EFFECTS OF INTENSIVE PHASE ANTITUBERCULOUS THERAPY ON HEPATIC AND HAEMATOLOGICAL PARAMETERS IN PATIENTS AT THE ADULT UNIVERSITY TEACHING HOSPITAL IN LUSAKA, ZAMBIA

By

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A dissertation submitted to the University of Zambia in partial fulfillment of the requirements of the degree of Masters in Clinical Pharmacy

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ABSTRACT

Zambia is a high tuberculosis (TB) burden country. Antituberculous medicines are mainstay of TB management. Several reports of antituberculous drug-related haematological and hepatic adverse effects have been noted in other settings. Adverse events have healthcare cost and morbidity implications. Prevalence and severity of these adverse effects is understudied in patients at the University Teaching Hospitals. The purpose of this study was to identify haematological and hepatic abnormalities and compare parameters before treatment and after completion of intensive phase among the TB patients. Factors associated with abnormalities were also determined. A prospective longitudinal study was undertaken at Chest Clinic. Study patients were followed up for 2 months. Full blood count (FBC) and liver function tests (LFTs) were recorded at base-line and at follow-up. Abnormalities were defined according to the 2017 Division of Acquired Immunodeficiency Syndrome Table for Grading the Severity of Adult and Paediatric Adverse Events. Data were analysed using GraphPad Prism version 8.0.1and SPSS version 22.0 Paired t-test and Wilcoxon matched-pairs signed ranks test were used to compare parameters. Logistic regression was performed to determine factors that were predictive of abnormalities. A $p < 0.05$ was considered statistically significant. A total of 37 patients were involved in the study. 56.8 percent of patients were male. The mean age of patients was 36.2 years (19 – 57 years) while mean body mass index (BMI) was 21.9 kg/m^2. Only 37.8 percent of patients were sputum smear positive at baseline. 56.8 percent of patients had human immunodeficiency virus (HIV) co-infection. 45.9 percent of patients were on antiretroviral therapy (ART). 45.2 percent of patients had grade 1-3 aspartate transaminase (AST) derangements at follow-up compared to 29.7 percent at baseline. 5.4 percent of the patients had grade 1-3 alanine transaminase (ALT) derangements at baseline while 9.7 percent of patients had grade 1 at follow-up. Fewer patients (16.1 percent) had grade 1-2 anaemia at follow-up while 62.2 percent of patients at baseline had grade 1- 4 anaemia. More patients (46.2 percent) had platelet derangements at follow-up compared to 25.8 percent at baseline. Fewer patients had differential white cell count (WCC) derangements at follow-up compared to baseline. Statistically significant differences in haematological parameters: haemoglobin concentration (Hb), haematocrit (HCT), red cell count, and white cell, eosinophil and neutrophil counts at baseline and follow-up were found ($p < 0.0001$, 0.0001, 0.0001 and $p = 0.0058$, $p < 0.0001$ and $p = 0.0005$ respectively). However, no statistically significant differences in red cell indices were observed. Changes in ALT levels at baseline and follow-up were statistically significant ($p = 0.0251$). Logistic regression was performed to determine the effects of age, gender, BMI, HIV infection, ART, sputum smear status, and appropriate baseline FBC/LFT parameters on the likelihood of study patients having deranged Hb, WCC and ALT at follow-up. Logistic regression models to predict deranged Hb and ALT were statistically insignificant. Logistic regression model for ALT was statistically significant, $\chi^2 = 18.597$, $p = 0.01$. The model explained 100 percent (Nagelkerke R^2) of variance in ALT derangement and correctly classified 100 percent of the cases. None of the predictor variables were associated with likelihood of ALT derangement. Findings of this study show that haematological and hepatic adverse effects were relatively fewer at follow-up and mostly grades 1-3, therefore, antituberculous therapy is relatively safe for patients during initial phase.

Key words: adverse effects, antituberculous, haematological, hepatic, initial phase

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ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ART	Antiretroviral therapy
AST	Aspartate transaminase
BMI	Body mass index
CDC	Centre of Disease Control and Prevention
DAIDS	Division of AIDS
EPTB	Extra pulmonary tuberculosis
ESR	Erythrocyte sedimentation rate
FBC	Full blood count
Hb	Haemoglobin
HCT	Haematocrit
HIV	Human immunodeficiency Syndrome
LFT	Liver function test
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration

MCV	Mean cell volume
MOH	Ministry of health
MPV	Mean platelet volume
MTB	*Mycobacterium tuberculosis*
NAT	N – acetyl aminotransferase
PDW	Platelet distribution width
PLT	Platelet
RCC	Red cell count
RDW	Red cell distribution width
RIF	Rifampicin
TB	Tuberculosis
WCC	White cell count
WHO	World Health Organization

CHAPTER 1: BACKGROUND AND INTRODUCTION

1.1. Introduction

Tuberculosis (TB) has existed for millennia and remains a major global problem (World Health Organization, 2017). TB is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS (World Health Organization, 2017).There has been a gradual reduction in the prevalence rate of TB since the 1990s (Glaziou *et al.*, 2015), however, one third of the world's population is still known to be infected with tubercle bacilli which can become active disease when conditions are favorable (World Health Organization, 2016, 2017). An estimated 10.4 million people (90 percent adults; 65 percent male; 10 percent people living with HIV) fell ill with TB in 2016 (incident cases). Most of the estimated number of incident cases occurred in South-East Asia (45 percent) while Africa accounted for 25 percent (World Health Organization, 2017).

Zambia is amongst the top 30 countries with a high per capita burden of TB, with its prevalence at 319/100000 in individuals aged 15 years and older. (World Health Organization, 2017). Kapata *et al*. (2016) reported that the prevalence of bacteriologically confirmed TB in Zambia was 638 (502 – 778) per 100,000 adult populations in 2013–2014. In addition, HIV positive individuals were found to be five times more likely to have tuberculosis infection compared to HIV negative individuals (Kapata *et al.*, 2016). There has been a gradual decline in the incidence of TB. For instance, the incidence was 391 per 100000 populations in 2015 (World Health Organization, 2016).

Mycobacterium tuberculosis and *Mycobacterium bovis* are the major bacilli that have been isolated in patients with tuberculosis. Infection with *Mycobacterium tuberculosis* accounts for over 98% of isolates (Walker and Whittlesea, 2012). Infection with the tubercle bacilli often occurs by the respiratory route by inhaling droplets from a person with the pulmonary infection. The droplets are produced during respiratory effort such as sneezing or coughing. After inhalation, the droplets containing *Mycobacterium tuberculosis* settle in the bronchioles and alveoli of the lungs.

The tubercle bacillus is an aerobic organism and thrives in tissues with a high partial pressure of oxygen and high arteriole blood flow such as the apices of the lungs. A person may develop active disease or latent infection depending on the inoculum of the bacilli, the state of cell mediated immunity of the host and the virulence of the organism (Narasimhan *et al.*, 2013). The ability of the host to respond to *M. tuberculosis* infection is reduced by certain disease such as diabetes mellitus, silicosis, chronic renal failure and HIV infection (American Thoracic Society, 2000).

Diagnosis, treatment and monitoring of TB

1. Microbiological investigations assess the infectious state of the patient and distinguish between infection with *Mycobacterium tuberculosis* and other mycobacterium species. They also determine the drug susceptibility patterns of the infecting organisms to ensure that drug prescribed will be effective in treating the individual patient. Direct microscopy of sputum uses the Ziehl – Nelson or other fluorescent stain to detect the infectious agent by looking for the acid fast bacilli. Two sputum samples are collected from a patient suspected with pulmonary TB. Currently, the Xpert® MTB/RIF assay, a

rapid test which can provide results within 2 hours is available for diagnosis of TB and it also detects *Mycobacterium tuberculosis* (MTB) susceptibility to rifampicin (World Health Organization, 2017).

2. Chest radiography is the other method of diagnosis used. The appearance of an opacity or opacities which are ill defined are usually seen in one or both of the upper lung lobes (organs with high blood flow and partial pressure of oxygen such as the apices of the lungs, kidneys, bones and brain are particularly favorable for growth of the organism, as it is an aerobic organism) (Walker and Whittlesea, 2012), these opacities are larger and more widespread in more advanced disease. Areas of translucency within the opacities indicate cavitation and in an untreated case the cavitation signify active disease.

3. Tuberculin testing is used to detect latent tuberculosis infection. A 0.1ml purified protein derivative of *M. tuberculosis* secreted proteins is intra - dermally injected into the volar surface of the forearm and a positive or negative delayed hypersensitivity reaction is evaluated (as millimeters of induration) after 48 – 72 hours (Broaddus et al., 2016). This method should be done by a person that has experience in its use. It is used in areas where the prevalence of TB is high and it does not confirm the diagnosis of TB, it confirms if someone has been previously infected with the tubercle bacilli, it cannot distinguish the different types of tubercle bacilli.

4**.** Culture – based method of diagnosis involves growing the bacterium in different mediums to find out which bacterium is present. Conventional culture use such as

Lowenstein - Jensen medium takes time for growth to occur, however modern liquid cultures produce results in 1 – 2 weeks (Walker and Whittlesea, 2012).

The WHO recommends that treatment of pulmonary TB should comprise intensive and continuation phases (World Health Organization, 2010). Treatment of pulmonary and extra pulmonary TB other than severe forms of TB meningitis, osteo - articular and spinal TB is achieved by using a two month intensive phase regimen of rifampicin, isoniazid, pyrazinamide and ethambutol followed by a continuation phase (Ministry of Health, 2017). The continuation phase is four months therapy with rifampicin and isoniazid. In countries with a high prevalence of isoniazid resistant TB, addition of ethambutol as part of the continuation phase regimen is recommended (World Health Organization, 2010). For TB relapse, the duration of treatment is eight months with a three months intensive phase and a five months continuation phase (World Health Organization, 2010).

Monitoring of TB treatment outcomes is achieved by examining sputum smears, which are the gold standard. Sputum is checked at the end of the intensive phase and samples are collected when the patient is given the last dose of the intensive phase (World Health Organization, 2010). Patients with smear positive drug-susceptible TB are expected to become smear negative by the end of the intensive phase of treatment.

Abnormalities in hepatic and haematological parameters due to antituberculous therapy

Treatment of TB with first line medicines has been successful. However, the drugs are associated with a number of side effects. Among the side effects caused by first line anti

– tuberculous medicines are haematological disorders and hepatotoxicity. A study conducted in Mumbai noted that haematological and biochemical abnormalities were common among TB patients on antituberculous therapy (Morris *et al*., 1990). These abnormalities may cause significant morbidity and compromise treatment regimens (Yee *et al.*, 2003). They may also lead to use of drugs that are less active and occasionally more toxic (Schaberg *et al*., 1996; Lv *et al.*, 2013). Side effects can also result in increased treatment costs, hospital visits and hospitalizations (Yee *et al.*, 2003).

A rise in the liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are a common adverse effect leading to termination of therapy in patients treated with isoniazid, rifampicin and pyrazinamide (Schaberg et al., 1996). When the serum liver enzyme concentration is less than 3 times above the upper limit, the drugs may be continued and patient monitored closely. If the liver enzyme concentration is 3 times above the upper limit and patient has symptoms of hepatotoxicity such as nausea, vomiting and jaundice, the drugs are withdrawn and reintroduced one by one when the liver enzymes revert to normal. The reintroduction starts with the least hepatotoxic drug. If hepatotoxicity recurs after initiation of a particular drug, the drug is permanently withdrawn. However, treatment with less than four drugs may delay time to smear conversion (Long *et al*., 2003).

Mechanisms of antituberculous drug induced abnormalities

There are a number of mechanisms by which antituberculous drugs cause abnormalities in haematological and hepatic parameters, ranging from immune reactions to hypersensitivity reactions.

Pyrazinamide is the most common cause of hepatotoxicity amongst the first line antituberculous drugs. The mechanism of pyrazinamide -induced hepatotoxicity is not well known (Chang *et al.*, 2008). It causes transient increases in serum amino transaminase levels and causes clinically apparent liver disease with symptoms of liver disease and jaundice. It is also known to cause thrombocytopenia, although the mechanism is not known.

Isoniazid metabolism is associated with isoniazid - induced liver injury. The major metabolites of isoniazid are acetyl hydrazine, acetyl isoniazid and hydrazine. Studies on the adverse effects of isoniazid in rats showed that acetyl isoniazid and acetyl hydrazine can cause hepatic necrosis (Wang *et al.*, 2016). A class of enzymes called N-acetyl aminotransferase (NAT) is responsible for metabolizing the metabolites of isoniazid. There are two types of NAT namely NAT 1 and NAT2. NAT 2 is responsible for metabolizing acetyl isoniazid, acetyl hydrazine and hydrazine. There is a wide range of genetic polymorphism of this enzyme. Slow metabolizers usually have accumulation of the metabolites and consequently hepatotoxicity.

Rifampicin - induced hepatotoxicity has been postulated to occur due to oxidative stress and elevated toxic metabolites caused by Cytochrome P450 (CYTP450) induction (Kim *et al.*, 2017). Rifampicin also induces hepatotoxicity of isoniazid (Sharma and Mohan, 2005).

Haematological adverse effects present as derangements in any one or a number of the blood cells (Kassa *et al.*, 2016). There have been variations in the reported incidence and prevalence of these adverse effects. Some studies report high prevalence whilst

other studies found very low prevalence of haematological adverse effects (Kassa *et al.*, 2016; Enoh *et al.*, 2017). Hepatic adverse effects are more prevalent than haematological adverse effects.

Factors associated with abnormalities in hepatic and haematological parameters

A number of factors have been identified as risks to having an antituberculous therapy – related adverse effect (Pande *et al.*, 1996; Yee *et al.*, 2003; Marra *et al.*, 2007). Female sex, cigarette smoking, initial high transaminase concentration, older age, prior liver damage, alcohol and drugs that increase CYTP450 activity are amongst the risk factors identified for adverse drug reaction occurrence. Knowledge of these risk factors may optimize management of patients with known risk factors through close monitoring thus some unwanted adverse outcomes may be prevented.

The present study looked at the changes in the haematological and hepatic parameters during the first two months of antituberculous therapy. It investigated factors associated with hepatic and haematological abnormalities among patients on intensive phase antituberculous therapy.

1.2. Theoretical Framework

Figure 1 below shows the adverse effects that result from use of antituberculous drugs and the problems that result if not detected and dealt with.

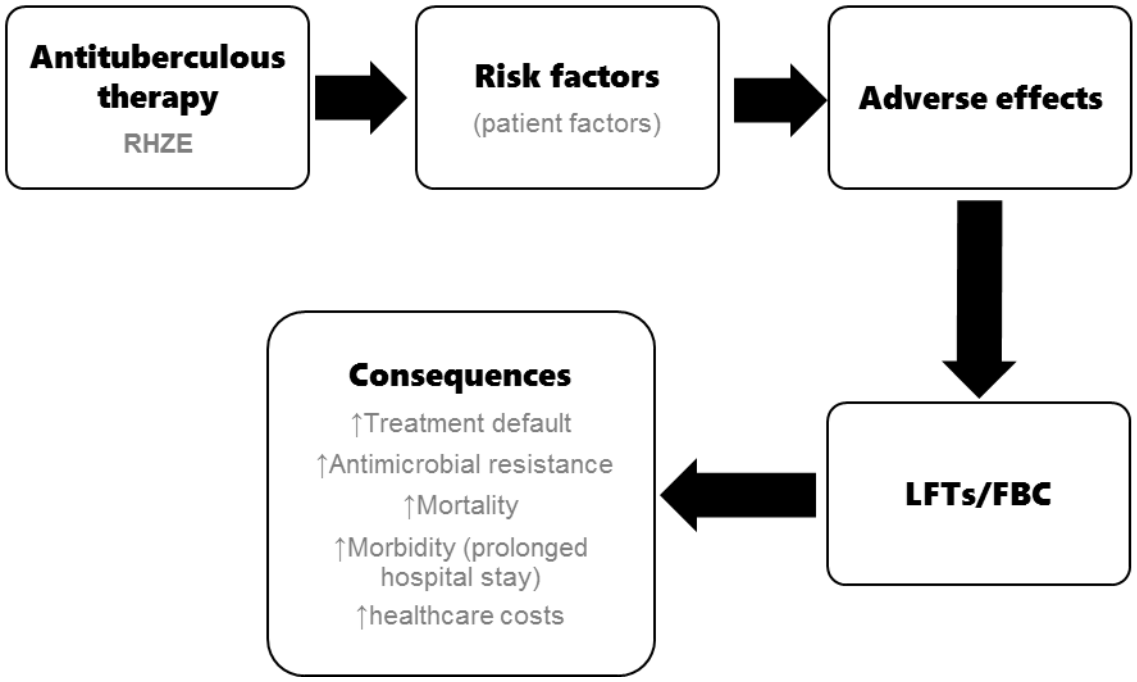

Figure1. Theoretical framework

1.3. Statement of the problem

The first line antituberculous regimen comprising a combination of isoniazid, rifampicin, ethambutol and pyrazinamide for the treatment of active tuberculosis is associated with significant adverse drug reactions resulting in abnormalities in various body systems. These adverse drug reactions range from effects on the liver, blood, skin, eyes and brain.

Some studies have shown that antituberculous drugs induce haematological and hepatic abnormalities (Kassa *et al.*, 2016) Hepatic abnormalities are amongst the common documented adverse effects (An *et al.*, 2013; Hassen Ali *et al.*, 2013; Abera *et al*., 2016)

while haematological abnormalities are rare but both are serious adverse effects (Farazi *et al.*, 2014).

There is variation in the reported prevalence of antituberculous therapy - related abnormalities in hepatic and haematological parameters and their associated risk factors. Variation in risk factors has been observed in different races. At the University Teaching Hospitals in Lusaka, there was no data on these adverse effects and the associated risk factors. Consequently, patients at higher risk of developing these abnormalities may not have been identified. Non identification of such patients could lead to poor outcomes of therapy including prolonged hospital stay, morbidity and mortality. Occurrence of adverse effects may lead to, increased healthcare costs, increased default rates and development of resistance to antituberculous therapy.

1.4. Justification of the study

There has been mixed reports regarding the extent of abnormalities in hepatic and haematological parameters in patients on the intensive phase of antituberculous drugs and the factors that are associated with increased risk of their occurrence.

This study was envisaged to identify the extent of the problem of haematological and hepatic parameter abnormalities induced by first line antituberculous drugs. The study also aimed to determine association of the abnormalities with individual patient factors. The study would further make recommendations based on its findings regarding appropriate monitoring of patients at higher risk of hematologic and hepatic abnormalities which would in turn result in reduction of morbidity and mortality, default rates and reduced hospital stay.

Lastly, the findings of the study would also serve as the basis for future studies.

1.5. Research questions

1. What are the haematological and hepatic abnormalities among patients in the intensive phase of antituberculous treatment?

2. What is the relationship of the haematological and hepatic abnormalities with intensive phase antituberculous therapy?

3. What are the factors associated with haematological and hepatic abnormalities in patients on intensive phase antituberculous therapy?

1.6. General objective

To explore the effects of intensive phase antituberculous therapy on hepatic and haematological parameters in patients at the University Teaching Hospitals in Lusaka.

1.7. Specific objectives

1. To identify the haematological and hepatic abnormalities among patients in the intensive phase of antituberculous treatment
2. To compare the haematological and hepatic parameters before and after treatment with intensive phase antituberculous drugs
3. To determine the factors associated with haematological and hepatic abnormalities in patients on intensive phase anti - tuberculous therapy

CHAPTER 2: LITERATURE REVIEW

This review of literature explored aspects of hepatic and haematological abnormalities among patients on intensive phase antituberculous therapy. The study within this review focused on objectives 1-3.

2.1 Haematological and hepatic abnormalities among patients on intensive phase antituberculous therapy

2.1.1. Global perspective

2.1.1.1. Hepatic abnormalities

Numerous studies have been conducted which have reported alterations in liver function tests (LFTs) and haematological parameters in patients after initiation of antituberculous drugs. The haematological parameters that were measured in these studies include: white cell count including differential counts (neutrophils, monocytes, eosinophils and basophils), red cell count (RCC), hemoglobin concentration (Hb), hematocrit, red cell indices and platelet count. Hepatic parameters measured were the liver enzymes - alanine transaminase and aspartate transaminase.

A study conducted in Nepal by Koju *et al.* (2005) showed a significant increase (abnormality) in some hepatic parameters after two months of therapy with antituberculous drugs. The hepatic parameters were ALT, AST, ALP, total and direct bilirubin. These findings are similar to those observed by Shang *et al.* (2011) . They found that 28.15 percent of the patients had mild elevation (grade 1), 28.64 percent of the patients had moderate elevation (grade 2), 23.79 percent of the patients had severe elevation (grade 3) and 19.42 percent of the patients had potentially life - threatening

elevation (grade 4) in ALT levels. They also found that 55.17 percent of the patients had mild elevation (grade 1), 31.17 percent had moderate elevation (grade 2), 12.07 percent had severe elevation (grade 3) and 1.72 percent had potentially life - threatening elevation (grade 4) in AST levels. They further reported that 1.89 percent died from the abnormalities, 69.81 percent changed their anti – TB treatment while 16.9 percent of the patients with hepatic abnormalities improved. Xiang *et al.* (2014) reported different findings in the extent of elevation of the hepatic parameters. Like Koju *et al.* (2005), the abnormal hepatic parameters noted were ALT, AST and bilirubin. Xiang *et al.* (2014) observed that 13 percent had elevations in the hepatic enzymes but were less than one times the upper limit of normal, four percent had significant elevation in the hepatic enzymes. Of the four percent, 83.15 percent had ALT levels elevated more than two times above the upper limit whereas 16.85 percent had AST and bilirubin levels elevated more than two times upper limit. 24.72 percent had three to four times increase in hepatic parameters above the upper limit of normal. Out of the 24.72 percent, 20.22 percent had increase in ALT and 4.5 percent increase in AST and bilirubin , 10.11 percent had more than five times increase in ALT levels which is about nine times higher than what Shang *et al.* (2011) observed. In a study undertaken by Chang *et al.* (2008), 150 cases (five percent) of the patients that were in the cohort were diagnosed with hepatic abnormalities. The patients were found to have elevations more than three times and up to five times the upper limit of normal in 22 cases (14.67 percent), of whom ten (6.7 percent) had hepatitis symptoms; elevations of more than five times and up to 10 times the upper limit of normal were noted in 14 cases (9.33 percent); and elevations of more than 10 times the upper limit of normal were found in 12 cases (eight

percent). Total bilirubin levels were elevated more than two times the upper limit of normal in seven cases, of whom six had serum ALT that was elevated more than five times the upper limit of normal. Two out of the 12 cases with ALT exceeding 10 times the upper limit of normal died of liver failure whereas all of the 36 cases with lower ALT survived.

2.1.1.2. Haematological abnormalities

In the study by Koju *et al.* (2005), a number of haematological parameters were in the reference range after treatment with anti - tuberculous drugs. The white cell count and the neutrophil count were noted to have been slightly reduced after treatment. The thrombocytes (platelets) and erythrocyte sedimentation rate (ESR) were also reduced while the eosinophil count was raised. There was no change in the concentration of lymphocytes and monocytes. The haematological findings reported by Koju *et al.* (2005) were similar to those observed in a study that was undertaken in China by An *et al*. (2013). An *et al.* (2013) noted that of the 267 patients that had abnormalities due to adverse drug reactions, 19 (7.1 percent) patients had an increase in white cell count while 59 (22.1 percent) had a reduced white cell count. The proportion of patients with leukopenia was higher than that of those who had leukocytosis. This finding is different from that reported by Koju *et al.* (2005) which showed that all patients had leukopenia. Like findings by Koju *et al.* (2005), 33 (12.4 percent) of the patients had eosinophilia whereas 28 (20.75 percent) had thrombocytopenia.

Al-muhammadi and Al - Shammery (2011) undertook a prospective cohort study in which they enrolled cases and controls. Cases comprised newly diagnosed TB patients

before initiating therapy and the patients who had been on antituberculous therapy for two months. Controls were healthy individuals without TB. They observed that the haematocrit and haemoglobin concentration were significantly increased in patients that had been on therapy while the ESR and thrombocyte count were significantly reduced. Thrombocytopenia was considered an abnormality arising from the use of antituberculous therapy.

2.1.2. Regional perspective

2.1.2.1. Hepatic abnormalities

Abera *et al*. (2016), in a study conducted in Ethiopia, looked at ALT, AST and Bilirubin levels. Abera *et al*. (2016) found that ten (eight percent) out of 124 patients included in the study had hepatic abnormalities. All hepatic parameters reported were five times above the upper limit of normal.

2.1.2.2. Haematological abnormalities

Enoh *et al.* (2017), in a cross sectional study carried out in Cameroon reported agranulocytosis, leukopenia, anaemia and thrombocytopenia. They did not find any patients with leukocytosis. Of the 62 patients that had haematological adverse effects, 24 (38.71 percent) had leukopenia, 35 (56.35 percent) had neutropenia, reduced basophil and eosinophil counts (agranulocytosis), 23 (37.10 percent) had anaemia while 17 (27.42 percent) had thrombocytopenia.

The study conducted by Kassa *et al.* (2016) in Ethiopia included other haematological parameters in their study, they included haematocrit, platelet as well as haemoglobin,

Red cell distribution width and platelet distribution width. Kassa *et al.* (2016) found significant reduction in haemoglobin concentration, platelet count and hematocrit ($p < 0.001$) after completion of the intensive phase treatment. In addition, they also found a significant difference in red cell count among patients in the age group 45–65 years ($p = 0.030$). The red cell distribution width of female patients and the platelet count of male patients were also significantly different ($p = 0.002$ and 0.031 respectively). However, there was no significant difference in white cell count and platelet distribution width.

2.1.2. Local perspective

There is paucity of data concerning hepatic and haematological abnormalities in patients on intensive phase antituberculous therapy, but anecdotal data shows that a majority of patients that do not complete treatment are defaulters. One of the reasons that could lead to defaulting would be adverse drug reactions.

As stated earlier, TB is a chronic infection which gives rise to a number of haematological abnormalities which include anaemia, leukocytosis, increase in erythrocyte sedimentation rate etc. (Yaranal *et al*., 2013; Bashir *et al*., 2015). It is known that various inflammatory cells, cytokines and mediators are involved in the formation of granulomatous lesions encountered in tuberculosis (Yaranal *et al*., 2013). Research has shown that these abnormalities improve as treatment is commenced and continues. Any further derangements may be attributed to adverse drug reactions (Kassa *et al.*, 2016).

As reviewed in the literature reviewed above, hepatic and haematological abnormalities have been noted in patients on intensive phase antituberculous therapy but the extent to

which they have occurred have varied. There has been no record of the type and extent to which these abnormalities occur in the setting where the current study was undertaken.

2.2 Relationship of haematological and hepatic abnormalities with intensive phase antituberculous therapy

2.2.1. Global perspective

A number of studies have shown that adverse drug reactions resulting in systemic abnormalities due to intensive phase antituberculous therapy occur within the first two months of therapy. Lv *et al.* (2013) explained that the median number of days to having haematological and hepatic abnormalities in patients was 55 days and 53 days respectively, with an average of 55.5 days. Furthermore, Lv *et al.* (2013) reported hepatic and hematologic abnormalities in 35.9 percent and 3.9 percent of the patients, respectively. Patients with these abnormalities were more likely to develop unsuccessful outcomes (adjusted OR, 2.58; 95 percent CI, 1.43– 4.68). PAR% for positive smear results at the end of the intensive phase and unsuccessful outcomes attributed to ADRs in TB patients was 10.75 percent (95 percent CI, 9.82 percent –11.67 percent). These findings are similar to those reported by Shang *et al.* (2011), Xiang *et al.* (2014) and An *et al.* (2013) who found that time to the onset of hepatic abnormalities was within two months. Shang *et al.* (2011) suggested a median interval time of 52.5 days. They reported 16.58 percent of unsuccessful treatment outcomes, which were attributed to hepatic abnormalities. They stated that the abnormalities resulted in 69.81 percent of the patients changing their treatment regimen, 19.81 percent having drug replacement, 50.94 percent interrupting the TB treatment, and 11.32 percent discontinuing treatment.

Yee *et al.* (2003b) reported that the time onset of antituberculous therapy - induced adverse effects occurred within 60 days of therapy. This finding was confirmed by Shang *et al.* (2011) and Lv *et al.* (2013). However, Chang *et al.* (2008) found a median time to the occurrence of hepatic abnormalities of 20.4 weeks (144 days).

2.2.2. Regional perspective

Abera *et al*. (2016), in a study conducted in Ethiopia reported a shorter time to onset of hepatic abnormalities of 13 – 58 days with median of 26 days.

2.2.3. Local perspective

There has been no documented data on the relationship of haematological and hepatic abnormalities with intensive phase antituberculous therapy; this study will serve as a base for other studies.

2.3 Factors associated with hepatic and hematologic abnormalities in patients on intensive phase antituberculous therapy

2.3.1. Global perspective

Farazi *et al.* (2014) identified female sex, age more than 50 years, smoking and Iranian nationality as factors associated with hepatic abnormalities in a study undertaken in Iran. The findings by Farazi *et al.* (2014) are in contrast to the findings of Gülbay *et al.*(2006) who found that elderly patients were not at increased risk of hepatotoxicity and male sex was associated with hepatic abnormalities. Radiological extension of TB and history of alcohol consumption were not risk factors (Gülbay *et al.*, 2006). Hassen Ali *et al.*(2013) also found that age was not associated with hepatic abnormalities.

Contrary to the findings by Gülbay *et al.*(2006), Hassen Ali *et al.*(2013) observed that low body mass index and disseminated TB were associated with hepatic abnormalities. Hassen Ali *et al.* (2013) cited low CD4 count as a risk factor for adverse effects among patients with TB and HIV co-infection.

Pande *et al.* (1996) undertook a case control study in India to assess the role of age, sex, and disease extent, nutritional status, past history of liver disease, infection with hepatitis virus, acetylator status and high alcohol intake as risk factors in the development of hepatic abnormalities in patients with pulmonary tuberculosis receiving antituberculous treatment. The findings were that advanced age, high alcohol intake, slow acetylator phenotype and extensive disease were factors associated with development of hepatic abnormalities.

Marra *et al.* (2007) in a population based study carried out in British Columbia cited female sex as a risk factor for developing hepatic abnormalities. Further, Marra *et al.* (2007) stated that increased age and baseline AST levels equal to or greater than 80U/L were predictors of hepatic abnormalities.

2.3.2. Regional perspective

Abera *et al*. (2016) identified alcoholism as a significant risk factor for hepatic abnormalities. Nonetheless, body mass index, extent of TB disease, gender and age were not significantly associated with the abnormalities (Abera *et al*., 2016).

Ekhabbazi *et al.* (2015) reported that age and female sex were significant risk factors for hepatic abnormalities. In addition, Ekhabbazi *et al.* (2015) also identified other risk

factors such as pregnancy, hypoalbuminaemia, multifocal tuberculosis, high transaminase concentration, HIV positive status and chronic liver disease.

2.3.3. Local perspective

There is no documented data regarding factors associated with abnormalities. The current study will provide a baseline for other studies to be undertaken.

CHAPTER 3: METHODOLOGY

This chapter provides details of the study design adopted to address objectives 1 – 3 which required collection of empirical data, together with the research setting, including the study population, sample size, outcome measures, data collection, data analysis and ethical considerations.

3.1. Study design

This was a prospective longitudinal study. This research design is suited for the study because it involved observation of the same subjects over a period of two months. The subjects' sputum samples, hepatic and haematological parameters were observed before commencing anti tuberculous therapy and two months after commencing the therapy.

3.2. Study setting

The study was conducted at Chest Clinic in the Adult Hospital of the University Teaching Hospitals, an 1863 - bed, referral and tertiary - care hospital with about 80,400 admissions per year.

3.3. Study population

The study population comprised all adults aged 18 years old and above enrolled on TB care. Both women and men were a part of the study population. The population was 114.

3.4 Sample size

The sample size for the study was determined as follows:

$$\mathbf{N=Za^2 \times s^2/d^2}$$

$\mathbf{Za^2}$=Confidence interval at 95% CI = 1.96

s = estimated standard deviation (0.15) (Kassa *et al.*, 2016); **d** = desired precision (0.05)

$=1.96^2 \times (0.15)^2 / (0.05)^2 = 3.8416 \times 0.0225/0.0025$

$= 0.086436/0.0025$

$= 34$

Plus 10% loss - to - follow up = 38 participants.

However, due to unforeseen loss to follow-up, one patient had missing data; only 37 participants with complete paired data were included in the data analysis.

3.5. Inclusion criteria

The following patients were included in the study:

- Adults who are 18 years old and above
- New TB patients (first incidence of tuberculosis)
- Pulmonary Tuberculosis
- HIV positive or negative patients

3.6. Exclusion criteria

Patients were excluded from the study at enrollment or at the end of two months following the criteria below.

3.6.1 Exclusion criteria at enrollment

- Patients with hepatitis, cancers
- Patients who took alcohol
- Patients who refused to participate in the study

- Patients with extra pulmonary TB
- Patients with multi-drug resistant TB

3.6.2. Exclusion criteria at the end of 2 months

- Patients who defaulted from treatment
- Patients who dropped out of the study
- Patients who died before follow – up results were obtained
- Patients with missing data

3.7. Sampling method

Patients were enrolled using the systematic sampling method. Every 3rd patient who met the selection criteria was enrolled. The sampling interval was arrived at by dividing the study population by the sample size.

3.8. Data collection and tool

The demographic information such as the age, weight, gender was obtained from the clinical records of the patient and patients themselves. This was entered on a data collecting tool (Appendix C). Venous blood samples were drawn before initiation of the antituberculous medicines for FBC and LFTs (ALT/AST). Sputum samples were also obtained for Xpert® MTB/RIF assay and Sputum direct microscopy. Results were noted in the data collection tool. The patients' current therapy and HIV status were also noted.

The patients were then initiated on antituberculosis medicines. After two months, venous blood samples were again collected for FBC and LFTs. Clinical laboratory test results were noted and written down on the data collection sheet.

The DAIDS (Version 2.1 – March 2017) adverse events grading system shown in table 1 was used to grade the severity of the abnormalities (Division of AIDS, 2017).

Table1. The 2017 DAIDS Table for Grading the Severity of Adult and Paediatric Adverse Events

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
ALT/AST, High	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Hemoglobin, Low (g/dL) ≥ 13 years of age (male only)	10.0 to 10.9	9.0 to < 10.0	7.0 to < 9.0	< 7.0
≥ 13 years of age (female only)	9.5 to 10.4	8.5 to < 9.5	6.5 to < 8.5	< 6.5
WBC, Decreased (cells/L) > 7 days of age	2.000 x 10^9 to 2.499 x 10^9	1.500 x 10^9 to 1.999 x 10^9	1.000 x 10^9 to 1.499 x 10^9	< 1.000 x 10^9
Platelets, Decreased (cells/L)	100 x 10^9 to < 125 x 10^9	50 x 10^9 to < 100 x 10^9	25 x 10^9 to < 50 x 10^9	< 25 x 10^9

Source: DAIDS AE Grading Table Version 2.1- March 2017 (Division of AIDS, 2017).

3.9. Outcome measures

In this study, the outcome measures were the LFTs (ALT/AST) and FBC - level of white cell count and differential counts, haemoglobin concentration, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, and packed cell volume, red cell count and red cell distribution width, platelet count, platelet distribution width and mean platelet volume. The measures are summarized in table 2 below.

Table 2: Table showing outcome measures

Outcome measure	Definition	Scale of Measurement
Age	18 years and above	Continuous
Gender	Male or female	Categorical
White blood cell count	Reduced or increased	Continuous/categorical for grading
Monocyte count	Reduced or increased	Continuous
Lymphocyte count	Reduced or increased	Continuous
Eosinophil count	Reduced or increased	Continuous
Red blood cell count	Reduced or increased	Continuous
Platelet count		Continuous/categorical for grading
Hemoglobin concentration	Less than12g/dl in females Less than 13g/dl in males	Continuous/categorical for grading
Mean cell hemoglobin concentration	Reduced or increased	Continuous
Mean cell volume	Reduced or increased	Continuous
Aspartate aminotransferase	Normal or > 40mmol/l	Continuous/categorical for grading
Alanine aminotransferase	Normal or > 45mmol/l	Continuous/categorical for grading
Body mass index	Underweight, Normal, overweight	Continuous
Sputum smear status	Smear positive or negative	Categorical
HIV status	HIV positive or negative	Categorical
HIV treatment status	On treatment or not on treatment	Categorical

3.10. Data management and storage

During data collection, completed data collection tools were checked regularly to rectify any discrepancy, logical errors or missing values. Participants' data were entered into a log book and later keyed into a computer using Microsoft excel 2013 and verified for the possibility of entering errors. The data were encrypted to protect it from theft/unauthorized access then backed up to avoid loss.

3.11. Data analysis

Data were analysed using GraphPad Prism version 8.0.1 (GraphPad Software Inc., La Jolla, CA, USA). The age, liver function tests and haematological parameters being continuous parameters were measured using measures of central tendency and central dispersions. Their means, medians, and standard deviations were calculated.

Categorical parameters such sputum smear status, HIV status, HIV treatment status and gender were expressed using proportions and percentages.

The hepatic and haematological parameters at baseline and follow-up were compared using paired t – test for normally distributed continuous variables and Wilcoxon matched - pairs signed rank test for non – normally distributed continuous variables. Shapiro – Wilk test was used to test for normality of the data distribution. The ROUT (robust regression followed by outlier identification) method was used to detect outliers with Q (maximum desired false discovery rate) set to 1 per cent. Significant outliers were excluded from the data set and the results of the analyses of data without outliers were also reported. Binary logistics regression was performed using SPSS version 22.0 (IBM Corp. Released 2013.Armonk, NY, USA) to determine the effects of age, gender,

BMI, HIV infection, ART, sputum smear status, and appropriate baseline FBC/LFT parameters on the likelihood of study patients having deranged Hb, WCC and ALT at follow-up. A p value < 0.05 was considered statistically significant.

3.12. Ethical considerations

Permission to conduct the research project was sort from the director at the Adult Hospital of the University Teaching Hospitals. The research proposal was reviewed by UNZABREC and formal ethical approval was granted (Reference number 002-12-17). To ensure confidentiality of participants, names of participants were not written on the data collection sheet. To identify participants during the follow - up visit at the end of the intensive phase and link participants to their baseline results, file numbers were written down on the data collection tool. The data collection tools were kept securely by the researcher. All the data were collected after written informed consent was obtained from each study participant.

3.13. Limitation of the study

One patient was lost to follow hence there was missing data which was not included in the data analysis. Data analysis was only carried out on complete paired data.

The researcher had no control on influence of other drugs taken by patients during follow-up which might affect hepatic and haematological parameters including other unidentified confounders.

The effects of individual antituberculous drugs on hepatic and haematological parameters were not explored.

CHAPTER 4: RESULTS

4.1. Demographics of study patients

A total of 37 patients were included in this study. 21 (56.8 percent) patients were male. The mean age of the patients was 36.2 years (19 – 57 years) while their mean BMI was 21.9 kg/m^2 (17 – 29.3kg/m^2). Only 14 (37.8 percent) patients were sputum smear positive at baseline. 21 (56.8%) patients had HIV co-infection. Only 17 (45.9 percent) patients were on ART. Table 3 below summarizes the demographics of the patients and figures 2 and 3 show the age and BMIs of the patients.

Table3: Summary of patient demographics.

Gender	**N (%)**
Male	21 (56.8)
Female	16 (43.2)
Sputum smear status at baseline	
Positive	14 (37.8)
Negative	23 (62.3)
HIV status	
Positive	21 (56.8)
Negative	16 (43.2)
HIV treatment status	
On ART	20 (54.1)
Not on ART	17 (45.9)

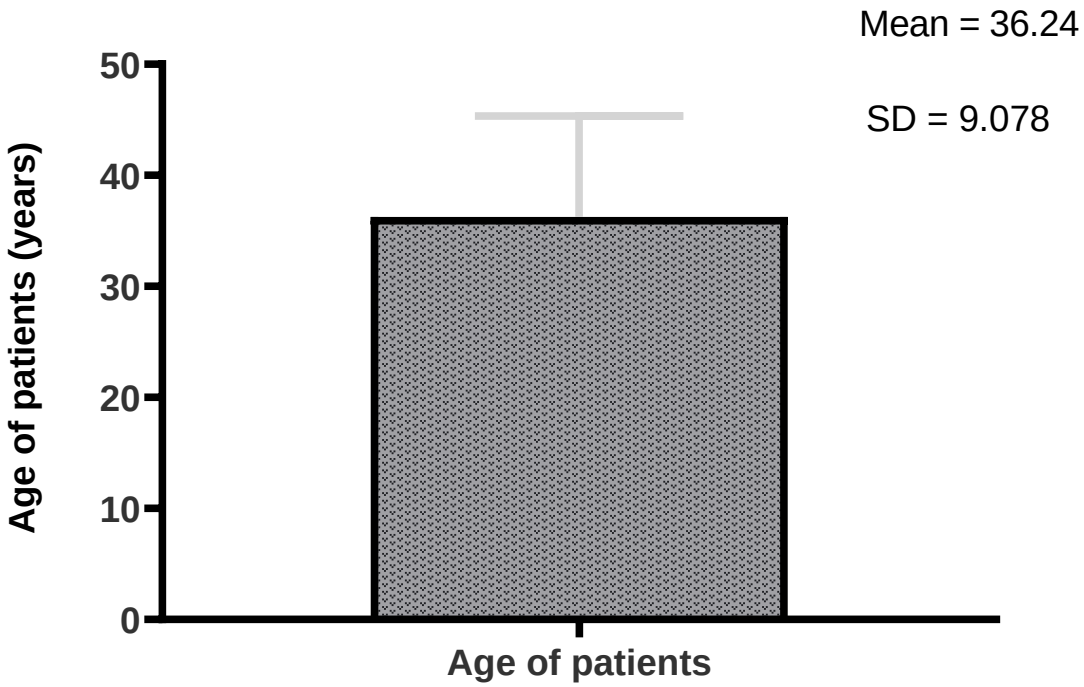

Figure 2: Age (mean with standard deviation) of patients

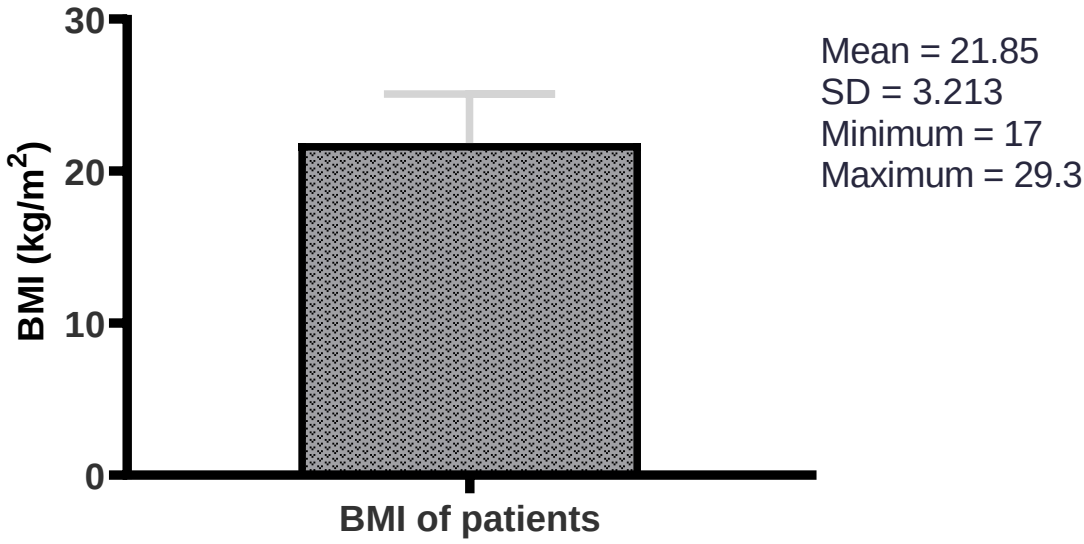

Figure 3: BMI (mean with standard deviation) of patients

4.2 Haematological and hepatic abnormalities in patients

4.2.1 Hepatic abnormalities at baseline and follow-up

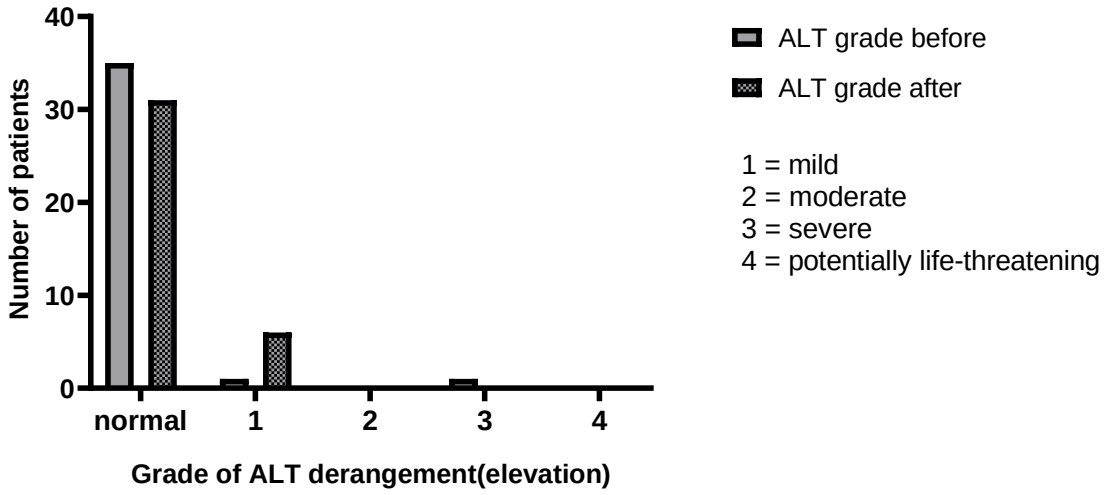

Figure 4: ALT grades of patients before and after treatment

Of the 37 patients that were included in the study, 35 (94.6 percent) of them had normal ALT levels at baseline. At follow – up, the number of patients with normal ALT levels reduced to 31 (83.8 percent) and more patients had an elevation 6 (16.2 percent) compared to the number at baseline 2 (5.4 percent). None of the patients that had elevated ALT concentration at baseline had an elevation at follow – up (Figure 4).

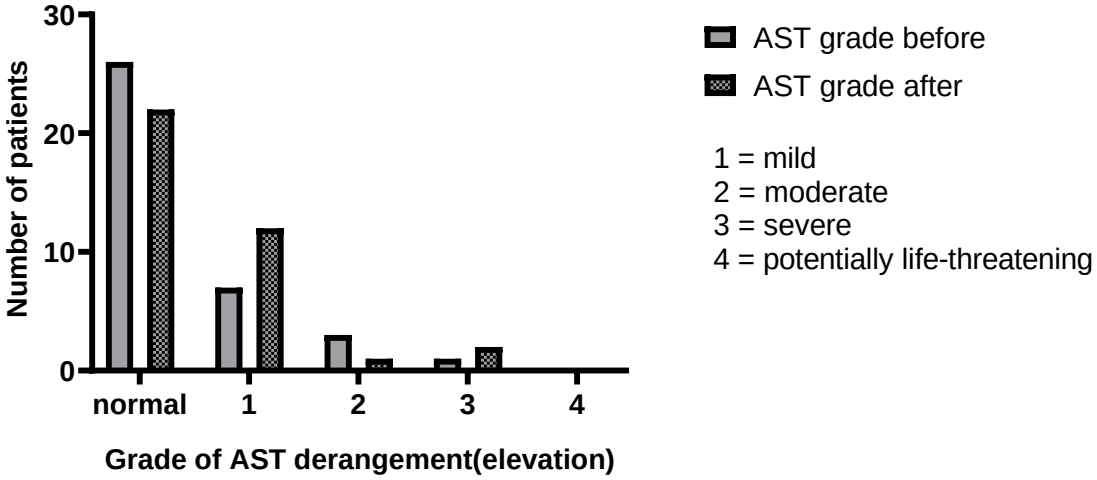

Figure 5: AST grades of patients before and after treatment

A reduction in the number of patients with normal AST concentration at follow – up 22 (59.5 percent) was recorded. This is compared to the higher number at baseline 26 (70.3 percent). The proportion of patients who had elevated AST concentration at baseline was 11 (29.7 percent).This increased to 15 (40.5 percent) at follow - up. Of the patients that had increased AST concentration at follow - up, 7 had already elevated AST at baseline and 8 developed an increase during therapy.

Of the 15 patients with elevated AST at follow – up, 6 (40 percent) were HIV negative and 9 (60 percent) were HIV positive, of the HIV positive patients, 7 Commenced HAART a few weeks before initiating anti – tuberculous therapy and 2 commenced a few weeks after starting TB treatment.

4.2.2 Haematological abnormalities at baseline and at follow-up

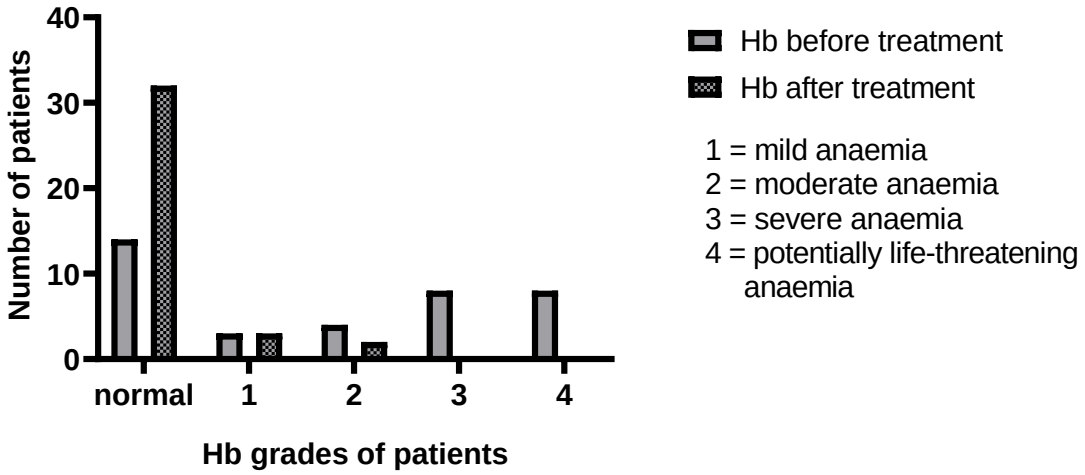

Figure 6: Hb grades of patients before and after treatment

A majority of the patients presented with anaemia at baseline 23 (62.2 percent) and the number reduced at follow - up 5 (13.5 percent), among the patients presenting with anaemia at follow – up, only one was newly diagnosed with anaemia. Out of the 6 patients who had elevated ALT at follow – up, 3 (50 percent) were HIV negative and 3 (50 percent) were HIV positive, of the HIV positive patients, two were on HIV treatment at the time of initiating anti – tuberculous drugs and one was initiated on HAART a few weeks after commencing TB treatment.

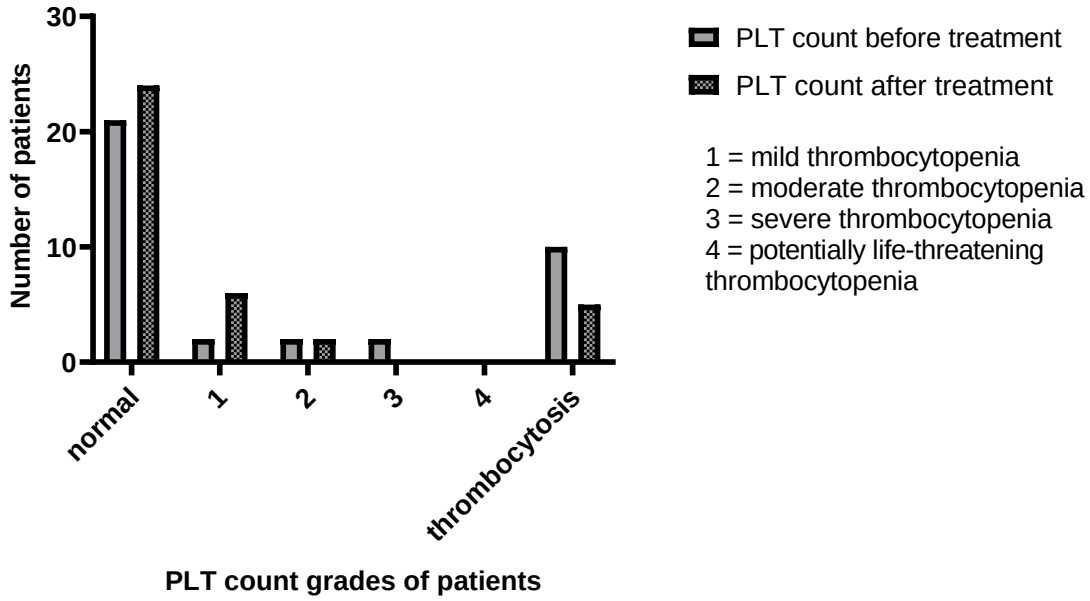

Figure 7: PLT grades of patients before and after treatment

More patients had thrombocytopenia at follow – up 13 (35.1 percent) compared to the number at baseline 6 (16.2 percent) and less patients had thrombocytosis at follow – up 5 (13.5 percent) compared to the proportion 10 (27 percent) at baseline. Of the patients that had thrombocytopenia, 4 had normal platelet count at baseline and among the patients that had thrombocytosis at follow – up, 2 had normal platelet count.

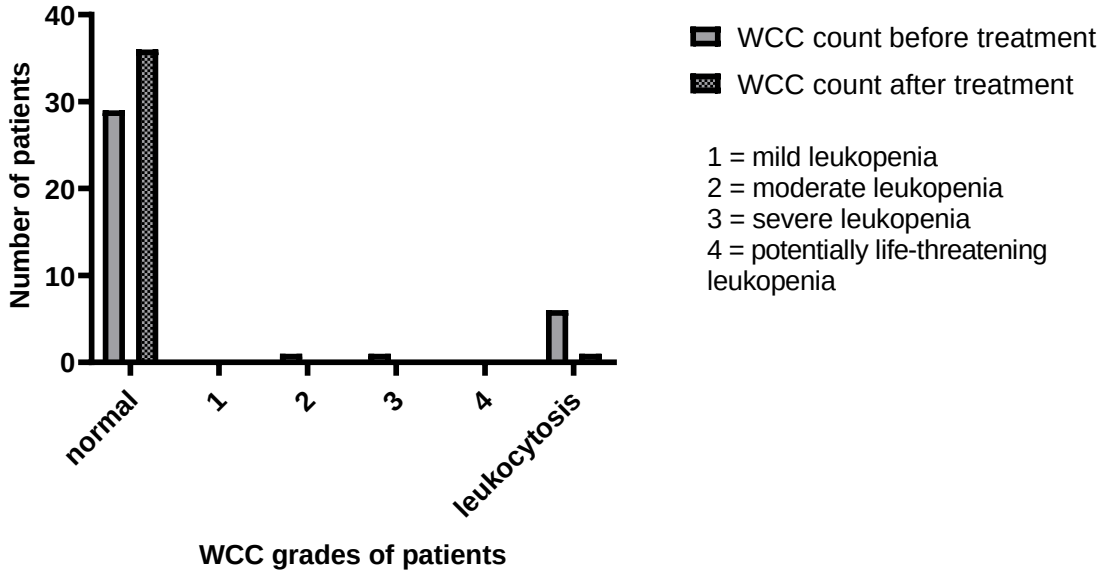

Figure 8: WCC grades of patients before and after treatment

Twenty nine (29) patients (78.4 percent) had normal white cell counts at baseline. And at follow - up, the number of patients with normal white cell count had increased to 36 (97.3 percent). At baseline 2 (5.4 percent) of the patients had leukopenia but none of them had it at follow – up. Leukocytosis was present in 6 (16.2 percent) of the patients at baseline and only 1 (2.7 percent) had it at follow – up.

4.3. Differences in hepatic and haematological parameters among patients at baseline and follow - up

4.3.1. Differences in hepatic parameters among patients at baseline and follow-up

The Wilcoxon matched-pairs sign ranked test was performed on ALT and AST concentrations to compare their medians at baseline and follow - up.

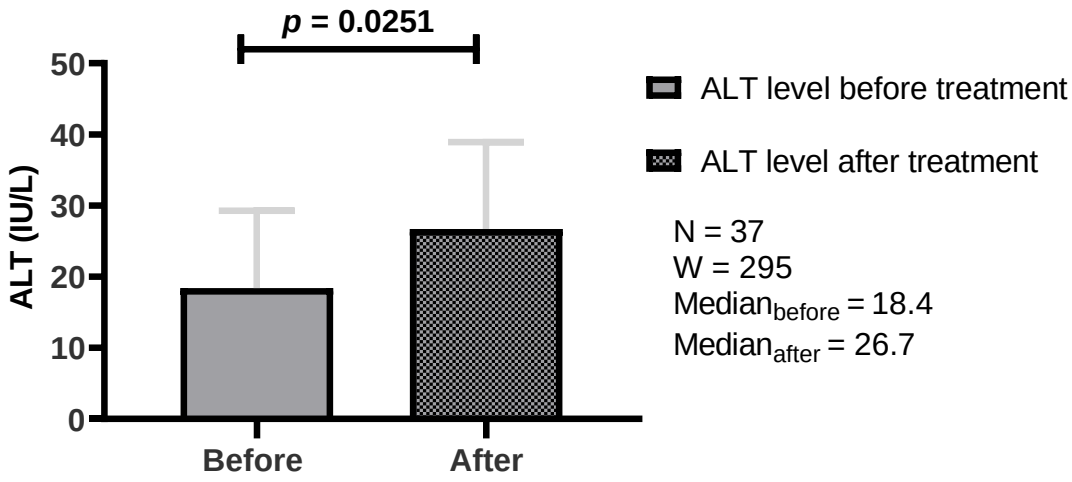

Figure 9: ALT level (median with interquartile range) of patients before and after treatment

A statistically significant increase in ALT was observed (p = 0.0251, baseline median 18.4, follow – up median 26.7). Further, when the analysis was repeated after two outliers were identified and removed, the increase in ALT was still statistically significant (p = 0.002).

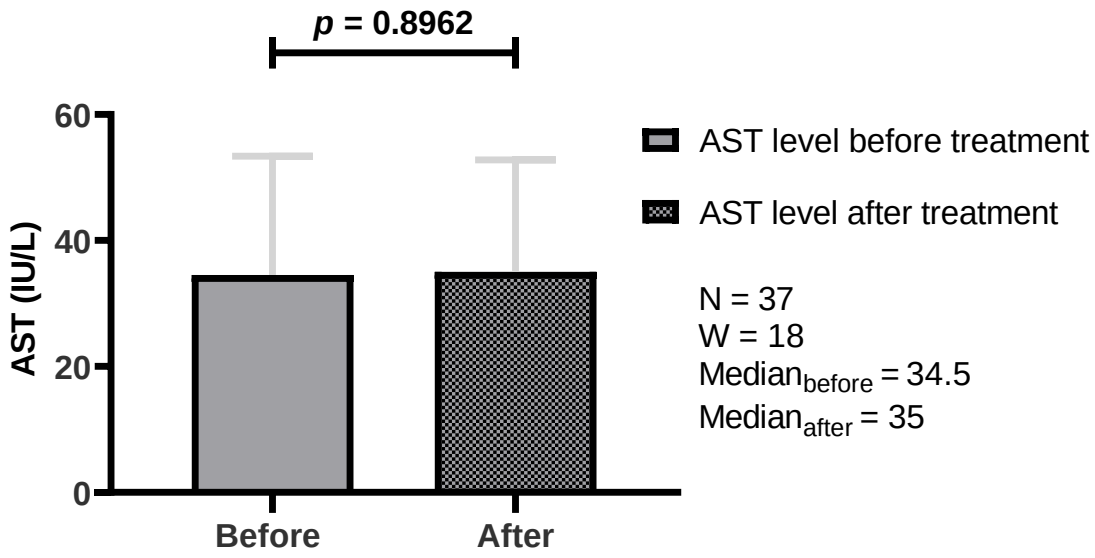

Figure 10: AST level (median with interquartile range) of patients before and after treatment

An increase in AST was observed but it was not statistically significant (p = 0.8962, median before treatment = 34.5, median after treatment = 35). There was no statistically significant difference in AST before and after treatment (p = 0.7503) following exclusion of five outliers from the analysis.

4.3.2. Differences in haematological parameters at baseline and follow-up

Paired t – test was performed on Hb concentration, HCT and RCC to compare their means at baseline and at follow – up.

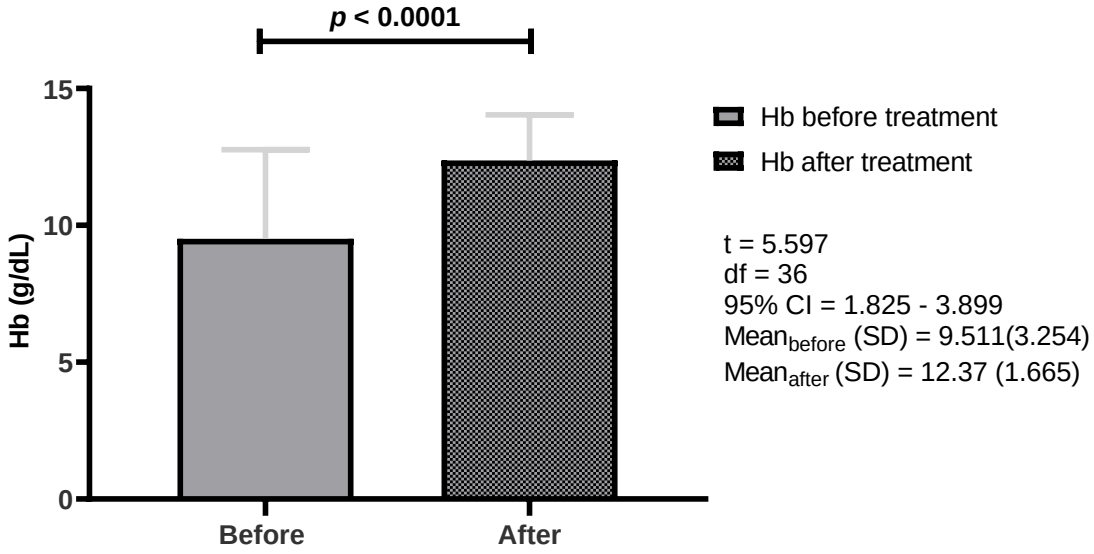

Figure 11: Hb level (mean with standard deviation) of patients before and after treatment

Statistically significant increase in haemoglobin concentration was recorded (baseline mean 9.51, follow – up mean 12.37, $p < 0.0001$).

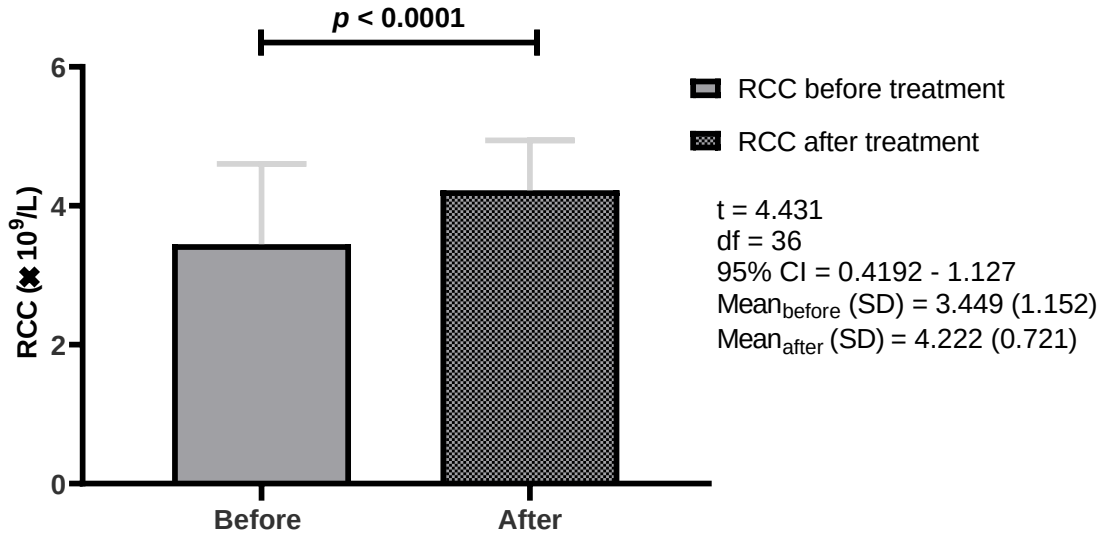

Figure 12: Red cell count (mean and standard deviation) of patients before and after treatment

Statistically significant increase in red cell count (baseline mean 3.25, follow – up mean 4.22, $p < 0.0001$).

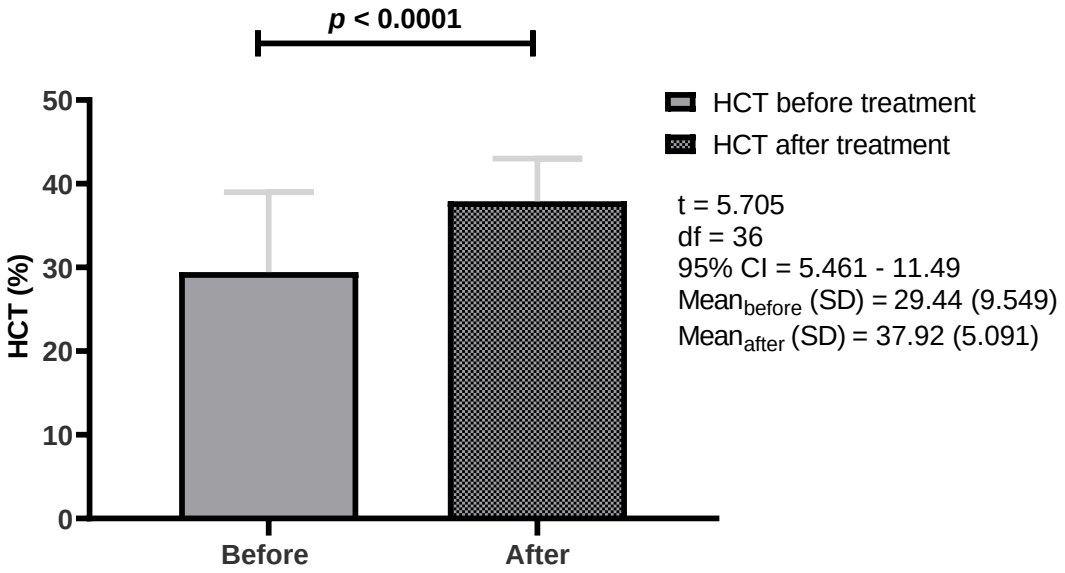

Figure 13: Haematocrit (mean with standard deviation) of patients before and after treatment

Statistically significant increase in hematocrit was recorded (baseline mean 29.44, follow – up mean 37.92, $p < 0.0001$).

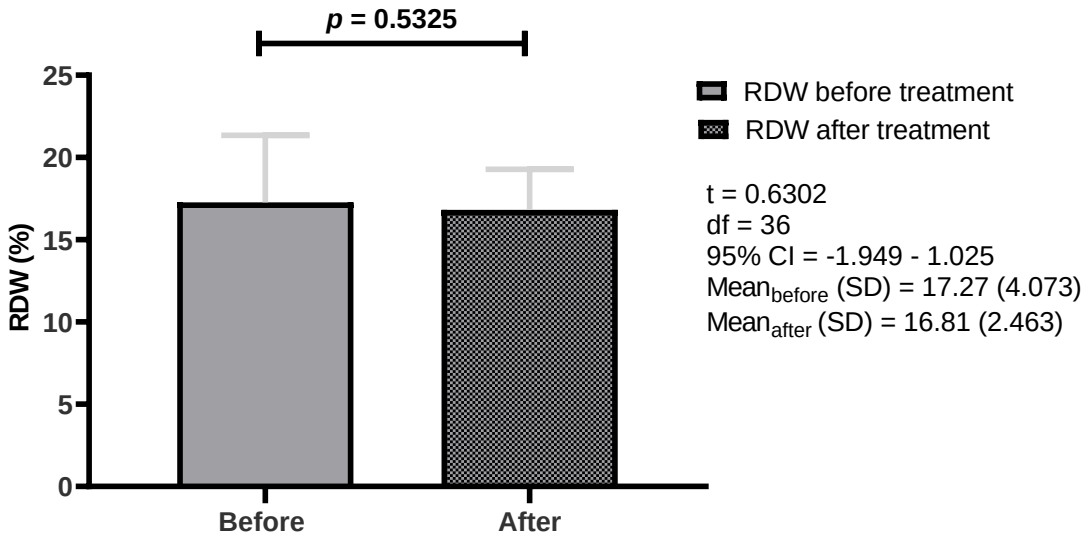

Figure 14: Red cell distribution width (mean with standard deviation) of patients before and after treatment

A non-statistically significant decrease in the red cell distribution width was recorded (baseline mean 17.27, follow – up mean 16.81, $p = 0.5325$).

4.3.2.1. Differences in red cell indices at baseline and follow-up

Paired t – test was performed on MCHC and MCV and Wilcoxon matched - pairs signed rank test was performed on MCH.

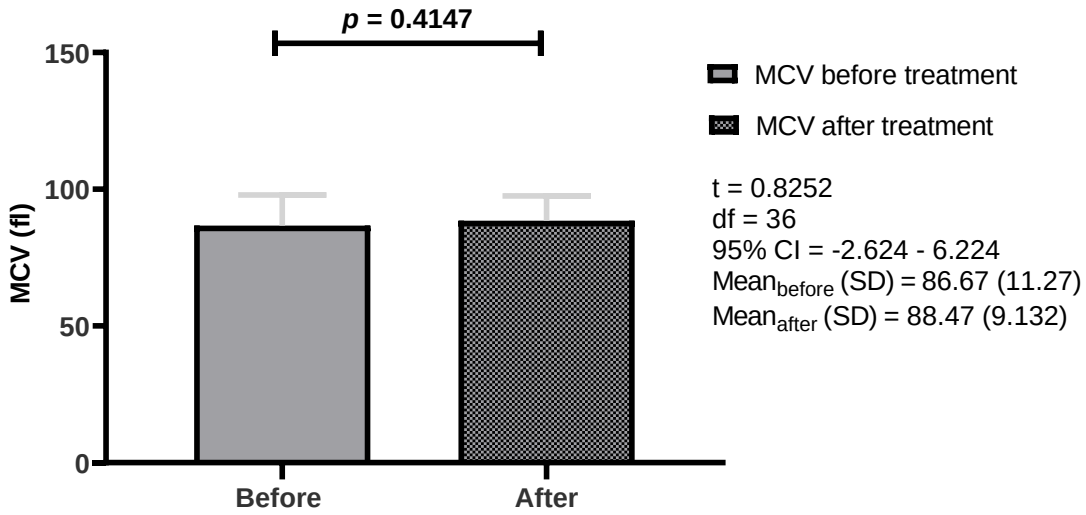

Figure 15: Mean cell volume (mean with standard deviation) of patients before and after treatment

No statistically significant differences were found in the red cell indices MCV was recorded (baseline mean 86.67, follow – up mean 88.47, $p = 0.4147$).

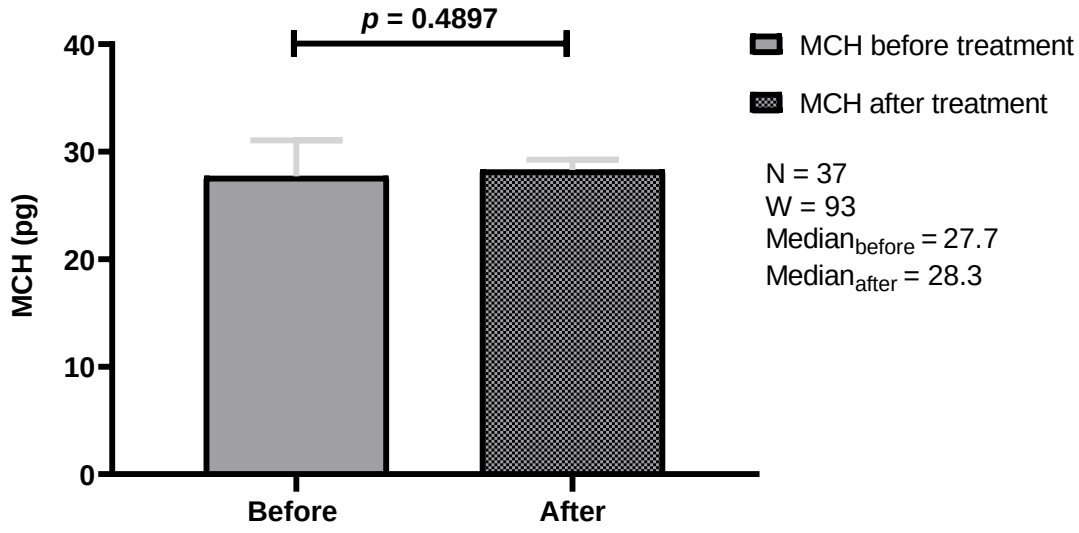

Figure 16: Mean cell haemoglobin (median with interquartile range) of patients before and after treatment

Non – statistically significant increase in MCH was recorded at follow – up (baseline median 27.7, follow – up median 28.3, p = 0.4897). Two outliers were detected in MCH, however, results of the analysis after exclusion of these outliers revealed that there was still no statistically significant difference in MCH before and after treatment (p = 0.4577).

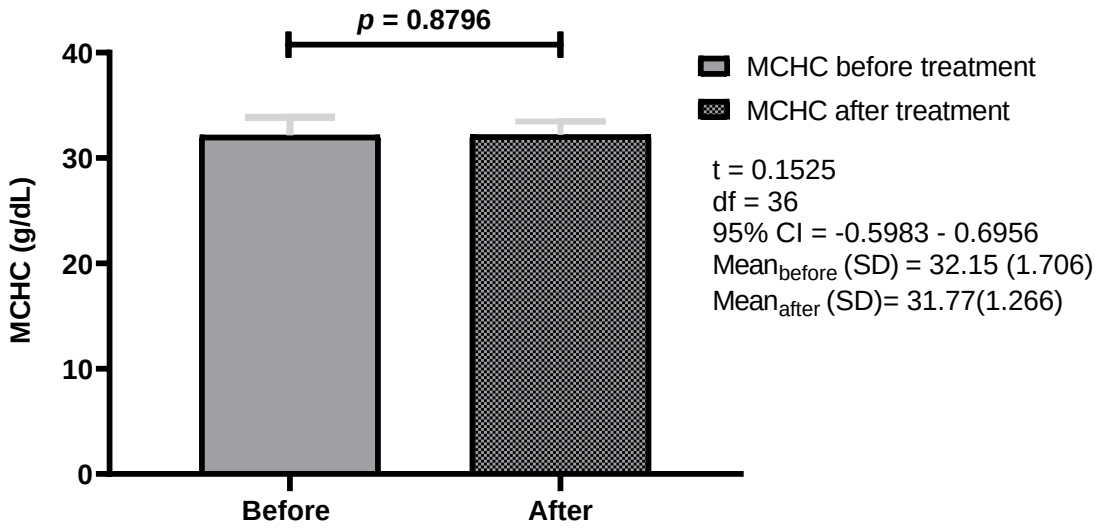

Figure 17: Mean cell haemoglobin concentration (mean with standard deviation) of patients before and after treatment

Non – statistically significant reduction in MCHC was recorded (baseline mean 32.15, follow – up mean 31.77, p = 0.8796).

4.3.2.2. Differences in platelet counts and platelet indices at baseline and follow-up

Paired t – test was performed on the platelet count, mean platelet volume and platelet distribution width.

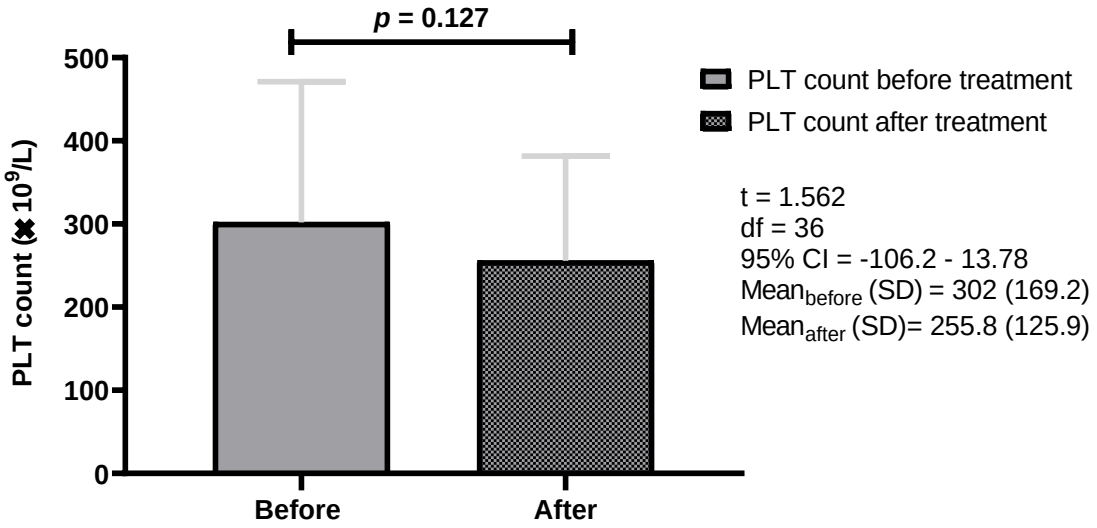

Figure 18: Platelet count (mean with standard deviation) of patients before and after treatment

Non – statistically significant reduction in the platelet count was observed (baseline mean 302, follow – up mean 255.8, $p = 0.127$)

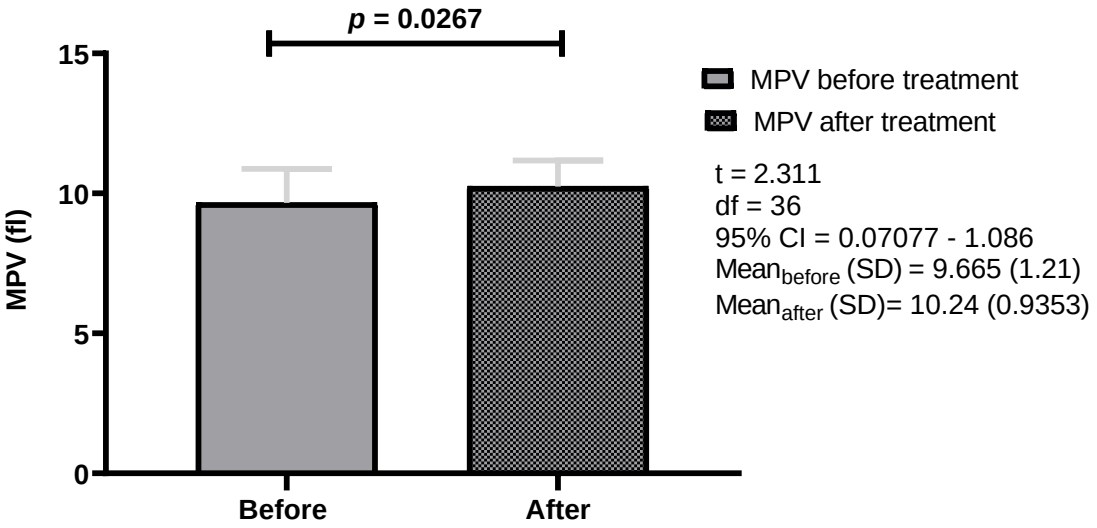

Figure 19: Mean platelet volume (mean with standard deviation) of patients before and after treatment

There was a statistically significant increase in the mean platelet volume (baseline mean 9.67, follow – up mean 10.24, $p = 0.027$).

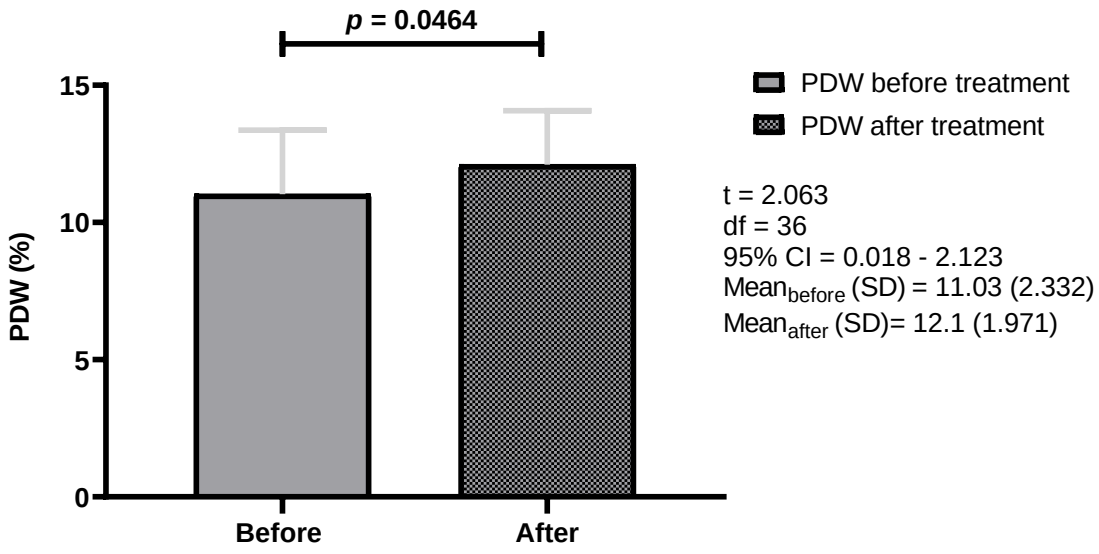

Figure 20: Platelet distribution width (mean with standard deviation) before and after treatment

There was a statistically significant increase in the platelet distribution width (baseline mean 11.03, follow – up mean 12.10, $p = 0.046$).

4.3.2.4 Differences in white cell counts and differential white cell counts at baseline and follow-up

Paired t – test was performed on the white cell count and eosinophil count and the Wilcoxon matched – pairs signed rank test was performed on the neutrophil, basophil, lymphocyte and monocyte counts.

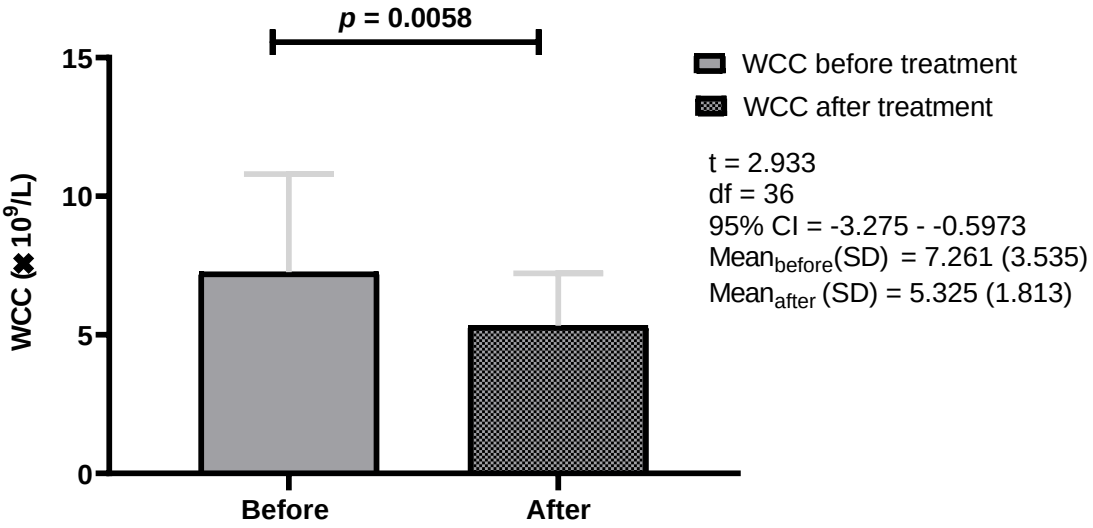

Figure 21: White cell count (mean with standard deviation) of patients before and after treatment

A statistically significant reduction in white cell (baseline mean 7.26, follow - up mean 5.33, $p = 0.0058$) was observed after treatment.

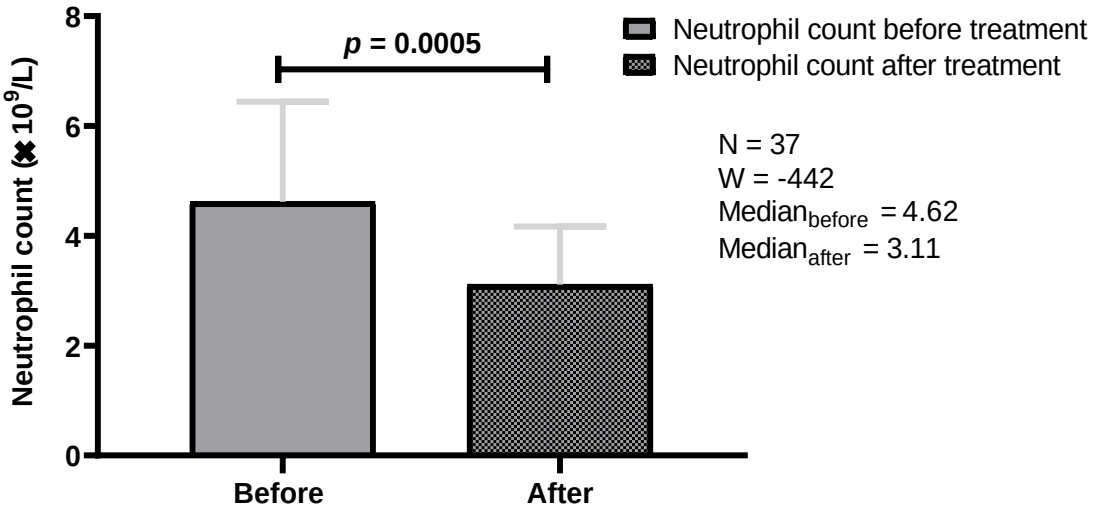

Figure 22: Neutrophil count (median with interquartile range) of patients before and after treatment

A statistically significant reduction in neutrophil count (baseline median 4.62, follow – up median 3.11, $p = 0.0005$) was observed after treatment. The difference in neutrophil count before and after treatment was still statistically significant ($p = 0.0022$) even after exclusion of two outliers from the analysis.

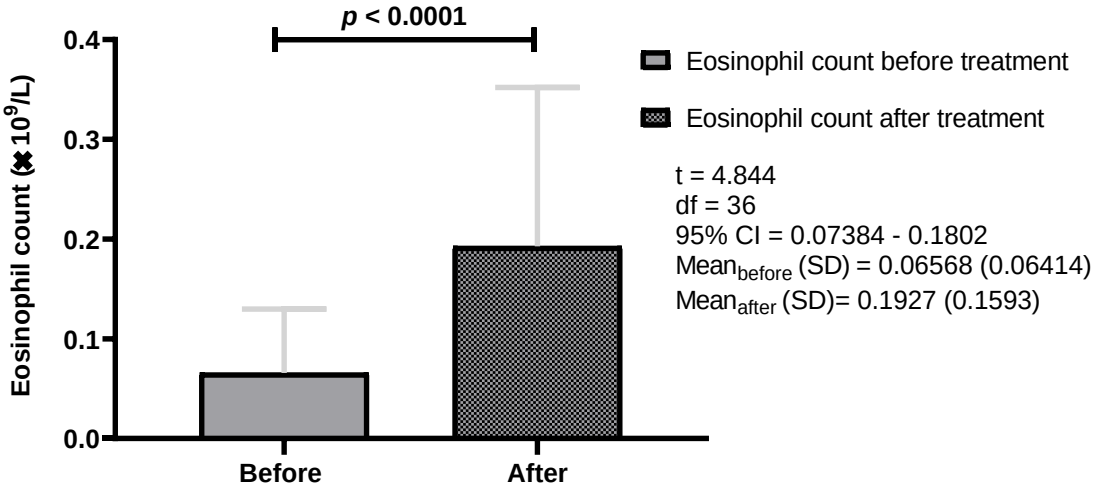

Figure 23: Eosinophil count (mean with standard deviation) of patients before and after treatment

A statistically significant increase in eosinophil count (baseline mean 0.07, follow – up mean 0.19, $p < 0.0001$) was observed after treatment. The increase in eosinophil count was statistically significant ($p < 0.0001$) even after exclusion of one outlier from the analysis.

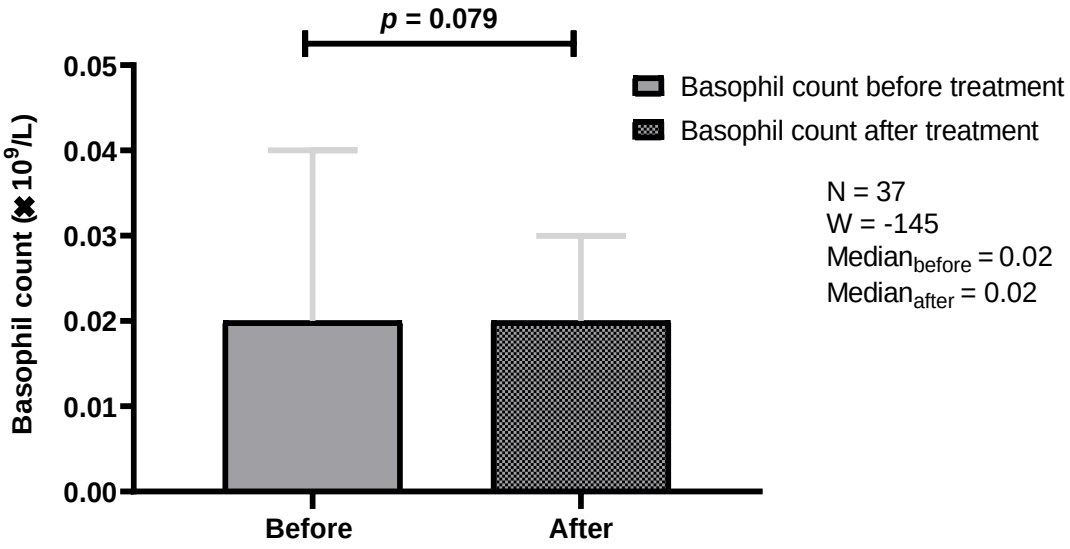

Figure 24: Basophil count (median with interquartile range) of patients before and after treatment

There was no change in the basophil count after treatment (baseline median 0.02, follow – up median 0.02, $p < 0.079$). The lack of change in the basophil count was statistically significant ($p < 0.034$) after exclusion of four outliers from the analysis.

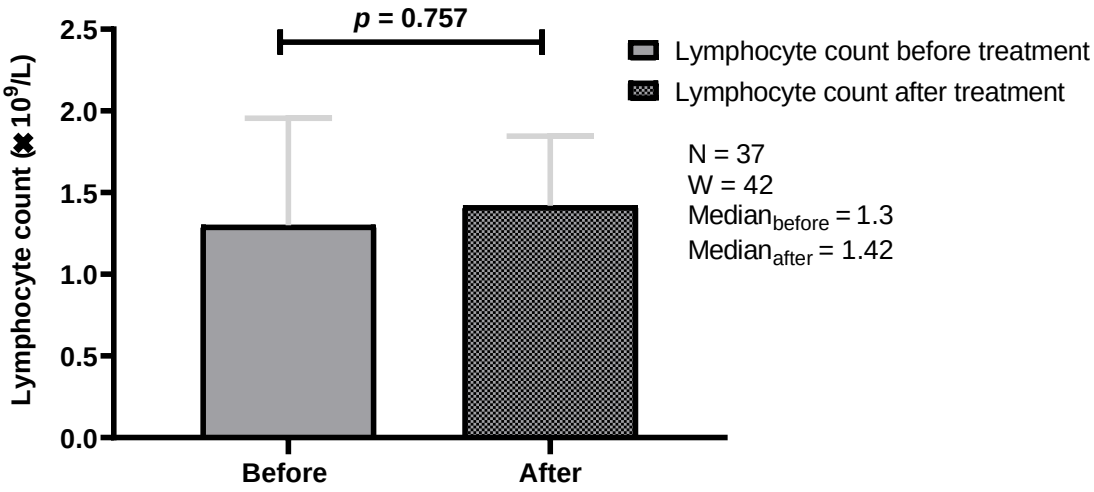

Figure 25: Lymphocyte count (median with interquartile range) of patients before and after treatment

The increase in lymphocyte count observed after treatment was not statistically significant (baseline median = 1.3, follow – up median = 1.42, p = 0.757). After exclusion of one outlier from the analysis, the increase in lymphocyte count was still not statistically significant (p = 0.816).

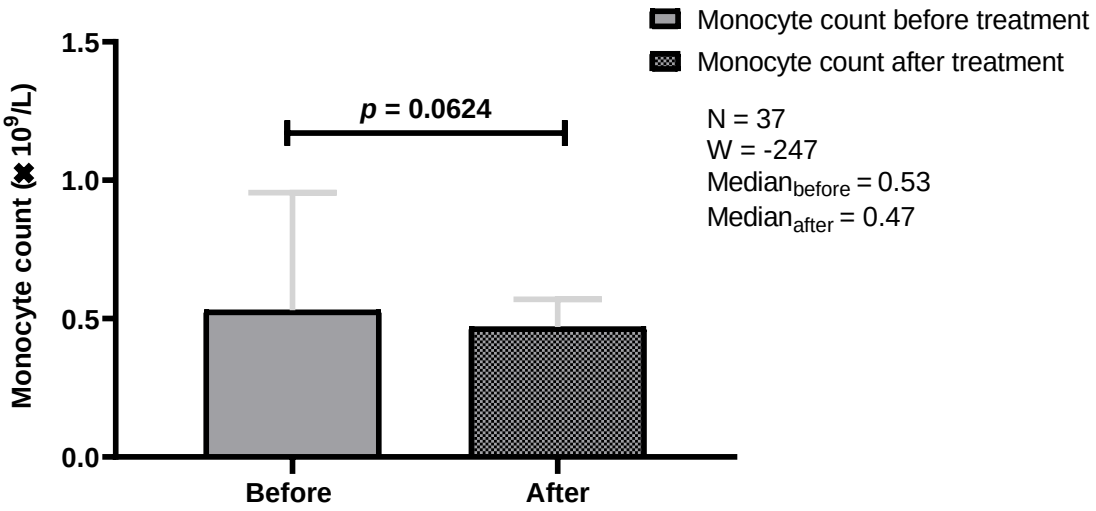

Figure 26: Monocyte count (median with interquartile range) of patients before and after treatment

The decrease in monocyte count (baseline median 0.53, follow - up median 0.47, p = 0.0624) observed after treatment was not statistically significant. After exclusion of one outlier from the analysis, the reduction in monocyte count was still not statistically significant (p = 0.0452).

4.4. Predicators of the abnormalities

Logistic regression was carried out to determine predictors of the statistically significant derangements in haemoglobin and ALT concentrations and white blood cell count. The model was not statistically significant for determining derangements in haemoglobin and white cell count (χ^2 =11.315, p =0.125, R^2 = 0.482, PAC=86.5 percent and χ^2 =9.195, p =0.239, R^2 = 1.000, PAC= 100.0 percent). The model was statistically significant for determining the association of covariates BMI, age, and sputum smear status at baseline, ALT level at baseline, HIV status and HIV treatment status. It correctly predicted 89.2% of the cases with a variance of 0.550 (χ^2 =14.441, p = 0.044, R^2 = 0.550, PAC=89.2 percent). There was no association between the increases in ALT concentration at follow - up with any of the covariates.

CHAPTER 5: DISCUSSION

5.1 Characteristics of study patients

The study showed that the majority of TB patients were young adults with a mean age of 36.2 years and age range of 19 – 57 years signifying the most productive population. This is similar to the findings by Bashir *et al*. (2015) and Kassa *et al.* (2016) whose studies included patients with mean age of 33 and 34.8 years, respectively. Yaranal *et al*. (2013) undertook a study in patients with a mean age of 41 years. The reasons for the higher prevalence of TB in the young adult age group could be due to HIV infection, a known immunosuppressant which is also more prevalent in this age group (Kapata *et al*., 2016). In addition, younger adults could be exposed to other factors that might predispose them to TB infection such as tobacco smoke and overcrowded environments.

Most of the patients were males accounting for 56.8 percent. A similar finding has been reported by several other studies (Koju *et al.*, 2005; Yaranal *et al*., 2013; Bashir *et al*., 2015; Enoh *et al.*, 2017). The findings of the present study are, however, different from those described by Marra *et al.* (2007) who included an equal number of male and female patients in their study. The findings of the present study with respect to gender distribution are also similar to those reported by the Zambia TB prevalence survey which found more males with tuberculosis (Kapata *et al.*, 2016). The similarity of the findings could be due to the fact that globally, more men have TB (Nhamoyebonde and Leslie, 2014). Nhamoyebonde and Leslie (2014) contend that the relationship between male sex and tuberculosis risk is less clear and is likely to involve a highly complex network of factors. The differences in the findings may be because the degree of male bias varies by geographic location and by year, however, the overall trend is clear, and

of the 20 high-burden countries for which data are available, more males have been recorded to have TB (Nhamoyebonde and Leslie, 2014).

A large number of the patients (56.8 percent) had HIV co – infection as highlighted by the Zambia TB prevalence survey. This could be due to lowered immune function among HIV patients which leads to activation of latent tuberculosis as HIV infection causes suppressed cell - mediated immunity (Getahun *et al*., 2015). The majority of patients (62.2 percent) had sputum smear negative tuberculosis. Montales *et al.* (2015) explain that HIV co-infection may alter the pathogenesis of tuberculosis and lead to negative sputum smear results.

Tuberculosis generally presents with constitutional signs and symptoms such as cough which may be dry initially then productive later, night sweats, loss of appetite and weight loss. Therefore, it is anticipated that TB patients may have lower BMIs. The present study found that patients' BMI ranged from 17 Kg/m^2 to 29.3 Kg/m^2 with a mean BMI of 21.9 Kg/m^2.These findings are similar to those reported by Abera *et al*. (2016) who found a mean BMI of 20.6 Kg/m^2 with a range of 17.08 Kg/m^2 to 24.06 Kg/m^2. The results of the present study, however, are in contrast to those found by Hassen Ali *et al.* (2013) and Nagu *et al.* (2014) where the majority of the patients (81.8 percent and 51 percent, respectively) had BMI less than 18.5 Kg/m^2. This could be because patients might have not promptly sought medical care before the effects of TB on loss of appetite were very pronounced.

5.2 Hepatic and haematological abnormalities among patients before and after treatment

Antituberculous therapy is known to cause a number of adverse effects that cause abnormalities in the haematological and hepatic parameters.

5.2.1 Hepatic abnormalities among patients before and after treatment

Antituberculous medicines are potentially hepatotoxic (Joint Formulary Committee, 2018). The medicines associated with hepatotoxicity include pyrazinamide, isoniazid and rifampicin in decreasing order of their hepatotoxic potential. Hepatotoxicity manifests as an elevation in liver enzymes namely AST and ALT. Elevated ALT is considered a more specific marker of hepatocellular injury (Giannini *et al*., 2005).

In the present study, 5.4 percent of the patients had deranged ALT before treatment while 16.2 percent of the patients were found to have elevated ALT levels after treatment. The median baseline ALT level was 18.4IU/L while the median follow-up ALT level was 26.7 IU/L. The findings of the present study are different from those highlighted by Hassen Ali *et al.* (2013) and Shang *et al.* (2011). In these two studies, only patients with normal baseline ALT levels were enrolled. Hassen Ali *et al.* (2013) found that 61.5 percent of the patients had elevated ALT at follow – up. This proportion of patients with elevated ALT was much higher than that noted in the present study. This could be because the study by Hassen Ali *et al.* (2013) had a larger sample size than the present study. Shang *et al.* (2011) found that 4.73 percent of the patients had elevated ALT at follow – up. The proportion of patients with elevated ALT was lower than that of the present study. This could be because of differences in the metabolism of

isoniazid as slow metabolizers are prone to hepatic abnormalities and fast metabolizers are less prone (Gonzalez *et al*., 2018). None of the patients in the present study had moderate to severe elevations in ALT after treatment.

The ALT level in the present study was non – normally distributed, therefore, median ALT levels were determined. This is different from the distribution reported in other studies (Pande *et al.*, 1996; Abera *et al*., 2016) which determined mean ALT levels. ALT levels observed in the present study were much lower than those found by Pande *et al.* (1996) and Abera *et al*. (2016). In these studies, the elevation in the ALT level was not graded.

With respect to AST, 29.7 percent of the patients in the present study had elevated AST levels before treatment whereas 40.5 percent of the patients were found to have raised AST levels after treatment. These findings are different from those reported by Hassen Ali *et al.* (2013) and Shang *et al.* (2011) who found the proportion of patients with elevated AST to be 11.1 percent and 1.3 percent, respectively.

AST was non - normally distributed hence the median baseline and follow – up levels were determined. The AST was normally distributed in the studies conducted by Pande *et al.* (1996) and, Abera *et al*. (2016) therefore they determined the mean baseline and follow – up AST levels. They both reported much higher values than those observed in the present study. The mean baseline values in the two studies were 44.7IU/L and 21.60IU/L respectively while their corresponding follow – up values were 411.2IU/L and 261.80IU/L respectively.

The elevation in ALT level was found to be statistically significant (p = 0.0251) in the present study. This finding was in agreement with that of Koju *et al.* (2005) who found statistically significant increase in ALT. Other studies (Pande *et al.*, 1996; Shang *et al.*, 2011; Hassen Ali *et al.*, 2013; Abera *et al*., 2016) did not state the statistical significance of their findings. The elevation in AST level was found to be statistically non – significant in the present study. This finding is in contrast with that of Koju *et al.* (2005).

Generally, the differences in the findings could be due to the difference in the adverse effect severity grading systems that were used. The present study applied the DAIDs system while other studies used the WHO grading system. The DAIDs grading system was preferred as it is more recent and has been used in several clinical trials.

5.2.2. Haematologic abnormalities among patients before and after treatment

Anti – tuberculous drugs have the potential to cause haematological abnormalities such as anaemias (hemolytic, aplastic and sideroblastic), thrombocytopenia and agranulocytosis (Joint Formulary Committee, 2018).

The findings of the present study show a decrease in the proportion of patients with anaemia at follow – up (13.5 percent) compared to 62.2 percent at baseline. This is in contrast with the findings of Enoh *et al.* (2017) and Kassa *et al.* (2016) who found that 37.1 percent and 72 percent of patients had anaemia at follow-up, respectively. The differences in the findings could be due to differences in patients' genetic predisposition to adverse effects due to variable metabolism of antituberculous drugs in the different settings. Patients' anaemia in the current study improved as they may have had anaemia

of chronic illness which improved as the infection resolved (Bashir *et al*., 2015). In the studies conducted by Enoh *et al*. (2017) and Kassa *et al*. (2016), the increase in the proportion of patients with anaemia at follow – up was attributed to the adverse effect of antituberculous drugs.

The findings of the present study showed an increase in the red cell count and hematocrit and a reduction in the red cell width. The findings are in line with those of Kassa *et al.* (2016) regarding red cell distribution width and contrasted regarding the hematocrit. Kassa *et al.* (2016) did not look at the red cell count while other studies reviewed did not reporting their findings on the RCC, RDW and HCT.

The present study analysed the changes in red cell indices namely MCV, MCH and MCHC. Increases in MCV and MCH, and a reduction in MCHC were observed. These findings are similar to those reported by Kassa *et al.* (2016) regarding MCV and MCHC. In contrast, Kassa *et al.* (2016) found a decrease in MCH. Other studies did not analyze the effects of antituberculous therapy on red cell indices.

Tuberculosis is among chronic infections that cause anaemia of chronic disease (Bashir *et al*., 2015). Means (2013) and Nemeth *et al.* (2004) propose that anaemia results from the activation of T – lymphocytes and macrophages in response to the presence of *Mycobacterium tuberculosis.* These immune cells produce cytokines such as Interleukin – 1 (IL-1), Interleukin - 6 (IL – 6), Tumor necrosis factor alpha (TNF – a) and Interferon gamma (IFN – y) which cause diversion of iron in the cells of the reticulo – endothelial system thus making it unavailable in the plasma for red cell synthesis.

Anaemia among patients at follow-up who had normal haemoglobin concentration before treatment could have resulted from the adverse effect of antituberculous therapy. Isoniazid, pyrazinamide and rifampicin have the potential to cause aplastic and hemolytic anaemia, sideroblastic anaemia, and hemolytic anaemia, respectively (Joint Formulary Committee, 2018).

Pyrazinamide, ethambutol and rifampicin are known to potentially cause thrombocytopenia (Joint Formulary Committee, 2018). In the current study, more patients (35.1 percent) had thrombocytopenia at follow – up compared to baseline (16.2 percent). Thrombocytopenia was noted in 21.8 percent of the patients while thrombocytosis was observed in 13.5 percent of the patients at follow-up. Kassa *et al.* (2016) in a prospective longitudinal study carried out in Ethiopia noted an increase in the proportion of patients with thrombocytopenia at follow - up (14.9 percent) compared to baseline (13.1 percent), however, the proportions were lower than those of the present study. The findings of the present study are similar to those reported by An *et al.* (2013) who found thrombocytopenia in 20.7 percent of patients and slightly lower than those found in the cross sectional study undertaken in Cameroon by Enoh *et al*. (2017) who noted thrombocytopenia in 27.4 percent of the patients. The finding of a decrease in platelet count in the present study was similar to the findings of several studies (Nagayama *et al.*, 2004; Koju *et al.*, 2005; Hadida *et al*., 2013) which also observed a decrease in platelet count following intensive phase antituberculous therapy.

Although there was a decrease in the mean platelet count at follow – up, there was an increase in the mean PDW and MPV. These findings are in contrast to those of Kassa *et*

al. (2016) and Tozkoparan *et al.* (2007) who found a decrease in PDW. However, Kassa *et al.* (2016) and Tozkoparan *et al.* (2007) did not report their findings on MPV.

The decrease in platelet count in the present study was not statistically significant (p = 0.127) while the increase in the PDW (p = 0.0464) and MPV (p = 0.0367) were statistically significant. The findings are different from those of Kassa *et al.* (2016) who noted a statistically significant decrease in platelets and a statistically significant decrease in PDW ($p < 0.001$).

Yakar *et al.* (2013) explains that anti - tuberculous drugs could cause thrombocytopenia by increasing platelet destruction or reducing production. Rifampicin causes thrombocytopenia by binding to membrane glycol proteins to form a non – covalent bond. The binding results in an epitope or conformational changes which are antibody specific. This stimulates the production of rifampicin specific anti – bodies which bind to platelets thus causing their destruction (George *et al.*, 1998). The mechanism by which pyrazinamide induces thrombocytopenia is not yet clearly elucidated but is suggested to be immunological (Chang *et al.*, 2008).

The present study showed a reduction in the proportion of patients with thrombocytosis at follow – up (0 percent) compared to 27 percent at baseline. This is similar to the findings of Kassa *et al.* (2016) who noted a reduction from 19.6 percent at baseline to 8.9 percent at follow – up. The explanation of this reduction in the proportion of patients with thrombocytosis could be due to immune destruction of platelets, an effect of anti – tuberculous drugs (Nagu *et al.*, 2014).

The present study revealed an increase in the proportion of patients with normal white cell count at follow-up. All the patients that had leukopenia at baseline (5.4 percent) were found to have normal white cell count at follow – up. The findings of the present study are similar to those reported by Koju *et al.* (2005) who also found that white cell count was within the reference range. However, several studies have found leukopenia among patients at follow-up, for instance, Enoh *et al.* (2017), Nagayama *et al.* (2004), Kassa *et al.* (2016) and An *et al.* (2013) found that 38.7 percent, 7.1 percent, 18.5 percent and 22.1 percent of patients had the derangement, respectively.

There was a reduction in the proportion of patients with leukocytosis, 16.2 percent at baseline and 2.7 percent at follow – up. These findings are different from those of Kassa *et al.* (2016) who noted an increase in the proportion of patients with leukocytosis from 17.3 percent at baseline to 18.5 percent at follow – up and An *et al.* (2013) who found leukocytosis in 7.1 percent of the patients.

The present study observed changes in the white cell differentials namely, neutrophils, eosinophils, basophils, lymphocytes and monocytes. The study observed a decrease in the median value of the neutrophils and monocytes. An increase in the mean eosinophil count and median lymphocyte counts was noted. These findings are similar to those s of Kassa *et al.* (2016) and Enoh *et al.* (2017) who found a reduction in the mean neutrophil count and an increase in the mean lymphocyte respectively and a decrease in the neutrophil count at follow – up. To the contrary, (Koju *et al.*, 2005) noted a decrease in the eosinophil count and a decrease in the neutrophil count.

The decrease in the mean white cell count was statistically significant ($p = 0.0058$) even though the mean white cell counts were within the reference range. The changes in the neutrophil and eosinophil counts were statistically significant ($p = 0.0005$ and $p < 0.0001$, respectively) but the changes in the basophil, lymphocytes and monocytes were statistically non – significant ($p = 0.079$, $p = 0.757$, $p = 0.0624$). In contrast to the findings of the present study, Kassa *et al.* (2016) did not find a statistically significant difference in the white cell count before and after treatment ($p = 0.224$) and similar to this study, they found no statistically significant differences in the basophil and eosinophil counts. The differences could be explained by differences in the sample sizes of the studies. The current study being relatively smaller than that of the other studies and the fact that the current study included patients who had HIV co-infection.

Tuberculosis like other bacterial infections may cause neutropenia and eosinophilia Nagayama *et al.* (2004). It is expected that after treatment of the disease there should be improvement in the condition resulting in resolution of neutropenia and eosinophilia. In the current study, there was a statistically significant reduction in the white cell count, decrease in neutrophil count and increase in eosinophil count. This could have been caused by anti – tuberculous drugs. Rifampicin is known to cause leukopenia and an increase in the eosinophil count whereas isoniazid is known to cause agranulocytosis.

5.3 Factors associated with hepatic and haematological abnormalities after treatment

The factors associated with derangements in haemoglobin concentration, white cell count and alanine aminotransferase levels were investigated in the present study.

The present study did not find any association between age, gender, HIV status, HIV treatment status, BMI, sputum smear status and baseline ALT with the increase in ALT level at follow- up. These findings are similar to those reported by Abera *et al*. (2016) who found that age, gender and nutritional status were not associated with elevated liver enzymes. The findings of the present study were, however, different from the findings of numerous other studies which found that age above 60 years was associated with increase in the ALT levels (Pande *et al.*, 1996; Schaberg *et al*., 1996; Yee *et al.*, 2003; Marra *et al.*, 2007), although none of the patients in the current study was above 60 years old. Marra *et al.* (2007) also found that higher baseline ALT levels were associated with elevated ALT levels at follow - up. Hassen Ali *et al.* (2013) established that lower BMI was a predictor of elevated ALT levels whereas An *et al.* (2013) stated that female sex was associated with deranged ALT levels. The differences in the findings of the present study and those of the other studies could be due to the fact that the oldest patient in the study was 57 years and as a consequence, none of the patients were above 60 years.

Although it is expected that the findings of the present study would be similar to those reported by other studies undertaken among African patients, who are generally considered slow acetylators of isoniazid and prone to developing isoniazid – induced hepatotoxicity which is characterised by raised liver enzymes (Gonzalez *et al*., 2018), the findings of this study were contrary to this assertion.

The differences in the findings were probably due to the use of different study designs, however, the results of the current study would be expected to be similar to those found by Ekhabbazi *et al.* (2015) who carried out a prospective longitudinal study like the

present study but they found that age, gender, HIV status and baseline transaminase level were associated with elevated liver enzymes. The findings of a cohort study undertaken by Abera *et al*. (2016) in Ethiopia were similar to those of the present study.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The use of intensive phase antituberculous drugs was associated with haematological abnormalities such as anaemia (grade 1-3), leukocytosis and thrombocytopenia (grade 1-3) and elevation in ALT and AST levels. Statistically significant differences in haemoglobin concentration ($p < 0.0001$), white cell count ($p = 0.006$) and ALT level ($p = 0.025$) were observed. However, the proportion of patients with derangements in haemoglobin level and white cell count decreased at follow – up. Factors such as age, gender, BMI, HIV infection, ART, sputum smear status, and appropriate baseline FBC/LFT parameters did not predict the likelihood of ALT derangements at follow-up.

6.2. Recommendations

In view of the findings of this study, the following recommendations are made:

1. Routine monitoring of LFTs during intensive phase of anti – tuberculous therapy may be necessary for ALT.
2. Routine monitoring of haematological parameters should only be considered in patients manifesting clinical signs and symptoms suggestive of anaemia.
3. A larger well - designed study is necessary to assess causality and validate the findings of this study.

REFERENCES

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

Al-muhammadi, M. O. and Al - Shammery, G. H. (2011) 'Studying some hematological changes in patients with pulmonary tuberculosis in Babylon Governorate', *Medical Journal of Babylon*, 8(4), pp. 608–618.

American Thoracic Society (2000) 'Diagnostic standards and classification of tuberculosis in Adults and Children', *American Journal of Respiratory and Critical Care Medicine*, 161, pp. 1376-1395.

An, H., Wu, X., Wang, Z., Xu, J., Zheng, S. and Wang, K. (2013) 'The clinical characteristics of anti-tuberculosis drug induced liver injury in 2457 hospitalized patients with tuberculosis in China', *African Journal of Pharmacy and Pharmacology*, 7(13), pp. 710–714.

Bashir, B. A., Abdallah, S. A. and Mohamedani, A. A. (2015) 'Anemia among patients with pulmonary tuberculosis in Port Sudan, Eastern Sudan', *International Journal of Recent Scientific Research*, 6(5), pp. 4128–4131.

Broaddus, V., Mason, R., Ernst, J., King, T., Lazarus, S., Murray, J., Nadel, J., Slutsky, A. and Gotway, H. (2016) *'Tuberculosis', in Murray & Nadel's Textbook of Respiratory Medicine*. 6th edn. Elsevier, pp. 593 - 628.

Chang, K. C., Leung, C. C., Yew, W. W., Lau, T. Y. and Tam, C. M. (2008) 'Hepatotoxicity of pyrazinamide: Cohort and case-control analyses', *American Journal of Respiratory and Critical Care Medicine*, 177(12), pp. 1391–1396.

Division of AIDS (2017) *Division of AIDS (DAIDS) table for grading the severity of adult and pediatric adverse events, DAIDS AE Grading Table Version 2.1-March 2017*.

Ekhabbazi, H., Benkirane, R., Khadmaoui, A., Sefiani, H., Quyou, A., Mokhtari, A., Soulaymani, A. and Bencheikh, S. (2015) 'Evaluation of adverse effects of antituberculosis in El-Idrissi Hospital, Kenitra, Morocco', *IOSR Journal of Pharmacy*, 5(1), pp. 6–11.

Enoh, J. E., Pokam, B. D. T., Eyo, A. O., Okafor, I. M., Nguedia, C. A., Fominyam, B. T. and Ngowe, M. N. (2017) 'Drug induced hematological disorders in patients on antituberculosis drugs in the South West Region of Cameroon', *European Journal of Pharmaceutical Medical Research*, 4(1), pp. 155–161.

Farazi, A., Sofian, M., Jabbariasl, M. and Keshavarz, S. (2014) 'Adverse reactions to antituberculosis drugs in Iranian tuberculosis patients', *Tuberculosis Research and Treatment*, 2014, pp. 1–6.

George, J. N., Raskob, G. E., Shah, S. R., Rizvi, M. A., Hamilton, S. A., Osborne, S. and Vondracek, T. (1998) 'Drug-induced thrombocytopenia: A systematic review of published case reports', *Annals of Internal Medicine*, 129(11), pp. 886–890.

Getahun, H., Chaisson, R. E. and Raviglione, M. (2015) 'Latent mycobacterium tuberculosis infection: Reply', *New England Journal of Medicine*, 373(12), pp. 1179–

1180.

Giannini, E. G., Testa, R. and Savarino, V. (2005) ‘Liver enzyme alteration: A guide for clinicians’, *Canadian Medical Association Journal*, 172(3), pp. 367–379.

Glaziou, P., Raviglione, M., Falzon, D. and Floyd, K. (2015) ‘Global epidemiology of tuberculosis’, *Cold Spring Harbor Perspectives in Medicine*, 5(a017798), p. 7.

Gonzalez, F., Coughtrie, M. and Tukey, R. (2018) 'Drug Metabolism', in Brunton, L., Hilal - Dandan, R., and Knollmann, B. (eds) *Goodman & Gilman's Pharmacological Basis of Therapeutics*. 13th edn. New York: McGraw - Hill, pp. 85 - 100.

Grobler, L., Nagpal, S., Sudarsanam, T. and Sinclair, D. (2016) 'Nutritional Supplements for People Being Treated for Active Tuberculosis', Cochrane Database of Systematic Reviews, CD006086(6), pp. 1 - 119.

Gülbay, B. E., Gürkan, Ö. U., Yildiz, Ö. A., Önen, Z. P., Erkekol, F. O., Baççioğlu, A. and Acican, T. (2006) ‘Side effects due to primary antituberculosis drugs during the initial phase of therapy in 1149 hospitalized patients for tuberculosis’, *Respiratory Medicine*, 100, pp. 1834–1842.

Hadida, M., Abo-smhadana, A. and Draid, M. (2013) ‘Effects of anti-tuberculous drugs on liver function profile in Libyan patients with tuberculosis’, *The Egyptian Journal of Hospital Medicine*, 52, pp. 740–751.

Hassen Ali, A., Belachew, T., Yami, A. and Ayen, W. Y. (2013) ‘Anti-tuberculosis drug induced hepatotoxicity among TB/HIV co-infected patients at Jimma University Hospital, Ethiopia: Nested case-control study’, *PLoS ONE*, 8(5), pp. 1–8.

Joint Formulary Committee (2018) *British National Formulary*. 75th ed. London: BMJ Publishing Group Ltd and Royal Pharmaceutical Society.

Kapata, N., Chanda-Kapata, P., Ngosa, W., Metitiri, M., Klinkenberg, E., Kalisvaart, N., Sunkutu, V., Shibemba, A., Chabala, C., Chongwe, G., Tembo, M., Mulenga, L., Mbulo, G., Katemangwe, P., Sakala, S., Chizema-Kawesha, E., Masiye, F., Sinyangwe, G., Onozaki, I., Mwaba, P., Chikamata, D., Zumla, A. and Grobusch, M. P. (2016) 'The prevalence of tuberculosis in Zambia: Results from the first national TB prevalence survey, 2013-2014', *PLoS ONE*, 11(1), pp. 1–14.

Kassa, E., Enawgaw, B., Gelaw, A. and Gelaw, B. (2016) 'Effect of anti-tuberculosis drugs on hematological profiles of tuberculosis patients attending at University of Gondar Hospital, Northwest Ethiopia', *BMC Hematology*. BMC Hematology, 16(1), pp. 1–11.

Kim, J., Nam, W. S., Kim, S. J., Kwon, O. K., Seung, E. J. and Lee, S. (2017) 'Mechanism investigation of rifampicin-induced liver injury using comparative toxicoproteomics in mice', *International Journal of Molecular Sciences*, 18(1417), pp. 1–13.

Koju, D., Rao, S. B., Shrestha, B., Shakya, R. and Makaju, R. (2005) 'Occurrence of side effects from anti - tuberculosis drugs in urban Nepalese population under DOTS treatment', *Kathandu University Jounal of Science and Technology*, 1(1), pp. 1–4.

Lv, X., Tang, S., Xia, Y., Wang, X., Yuan, Y., Hu, D., Liu, F., Wu, S., Zhang, Y., Yang, Z., Tu, D., Chen, Y., Deng, P. and Ma, Y. (2013) 'Adverse reactions due to directly observed treatment strategy therapy in Chinese tuberculosis patients : A prospective

study', *PLoS ONE*, 8(6), pp. 1–8.

Marra, F., Marra, C. A., Bruchet, N., Richardson, K., Moadebi, S., Elwood, R. K. and FitzGerald, J. M. (2007) 'Adverse drug reactions associated with first-line anti-tuberculosis drug regimens', *International Journal of Tuberculosis and Lung Disease*, 11(8), pp. 868–875.

Means, R. T. (2013) 'Anemia of inflammation : clinical insights from mechanisms', in *The 75th Annual Meeting of the Japanese Society of Hematology*. Sapporo, pp. 1596–1602.

Ministry of Health (2017) *The national tuberculosis and leprosy control program - Tuberculosis manual*. Lusaka.

Montales, M. T., Beebe, A., Chaudhury, A. and Patil, N. (2015) 'Mycobacterium tuberculosis infection in a HIV-positive patient', *Respiratory Medicine Case Reports*. Elsevier Ltd, 16, pp. 160–162.

Morris, D. W. C., Bird, R. A. and Nell, H. (1990) 'The haematological and biochemical changes in severe pulmonary tuberculosis', *The Quarterly Journal of Medicine*, 73(272), pp. 1151–9.

Nagayama, N., Shishido, Y., Masuda, K., Baba, M., Tamura, A., Nagai, H., Akagawa, S., Kawabe, Y., Machida, K., Kurashima, A., Komatsu, H. and Yotsumoto, H. (2004) 'Leukopenia due to anti-tuberculous chemotherapy including rifampicin and isoniazid', *Kekkaku*, 79(5), pp. 341–348.

Nagu, T. J., Spiegelman, D., Hertzmark, E., Aboud, S., Makani, J., Matee, M. I., Fawzi,

W. and Mugusi, F. (2014) ‘Anemia at the initiation of tuberculosis therapy is associated with delayed sputum conversion among pulmonary tuberculosis patients in Dar-es-Salaam, Tanzania’, *PLoS ONE*, 9(3), pp. 1–8.

Narasimhan, P., Wood, J., Macintyre, C. R. and Mathai, D. (2013) ‘Risk factors for tuberculosis’, *Pulmonary Medicine*, 2013, pp. 1–12.

Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B. K. and Ganz, T. (2004) ‘IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin’, *Journal of Clinical Investigation*, 113(9), pp. 1271–1276.

Nhamoyebonde, S. and Leslie, A. (2014) ‘Biological differences between the sexes and susceptibility to tuberculosis’, *The Journal of Infectious Diseases*, 209(S3), pp. S100-6.

Pande, J. N., Singh, S. P., Khilnani, G. C., Khilnani, S. and Tandon, R. K. (1996) ‘Risk factors for hepatotoxicity from antituberculosis drugs: A case-control study.’, *Thorax*, 51(2), pp. 132–6.

Schaberg, T., Rebhan, K. and Lode, H. (1996) ‘Risk factors for side-effects of isoniazid, rifampin and pyrazinamide in patients hospitalized for pulmonary tuberculosis’, *European Respiratory Journal*, 9(10), pp. 2026–2030.

Shang, P., Xia, Y., Liu, F., Wang, X., Yuan, Y., Hu, D., Tu, D., Chen, Y., Deng, P., Cheng, S., Zhou, L., Ma, Y., Zhu, L., Gao, W., Wang, H., Chen, D., Yang, L., He, P., Wu, S., Tang, S., Lv, X., Shu, Z., Zhang, Y., Yang, Z., Chen, Y., Li, N., Sun, F., Li, X., He, Y., Garner, P. and Zhan, S. (2011) ‘Incidence, clinical features and impact on anti-

tuberculosis treatment of anti-tuberculosis drug induced liver injury (ATLI) in China', *PLoS ONE*, 6(7), pp. 1–7.

Sharma, S. and Mohan, A. (2005) 'Antituberculosis treatment-induced hepatotoxicity: From bench to bedside', *Medicine Update*, pp. 479–484.

Suresh, K. and Chandrashekara, S. (2012) 'Sample size estimation and power analysis for clinical research studies', *Journal of Human Reproductive Sciences*, 5(1), p. 7.

Tozkoparan, E., Deniz, O., Uçar, E., Bilgic, H. and Ekiz, K. (2007) 'Changes in platelet count and indices in pulmonary tuberculosis', *Clinical Chemistry and Laboratory Medicine*, 45(8), pp. 1009–1013.

Umeki, S. (1989) 'Adverse effects of antitubercular drugs and significance of measurement of the drug-stimulating lymphocyte transformation rate', *Japanese Journal of medicine*, 28(3), pp. 335–340.

Walker, R. and Whittlesea, C. (2012) *Clinical Pharmacy and Therapeutics*. 5th edn. Edited by R. Walker and C. Whittlesea. London: Churchill Livingstone Elsevier.

Wang, P., Pradhan, K., Zhong, X. and Ma, X. (2016) 'Isoniazid metabolism and hepatotoxicity', *Acta Pharmaceutica Sinica B*. Elsevier, 6(5), pp. 384–392.

World Health Organization (2010) *Treatment of tuberculosis: guidelines*. Geneva.

World Health Organization (2016) *Global tuberculosis report 2016*. Geneva.

World Health Organization (2017) *End TB global tuberculosis report 2017*. Geneva.

Xiang, Y., Ma, L., Wu, W., Liu, W., Li, Y., Zhu, X., Wang, Q., Ma, J., Cao, M., Wang,

Q., Yao, X., Yang, L., Wubuli, A., Merle, C., Milligan, P., Mao, Y., Gu, J. and Xin, X. (2014) 'The incidence of liver injury in Uyghur patients treated for TB in Xinjiang Uyghur Autonomous Region, China, and its association with hepatic enzyme polymorphisms NAT2, CYP2E1, GSTM1 and GSTT1', *PLoS ONE*, 9(1), pp. 1–8.

Yakar, F., Yildiz, N., Yakar, A. and Kiliçaslan, Z. (2013) 'Isoniazid- and rifampicin-induced thrombocytopenia', *Multidisciplinary Respiratory Medicine*, 8(2), pp. 1–3.

Yaranal, P. J., Umashankar, T. and Govindareddy, S. (2013) 'Hematological profile in pulmonary tuberculosis', *International Journal of Health and Rehabilitation Services*, 2(1), pp. 50–55.

Yee, D., Valiquette, C., Pelletier, M., Parisien, I., Rocher, I. and Menzies, D. (2003) 'Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis', *American Journal of Respiratory and Critical Care Medicine*, 167, pp. 1472–1477.

APPENDICES

Appendix A: Participants information sheet

Young Women's Christian Association Hostels,

Nationalist road,

P.O.BOX, 50115,

Lusaka.

Contact numbers: +260977891961/+260963773561

I am Glorious Mwaba, a 3rd year clinical Pharmacy student at the University of Zambia. I am carrying out a study entitled "Effect of first line anti – tuberculous medicines on haematological and hepatic Parameters".

This research will look at the sputum smears, the concentration of the various blood cells in the plasma (the liquid part of blood) and the concentration of 3 enzymes that are found in the liver, before TB drugs are started and at the end of the first 2 months. This will be done by collecting sputum samples and blood (5mls) which will be taken to the laboratory as it is routinely done. There will be no serious consequences except slight pain when drawing blood.

The basic information will be obtained from the drug charts. No names will be written on the information sheet to maintain confidentiality.

Participation in this study is voluntary and you are free to withdraw at any time. Withdrawal from the study will not affect provision of standard care.

If you need further clarification, you may contact me on the address and phone numbers given above or UNZAREC whose contact details are given below:

University of Zambia,

Biomedical Research Ethics Committee,

Ridgeway Campus,

P.O.BOX, 50110,

Lusaka, Zambia.

Telephone: 256067

Telegrams; UNZA, LUSAKA

Telex: UNZALUZA 44370

Fax: unzarec@zamtel.zm

Appendix B: Consent form

Glorious Mwaba

Young Women's Christian Association Hostels,

Nationalist road,

P.O.BOX, 50115,

Lusaka.

I have read, or have had read to me in my first language, and I understand the Participant Information Sheet. Yes □ No □

I have been given time to consider whether or not to participate in this study. Yes □ No □

I have had the opportunity to ask questions concerning the study to help me understand the study. Yes □ No □

I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet. Yes □ No □

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care. Yes □ No □

I understand that the risk associated with taking part in this study is minimal. Yes □ No □

I consent to the researcher collecting and processing my information, including information about my health and samples required for the study. Yes □ No □

I understand that my participation in this study is confidential and that the file number indicated on the information sheet is solely for linking the results at enrolment to the results at two months but will not be used in any reports on this study. Yes □ No □

I know who to contact if I have any questions about the study in general. Yes □ No □

I hereby consent to participate in this research.

Participant's name…….

Signature……

Date…….

Please sign or print thumb in the lines above if consented.

Data collector's name……

Signature……

Date…….

Witness' name……

Signature……

Date…….

Appendix C: Data collection sheet

Effects of Intensive Phase Anti Tuberculous Therapy on Hepatic and Haematological Parameters and in Patients at the University Teaching Hospital

A. Social demographic data

1. File Number…….

2. Age

3. Sex a. Male b. Female

4. Body mass index (BMI)

5. Marital status

a. Single b. Married c. Divorced d. Separated e. Widow/widower

6. Occupation

7. Residential area

a. Low density b. Medium density c. High density

B. Other details

8. Date of commencing anti - tuberculosis drugs

9. Sputum results at start of therapy

Sputum Smear Results

Sputum	At start of therapy	At 2 months of therapy
Smear		
	Date:	Date
Results		

10. HIV Status

a. Reactive b. Non - reactive

11. Liver Function test results

LFTs	Results		Reference range	Grade of derangement
Investigation	At start of therapy Date:	At 2 months of therapy Date:		
AST				
ALT				

12. Full blood count results

FBC	Results		Reference range	Grade of derangement
Investigations	At start of therapy Date:	At 2 months of therapy Date:		
WCC				
RCC				
PCV				
MCV				
Hb				

MCH				
RDW				
PLT				
MPV				
Lymphocytes				
Neutrophils				
Basophils				
Eosinophils				
Monocytes				

13. Antiretroviral drugs being taken

14. Duration of taking Anti – retroviral drugs

15. Any other medications being taken

16. Duration of taking

Appendix D: Ethical approval

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
E-mail: unzarec@unza.zm
Assurance No. FWA00000338
IRB00001131 of IORG0000774

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

9th April, 2018.

Ref: 002-12-17.

Ms. Glorious Mwaba,
University of Zambia,
Department of Pharmacy,
P.O Box 50110,
Lusaka.

Dear Ms. Mwaba,

RE: SUBMITTED RESEARCH PROPOSAL: "EFFECT OF INTENSIVE PHASE ANTI TUBERCULOUS THERAPY ON HEPATIC AND HEMATOLOGICAL PARAMETERS IN PATIENTS AT THE UNIVERSITY TEACHING HOSPITAL" (REF.002-12-17)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee (UNZABREC) on 26th March, 2018. The proposal is approved. The approval is based on the following documents that were submitted for review:

a) **Study proposal**
b) **Questionnaires**
c) **Participant Consent Form**

APPROVAL NUMBER : REF. 002-12-17

This number should be used on all correspondence, consent forms and documents as appropriate.

- **APPROVAL DATE : 9th April, 2018**
- **TYPE OF APPROVAL : Standard**
- **EXPIRATION DATE OF APPROVAL : 8th April, 2019**
  After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the UNZABREC Offices should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING**: All SAEs and any other serious challenges/problems having to do with participant welfare, participant safety and study integrity must be reported to UNZABREC within 3 working days using standard forms obtainable from UNZABREC.
- **MODIFICATIONS**: Prior UNZABREC approval using standard forms obtainable from the UNZABREC Offices is required before implementing any changes in the Protocol (including changes in the consent documents).
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the UNZABREC using standard forms obtainable from the UNZABREC Offices.
- **NHRA:** Where appropriate, apply in writing to the National Heath Research Authority for permission before you embark on the study.
- **QUESTIONS**: Please contact the UNZABREC on Telephone No. 256067 or by e-mail on unzarec@unza.zm.
  Other: Please be reminded to send in copies of your research findings/results for our records. You're also required to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study.

Yours sincerely,

Dr. S.H Nzala
VICE-CHAIRPERSON

Appendix E: Permission letter

University Of Zambia

School of Health Sciences

P.O.BOX, 50110.

Department of Pharmacy.

Lusaka.

16th September, 2017.

The Senior Medical Superintendent,

Adult Hospital of the University Teaching Hospital,

P/Bag RW 1X

Ridgeway

Lusaka.

REPUBLIC OF ZAMBIA
HEAD CLINICAL CARE
27 OCT 2017
UNIVERSITY TEACHING HOSPITAL
P/BAG RW 1X, LUSAKA

REPUBLIC OF ZAMBIA
MINISTRY OF HEALTH
UNIVERSITY TEACHING HOSPITAL
27 OCT 2017
SENIOR MEDICAL SUPERINTENDENT
PRIVATE BAG RW1X, LUSAKA

Dear sir/madam,

REF: REQUEST FOR PERMISSION TO CONDUCT A RESEARCH

Reference is made to the above headline. I am a 3rd year student pursuing a masters degree in clinical Pharmacy.

I would like to carry out a research entitled "Effects of intensive phase anti – tuberculous therapy on hepatic and hematological parameters in patients at the University Teaching Hospital. I write to request for permission to carry out this study.

The study will be a descriptive cross sectional study at two time points. Participants will be enrolled using a systematic sampling method, baseline data obtained before commencing TB treatment and at 2 months of treatment.

Ethical approval will be sort from the Biomedical Research Ethics Committee.

Your positive response will be highly appreciated.

Yours Faithfully

G. M.

Glorious Mwaba

PG Cert (Clinical Pharmacy)