Drug induced liver injury in patients on anti-tubercular therapy and/or anti-retroviral therapy at Helen Joseph Hospital, Johannesburg, South Africa

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DECLARATION

I, Ruchika Mehta, declare that this research report is my own, unaided work. It is being submitted for the Degree of Masters in Internal Medicine (in the submissible format with my protocol and an extended literature review) at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

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……day of ……. 2019

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DEDICATION

Without the support of my family I would not have achieved anything I have today and I would like to thank them for their endless support and encouragement; Akash Singh, my wonderful parents Arvinder Jit Singh and Neerja Mehta who encouraged me to follow my dreams regardless of how impossible they may seem, my siblings Kanika and Abhinav who have been there every step of the way, cheering me on at the finish line.

PRESENTATIONS ARISING FROM THIS PROJECT

Poster presentation

1. Drug induced liver injury in patients on anti-tubercular and/or anti-retroviral therapy at Helen Joseph Hospital.

   Wits Health Sciences Research day, 06/09/2018

2. Drug induced liver injury in patients on anti-tubercular and/or anti-retroviral therapy at Helen Joseph Hospital.

   4th Southern African HIV Clinicians Society conference,

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ABSTRACT

Background

South Africa has one of the highest prevalence rates of human immunodeficiency virus (HIV) and tuberculosis (TB) globally, and an estimated 73% of patients with TB are co-infected with HIV. The anti-tubercular drugs and anti-retroviral drugs are known to cause drug induced liver injury (DILI). In addition, these patients may be on other hepatotoxic medication and acquire other opportunistic infections which can also cause liver injury. The consequences are treatment failure, disease relapse and drug resistance.

Objectives

To study patients on anti-tubercular and/or anti-retroviral therapy with DILI to establish their demographics, clinical presentation and severity, time to presentation from initiation of drug therapy, management and outcomes.

Methods

A retrospective review of patients who presented with DILI to Helen Joseph hospital over a 17-month period from October 2015 to February 2017 was done. The records of 129 patients were analysed and data collected on drug history, biochemical investigations and any relevant imaging studies available.

Results:

Of the 129 patients, 61% were male (n=79) and 39% female (n=50). Forty-six patients had anti-tubercular DILI (36% of patients), 29 had anti-retroviral DILI (22% patients) and 54 patients (42%) were on both anti-tubercular and anti-retroviral therapy. The median age of patients was 35.4 years (IQR 31.4-43.5 years). Twenty percent of the patients were on a concomitant hepatotoxic drug, cotrimoxazole ($p < 0.05$).

None of the patients were co-infected with Hepatitis C and 4.7% patients had co-infection with Hepatitis B. The commonest presenting symptom was nausea and vomiting followed by jaundice.

Of the patients with anti-retroviral DILI, 89% were on first line treatment with tenofovir/emtricitabine/efavirenz (TDF/FTC/EFV), with a median CD4 count of 549 cells/microlitre and median viral load of 549 copies/ml. The median time to development of DILI was 85 days from initiation of anti-retroviral therapy.

Of the patients with anti-tubercular DILI, 76% were on a regimen with rifampicin/isoniazid/pyrazinamide/ethambutol (RIF/INH/PZA/ETH), with a median CD4 count of 37.5 cells/microlitre and median viral load of 57,400 copies/ml. The median time to development of DILI from initiation of anti-tubercular therapy was 14 days.

In the patients with mixed DILI, 89% were on first line anti-retroviral therapy (TDF/FTC/EFV) and 70.4% on RIF/INH/PZA/ETH containing anti-tubercular therapy. Their median CD4 count was 56 cells/microlitre and median viral load 1230 copies/ml. In this group anti-retroviral therapy was used for a median 168 days prior to presentation and anti-tubercular therapy for a median of 41 days.

Overall the in-hospital mortality of these patients was 16.3% (n=21).

Conclusion

Patients with anti-tubercular DILI presented earlier (within intensive phase of therapy) whereas those anti-retroviral DILI presented up to 1 year after therapy initiation; this highlights the importance of maintaining a high index of suspicion in this group of patients and regular surveillance for earlier diagnosis of DILI to reduce associated morbidity.

Those with anti-retroviral DILI presented with lower viral loads and higher CD4 counts likely reflecting adequate HIV therapy. Only 27% of those with anti-retroviral DILI and 35% of those with anti-tubercular DILI were successfully re-challenged to their original drug regimen by discharge.

Among those admitted with DILI, those with severe DILI and mixed pattern of liver injury were at increased risk of all-cause mortality during the admission.

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LIST OF ABBREVIATIONS

HIV – human immunodeficiency virus

AIDS – acquired immune-deficiency syndrome

CD4 – cluster of differentiation 4

VL – viral load

TB - tuberculosis

DILI – drug induced liver injury

ADR – adverse drug reaction

WHO - World Health Organisation

SAHIVCS – Southern African HIV Clinicians Society

LFTS – liver function tests

FDC – fixed dose combination

ART – anti-retroviral therapy

NNRTIS – non-nucleoside reverse transcriptase inhibitor

NRTIS – nucleoside reverse transcriptase inhibitor

PI – protease inhibitor

EFV – efavirenz

FTC – emtricitabine

3TC – lamivudine

AZT – zidovudine

TDF – tenofovir

LPV-r – lopinavir-ritonavir

ATV-r – atazanavir-ritonavir

ABC - abacavir

NVP – nevirapine

RAL – raltegravir

D4T – stavudine

RIF – rifampicin

INH – isoniazid

PZA – pyrazinamide

ETH – ethambutol

MOXI – moxifloxacin

ULN – upper limit of normal

ALT – alanine aminotransferase

AST – aspartate aminotransferase

GGT – gamma glutamyl transferase

ALP – alkaline phosphatase

INR – international normalised ratio

GXP – gene xpert

CSF – cerebrospinal fluid

LAM – lipoarabinomannan

IQR – inter quartile range

HR – hazard ratio

CI – confidence interval

CHAPTER 1: PROTOCOL WITH EXTENDED LITERATURE REVIEW

1.1: INTRODUCTION AND EXTENDED LITERATURE REVIEW

1.1.1:Background of the problem

South Africa has one of the highest prevalence rates of human immunodeficiency virus (HIV) in the world - 11.2% of the general population and 16.6% of the population aged 15-49 years who are in their most productive years - and its associated opportunistic infections, the commonest being tuberculosis (TB) (1). In 2016, South Africa had 270,000 new HIV infections and 110,000 AIDS related deaths(2). HIV infection is also associated with significantly increased risk of progression from latent to active TB. TB is the leading cause of death among people living with HIV, causing more than one third of all AIDS-related deaths in 2015. According to World Health Organisation (WHO), South Africa ranks third globally in TB prevalence (0.4-0.59 million) after India and China (3). According to the South African Department of Health, 73% of patients with TB are HIV positive (4).

Of the 7,100,000-people living with HIV in South Africa in 2016, 56% were accessing anti-retroviral therapy. South Africa has the largest treatment programme in the world, accounting for 20% of people on antiretroviral therapy globally (2). The current anti-retroviral therapy guidelines in South Africa have been updated to a 'test and treat' policy which will translate to a higher number of patients using anti-retroviral therapy which can potentially cause a higher number of drug induced liver injury (DILI) cases than we are already witnessing (5) since the number of TB and hence anti-tubercular DILI cases may decrease however the anti-retroviral DILI cases may rise.

Anti-retroviral therapy and anti-tubercular therapy are known to cause many adverse effects such as liver injury, skin reactions, neurological and gastrointestinal disorders; drug induced liver injury (DILI) is one of the most important and serious adverse effects. (6)

It is estimated that drug induced liver injury affects 5-33% of patients on treatment for TB, although local data are limited (7). Depending on the regimen, 9-30% patients on anti-retroviral therapy develop DILI (8). Anti-tubercular DILI in HIV co-infection is the most common adverse effect of treatment necessitating therapy interruption (9). The subsequent adherence problem may cause treatment failure, relapse or drug resistance (10)((11, 12).

In the setting of HIV infection, the diagnosis of TB can be complex due to variable sites of infection, paucity of specimens in some cases and the sensitivity and specificity of diagnostic tests being variable (13) (14). In addition, TB in patients with HIV is often difficult to

diagnose due to its pauci-bacillary nature. The commonest site of TB infection is the lungs and sputum from infected patients is analysed using a polymerase chain reaction (PCR) technology, known as Gene Xpert (GXP) which detects the genetic sequence of the mycobacterium that causes TB and additionally can test for resistance to rifampicin which is the main anti-tubercular drug (15). Other commonly used specimens used to diagnose TB include pleural fluid, cerebrospinal fluid, ascitic fluid, lymph node aspirates and tissue specimens (14). In many cases, however, a culture of the specimen is required which can take up to 42 days to be reported.

In some cases, patients are put on empiric treatment based on their clinical presentation and examination findings, especially in resource limited areas which may not have access to the diagnostic kits.

Treatment of TB varies from 6 months to 1 year, using 4 drugs in the first 2 months of treatment (intensive phase) and 2 drugs for the remainder of treatment (continuation phase), in patients who have strains of TB which are susceptible to the standard first line drugs (11). HIV infection requires lifelong treatment with anti-retroviral drugs, the standard first line medication in South Africa being efavirenz (EFV), emtricitabine (FTC) and tenofovir (TDF), all of which are known to cause liver enzyme elevation (16), although in most cases EFV is the culprit drug.

Although newer and safer drugs are continuously being sought after and used in the treatment of HIV, the mainstay of drugs used in the treatment of TB still includes the drugs identified 5-6 decades ago, namely isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (ETH) (17). Drug development for the treatment of tuberculosis stopped in the 1970s due to a decline in the incidence of TB in Europe and America (18). Although a vast majority of patients tolerate these drugs, the adverse effect of hepatotoxicity is the most significant (19). The drugs used in first line anti-tuberculous therapy that are associated with hepatotoxicity are RIF, INH and PZA (12).

The natural resistance of *Mycobacterium tuberculosis* against INH, RIF and PZA is very low (approximately 1 resistant bacterium in a population of 10^8 bacteria), hence a lot of resistance that is seen is acquired; some of the reasons include poor compliance (partly due to adverse effects), non-adherence to medication and failure to monitor therapy. This is evident by the fact that prior treatment for TB is the most important risk factor for occurrence of drug

resistant TB. However, in areas where acquired resistance becomes epidemic, new infections with resistant strains can occur and contribute to the resistance problem (18).

The incidence of hepatotoxicity of INH when used at a dose of 5mg/kg body weight is 0.5-3% for clinical hepatotoxicity (symptomatic DILI) and 10-25% for raised transaminases (asymptomatic DILI). For RIF, when used at a dose of 10mg/kg, 2-5% patients develop clinical hepatotoxicity and 10-15% develop altered liver functions (20). When PZA is used at a dose of 25-30 mg/kg, 5% of the patients develop clinical hepatotoxicity whereas 10% have altered liver functions (21). According to Daphnee Yee *et al*, the incidence of PZA induced hepatotoxicity and rash during treatment for active TB is substantially higher than with other first line anti-tubercular drugs (6).

When a patient is treated with a regimen excluding INH and PZA, the duration of treatment is prolonged to 9-12 months and when a patient is treated with a regimen without RIF, treatment continues for 18 months. This is likely to contribute to the existing adherence problem and pill burden faced by these patients.

According to a study on anti-tubercular DILI in China, compared to patients who don't develop DILI, those who do have a 9.25-fold increased risk of developing unsuccessful treatment outcomes and 2.1-fold increased risk of prolonging intensive treatment phase. Seventeen percent of unsuccessful treatment outcomes in this study were attributed to the occurrence of DILI (22).

This is further complicated by the fact that HIV infection can be co-acquired with other infections such as hepatitis B and hepatitis C which are risk factors for acute or chronic liver disease and progression to hepatocellular carcinoma.

Patients with HIV who have a CD4 count of <200 cells/microlitre are also on Cotrimoxazole prophylaxis for the prevention of *pneumocystis jirovecii* pneumonia and toxoplasmosis, which is also hepatotoxic. In patients with history of cryptococcal meningitis and CD4 count <200, prophylactic fluconazole therapy is an additional risk factor for liver dysfunction (23).

1.1.2:Definition

Drug induced liver injury is one of the adverse effects of anti-retroviral therapy and anti-tubercular therapy. There is no globally agreed definition of anti-tubercular DILI; most definitions focus on alanine transaminase (ALT). The DILI Expert Working group have called for a consensus criterion for any drug related DILI. (24)

The criteria for anti-tubercular DILI in this study is defined as per the Southern African HIV Clinicians Society (SAHIVCS) as: elevation of liver enzyme alanine aminotransferase (ALT) >120 IU/l (>3 times upper limit normal) (ULN) in symptomatic patients (who may complain of nausea, vomiting, malaise, loss of appetite, abdominal pain or jaundice) or >200 IU/l (>5 times ULN) in asymptomatic patients; or total serum bilirubin concentration of >40 mmol/l, provided other competing aetiologies such as acute viral hepatitis, autoimmune hepatitis and other liver diseases are ruled out (7). The upper limit of normal for ALT and total bilirubin as per South African National Health Laboratory service (NHLS) reference range is 40 IU/l and 21 mmol/l respectively.

1.1.3:Incidence

The hepatotoxic potential of a drug was until recently monitored mainly by the pharmaceutical industry and there was paucity of available data. Also, due to lack of diagnostic markers, DILI remains a diagnosis of exclusion, which further limits accurate information for many drugs (25). This, however, may change as based on genetic studies and DILI patterns in patients, susceptibility genotypes are being identified and biomarkers and prediction tools may become available to identify and monitor patients at high risk of developing DILI (25). However, although there is a genetic susceptibility on occurrence of idiosyncratic DILI, it is difficult to extrapolate the estimated DILI risk of a specific drug in individuals from different genetic backgrounds, hence screening is not yet a viable option

According to the American Thoracic Society, DILI accounts for approximately 7% of reported adverse drug events, 2% of jaundice in hospitals and approximately 30% of fulminant liver failure(12).

There are very few true population-based prospective studies looking at determining the incidence of DILI. A prospective study in northern France monitored 81000 individuals revealing an incidence of 14 cases per 100,000 persons per year. In these individuals, the drugs implicated in order of frequency were: antibiotics 25% (mostly amoxicillin-clavulanic acid), psychotropics 22% and hypolipidaemics 12%. In these patients, 10% died of liver disease. This is consistent with data from other developed countries in which the commonest drugs implicated in causing DILI include amoxicillin-clavulanate and paracetamol (25).

Similarly, a study in Iceland done with 250,000 adult patients over a 2-year period showed an incidence of DILI of 19 cases per 100,000 per year. The drugs implicated in this study were: single prescription drug (75%), dietary supplements (16%) and multiple agents (9%). This

study is considered to provide better incidence data due to good record keeping with information of prescriptions and DILI events. The commonest causative drug in this study was amoxicillin-clavulanate (1 per 2350 users); however, a majority of these patients were asymptomatic (25).

Anti-tubercular therapy or anti-retroviral therapy-associated DILI is a common reason for presentation at a referral hospital in South Africa and in-hospital and 3-month mortality are high (26). In a recent survey of medical wards at four South African hospitals, there were 164 of 1951 (8.4%) adverse drug reaction (ADR) related admissions and anti-retroviral therapy, anti-tubercular therapy and /or cotrimoxazole were implicated in 56 of 164 (34%) of ADR related admissions (27).

In a study in Tanzania done in patients with HIV on efavirenz-based anti-retroviral therapy or HIV-TB co-infected patients on RIF based anti-tubercular therapy, the overall incidence of DILI was 7.8%, being non-significantly higher among patients receiving concomitant combination based anti-tubercular therapy and anti-retroviral therapy (10%) than those receiving anti-retroviral therapy alone (5.9%) (10).

A study looking at the incidence of DILI among patients with anti-retroviral therapy and/or anti-tubercular therapy DILI in South Africa concluded that the incidence of DILI was higher in patients on both anti-retroviral and anti-tubercular therapy (71.2%) compared to patients on anti-tubercular therapy only (51.2%). Standard TB treatment was re-introduced in 71.8% of these patients. More patients with anti-retroviral and anti-tubercular DILI than anti-tubercular DILI required modified TB treatment (37.2% vs 17.1%) (28).

DILI due to anti-tubercular therapy can occur any time during treatment duration, 75% occurring within the first 2 months of treatment (29). Progression to acute liver failure occurs in a quarter of patients, the overall mortality is 22.7%, which is higher when accompanied by jaundice, encephalopathy and ascites(29). A study in Brazil on hepatotoxicity due to anti-tubercular therapy in TB/HIV co-infected patients also showed that more than 90% of hepatoxicity occurred within the first 6 weeks of treatment (30).

According to a study done in Tanzania, the overall median time to DILI in HIV positive patients on efavirenz based anti-retroviral therapy was 2 weeks while in TB-HIV co-infected patients, the median time to DILI was 5 weeks, corresponding to 1 week after anti-retroviral therapy was added on the anti-tubercular therapy. However, the difference was not statistically significant and none of the study patients had DILI onset after 12 weeks (10).

The impact of DILI on patients is many-fold, including but not limited to additional costs due to added outpatient visits and tests or hospitalisation in more severe cases. A study of patients on TB treatment with standard first line regimen in Montreal, Canada, 29 patients who developed DILI and were discharged subsequently made a total of 91 extra clinic visits (6).

Alternative drugs may be more toxic and/or less effective, resulting in prolonged treatment with further strain on compliance. This results in higher risk of treatment failure and relapse of TB (6). Hepatotoxicity is also associated with reduction in HIV viral suppression over time due to treatment discontinuation (31).

1.1.4:Pathogenesis

DILI can be classified as idiosyncratic and non-idiosyncratic (predictable). Idiosyncratic reactions are considered to be unpredictable and not dose related.

Pathogenesis of DILI is complex and not entirely understood. It is thought to be due to an interplay of factors including the individual patient, drug and environment (32). Either the parent drug molecule or its metabolite(s) may cause the initial insult culminating in hepatic injury. Adaptation may explain why DILI occurs in a small percentage of individuals; it is characterised by transient minor abnormalities in liver enzymes without symptoms of DILI, occurring shortly after drug initiation and resolving in the presence of continued drug exposure. Mechanisms thought to explain adaptation include optimised drug processing by the liver, development of tolerance by the adaptive immune system and recruitment of antioxidant defences(32) (17).

Although several antiretrovirals have been reported to cause fatal acute hepatitis, they most often cause asymptomatic elevation of transaminases. The possible pathogenic mechanisms include direct drug toxicity, immune reconstitution inflammatory syndrome (IRIS) in the presence of hepatitis B/C, hypersensitivity reactions and mitochondrial toxicity (31). Some anti-retrovirals also cause insulin resistance and ultimately steatohepatitis. Multiple pathogenic pathways probably occur simultaneously in some patients thus it may be difficult to identify the exact mechanism involved in the development of hepatotoxicity. (31)

1.1.5:Risk Factors

Some of the risk factors associated with the development of DILI are advanced age, female gender, slow acetylator status, malnutrition, HIV and pre-existent liver disease(11). Pedral-Sampaio *et al* also concluded that the incidence of hepatitis in patients on anti-tubercular therapy was higher in HIV positive patients than in HIV negative patients (33).

A study conducted in South Africa found that the concomitant treatment of TB increased the risk of anti-retroviral therapy associated DILI by 8.5-fold and hepatitis B surface antigen (Hep B S Ag) positivity increased the risk by 3-fold and patients with a nadir CD4 count <100 were 1.9 times more likely to get a DILI (34).

The SLATIN DILI network looked at 250 patients with DILI due to various drugs, in a prospective study, and found there to be a female predominance of patients with a mean age of patients being 51 years (25). This is consistent with other studies which report female

predominance in DILI patients. However, studies done by Alima *et al*, Pukenyte *et al* and Lima *et al* found no co-relation between drug induced hepatotoxicity and gender (30, 35, 36).

A study in Ethiopia analysing the risk factors for development for DILI concluded HIV positivity is a significant risk factor especially with lower CD4 counts and concomitant anti-tubercular therapy but their study was inconclusive for the role of alcohol use, body mass index, hepatitis B surface antigen (Hep B S Ag), hepatitis C antibodies or age as risk factors (37).

Co-infection with hepatitis B and C viruses appears to significantly increase the risk of toxicity of non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as efavirenz (EFV), which is part of the current first line therapy for HIV (38).

Mugusi *et al* studied HIV positive patients on EFV based anti-retroviral therapy with or without TB co-infection and concluded that the use of fluconazole, alcohol consumption and older age were not associated with an increased incidence of DILI. Hepatitis C co-infection, history of weight loss, WHO clinical stages and CYP2B6*6 genotype are significant predictors of DILI (10).

1.1.6:Clinical Presentation

The clinical spectrum of DILI can vary from asymptomatic hepatitis to acute liver failure (23, 39). The DILI can be categorised as being mild if they are clinically well with elevation of ALT >120 but <200 IU/l and total bilirubin <40 mmol/l; moderate if they are well but with ALT>200 IU/l or total bilirubin >40 mmol/l and severe if they are clinically unwell (e.g. vomiting, abdominal pain) and meet the DILI definition (7).

There are 3 recognised patterns of liver injury; predominantly hepatocellular (markedly elevated ALT), predominantly cholestatic (markedly elevated gamma glutamyl transferase, GGT) and mixed pattern - raised ALT and GGT (12) (40) (41). Bilirubin may be elevated across both presentations.

In a prospective 4 arm observational study conducted in Ethiopian patients, patterns of liver toxicity in patients on anti-retroviral therapy and anti-tubercular therapy were evaluated in treatment naïve patients; 1060 patients were enrolled in 4 treatment groups and liver function tests (LFTs) monitored at baseline, 1, 2, 4, 8, 12 and 24 weeks during treatment; CD4 count and HIV viral load (VL) were measured at baseline, 24 and 48 weeks (42).

Overall 15% patients in this study developed DILI; incidence of DILI in order of frequency was: 24.2% in TB-HIV co infected patients with CD4<200 receiving RIF based TB

medication and EFV based anti-retroviral therapy; 10.8% in TB-HIV co-infected patients with CD4>200 receiving anti-tubercular therapy alone, 8.8% in HIV patients receiving anti-retroviral therapy alone followed by 2.9% in TB patients taking anti-tubercular therapy alone. Concomitant TB and HIV medication increased the risk of DILI by 10-fold compared to TB medication alone. HIV co-infection increased risk of DILI by 4-fold. Anti-retroviral therapy associated DILI was 3-fold higher than anti-tubercular therapy.

The DILI patterns were cholestatic in 61% cases, hepatocellular in 15% and mixed in 24% cases. Anti-retroviral therapy was associated with a cholestatic pattern and mild DILI whereas anti-tubercular therapy was associated with hepatocellular pattern and more severe disease (42).

1.1.7:Management

Management of patients who develop DILI is in accordance with the Southern African HIV Clinicians Society guidelines and depends on the severity of elevation of ALT and total bilirubin and drug regimen (7). Treatment options for anti-tubercular DILI include interruption of therapy with introduction of bridging therapy with moxifloxacin, ethambutol and an injectable like kanamycin. The liver functions are monitored serially till the ALT is less than 100 IU/l and/or total bilirubin is less than 25mmol/l and the first line anti-tubercular drugs are re-introduced serially as tolerated, starting with RIF, followed by INH and PZA provided ALT and total bilirubin monitored 3 days after introduction of each drug remain <100 IU/l and <25mmol/l respectively (7). Therefore, at discharge, patients may be on a regimen of moxifloxacin, ethambutol and kanamycin, or 2 of the first line anti-tubercular drugs successfully re-introduced or 3 of the first line anti-tubercular drugs re-introduced or successful re-introduction of the full first line regimen.

In the case of anti-retroviral therapy DILI developing on an EFV based regimen, if the DILI is mild and LFTs resolve within 5-7 days after interruption of EFV, it may be re-started before re-challenging the TB medication. Otherwise, its re-challenge can be attempted after TB drug re-challenge if it was a mild DILI. If, however, the DILI is severe, a protease inhibitor regimen with lopinavir-ritonavir can instead be initiated. If the DILI develops in patients on a nevirapine (NVP) based regimen, after the TB drugs have been re-challenged, an anti-retroviral therapy regimen based on EFV can be attempted.

According to data analysed from King Edward Hospital in Durban, the median time to ALT normalisation was 28 days and 42 of 53 patients were successfully re challenged with either a

bridging therapy drug plus one of the first line anti-tubercular drugs or 2 or more of the first line anti-tubercular drugs and in 5 patients the DILI recurred; the recurrence was not associated with the method of re-challenge. This study concluded that anti-tubercular therapy can be safely re started in majority of subjects with recurrence in approximately 12% of subjects (43).

A small randomised controlled trial conducted in 175 HIV negative patients in India found no difference in recurrence rates of anti-tubercular DILI between 3 approaches; re-challenging with a single agent one by one with gradual increment of dose, re-challenge each agent at full dose at a time or re-challenge with all drugs together at full dose (44).

This retrospective study therefore looks at patients who present with DILI in an in-patient setting and various characteristics such as symptoms at presentation, clinical and biochemical severity, concomitant infections, medications used and the outcome of medication re-challenge.

1.2: STUDY OBJECTIVES

1) To describe the demographics of patients presenting with DILI on admission
2) To determine the patterns of clinical presentation of patients with DILI
3) To determine the median time from start of treatment to presentation with DILI
4) To assess the proportion of patients presenting with DILI on anti-tubercular therapy alone versus anti-retroviral therapy alone versus combination therapy
5) To determine the number of patients successfully re-challenged on first line drugs or remaining on alternative regimens at discharge
6) To determine the in-hospital mortality rate amongst the study patients, either as a direct or indirect result of the DILI, and identify predictors of all-cause mortality

1.3: MATERIALS AND METHODS

The study is a retrospective review of the database of patients who presented with drug induced liver injury as in-patients to the division of Infectious Diseases at Helen Joseph hospital, Johannesburg between October 2015 and February 2017. Due to the retrospective nature of the study, convenience sampling was used where all patients who presented over the study period were included with the exception of one due to the age criterion.

The participants (aged 18 years and older) were admitted to the medical wards with TB and/or HIV co-infection, on therapy with anti-tubercular drugs and/or anti-retroviral drugs and presented with DILI.

Anti-tubercular DILI is defined by the Southern African HIV Clinicians Society as: elevation of liver enzyme alanine aminotransferase (ALT) >120 IU/l (>3 times upper limit normal) (ULN) in symptomatic patients (who may complain of nausea, vomiting, malaise, loss of appetite, abdominal pain or jaundice) or >200 IU/l (>5 times ULN) in asymptomatic patients; or total serum bilirubin concentration of >40 mmol/l, provided other competing aetiologies such as acute viral hepatitis, autoimmune hepatitis and other liver diseases are ruled out (7). The upper limit of normal for ALT and total bilirubin as per South African National Health Laboratory service reference range is 40 IU/l and 21 mmol/l respectively.

Approval was obtained from the human research ethics committee (HREC) of the University of Witwatersrand and the ethics committee at Helen Joseph hospital. The database is maintained by the division of Infectious diseases at Helen Joseph Hospital. Each patient was assigned a random number and various parameters were entered into an excel sheet. This included:

- patient demographics - age and gender

- date of admission and date of discharge/death – this was used to calculate the length of hospital stay

- symptoms on admission: nausea, vomiting, abdominal pain, jaundice
- HIV status, CD4 count and HIV viral load results

-if patient was on treatment for TB, the site of infection (pulmonary, meningitis, bone, abdominal or disseminated) and how diagnosis was made (sputum GXP, cerebrospinal fluid findings, abdominal ultrasound findings, urine lipoarabinomannan (LAM) results or TB Bactec) was recorded

-use of other concomitant hepatotoxic drugs by the patient - cotrimoxazole, fluconazole, paracetamol or co-amoxiclav

-results of liver function tests: alanine transaminase (ALT), total bilirubin, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), serum albumin, INR;

-Hepatitis B surface antigen and Hepatitis C antibody results, autoimmune work up (if done) such as anti-liver kidney microsomal antibodies (anti-LKM), anti-nuclear antibody (ANA) and anti-smooth muscle antibody (ASMA)

- for those patients in whom an abdominal ultrasound was done, findings documented include liver size, presence of splenic micro-abscesses, intra-abdominal lymphadenopathy or presence of ascites

-in patients on anti-tubercular therapy, the drug regimen was documented as being on one or more of: RIF, INH, PZA, ETH or other (Moxifloxacin, Kanamycin). The date of onset of therapy was used to calculate the duration of treatment prior to presentation with DILI

-in patients on anti-retroviral therapy the drug regimen was documented as being on one or more of efavirenz (EFV), emtricitabine/lamivudine (FTC/3TC), tenofovir (TDF), lopinavir-ritonavir (LPV-r), atazanavir-ritonavir (ATV-r), stavudine (d4T), abacavir (ABC), nevirapine (NVP), zidovudine (AZT), raltegravir (RAL). The date of onset of therapy was used to calculate the duration of treatment prior to presentation with DILI

-similarly, the discharge regimen was documented for all patients; however, if a patient presented in fulminant liver failure necessitating interruption of therapy, the patient may be discharged with no drug therapy and followed up as an outpatient and drug therapy re challenged as an out-patient when feasible

The liver function tests were used to derive the R value (patient's ALT/40 divide by patient's ALP/120) which would classify the pattern of liver enzyme elevation as hepatocellular, cholestatic or mixed, where:

-hepatocellular: R > or equal to 5

-cholestatic: R < or equal to 2

-mixed: R >2 but <5

Based on the SAHIVCS definition of DILI, the patient's symptoms and liver enzymes were used to categorise the DILI as mild, moderate or severe.

-mild DILI - patient clinically well, with ALT >120 but <200 IU/l, total bilirubin <40mmol/l

-moderate DILI - patient clinically well, with ALT >200 IU/l regardless of bilirubin results

-severe DILI - patient who is clinically unwell and meets the DILI criteria

1.3.1:Statistical analysis

Patient demographic and clinical characteristics were summarized using frequencies for categorical variables and means with standard deviation for normally distributed or the median and interquartile range (IQR) for not normally distributed data. We present patient demographics and clinical characteristics at admission, stratified by type of DILI. To compare demographic and clinical characteristics at baseline between the different types of DILI, we used Wilcoxon rank sum or Kruskal-Wallis test for non-parametric data, student t test for parametric or normally distributed data and chi-square (or Fischer's exact test for sparse data) for proportions.

To assess predictors of all-cause mortality we used Cox proportional hazards regression among patients admitted to the medical wards for DILI. Follow-up time was calculated from the date of admission to the ward for DILI until the earliest of death, discharge or close of the dataset (date). Variables with a p value less than 0.25 in the univariate analysis along with *a priori* variables (e.g. age and gender) were included in the final multivariate model. We present the hazard ratio and corresponding 95% confidence interval. Because the number with the outcome of interest was limited (n=21) and to minimize the risk of overfitting, the final model was restricted by the number of predictors that could be added (i.e. one predictive variable for every five to ten outcomes observed) (45). All analyses were conducted in Statistica and SAS version 9.3 (SAS Institute, Cary, North Carolina, USA).

1.4: ETHICS APPROVAL

Ethics approval was obtained from the Human Research Ethics Committee of the University of Witwatersrand. Permission to conduct the research was also obtained from the management of Helen Joseph hospital. Every patient was assigned a random number and patient identity remained anonymous. As the study was retrospective, there is no risk to any patient involved in the study and a waiver of informed consent was granted to retrospectively review the records.

1.5: LIMITATIONS OF STUDY

The data set was based on information entered into the database by several different users of the system which may result in some variability of information. There were incomplete sets of results for variables such as Hepatitis C serology and findings of abdominal ultrasound. Not all

patients had information regarding concomitant hepatotoxic drug use and dates of initiation of anti-tubercular or anti-retroviral therapy were not available for all patients. No information was available regarding over-the-counter or herbal medication use or ethanol use.

Only a minority of patients had a liver biopsy since it is not routinely performed in evaluation of patients with liver injury.

To calculate the actual incidence of DILI due to various drugs a prospective trial enrolling all patients in the hospital and using that as a denominator for patients developing hepatotoxicity and further analysing those patients will provide more accurate information regarding incidence, risk factors, high risk groups and patient characteristics for DILI.

1.6: FUNDING

The study is self-funded including all costs on printing of materials, transport to collect data and internet costs associated.

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CHAPTER 2: SUBMISSIBLE ARTICLE

Title: Drug induced liver injury in patients on anti-tubercular and/or anti-retroviral therapy at Helen Joseph Hospital, Johannesburg

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ABSTRACT

Background

In South Africa, an estimated 73% of patients with TB are co-infected with HIV. The anti-tubercular and anti-retroviral drugs are known to cause drug induced liver injury (DILI), the consequences of which include treatment failure, disease relapse and drug resistance.

Objectives

To study patients on anti-tubercular and/or anti-retroviral therapy with DILI to establish their demographics, clinical presentation and severity, time to presentation from initiation of drug therapy, management and outcomes.

Methods

A retrospective review of 129 patients who presented with DILI to Helen Joseph hospital from October 2015 to February 2017 was done and data collected on drug history, biochemical investigations and relevant imaging.

Results:

Overall, 61% patients were male (n=79) and 39% female (n=50). 46 patients had anti-tubercular DILI, 29 had anti-retroviral DILI and 54 patients were on both anti-tubercular and anti-retroviral therapy. The median age of patients was 35.4 years (IQR 31.4-43.5 years). 20% patients were on a concomitant hepatotoxic drug, co-trimoxazole ($p < 0.05$).

None of the patients were co-infected with Hepatitis C and 4.7% patients had co-infection with Hepatitis B. The commonest presenting symptom was nausea and vomiting.

Of the patients with anti-retroviral DILI, 89% were on first line treatment (TDF/FTC/EFV), with a median CD4 count of 549 cells/microlitre and median viral load of 549 copies/ml. The median time to development of DILI was 85 days from drug initiation.

Of the patients with anti-tubercular DILI, 76% were on first line regimen (RIF/INH/PZA/ETH) with a median CD4 of 37.5 cells/microlitre and median viral load of 57,400 copies/ml. The median time to DILI from initiation of therapy was 14 days.

In the patients with mixed DILI, 89% were on first line anti-retroviral therapy and 70.4% on first line anti-tubercular therapy. Their median CD4 count was 56 cells/microlitre and median viral load 1230 copies/ml. In this group anti-retroviral therapy was used for a median 168 days prior to presentation and anti-tubercular therapy for a median of 41 days.

Overall the in-hospital mortality of these patients was 16.3% (n = 21).

Conclusion

Patients with anti-tubercular DILI presented earlier (within 2 months) whereas those anti-retroviral DILI presented up to 1 year after therapy initiation.

Those with anti-retroviral DILI presented with lower viral loads and higher CD4 counts. Only 27% of those with anti-retroviral DILI and 35% of those with anti-tubercular DILI were successfully re-challenged to their initial drug regimen by discharge

Patients with severe DILI and mixed pattern of liver injury were at increased risk of all-cause mortality during the admission.

Introduction:

South Africa has one of the highest prevalence rates of human immunodeficiency virus (HIV) in the world - 11.2% of the general population and 16.6% of the population aged 15-49 years who are in their most productive years - and its associated opportunistic infections, the commonest being tuberculosis (TB) (1). South Africa ranks third globally in TB prevalence (0.4-0.59 million) after India and China. TB is the leading cause of death among people living with HIV, causing more than one third of all AIDS-related deaths in 2015 according to World Health Organisation (WHO).(3). According to the South African Department of Health, 73% of patients with TB are HIV positive.(46)

Anti-retroviral therapy and anti-tubercular therapy are known to cause many adverse effects such as liver injury, skin reactions, neurological and gastrointestinal disorders. Drug induced liver injury (DILI) is one of the most important and serious adverse effects. (6)

It is estimated that DILI affects 5-33% of patients on treatment for TB, although local data are limited (7). Depending on the regimen, 9-30% patients on anti-retroviral therapy develop DILI (8). Anti-tubercular DILI in HIV co-infection is the most common adverse effect of treatment necessitating therapy interruption (9) which can consequently lead to treatment failure, relapse or drug resistance. This presents a challenge in the management of both HIV and TB, with South Africa having the largest treatment programme in the world, accounting for 20% of people on antiretroviral therapy globally. (2). Hepatotoxicity leading to treatment interruption is associated with reduction in HIV viral suppression over time. (31)

This is further complicated by the fact that HIV can be co-acquired with infections such as hepatitis B and hepatitis C which are risk factors for acute or chronic liver disease and progression to hepatocellular carcinoma (23). Patients with HIV who have a CD4 count of <200 cells/microlitre are also on cotrimoxazole prophylaxis for the prevention of *pneumocystis jirovecii pneumonia* and toxoplasmosis, which is also hepatotoxic. In patients with history of cryptococcal meningitis and CD4 count <200, prophylactic fluconazole therapy is an additional risk factor for liver dysfunction (23).

Aims

To determine the demographic, clinical and biochemical characteristics of patients presenting with DILI to Helen Joseph hospital as in-patients, time between drug initiation and liver injury and determine rate of successful re-challenge to medication and outcome in these patients.

Materials and methods

A retrospective review of an electronic database of patients who presented with drug induced liver injury as in-patients to the division of Infectious Diseases at Helen Joseph hospital, Johannesburg between October 2015 and February 2017. In view of the retrospective nature of the study, convenience sampling was used whereby all the patients who presented over the study period were included with the exception of one due to the age criterion (age 18 years and older who were admitted to the medical wards with TB and/or HIV co infection, on therapy with anti-tubercular drugs and/or anti-retroviral drugs who presented with DILI).

The patients were managed according to the Southern African HIV Clinicians Society (SAHIVCS) guidelines.

Data Collection

Upon admission of patients, information regarding patient demographics (age, gender), co-morbidities, presenting symptoms, date of onset of drug therapy, drug regimen (including other hepatotoxic drugs), mode of diagnosis of tuberculosis (if present) was recorded.

Liver function tests were monitored (ALP, GGT, total bilirubin, ALT, AST, INR) on admission and serially as indicated to monitor response to therapy and/or re-challenge. In addition, other laboratory investigations such as CD4 count, HIV viral load, hepatitis B and hepatitis C status, any autoimmune work up and liver biopsy results (done to rule out other causes of liver injury) and radiological investigations such as ultrasound of the liver were also collected.

The liver enzymes were used to derive the pattern of DILI (hepatocellular, cholestatic or mixed) and severity of DILI was categorised as mild, moderate or severe.

The anti-retroviral and anti-tubercular drug regimen on discharge was also documented to establish how many patients were successfully re-challenged back to the initial drug regimen. The length of stay and mortality rate of the patients was derived using this data.

Definitions

Anti-tubercular DILI is defined by the Southern African HIV Clinicians Society as: elevation of liver enzyme alanine aminotransferase (ALT) >120 IU/l (>3 times upper limit normal) (ULN) in symptomatic patients (who may complain of nausea, vomiting, malaise, loss of appetite, abdominal pain or jaundice) or >200 IU/l (>5 times ULN) in asymptomatic patients; or total serum bilirubin concentration of >40 mmol/l, provided other competing aetiologies such as acute viral hepatitis, autoimmune hepatitis and other liver diseases are ruled out (7).

The upper limit of normal for ALT and total bilirubin as per South African National Health Laboratory Service (NHLS) reference range is 40 IU/l and 21 mmol/l respectively.

Anti-tubercular DILI was defined as DILI occurring in a patient with TB/HIV co-infected patient who was exclusively on anti-tubercular therapy; anti-retroviral DILI as DILI occurring in a patient only on anti-retroviral therapy and mixed DILI was defined as occurring in a patient using both anti-retroviral therapy and anti-tubercular therapy.

The liver function tests were used to derive the R value (patient's ALT/40 divide by patient's ALP/120) which would classify the pattern of liver enzyme elevation as hepatocellular, cholestatic or mixed, where:

-hepatocellular: R > or equal to 5

-cholestatic: R < or equal to 2

-mixed: R >2 but <5

Based on the SAHIVCS definition, the patients' symptoms and liver enzymes were used to categorise the DILI as mild, moderate or severe:

-mild DILI- patient clinically well, with ALT >120 but <200 IU/l, total bilirubin <40mmol/l

-moderate DILI- patient clinically well, with ALT >200 IU/l regardless of bilirubin results

-severe DILI- patient who is clinically unwell and meets the DILI criteria.

The anti-retroviral and anti-tubercular drug regimen on discharge was also documented to establish how many patients were successfully re-challenged back to the initial drug regimen. Patients were categorised as either being discharged on the same or a modified regimen. The outcome was recorded (discharged or died) and time from admission to the outcome was calculated.

Statistical analysis

Patient demographic and clinical characteristics were summarized using frequencies for categorical variables and means with standard deviation for normally distributed or the median and interquartile range (IQR) for not normally distributed data. We present patient demographics and clinical characteristics at admission, stratified by type of DILI. To compare demographic and clinical characteristics at baseline between the different types of DILI, we used Wilcoxon rank sum or Kruskal-Wallis test for non-parametric data, student t test for

parametric or normally distributed data and chi-square (or Fischer's exact test for sparse data) for proportions.

The primary outcome of the study was all-cause mortality. To assess predictors of all-cause mortality we used Cox proportional hazards regression among patients admitted to the ID ward for DILI. Follow-up time was calculated from the date of admission to the ID ward for DILI until the earliest of death, discharge or close of the dataset (date). Variables with a p value less than 0.25 in the univariate analysis along with *a priori* variables (e.g. age and gender) were included in the final multivariate model. We present the hazard ratio and corresponding 95% confidence interval. Because the number with the outcome of interest was limited (n=21) and to minimize the risk of overfitting, the final model was restricted by the number of predictors that could be added (i.e. one predictive variable for every five to ten outcomes observed) (45). All analyses were conducted in Statistica and SAS version 9.3 (SAS Institute, Cary, North Carolina, USA).

RESULTS

The records of 137 patients was reviewed; HIV negative patients were excluded from analysis due to the small number (n=8). The other 129 patients were divided into 3 categories of patients: anti-tubercular DILI, anti-retroviral DILI and mixed DILI.

These subgroups were further analysed for various characteristics.

Patient demographics:

There were 79 male (61%) and 50 female (39%) patients. Of these patients, 46 had anti-tubercular DILI (35% patients), 29 had anti-retroviral DILI (22%) and 54 patients had mixed DILI (42% patients). In these patients, the median age was 35.4 years (inter quartile range 31.4 – 43.5 years). A pictorial representation is presented in Figure 1.

Information regarding concomitant use of medication was only available from 123 patients and amongst them, the commonest drug with hepatotoxic potential being used was co-trimoxazole, in 20% of all patients (p value <0.05). Other drugs include fluconazole in 7% patients and amoxicillin-clavulanate in 10% patients. Inadequate information was available regarding the use of other hepatotoxic drugs, over-the-counter and herbal medication.

Only 6 patients had hepatitis B infection; however, no results were available for 12 patients. None of the study patients were found to be hepatitis C positive.

Presentation:

The commonest symptom on presentation was nausea and/or vomiting in 46.5% patients (n=60), jaundice in 42.6% patients (n=55) and abdominal pain in 19% patients (n=24). Sub-group analysis however showed that in patients with anti-tubercular DILI or mixed DILI, nausea and/or vomiting was more common and in the group with anti-retroviral DILI, jaundice was more common.

Patients with anti-retroviral DILI:

On admission, 89% patients (n=83) were using anti-retroviral therapy; 89% of them were on the FDC (FTC/TDF/EFV). 65% of them presented with a mixed DILI (i.e. concomitant use of anti-tubercular therapy) and 35% with DILI due to anti-retroviral therapy only. Of these patients, only 27.7% (n=23) were discharged on the FDC and 83.3% (n=60) required a modified regimen (whereby EFV was substituted with a protease inhibitor such as Lopinavir-Ritonavir). The median duration between anti-retroviral therapy initiation and time to DILI was 85 days in the anti-retroviral DILI group versus 168 days in the mixed DILI group.

Patients with anti-tubercular DILI:

Of those on anti-tubercular therapy, 76% had a confirmed diagnosis and 24% were on empiric therapy. The commonest site of TB in these patients was pulmonary (56.1% patients); 35.6% had disseminated TB and 8.2% had extra-pulmonary TB.

On admission, 100 patients were on anti-tubercular therapy; a majority (n=73) were on first line regimen (RHZE) for drug sensitive TB, 13 patients were on RIF and INH only. Of these patients, 46 presented with anti-tubercular DILI and 54 with mixed DILI. Only 12 were discharged on the first line regimen (RHZE) after successful re-challenge as in-patients and 23 patients were discharged on a regimen containing RIF and INH only. Sixty-five patients were discharged on a modified regimen containing only one first line drug or bridging therapy regimen.

The median duration between TB treatment and DILI was 14 days for patients on anti-tubercular therapy only versus 41 days for patients on both antiretroviral therapy and anti-tubercular therapy.

Biochemical characteristics:

The median CD4 count was 66 cells/microlitre among all patients; 37.5 (IQR 13-70) cells/microlitre in anti-tubercular DILI patients, 549 (IQR 345-741) cells/microlitre in anti-retroviral DILI patients and 56 (IQR 19-108) cells/microlitre in mixed DILI patients. The median viral load amongst all patients was 611 copies/ml (IQR 64-332500); 57400 (IQR 11300-2,180,000) in patients with anti-tubercular DILI, 549 (IQR 20-2480) in patients with anti-retroviral DILI and 1230 (IQR 130-143,000) in patients with mixed DILI.

The DILI was classified as severe among 56% of all patients; of those with TB DILI, 52.3% were severe and 29.6% were moderate; in those with anti-retroviral DILI, 62.1% were severe, 37.9% moderate and none had mild DILI. In patients with mixed DILI, 55.6% patients were classified as severe.

The median ALT was highest in the anti-retroviral DILI group at 626 units/litre (IQR 292-1293) versus 135 (IQR 79-438) in those with anti-tubercular DILI and 125 (IQR 45-291) in those with mixed DILI. The median total bilirubin was highest in patients with anti-retroviral DILI, 193mmol/litre (IQR 75-286) versus 60mmol/l (IQR 17-208) in anti-tubercular DILI patients and 64 (IQR 21-127) mmol/l in those with mixed DILI. GGT was also highest in the anti-retroviral DILI group at 322 units/litre (IQR 131-519), 200 units/litre (IQR 122-304) in the anti-tubercular DILI group and 294 units/litre (IQR 172-497) in the mixed DILI group.

In all the DILI patients, 31% had a predominantly hepatocellular pattern whereas 42.6% had a predominantly cholestatic pattern and 26.4% patients had a mixed pattern. In those with anti-tubercular DILI, the LFTS in 26.1% were hepatocellular whereas 43.5% were cholestatic and 30.4% mixed. In the anti-retroviral DILI group, hepatocellular pattern was most common in 58.6% patients versus a mixed pattern in 27.6% patients and cholestatic in 13.8% patients. In those with mixed DILI, majority patients (57.4%) had a predominantly cholestatic pattern, 20.4% had a hepatocellular pattern and 22.2% had a mixed pattern. This is illustrated in Figure 2.

Other investigations:

Sixteen patients had an autoimmune screen; 3 patients had a positive anti-nuclear factor whereas all anti-microsomal antibodies and anti-liver-kidney antibodies were negative.

6 patients had a liver biopsy; 2 were reported as drug induced hepatitis, 1 as autoimmune hepatitis, 1 as granulomatous hepatitis, 1 patient had liver cirrhosis and 1 specimen was reported as being inadequate.

Outcomes:

The median length of hospital stay was longest in those presenting with anti-tubercular DILI at 19 days (IQR 12-35 days) and shortest in those with anti-retroviral DILI at 9 days (IQR 6-13 days) and 15 days in those with mixed DILI (IQR 10-23 days).

Of all the patients admitted with DILI, 84% (n=108) were discharged home and 16% (n=21) died. Of those who died, 10 were admitted with anti-tubercular DILI, 2 with anti-retroviral DILI and 9 with mixed DILI.

The mortality rate was 15.5% in patients <35 years old versus 16.9% in those >35 years of age; this was not found to be statistically significant. A similar percentage of male and female patients died (16.4% and 16% respectively).

Of those who died, 3 had a hepatocellular pattern of liver enzyme elevation (HR 1.0), 9 had a cholestatic pattern (HR 1.48) and 9 had a mixed pattern with a hazard ratio of 2.43 (0.66-9.03).

Only 1 patient classified as mild DILI died whereas 16 patients who died were classified as severe DILI (hazard ratio with 95% confidence interval = 8.58) and 4 patients who died had moderate DILI.

Table 1 presents the Predictors of death (n=21) among patients admitted for drug induced liver injury between Feb 2015 and Feb 2017 (n=129).

Discussion

The majority of patients in this study were male and median age of patients was 35.4 years; this is in contrast to the SLATIN DILI network whose patients had a mean age at DILI diagnosis of 51.4 years with a female predominance. (25)

Also, in a DILI study conducted in Montreal, female sex was consistently found to be associated with increased incidence of adverse effects, even after adjusting for potential confounding factors (6) which is in contrast to our findings. This is despite the higher prevalence of HIV in females in our population, thus likely reflecting altered propensity to adverse drug reactions or healthcare seeking attitudes in our setting.

In the study by Pedral Sampaio *et al*, patients with HIV/TB co infection whose nadir CD4 <50 were 1.9 times more likely to get DILI (33). This is similar to our findings whereby the patients with mixed DILI had a CD4 count less than 50 cells/microlitre in 48% of patients.

Use of concomitant hepatotoxic medication, co-trimoxazole was statistically significant ($p<0.05$) but co-infection with hepatitis B was not significantly associated with DILI.

The incidence of DILI was highest in the group on combination anti-tubercular and anti-retroviral therapy (42% patients) followed by those on anti-tubercular therapy followed by patients on anti-retroviral therapy only. This is consistent with other studies which showed higher incidence of DILI in patients on both drugs versus either drug alone (28, 37).

Those with anti-tubercular DILI presented a median of 14 days after drug initiation or 41 days if they were on anti-tubercular and anti-retroviral therapy whereas in the study by S Naidoo *et al*, which looked at out-patients presenting with anti-tubercular DILI, the average duration between TB treatment and time to DILI diagnosis was 31 days (28). This probably reflects the more severe DILI presenting earlier in our study and requiring in patient care. The longest duration between treatment initiation and presentation with DILI in this group was 92 days.

The anti-retroviral DILI patients presented later on average, at a median of 85 days if on anti-retroviral therapy only versus a median of 168 days if on anti-retroviral and anti-tubercular therapy. Some patients on anti-retroviral therapy presented as late as 355 days after initiation of therapy.

Those with anti-retroviral DILI had higher CD4 counts and lower viral loads than those anti-tubercular or mixed DILI and this was found to be statistically significant ($p<0.05$).

Patients with anti-tubercular and mixed DILI were more likely to have cholestatic LFTs, consistent with the study by Naidoo *et al* who reported a predominantly cholestatic picture in anti-tubercular DILI (28). In contrast, the predominant pattern of liver enzyme elevation in anti-retroviral DILI patients was hepatocellular.

Most patients in our study presented with severe DILI, regardless of drug implicated whereas the study by Yimer *et al* suggested that anti-retroviral drugs are associated with a mild DILI and anti-tubercular therapy with a severe DILI (42).

Among those presenting with anti-tubercular DILI, only 12% were successfully re-challenged while in hospital to full first line treatment (RHZE) whereas in the study by Naidoo *et al* 72% of their patients who presented with anti-tubercular DILI were successfully re-introduced to first line therapy; however, it was a study done in an out-patient setting hence they had a different follow up period to our study whose findings are limited to their in-patient stay.

In patients presenting with anti-retroviral DILI, 27% patients were successfully re-challenged back to the original drug regimen at discharge whereas the majority were discharged on a modified regimen (as per the methods discussed earlier).

The small sample size limits the statistical significance of some of the findings and some patients were lost to follow up therefore how many were eventually re-challenged is not clear. Being retrospective in nature, some questions could not be answered if not included on the original data collection form. There was also inadequate information available from some patients which limits accurate assessment of these results.

Yet, this study is unique in that it is one of few in-patient analyses of patients with DILI in South Africa and variability from other African study results points to a potential difference in genetic or environmental susceptibility of these patients to DILI and its outcomes.

Conclusion

Patients with anti-tubercular DILI presented earlier (within intensive phase of therapy) whereas those anti-retroviral DILI presented up to 1 year after therapy initiation; this highlights the importance of maintaining a high index of suspicion in this group of patients and regular surveillance for earlier diagnosis of DILI to reduced associated morbidity.

Antitubercular and mixed DILI patients were more likely to present with cholestatic enzymes whereas antiretroviral DILI patients were more likely to present with transaminitis. Most patients presented with severe DILI and it was associated with the highest mortality.

Among those admitted with DILI, those with severe DILI and mixed pattern of liver injury were at increased risk of all-cause mortality during the admission.

Recommendations

Further prospective studies and data are required to establish which patients are at highest risk and which drugs are more likely to cause their DILI so that closer monitoring can be instituted for high risk patients. Adverse drug reactions remain notifiable. A national registry of these patients should be maintained and better adverse drug reaction monitoring should be in place to gauge the extent of drug induced liver injury in order to make a case for development of safer drugs. In the interim, therapeutic drug level monitoring can guide dose adjustments of current medications.

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Tables &Figures:

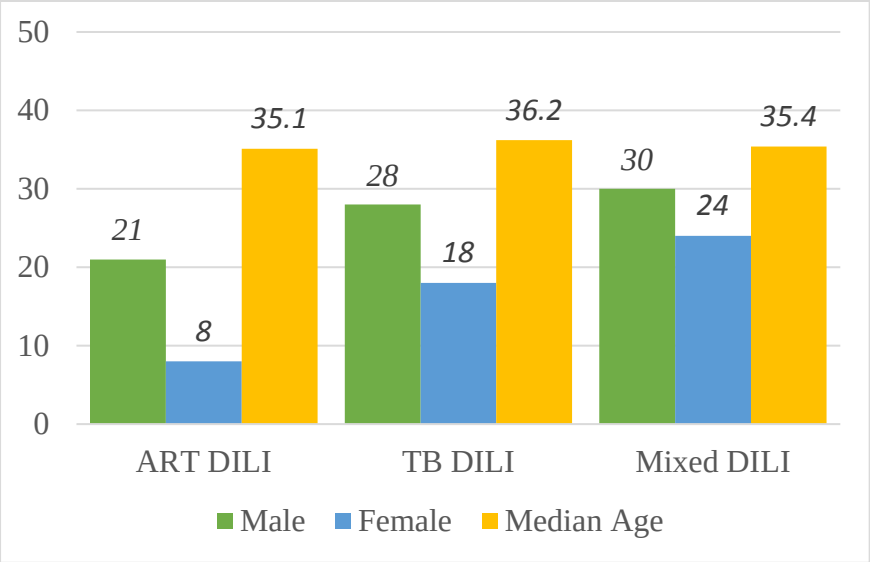

Figure 1: Demographics of Presenting Patients (n=129)

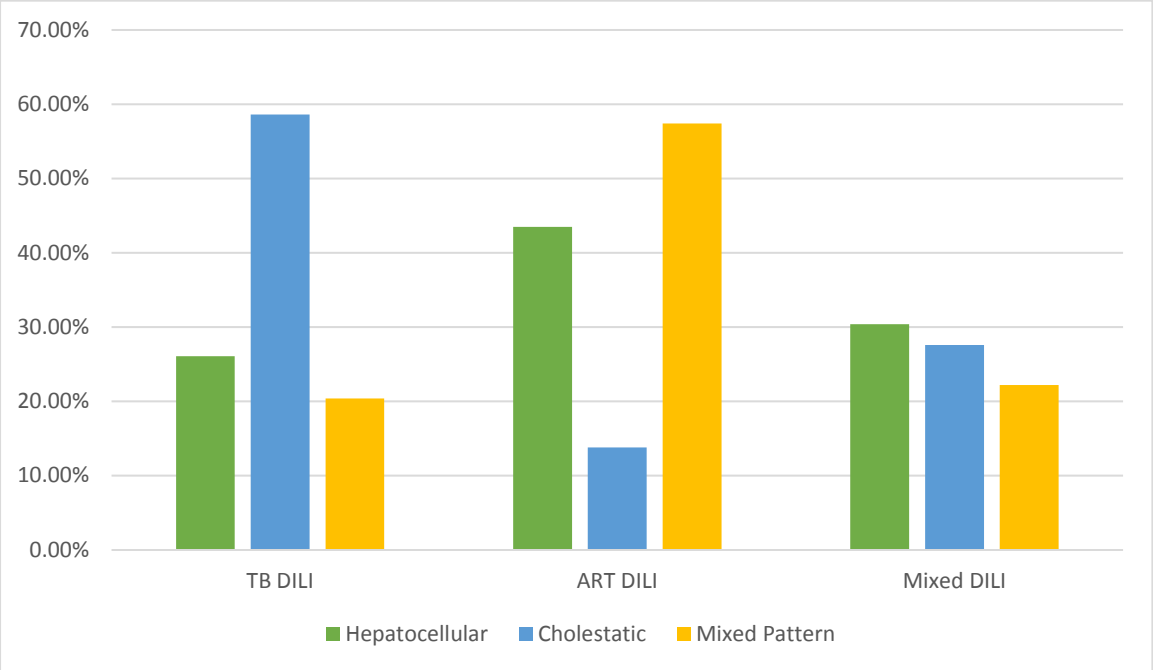

Figure 2: Patterns of liver injury

Table 1: Predictors of mortality (n=21)

		Outcome (n/N)	Crude HR 95% CI	Adjusted HR 95% CI
		N = 21		
Exposure	TB alone	10/46 (21.7%)	0.92 (0.37 – 2.30)	
	ART alone	2/29 (6.9%)	0.84 (0.18 – 3.97)	
	TB and HIV	9/54 (16.7%)	1.0	
Age	<35	9/58 (15.5%)	1.0	1.0
	≥35	12/71 (16.9%)	0.97 (0.41 – 2.32)	0.76 (0.30 – 1.89)
Gender	Female	8/50 (38.1%)	1.0	1.0
	Male	13/79 (61.9%)	0.91 (0.38 – 2.21)	0.77 (0.31 – 1.90)
CD4 count at presentation	≤50	7/56 (12.5%)	0.5 (0.18 – 1.45)	
	51 – 100	7/24 (29.2%)	1.23 (0.43 – 3.54)	
	> 101	7/49 (14.3%)	1.0	
Type of DILI	Mild	1/21 (4.8%)	1.0	1.0
	Moderate	4/35 (11.4%)	3.38 (0.38 – 30.25)	3.31 (0.36 – 29.2)
	Severe	16/71 (22.5%)	8.37 (1.10 – 63.65)	8.58 (1.13 – 65.3)
Type of Liver patter	Hepatocellular	3/40 (7.5%)	1.0	
	Cholestatic	9/55 (16.4%)	1.48 (0.40-5.51)	
	Mixed	9/34 (26.5%)	2.43 (0.66 – 9.03)	

CHAPTER 3: APPENDICES

3.1: DATA COLLECTION FORM

Drug induced livery injury in patients on anti-tubercular therapy and/or anti-retroviral therapy at Helen Joseph Hospital:

1) Unique Patient Identifier Number: _____

2) Demographics

   Age: _____

   Gender

   a) Male _____

   b) Female _____

3) HIV:

   a) Positive _____

   b) Negative _____

4) TB:

   a) Present _____

   b) Absent _____

5) In patients with TB, diagnosis:

   a) Confirmed _____

   b) Empiric _____

5.1) In patients with TB, site of disease:

   a) Pulmonary _____

   b) Abdomen _____

   c) Meningitis _____

   d) Bone _____

   e) Disseminated _____

f) Other _____

6) In patients with HIV

a) CD4 Count (cells/microlitre) _____

b) Viral Load (copies/ml): _____

7) Hep B S Ag Status: _____

8) Hep C Ab status: _____

9) TB regiment at presentation:

a) Rifampicin _____

b) Isoniazid _____

c) Ethambutol _____

d) Pyrazinamide _____

e) Other _____

10) HIV medication at presentation:

a) Efavirenz _____

b) Emtricitabine/Lamivudine _____

c) Tenofovir _____

d) Stavudine _____

e) Abacavir _____

f) Zidovudine _____

g) Nevirapine _____

h) Lopinavir-Ritonavir _____

i) Raltegravir _____

j) Atazanavir-Ritonavir _____

k) Other _____

11) Duration since onset of anti-tubercular drugs: _____

12) Duration since onset of anti-retroviral therapy: _____

13) Other drugs:

a) Bactrim _____

b) Fluconazole _____

c) Paracetamol _____

d) Amoxicillin-Clavulanate _____

14) DILI Symptoms (at presentation):

a) Nausea _____

b) Vomiting _____

c) Abdominal Pain _____

d) Jaundice _____

15) At Presentation of Dili:

a) ALT: _____

b) T Bilirubin: _____

c) GGT: _____

d) ALP: _____

e) Albumin: _____

f) INR: _____

16) Liver Functions Predominantly:

a) Cholestatic _____

b) Hepatocellular _____

c) Mixed _____

17) Results of Autoimmune Screen:

a) Anti-liver kidney microsomal antibodies: _____

b) Anti-smooth muscle antibodies: _____

c) Anti-nuclear antibodies: _____

18) Ultrasound done:

a) Yes _____Findings:

b) No _____

19) Severity of Dili:

a) Mild _____

b) Moderate _____

c) Severe _____

20) Liver Biopsy done:

a) Yes _____Findings:

b) No _____

21) Discharge Drugs for Patients with TB:

a) Rifampicin _____

b) Isoniazid _____

c) Ethambutol _____

d) Pyrazinamide _____

e) Moxifloxacin _____

f) Kanamycin _____

g) Other _____

22) Time to re-introduction anti-tubercular regimen at discharge: _____

23) Discharge Drugs for Patients with HIV:

a) Efavirenz _____

b) Emtricitabine/Lamivudine _____

c) Tenofovir _____

d) Stavudine _____

e) Abacavir _____

f) Zidovudine _____

g) Nevirapine _____

h) Lopinavir-Ritonavir _____

i) Raltegravir _____

j) Atazanavir-Ritonavir _____

k) Other _____

24) Time to re-introduction of anti-retroviral regimen at discharge: _____

25) Length of hospital stay: _____

26) Outcome

a) Discharge: _____

b) Death: _____

3.2: ETHICS CLEARANCE:

3.2.1:WITS HREC Ethics Clearence

R14/49 Dr Rushika Mehta

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170324

NAME: (Principal Investigator)	Dr Rushika Mehta
DEPARTMENT:	Internal Medicine Helen Joseph Hospital
PROJECT TITLE:	Drug Induced Liver injury in Patients on Anti-tubercular and/or Anti-retroviral Therapy at Helen Joseph Hospital
DATE CONSIDERED:	31/03/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Prudence Ive and Prof Colin Menezes
APPROVED BY:	Prof P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/04/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. I agree to submit a yearly progress report. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in March and will therefore be due in the month of March each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

3.2.2:Helen Joseph Hospital Ethics Clearance

GAUTENG PROVINCE
REPUBLIC OF SOUTH AFRICA

Gauteng Department of Health
Helen Joseph Hospital
Enquiries: Dr. M.R. Billa
Chief Executive Officer
Tel :(011) 489-0306/1087
Fax :(011) 726-5425
E mail: Raymond.Billa@gauteng.gov.za
Date: 10 May 2016

Dr.M.R.Billa
Chief Executive Officer
Helen Joseph Hospital

Dear Dr.Billa

STUDY: Drug included liver injury in patients on Anti-tubercular and Anti-retroviral Therapy at Helen Joseph Hospital.

RESEARCHERS: Dr Ruchika Mehta

The above was discussed at the Research Committee Meeting. We recommend that permission be granted for Helen Joseph Hospital to be used as a site for the above research. However, since this is a research project involving voluntary participation. We cannot guarantee participation of individuals/patients.
Upon completion of the study, a copy thereof should be submitted to Helen Joseph Hospital.

Thank you

DR. Murimisi Mukansi
CHAIRPERSON
DATE:

Approved

DR. M.R. BILLA
CHIEF EXECUTIVE OFFICER
DATE: 15.05.2016