ASSESSMENT OF THE LIVER IN AN HIV ERA: CLINICAL, LABORATORY AND RADIOLOGICAL ABNORMALITIES

By

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As the candidate's supervisor I have approved this thesis for submission.

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Declaration

I, Dr Bavumile Mbanjwa declare that:

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

(ii) This dissertation has not been submitted for any degree or examination at any other university.

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Dedication

To the almighty God, nothing would have been possible without you in my life.

To my dear husband Vuyani and my lovely son Okuhle, my father (the late) Mr Sabelo Njiyela, my grandmother Mrs. Eunice Njiyela, my guardians Mrs. Nontobeko Mtshengu and Mr Nkosinathi Mtshengu. Thank you for the support you have given me.

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List of abbreviation and nomenclature

AFB	Acid Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ART	Antiretroviral Therapy
AST	Aspartate Aminotransferase
$CD4^+$	Cluster of differentiation 4
DILI	Drug Induced Liver Injury
GGT	Gamma Glutamyl Transferase
GXP	GeneXpert
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency virus 1 and 2
INR	International Normalised Ratio
TB	Tuberculosis
IREP	Rifampicin, Isoniazid, Ethambutol and Pyrazinamide
IR	Isoniazid and Rifampicin

Abstract

Background: Liver – related mortality and morbidity are an increasing burden worldwide.

Aim: To outline the pattern of liver abnormalities at a tertiary hospital in KwaZulu Natal (KZN), Durban, South Africa, during the era of the HIV epidemic.

Methods: This cross-sectional, retrospective study conducted medical records review of all patients found to have liver abnormalities based on clinical, laboratory, and radiological profile, admitted to the medical wards for the period between June 2016 to December 2016.

Results: A total of 157 patients were included, of which 63.1% were males, and 91.7% were black, with a median age of 41 years (IQR, 32–54). Sixty – six (42.0%) patients were HIV negative; 91 (57.9%) were HIV infected, of which 51 (56.0%) were on antiretroviral therapy. Only 15 (29.4%) had an HIV viral load of < 50 copies/mL and 21 (30.9%) with a $CD4^+$ count of ≥ 200 cells/mm^3. In HIV negative, heart failure (48.5%) was the main cause of liver abnormalities (p-value < 0.001), whereas in HIV infected, abdominal TB (24.2%) and DILI (18.7%) were the commonest. Sixty- seven (42.7%) patients died while admitted, and leading causes were HIV/AIDS (40.3%), hypertension (13.4%), and metastatic cancer (13.4%).

Conclusion: In HIV infected patients, abdominal TB was common, which was consistent with the common presenting symptoms of fever and vomiting in this group; whereas in HIV negative, heart failure was the commonest which was also consistent with the leading presenting symptoms of abdominal distension and ascites, and comorbid conditions of hypertension, diabetes mellitus, and dyslipidaemia which are all risk factors of cardiac diseases. Also, mortality was significantly high, and the leading causes were HIV/AIDS, hypertension, and advanced malignancy, which underscores the need to strengthen community-based screening programs for both communicable and non-communicable disease for early detection and referral to care.

Keywords: Liver abnormalities, HIV/AIDS, Antiretroviral therapy, Tuberculosis

Chapter 1: Background and literature review

1. Introduction

Although significant work on various aspects of liver pathology has been done worldwide, including South Africa, a new spectrum of diseases is emerging mainly because of health evolution. We are now seeing an increasing prevalence of liver-related lifestyle disorders, namely non-alcoholic fatty liver disease (NAFLD) with resultant liver cirrhosis and thus the rising frequency of hepatocellular carcinoma (HCC) (1, 2).

Furthermore, the HIV/AIDS epidemic and access to antiretroviral therapy continue to change the disease profile. Amongst other conditions, liver pathology continues to burden HIV infected population with a mortality rate of about 15% according to the Data Collection on Adverse Events of Anti- HIV Drugs study (3).

1.1. Liver abnormalities

1.1.1. Anatomical overview and physiology of the liver and biliary system

The liver is the biggest vital organ in the body, representing 1.5 – 2.5% of body weight, which amounts to 1400 – 1800g in adults (4, 5). It has numerous functions, including bile production, fat-soluble vitamin storage, and metabolism of drugs and bilirubin (6). The liver receives nearly 25% of the cardiac output via two sources; oxygen-rich blood supply through the hepatic artery and nutrient-rich blood from the stomach, intestines, pancreas, and spleen through the portal vein (7).

Macroscopically, the liver is divided into right and left lobes and further subdivided into eight independent segments. Each segment has its portal pedicle, consisting of the hepatic arterial branch, portal branch, and the bile duct with a separate hepatic venous branch (4).

Microscopically, the liver is organized into structural units called lobules. However, functionally it is organized into acini, the smallest functional units containing a small portal tract at the center and terminal hepatic venules at the periphery. The acinus is divided into three zones; zone 1 surrounds the portal tract, zone 3 surrounds the hepatic venule, and the intervening hepatocytes constitute zone 2. Blood from the portal tract flows through these zones to the venule with a decreasing oxygen and nutrient gradient (8, 9). The biliary tract collects, stores, concentrates and delivers bile secreted by the liver, and the bile ducts anatomy follows that of the liver's portal system and segmentation (10, 11).

1.2.2. Mechanism and causes of liver abnormalities

As an organ with a complex structure, high vascular supply, and increased metabolic activity, the liver is particularly susceptible to damage (9, 12). Liver abnormalities generally occur via five mechanisms, namely, inflammation (viral hepatitis, drug-induced liver injury (DILI), alcoholic hepatitis, autoimmune hepatitis), excessive storage (non-alcoholic fatty liver, Wilson's disease, hemochromatosis), infiltration (hepatocellular carcinoma, metastatic tumour, tuberculosis), vascular congestion (congestive cardiac failure, cor-pulmonale, Budd-Chiari syndrome) and biliary obstruction (choledocholithiasis, cholangiocarcinoma, choledochal cyst) (13, 14). Ischemic hepatitis is another well-recognized entity resulting from reduced oxygen delivery to the liver, and the term hepatitis is somewhat of a misnomer since an inflammatory process does not mediate the injury but hepatocytes necrosis (15).

1.1.3. Clinical features

In the acute phase, patients may present with non-specific symptoms such as malaise, anorexia, fever, and jaundice appear as the illness progresses; and clinical examination may be normal or reveal jaundice, hepatomegaly, and hepatic pain. If the insult to the liver is not removed, fibrosis may occur and eventually cirrhosis. Clinical signs such as spider neavi, palmar erythema may be seen, but these are not specific as they may be seen in normal people and pregnant women, and may occur in both acute and chronic liver disease. In males with chronic liver disease, particularly related to alcohol use, signs of hyperestrogenemia, namely gynecomastia, testicular atrophy, loss of male pattern hair distribution, may be seen. With advanced liver disease, ascites, oedema, dilated abdominal veins, feto-hepaticus, asterixis, confusion, and coma may be found (16)

1.1.4. Diagnosis and grading of liver abnormalities

Liver function test

There are several blood tests available that are useful in assessing the status of the liver. The most commonly used tests in clinical practice include the serum aminotransferases, bilirubin, alkaline phosphatase, albumin, and prothrombin time; collectively termed “liver function tests”. These tests are useful in guiding management by determining the pattern of liver disease, classified as either hepatocellular, cholestatic (obstructive), or mixed; and the degree of severity (1)

Imaging

Ultrasound is a non-invasive and inexpensive screening tool in patients presenting with abnormal liver function tests. It may hint on liver morphology, biliary system abnormalities, infiltrative processes, and vascular dopplers (18, 19). Other imaging techniques are Computed tomography CT scan and

Magnetic resonance cholangiopancreatography (MRCP), both of which are minimally invasive. They provide precise depictions of the biliary system, characterize the liver lesion, and delineate vascular anatomy (20). However, in a patient with a cholestatic pattern on liver function test, with or without dilated MRCP is preferable to CT since it can also provide additional information regarding biliary flow (21, 22)

The Fibroscan (ultrasound elastography) is a simple, non-invasive test to rule in/out advanced fibrosis and cirrhosis. It works by measuring the sound wave's velocity passing through the liver and then converts those into a liver stiffness measurement. Since Fibroscan test results are promptly obtained, clinicians can use them to make decisions during the patient's visit; but technical limitations of the test preclude its use in patients with ascites and morbid obesity (23).

Liver Biopsy

Liver biopsy is viewed as the gold standard in diagnosing liver pathology since it provides otherwise unobtainable qualitative information regarding the liver's structural integrity and the type and degree of injury (24). However, several factors are limiting its use; (i) It is contraindicated in the presence of severe coagulopathy, infection of the hepatic bed, extrahepatic biliary obstruction, possible vascular lesions, and hydatid disease. (ii) There may be a misdiagnosis and incorrect staging if the biopsied samples are not representative of the liver's rest. (iii) The limited number of experienced clinicians to perform this invasive procedure and experienced histopathologists to analyze the sample once obtained, particularly in the developing countries. (v) Histology results may be non-specific and non-diagnostic, for example, in drug and toxin-induced injury, overwhelming sepsis (24, 25)

Miscellaneous investigations

Other useful specific tests include toxicology screen and drug tests such as paracetamol level, viral hepatitis serology, autoimmune markers, ceruloplasmin, urine copper, iron saturation, serum ferritin, and alpha-fetoprotein (26, 27).

Those patients with abnormal liver function and morphology presenting with ascites, peritoneal fluid analysis helps determine whether the ascites is due to portal hypertension-related causes or secondary causes. Apart from the fluid's macroscopic analysis, the initial step is to determine the serum-ascites albumin gradient (SAAG). A gradient of more than 1.1g/dl indicates portal hypertension, and that of less than 1.1g/dl indicates nonportal hypertension and suggests the peritoneal cause.

If abdominal tuberculosis (TB) is suspected, peritoneal fluid Adenosine deaminase (ADA) may help. ADA of more than 30 U/L has a sensitivity of 100% (28). Microbiology investigations, namely smear for acid-fast bacilli (AFB), modified polymerase chain reaction (PCR) technique for mycobacterium tuberculosis DNA (GeneXpert MTB/RIF assay), and mycobacterial culture may be performed.

However, AFB smear has a low yield with a reported sensitivity of 0%–6%. GeneXpert is always useful for the early detection of mycobacterium tuberculosis. A systemic review by Maynard-Smith et al. revealed gene Xpert median sensitivities of 0.85 (IQR, 075 -1.00) when testing non-pleural serous fluids, including ascites. Although present data support the current implementation of this assay for EPTB diagnosis, further studies are needed to expand the evidence base to use this assay. (29). Mycobacterial culture has a sensitivity of up to 50%; however, results may take 3-6 weeks to obtain, delaying therapy (30-32).

1.1.5. Epidemiology

It is estimated that 844 million people have liver disease, with a global mortality rate of 2 million deaths per year (33). Liver disease has been ranked the 2nd commonest cause of mortality amongst all digestive diseases in the United States of America, and number five in Europe (34, 35). In China, approximately 300 million people have liver disease, and leading causes are non-alcoholic fatty liver disease (NAFLD) (49.3%), viral hepatitis B (HBV) (22.9%), alcoholic liver disease (ALD) (14.8%), and viral hepatitis C (3.2%) (36). It is noted that cirrhosis-related mortality doubled between 1980 and 2010 in Sub-Saharan Africa, and most cases of cirrhosis were due to hepatitis B virus (HBV), alcohol misuse, and hepatitis C virus (HCV) (37). However, recent data on the overall burden of liver abnormalities in Africa is limited. A descriptive study by Kruger et al. conducted in Western Cape province in South Africa revealed that Non-alcoholic fatty liver disease (NAFLD) is common in this population, confirmed in 111(87%) patients (2). A recent cohort study of 301 biopsied patients with HIV/AIDS conducted in Cape Town, South Africa, showed that drug-induced liver injury (42%), granulomatous inflammation (29%), non-alcoholic steatosis or steatohepatitis (19%), and hepatitis B (19%) were the most common findings (38).

1.2. HIV/AIDS and Liver

In 2017, approximately 36.9 million people were living with human immunodeficiency virus (HIV) worldwide, of which 7.7 million were South Africans; and access to antiretroviral therapy (ARTs) has decreased mortality due to acquired immunodeficiency syndrome (AIDS) (39-41).

Susceptibility to tuberculosis, hepatitis B virus (HBV), and hepatitis C virus (HCV) co-infection, medication-related hepatotoxicity, alcohol abuse, and non-alcoholic fatty liver disease (NAFLD) are an increasing global burden in HIV infected patients (3, 42).

Globally, an estimated 2-4 million people living with HIV have the hepatitis B virus (HBV) co-infection, and 4-5 million have hepatitis C virus (HCV) co-infection (43). In Sub-Saharan Africa, the mean prevalence of HBV is 15%, and HCV is 7% (44, 45). Dual infection with HIV and HBV or HBC may complicate antiretroviral therapy delivery by increasing the risk of drug-related

hepatotoxicity and liver disease; this may also impact the selection of specific drugs dually active against HBV and HIV (43).

Tuberculosis (TB) is the most common opportunistic infection leading to morbidity and mortality in HIV infected people (46). Abdominal TB with liver involvement is one of the common extrapulmonary manifestations in advanced HIV infected patients with mortality due to diagnostic and treatment challenges (47-49)

Drug-induced liver injury (DILI) is common, complicating the introduction of TB treatment in 5- 33% and that of ARTs in 9 - 30% (50). Commonly used antiretroviral drugs (Efavirenz, Nevirapine, and Lopinavir/Ritonavir), anti-TB drugs (Rifampicin, Rifabutin, Isoniazid, Pyrazinamide, Bedaquiline and many second-line drugs, including quinolones) and Sulfamethoxazole & Trimethoprim may cause hepatitis, steatohepatitis, and transaminitis (51). The mechanism of liver injury due to these drugs is mostly due to idiosyncratic drug reaction. The occurrence of hepatitis is usually unpredictable, may occur at any time during or shortly after exposure to the drug, and the response is not as dose-dependent as the injury associated with direct hepatotoxicity (16)

Non-Alcoholic Fatty Liver Disease (NAFLD) is significant, with a prevalence of up to 50%. NAFLD's pathogenesis and the reasons for progression to non-alcoholic steatohepatitis (NASH) are still not fully elucidated, but insulin resistance, mitochondrial dysfunction, and dyslipidaemia seem to be the main drivers. Both HIV-infection itself and combination antiretroviral therapy can contribute to the development of NAFLD/NASH (52).

Although the role of alcohol abuse on liver disease in HIV infected population has not been well defined, evidence suggests that alcohol abuse is prevalent among this population and can independently contribute to liver disease progression. As a modifiable risk factor for liver disease, clinicians must provide counselling regarding alcohol consumption in this group (42, 53)

1.3. Problem statement

Although significant work on various aspects of liver pathology has been done worldwide, including in South Africa, a new spectrum of diseases is emerging because of health evolution. We are seeing an increasing prevalence of liver-related lifestyle disorders, namely non-alcoholic fatty liver disease (NAFLD) with resultant liver cirrhosis and thus the rising frequency of hepatocellular carcinoma (HCC) (1, 2). Furthermore, the HIV/AIDS epidemic and access to antiretroviral therapy continue to change the disease profile. Amongst other conditions, liver pathology continues to burden HIV infected population (38, 54-56). We hypothesize that, liver abnormalities are common in patients admitted to the medical ward, and prevalent causes may vary in HIV infected versus HIV negative.

This study proposes to outline the pattern of liver abnormalities in Durban, South Africa, during the HIV epidemic era, to help clinicians working in resource constrained environments to formulate a rational management approach for prevention and early detection of clinical conditions associated with liver abnormalities and curb associated morbidity and mortality.

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Chapter 2: A submitted ready manuscript

Assessment of the liver in an HIV era: Clinical, laboratory and radiological abnormalities

Abstract

***Background*:** Liver – related mortality and morbidity are an increasing burden worldwide.

Aim: To outline the pattern of liver abnormalities at a tertiary hospital in KwaZulu Natal (KZN), Durban, South Africa, in the HIV era.

Methods: This cross-sectional, retrospective study conducted medical records review of all patients found to have liver abnormalities based on clinical, laboratory, and radiological profile, admitted to the medical wards for the period between June 2016 to December 2016.

***Results*:** A total of 157 patients were included, of which 63.1% were males, and 91.7% were black, with a median age of 41 years (IQR 32–54). Sixty – six (42.0%) patients were HIV negative; 91 (57.9%) were HIV infected, of which 51 (56.0%) were on antiretroviral therapy. Only 15 (29.4%) had an HIV viral load of < 50 copies/mL and 21 (30.9%) with a $CD4^+$ count of ≥ 200 cells/mm^3, with median of 426 cells/mm3 (IQR, 268 – 780). In HIV negative, heart failure (48.5%) was the main cause of liver abnormalities (p-value < 0.001), whereas in HIV infected, abdominal TB (24.2%) and DILI (18.7%) were the commonest. Sixty-seven (42.7%) patients died while admitted, and leading causes were HIV/AIDS (40.3%), hypertension (13.4%), and metastatic cancer (13.4%).

Conclusion: Heart failure, abdominal TB, and DILI were the leading causes of liver abnormalities in this cohort. Also, mortality was significantly high and main causes were HIV/AIDS, hypertension, and advanced cancer, which underscores the need to reinforce community-based screening programs for communicable and non-communicable disease, for early detection and referral to care.

Keywords: Liver abnormalities, HIV/AIDS, Antiretroviral therapy, Tuberculosis

Introduction

Significant strides have been made over the years on various aspects of liver pathology worldwide. However, most of this progress is undermined by the emerging increase in the prevalence of lifestyle-related liver disorders and the HIV/AIDS epidemic and related disorders.

The liver pathology burdens the HIV infected population with a mortality rate of about 15% per year based on data from first world counties (3) Tuberculosis (TB) is the most common opportunistic infection in HIV infected patients, and disseminated TB with liver involvement is one of the most typical extrapulmonary manifestations in those with advanced disease, associated with high mortality owing to diagnostic and treatment challenges (46) (47-49).

Dual infections affecting the liver complicate therapy; an estimated 2-4 million people living with HIV have hepatitis B virus (HBV) co-infection and 4-5 million have hepatitis C virus (HCV) co-infection (43); such dual infections complicate therapy with looping adverse effects of drug-related hepatotoxicity and progression to liver disease (43).

Drug-induced liver injury (DILI) complicates the introduction of TB treatment in 5- 33% of patients and that of antiretroviral therapy in 9 - 30% (50). Commonly used ARTs, anti-TB drugs, and Sulfamethoxazole & Trimethoprim (co-trimoxazole) may cause hepatitis, steatohepatitis, and transaminitis (51). Liver injury from these drugs is mostly due to idiosyncratic drug reaction; the occurrence of hepatitis is usually unpredictable and may occur at any time after exposure. The response is not as dose-dependent as the injury associated with direct hepatotoxicity (16).

There is an increasing prevalence of lifestyle-related liver disorders such as non-alcoholic fatty liver disease (NAFLD) with resultant liver cirrhosis and thus the rising frequency of hepatocellular carcinoma (HCC) (1, 2). In HIV-infected, NAFLD has a significant prevalence of up to 50%. Both HIV-infection and combined antiretroviral therapy contribute to the development of NAFLD (52).

This study aims to outline the pattern of liver abnormalities in KwaZulu Natal (KZN), South Africa, in the era of the HIV epidemic.

Methodology

This study conducted a medical records' appraisal of admissions with evidence of liver abnormalities from June 2016 to December 2016 at a tertiary public hospital in Durban, South Africa. Records of patients aged 13 years and older found to have liver abnormalities based on clinical, laboratory, or radiological findings were selected.

Data collection (Appendix D) included the following information:

Demographic: Age, gender, and race

Clinical profile: the presence of symptoms of fever, vomiting, jaundice, abdominal pain, abdominal distension, signs of jaundice, oedema, lymphadenopathy, liver consistency, ascites, admission blood pressure, pulse, and temperature. Relevant medical history including HIV/AIDS status, medication such as Trimethoprim-Sulfamethoxazole, antiretroviral therapy, anti-tuberculous treatment, and alcohol use

Laboratory parameters: Admission full blood count (FBC), urea and electrolytes, Liver function test (LFT), and the international normalized ratio (INR). Hepatitis A, B, and C serology, most recent CD4 cell count and HIV viral load (within 6-12 months) where applicable. Microbiology and histopathology results

Imaging: Ultrasound of the liver and its findings

Study implementation and recruitment

Names and hospital numbers of all patients were obtained from the hospital admissions register. Each patient's file was retrieved and reviewed by the principal investigator for eligibility. Files found to have records of liver abnormalities were selected and audited. Histological and other laboratory results, if missing, were obtained from the National Health Laboratory System (NHLS) database. Names and hospital numbers were removed when capturing the data to the collection sheet, and the new numbering system was used to avoid duplication. All data was handled by the research team only.

Statistical analysis

SPSS® 23.0 software (IBM Corp, Armonk, NY, USA) was used for data analysis. Data were assessed for normality, and non-parametric tests were used where relevant. Medians and interquartile ranges (IQR) were used for data not normally distributed. Pearson's chi-square test or Fisher's exact test were used in assessing the association between categorical variables. The significance for all tests was set at $p < 0.05$.

Ethics approval

The ethics approval was obtained from the University of Kwa-Zulu Natal (UKZN) Biomedical Research Ethics committee (BREC reference no. BE401/16). Permission to conduct the study was obtained from the health system gatekeepers.

Results

There were 1849 admitted patients to the medical wards at King Edward VIII Hospital as per the admission register during the period under review. However, only 1277 patient's files were retrievable from the archives and audited for eligibility. In 1277 files, 157 (12%) met the inclusion criteria (**Figure 1**).

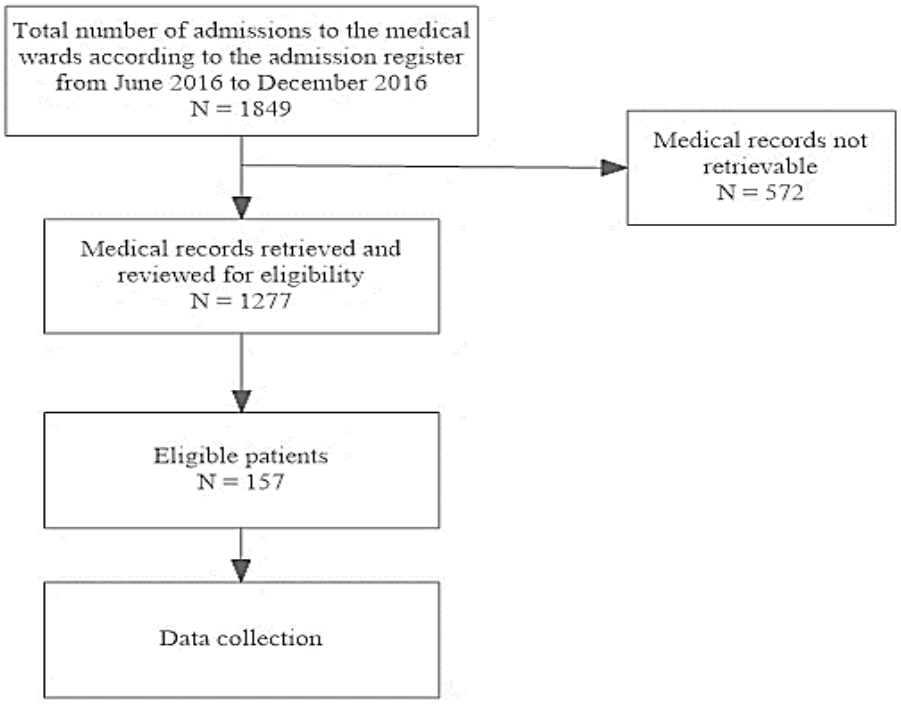

Figure 1: A flowchart of our study

Of 157 patients, 99(63.1%) were males, and 144(91.7%) were black with a median age of 41 years (IQR, 32 – 54) Ninety -one (58.0%) were HIV infected, and sixty-six (42.0%) were negative. The difference observed in systolic blood pressure (SBP) between the two groups was statistically significant using Independent-Samples Mann-Whitney U Test (p-value = 0.024). Fever (36.3%), vomiting (44%), pallor ((58.2%) were common clinical findings in HIV infected, whereas, in HIV negative, abdominal distention (56.1%), ascites (51.5%) were common. Comorbidities of hypertension, diabetes, and dyslipidemia were common in HIV negative, found in 45.5%, 24.2%, and 28.2%, respectively compared to HIV infected (p - values < 0.001) (**Table 1**).

Table 1: Demographic and clinical characteristics (n =157)

Characteristics	**All patients (*n* = 157)**	**HIV negative (*n* = 66)**	**HIV infected (*n* = 91)**	**p - value (HIV negative vs HIV infected)**
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	
Age (years):	41 (32 - 54)	53 (38 – 65)	37 (29 – 46)	0.030
	Frequency (%)	**Frequency (%)**	**Frequency (%)**	
Sex:				0.019
Male	99 (63.1)	49 (74.2)	50 (54.9)	
Female	58 (36.9)	17 (25.8)	41 (45.1)	
Race:				0.009
Black	144 (91.7)	56 (84.8)	88 (96.7)	
Indian	4 (2.5)	3 (4,4)	1 (1.1)	
White	4 (2.5)	2 (3.0)	2 (2.2)	
Coloured	5 (3.2)	5 (7.6)	0 (0)	

BP systolic (mmHg):				
<120	98 (62.4)	35 (53.0)	63 (69.2)	0.024**
120 - 139	43 (27.4)	20 (30.3)	23 (25.3)	
≥ 140	16 (10.2)	11 (16.7)	5 (5.5)	
BP diastolic (mmHg):				
< 80	126 (80.3)	54 (81.8)	72 (84.6)	0.770
80 - 89	16 (10.2)	7 (10.6)	9 (9.9)	
≥ 90	15 (9.6)	5 (7.6)	10 (11)	
Symptoms:				
Fever	41 (26.1)	8 (12.1)	33 (36.3)	0.001
Vomiting	56 (35.7)	16 (24.2)	40 (44)	0.012
Jaundice	72 /45.9)	25 (37.9)	47 (51.6)	0.105
Abdominal pain	107 (68.2)	45 (68.2)	62 (68.1)	1.000
Abdominal distension	71 (45.2)	37 (56.1)	34 (37.4)	0.024
Right abdominal mass	2 (1.3)	1 (1.5)	1 (1.1)	1.000
Pale stool	8 (5.1)	5 (7.6)	3 (3.3)	0.282
Dark urine	33 (21.0)	14 (21.2)	19 (20.9)	1.000
Signs:				
Jaundice	74 (47.1)	29 (43.9)	45 (49.5)	0.521
Ascites	59 (37.6)	34 (51.5)	25 (27.5)	0.003
Hepatomegaly	104 (66.2)	45 (68.2)	59 (64.8)	0.730
Pallor	79 (50.3)	26 (39.4)	53 (58.2)	0.024
Comorbidities:				
Hypertension	36 (22.9)	30 (45.5)	6 (6.6)	<0.001
Diabetes mellitus	19 (12.1)	16 (24.2)	3 (3.3)	<0.001
Dyslipidaemia	20 (12.7)	19 (28.2)	1 (1.1)	<0.001
Chronic HBV/HCV*	3 (1.9)	0 (0.0)	3 (3.3)	0.264
Abdominal TB	3 (1.9)	0 (0.0)	3 (3.3)	0.264
Alcohol consumption	42 (27.6)	16 (24.6)	26 (29.9)	0.583

Data expressed as frequency (%) or median (Interquartile ratio). TB = tuberculosis, BP = blood pressure, HBV = hepatitis B virus, HCV = hepatitis C virus. Known with positive hepatitis B surface antigen/anti – HCV before admission*. Statistically significant using Independent-Samples Mann-Whitney U Test**.
TDF = tenofovir, 3TC = emtricitabine, EFV = efavirenz, NVP = nevirapine, AZT = zidovudine, LPV/rit = Lopinavir/ritonavir, ABC = abacavir.

HIV infected had mean hemoglobin (Hb) of 9.34 (SD ± 2.66) g/*dl* (p-value = 0.001), white cell count (WCC) median of 7.10 x 109/L (IQR, 4.60 – 11.25) (p-value = 0.014), globulin fraction of 44 g/L (IQR, 39 – 55) (p-value = 0.027, and serum albumin median of 20 g/L (IQR, 16 – 27) (p-value = 0.001 compared to HIV negative; all statically significant using Independent samples Mann-Whitney U Test (**Table 2**).

Table 2: Laboratory characteristics on admission (n = 157)

Parameters	**HIV negative (*n* = 66)**	**HIV infected (*n* = 91)**	**P value (HIV negative vs HIV infected)**
	Mean ± SD	**Mean ± SD**	
Hb (g/*dl*)	11.06 ± 3.05	9.34 ± 2.66	0.001**
	Median (IQR)	**Median (IQR)**	
WCC (10^9/L)	9.47 (5.98 – 13.87)	7.10 (4.60 – 11.25)	0.014**
Platelets (10^9/L)	240 (137 – 366)	227 (127 – 340)	0.579
Total protein (g/L)	68 (62 – 78)	68 (59 – 77)	0.758
Globulin fraction (g/L)	42 (35 -49)	44 (39 – 55)	0.027**
Albumin (g/L)	26 (20 – 33)	20 (16 – 27)	0.001**
Total bilirubin (µmol/L)	35 (22 – 78)	45 (11 – 164)	0.773
ALT (U/L)	46 (23 – 219)	84 (33 – 222)	0.065
AST (U/L)	69 (33 – 300)	369 (83 – 460)	0.164
ALP (U/L)	203 (145 – 337)	214 (145 – 354)	0.676
GGT (U/L)	236 (100 – 344)	205 (96 – 300))	0.208
INR	1.30 (1.12 – 1.62)	1.32 (1.12 – 1.77)	0.518

Data expressed as mean ± standard deviation or median (Interquartile ratio). Statistically, significant using Independent samples Mann-Whitney U Test. Significant level is 0.05** Hb = hemoglobin, WCC = white cell count, ALT = alanine aminotransferase, AST = aspartate transaminase, ALP = alkaline phosphatase, GGT = gamma-glutamyl transpeptidase, INR = international normalized ratio.

Of 91 patients who were HIV infected, 51(56.0%) were on ART most of which were on "first – line "regimen comprising of TDF+3TC+EFV (90.2%). Twenty - one (30.9%) had a $CD4^+$ count of $\geq$ 200 cells/mm^3, with median of 426 cells/mm^3 (IQR, 268 – 780), and 15 (29.4%) had an HIV viral load of < 50 copies/mL. Whereas 36 (70.6%) had detectable viral load with a median of 214000 copies/ mL (IQR, 11050 – 122000) (**Table 3**).

Table 3: CD4+ cell count, HIV viral load and ART use in HIV infected (n = 91)

Parameters	**Frequency (%)**	**Median (IQR)**
$CD4^+$ count (cells/mm^3):		
< 200	47 (69.1)	73 (39 – 103)
$\geq$ 200	21 (30.9)	426 (268 – 780)

HIV viral load (copies/ mL): < 50 ≥ 50	 15 (29.4) 36 (70.6)	 0 (0 – 0) 214000 (11050 – 122000)
Antiretroviral drug use:	51 (56.0)	N/A
ART combination: TDF+3TC+EFV TDF+3TC+NVP AZT+3TC+LPV/r ABC+3TC+LPV/r	 46 (90.2) 1 (2.0) 1 (2.0) 3 (5.8)	 N/A N/A N/A N/A
Data expressed as frequency (%) or median (Interquartile ratio). ART = Antiretroviral therapy, TDF = tenofovir, 3TC = emtricitabine, EFV = efavirenz, NVP = nevirapine, AZT = zidovudine, LPV/rit = Lopinavir/ritonavir, ABC = abacavir.		

Overall, heart failure (30.6%), abdominal TB (14.6%), and drug-induced liver injury (14.0%) were common causes of liver abnormalities. Compared to HIV negative, abdominal TB (24.2%) and drug-induced liver injury (18.7 %) were the commonest in HIV infected, while in HIV negative, heart failure (48.5%) was the leading cause (p-value < 0.001). Other conditions including liver ischemia, choledocholithiasis, non-alcoholic fatty liver disease (NAFLD), autoimmune hepatitis, lymphoma, cholangiocarcinoma, AIDS-associated cholangiopathy, and disseminated histoplasmosis accounted for 7% of all causes. In 21 (13.4%) patients, causes of liver abnormalities were not established (**Table 4**).

Table 4: Causes of liver of liver abnormality (n = 157)

Parameters	**All patients (*n* = 157)**	**HIV negative (*n* = 66)**	**HIV infected (*n* = 91)**	**P value (HIV negative vs HIV infected)**
	Frequency (%)	**Frequency (%)**	**Frequency (%)**	
Heart failure	48 (30.6)	32 (48.5)	16 (17.6)	< 0.001
Abdominal TB	23 (14.6)	1 (1.5)	22 (24.2)	< 0.001
DILI	22 (14.0)	5 (7.6)	17 (18.7)	0.048
Acute hepatitis B	16(10.2)	3 (4.5)	13 (14.3)	0.046
Alcoholic liver disease	8 (5.1)	7 (10.6)	1 (1.1)	0.010
Liver metastasis	4 (2.5)	3 (4.5)	1 (1.1)	0.310
HCC	3 (1.9)	2 (3.0)	1 (1.1)	0.573
Other	11 (7.0)	5 (7.6)	6 (6.6)	1.000
Unknown	21 (13.4)	7 (10.6)	14 (15.4)	0.385
Data expressed as frequency (%). For counts less than 5 Fisher's exact tests were used to assess association between categorical variables and Pearson's Chi square test where appropriate. TB = tuberculosis, DILI = drug induced liver injury, HCC = hepatocellular carcinoma, NAFLD = non-alcoholic fatty liver disease Other = (liver ischaemia, choledocholithiasis, NAFLD, autoimmune hepatitis, lymphoma, cholangiocarcinoma, AIDS -associated cholangiopathy and disseminated histoplasmosis)				

The diagnosis of abdominal TB was made based on constellation of tests, and the most utilised test was ultrasound (20), followed by ADA (4) and GXP (3). Liver biopsy was utilised in 2 patients (**Table 5**).

Table 5: Investigations used to diagnose abdominal tuberculosis (n = 23)

Parameters	**Frequency**
Ultrasound	20
ADA	4
GXP	3
Liver biopsy	2
Data expressed as frequency. ADA = adenosine deaminase. GXP = Gene Xpert	

In patients with drug-induced liver injury (DILI), associated drugs were Isoniazid, Rifampicin, Ethambutol, and Pyridoxine (IREP) combination therapy (54.6%), efavirenz (22.7%), INH (13.6%), and paracetamol overdose (9.1%).

Table 6: Causes of death (n = 67)

Parameters	**Frequency (%)**
HIV/AIDS	27 (40.3)
Hypertension	9 (13.4)
Metastatic cancer	9 (13.4)
Alcoholic liver disease	5 (7.5)
Severe sepsis	4 (6.0)
Diabetes mellitus	3 (4.5)
Hepatitis B	3 (4.5)
DILI	2 (3.0)
Other	5(7.5)
Data expressed as frequency (%). DILI = drug induced liver injury	

There were 67 (43%) deaths in this cohorts, of which HIV/AIDS (40.3%), hypertension (13.4%) and metastatic cancer (13.4%) were the common documented causes. Alcoholic liver disease, hepatitis B infection and drug – induced liver injury were documented causes of death in 7.5%, 4.5% and 3.0% respectively.

Discussion

Liver abnormalities are common in patients admitted to the medical wards. In this cohort of 12% of patients whose files were accessed and reviewed had liver abnormalities.

The majority of patients were young and HIV infected. This finding is comparable to other studies conducted in other facilities in South Africa (21, 57). Most patients were males (p-value = 0.019), but the exact reason for male predominance is beyond this study's scope. The racial demographic differences likely reflect the general provincial demographics that access the public health system.

In patient with HIV infection fever (p value = 0.001), vomiting (p value = 0.012) and pallor (p value = 0.025) were common compared to those who were HIV negative; this is consistent with the clinical manifestation of TB which was high in HIV infected group (p value < 0.001) compared to HIV negative. HIV negative patients had abdominal distention (p value = 0.024) and ascites (p value = 0.003); and hypertension (p value <0.001), diabetes mellitus (p value <0.001) and dyslipidaemia (p value <0.001) were common, all of which were consistent with the common diagnosis of heart failure which was high compared to HIV infected (p value <0.001).

In this study, DILI was expected, although frequency was low compared to other studies conducted in South Africa (21, 57). Anti-tuberculous medication (IREF and INH prophylaxis) and EFV were the significant causes of DILI in this study; however, for the treatment of TB, it was not clear which specific anti-tuberculous drug was the cause since patients were on combination therapy.

Liver biopsy is viewed as the gold standard in diagnosing liver pathology, but due to the invasive nature of this procedure and the need for experienced personnel to analyses the biopsied specimen, it is proven to be the challenge in clinical practice (30, 31). The majority of documented diagnoses were based on other supplementary tests; liver biopsy was performed in only two patients in this study's tuberculosis diagnosis. The reason for a liver biopsy to be rarely performed is not known, but thought to be due to several factors, (i) less invasive procedures may have been adequate to make the diagnosis (liver congestion due to heart failure) (ii) those who required biopsy may have been unstable for the procedure to be performed; this is supported by looking at the number of deaths in this study.

The number of HIV infected patients on ART (56.0%), and those who had an undetectable HIV viral load (29.4%) were far below the aimed targets for 90-90-90 strategy for eliminating HIV/AIDS disease burden in this cohort. Even though data on ART duration was not collected, the frequency of patients with $CD4^+$ <200 cells/mm^3, low mean haemoglobin of 9.34 (SD ± 2.66) g/dl, and low median albumin of 20 g/L (IQR, 16 – 27) suggest that most patients infected with HIV had the advanced disease; which may explain in part high mortality observed, with HIV/AIDS (40.3%) as a leading cause.

This cohort's limitations were that it was impossible to access histology that confirms liver abnormalities for the majority of patients, data on body mass index (BMI), serum lipids levels, the quantity of alcohol intake, duration, and doses of hepatotoxic drugs, including herbal medication. However, this study does report on the clinical characteristics of patients with liver abnormalities, both HIV-infected and HIV-negative.

Conclusion:

The majority of patients with HIV infection had a symptom of fever, vomiting, and were found to have to abdominal TB as the commonest cause of liver abnormality; while the majority of those that were HIV negative had abdominal distention and ascites, with risk factors of heart failure, such as hypertension, diabetes mellitus, and dyslipidaemia. These patients had heart failure as the commonest cause of liver abnormalities. Also, there was a high number of mortalities in this cohort, and the commonly reported causes were HIV/AIDS, hypertension, and advanced cancer. The findings in this cohort could assist health care officials to formulate strategies to strengthen community-based programs for screening of communicable diseases such as HIV/AIDS and TB including non-communicable disease such as hypertension and diabetes mellitus at a community level, for early detection and referral to care in addition to emphasizing treatment adherence; and clinicians working in this resource-constrained environment may utilise this data to formulate a rational approach for early detection and management of common clinical conditions associated with liver abnormalities.

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Competing interests

The authors declare that they have no competing interests.

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Appendix A: Research protocol

Title of the study: Assessment of the liver in an HIV era: Clinical, laboratory and radiological abnormalities

Aims of the study

- To determine causes of liver abnormalities, including histological diagnosis in patients admitted to the medical wards at King Edward VIII hospital
- To compare the prevalent causes of liver abnormalities in HIV infected and HIV negative

Objectives of the study

- To identify patients with liver abnormalities admitted to the medical wards
- To ascertain demographic, clinical, laboratory and radiological characteristics of patients with liver abnormalities
- To identify patients with HIV infection and HIV negative with liver abnormalities

Background and literature review

1.Introduction

Globally, liver-related morbidity and mortality are an increasing burden. Although significant work on various aspects of liver pathology has been done worldwide, a new spectrum of diseases is emerging because of health evolution. We are seeing an increasing prevalence of liver-related lifestyle disorders, namely non-alcoholic fatty liver disease (NAFLD) with resultant liver cirrhosis and thus rising frequency of hepatocellular carcinoma (HCC) (1, 2).

Furthermore, the HIV/AIDS epidemic and access to antiretroviral therapy continue to change the disease profile. Amongst other conditions, liver pathology continues to burden HIV infected population with a mortality rate of about 15% according to the Data Collection on Adverse Events of Anti- HIV Drugs study (3).

1.1. Liver abnormalities

1.1.1. Anatomical overview and physiology of the liver and biliary system

The liver is the biggest vital organ in the body, representing 1.5 – 2.5% of body weight which amounts he liver is the biggest vital organ in the body, representing 1.5 – 2.5% of body weight, which amounts to 1400 – 1800g in adults (4, 5). It has numerous functions, including bile production, fat-soluble vitamin storage, metabolism of drugs, and bilirubin (6). The liver receives nearly 25% of the cardiac

output via two sources; oxygen-rich blood supply through the hepatic artery and nutrient-rich blood from the stomach, intestines, pancreas, and spleen through the portal vein (7). Macroscopically, the liver is divided into right and left lobes and further subdivided into eight independent segments. Each segment has its portal pedicle, consisting of the hepatic arterial branch, portal branch, and the bile duct with a separate hepatic venous branch (4).

Microscopically, the liver is organized into structural units called lobules. However, functionally it is organized into acini, the smallest functional units containing a small portal tract at the center and terminal hepatic venules at the periphery. The acinus is divided into three zones; zone 1 surrounds the portal tract, zone 3 surrounds the hepatic venule, and the intervening hepatocytes constitute zone 2. Blood from the portal tract flows through these zones to the venule with a decreasing oxygen and nutrient gradient (8, 9). The biliary tract collects, stores, concentrates, and delivers bile secreted by the liver, and the bile ducts anatomy follows that of the portal system and segmentation of the liver (10, 11).

1.1.2. Mechanism and causes of liver abnormalities

As an organ with a complex structure, high vascular supply, and increased metabolic activity, the liver is particularly susceptible to damage (9, 12). Liver abnormality generally occurs via five mechanisms, namely, inflammation (viral hepatitis, drug-induced liver injury, alcoholic hepatitis, autoimmune hepatitis), excessive storage (non-alcoholic fatty liver, Wilson's disease, hemochromatosis), infiltration (hepatocellular carcinoma, metastatic tumor, tuberculosis), vascular congestion (congestive cardiac failure, cor-pulmonale, Budd-Chiari syndrome) and biliary obstruction (choledocholithiasis, cholangiocarcinoma, choledochal cyst) (13, 14). ischemic hepatitis is another well-recognized entity resulting from reduced oxygen delivery to the liver, and the term hepatitis is somewhat of a misnomer since an inflammatory process does not mediate the injury but hepatocytes necrosis (15).

1.1.3. Clinical features

In the acute phase, patients may present with non-specific symptoms such as malaise, anorexia, fever, and jaundice appear as the illness progresses; and clinical examination may be normal or reveal jaundice, hepatomegaly, and hepatic pain. If the insult to the liver is not removed, fibrosis may occur and eventually cirrhosis. Clinical signs such as spider naevi, palmar erythema may be seen, but these are not specific as they may be seen in normal people and pregnant women, and may occur in both acute and chronic liver disease. In males with chronic liver disease, particularly related to alcohol use, signs of hyperestrogenemia, namely gynecomastia, testicular atrophy, loss of male pattern hair distribution, may be seen. With advanced liver disease, ascites, edema, dilated abdominal veins, feto-hepaticus, asterixis, confusion, and coma may be found (16)

1.1.4. Diagnosis and grading of liver abnormalities

Liver function test

There are several blood tests available that are useful in assessing the status of the liver. The most commonly used clinical practice tests include the serum aminotransferases, bilirubin, alkaline phosphatase, albumin, and prothrombin time, collectively termed "liver function tests." These tests are useful in guiding management by determining the pattern of liver disease, classified as either hepatocellular, cholestatic (obstructive), or mixed; and the degree of severity (17)

Imaging

Ultrasound is a non-invasive and inexpensive screening tool in patients presenting with abnormal liver function tests. It may hint on liver morphology, biliary system abnormalities, infiltrative processes, and vascular dopplers (18, 19). Other imaging techniques are Computed tomography CT scan and Magnetic resonance cholangiopancreatography (MRCP), both of which are minimally invasive. They provide precise depictions of the biliary system, characterize the liver lesion, and delineate vascular anatomy (20). However, in a patient with a cholestatic pattern on liver function test, with or without dilated MRCP is preferable to CT since it can also provide additional information regarding biliary flow (21, 22)

The Fibroscan (ultrasound elastography) is a simple, non-invasive test to rule in/out advanced fibrosis and cirrhosis. It works by measuring the sound wave's velocity passing through the liver and then converts those into a liver stiffness measurement. Since Fibroscan test results are promptly obtained, clinicians can use them to make decisions during the patient's visit; but technical limitations of the test preclude its use in patients with ascites and morbid obesity (23).

Liver Biopsy

Liver biopsy is viewed as the gold standard in diagnosing liver pathology since it provides otherwise unobtainable qualitative information regarding the liver's structural integrity and the type and degree of injury (24). However, several factors are limiting its use; (i) It is contraindicated in the presence of severe coagulopathy, infection of the hepatic bed, extrahepatic biliary obstruction, possible vascular lesions, and hydatid disease. (ii) There may be a misdiagnosis and incorrect staging if the biopsied samples do not represent the liver's structural integrity. (iii) The limited number of experienced clinicians to perform this invasive procedure and experienced histopathologists to analyze the sample once obtained, particularly in the developing countries. (v) Histology results may be non-specific and non-diagnostic, for example, in drug and toxin-induced injury, overwhelming sepsis (24, 25)

Miscellaneous investigations

Other useful specific tests include toxicology screen and drug tests such as paracetamol level, viral hepatitis serology, autoimmune markers, ceruloplasmin, urine copper, iron saturation, serum ferritin, and alpha-fetoprotein (26, 27).

Those patients with abnormal liver function and morphology presenting with ascites, peritoneal fluid analysis helps determine whether the ascites is due to portal hypertension-related causes secondary causes. Apart from the fluid's macroscopic analysis, the initial step is to determine the serum-ascites albumin gradient (SAAG). A gradient of more than 1.1g/dl indicates portal hypertension, and that of less than 1.1g/dl indicates nonportal hypertension and suggests the peritoneal cause.

If abdominal tuberculosis (TB) is suspected, peritoneal fluid Adenosine deaminase (ADA) may help. ADA of more than 30 U/L has a sensitivity of 100% and a specificity of 92-97% (28). Microbiology investigations, namely smear for acid-fast bacilli (AFB), modified polymerase chain reaction (PCR) technique for mycobacterium tuberculosis DNA (GeneXpert MTB/RIF assay), and mycobacterial culture may be performed. However, AFB smear has a low yield with a reported sensitivity of 0%–6%. GeneXpert is always useful for the early detection of mycobacterium tuberculosis. A systemic review by Maynard-Smith et al. revealed GeneXpert median sensitivities of 0.85 (IQR, 075 -1.00) when testing non-pleural serous fluids, including ascites. Although present data support the current implementation of this assay for EPTB diagnosis, further studies are needed to expand the evidence base to use this assay. (29). Mycobacterial culture has a sensitivity of up to 50%. However, results may take 3-6 weeks to obtain, which may delay therapy (30-32).

Epidemiology

It is estimated that 844 million people have liver disease, with a global mortality rate of 2 million deaths per year (33). Liver disease has been ranked the 2nd commonest cause of mortality amongst all digestive diseases in the United States of America, and number five in Europe (34, 35). In China, approximately 300 million people have liver disease, and leading causes are non-alcoholic fatty liver disease (NAFLD), viral hepatitis B (HBV) (22.9%), alcoholic liver disease (ALD), and viral hepatitis C at 49.3%, 22.9%, 14.8%, and 3.2% respectively (36).

In Africa, data demonstrating the overall burden of liver disease is limited; however, it has been noted that cirrhosis-related mortality has doubled between 1980 and 2010 in Sub Saharan Africa; and most cases of cirrhosis were due to hepatitis B virus (HBV), alcohol misuse, and hepatitis C virus (HCV) (37). A descriptive study by Kruger et al. conducted in Western Cape province in South Africa revealed that Non-alcoholic fatty liver disease (NAFLD) is common in this population, confirmed in 111(87%) patients (2). A recent cohort study of 301 biopsied patients with HIV/AIDS conducted in Cape Town, South Africa, showed that drug-induced liver injury (42%), granulomatous inflammation (29%), non-alcoholic steatosis or steatohepatitis (19%), and hepatitis B (19%) were the most common findings (38).

1.2. HIV/AIDS and Liver

In 2017, approximately 36.9 million people were living with human immunodeficiency virus (HIV) worldwide, of which 7.7 million were South Africans; and access to antiretroviral therapy (ARTs) has decreased mortality due to acquired immunodeficiency syndrome (AIDS) (39-41).

Susceptibility to tuberculosis, hepatitis B virus (HBV), and hepatitis C virus (HCV) co-infection, medication-related hepatotoxicity, alcohol abuse, and non-alcoholic fatty liver disease (NAFLD) are an increasing global burden in HIV infected patients. Liver-related mortality amongst these patients is about 15%; however, this data is from high-income countries (3, 42).

Globally, an estimated 2-4 million people living with HIV have the hepatitis B virus (HBV) co-infection, and 4-5 million have hepatitis C virus (HCV) co-infection (43). In Sub-Saharan Africa, the mean prevalence of HBV is 15%, and HCV is 7% (44, 45). Dual infection with HIV and HBV or HBC may complicate antiretroviral therapy delivery by increasing the risk of drug-related hepatotoxicity and liver disease; this may also impact the selection of specific drugs dually active against HBV and HIV (43).

Tuberculosis (TB) is the most common opportunistic infection leading to morbidity and mortality in HIV infected people (46). Abdominal TB with liver involvement is one of the common extrapulmonary manifestations in advanced HIV infected patients with mortality due to diagnostic and treatment challenges (47-49)

Drug-induced liver injury (DILI) is a common, complicating introduction of TB treatment in 5- 33% and ARTs in 9 - 30% (50). Commonly used antiretroviral drugs (Efavirenz, Nevirapine, and Lopinavir/Ritonavir), anti-TB drugs (Rifampicin, Rifabutin, Isoniazid, Pyrazinamide, Bedaquiline and many second-line drugs, including quinolones) and Sulfamethoxazole & Trimethoprim may cause hepatitis, steatohepatitis, and transaminitis (51). The mechanism of liver injury due to these drugs is mostly due to idiosyncratic drug reaction. The occurrence of hepatitis is usually unpredictable, may occur at any time during or shortly after exposure to the drug, and the response is not as clearly dose-dependent as the injury associated with direct hepatotoxicity (16)

Non-Alcoholic Fatty Liver Disease (NAFLD) is significant, with a prevalence of up to 50%. NAFLD's pathogenesis and the reasons for progression to non-alcoholic steatohepatitis (NASH) are still not fully elucidated, but insulin resistance, mitochondrial dysfunction, and dyslipidaemia seem to be the main drivers. Both HIV-infection itself and combination antiretroviral therapy can contribute to NAFLD/NASH (52).

Although the role of alcohol abuse on liver disease in HIV infected population has not been well defined, evidence suggests that alcohol abuse is prevalent among this population and can independently

contribute to liver disease progression. As a modifiable risk factor for liver disease, clinicians must provide counselling regarding alcohol consumption in this group (42, 53)

The purpose of this study is to outline the pattern of liver abnormalities in patients admitted to the medical wards at a tertiary teaching hospital in Durban, KwaZulu Natal, in the HIV era. The purpose of this study is to outline the pattern of liver abnormalities in patients admitted to the

Methodology

Study design

Cross- sectional, retrospective study. Medical records review of all patients admitted to the medical wards, found to have liver abnormalities from June 2016 to December 2016 will be performed

Setting

King Edward VIII Hospital in Durban, South Africa, which is a tertiary level public hospital and a teaching hospital complex supported by the University of KwaZulu Natal.

Study population

Patients age 13 years and older, both men and women from different racial groups

Study sample

Convenient sample depending on the number of patients identified and have met the inclusion criteria

Inclusion criteria

Patients from age 13 years and above
Patients found to have abnormal liver, either biochemically, structurally or both

Exclusion criteria

Pregnant women

Study measurements

Data collection will include the following information:

Demographic: Age, gender and race

Clinical: the presence of symptoms such as, fever, vomiting, jaundice, abdominal pain, abdominal distension; signs of jaundice, pallor, edema, lymphadenopathy, liver consistency, ascites if present and admission blood pressure, pulse and temperature. Relevant medical history including HIV/AIDS

status, medication such as Trimethoprim- Sulfamethoxazole, antiretroviral therapy, anti- tuberculous treatment and alcohol use

Laboratory parameters: Admission full blood count (FBC), urea and electrolytes, Liver function test (LFT) and the international normalized ratio (INR). Hepatitis A, B and C, most recent CD4 cell count and HIV viral load (within 6-12 months) where applicable. Serology, microbiology and histopathology results

Ultrasound of the liver and its findings

Implementation and recruitment

Names and hospital numbers of all patients admitted to the medical wards during the period between June 2016 to December 2016 will be obtained from the hospital admission register. Each patient's file will be retrieved and reviewed by the principal investigator for eligibility to be included in the study based on selection criteria. Those found to have evidence of liver abnormalities based on clinical, laboratory, and or radiological investigations will be further reviewed for required information, namely, clinical presentation, comorbid conditions, medication, laboratory and ultrasound findings, and outcomes. If missing on the patient's file, histological and other laboratory results will be obtained from the National Health Laboratory System (NHLS) database. Once retrieved, all data will be captured on the data collection sheet (Appendix D). Patient's names and hospital numbers will be removed between initial retrieval and recording in the database, and all patients will be identified by the numbering system to avoid duplication of information. Anonymous information will be captured into the Microsoft Excel database.

Ethical considerations

The study's approval will be obtained from the University of Kwa-Zulu Natal (UKZN) Biomedical Research Ethics committee (BREC).

Since there will be no interaction with the patients, patient consent will not be taken. Permission to conduct the study will be obtained from King Edward VIII Hospital, and patients' information will be kept confidentially.

Statistical analysis

SPSS® 23.0 software (IBM Corp, Armonk, NY, USA) will be employed for data analysis. Data were assessed for normality, and non-parametric tests were used where relevant. Medians and interquartile ranges (IQR) were used for data not normally distributed. Pearson's chi-square test or Fisher's exact

Limitations and strengths of the study

Since this is a small study, it may not show the true reflection of the general population; and missing data is expected since this is a retrospective study. This study will be able to report on clinical data for patients, in addition to the laboratory and radiological data.

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Appendix B: Instructions to the Author for the Journal selected for submission

Manuscript Preparation

The SAJID complies with the Uniform Requirements for Manuscripts Submitted to Biomedical Journal Journals (Ann Intern Med 2000; 133:229-231 [editorial]; http://www.icmje.org, full text). Text, tables, references, and legends must be double- spaced. Italics should be used for genus and species names and for genes but not for in vivo, in vitro, in situ, et al., or other Latin-derived expressions. For layout of manuscript and appropriate style see a recent issue of SAJID.

Title page. On the title page, please supply a running head of not more than 40 characters and spaces, a title of not more than 160 characters and spaces, the names and affiliations of all the authors, and word counts of the abstract and text. Each author's first name, subsequent initials and surname must be used.

Footnote page. Footnotes must include:

- Statement that authors either have or have not a commercial or other association that might pose a conflict of interest (e.g. pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding)
- Statement naming sources of financial support (including grant numbers)
- Name, date (month and year), and location (city, and country if not South Africa) of a meeting at which all or part of the information has been presented (include an abstract number, if available)
- Name and e-mail address of the person to whom correspondence should be addressed
- Current affiliations for authors whose affiliations have changed since completion of the study

Abstract. The abstract for an Article may be structured with the headings Background, Methods, Results, and Conclusions (250-word limit) or unstructured (200-word limit). Abstracts of Brief Reports should be no more than 100 words. Whether structured or unstructured, the abstract must state the purpose of the research, the methods used, the results, and the conclusions. Do not cite references in the abstract. Include up to 10 key words, separate from the abstract. Please remember that the abstract is particularly useful for literature retrieval purposes.

Text. The text of Articles must be no longer than 3500 words, and that of Brief Reports no longer than 2000 words. The Methods section must include a statement that informed consent was obtained from patients or their parents or guardians, and human experimentation guidelines of the National Department of Health (http://www.doh.gov.za) or the South African Medical Research Council

(MRC; http://www.sahealthinfo.org/ethics/index.htm) and /or those of the authors' institution(s) were followed in the conduct of clinical research or that animal experimentation guidelines (see MRC website above) were followed in animal studies.

References. Articles are generally limited to 50 references, Brief Reports to 15 references. Only works that have been published or accepted for publication can be included in the reference list. Unpublished observations by the authors (authors' unpublished data) personal communications (SP Stanley, personal communication), and manuscripts submitted for publication (J Odendaal, S Coovadia and J Radebe, submitted) should be mentioned parenthetically in the text Please number references in order of appearance; those cited only or first in tables or figures are numbered according to the order in which the table or figure is cited in the text. Example: If table 3 is cited in the text after reference 20, a new reference cited in table 3 will be reference 21.

References must follow the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org, full text). Provide all authors' (or editors') names when there are fewer than 7; for 7 or more, list the first 3 and add "et al." Titles of journals not listed in *Index Medicus* should be spelt out in full. Reference to a doctoral thesis or Master's dissertation should include the author, title, institution, location, year and publication information, if published. For online resources, include a URL and date accessed. Accuracy of references is the responsibility of the authors.

Acknowledgment(s). The page preceding the references may include a statement thanking those who assisted substantially with work relevant to the study.

Statistical analysis. The statistical analyses used should be identified both in the text and in all tables and figures where the results of statistical comparison are shown.

Units of measure. All Data should be expressed in metric units; use of SI units is encouraged. Use ºC for temperature.

Tables and figures. Articles are limited to a maximum of seven inserts (tables and figures combined), Brief Reports to a maximum of two inserts. Data should not be repeated in both a table and a figure. Abbreviations and acronyms used in tables and figures must be explained in the table footnotes and figure legends, even if already defined in the text.

Tables should be numbered in the order of mention in the text. Tables should be typed double-spaced throughout, with no vertical or internal rules. Footnotes and accompanying explanatory material should be kept to a minimum. Footnotes should be placed below the table and designated by

superscript lowercase letters (listed in order of location when the table is read horizontally). Each column must have an appropriate heading describing the data in the column below, and units of measure must be clearly indicated. For further instructions on the preparation of tables in Word, consult the Special Instructions for Tables.

Figures should be also numbered in the order of mention in the text and should appear at the end of the manuscript and references. Your figures should be prepared in accordance with the Guidelines for Submission of Artwork. Letters, numbers, and symbols should be clear and of sufficient size to be legible when the figures are reduced. Photomicrographs should have internal scale markers. Figures reproduced from other publications must be accompanied by permission from the copyright holder. If the manuscript is accepted, the author will be required to send one complete set of glossy, hard-copy figures.

Figure legends should be double-spaced and appear on a separate page preceding the figures. Any abbreviations or symbols used but not defined in the figure itself must be defined in the legend.

Style. Authors are referred to the *American Medical Association Manual of style: A Guide for Authors and Editors* (9th ed., Williams& Wilkins, 1997) and the *Chicago Manual of Style* (15th ed., University of Chicago Press, 2003).

For commercially obtained products mentioned in the text, list the full names of manufacturers. Generic names of drugs and other chemical compounds should be used.

Nomenclature. SAJID recommends the latest widely accepted nomenclature, as set out in documents prepared by recognized international agencies e.g. the *International Journal of Systematic and Evolutionary Microbiology, Bergey's Manual of Determinative Bacteriology* (9th ed., revised, Williams& Wilkins, 1993), *Virus Taxonomy – The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses* (Springer-Verlag, 1995). The latter document also supplies standard abbreviations for virus species.

Appendix C: Ethical approval

(i) Biomedical Research Ethics Committee (BREC) approval

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI
RESEARCH OFFICE
Biomedical Research Ethics Administration
Westville Campus, Govan Mbeki Building
Private Bag X 54001
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Tel: 27 31 2604769 - Fax: 27 31 2604609
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Website http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

14 November 2018

Dr B Njiyela (215082526)
Department of Medicine
School of Clinical Medicine
NRMSM
b.kanjiyela@gmail.com

Title: A retrospective study of patients with liver disease.
Degree: MMed
BREC REF NO: BE401/16

NEW TITLE: A retrospective study of patients with liver disease in a HIV era

We wish to advise you that your Application for Amendments received on 08 November to change the title to the above for the above study has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee.

The committee will be notified of the above approval at its next meeting to be held on 11 December 2018.

Yours sincerely

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

cc supervisor: magilan@ukzn.ac.za cc postgraduate administrator: ter'ai@ukzn.ac.za

(ii) King Edward VIII hospital letter of approval

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

OFFICE OF THE HOSPITAL CEO
KING EDWARD VIII HOSPITAL

Private Bag X02, CONCELLA, 4013
Corner of Rick Turner (Francois Road) & Sydney Road
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Ref.: KE 2/7/1/(40/2016
Enq.: Mrs. R. Sibiya
Research Programming
15 August 2016

Dr. B Njiyela
Department of Medicine
Nelson R. Mandela School of Medicine
UNIVERSITY OF KWAZULU-NATAL

Dear Dr. Njiyela

Protocol: "The prevalent causes of hepatomegaly in patients admitted to medical wards at King Edward VIII Hospital, Durban in the HIV era: A cross sectional study". Degree: MMed. BREC Ref. No. BE401/16

Permission to conduct research at King Edward VIII Hospital is provisionally granted, pending approval by the Provincial Health Research Committee, KZN Department of Health.

Kindly note the following:-

- The research will only commence once confirmation from the Provincial Health Research Committee in the KZN Department of Health has been received.
- Signing of an indemnity form at Room 8, CEO Complex before commencement with your study.
- King Edward VIII Hospital received full acknowledgment in the study on all Publications and reports and also kindly present a copy of the publication or report on completion.

The Management of King Edward VIII Hospital reserves the right to terminate the permission for the study should circumstances so dictate.

Yours faithfully

SUPPORTED/~~NOT SUPPORTED~~ 01/09/16

DR. S. CHINASAMY
CLINICAL HEAD: MEDICINE
DATE

SUPPORTED/~~NOT SUPPORTED~~ 01/09/16

DR. SA MOODLEY
ACTING SENIOR MEDICAL MANAGER
DATE

Fighting Disease, Fighting Poverty, Giving Hope

(iii) Department of health research and knowledge management

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

DIRECTORATE:
Health Research & Knowledge Management

Physical Address: 330 Langalibalele Street, Pietermaritzburg
Postal Address: Private Bag X9051
Tel: 033 395 2805/ 3189/ 3123 Fax: 033 394 3782
Email:
www.kznhealth.gov.za

HRKM Ref: 307/16
NHRD Ref: KZ_2016RP12_G14

Date: 23 September 2016
Dear Dr B. Njiyela
UKZN

Approval of research

1. The research proposal titled '**The prevalent causes of hepatomegaly in patients admitted to the medical wards at King Edward VIII Hospital, Durban in the HIV era. A cross sectional study**' was reviewed by the KwaZulu-Natal Department of Health.

   The proposal is hereby **approved** for research to be undertaken at King Edward VIII Hospital.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mr X. Xaba on 033-395 2805.

Yours Sincerely

Dr E Lutge
Chairperson Health Research Committee
Date: 26/09/16

Fighting Disease, Fighting Poverty, Giving Hope

Appendix D: Data collection sheet

Case number:	
Age:	
Gender:	□ Female □ Male
Race	□ Black □ White □ Asian □ Coloured
Date of admission	

Presenting symptoms
□ Fever □ vomiting □ hematemesis □ diarrhoea
□ yellow eyes □ abdominal pain □ abdominal distension/mass
□ Dark urine □ asymptomatic

Presenting clinical signs
Vital signs BP ……mmHg HR ……bpm T ……ºC HGT ……mmol/L □ Jaundice □ Pallor □ Clubbing □ Oedema □ Lymphadenopathy, site(s): …… □ Ascites □ Liver span ……cm

□ Liver consistency, explain…….
□□Bruits(liver)
□□ Splenomegaly

Comorbidities
□ Hypertension □ Dyslipidaemia □ Diabetes Mellitus
□ Heart failure □ abdominal TB Hepatitis B/C □ Other, Specify ……
RVD □Positive □ Negative □ unknown If Positive CD4+ count _____ VL _____ copies ARVs:
Alcohol intake □ yes □no

Laboratory investigations	
Parameters	Results
Hb	
WCC	
Plts	
Total bilirubin	

unconjugated bilirubin	
Alb	
ALT	
AST	
ALP	
GGT	
INR	
Total Cholesterol	
Triglycerides	
HDL Cholesterol	
LDL Cholesterol	
VCT (Rapid HIV test)	
HIV ELISA (if VCT negative)	
IF positive: CD4 count	
Viral load	
Hepatitis B: HBsAg Anti-HBc IgM anti-HBc Anti-HBs Titre	
Hepatitis C: HCV RNA Anti-HBV	
ANA	
Rheumatoid factor	

Anticardiolipin antibody	
Antithyroid antibody	
Anti-smooth muscle antibody	
Other(s)	

Paracentesis

SAAG:

Ultrasound abdomen

Liver size: …….cm

Other significant findings:

Biopsy/Histology:

Final diagnosis:

Assessment of the liver in an HIV era: Clinical, laboratory and radiological abnormalities

by DR B MBANJWA

General metrics

44,386	6,484	425	25 min 56 sec	49 min 52 sec
characters	words	sentences	reading time	speaking time

Writing Issues

No issues found

Plagiarism

33 sources

5% of your text matches 33 sources on the web or in archives of academic publications