or disseminated without your written consent.

In the event that you do not wish to participate in the study, you will be evaluated and treated along with other patients routinely. Treatment will not be denied and hospital policy will prevail as stipulated.

The participant shall indicate in the box by ticking in space indicated.

I agree to take part in the study ☐

I do not agree to take part ☐

Name _____ Signature. _____ IP no._____

APPENDIX V: CONSENT- KISWAHILI

Jina langu ni Daktari Lusweti Carolyne Khisa. Ninasomea shahada ya pili katika chuo kikuu cha Moi Kitivo cha Afya.

Nina nia ya kufanya utafiti kwa ajili ya kukusanya takirimu kuhusu madhara ya dawa zinazo tumiwa kutibu ugonjwa ya kifua kikuu yanii TB ya maini. Matarajio ya utafiti huu ni kujumlisha watu wanaopata madhara ya maini wanapo tumia dawa hizi za kutibu kifua kikuu. Utafiti huu utahusisha kujibu maswali kadhaa kuhusu kifua kikuu, magonjwa mengine yanayo uguzwa na pia dawa zinazotumika. Hakuna madhara tunatarajia kutokana na utafiti huu.

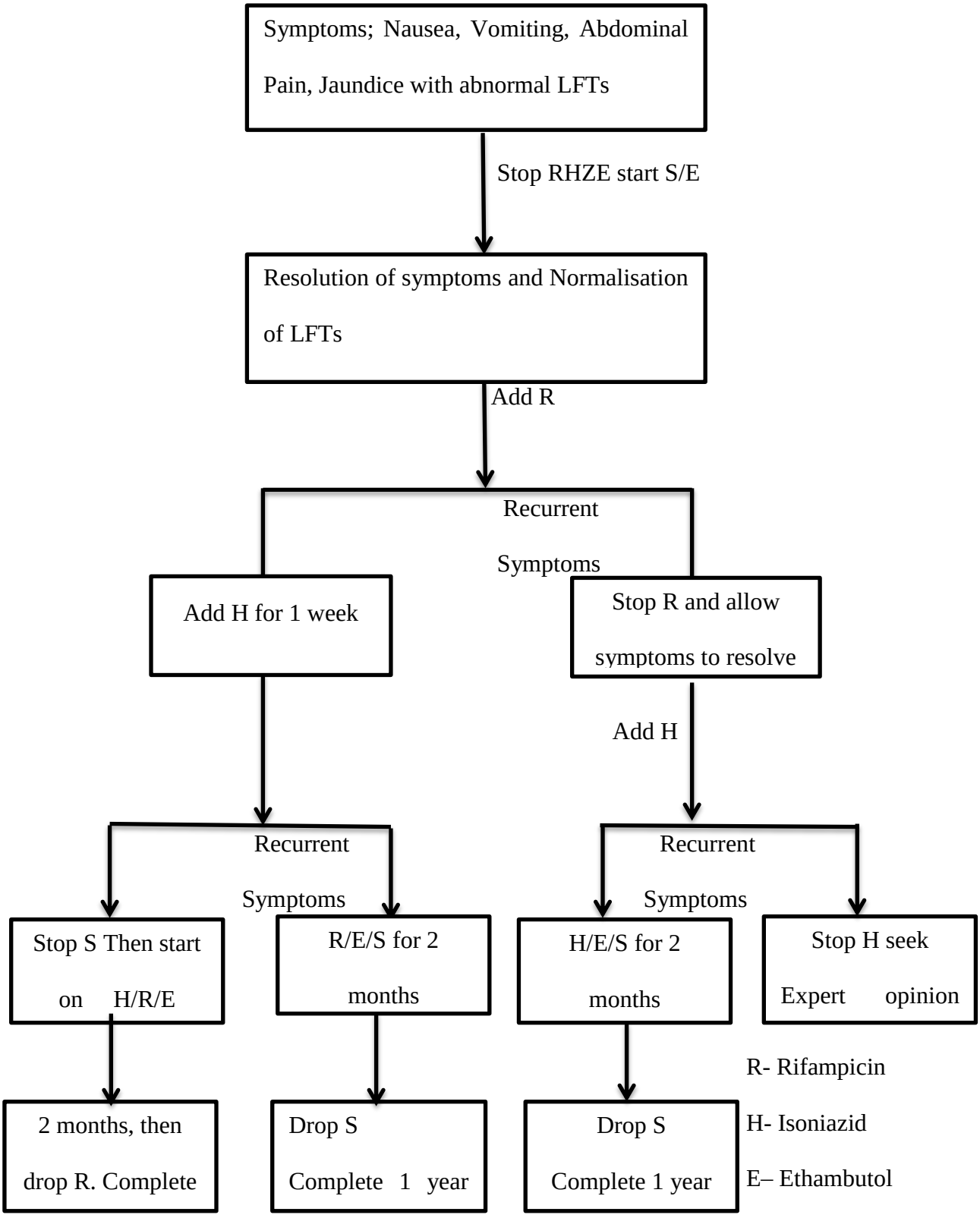
Maswala yote yanayokuhusu yatawekwa vyema na wala hautatambuliwa kwa jina au njia nyingine yeyote.

Ninacho kuomba ni kwaba uniruhusu uwe mmoja wa wale watu nitakao kusanya takirimu.

Kama unakubali kuwa kwa takirimu hii, tafadhali weka sahihi yako kwa nafasi iliyoko;

JINA---------- SAHIHI----------I/D NO.----------

APPENDIX VI: FLOW CHART SHOWING AMPATH TB HEPATITIS COMPLICATION PROTOCOL



APPENDIX VII: PROCEDURE FOR HBsAg DETECTION

HEPATITIS B DETECTION USING ELISA KITS TO DETECT HBsAg IN HUMAN SERUM AND PLASMA(GS, 1981; HA, 1983)

Manufacturer of Kit: ERBRA Diagnosis Mannheim GmBh

Kit Name; EBRA LISA HEPATITIS B

Introduction

The test kits detect HBsAg which is set of lipoproteins of molecular weights ranging from 22Kd to 96Kd that constitute the envelope of the virus.

HBsAg is the first detectable marker in HBV infected serum and is detectable during the whole jaundice phase and becomes undetectable after the appearance of anti- HBs Ag in serum.

ERBA LISA HEPATITIS B use polyclonal antibodies to HBs Ag as coating materials

Principle

The test kit is a solid phase immunoassay for the qualitative detection of HBs Ag in human serum and plasma.

The additional of positive control or HBs Ag containing serum or plasma will form a stable complex with the bound antibody present in the well and with anti-HBsAg-HRPO.

A washing step will remove the unbound conjugate molecule.

Addition of color reagent will develop blue in positive control wells and wells containing HBsAg in test specimen.

Upon addition of a stopping solution the blue changes to Yellow and the intensity of the yellow is directly proportional to the presence of unbound HBsAg in the respective wells.

Contents of the kit

Anti HBsAg coated plate

Conjugate

HBsAg positive control

HBs Ag negative control

Color reagent

Sample diluents E

Stopping solution

Washing solution

Washing solution

Washing solution D(20x) concentrate

Black cover

Adhesive strips

Additional materials required

0 to 20 and 50 to 200 microliter micropipettes and disposable tips

Automatic micropipette washing instrument

Precision ELISA reader

Disposable gloves

Timer

Measuring cylinder 500ml

Storage

Store at 2-8^{0}c

Immediately after use return the reagent at 2-8^{0}c

Precautions

Use disposable gloves throughout the procedure

The test is for in vitro diagnostic use only

Treat all serum as potentially infectious material

Prior to disposal collect all waste ad keep in 5% sodium hypochlorite solution for 30 minutes

Don’t use expired kits

Don’t interchange reagents between different lots

Use clear serum- remove particulate matter by centrifuging

Use separate tips for controls and individual test specimens

Don’t expose color reagents to sunlight

Use distilled or deionized water for dilution of the washing buffer

After using the required strips the rest of the strips along with the activated silica gel should be kept in a sealed condition in the polythene zip lock bag

Specimen collection and storage

All test specimens should be handled as potentially bio-harzardous

Early separation of serum from the clot prevent hemolysis of serum

Use aseptic techniques to collect serum

Store undiluted serum at 2-8^{0}c

Frozen specimen should be thawed properly

Microplate washing procedure

Dilute the washing solution in the ratio 1:20 with distilled or deionized water

The washing solution may be crystallized at cool storage conditions. If so then use after thawing at 37^{0}c in a water bath

At least 6 cycles with at least 0.35ml wash buffer per well per wash and a soak time of 30 seconds are recommended

The plate should be inverted and tapped on an absorbent pad to remove the remaining solution

Test procedure

Bring all test reagents and test specimens to room temperature before use.

Add 50 microliters of sample diluents to each to each well and in each run maintain 1 blank (100 microliter sample diluents +50 microliter of conjugate), 3 negative controls and 1 positive control

Add 50 microliters of color reagent. Cover the plate with a black color and incubate for 15 minutes in the dark at 20-30^{0}c

Add 100 microliters of stopping buffer to each well

Read the absorbency at 450mm. Deduct the blank absorbency from the control and test wells

Calculation for cut off value determination

Blank value: Absorbency of the blank value should be <0.2

Positive control: Absorbency of individual positive control should be>1.0

Negative control: Absorbency of individual negative control should be <0.1

NCx = Average of the negative controls

Cut off value formula = 0.1 + NCx

Interpretation of results

Non-reactive: If the absorbency of the test serum is less than the cut off value

Reactive: If the absorbency of the test serum is equal to or greater than the cut off value then it is considered as initial reactive

The sample should be retested as duplicate. If the absorbency of the retest is less than the cut off value then the sample is considered to be non-reactive.

If both the duplicate retest are reactive then the specimen is repeatedly reactive

APPENDIX VIII: PROCEDURE FOR HCV ANTIBODY TEST

HEPATITIS C DETECTION USING ELISA KITS TO DETCT anti HCV in HUMAN SERUM AND PLASMA(GS, 1981; HA, 1983)

Manufacturer of Kit:

ERBA Diagnostics Mannheim GmBh

Kit Name: ERBA LISA HEPATIS C

ERBA LISA HEPATITIS C: Use synthetic and recombinant protein of Hepatitis C

PRINCIPLE

The test kit is a solid phase immunoassay, utilizing a mixture of synthetic peptides and recombinant proteins of HCV i.e. CORE, NSE3, NS4 and NS5 for the detection of HIV antibodies present in human serum and plasma. When human serum is added to the well the bound antigen present in the well will form a stable complex with the anti-HCV present in the test or positive control specimen. After washing, anti-human IgG –HRPO is added to the wells and only the bound antigen-antibody complex present in the well will react with the conjugate molecule. A second washing step will remove molecule. Addition of color reagent will develop color only in positive control wells and wells containing anti- HCV in test specimen. The intensity of development of color is directly proportional to the presence of bound anti-HCV in the respective wells.

Contents of the kit

HCV antigen coated plate

Conjugate

Anti HCV positive control

HCV negative control

Color reagent

Sample diluents

Stopping solution

Washing solution

Washing solution

Washing solution D(20X) concentrate

Black cover

Adhesive strips

Additional materials required:

0 to 50 and 50 to 200 microliter micropipettes and disposable tips

Automatic micropipette washing instrument

Precision ELISA reader

Disposable gloves

Timer

Measuring cylinder 500ml

Storage

Store at 2-8^{0}c

Immediately after use return the reagents at 2-8^{0}c

Precautions

Use disposable gloves throughout the procedure

The test is for in vitro diagnostic use only

Treat all serum as potentially infectious material

Prior to disposal collect all waste and keep in 5% sodium hypochlorite solution for 30 minutes

Don’t use expired kits

Don’t interchange reagents between different lots

Use clear serum-remove particulate matter by centrifuging

Use separate tips for control and individual test specimens

Don’t expose color reagents to sunlight

Use distilled or deionized water for dilution of the washing buffer

After using the required strips the rest of the strips along with the activated silica gel should be kept in sealed condition into the polythene zip lock bag

Specimen collection and storage

All test specimens should be handled as potentially bio-harzardous

Early separation of serum from the clot prevents hemolysis of serum

Use aseptic techniques to collect serum

Store undiluted serum at 2-8^{0}c

Frozen specimen should be thawed properly

Microplate washing procedure

Dilute the washing solution in the ratio 1:20 with distilled or deionized water

The washing solution may be crystallized at cool storage conditions. If so then use after thawing at 37^{0}c in a water bath

At least 6 cycles with at least 0.35ml wash buffer per well per wash and a soak time of 30 seconds are recommended

The plate should be inverted and tapped on an absorbent pad to remove the remaining washing solution

Test procedure

Bring all test reagent and tests and test specimens to room temperature before use

Expect for the blank add 100 microliter of sample diluents F to each well and in each run maintain 1 blank (100 microliter sample diluent+ 50 microliter of conjugate), 3 negative controls and 1 positive control.

Add 10 microlitres of control and test specimen to the respective wells.

Mix with a pipette and cover the plate with a black cover then incubate for 45 minutes at 20-30^{o}C.

Wash the plate as per micro plate washing procedure.

Add 50 microlitres of the conjugate to each well except the blank. Cover the plate with a black cover and incubate for 15 minutes at 20-30^{0}C.

Wash the plate as per the micro plate washing procedure.

Add 50 microliters of color regent. Cover the plate with a black cover and incubate for 15 minutes in the dark at 20-30

Add 100 microlitres of stopping buffer to each well.

Read the absorbency at 450nm. Deduct the black absorbency from the control and test wells.

Calculation for cut off value determination

Black value: Absorbency of the black value should be <0.2.

Positive control: absorbency of individual negative control 1 should be <0.1.

NCx = Average of the negative controls.

Cut off value formula = 0.1 + NCx.

Interpretation of results

Non-reactive: if the absorbency of the test serum is less than the cut off value.

Reactive: if the absorbency of the test serum is equal to or greater than the cut off value, then it is considered as initially reactive.

Limitations

A non-reactive result with this test does not preclude the possibility of HCV infection.

Repeatedly reactive tests should be further confirmed with other like western blot or indirect immune-fluorescent assay.

APPENDIX IX: IREC LETTER OF STUDY APPROVAL

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2013/121
Approval Number: 0001069

24th September, 2013

Dr. Lusweti Caroline Khisa,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Dr. Lusweti,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee have reviewed your research proposal titled:-

"Incidence and Risk Factors for Anti-Tuberculosis Drug Associated Hepatitis at the Moi Teaching and Referral Hospital, Kenya".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1069*** on 24th September, 2013. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 23rd September, 2014. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
APPROVED
24 SEP 2013
P.O. Box 4606 ELDORET

cc Director - MTRH Dean - SOM Dean - SON
Principal - CHS Dean - SPH Dean - SOD

APPENDIX X: MTRH APPROVAL

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
P. O. Box 3
ELDORET

Ref: ELD/MTRH/R.6/VOL.II/2008

24th September, 2013

Dr. Lusweti Caroline Khisa,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Incidence and Risk Factors for Anti-Tuberculosis Drug Associated Hepatitis at the Moi Teaching and Referral Hospital, Kenya".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

DR. J. KIBOSIA
DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM