SERUM ANALYSIS OF MAJOR BIOCHEMICAL CHANGES AMONG PATIENTS WITH MULTI-DRUG RESISTANT TUBERCULOSIS (MDR-TB) IN MEKELLE HOSPITAL, TIGRAY, ETHIOPIA

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

By

DESALEGN TEKLU (DVM)

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By

DESALEGN TEKLU

Under the supervision of

Advisor-Dr. Daniel Seifu, PhD (Department of Biochemistry, School of Medicine, Addis Ababa University)

Co-advisor-Dr.FikereselamDesalegn,MD,MSc,MekeLLe Hospital

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School of Graduate studies

This is to certify that the thesis prepared by **Desalegn Teklu Berhe** entitled: "**serum analysis of major biochemical changes among patients with multi-drug resistant tuberculosis (MDR-TB) in Mekelle Hospital, Tigray, Ethiopia"** and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Medical Biochemistry complies with regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner:_____Signature: _____Date:_____

Advisor:Dr. Daniel Seifu_____Signature: _____Date:_____

Co-Advisor:Dr.FikreselamDesalegn_Signature: _____Date:_____

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ABBREVIATIONS

ADRs- Adverse drug reactions

ALT- Alanine aminotransferase

ART-Anti-retroviral therapy

AST-Aspartate aminotransferase

AZT-Zidovuidine

CL-Confidence interval

FLDs- First line drugs

HIV- Human immune deficiency virus

LFT-Liver function test

MDR-TB-Multi-drug resistance tuberculosis

OR-odds ratio

RFT-Renal function test

SLDs- Second line drugs

TB-Tuberculosis

TDF- Tenofovird iso-proxil fumarate

TFT-Thyroid function test

TSH-Thyroid stimulating hormone

WHO-World Health Organizations

XDR-TB-Extensive drug resistance tuberculosis

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ABSTRACT

Introduction: Adverse drug reaction (ADRs) defined as: any unintended adverse response to a drug occurring at a therapeutic dose and resulting in either death, drug withdrawal, change in the administration of the frequency or dose of the drug, is one of the major concerns about SLDs to treat MDR-TB. Hypothyroidism, liver toxicity, renal dysfunction and electrolyte disturbances have been reported among the prevalent ADRs during MDR-TB treatment. However, these ADRs had not investigated in Ethiopia.

Objectives: The aim of this study was to investigate the sero- prevalence of adverse drug reactions (ADRs) and associated risk factors among MDR-TB patients with second line drugs.

Methods: this prospective, observational cohort study was carried out from August 2013 to April 2014 in Mekellle Hospital. 68 confirmed pulmonary MDR-TB patients by Tigray Regional Laboratory were followed and evaluated for ADRs development during the study period. Routine laboratory test according to ADRs monitoring schedule was done in Ayder Referral Hospital in collaboration with Mekelle Hospital. Demographic and clinical information were extracted from standardized clinical files.

Results: There were 67.6% male and 32.4% female patients. The mean patient age was 32.62 ± (11.173) years. Among the 68 patients screened for adverse drug reactions, electrolyte related abnormality (hypokalemia 47/68(69.11%), hypomagnesaemia18/68(26.47%) and hypocalcaemia 8/68(11.76 %) was 83.80%, nephrotoxicity 22/68(32.35%), hypothyroidism16/68(23.53%) and hepatotoxicity 10/68(14.71%) were recorded.

Conclusions: High rate of hypokalemia, nephrotoxicity, hypothyroidism and hepatotoxicity was observed in this cohort. This is a treatable and reversible; however, it may go undiagnosed in the absence of regular monitoring. Care providers should not wait for clinical symptoms, as this risks compromising treatment adherence. Electrolyte related abnormalities and renal toxicity was the most prevalent among others. Higher magnitude of ADRs was observed among older, females and HIV co-infected patients on ART/anti-TB.

Key words: adverse drug reactions, MDR-TB, second line ant-TB drugs

1. INTRODUCTION

1.1. Tuberculosis (TB)

Tuberculosis remains a major global health problem (Liu *et al*., 2013). It causes ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV) (Santha *et al*., 2002). Mycobacterium tuberculosis complex includes several species, namely *mycobacterium tuberculosis* (MTB), *Mycobacterium bovis, Mycobacterium africanum, Mycobacterium microti, Mycobacterium caneti and Mycobacterium pinnipedi*, all belonging to the Mycobacterium genus and the Mycobacteriaceae family (Chiang,2013). MTB transmits through small airborne droplets generated by the coughing, sneezing, talking, or singing of a person with pulmonary TB is the usual causative agent of human TB (Knechel, 2009). Definitive diagnosis of TB requires identification of MTB in a culture of a diagnostic specimen (Knechel, 2009).

Access to TB care has been expanded substantially and major progress has been made in reducing TB cases and deaths since the mid-1990s (Dias *et al*., 2012). Between 1995 and 2012, 56 million people were successfully treated for TB in countries that had adopted directly observed treatment, short course(DOTS)/Stop TB Strategy, saving 22 million lives (Baddeley *et al*., 2013). By 2012, the TB mortality rate had been reduced by 45% since 1990 and the target of a 50% reduction by 2015 is within reach and incidence is declining at a rate of 2.2% between 2010 and 2011. Seven of the 22 high burden countries have met all of the 2015 targets for reductions in TB cases and deaths and Ethiopia is on track to fulfill (Baddeley *et al*., 2013). One of the most important indicators of global progress is the 87% treatment success rate in 2011, up from 69% in 2000 (Baddeley *et al*., 2013). This demonstrates huge improvement in the provision of high quality TB care in most countries.

Despite this encouraging progress and the availability of treatment that will cure most cases of TB, the global burden of TB remains enormous. For instance, the latest estimate indicated that there were almost 8.6 million new cases with 1.3 million deaths globally in 2012 (Lomtadze *et al*., 2013). Data from drug resistance surveys and continuous surveillance among notified TB cases suggested that 3.7% of new cases and 20% of previously treated cases are estimated to

have multi drug resistant tuberculosis (MDR-TB) (Kassa *et al*.,2013). Therefore, more effort is still required by the international community to impose TB, the curable disease.

World Health Organization (WHO) currently-recommended approach to TB care and control is the Stop TB Strategy launched in 2006 (Enarson & Harries,2013). The strategy will have been intended to target a 50% reduction in prevalence and death rates by 2015 compared with their levels in 1990 and to decline the global incidence of active TB cases to less than one case per million population per year By 2050. Therefore, to successfully accomplish the WHO target, patients must adhere to second line drugs (SLDs) (Lawn *et al*., 2013). However, SLDs have been associated with more Adverse drug reaction (ADRs), by which many of them are poorly tolerated (Rodriguez *et al*., 2013)

Adverse drug reaction (ADRs) defined as: any unintended adverse response to a drug occurring at a therapeutic dose and resulting in either death, drug withdrawal, change in the administration of the frequency or dose of the drug, is one of the major concerns about SLDs to treat MDR-TB (Van der Walt *et al*., 2013).Therefore, ADRs induced by SLDs could have a severe impact on adherence, the critical factor in the management of MDR-TB (Weyer *et al*., 2004.)

During MDR-TB treatment, generally there are two phases, the intensive and the continuous phase (Monedero & Caminero, 2010). The intensive phase is characterized by high pill burden and more toxic drugs (Sagwa *et al*., 2012). Thus, investigation of ADRs prevalence and severity among MDR-TB patients in the intensive phase help, physicians to monitor them early and properly, as result its impact on adherence will be eliminated . Hypothyroidism, liver toxicity, renal dysfunction and electrolyte disturbance (Table 1.1) are among the prevalent ADRs encountered during MDR-TB treatments (kassa *et al*., 2012).

Table 1.1.Common adverse effects of SLDs (Chiang,2013)

Types of drugs	Drug Adverse effects
Ethionamide, prothionamide and Para-aminosalicylic acid	Hypothyroidism, Gastrointestinal disturbance, hepatitis,
Cycloserine/terizidone	Neurological and psychiatric disturbances: suicidal ideation headache, irritability, depression, seizures,
Kanamycin, amikacin, capreomycin	Pain at injection site, hypokalemia and hypomagnesaemia, nephrotoxicity, ototoxicity, peripheral neuropathy
floxacin, levofloxacin, gatifloxacin, moxifloxacin	Generally well tolerated, occasional gastrointestinal disturbance, joint pain

In general, the magnitude of ADRs prevalence on MDR-TB patients treated under SLDs from previous reports was variable. This might be due to the variation in setting of researchers, the methodology used, level of awareness in health worker, health professional and community as a whole, drugs used and in the system of ADRs monitoring and management(Sagwa *et al*., 2012). Accordingly, some of the following prevalence was reported: 96% (Tupasi *et al*., 2006); 90% (Sagwa *et al*., 2012); 95% (Delgado *et al*., 2011); 60.6 % (Vishakha & Sanjay 2014); 60.9% (Ünsal *et al*., 2013); 69.2% (Törün *et al*., 2005); 73.3% (Shin *et al*., 2007); 79% (Bloss *et al*., 2010).

1.2. Tuberculosis in Ethiopia

Tuberculosis is widespread in Ethiopia and other sub-Saharan countries and it has emerged as a major public health problem, including a high prevalence of MDR-TB (Habtewold, 2013). Ethiopia is among the 22 high HIV burden countries (Habtewold, 2013) and among the 27 high MDR TB burden countries in the world (Abate et al., 2012). According to the 2013 TB report the total estimated TB prevalence in 2012 was 210,000 (Baddeley et al., 2013). From the same report the estimated incident and total notified TB cases were 230,000 and 147, 592 respectively.

Notified TB cases, 1.6 % new and 12 % of previously treated have had MDR-TB (Kassa et al., 2013).

In Ethiopia, a standardized TB prevention and control program incorporating DOTs was started as a pilot in 1992 and is now widely in use (Getahun et al., 2013). The 2015 DOTs/Stop TB targets for 50 % reductions in incidence and mortality has met and appears on track to reach the target of halving the 1990 prevalence rate by 2015 (Dias et al., 2012). Cases enrollment and treatment success in Ethiopia is getting better and better since 2009 (Baddeley et al., 2013). MDR-TB Cases enrolled to treatment since 2009-2012 were 88,120,199,289 respectively (Baddeley et al., 2013). The Treatment success rate in 2011 for new smear-positive and/or culture-positive, new smear-negative/extra pulmonary and retreatment were 90%, 87%, 78% respectively (Baddeley et al., 2011). Therefore, to keep this peace and raise patients' enrolment and treatment success, early ADR monitoring is necessary.

1.3. Biological characteristics of *Mycobacterium tuberculosis* (MTB)

Human Tuberculosis is an infection mostly caused by the rod-shaped, non–spore-forming, aerobic bacterium MTB (Knechel, 2009). It is characterized by a unique, lipid-containing cell wall which is responsible for the acid-alcohol fast properties (Chiang, 2013). The rigid core of the cell wall is composed of three macromolecules: peptidoglycan, arabinogalactan, and mycolic acids. External to the peptidoglycan and the arabinogalactan are the mycolic acids, which together with free lipids act as a hydrophobic permeability barrier (Knechel, 2009). This explains why MTB naturally resistant to many antibiotics including penicillin and sulfonamides (Chiang, 2013).

1.4. Drug resistance mechanism

Globally, the emergence of multidrug-resistant strains of MTB is an increasing problem which adversely affects patient care and public health (Chiang ,2013). Several bacterial species acquire resistance through mobile genetic elements, while resistance to anti-TB drugs is caused by spontaneous chromosomal mutation (Chhabra *et al*., 2011). The proportion of wild-type resistant mutants in an untreated MTB population is usually very small (Habtewold, 2013***)***. Treatment with anti-TB drugs imposes selection pressure, resulting in a decline of drug-susceptible bacilli

and rise of drug-resistant mutants (Chiang,2013). To date, there is no single chromosomal mutation that has been found to cause resistance to two or more anti-TB drugs (Chhabra *et al*., 2011).Inappropriate regimens, use of lower-than-recommended dosage, poor drug quality and poor adherence to treatment are commonly associated with emergence of drug resistance in TB patients (Pfyffer,2000). The cellular targets of anti-TB drugs and the gene responsible for resistance are summarized in (Table1.2) below.

Table 1.2.Cellular targets for anti-TB drugs and gene mutations responsible for resistance in MTB (Pfyffer,2000)

Drug	**Cellular target**	**Gen**	**Gen product /functional role**
Isoniazid	Cell wall	katG	catalase-peroxidase / activation of pro-drug
		inhA	enoyl-acyl carrier protein reductase / mycolic acid biosynthesis
		kasA	-ketoacyl acyl carrier protein synthase / mycolic acid biosynthesis
Rifampicin	nucleic acids	rpoB	subunit of RNA polymerase / transcription
Streptomycim	protein synthesis	rpsL	ribosomal protein S12 / translation
Pyrazinamide	Unknown	pncA	pyrazinamidase-nicotinamidase/activation of pro-drug
Ethambutol	cell wall	embB	arabinosyl transferase / arabinan polymerisation
Flouroquinolones	nucleic acids	DNA gyr A	DNA gyrase subunit A / DNA replication

1.5. Multi drug resistance tuberculosis treatment

1.5.1. Principles of treatment

Effective TB treatment started in 1946, with the introduction of streptomycin in United Kingdom (Caminero, 2013). Unfortunately after some years, MBT became resistant to it (Chiang, 2013). Afterward, para-aminosalicylic acid was combined with streptomycin and their synergetic effect greatly reduced the incidence of resistance (Dooley *et al*., 2008). In 1952, isoniazid was introduced as a new wonder drug and treatment of TB was successful for some years again (Caminero, 2013). Isoniazid and rifampcin resistance MTB became a growing issue for the development of MDR-TB (Abanna & Menzie, 2011).

MDR-TB is now a health problem faced by most developing and developed countries across the globe (Albanna & Menzies, 2011). As MDR-TB development is due to improper treatment (Chiang, 2013). Therefore, the broad objective of TB treatment is to produce rapid reduction in bacillary load, prevent emergency of drug resistant mutants and relapse (Pinto & Menzies, 2011). Consequently, potent bactericidal drugs such as flouroqunnols and the second line injectants and multiple bacteriostatic drugs with proven efficacy by drug sensitivity test or likely never previously used, is issued (Monedero & Caminero, 2010). In general, adequate drug regimen with sufficiently long duration to eliminate residual surviving organisms responsible for disease relapse is recommended (Caminero, 2013).

1.5.2. Drug selection and combination during MDR-TB treatment

Second line drugs (SLDs) used to treat MDR-TB includes: fluoroquinolones, second-line injectable drugs, ethionamide, prothionamide, cycloserine and Para-aminosalicylic acid (Table 1.3) (Chiang, 2013). According to WHO guidelines for the programmatic management of DR-TB, 2011 update recommendation, a combination of at least four SLDs likely to be effective as well as pyrazinamide from first line drugs(FLDs) should be incorporated during MDR-TB treatment (Falzon *et al*., 2011).

Traditionally for years ant-TB drugs have been classified as FLDs or SDLs (Shim & Jo, 2013). Nowadays, as the incidence of MDR-TB has increased, they have been categorized into 5 groups (Table 1.3) (He *et al*., 2011). Drugs are chosen with a stepwise selection process from the five

groups on the basis of efficacy, safety, and cost (Chang & Yew, 2013). Among the first group; the oral FLDs: high-dose isoniazid, pyrazinamide, and ethambutol are thought of as an adjunct for the treatment of MDR -TB (Caminero, 2013). Only one drug from the second group is recommended to incorporate because they have the same mechanism and prioritized in the order of capreomycin, kanamycin, then amikacin (Caminero *et al*., 2010). The third group consists of fluoroquinolones (Kassa *et al*, 2012). However, among these agents, ciprofloxacin is no longer considered to be an anti-TB drug because of the dyglysemic effec(Lee *et al*., 2011). Moxifloxacin and levofloxacin are the newer fluoroquinolones in this group (Ho *et al*., 2009) and almost have the same efficiency in MDR-TB treatment (Lee *et al*., 2011). The fourth group is called the second line oral bacteriostatic drugs and should be used in the order of, prothionamide, ethionamide, cycloserine, para-aminosalicylic acid (Chiang, 2013). The fifth group includes drugs with unproven efficacy in MDR-TB (Shim & Jo, 2013) and should be used in the following order: clofazimine, amoxicillin with clavulanate, linezolid, carbapenems, thioacetazone, then clarithromycin (Caminero *et al.*, 2010).

Table 1.3 . Anti-tuberculosis drugs classification (kassa et al., 2012)

Groups	Description	Drugs
1	First-line oral anti-TB agents	Pyrazinamide, Ethambutol, Rifampicin and Isoniazid
2	Injectable anti-TB agents	Streptomycin, Kanamycin, Amikacin,Capreomycin and Viomycin
3	Second-line fluoroquinolones	Ciprofloxacin, Ofloxacin, Levofloxacin, Moxifloxacin, Gatifloxacin and Sparfloxacin
4	Oral bacteriostatic second-line anti-tuberculosis agents	Ethionamide, Protionamide, Cycloserine, Terizidone, P-amino salicylic acid and Thioacetazone
5	Agents with unclear role in DR-TB treatment	Amoxicillin/clavulanate ,Clarithromycin , High-dose isoniazid ,Clofazimine

1.5.3. Treatment regimen

Multi-drug resistant tuberculosis (MDR-TB) treatment is based on expert opinion and requires the combination of drug regimens chosen from five hierarchical groups of first-line and SLDs (Karagöz *et al*., 2009). Regimens may be chosen on a standardized or empirical basis and then switched to individualized therapy after data regarding drug susceptibility testing become available (Naidoo *et al*., 2013). However, reliable drug-susceptibility testing is not widely available in regions in which TB is endemic, particularly for SLDs (Amuha *et al*., 2009). In addition, drug like cycloserine even difficult to test, as a result in vitro testing has been abandoned (Falzon *et al*., 2011). Regardless of drug susceptibility profiles, the most successful treatment regimen for MDR- TB is the standardized regimen (Pinto & Menzies, 2011).This regimen is based on country-specific profiles of drug resistance and is expected to yield good results in resource limited countries where SLDs have not been previously used for TB treatment (Shim & Jo, 2013).

1.5.4. Durations of MDR-TB treatment

Traditionally, the recommended treatment duration for MDR-TB has been 18 to 24 months and requires daily administration of drugs that are more toxic and less effective than those used to treat drug-susceptible forms of TB (Monedero & Caminero, 2010).The update 2011 WHO guidelines for programmatic management of DR-TB recommended that an intensive phase of 8 months' and total treatment duration of 20 months in patients without any previous history of MDR-TB treatment (Falzon *et al*., 2011). However, such durations yet presents problem in terms of costs and adverse events (Shim & Jo, 2013). A Prospective cohort study conducted by Van Deun *et al*., 2010, in Bangladesh on 206 patients with MDR-TB and not previously treated with SLDs using a 9-month regimen (an intensive and continuous phase of 4 and 5 months respectively) found relapse-free cure rate of 87.9%. In addition, recent guidelines for MDR-TB therapy published by the International Union against Tuberculosis and Lung Disease recommend the 9-month Bangladesh regimen for MDR-TB cases showing susceptibility to fluoroquinolones and injectable drugs (Shim & Jo, 2013). Further, the 9 months MDR-TB regimen reported in Bangladesh is now being evaluated in Ethiopia, South Africa and Vietnam (Lawan *et al*., 2013).

1.6. MDR-TB treatment regimen in Ethiopia

Fully standardized regimen is currently is in use in Ethiopia (Kassa *et al*., 2013). Standardization is done according to WHO recommendation (Falzon *et al*., 2011).the following five drugs: Levofloxacine, capreomycin, Ethionamide, Cycloserine and pyrazinamide were used to treat MDR-TB in the study area (Kassa *et al*., 2013).

1.8. Prevalence of adverse drug reaction during MDR-TB treatment

1.8.1. Hypothyroidism

Drug induced primary hypothyroidism defined as serum level of TSH ≥ 10 mIU/L (Satti *et al*.,2012) is a known reversible adverse effect of prolonged therapy with ethionamide ,prothionamide and para-aminosalicylic acid (Deary *et al*., 2013). Drug-induced hypothyroidism may or may not be associated with goiter and can be virtually asymptomatic (Chhabra *et al*., 2012). As a result the WHO recommended that patients on second line anti- TB drugs should be screened for hypothyroidism every 3 months through out treatment or sooner if symptoms arise (Kassa *et al*., 2012). Hypothyroidism responds to thyroxin (Andries *et al*., 2013).Therefore, the Clinical and biochemical levels back to normal after administration of thyroxin with out treatment interuption(Chhabra *et al*., 2012). Recently in most countries screening for hypothyroidism among patients on MDR-TB treatment is recommended and a national guideline has been prepared (Falzon *et al*., 2011).

According to the 2012 clinical practice guidelines for the management of hypothyroidism in adults, co-sponsored by the American Association of Clinical Endocrinologists and the American Thyroid association, serum thyroid stimulating hormone(TSH) is the single best screening test recommended for primary thyroid dysfunction for the vast majority of inpatient and outpatient clinical situations (Deary,et al., 2013).

Tthyroid stimulating hormone (TSH), is a glycoprotein with a molecular weight of approximately 28,000 daltons (Soldin *et al*., 2013). The beta subunit determines the specific biological and immunological properties (Deary *et al*., 2013). TSH promotes expression of thyroid hormones T3 and T4 which are essential for metabolism, growth, and development (Soldin *et al*., 2013).

So far quite variable magnitude of hypothyroidism was reported worldwide, despite the magnitude of the problem is not yet investigated in Ethiopia where Ethionamide has been extensively used in MDR-TB treatment (Kassa *et al*., 2013). SLDs like ethinomide/prothnomide and para-aminosalicylic acid used to treat TB can cause hypothyroidism by suppressing thyroid hormone synthesis (T3 and T4) through the mechanism of inhibiting the uptake of iodine into thyroid cells and the activity of thyroid peroxidisase respectively (Thee *et al*., 2011). In a retrospective cohort of 186 adults with MDR-TB in which 96.2% were treated with ethionamide/ prothionamide and Paraminosalicylic acid, 129/186 (69%) had hypothyroidism defined as a TSH >10mIU/L (Satti *et al*.,2012) in Lesotho. From other retrospective study in Pakistan and Botswana, carried out by Qayyum *et al*., 2012 and Modongo & Zetola 2012,15/440 (3.4%**)** and 73/213(34.2% hypothyroidism respectively was reported.

From a prospective observational cohort studies conducted by Andries *et al*.,2013; Dutta *et al*., 2012 ;Isaakidis *et al*.,2012, found 54% , 11/52 (21%) and 22/67 (32%) drug induced hypothyroidism respectively. Shin *et al*., 2007,from Rusia,Tomsk also reported about 42 (17.2% drug induced hypothyroidism. In children drug induced hypothyroidism is common (Thee *et al*., 2011). From Ethionamide treated children with MDR-TB, 79/137 (58%) had abnormal thyroid functions, with at least 41% of those with abnormalities likely due to Ethionamide treatment. The association of HIV co-infection and hypothyroidism in adults (Satti *et al*., 2012) was not as significant as in pediatrics (Thee *et al*., 2011).

1.8.2. Renal Insufficiency

Kanamycin/amikacin and capreomycin are among the nephrotoxic anti TB drugs used to treat MDR-TB (Sturdy *et al*, 2011). Aminoglycoside and capreomycin-induced nephrotoxicity is reversible and is related to uptake of these drugs by renal tubular cells after glomerular filtration, with intracellular accumulation and subsequent tubular necrosis generally resulting in oliguric renal failure (de Jager & Altena, 2002). However, this is often asymptomatic in the early stages and can only be diagnosed with routine laboratory monitoring otherwise permanent nephrotoxic damage can happen (Gupta *et al*., 2005).

Serum creatinine concentration is widely interpreted as a measure of the glomerular filtration rate and is used as an index of renal function in clinical practice (Kotask *et al*., 2008)..Therefore, any condition that reduces the glomerular filtration rate will result in a lessened excretion from the body; with a consequent rise in the concentration of creatinine in the blood (Bowers & Edward, 1980). Adult men and women with no kidney disease have serum creatinine of (0.6 to 1.1 mg/dL) and (0.5 to 0.9 mg/dL) respectively (Bartels & Bohmer, 1971). Monitoring is carried out monthly for those with normal baseline renal function test (RFT) and more frequently with abnormal test (kassa *et al*., 2012). Moreover, Patients with diabetes, HIV co-infection, especially on antiretroviral therapy (ART) haven been taught at high risk of injectable nephrotoxicity therefore, monitoring is more frequent for such patients (Isaakidi *et al*., 2012). Tenofovir disoproxil fumarate (TDF) overlapping toxicity at the level of the proximal renal tubules was documented when given together with capreomycin (Lawan *et al*., 2013).

Injectable anti-TB drugs effect on the renal tubules is asymptomatic in early stages (Gupta *et al*., 2005). In addition, the mild and moderate kidney injuries are poorly inferred to serum creatinine alone (Dheda *et al*., 2010). Consequently, the national kidney disease education program in the United State of America encourage clinical laboratories to routinely estimate creatinine clearance rate using the Cockcroft-Gault equation (Gupta *et al*., 2005) and interpreted as Creatinine clearance rate (ml/min) <15 end stage kidney disease, between -15-29 / 30-59 sever renal impairment and moderate chronic kidney abnormality respectively, while creatinine clearance rate >60ml/min shows normally functioning kidney using the following formula (Kimberly *et al*., 2011).

$$\text{Creatinine clearance (mL/min)} = \frac{[140 - \text{age (years)}] \times \text{weight (Kg)} \times [0.85 \text{ if female}]}{72 \times \text{serum creatinine (mg/dL)}}$$

Some of the drug induced nephrotoxicity reported from previous works are summarized here under .In 2 adult cohorts treated for MDR-TB, 9.8% (Shin *et al*., 2007) and 9.3% (de Jager & van Altena, 2002) had nephrotoxicity. In addition, in retrospective studies conducted by Baghaei *et al*., 2011, 3.8% renal toxicity was reported in Iran. Among nephrotoxic MDR-TB patients, 3% renal failures were observed (Dziusmikeyeva *et al*., 2011). 3% and 21% severs renal impairment was recorded respectively in Indian and Lesotho MDR-TB/HIV co-infected cohort

on ART (Isaakidi *et al*., 2012). In the Indian cohort mentioned above out of the 3% patients with severe renal impairment two patients were concomitantly treated with TDF/ injectable anti-TB. In another cohort of XDR-TB patients conducted in South Africa, five persons were died due to the presumed capreomycin-associated progressive acute renal failure (Shean *et al*., 2013).

1.8.3. Hepatotoxicity

The liver is the commonest organs that can be affected by anti TB drugs (Hsu *et al*., 2010). Drug induced Hepatotoxicity defined as elevation of serum transaminase at least 3 times the upper limit of normal values is a very common phonemona in both inpatient and outpatient clinical situations(Chhabra *et al*., 2012) . Anti-TB drug-induced hepatitis is one of the serious adverse drug reactions, which often impedes scheduled treatment and cure (Younossian *et al*., 2005). It encompasses a wide spectrum of liver injury, ranging from asymptomatic minimal elevation of liver enzymes to acute liver failure, and often leads to death or liver transplantation (Dufou *et al*., 2000)

Generally, drugs induced hepatotoxicity is reversible upon dose adjustment or complete discontinuation of the suspected drug (Okonkwo *et al*., 2012). Biochemical results will guide the decision (Keshavjee *et al*., 2012). Serum enzymes are the most commonly used and sensitive biochemical markers for the assessment of hepatocellular injury and its resultant liver disease (Keshavjee *et al*., 2012).The enzymes most commonly used are aminotransferases(Dufou *et al*., 2000). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are found in the cytoplasm and mitochondria of liver cells in high concentrations but low in blood (Tostmann *et al*.,2008). The normal concentration of AST and ALT in blood is 37 U/ L and 42 U /L respectively (Tam *et al*., 2002). However, increased activities of these enzymes in serum are due to increased membrane permeability and leakage in to the blood circulation when hepatocytes are injured (Huang *et al*., 2006).

The degree of severity of hepatotoxicity could be evaluated based on WHO Toxicity Classification Standards (Hassen et al., 2013). Mild hepatotoxicity is defined as AST and/or ALT elevations of < 3 fold the Upper Limit Unit <121 IU/L , moderate hepatotoxicity as elevations of 3–5 fold the upper limit unit 121–200 IU/L , severe hepatotoxicity as elevations 5–10 times the upper limit unit 201–400 IU/L (Hassen et al., 2013).

According to, WHO recommendation, pyrazinamide from the FLDs is included in MDR-TB treatment (Frazon *et al*., 2011) and was proved to be hepatotoxic (Younossian *et al*., 2005). From a clinical trial reported by Shih *et al*., 2013 hepatotoxicity can be induced by oral administration of pyrazinamide or pyrazinoic acid at a dose of 500 mg/kg/day over 7 weeks in rats and this finding can be extrapolated to human being. The current minimum recommended dose of parazinamide for MDR-TB patients ≥33 is 1000mg/kg/day which is twice of the dose taught to induce hepatotoxicity (Kassa *et al*., 2012). However,the mechanism of hepatotoxicity is not yet clear (Shih *et al*.,2013). A review by Pandit *et al*., 2012, indicated that pyrazinamide can inhibits the activity of several cytochromeP450 (CYP450) isoenzymes such as CYP2B, CYP2C, CYP2E1, and CYP3A that involve in the detoxification process of pyrazinoic acid and 5-hydroxypyrazinoic acid. Ethionamide, Protonamide and para aminosaselic acid also have hepatotoxic effect, although less severe than pyrazinamide (Hsu *et al*., 2010). Metabolism of pyrazinamide in liver to pyrazinoic avcid and 5-hydroxypyrazinoic acid (Figure 1.1).

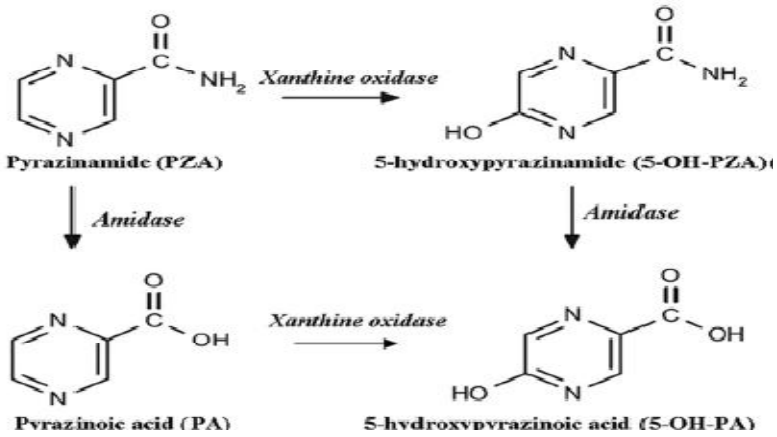


Figure 1.1. *Metabolic pathway of pyrazinamide (Shih et al.,2013)*

Previously reported rates of hepatitis associated with SLDs treatment in patients with MDR-TB have varied considerably ranging from 1.7% to 16.8% (Hsu *et al*., 2010). In one study conducted by Papastavros *et al*., 2002 to assess adverse events associated with pyrazinamide and levofloxacin in the treatment of latent multidrug-resistant tuberculosis in toronto,canada 47%

hepatotoxicity was reported. In a retrospective study conducted by Masjedi, et al., 2008, 9.3%; Dziusmikeyeva,et al., 2011, 4%; shin *et al*., 2007, 16%; Baghaei, 2011, 5%;Keshavjee, 2012, 16.5%, were some of drug induced hepatotoxicity reported.In addition, From prospective cohort study, 15.9% hepatotoxicity was reported in Taiwan (Hsu *et al* 2010) . Co-infection with HIV or/ and concurrent treatment with ART increase the risk of developing drug-induced hepatotoxicity (Lomtadze *et al*., 2013).

1.8.4. Electrolyte disturbance

Electrolyte disturbance is one of the most challenging adverse reactions related to MDR-TB management, in particular because of the paucity of presenting symptoms and potential morbidity associated with this disorder (Shin *et al*., 2004). Drug induced disturbance of electrolytes are common in patients receiving MDR-TB treatment (Jenh *et al*., 2011). Common causes are Vomiting and diarrhea due to the anti-TB drugs induced gastro intestinal upset and the injectants (capreomycin and aminoglycosides) induced nephrotoxicity (Isaakidi *et al*., 2012). This syndrome is more common and severe in HIV co-infection treated with TDF (Dheda *et al*., 2010).

Drug induced hypokalemia and hypomagnesemia defined as a serum potassium level of < 3.5 mEq/L(Huang & Kuo 2007) and serum magnesium concentration of less than 1.3mEq/L respectively are among the most frequently encountered fluid and electrolyte abnormalities in clinical medicine (Kassa *et al*.,2013). In healthy individuals the concentration of potassium and magnesium in blood falls within a very narrow interval ranging from 3.5 -5.5 mEq /L (Shin *et al*., 2004) and 1.3 - 2.2 mEq/L (AHA,2000) respectively. Patients with mild hypokalemia (serum potassium 3.1-3.5mEq/L) are asymptomatic, while mild hypokalemia (Serum potassium level from 2.6-3.0mEq/L) may characterized by constipation and weakness (Kassa *et al*., 2013). Severe hypokalemia defined as serum potassium less than 2.5mEq/L or symptomatic hypokalemia may cause an ascending muscle paralysis and subsequent respiratory failure (AHA, 2000).

Hypocalcemia is also the commonly encounter electrolyte abnormality in MDR-TB patients. Symptoms of hypocalcemia usually occur when ionized calcium fall below 2.5 mg/dL(Miller & Graham,2006). Severe hypomagnesemia (serum magnesium level lower than 1mEq/L) is also

associated with hypocalcaemia, defined as an ionized serum calcium concentration below the normal range of 4.2 - 4.8 mg/dL(Miller & Graham, 2006).

Aminoglycosides and capreomycin are thought to produce hypokalemia, hypomagnesemia, and hypocalcemia by causing necrosis of the proximal renal tubules (Shin *et al*.,2007). Electrolyte abnormalities have been associated with high cumulative doses of these drugs, and occur at a frequency of about 4.5% for aminoglycosides (Jenh *et al*., 2011) and between 4-15% of patients on prolonged capreomycin (Shin *et al*.,2004). In a cohort of 115 adults with MDR-TB, 34.8% had an electrolyte abnormality during the course of treatment, with 31.3% having hypokalemia, 15.7% hypomagnesemia, and 12.2% both (Shin *et al*., 2004). In other study conducted by Holmes *et al*., 1970, to asses the effect of capreomaycin, 68.2% have had hypokalemia. Hypokalemia was also noted in 33.2% of an adult MDR-TB cohort in Russia,Tomsk resulting in the discontinuation of capreomycin in 7.4% of patients (Shin *et al*., 2007). Moreover, in a cohort of XDR-TB patients in South Africa, one person was died due to the presumed capreomycin-associated hypokalemia (Shean *et al*., 2013).

1.9. Management of adverse drug reactions

The first principle in the management of MDR-TB is preventing its emergence (Nathanson *et al*.,2005).National policies and practices differ in the model of care utilized for MDR-TB management (Dias *et al*., 2012). WHO recommend ambulatory care rather than models of care based principally on hospitalization in patients with MDR-TB treatment (Falzon *et al*., 2011). In Africa model of care based on hospitalization ranges from 10% to 100%, the lowest in Democratic Republic of Congo and the highest in Ethiopia and Nigeria respectively in 2012 (Baddeleyt *et al*., 2013). However, currently Ethiopia has implemented both hospitalization and ambulatory model of care (Kassa *et al*.,2013).

It has been repeatedly mentioned that adverse events associated with SLDs become an obstacle in MDR-TB management (Mitnick *et al*., 2003) . It negatively affects therapy adherence, decrease treatment success rates and increase treatment failure, relapse or drug resistance (Deun & Caminero 2013). For instance, data on adverse events collected from five DOTS-Plus sites in Estonia, Latvia, Peru (Lima), the Philippines (Manila) and the Russian Federation (Tomsk ,Oblast) (Table l.4) indicated that patients who required drug removal from the regimen due to

ADRs were high, ranging from 24.2% - 49.4% (Nathanson *et al*., 2005). Thus, it is important to routinely monitor the occurrence of ADRs in MDR-TB patients' during treatment (Chang & Yew, 2013). Otherwise, drugs may be stopped unnecessarily or treatment may be terminated prematurely by inexperienced health workers and also patients may refuse to continue treatment if discomfort caused by adverse ADRs is not properly managed resulting in a high proportion of failure and further development of unnecessary resistant strains (Dziusmikeyeva *et al*.,2011).

Table 1.4.*The effect of adverse drug reaction on Treatment continuity in patients enrolled on MDR-TB treatment collected from five countries (Nathanson et al., 2005).*

	Estonia n(%)	Lativia n (%0	Peru (lima) n (%)	Philippnes(Manila) n (%)	Russia(To msk, blast) n (%)
Patients enrolled in MDR-TB treatment	136 16.6)	367 (44.9))	73 (8.9)	85 (10.4)	157(19.2)
Patients who stopped treatment due to adverse reaction	4 (2.9)	6 (1.6)	0 (0)	7 (8.2)	0 (0)
Patients who required drug removal from the regimen due to adverse reactions	58 (42.6)	89 (24.2)	25 (34.2)	42 (49.4)	31 (19.7)

Regular laboratory screening is important for occult adverse effects using serum indicators such as creatinine, potassium, TSH and AST/ALT (Chiang,2013). Laboratory tests have been scheduled according to WHO and National Tuberculosis Control Program recommendation (Table 1.5) (Kassa *et al*.,2012).

Table 1.5. *Laboratory follow-up Schedule during MDR-TB treatment in Ethiopia (Kassa et al., 2013)*

Parameter	Baseline	Intensive phase	Continuous phase
Serum creatinine and or BUN	√	monthly	If clinically indicated
Serum potassium and associated electrolytes	√	monthly	If clinically indicated
Thyroid stimulating hormone(TSH)	√	3rd and 6th	Every 6 month
Aminotransfarase(ALT /AST	√		If clinically indicated

1.10. Potential risk factors

1.10.1. Challenges of ART/anti-TB concomitant use

Antiretroviral therapy (ART) has revolutionized the care of patients with HIV infection (Lawn *et al*.,2013). ART is a combination of at least three drugs (Dlodlo *et al*., 2013). The use of two- and three drug regimens that include agents to inhibit the HIV enzymes, reverse transcriptase and protease, can markedly suppress HIV replication (Tweya *et al*., 2014) resulting in unprecedented declines in the rates of death and opportunistic illnesses among patients with AIDS (Reust, 2011).

For a reason mentioned above, the 2011 update WHO guidelines for the programmatic management of MDR-TB strongly recommended MDR-TB patients who are already on ART should continue it and these who are not, should start ART for all MDR-TB/HIV co-infected patients requiring second-line anti-TB drugs, irrespective of CD4 cell count, as early as possible (within the first 8 weeks) following initiation of anti-TB treatment (Dlodlo *et al*., 2013). However, concomitant anti-TB / ART drugs use might be complicated by adherence challenge of overlapping toxicity (Table 1.6) (Dlodlo *et al*., 2013).

Table 1.6 *Potential overlapping toxicity from ART and anti-TB medicines (Dlodlo et al., 2013)*

Potential toxicity	Antiretroviral therapy	Anti tuberculosis therapy
Hepatitis	Nevirapine,Efavirenz ritonavirboosted -protease inhibitors, etravirine, maraviroc	Pyrazinamide, isoniazid, rifampicin/ rifabutin,p-aminosalicylicacid, ethionamide/prothionamide,fluoroquinolones
Gastrointestinal intolerance	Zidovudine, protease inhibitors, didanosine	Ethionamide/prothionamide,p-minosalicylicacid, pyrazinamide, isoniazid, rifampicin, ethambutol, clofazimine
Renal toxicity	Tenofovir, indinavir	Capreomycin, Streptomycin, kanamycin, amikacin,viomycin, rifampicin Linezolid

In addition, the MDR-TB/HIV co infected patients take a number of drugs. As a result they may be subjected to a high Pill burden (Table 1.7) and dug-drug interactions (Dlodlo *et al.*, 2013).

Table1.7.A typical MDR-TB/HIV co-infected patient > 60 kg often has a large pill burden (Kassa et al., 2012)

Morning dose	**Evening dose**
Pyrazinamide (500mg): 4 tablets	Ethionamide (250 mg): 2 tablets
capreomycin (1-gram vial): 1 g IM	Cycloserine (250 mg): 2 capsules
Levofloxacin (500 mg): 2 tablets	
Ethionamide (250 mg): 1 tablet	Pyridoxine (50 mg): 4 tablets
AZT/3TC combination: 1 tablet	AZT/3TC combination: 1 tablet EFV (600 mg): 1 tablet
Cotrimoxazole: 1 tablet	

Modified from the guideline for programmatic management of MDR-TB in Ethiopia

1.10.1.1. ART treatment regimen

According to the current WHO, recommendation the first-line ART regimen should contain two nucleoside reverse transcriptase inhibitors plus one non-nucleoside reverse transcriptase inhibitor (Habtewold, 2013). In many countries the preferred nucleoside reverse transcriptase inhibitors backbone is zidovudine (AZT) or TDF, combined with either lamivudine (3TC) or emtricitabine (Tweya *et al*., 2014). Nucleoside reverse transcriptase inhibitors remain the backbone of ART during TB/HIV co-treatment due to their safety, efficacy and favorable drug-drug interaction profiles (Dooley *et al*., 2008). Despite, definitive recommendation cannot be made in patients co-infected with MDR-TB; TDF is generally avoided in the intensive phase due to the possibility of overlapping renal toxicity with capreomycin (Kenyon *et al*., 2011). In recently published systematic review and meta-analysis by Kenyon *et al*., 2011, acute renal failure in patients on TDF compared to AZT and stavudine was a statistically significant with 0.7 times higher renal toxicity. TDF is therefore reserved for cases with ART drug resistance or if AZT and stavuidine

are not appropriate due to hematological toxicity and peripheral neuropathy they respectively cause. AZT should not be started in patients with a hemoglobin value less than 7 g/dL. Nevirapine is generally avoided due to the risk of hepatotoxicity when used concurrently with pyrazinamide. Efavirenz (EFV) is the preferred drug in the setting of MDR-TB treatment with pyrazinamide containing regimens (Isaakidis *et al*., 2012). Therefore, the most commonly used ART regimen for MDR-TB patients infected with HIV is AZT + 3TC + EFV (Dooley *et al*., 2008).

1.10.2. Mechanism of antiretroviral drugs induced liver toxicity

1.10.2.1. Direct toxicity

Highly active antiretroviral therapy can induce direct toxicity in the liver (Pandit *et al*.,2012). Drugs metabolized in the liver through the cytochrome pathways may cause liver toxicity when there are polymorphisms in the enzymes (Bissell *et al.,* 2001). Since many of the ART drugs are metabolized in the liver through the cytochrome pathways, idiosyncratic polymorphisms of the enzymatic complexes might lead to significant heterogeneity in drug metabolism, predisposing to the development of hepatotoxicity in certain individuals (Pandit *et al*.,2012). Drugs that induce or inhibit CYP450 enzymes may decrease or increase, respectively, concentrations of concurrently administered drugs that are CYP450 substrates. Changes in drug concentrations resulting from drug interactions can lead to treatment failure or toxicities (Dooley *et al* 2008).for instance, EFV is largely metabolized by CYP2B6 and, to a lesser extent, by CYP3A4 (Habtewolde 2013). EFV is a moderate inducer of CYP3A4; however, it can act as an inhibitor, leading to increased concentrations of some drugs (Dooley *et al*., 2008). Some drugs may potentiate the activation of death receptors and/or intracellular stress pathways (Leist *et al.,* 1998).

1.10.2.2. Mitochondrial toxicity

It is infrequent but a distinctive type of hepatotoxicity that may evolve to acute liver failure (Pandit *et al*.,2012). The main feature of the hepatic lesion is the accumulation of micro vesicular steatosis in liver cells and mitochondrial depletion (Chitturi & George,2002). This early lesion may evolve to macro vesicular steatosis with focal necrosis, fibrosis, cholestasis, proliferation of

biliary ducts. Of interest, the underlying liver disease does not predispose to this type of lesion (Bissell *et al.*, 2001).

1.10.3. Age and gender

Female has been shown to be a risk factor for clinically relevant ADRs with a 1.5 to 1.7-fold greater risk of developing ADRs compared to male patients (Carrasco-Portugal & Flores-Murrieta,2011). There are clear physical difference between men and women which can modify pharmacokinetic and pharmacodynamics activity (Ciccon & Holdcroft,1999). However, in the tradition of MDR-TB treatment Dosage regimen are given usually without normalizing by the body weight, having as a consequence higher doses in women in comparison with men (Table1.8) (Carrasco-Portugal & Flores-Murrieta, 2011). In addition, glomerular filtration rate in female is slower than male (Carrasco-Portugal & Flores-Murrieta, 2011).

Table1.8. Weight based dosing of injectable drugs during MDR-TB treament (Kassa et al., 2012)

Drugs	<33Kg	33-50Kg	50-70Kg	>70Kg
Streptomycin(1gram vial)	15-20mg/Kg	500-750mg	1000mg	1000mg
Kanamycin(1 gram vial)	15-20mg/Kg	500-750mg	1000mg	1000mg
Amikacin(1 gram vial)	15-20mg/Kg	500-750mg	1000mg	1000mg
Capreomycin(1 gram vial	15-20mg/Kg	500-750mg	1000mg	1000mg

.

In previous reports, the occurrence of any major ADRs has been associated with age; especially amongst the elderly (Yee *et al.*,2003; Ormerod & Horsfield,1996).Vulnerability to ADRs are more probable in hepatotoxic patients due to a significant reduction in clearance rate of metabolized drug agents by the cytochrome P450 enzyme, changes in the hepatic blood flow

distribution, as well as other factors affecting liver function (Forget & Menzies 2006; Tostmann *et al*., 2008). One study reported drug induced hepatotoxicity rate in TB patients from 2 to 8% as age increased, with an average of 5% (Saukkonen *et al*., 2006). Babalık *et al* 2012, also stated that the development of hepatotoxicity was more common in patients > 40 years old than ≤ 40. In addition, in a study carried out by Delgad *et al*., 2011, in Lima, Peru to asseses risk factors associated with Anti-TB Medication adverse Effects by grouping age<39 and ≥40, age was found statistically significant .The risk of ADR occurrence was 3.93 higher in patients greater or equal to 40. Conversly , in a Nested Case-Control Study conducted by Hassen *et al* 2013 at Jimma University Hospital in TB/HIV co-infected patient between age groups younger (<35 years) and older (>35 years) they found nothing difference in the incidence of anti-TB drugs induced hepatotoxicity between cases and controls.

1.11. Statement of the problem

Nowadays, DR-TB like MDR-TB and XDR-TB has been threatening the global TB control and is a major public health concern in several countries (Kapadia & Tripathi,2014). Globally, in 2012 it was estimated that there were approximately half a million MDR-TB, defined as resistance to isoniazid and rifampicin with or without resistance to other anti-TB drugs (Carroll *et al*., 2012).This resistant developed has threaten DOTs, WHO recommended strategy for TB control (Delgado *et al*., 2011). For that matter, treatment designs of DR-TB such as MDR-TB involves the use of multiple medications for long duration; as a result most patients will experience some difficulty in tolerating these (Chang & Yew,2013).

It was very common phenomenon to come across patients become unwilling to continue their treatment duration (Karagöz *et al*., 2009) and/or physician withdraw the responsible drugs due to adverse effects (Nathanson *et al*.,2005). For instance, only 48% of the MDR-TB patients detected in 2010 cohort were reported successfully treated globally, reflecting high mortality rates and loss to follow-up (Kassa *et al*.,2031). Non-adherence to treatment partly due to ADRs is among the commonly cited problem in TB management, especially in MDR-TB (Amuha *et al*., 2009). Failure to achieve patient adherence to the recommended treatment imposed by ADRs, not only lower the cure rates ,raise both transmission and mortality rates, but also leads to development of MDR-TB resistant strains of MTB to SLDs (Ginsberg,2008). Recently due

to poor case management, often because of non-adherence to treatment, resurgence of TB and the appearance of multiple drug resistance like XDR-TB is common (Ansari *et al*., 2013). The proportion of MDR-TB resistance to both fluoroquinolones and/or second-line injectable agents, the two most bactericidal SLDs has been increasing (Kassa *et al*., 2013). By 2012 approximately, 30080 MDR-TB patients have had resistance to a fluoroquinolone, second-line injectants agent, or both (Baddeley *et al*., 2013).Therefore, to eliminate the above problems by early detection and monitoring of drug induced adverse events in MDR-TB patients under SLDs it is important first to know the magnitude and characteristics of these ADRs.

Albeit, ADRs during MDR-TB treatment is eminent, to the level of our knowledge there was no work previously published concerning this title in PubMed or in the Ethiopian medical journal? Therefore, aim of this study is to investigate the characteristics and magnitude of ADRs among MDR-TB patients in Mekelle Hospital, MDR-TB center.

1.12. Hypothesis

In MDR-TB patients, drug induced electrolyte disturbance, nephrotoxicity, hypothyroidism and hepatotoxicity are among the commonly encountered problems during treatment with second line drugs.

2. OBJECTIVES

2.1. General objective

The aim of this study was to investigate the prevalence of adverse drug reactions among MDR-TB patients with second line drugs in Mekelle Hospital MDR-TB center

2.2. Specific objectives

To determine magnitude and characteristics of adverse drug reactions among the study subjects

To describe the possible influences of risk factors such as age, gender, ant-TB /ART concomitant use on the occurrence of adverse drug reactions

3. MATERIALS AND METHODS

3.1. Study area and period

This study was carried out from August 2013 to April 2014 by Department of Biochemistry, Addis Ababa University in Mekelle Hospital, MDR-TB center. The center was established since March 2013 with purpose of MDR-TB patients cure and care in a physically isolated ward. It has 12 staffs with one specialist and one medical director.

3.2. Study participants

68 MDR-TB confirmed by Tigray Regional Laboratory were followed and evaluated for ADRs development during the study period.They were both males and females with normal base line electrolyte (K^+) LFT, thyroid function test (TFT), RFT before the treatment is initiated.

3.3. Inclusion and exclusion criteria

3.3.1. Inclusion criteria

- ✓ Confirmed pulmonary MDR-TB patients
- ✓ Patients having normal base line serum electrolyte (K+), LFT, TFT and RFT test result
- ✓ No previously liver / renal insufficiency
- ✓ Age ≥18

3.3.2. Exclusion criteria

- ✓ Patient with extra-pulmonary TB
- ✓ Patients having previously liver / renal insufficiency
- ✓ Age<18 and patients with abnormal base line serum potassium(K+),LFT,RFT,TFT test results were excluded

3.4. Study design

This was a prospective, observational cohort study, using data routinely collected in Ayder educational and referral hospital in collaboration with Mekelle Hospital, Mekelle, Ethiopia

3.5. Sample size determination

Sample size for serum collection was determined using the formula given by (Makita, 2009) as follows. $\mathbf{n = \frac{(z^*)^2 \times P(1-P)}{d^2}}$

n= the required sample size; P=expected prevalence; d= desired absolute precision; z*= the 90% critical value=1.456

Accordingly, using expected prevalence of 50% at 90% confidence interval and 10% desired absolute precision a sample size of 68 subjects were used. However, for statistical analyses the conventional and widely accepted significance level of p=0.05 was used.

3.6. Ethical clearance

Ethically it was approved by Research and Ethics Review Committee (RERCD) of Department of Biochemistry, School of Medicine, and Addis Ababa University with hosting permission from Mekelle Hospital. Since there was no direct contact with patients informed consent was not required.

3.7. Blood sample collection, handling and storage

Fasting venous blood samples were obtained by vein puncture from theante-cubital vein using syringes and needles, after initial sterilization of the cubital fossa with a cotton wool soaked in 70% alcohol. A 5ml blood was collected from each subjects at the begging before treatment was initiated and then monthly along the follow up periods and transferred into clean and dry tubes, then allowed for proper retraction and clotting for 15 minutes and centrifuged at 3000 rpm for 10 minutes. The serum was separated into clean and dry specimen bottles using clean Pasteur pipette for each specimen. Electrolytes were immediately tested while the rest frozen at -20 ^{0}c until they were analyzed.

3.8. Data collection

Demographic and clinical information were systematically recorded in standardized clinical files designed specifically for the program and extracted from it using a questionnaire. Routine

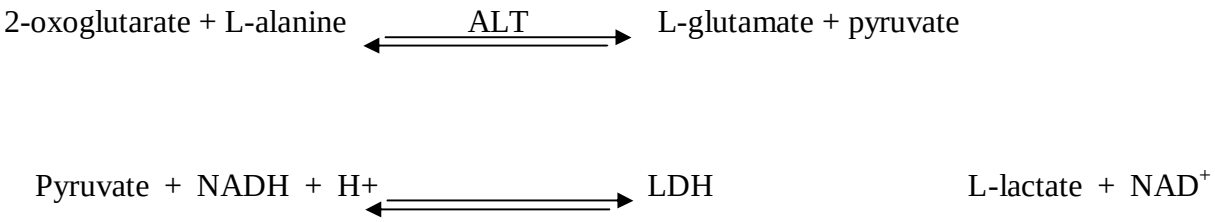
laboratory test were done in Ayder Hospital in collaboration with Mekelle Hospital according to the ADRs monitoring schedule. Information on HIV and antiretroviral therapy was collected in the same patient file. Each patient had a unique identification code. Trained personnel regularly recorded clinical, treatment, and laboratory data for individual patient.

3.9. Biochemical assays

3.9.1. Estimation of ALT and AST

Principles: Alanine aminotransferase (ALT) and AST activity in Serum specimen was estimated colorimetrically by modified IFCC kinetic method (Reitman and Frankel, 1957) using available commercial reagent kit, supplied by Roche, diagnostic laboratory, German.

Alanine aminotransferase (ALT) is measured by the reagent rate analysis by the coupled reaction with lactate dehydrogenase (LDH) to reduce NADH to NAD^+ at a wavelength of 340nm. The rate of decrease in absorbance at 340nm due to NADH depletion is proportional to the ALT activity in the sample.



Aspartate aminotransferase catalyzes the transamination of L-aspartate to 2-oxoglutarate forming L-glutamate and Oxaloacetate. The oxaloacetate formed is reduced to malate by malate dehydrogenase (MDH) with simultaneous oxidation of reduced NADH to NAD^+. The change in absorbance with time is due to the conversion of NADH to NAD which is directly proportional to AST activity. Measurement taken by spectrophotometer at 340nm using continuous kinetic produced (Reitman and Frankel, 1957).

$$\text{2-Oxoglutarate} + \text{L- aspartate} \xrightleftharpoons{\text{AST}} \text{L-glutamate} + \text{Oxaloacetate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightleftharpoons{\text{MDH}} \text{L-malate} + NAD^+$$

Procedure for ALT/AST: 1000µL of the working reagent was added into clean labeled test tubes (test and blank) and the tubes were incubated for approximately 5 minutes at 370c. Then 100µL of sample was added to the sample tubes, mixed and absorbance was read at 340nm. The initial absorbance was read against air after 1 minute and a timer was started and absorbance read again after exactly 1, 2 and 3 minutes against reagent blank and the activity was calculated

3.9.2. Creatinine Estimation

Principle: Creatinine and non-creatinine chromagen in the sample react with picric acid in alkaline media to form an orange-red colored complex solution (Bartels *et al*., 1971). During the first step (approximately 30sec), mostly the rapid non-creatinine chromagen react whereas during the following phase (approximate 2min) mainly true creatinine reacts, and finally the slow non-creatinine chromagen (Kotask *et al*., 2008). The change in absorption during the second step is measured at 492nm. The absorbance of this complex is proportional to the creatinine concentration in the sample (Bowers & Edward 1980).

$$\text{Creatinine} + \text{Picric acid} \xrightarrow{\text{NaOH}} \text{Creatinine-picrate complex}$$

Procedure: Creatinine activity in Serum specimen was estimated colorimetrically by the modified kinetic method using the available reagent kit, supplied by Roche diagnostic laboratory (Bartels *et al*., 1971; Bowers & Edward 1980]. 100µl sample and 1000µl working reagent was added, mixed and the stop watch was started. After 30 seconds the absorbance was read at 492nm.Then again the second absorbance was read at exactly 2min.

3.9.3. TSH estimation

TSH activity in Serum specimen was assayed using an automated VIDAS- instrument Mini immunoassay analyzer with principle of Enzyme Linked Fluorescence Assay (Beck,1986) using available commercial reagent kit, supplied by bio Mérieux, Inc.

Principle: The assay principle combines a one-step enzyme immunoassay sandwich method with the final fluorescent detection.

The solid phase receptacle serves as the solid phase as well as the pipetting device for the assay. Reagent for the assay is ready to use and pre-dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is recycled in and out of solid phase receptacle several times.

The sample is transferred into the well anti-TSH anti-body labeled with alkaline phosphatase (conjugate).The sample/conjugate mixture is cycled in and out of solid phase receptacle. The antigen binds to the antibody coated on the solid phase receptacle and to the conjugate forming a sandwich. Unbounded components are eliminated during the washing steps.

During the final detection step, the substrate (4-methyl-umbelliferyl phosphate) is cycle in and out of the solid phase receptacle. The conjugate enzyme catalyzes the hydrolysis of this substrate in to a fluorescent product (4-methylumbelliferone) the fluorescent of which is measured at 450nm. The intensity of the fluorescence is proportional to the antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory and then printed out

Procedure: The required reagents were first removed from the refrigerator and allowed to come to room temperature for at least 30 minutes. Then, one TSH strip and one TSH solid phase receptacle was used for each sample, control and calibrator.200µL of control, sera and calibrator was added into the well containing anti-TSH antibody conjugated with alkaline phosphatase and mixed using a vortex type mixer. The TSH strip and TSH solid phase receptacle were inserted in to the instrument. Finally; 300µl 4-Methylumbelliferyl phosphate was added and measured at 450nm. After approximately 40 minutes the strip and solid phase receptacle was removed from the instrument.

3.9.4. Electrolyte estimation

Serum potassium and ionized calcium was estimated using AVL 9180 Electrolyte Analyzer based on the ion selective electrode measurement principle to precisely determine the measurement value (Bishop *et al*., 1992).

Principle: There are six different electrodes used in the AVL 9181 Electrolyte Analyzer: sodium, potassium, chloride, ionized calcium, lithium and a reference electrode. Each electrode has an ion-selective membrane that undergoes a specific reaction with the corresponding ions contained in the sample being analyzed. The membrane is an ion exchanger, reacting to the electrical charge of the ion causing a change in the membrane potential, or measuring voltage, which is built up in the film between the sample and the membrane.

A galvanic measuring chain within the electrode determines the difference between the two potential values on either side of the membrane. The galvanic chain is closed through the sample on one side by the reference electrode, reference electrolyte and the "open terminal". The membrane, inner electrolyte and inner electrode close the other side. A difference in ion concentrations between the inner electrolyte and the sample causes an electrochemical potential to form across the membrane of the active electrode. The potential is conducted by a highly conductive, inner electrode to an amplifier. The reference electrode is connected to ground as well as to the amplifier.

The ion concentration in the sample is then determined by using a calibration curve determined by measured points of standard solutions with precisely known ion concentrations.

Principle for magnesium: serum magnesium was determined using ARCHITECT clinical chemistry analyzer (Burtis & Ashwood,1994) in international clinical laboratory. This method utilizes an arsenazo dye which binds preferentially with magnesium. The absorbance of the arsenazo-magnesium complex is measured at 572 nm and is proportional to the concentration of magnesium present in the sample. Calcium interference is prevented by incorporation of a calcium chelating agent.

Procedures: using the Automated Dilution Protocol, specimen dilution was made and its concentration was automatically corrected by multiplying the appropriate dilution factor. The 95µL and 2.4µLrespectively for potassium / calcium and magnesium was used. Finally results were calculated according the calibration curve.

3.10. Data Analysis

Data collected during the study period were stored in the Microsoft Excel spread sheet program and analyzed using SPSS 20.0 (Statistical Package for Social Sciences) version soft ware program. The total adverse drug reaction was calculated by dividing the number of test positive (patients with at least one adverse drug reaction of any type) by the total number

MDR-TB patients subjected in study and the frequency of ADR per person was counted and categorized. Chi square test was utilized to measure the association between the occurrence of adverse events and risk factors such as gender, age and concomitant use of anti-TB/ ART drugs. A p-value of less than 0.05 was considered for statistically significant difference.

4. RESULTS

4.1. Demographic and clinical characteristics

Table 4.1.Demographic and clinical characteristics of the study participants

Characteristics	**n (%)**
Gender	68(100)
male	46/68(67.65)
female	22/68(32.35)
Age	32.62 ±11.173
≥30	40.53 ± 8.385
<30	22.60 ± 3.597
Registration group	
New	1/68(1.47)
Retreatment	66/68(97.1)
unknown	1/68(1.47%)
Diabetes co-infection	2/68(2.94)
male	1/68(1.47)
Female	1/68(1.47)
HIV co-infection	18/68(18.37)
male	9/18(50)
female	9/18(50)
Proportion of HIV positive persons on ART	15/18(83.33)
TDF/3TC/EFV	13/15(86.67)
AZT/3TC/EFV	2/15(13.33)

4.2. Laboratory ADRS evaluation test results

The 68 MDR-TB patients, 18 of which were HIV co-infected were followed for six months. Laboratory test evaluations were done according to the ADRs evaluation schedule in Ethiopia. Every patient was checked whether if they have had abnormality of the liver, thyroid gland, renal system and system electrolytes using serum indicators and joined to the MDR-TB cure and care ward. Then, blood sample was taken monthly to check for any drug induced adverse reactions.”0” in the tables indicates laboratory test results made before the treatment was initiated and 1,2,3,4,6 represents for the first, second, third , four and six month

laboratory evaluation test results. After treatment was initiated Serum ALT/AST,creatinine and electrolytes monitoring was made monthly for four months while TSH test was done at the third and six month and the following results were found.

Table 4.2 ALT/AST,creatinine and TSH laboratory evaluation test results in MDR-TB/HIV co-infected patients

			ALT/AST(IU/L)					Creatinine(mg/dL)					TSH(mIU/L)		
NO.	CODE	ART	0	1	2	3	4	0	1	2	3	4	0	3	6
1	227229	NO	16/30	23/25	28.5/32	19/30	15/23	0.5	4.1	10	2.4	1.7	0.99	2.98	1.98
2	233204	Yes	15/20	19/32	123/157	176 /198	18.67/34	0.7	0.7	0.8	0.5	0.8	0.9	16.7	7.35
3	236787	No	12/40	10/22	25/34	16.3/19	21/27	0.8	0.8	0.9	0.6	0.76	1.32	1.98	2.34
4	223819	Yes	17.2/43	19/23	18/34	208.3/245	165/209.5	0.7	0.7	1.5	2.4	1.2	5.54	6.7	17.5
5	211319	Yes	17/31	20/21	17/24	15/28	10/30	0.9	0.8	0.8	0.6	0.9	2.3	1.34	3.23
6	233011	No	23.4/39.9	45/51	345/389	265.18/312.4	65/76	0.9	0.9	0.9	0.8	0.6	0.99	2.6	5.7
7	220804	Yes	12/26	123/66	200/198	45/78	34/67	0.6	0.5	1.2	3.5	1.7	3.6	2.5	1.99
8	234338	Yes	22.47/22	19/32	26/24.9	31/32	20/32	0.8	0.6	0.9	0.8	0.7	1.55	2.6	1.99
9	130528	Yes	14/24	16.7/25	12/32	14.6/12	20/28	0.75	0.8	1.3	0.7	0.7	4.5	5.45	4.85
10	251857	Yes	23/43.6	66/54	299/378	27/68	25.9/43	0.76	0.6	0.8	1.4	2.3	5.55	8.9	13.3
11	171427	Yes	31/37.4	33/24	12/23	22/18.9	15.7/24	0.87	0.5	0.8	0.6	0.7	2.67	1.76	4.6
12	198301	Yes	15/31.5	23/34	26/23.6	21.6/19.8	30/35	0.55	0.6	0.6	0.7	0.8	0.99	0.78	1.54
13	208928	Yes	18/16	16.7/25	12/32	14.6/12	20/28	0.79	0.8	0.8	2.1	1.3	4.84	20.1	6.87
14	226865	Yes	23/13	15/12	23/28	14/17	15/23	0.9	0.8	0.8	0.8	0.9	1.99	1.76	3.56
15	156564	Yes	16/24	255/275	201/367	231/235	69/102	0.9	0.9	0.8	1.6	0.9	2.87	3.78	17.8
16	230619	Yes	30/22.4	23/22	25/34	19/32	25/34	0.76	0.7	0.5	0.5	0.6	4.73	2.56	5.43
17	230473	Yes	24.5/13	15/12	23/28	14/17	15/23	0.9	0.8	0.8	0.8	0.9	1.99	1.76	3.56
18	265065	Yes	16/17	16.7/12	23.6/29	14/11	10/23	0.9	0.8	0.8	1.7	2.0	1.99	1.76	3.56

Table 4.3. Electrolyte laboratory evaluation test results in MDR-TB/HIV co-infected patients

			Potassium (mEq/L)					Magnesium (mEq/L)				Calcium (mg/dL)			
NO.	CODE	ART	0	1	2	3	4	1	2	3	4	1	2	3	4
1	227229	N0	3.7	2.2	3.6	2.5	3.3	1.78	1.56	2.2	1.4	4.21	4.45	4.89	4.36
2	233204	yes	3.79	2.7	2.8	2.76	3.5	1.65	1.56	2.2	1.4	4.21	4.45	4.89	4.36
3	236787	N0	3.56	3.25	3.34	3.32	3.5	1.99	1.56	2.2	1.4	4.5	4.5	4.34	4.78
4	223819	yes	3.62	2.4	2.1	2.0	2.3	1.6	0.98	1.2	1.3	3.5	2.3	4.1	3.5
5	211319	yes	4.23	2.8	2.67	2.98	3.5	1.7	1.7	2.0	1.43	4.37	4.7	4.3	4.77
6	233011	No	4.1	3.3	3.3	4.5	3.98	0.8	1.05	1.2	0.98	4.6	4.4	4.3	4.67
7	220840	yes	4.49	4.5	4.32	4.7	4.65	1.34	1.56	2.2	1.4	4.21	4.45	4.89	4.36
8	234338	yes	3.72	2.0	2.4	4.3	4.25	0.8	1.0	1.1	1.0	4.2	4.6	4.6	4.32
9	130528	yes	4.32	2.2	3.53	3.6	3.56	1.7	1.8	1.4	1.5	4.3	4.7	4.23	4.34
10	251857	yes	4.1	2.2	2.1	2.0	2.22	1.07	0.6	0.8	1.4	2.9	3.5	3.9	4.3
11	171427	yes	4.5	2.2	2.43	2.34	2.5	1.3	1.8	2.1	2.0	4.3	4.2	4.4	4.4
12	198301	yes	4.52	3.4	2.56	2.7	2.76	1.56	1.89	2.06	1.79	3.5	2.3	4.1	3.5
13	208928	yes	3.9	2.2	2.3	4.8	3.7	1.5	1.3	1.6	2.0	4.5	4.5	4.34	4.78
14	226865	yes	3.87	2.1	2.07	2.5	3.5	0.89	1.1	1.2	1.1	3.5	2.3	4.1	4.8
15	156564	yes	3.54	2.3	2.2	2.3	3.6	1.56	1.89	2.06	1.79	4.5	4.6	4.34	4.44
16	230619	yes	4.45	2.4	2.1	2.3	2.3	0.8	1.2	0.9	0.7	4.5	4.5	4.34	4.21
17	230473	yes	3.8	2.4	3.5	4.7	4	1.56	1.8	2.0	2.1	4.5	4.6	4.6	4.71
18	265065	yes	4.1	2.4	4.23	4	4	1.56	1.8	2.0	2.1	3.5	2.3	4.1	3.5

Table 4.4. ALT/AST,creatinine and TSH laboratory evaluation test results in MDR-TB patients not co-infected with HIV

		ALT/AST(IU/L)					Creatinine(mg/dL)					TSH(mIU/L)		
NO.	CODE	0	1	2	3	4	0	1	2	3	4	0	3	6
1	198302	15/30	18.5/24	17/16	12.5/31	23/34	0.8	0.7	0.7	1.4	2.4	3.46	2.34	4.76
2	231954	20/30	145/145	131/178	87/89	65.7/58	0.8	0.7	0.7	0.8	0.7	1.97	2.67	5.6
3	225810	17.33/15.3	18.7/12.	15/32	21/24	19.7/34	0.9	0.8	1.5	2.3	1.6	2.54	3.7	4.98
4	223473	14/30	377/365	234/365	305/276	89/175	0.8	0.6	0.9	0.8	0.7	6.7	10.9	12.6
5	209222	22.7/20	10/22	25/34	19/32	25/34	0.8	0.9	1.6	3.5	4	7.6	10.9	14.6
6	234354	31.48/32.9	18.7/12	17/24	16/15	21/27	0.6	0.7	1.3	1.5	0.9	4.8	2.6	5.3
7	238319	21/26	20/21	17/23	15/23	23/30	0.7	0.8	4	1.9	2.1	3.7	6.73	12.5
8	231120	13/20	13/20	13/20	20/21	15/20	0.6	0.7	0.9	0.7	0.9	2.54	3.7	4.98
9	227676	20.6/50.83	15/12	23/28	14/17	15/23	0.9	0.8	2.5	1.9	0.9	1.99	1.76	3.56
10	213110	25/22	19/32	26/24.9	31/32	20/32	0.6	0.7	0.6	0.8	0.7	1.5	1.23	1.43
11	202832	18/31.7	13/20	15/12	18.5/24	17/16	0.8	0.8	1.2	1.4	3.0	0.94	3.5	0.87
12	227810	19.64/21.9	10/22	25/23	19/30	25/35	0.7	0.9	0.9	0.8	0.6	1.55	1.32	1.67
13	222401	20.32/13.7	20/30	21/26	13/20	17/31	0.6	0.7	0.9	0.9	0.8	5.67	19.8	11.6
14	232634	20.3/18.90	14.5/23	21.2/19	20/24	23/26.7	0.8	0.7	0.5	0.8	0.8	6.7	1.99	2.65
15	230435	16/23	16/18	12.5/14	20.1/23	15/13	0.5	0.8	0.8	0.7	0.8	0.99	0.57	1.73
16	214401	21.5/27.84	194/167	187/154	86/101	56/87	0.9	0.9	1.3	4.5	7	1.34	4.76	14.8
17	198298	22/30.2	16/19	12.5/17	16.1/23	10/13	0.5	0.8	0.6	0.7	0.7	0.99	0.57	2.76
18	198299	30/29	12.23	22/18.9	15.7/24	21/20.1	0.5	0.8	0.6	0.7	0.7	1.76	4.6	1.99
19	198300	33/24	23/26.7	21.2/19	20/24	14.5/23	0.5	0.8	0.6	0.7	0.7	6.5	3.7	4.53
20	215252	31/22	16/19	12.5/17	16.1/23	10/13	0.5	0.7	0.5	0.5	0.7	3.32	2.99	0.98
21	193596	12/13	13/20	15/12	18.5/24	17/16	0.8	0.6	0.8	0.8	1.4	4.6	4.00	1.68
22	205077	15.5/17.8	14.6/12.	17.9/23	20/34	21/28	0.9	0.9	1.3	2.5	1.5	2.7	3.5	2.54
23	215150	35/20	10/12	28/21	23.2/17.9	19.5/19	0.8	0.9	1.9	2.5	3.0	0.96	1.55	3.45
24	166779	24.9/43.7	196/145	150/68.5	102/69.3	89/67	0.9	0.9	1.7	1.4	1.1	5.55	4.99	20.7
25	198860	19/36	23/26.7	21.2/19	20/24	14.5/23	0.8	0.8	0.6	0.7	0.9	1.99	1.76	3.56
26	242917	10/20	15/24	20/23	34/36	18.9/21	0.76	0.6	1.2	2.1	2.6	3.65	0.89	2.84
27	119680	13/17	16.7/25	12/32	14.6/12	20/28	0.87	0.5	0.8	0.6	0.7	2.67	1.76	4.6
28	200543	14/32	15/22	25/28	18/17	15/20	0.99	0.8	0.7	0.9	0.9	1.22	1.67	3.22
29	200538	15.6/17.9	23/26.7	21.2/19	20/24	14.5/23	0.76	0.5	0.6	1.4	0.7	0.88	2.15	3.54
30	234363	16/32	347/543	298/198	69.6/107	55.9/74	0.99	0.8	5.6	4.2	0.8	4.6	4.76	4.09
31	204621	10.41/17.94	16.7/12	23.6/29	14/11	10/23	0.87	1.4	0.6	0.7	0.7	1.45	1.43	1.87
32	250099	12.5/32	13/20	13/20	20/21	15/20	0.6	0.7	0.9	0.7	0.9	2.54	3.7	4.98
33	214403	15/23	15/24	20/23	34/36	18.9/21	0.89	1.4	1.6	3.2	3.5	3.65	4.77	5.65
34	213950	15/21	33/24	12/23	22/18.9	15.7/24	0.87	0.5	0.8	0.6	0.7	2.67	1.76	4.6

35	212588	21.77/26.51	33/24	12/23	22/18.9	15.7/24	0.87	0.5	0.8	0.6	0.7	2.67	1.76	4.6
36	200007	10/29	12/13	11.8/23	21/24	16.5/12.8	0.87	0.5	0.8	0.6	0.7	2.54	11.6	6.87
37	206897	10/12	32/23	13/26	17/18	15/24	0.76	0.8	0.6	0.5	0.7	3.53	2.67	4.76
38	227874	12.54/20.64	16.3/17.5	14/23	21/28	23/19	0.55	0.6	0.6	0.7	0.7	1.89	2.69	3.96
39	253074	18/30	24/27	28.6/34	33/37	19.9/23.2	0.8	1.2	1.5	1.9	2.0	3.43	4.5	3.78
40	249245	12/16	23.7/29	17.6/34	21/27	12/16	0.9	0.9	1.3	3.0	1.2	4.56	7.8	18.5
40	220804	36/14	16/17	24/30	26/28	32/45	0.6	0.5	0.8	1.4	1.6	1.67	1.69	1.46
42	214194	18/34	15/23	17/26	27/35	19.8/23	0.9	0.9	0.6	0.8	0.9	2.45	2.80	3.12
43	206295	19/22	24/27	28.6/34	33/37	19.9/23.2	0.9	0.9	0.6	0.8	0.9	3.43	4.5	3.78
44	219286	12/22.2	11/21	23/35	19/29	17/24	0.87	0.6	0.8	4.0	3.6	2.87	12.6	10.9
45	219386	18.28.1	23.7/29	17.6/34	21/27	12/16	0.45	0.7	1.8	1.1	0.9	2.54	9.88	18.6
46	209542	18/20	16.3/17.5	14/23	21/28	23/19	0.55	0.6	0.6	0.7	0.7	1.89	2.69	3.96
47	218134	17/41	16.3/17.5	14/23	21/28	23/19	0.55	0.6	0.6	0.7	0.7	1.89	2.69	3.96
48	198297	20/15	15/23	17/26	27/35	19.8/23	0.9	0.9	0.6	0.8	0.9	2.45	2.80	3.12
49	213783	16/30	24/27	28.6/34	33/37	19.9/23.2	0.86	0.9	1.3	1.5	0.9	5.76	16.8	15.6
50	223206	35.5/22.7	25/92	23/28	14/17	15/23	0.9	0.8	0.8	0.8	0.9	1.99	1.76	3.56

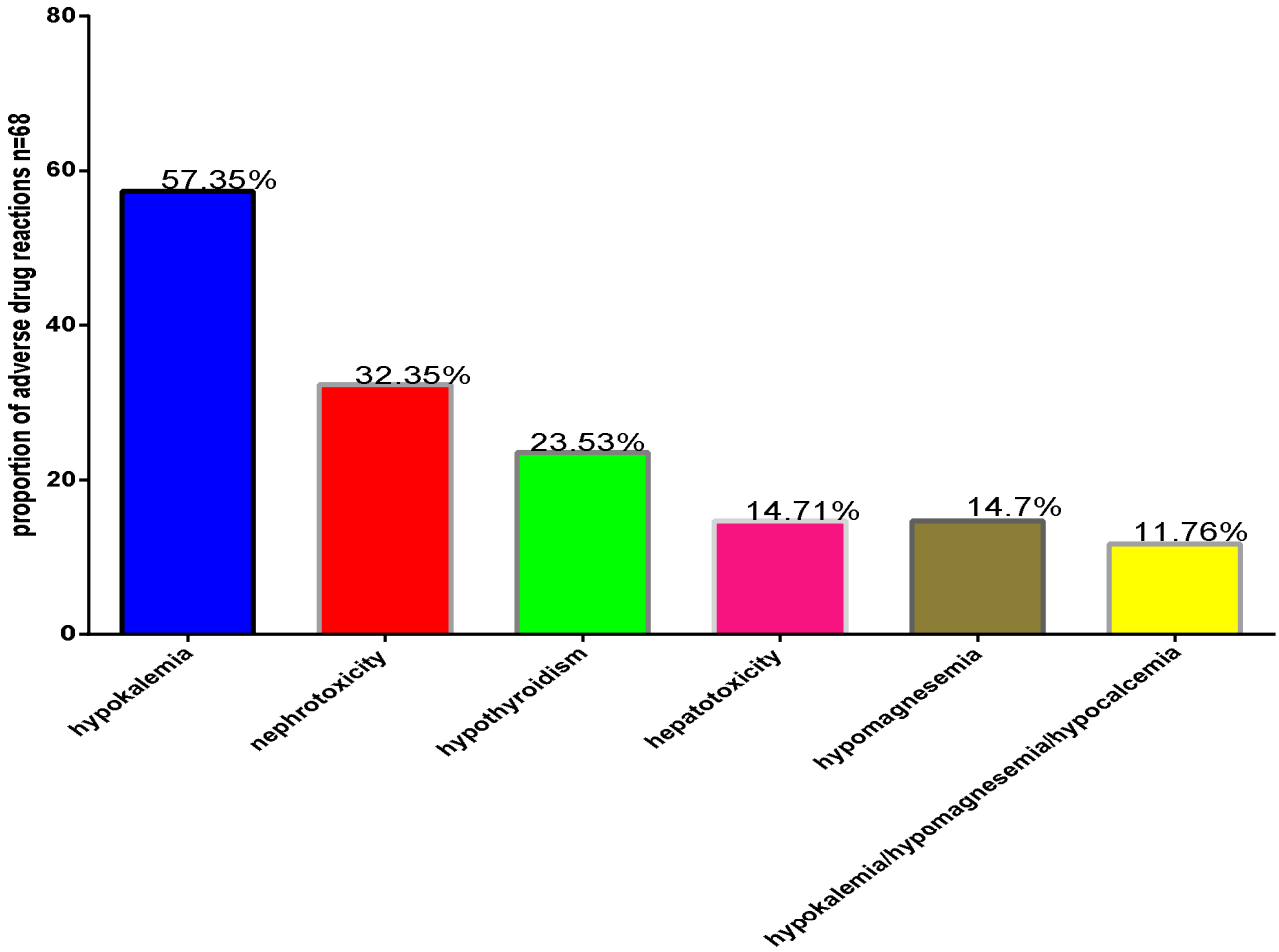
Table 4.5. Electrolyte laboratory evaluation test results in MDR-TB patients not co-infected with HIV

		Potassium (mEq/L)					Magnesium (mEq/L)				Calcium (mg/dL)			
NO.	CODE	0	1	2	3	4	1	2	3	4	1	2	3	4
1	198302	4.94	4.26	3.16	3.32	3.25	1.56	1.89	2.06	1.7	4.3	4.23	4.4	4.4
2	231954	3.49	2.91	2.98	3.09	2.78	0.98	0.99	1.2	1.1	4.8	4.56	4.6	4.5
3	225810	3.45	3.45	4.1	3.67	3.5	1.35	1.40	1.99	2.0	2.43	3.45	2.98	3.67
4	223473	4.1	3.91	3.7	3.32	3.32	1.35	1.40	1.99	2.0	3.23	2.67	4.06	4.01
5	209222	3.97	2.4	2.12	2.0	3.45	1.2	0.65	0.7	1.3	4.3	4.23	4.4	4.4
6	234354	3.58	2.72	2.57	3.01	3.56	0.98	0.99	1.2	1.1	3.5	4.09	2.99	4.11
7	238319	4.21	3.2	3.32	3.18	3.3	1.35	1.40	1.99	2.0	4.3	4.23	4.4	4.4
8	231120	3.45	3.45	3.23	3.43	3.5	1.35	1.40	1.99	2.0	4.3	4.23	4.4	4.4
9	227676	4.53	3.92	3.67	3.7	4.09	1.35	1.40	1.99	2.0	4.5	4.80	4.34	4.35
10	213110	4.5	3.7	3.98	3.65	3.67	1.56	1.89	2.06	1.7	4.34	4.7	4.8	4.9
12	202832	3.8	3.4	3.2	3.33	3.31	0.9	0.98	1.1	1.2	4.5	4.5	4.4	4.7
13	227810	3.84	3.54	3.43	4.6	4.7	1.67	1.98	2.09	1.6	4.26	4.8	4.67	4.65
14	222401	4.0	3.17	3.4	3.3	3.5	1.32	1.4	1.7	1.4	4.5	4.5	4.5	4.3
15	232634	4.5	4.0	5.0	4.5	3.98	1.36	1.7	2.1	2.0	4.3	4.23	4.4	4.4
16	230435	3.78	3.2	3.4	3.21	3.16	0.98	1.2	1.0	1.3	4.8	4.56	4.6	4.5
17	214401	4.1	2.2	2.1	2.0	2.22	1.07	0.6	0.8	1.4	2.9	3.5	3.9	4.3
18	198298	3.9	3.37	3.43	3.45	3.4	1.7	1.8	1.4	1.5	4.3	4.7	4.23	4.34
19	198299	4.19	3.68	3.76	3.8	3.7	1.36	1.7	2.1	2.0	4.3	4.23	4.4	4.4
20	198300	3.9	3.65	4.22	3.85	3.67	1.56	1.89	2.06	1.7	4.34	4.7	4.8	4.9
21	215252	4.1	2.33	2.4	2.1	2.3	1.07	0.6	0.8	1.4	4.8	4.56	4.6	4.5
22	193596	3.7	3.93	2.73	2.5	2.34	1.36	1.7	2.1	2.0	4.3	4.23	4.4	4.4
23	205077	3.7	2.89	2.1	2.6	2.6	1.35	1.40	1.99	2.0	3.8	2.9	3.56	4.3
24	215150	3.96	2.4	3.1	4.6	3.9	1.36	1.7	2.1	2.0	4.3	4.23	4.4	4.4
25	166779	3.93	4.23	4	4.2	4.5	1.35	1.4	1.87	2.3	4.4	4.5	4.02	4.7
26	198860	4.41	4.43	4.5	5.1	5.1	1.45	1.8	1.9	1.3	4.8	4.82	4.34	4.2
27	242917	3.78	3.5	3.54	3.67	5.1	1.45	1.8	1.9	1.3	4.8	4.82	4.34	4.2
28	119680	3.84	3.34	3.45	3.34	5.0	1.35	1.8	1.7	1.8	4.5	4.79	4.88	4.88
29	200538	4.5	3.23	3.15	3.4	3.45	1.3	1.2	1.1	1.5	4.7	4.6	4.34	4.8
30	234363	3.6	3.4	3.2	3.5	4.34	1.35	1.8	1.7	1.8	4.7	4.6	4.34	4.8
31	204621	4.04	3.22	3.19	3.34	3.8	1.3	1.2	1.1	1.5	4.7	4.6	4.34	4.8
32	250099	3.6	2.7	2.89	2.65	3.0	1.35	1.8	1.7	1.8	4.7	4.6	4.34	4.8
33	214403	3.9	2.77	2.9	2.78	2.67	1.3	1.2	1.1	1.5	4.7	4.6	4.34	4.8
34	213950	3.67	3.4	3.33	3.34	3.12	1.35	1.8	1.7	1.8	4.7	4.6	4.34	4.8
35	212588	4.69	3.83	3.9	4.1	4.34	1.3	1.2	1.1	1.5	4.7	4.6	4.34	4.8
36	200007	4.23	4.72	4.13	4.13	3.87	1.35	1.8	1.7	1.8	4.7	4.6	4.34	4.8
37	206897	3.87	2.7	2.57	3.0	3.49	1.3	1.2	1.1	1.5	4.7	4.6	4.34	4.8
38	227874	4.16	3.93	3.68	3.45	3.7	1.35	1.8	1.7	1.8	4.7	4.6	4.34	4.8
39	253074	4.5	4.6	4.2	3.97	4.6	1.35	1.8	1.7	1.8	4.7	4.6	4.34	4.8

40	249245	4.1	4.5	4.34	4.2	4.3	1.3	1.56	1.7	1.5	4.7	4.6	4.34	4.8
41	220804	4.3	3.8	3.52	3.75	3.2	1.3	1.6	1.76	1.5	4.7	4.6	4.34	4.8
42	214194	4.5	3.69	3.72	3.98	3.45	1.3	1.54	1.89	1.5	4.7	4.6	4.34	4.8
43	206295	4.5	3.69	3.72	3.98	3.45	1.35	1.8	1.7	1.8	4.7	4.6	4.34	4.8
44	219286	4.2	4.6	4	4.7	4.72	1.5	1.5	2	2.1	4.5	4.56	4.34	4.8
45	219386	4.5	4.5	4.2	3.9	3.5	1.5	1.5	2	2.1	4.5	4.56	4.34	4.8
46	209542	3.7	3.5	3.8	3.34	3.7	1.3	1.3	2.2	1.9	4.4	4.2	4.3	4.5
47	218134	3.56	3.3	3.08	3.22	3.4	1.5	1.5	2	2.1	4.5	4.56	4.34	4.8
48	198297	3.95	3.98	4.2	3.7	3.7	1.5	1.5	2	2.1	4.5	4.56	4.34	4.8
49	213783	3.56	3.33	4.56	3.2	3.7	1.5	1.5	2	2.1	4.5	4.56	4.34	4.8
50	223206	4.45	3.67	3.4	3.32	3.87	1.5	1.5	2	2.1	4.5	4.56	4.34	4.8

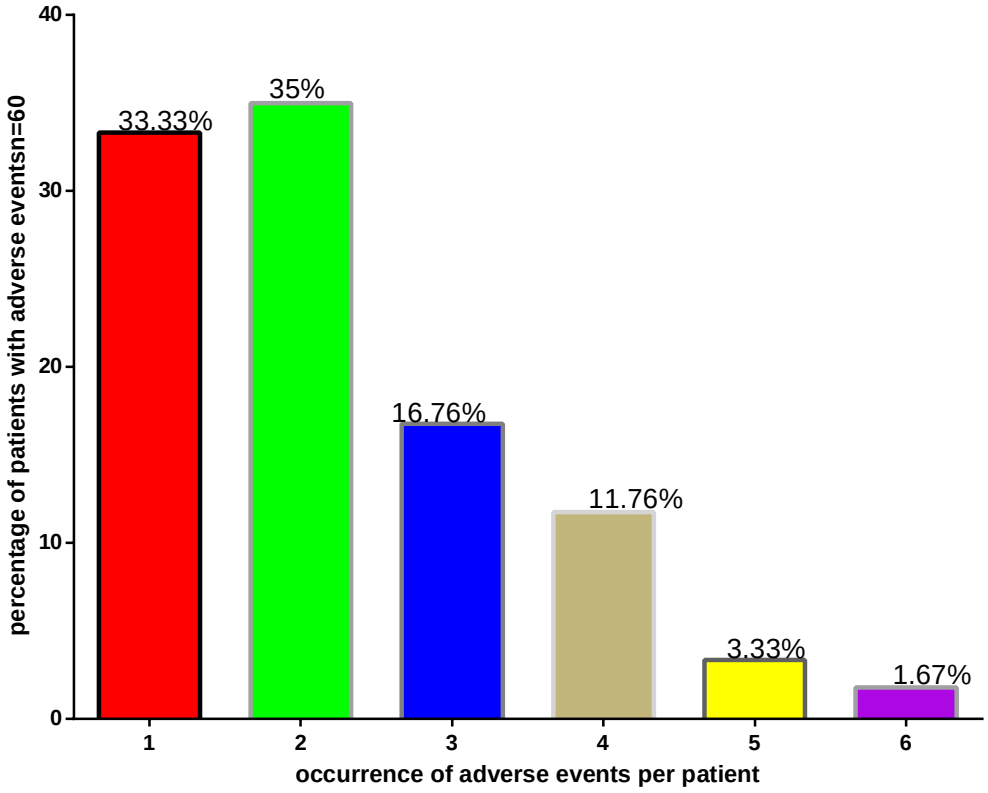
4.3. Prevalence of adverse drug reactions (ADRs)

Sixty patients experienced at least one adverse event of varying severity grading 88.24%. System electrolytes (potassium/magnesium and calcium) and liver/ thyroid gland and the urinary system dysfunctions were investigated in each patient in the intensive phase and the following results were found: Electrolyte related abnormality 57/68 (83.80%) (Hypokalemia 47/68(69.11%), hypomagnesaemia 18/68(26.47%) and hypocalcaemia 8/68 (11.76 %), nephrotoxicity 22/68(32.35%), hypothyroidism 16/68(23.53%) and hepatotoxicity 10/68(14.71%) (Figure4.1).



Figures 4.1. Medications related electrolyte and organ dysfunction problems

Six types of adverse drug reactions were investigated in the study and the number of adverse drug reaction per patient was detrmined.33.33%,35%,16%,3.33% and 1.67% of the patients were characterized by 1,2,3,4,5,and 6 number of the adverse events(Figure 4.2).



Figures 4.2. Distribution of percentage of patients by number of adverse events experienced per patient

4.4. Adverse drug reaction severity (ADRs)

The severity of ADRs in each organ and on serum potassium was also evaluated. Accordingly, 12/47 (25.53 %) of the 47 hypokalemic patients were found with a life threatening sever hypokalemia. 31.91 %(15/47) and 42.55 %(20/47) have had mild symptomatic and asymptomatic hypokalemia respectively. Creatinine clearance for all nephrotoxic patients were calculated using Cockcroft-Gault equation. As a result, 22.72 %(5/22) patients were found suffering from drug induced sever renal impairment, 63.63 %(14/22) with moderate problem and the rest 13.64 %(3/22) were at clinically end stage. Patients with liver problem also graded according to the extent of severity their liver was challenged and found 60 %(6/10) with serum AST/ALT level between 121- 200IU/L (moderate liver problem) and 40% (4/10) were between 201IU/L- 400 IU/L (sever hepatotoxicity).

4.5. Potential Risk Factors (Age, gender, ART/ant-TB concurrent use)

Comparison was made on the prevalence of ADRs in male and female patients to observe effect of gender in the abundance of the adverse events, the result of the current study shown

that the proportion of ADR found in female and male patients was 95.46% and 84.78% respectively (Table 5.1).

Age based association of ADR occurrence for age ≥30 and <30 was calculated and found 94.74% and 80% respectively (Table 5.2).

The association of ART concomitant treatment to MDR-TB patients under anti–TB agents was assessed. 93.33% (14/15) experienced a minimum of one ADR across their treatment duration in the intensive phase (Table 5.3).

5. DISCUSSION

In the previous studies variable magnitude of ADRs were reported, ranging from 60.6% - 96% (Vishakha & Sanjay, 2014;Ünsal *et al*., 2013;Törün *et al*., 2005; Shin *et al*.,2007;Tupasi *et al*., 2006; Delgado *et al*., 2011; Sagwa *et al*., 2012). In this study, treatment was standardized and the standardized drugs were Levofloxacine, capreomycin, Ethionamide, Cycloserine and pyrazinamide (Kassa *et al*., 2013). ADRs based on clinical symptoms were not included in the current study. However, in previous studies who reported higher ADRs prevalenc compare to the present studies, treament was indivualized based on drug sensitivity tes as a result, patients took different drug (Tupasi *et al*., 2006; Delgado *et al*., 2011; Sagwa *et al*., 2012). In these studies patients took both SLDs and FLDs. However no patient were treated by SLDs.Inaddition,the study designs were retrospective and sympthomatically documented ADRs were included in their reports (Tupasi *et al*., 2006; Delgado *et al*., 2011; Sagwa *et al*., 2012).Therefore,the variation observed might be due the variation in type and number of drugs used,the study design,whether sympthomatically observed ADRs are included.

Group statistics between patients previously treated with FLDs and new, diabetic/non diabetic and patients under TDF/3TC/FEV/ AZT/3TC/EFV regimen was not computed in this study. Because 66 of the 68 patients were treated previously by FLDs with only one new and the rest unknown treatment profile. Diabetes co-morbidity and patients under the AZT/3TC/EFV regimen were rare. There was only two diabetic and two on AZT/3CT/EFV regimen.

In the current study, treatment was according to existing guidelines, similar to other observational studies (Frazon *et al*., 2011; Kassa *et al*., 2013). However, the ART and anti-TB agents used differ from other cohort studies previously conducted (Adries *et al*., 2012; Isaakidis *et al*., 2012; Satti,et al., 2012). For instance, 13/15(86.67%) of the patients on ART in this study were under the regimen of TDF/3TC/EFV, where TDF is proved to pose overlapping renal toxicity with capreomycin (Dlodlo *et al*., 2013). All MDR-TB/HIV co-infected patients who initiated ART were under EFV regimen, which is characterized by liver and thyroid gland toxicity (Dooley *et al*., 2008; Dlodlo *et al*., 2013). Therefore , TDF and EFV might induce an additional toxicity on their respected organs.

The incidence of ADRs in this study was 88%. Electrolyte abnormality 57/68 (83.80%) was profoundly the highest adverse event encountered with (39/68(57.35%) hypokalemia, 10/68(14.7%)hypomagnesaemia and 8/68 (11.76 %) for

hypokalemia/hypomagnesaemia/hypocalcaemia) followed by 22/68(32.35%) nephrotoxicity, 16/68 (23.53%) hypothyroidism and 10/68(14.71%) hepatotoxicity of varying severity. 20/60(33%) and 21/60(35%) patients experienced respectively one and two adverse events. Patients with three and four types of ADRs were relatively small. Only one person characterized all the six adverse events.

Some differences in adverse events occurrence were observed between male and female, old and young and patients under anti-TB /ART. Hypokalemia, 13/68(19%) and hepatotoxicity 4/68(5.8%) were the highest and the lowest ADRs in countered in MDR-TB/HIV co-infected patients on ART/anti-TB. Nephrotoxicity and hypothyroidism respectively account for 7/68(10.29%) and 5/68 (7.35%) MDR-TB/HIV co-infected patients.

The frequency of hypothyroidism in the current study was 23.54 %. Drug induced primary hypothyroidism defined as serum level of TSH $\geq$ 10 mIU/L (Satti *et al.*,2012) is a known reversible adverse effect of prolonged therapy with ethionamide/prothionamide and para-aminosalicylic acid (Deary *et al.*, 2013). So far the definition of drug induced primary hypothyroidism was similar across t studies and this study also used the same definition. Hypothyroidism could be induced by suppressing thyroid hormone synthesis (T3 and T4) through the mechanism of inhibiting the uptake of iodine into thyroid cells by Ethinomide/prothnomid and the activity of thyroid peroxidase by para- aminosalicylic acid (Thee *et al.*, 2011).Therefore, it is clear that the Prevalence of hypothyroidism becomes high if Ethionamide and para-aminosalicylic acid are given together (Satti *et al.*, 2012). In addition, EFV /AZT (Dlodlo *et al.*, 2013) and stavuidine (Andries *et al.*, 2013) are among the ART drugs that could induce primary hypothyroidism. Therefore, EFV/ AZT and stavudine may have had an additive effec in hypothyroidism development.

The prevalence of hypothyroidism in MDR-TB patients from previous studies was variable ranging from 3.4%-69%(Qayyum *et al.*, 2012;Modongo & Zetola;Thee *et al.*, 2011;Andries *et al.*,2013; Dutta *et al.*, 2012 ;Isaakidis *et al.*,2012; Chhabra *et al.*, 2012 ;Satti,et al., 2012). Since the defintion of hypothyroidism was the same accros all studies, therefore, the variation might be due the type of drug/ /the nuber of drugs used and whether ART/anti-TB drugs is concurrently uesd. For instance, 69% hypothyroidism reported in Lesotho by Satti,et al., 2012,is higher compare to the current study 23.52%. In the Lesotho case, of 186 adults with MDR-TB 96.2% were treated with ethionamide/ prothionamide and Paraminosalicylic acid. However, in the current study all patients were treated by ethionamide only. 54% hypothyroidim was also reported from a prospective, observational cohort study carried out in Mumbai by Andries *et al.*, 2013, which is again higher in magnitud compare to the present

study. In the Mumbai cohort, 45 of the MDR-TB/HIV co-infected patients received ethionamide and para-aminosalicylic acid plus stavudine among the ART drugs. In the above two studies the MDR-TB patients were subjected to both ethionamide and para-aminosalisylic acid but in the current study all patients received ethionamide only. There was also a variation in types of ART used and HIV co-infection. All the study participants were MDR-TB/ HIV co-infected and on ART in the prevous two studies carried out by Andries *et al*.,2013 ; Satti,et al., 2012 while, in the current study 18 patients were MDR-TB /HIVco-infected out of which 15 were under ART/anti-TB regimen.However, in other Indian cohort where only ethionamide was given, 21% hypothyroidism was reported (Dutta *et al*., 2012).This is nearly similar to the hypothyroidism found in present study.

Conversly, Lower hypothiroidism was reported by ,Qayyum *et al*., 2012;Modongo & Zetola;Chhabra *et al*., 2011.Therefore, the observed variation might be due to the number of MDR-TB /HIV co-infection, the type of ART used, the number of anti TB drugs involved and the variation in patients managment.

Drug induced Hepatotoxicity defined as elevation to serum transaminase at least 3 times the upper limit of normal values is a very common phonemona in both inpatient and outpatient clinical situations(Chhabra *et al*., 2012). AST/ALT is found in the cytoplasm and mitochondria of liver cells in high concentrations but low in blood (Dufou *et al*.,2000). However, increased activities of these enzymes in serum are due to increased membrane permeability and leakage in to the blood circulation when the cytoplasm and mitochondria is get injured (Huang *et al*., 2006; Pandit *et al*.,2012; Chitturi & George,2002).

The frequencey of hepatotoxicity in this tsudy was 14.7%. The expected rates of hepatitis associated with SLDs treatment in patients with MDR-TB have varied considerably ranging from 1.7% to 16.8% (Hsu *et al*., 2010). The observed hepatotoxicity in this study is higher than previous reportes (Baghaei, 2011, 5%; Masjedi *et al*., 2008, 9.3%; Dziusmikeyeva *et al*., 2011, 4%). However, nearly similar drug induced hepatotoxicity were reported by shin *et al*., 2007, 16%; Keshavjee, 2012, 16.5%, from retrospective studies. In addition, in a prospective, observational cohort conducted by Hsu *et al* 2010,15.9% drug induced hepatotoxicity was observed ,which is also similar the present finding. The 47% hepatotoxicity reported by Papastavros *et al*., 2002, in toronto,canada was more prevalent compare to the presnt study and even goes beyond the expected rates of hepatitis (1.7% to 16.8%) associated with SLDs treatment in patients MDR-TB (Hsu *et al*., 2010).The variation observed might be due to the variation in definition of hepatotocity,the type and number of hepatotoxic drugs used,the exclusion and inclusion criteria set, HIV co- infection, drug -drug

interation between/among ART/anti-TB drugs (Younossian *et al*., 2005; Hsu *et al*., 2010; Lawn *et al*., 2013).

Among the anti TB drug used to treat MDR-TB, pyrazinamide, Ethionamide, Protonamide and para- aminosalcylic acid, flouroquinolones are proved to be hepatotoxic with pyarazinamide the most hapatotoxic and flouroquinolones mostly tolerated (Younossian *et al*., 2005; Hsu *et al*., 2010) . In recently reported clinical trial by Shih *et al*., 2013, oral administration of pyrazinamide itself or the intermediate product pyrazinoic acid at a dose of 500 mg/kg/day over 7 weeks in rats cause liver damage and this finding can be extrapolated to human being. In this study the minimum recommended dose of parazinamide for MDR-TB patients' with≥33Kg body weight is 1000mg/kg/day which is twice the dose taught to induce hepatotoxicity (Kassa *et al*., 2012). Therefore, the increased liver injury found in this study may partly be due to high pyrazinamide dose. Pyrazinamide also can inhibit the activity of several cytochromeP450 iso-enzymes such as CYP2B, CYP2C, CYP2E1, CYP3A, which involve in the detoxification process of pyrazinoic acid and 5-hydroxypyrazinoic acid the intermidate products pyrazinamide (Pandit *et al*., 2012). Therefore,if parazinamide was the drug of choice self induced liver hepatotoxicity is commmon.In addition,CYP2B6 and CYP3A4 are among the cytochrome P450 monooxygenases enzymes reponsible for the detoxification of EFV in liver (Dooley *et al*., 2008). However, if these enzymes are inhibited by pyrazinamide the EFV elimination from liver will be halted.Therefore, EFV can a induce liver toxicity (Dlodlo *et al*.,2013).In general,drug -drug interaction between pyrazinamid/ EFV and the additive liver damage imposed by EFV might contribute for the increased hepatotoxicity observed in this study. Furthermore, some ART drugs may also potentiate the activation of death receptors and/or intracellular stress pathways (Leist *et al*., 1998) consequently, cause necrosis of the parenchyma cell.

Electrolyte disturbance is one of the most challenging adverse reactions related to MDR-TB management, in particular because of the paucity of presenting symptoms and potential morbidity associated with this disorder (Shin *et al*., 2004). Hypokalemia, hypomagnesemia amd hypocalcemia were the dominant ADRs encountered in this study with hypokalemia the most frequent.

The frequency of hypokalemia in the current study was 69.11 %. This finding is similar to the findings of (Holmes *et al*., 1970), who reported capreomaycin induced 68.2% hypokalemia. In addition, the 11.76% electrolyte abnormality both for hypokalemia /hypomagnesaemia is also similar to the findings of Shin *et al*., 2004,who reported 12.2% hypokalemia /hypomagnesaemia. However, in the Holmes study all the patients were HIV

negative therefore, there was no additive TDF tubular necrosis. The increased renal toxicity observed in the Holmes study might be due to poor patient management, while in the current study in addition to patient's management the additive effect of TDF may also contribute. However, the hypokalemia found in this study is higher than the 31.3% and 33.2% hypokalemia respectively reported by Shin *et al*., 2004; Shin *et al*., 2007 in Russia ,Tomsk. It even goes beyond 4-15 % expectation of hypokalemia in MDR-TB patients treated under the drug capreomycin (Shin *et al*.,2004). Among the 33.2% hypokalemic patients reported by Shin *et al*., 2007, in Russia, Tomsk capreomycin was withdrawn in 7.4% patients. Moreover, in a cohort of XDR-TB patients in South Africa, one person was died due to the presumed capreomycin-associated hypokalemia (Shean *et al*., 2013). But in the present study permanet drug discontunation and presumed drug –associated death was not observed.

In general, Electrolyte abnormality during MDR-TB treatment is predominantly associated with the use of parenteral anti-TB agent like capreomycin and aminoglycosides (Shin *et al*.,2007). Electrolyte wasting is even much provoked in MDR-TB/ HIV co-infected patients under TDF regimen due to the TDF/ capreomycin additive renal toxicity (Dheda *et al*., 2010). In addition, ART/anti-TB pill burden could induce excessive diarrhea and vomiting through which electrolyte can be wasted (Isaakidi *et al*., 2012). Therefore, the prevalence of electrolyte abnormalities observed in the present study is more than any other study reported previously and is also dominant among the ADRs investigated in this study. The reason for this might be the extensive use of TDF/capreomycin in the intensive phase and the high pill burden induced vomiting and diarrhea.

Kanamycin/amikacin and capreomycin are among the nephrotoxic drugs which are commonly used to treat MDR-TB patients (Sturdy *et al*, 2011). Kanamycin/amikacin and capreomycin induce nephrotoxicity through intracellular accumulation and subsequent renal tubular necrosis generally (Jager & Altena, 2002).MDR-TB/HIV co-infection on ART haven been taught at high risk of nephrotoxicity development (Isaakidi *et al*., 2012) due to TDF/ capreomycin overlapping toxicity at the level of the proximal renal tubules (Lawan *et al*., 2013). Variable magnitude of renal toxicity might have be reported due the variation in the definition of nephrotoxicity, whether Kanamycin/amikacin or capreomycin is the drug of choice, ART drugs additive effect , specially TDF, prior renal insufficiency etc.

The frequency of renal toxicity in the present was 32.35 %. This finding was profoundly higher than any of the previous works carried out by (Shin *et al*., 2007 with 9.8%; de Jager & van Altena, 2002 with 9.3%; Baghaei *et al*., 2011 with 3.8%. This might be due to the extensive TDF/ capreomycin incorporation. Despite, it is recommended to replace TDF by

AZT in MDR-TB; TDF in the intensive phase in order to avoid possibility of overlapping renal toxicity with capreomycin (Kenyon *et al*., 2011) 13 of the 15 MDR-TB/HIV co-infected patients under ART/anti-TB were subjected to TDF/3TC/EFV. In addition to the capreomycin induced nephrotoxicity, the additive effect of TDF may also account for the observed variation between this study and the previous reports. The 21% sever renal impairment observed in MDR-TB / HIV co-infected patients on ART (Isaakidi *et al*., 2012) was in comparison to 22% sever renal impairment found in this study. However, higher than 3 % (Isaakidi *et al*., 2012) reported in India.

Associated risk factors

Table 5.1.*Prevalence of ADRs by age in Mekelle Hospital, MDR-TB center*

Age	**Number tested**	**Occurrence adverse event (%)**	**95%CI**
≥30	38	36(94.74%)	0.975-1.438
<30	30	24(80%)	0.057-1.212
Total	68	60(88.24%)	0.837-24.183

$\chi^2 = 3.507$, OR (4.5), $P > 0.05$

Age has been one of the risk factors affecting the development and frequency of ADRs occurrence in MDR-TB patients during treatment (Isaakidis *et al*., 2012; Dziusmikeyeva,*et al*.,2011; Forget & Menzies 2006); Tostmann *et al*., 2008). In the present study, prevalence of the ADRs based on age was 94.74% in 30 ≥ and 80% in < 30. However, the observed difference was not statically significant ($P > 0.05$).This finding is in agreement with the work done by (Delgad *et al*., 2011, Saukkonen *et al*., 2006 , Babalık *et al* 2012, Yee, 2003, Ormerod & Horsfield, 1996) who reported higher prevalence in aged patients than youngsters. The higher prevalence of older patients in this study could be due to a significant reduction in clearance rate of metabolized drug agents by the cytochrome P450 enzyme and /or changes in the hepatic blood flow distribution into liver, as age increase[(Forget & Menzies, 2006 and Tostmann *et al*., 2008).

Table 5.2.*Prevalence of ADR differentiated by gender in Mekelle Hospital, MDR-TB center*

Gender	**Number tested**	**Occurrence of adverse event (%)**	**95%CI**
male	46	39(84.78)	0.762-1.035
female	22	21(95.46)	0.438-25.561
total	68	60(88.24)	0.31-2.304

$\chi^2 = 1.633$, OR (0.265), $P > 0.05$

Gender is supposed to have some association the occurrence of ADR in MDR-TB patient under anti TB drugs treatment (Modongo & Zetola, 2012). Females have been shown to be a risk factor for clinically relevant ADRs with a 1.5 to 1.7-fold greater risk of developing ADRs compared to male patients (Carrasco-Portugal & Flores-Murrieta, 2011). In this study, prevalence of ADRs based on gender was 95.46% in female and 84.78% in male. The high prevalence found in female than male patients in this study could be explained due to anatomical difference; females are less muscular than males (Ciccone & Holdcroft, 1999). despite,this variation, the tradition of MDR-TB treatment Dosage usually given without normalizing by body weight, rather simply put a bench mark above or below would receive the same dosage (Table 1.8). Consequently, high dose, a dose beyond the recommended might be given to women in comparison to men and as a rresult the more ADRs may occurre in famels (Carrasco-Portugal & Flores-Murrieta, 2011).Inaddition, the glomerular filtration rate in female is slower than male (Carrasco-Portugal & Flores-Murrieta, 2011).Therefore,drugs eliminated through the urinary system might be delayed in female than male and as a result more insult will happen to the proximal tubules (De Jager & Altena, 2002).

Table 5.3. *Table 5.3.Adverse drug reactions associated with ART/anti-TB concurrent use among MDR-TB/HIV co-infected patients in Mekelle Hospital,MDR-TB center*

ART/ anti-TB treatment status	**Number tested**	**Occurrence of adverse events %**	**95%CI**
ART / anti-TB drugs co – treated	15	14(93.33%)	0.282-12.355
Treated only by anti-TB drugs	53	46(86.79%)	0.651-1.179
Total	68	60(88.24%)	0.241-18.828

χ^2 =0.48, OR=2.31,P >0.05

Anti retroviral / anti-TB concurrent therapy for MDR-TB/HIV co-infected patients were reported as risk factor for ADRs occurrence in these patients (Dlodlo *et al*., 2013). In this study the prevalence of adverse events based on ART/anti-TB drugs treatment was 93.33% compare to 86.79% HIV uninfected patients.TDF because renal toxicity through the proximal tubules necrosis (Kenyon *et al*., 2011) and this is more pronounced if it is given with capreomycin(Isaakidi *et al*., 2012). Inadditon, EFV and EFV/AZT respectively induce hepatotoxicity and primary hypothyroidism (Dlodlo *et al*., 2013;Andries *et al*., 2013). EFV also involve in drug–drug interactions with the anti–TB drugs (Dooley *et al* 2008). Nucleoside reverse transcriptase inhibitors remain the backbone of ART during MDR-TB/HIV co-treatment due to their safety, efficacy and favorable drug-drug interaction profiles (Dooley *et al*., 2008) and in many countries the preferred nucleoside reverse transcriptase inhibitors backbone is zidovudine (AZT) or TDF (Tweya *et al*., 2014). However, TDF is generally avoided in the intensive phase due to the possibility of overlapping renal toxicity with capreomycin (Kenyon *et al*., 2011). In recently published systematic review and meta-analysis by Kenyon *et al*., 2011, indicated that the risk of acute renal failure development induced by TDF was a statistically significant compared to AZT and stavuidine with a risk difference of 0.007. In this study 13 of 15 MDR-TB/HIV co-infected patients were under TDF/3TC/EFV regimen and all MDR-TB/HIV co-infected patients were treated with EFV. Therefore the increased ADRs prevalence observed in the current study might be due to the contribution of TDF on renal toxicity and the additive effect of EFV on liver and the thyroid gland. Furthermore, electrolyte related abnormality, which was the most frequent ADRs

found in this study could be wasted due ART induced diarrhea and/ or vomiting when ART was given with anti TB drug due to drug pill burden to the gastro intestinal tract (Isaakidi *et al*., 2012).

6. CONCLUSION

High rate of hypokalemia, nephrotoxicity, hypothyroidism and hepatotoxicity was observed in this prospective cohort. This is a treatable and reversible; however, it may go undiagnosed in the absence of regular monitoring. Care providers should not wait for clinical symptoms, as this risks compromising treatment adherence. Electrolyte related abnormalities and renal toxicity was the most prevalent among others. The increased electrolyte/ renal related ADRs observed in this study might be due to capreomycin/TDF additive effect on proximal renal tubules. 24/60(40%) of the patients were with a life threatening situation. 36/60(60%) were clinically occult or in mild clinical manifestation. ADRs were more prevalent (14/15) in MDR-TB/ HIV co-infected patients on ART/anti-TB. Relatively high ADRs were also observed among older and female patients.

7. RECOMMENDATION

- ✓ Higher magnitude of ADRs was observed among older, females and MDR-TB/ HIV co-infected patients on ART/anti-TB. Therefore, MDR-TB /HIV co-infected, older and female patients should be closely followed for the Occurrence of ADRs during the intensive phase to prevent morbidity and mortality

- ✓ Patients with prior liver / renal insufficiency were excluded in this study. Therefore, subsequent studies should incorporate and thoroughly examined the characteristics and magnitude ADRs in these patients

- ✓ Since the current study determined only ADRs limited to the intensive phase. Subsequent studies should deal with the continuous phase as well.

- ✓ According to the WHO guideline for MDR-TB /HIV co-infected patients management recommendation, the best first line regimen during the intensive phase is AZT/3TC/EFV. However, 13 of the 15 patients on ART in this study were under the TDF/3TC/EFV regimen. Therefore, clinical authority should give attention in designing ART regimen to minimize the capreomycin/TDF additive effect on the proximal tubules.

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9. ANNEX

9.1 Questionnaire

Table 9.1. Demographic and clinical information of the study participant

I. Questionnaire for general information of the study participants		
1	Interviewee code number	
2	Age of the respondent (in years)	
3	Sex of the respondent(male-m) or (female-f)	
4	Weight(in Kg)	
5	Model of care used	1.ambulatory
		2.hospitalization
6	Type of TB	1.pulmonary
		2.Extra-pulmonary
7	Types of the anti-TB drugs given at the movement	
8	Treatment approach used	1.Standardized
		2.Individualized based on drug sensitivity test
II. Questionnaire for history of previous treatment		
9	What is the previous treatment notification of him/her?	1.New
		2.retreatment
10	If he/she was retreatment case, what is its category?	1.relapse
		2.treament after failure
		3.defualted
		4.unknown
11	What was the Category of drugs given	1.First line drugs
		2.second line drugs
III. Questionnaire for base line serum organ and electrolyte test results(before treatment is initiated)		
12	Liver function test(LFT)	ALT(IU/L)=
		AST(IU/L)=
13	Renal function test(RFT)	Creatinine(mg/dL)=
14	Thyroid function test(TFT)	TSH(mIU/L)=
15	Electrolyte test results(potassium-K+)	K+(mEq/L)=
IV. Questionnaire for co-morbid illness		
16	HIV status of the respondent	Positive
		negative
17	If HIV positive is he/she taking ART	1.Yes, he/she is taking ART
		2.No ,he/she did not started ART
		3.He/she started but defaulted now
18	Hepatitis B virus	1.Present
		2.absent
19	Hepatitis C virus	1.Present
		2.absent
20	Prior liver/renal insufficiency	1.Present
		2.absent
21	Diabetes status of patients	1.Diabetic
		2.Non-diabetic
V. Questionnaire for social history of patients		
22	Smoking habits	1.Smoker
		2.Non-smoker
23	Alcohol drinking habits	1.Drunker
		2.Non-drunker
24	If he/she is a drunker what was his/her drinking frequency	2.Every day
		2.weekly
		3.occasionally