# HEPATITIS B AND C VIRAL CO-INFECTION IN HIV INFECTED PATIENTS ENROLLED IN THE ANTIRETROVIRAL TREATMENT PROGRAM AT THE UNIVERSITY TEACHING HOSPITAL IN LUSAKA, ZAMBIA.



By

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**A Dissertation submitted to the University of Zambia in a partial fulfillment of the requirement for the degree of Master of Medicine in Internal Medicine.**

(School of Medicine)
**THE UNIVERSITY OF ZAMBIA**
**December, 2008**

**DECLARATION**

I declare that this dissertation represents my own work and that it has not previously been submitted for a degree, diploma or other qualification at this or another University.

Signed.....  ..... Date..25/06/2009.....

Candidate

## APPROVAL

This dissertation of **Kenneth Kapembwa** is approved as fulfilling the requirements for the award of the Master of Medicine in Internal Medicine of the University of Zambia.

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## ABSTRACT

**Title**: Hepatitis B and C viral co-infection in HIV infected patients enrolled in the antiretroviral treatment program at the University Teaching Hospital in Lusaka, Zambia.

**Background:** There are currently few studies describing the prevalence of HIV and viral hepatitis co-infection in Zambia. Such epidemiologic data are critically needed to guide national health policy in the areas of routine hepatitis screening and optimized antiretroviral therapy (ART) for co-infected patients.

**Methods:** In this cross-sectional study, we screened HIV-infected ART-eligible adults for hepatitis B surface antigen (HBsAg) and hepatitis C antibody (HCV) at the University Teaching Hospital in Lusaka, Zambia. We collected basic demographic, medical, and laboratory data to determine predictors for hepatitis and HIV co-infection.

**Results:** From December 2007 to June 2008, we enrolled 323 consecutive patients eligible for HIV treatment. The median age was 37 years (IQR=32.0, 44.0), median CD4 count was 118 cells/μl (IQR= 59, 199) and 174 (54%) were women. Overall, 32 (10%) were found to have active hepatitis B (i.e. HBsAg-positive) while 4 (1%) were diagnosed with hepatitis C. In univariate analysis, patients with hepatitis B co-infection were more likely to be younger than 40 years (84.4% vs. 61.4%; p=0.01) when compared to those who were not co-infected. No differences were noted in baseline WHO stage, CD4 count, or sex of subject as well as exposures to blood transfusion, tattooing and number of sexual partners. Patients with hepatitis B we more likely to have mild to moderately elevated AST/ALT (40-199 IU/L, 15.8% vs. 5.4%; p=0.003). A severe elevation of liver enzymes (> 200 IU/L) was uncommon in this population (2.2%) and not different between the two groups (14.3% vs. 9.7%; p=0.5192). We were unable to determine predictors of hepatitis C infection due to the low prevalence of the disease.

**Conclusion**: HIV and hepatitis B co-infection was common among patients initiating ART at this tertiary care facility in Zambia. Programs to routinely screen for hepatitis B should be considered in this population, given the proven efficacy of optimized ART regimens including tenofovir or lamivudine.

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## DEDICATION

This study is dedicated to my wife, Kunda, daughters Mutale and Musawa, and to my son, Chali, for their unwavering support during the execution of this study.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AIDS | Acquired immunodeficiency syndrome |
| ACTG | AIDS Clinical Trials Group |
| ALT | Alanine aminotransferase |
| ALP | Alkaline phosphatase |
| AST | Aspartate aminotransferase |
| ARV | Anti-retroviral |
| DNA | Deoxyribonucleic acid |
| gGT | Gamma glutamyltransferase |
| HAART | Highly Active Anti-Retroviral Therapy |
| HBV | Hepatitis B virus |
| HBeAg | Hepatitis B envelope antigen |
| HBsAg | Hepatitis B surface antigen |
| HCV | Hepatitis C virus |
| HIV | Human immunodeficiency virus |
| LEE | Liver enzyme elevation |
| RNA | Ribonucleic acid |
| TTI | Transfusion Transmitted Infections |
| ULN | Upper limit of normal |
| UTH | University Teaching Hospital |
| WHO | World Health Organization |
| ZNBTS | Zambia National Blood Transfusion Service |

# CHAPTER 1

## 1.0 Background

Co-infection with human immunodeficiency virus (HIV) and hepatitis B and/or C viruses occurs frequently since these viruses share similar modes of transmission (parenteral, sexual, and vertical). In the Zambian context, HIV and hepatitis B virus (HBV) are predominantly transmitted through the heterosexual route. It is also well known that sexual transmission of HIV is more efficient than that of hepatitis C virus (HCV). In Zambia, HCV infection is uncommon and this has been shown in blood donors at the Zambia National Blood Transfusion Service (ZNBTS) laboratory were rates are as low as 0.41% to 0.5%.[1-2] However, for some countries, HCV infection is common due to intravenous drug use, a more efficient mode of transmission of HCV compared to the sexual route.[3]

The roll-out of Highly Active Antiretroviral Therapy (HAART) in Zambia has been done without due regard to co-infection with hepatitis B and C viruses. No prevalence studies looking at HIV co-infection with Hepatitis B and C in the era of HAART roll-out have been published in Zambia. Previous Zambian studies examining Hepatitis B and C viral prevalence have varied greatly from 3.3% to 31.3% depending on the year of study, geographical location and HIV status of population sampled.[4-6] However, the true prevalence of co- infection of HIV and HBV is unknown in patients who are yet to commence on HAART and may be important as antiretroviral therapy rolls out nationwide.

Liver dysfunction is known to occur in patients that are on HAART, and severe hepatotoxicity has been documented in up to 6 % of patients.[7] Drug induced hepatotoxicity as a result of antiretroviral therapy depends on the drug classes or agents used as well as on pre-existing liver dysfunction.[8] Severe hepatotoxicity and hepatic failure have been observed during treatment with nevirapine and ritonavir.[9-14] Case reports also exist about liver failure occurring with indinavir, efavirenz, nelfinavir and different nucleoside analogs.[15] Some causes of pre-existing conditions include infection due to hepatitis B and C viruses, malnutrition, chronic alcoholism or interaction with other hepatotoxic drugs such as anti-tuberculosis drugs.[16] The level of liver toxicity

ranges from mild and fully reversible liver enzyme elevation (LEE) to rare, but rapidly progressive fulminant and occasionally fatal, liver failure.[16, 17]

While antiretroviral drugs can lead to increased hepatotoxicity in patients with pre-existing liver disease due to HBV and/or HCV, it has also been shown that the unfavorable course of HBV and/or HCV viral infections in HIV infection can be improved by treatment of HIV infection with HAART. It has also been suggested that drugs such as stavudine, didanosine, and nevirapine should be avoided in HIV and HBV co-infected patients. Furthermore, certain antiretroviral drugs, such as tenofovir and lamivudine, have proven efficacy against HBV.[18]

The commonly used antiretroviral drugs in Zambia are stavudine, lamivudine, nevirapine, zidovudine and efavirenz. Other antiretroviral drugs include abacavir, didanosine, tenofovir and emtricitrabine. Protease inhibitors are also used, especially as second-line drugs. The antiretroviral drugs are administered according to the current protocol regardless of hepatitis viral profile status.[19]

### 1.1 Statement of the Problem

In Zambia, there is no routine testing for HBV and/or HCV in HIV infected patients who are about to commence on antiretroviral (ARV) drugs. Consequently, potentially vulnerable co-infected individuals who could develop hepatotoxicity or do poorly on antiretroviral drugs have not been identified.

### 1.2 Study Justification

Knowing the levels of HIV co-infection with hepatitis B and C viruses would lead to optimization of antiretroviral drug choice which would likely improve the treatment outcome of the patients.

### 1.3 Research Question

Is co-infection of HIV with hepatitis B and C viruses common in HIV patients prior to commencement of HAART in Zambian patients?

### 1.4.0 Objectives

### 1.4.1 Primary Objective

To establish the prevalence of Hepatitis B and C viral infections among HIV infected patients seeking outpatient care at the University Teaching Hospital in Lusaka.

### 1.4.2 Secondary Objectives

1.4.2.1 To determine prevalence of liver dysfunction in patients co-infected with HIV and Hepatitis B and/or C viruses.

1.4.2.2 To determine the predictors for hepatitis B and/or C virus co-infections in a Zambian cohort of HIV positive patients.

# CHAPTER 2

## 2.0 Literature Review

HIV, HBV and HCV have been known to co-exist in an individual. The three viruses have similar routes of transmission, though the efficiency of transmission differs. This co-existence of the viruses increases the morbidity and mortality of the affected patient since HIV increases the progression of HBV and HCV.[20- 21] The treatment of HIV in co-infected patients can worsen the outcome if the antiretroviral drugs are not optimally chosen.[9-14] Equally, optimal choice of antiretroviral drugs in co-infected patients can improve the outcome of liver dysfunction.[18] Liver dysfunction can result from the use of certain antiretroviral drugs. Other factors known to cause liver dysfunction in HIV infected patients include among others, alcohol, anti-TB therapy and the use of herbal medication.

### 2.1.0 Epidemiology

HIV, HBV and HCV share similar routes of transmission. All three viruses can be transmitted vertically, sexually and through contact with HIV contaminated blood, blood products and body organs. The prevalence of co-infection varies according to the risk factors of acquiring HIV as well as the endemicity of HBV or HCV and the predominant age at acquisition.[22- 23]

### 2.1.1 Hepatitis B virus

Hepatitis B virus is cosmopolitan and endemic in Southeast Asia, China and Africa where more than half the population has been infected at some point in their lives and with more than 8% of the population being carriers, whereas its prevalence is very low in North America, Western Europe and Australia.[24]

About 350 million people worldwide are infected with HBV while over one million people die annually from HBV related liver disease such as liver cirrhosis and hepatocellular carcinoma.[23]

In sub-Sahara Africa, the main route of transmission depends on the age of the patient. Vertical transmission of HBV was not common as most infants of hepatitis B surface antigen (HBsAg) positive mothers. This is due to protection by maternal anti-hepatitis B surface antigen antibodies (anti-HBs antibodies) that are passively transmitted from mother to child and last for six to twelve months and also because 81.4% to 100% these

mothers were negative for hepatitis B envelope antigen (HBeAg).[25] HBeAg when present increases infectiousness of HBV such that HBsAg positive mothers who are HBeAg negative are less likely to pass on the infection to their babies. In children under five years old, transmission is mainly horizontal while in adolescents and adults, sexual transmission is the common route. In a study done in Zambia in the pre-HIV era, horizontal transmission of hepatitis B virus was shown to occur in both children and adults living in rural Zambia.[26]

In patients with poor immunity such as HIV immunosuppression and children under five years old, HBV infection can persist leading to chronic carriage of the HBV. This is because poor immunity leads to failure of the body to clear the infection.[22, 25] The rate of spontaneous HBV clearance is over 90% in immunocompentent patients while chronic infection develops in 20% of HIV infected patients exposed to HBV.[22] HBsAg carriage has been found more commonly in males than females despite both sexes being equally exposed to the virus.[27-28]

Studies done in Zambia showed that the prevalence of Hepatitis B depended on location, time and HIV status of the patient. In the era before HIV, the prevalence of HBV among healthy blood donors was found to be 5.0% though more recent unpublished records at the ZNBTS show a prevalence of 8.0% in healthy blood donors.[29] The prevalence was higher in HIV positive patients than in HIV negative patients with rates of 31.3% and 16.8% respectively (p=0.003).[6] However, earlier studies had shown a lower prevalence of HBV in both HIV positive and HIV negative patients with prevalence of 7.1% and 5.4% (p=0.23) among pregnant women attending prenatal clinic respectively.[5] It was also found that the virus was more common in some rural parts of the country compared to urban areas as illustrated in a study of 2,098 antenatal women, the overall prevalence of HBV was 6.5% while that in urban areas was found to be less than rural areas.[4]

### 2.1.2 Hepatitis C

Hepatitis C virus also has a worldwide distribution and affects about 170 million people globally.[30-31] Co-infection with HIV occurs commonly as the two viruses share similar modes of transmission. In Europe and USA, the prevalence of co-infection is as high as 30%.[32-34] The prevalence of co-infection in Western countries, however, is highest in intravenous drug users, with rates of 80-90% since this route of transmission is efficient

for both HCV and HIV.[35-37] This pattern is broken in the Netherlands where as a result of an effective needle exchange and education programme, the prevalence rates of co-infection is 8%.[38-39]

In Zambia, intravenous drug abuse is not a common act and as such, Hepatitis C virus infection is not common. The prevalence of hepatitis C virus was shown to be 0.41% in a 1996 study done in Zambia, while a more recent unpublished study done at the ZNBTS showed a prevalence of 0.5%.[1-2] The former study comprised of 735 samples from three groups of adults labeled respectively as (i) blood donors, (ii) hospital patients with jaundice and (iii) hospital patients without jaundice. It was found that none of the blood donors tested positive for hepatitis C while two and one patients respectively from group (ii) and (iii) tested positive. This picture seen in Zambia can also be attributed to poor transmission of HCV sexually; the main mode through which many Zambians acquire HIV.[40] However, the true mode of acquisition of hepatitis C virus remains unknown in Zambia.[1]

**2.2 Diagnosis**

Various tests are available to detect HBV and HCV infection. The test applied depends on the purpose of the test and include:

i) Serological tests to detect antibodies or antigens for HVC and HBV respectively

ii) Molecular tests to detect and quantitiate viral ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) for HCV and HBV respectively

iii) Genotyping techniques

Serological tests are mainly employed as screening tests. The molecular and genotypic tests become useful during treatment of patients with confirmed HBV or HCV infection.

HBV infection can be confirmed by serological tests that look for the presence of HBsAg while HCV infection can be confirmed by testing for the presence of anti- HCV antibodies.[41-42]

HCV can be detected by any one of three serological tests denoted as first, second or third generation tests. Third generation tests are a better test than first generation tests as they are more sensitive and specific.[41]

### 2.3. **Effects of HIV/HBV co-infection.**

Co-infection with HIV and Hepatitis B virus leads to the rapid progression of HBV infection. It has been seen that immunosuppression may reduce liver damage as a result of a less aggressive HBV-specific immune response.[43] However, HIV has been found to worsen liver disease in HBV infected patients who are more likely to proceed to cirrhosis or hepatocellular carcinoma faster than in HIV negative patients.[44- 45] HBV and HIV co-infected patients are more likely to die from liver disease and this risk is increased upon commencement of HAART.[46] The reason for this is due to the resulting hepatotoxicity from the anti-retroviral drugs. On the other hand, Hepatitis B does not seem to have any effect on the progression of HIV to Acquired immunodeficiency syndrome (AIDS).[47]

### 2.4. Effects of HIV/HCV co-infection.

HIV increases the progression of liver disease caused by HCV infection.[21] Progression to hepatic cirrhosis occurs more readily at lower CD4 counts.[48-49] In HCV mono-infected patients, the progression to liver cirrhosis or hepatic decompensation takes decades to occur while in HIV/HCV co-infected patients, a higher rate of hepatocellular carcinoma occurs and the progression to hepatic failure is shortened to 6 to 10 years.[50-52] The exact mechanism for this is not clear. However, it has been found that HCV viraemia is higher in HIV infected patients and the clearance of HCV is less effective in co-infected patients. In mono-infected patients, spontaneous clearance of HCV occurs in 15-20% of patients while the rates of spontaneous clearance are lower in co-infected patients.[37] The immune mechanisms through which HCV clearance occurs involves the use of CD4 T helper lymphocytes, cytotoxic T lymphocytes and interferons[53, 54]. Thus, as HIV disease advances with lower CD4 cell counts, the body clearance mechanisms of HCV become impaired with a higher viraemia and increased hepatic damage and liver failure. It has actually been shown that patients with CD4 counts less than 500 cells/mm$^3$ have a higher progression to hepatic fibrosis.[49] On the other hand, the scale up of HAART world wide is leading to changes in the contribution of HCV to HIV morbidity and mortality. In the developed world, the treatment of HIV since the introduction of HAART has changed the presentation of disease from common opportunistic infections to cardiovascular and liver

related pathologies as HIV infected patients are now living longer.[55-56] As a result, HIV and HCV co-infected patients are presenting more with complications of cirrhosis, hepatic failure and hepatocellular carcinoma.[57-58]

**2.5. Liver dysfunction**

Liver dysfunction can be assessed through the laboratory measurement of certain enzyme markers. The liver enzyme markers include alanine aminotransferases (ALT), aspartate aminotransferases (AST), gamma glutamyltransferase (gGT) and alkaline phosphatase (ALP).

ALT is found in highest concentrations in the liver as compared to any other tissue and for this reason it is a more specific indicator of liver dysfunction.[59] AST is also found in the liver, but is also widely distributed in other tissues. AST is thus a less sensitive indicator of hepatic dysfunction.[59] gGT is not useful on its own and has to be interpreted with respect to AST and ALT in patients who have liver impairment due to alcohol abuse. It is a non-specific enzyme as it is elevated in many situations such as myocardial infarction, diabetes, pancreatitis and chronic obstructive pulmonary disease.[60] ALP is most important in assessing obstructive liver disease as well as infiltrative liver diseases. Infiltrative diseases include granulomatous diseases such as sarcoidosis and metastatic cancer to the liver.[59] For these reasons, in this study, only transaminases (ALT and AST) were used to assess for liver dysfunction in the subjects enrolled.

Liver dysfunction can be graded according to the AIDS Clinical Trials Group (ACTG) criteria as follows: [61]

| Grade | Enzyme marker level | Description |
|---|---|---|
| 1 | 1.25- 2.5X ULN* | Mild |
| 2 | 2.6X- 5.0X ULN | Moderate |
| 3 | 5.1-10X ULN | Severe |
| 4 | > 10 X ULN | Severe |

* ULN= Upper limit of normal

Severe hepatotoxicity or liver enzyme elevation has been described as either grade 3 and 4 ALT and AST elevations, or occasionally only grade 4 elevations.

Liver dysfunction can result from a number of factors in HIV infected patients. More commonly, HCV and HBV are behind the elevated liver enzymes in HIV patients. However, other factors that can cause liver dysfunction have to be excluded as necessary. Factors like autoimmune diseases (autoimmune hepatitis, primary biliary cirrhosis), Wilson's disease and haemochromatosis, and drugs (antiretroviral drugs, isoniazid, paracetamol and almost any drug) can also cause elevation of liver enzymes.

Liver dysfunction resulting from Hepatitis B and Hepatitis C infection tends to occur a few weeks after infection. The causes of hepatic dysfunction in co-infected patients can be directly as a result of the viruses inducing an immune reaction that leads to liver damage or indirectly through drugs that may be used to treat HIV.

Hepatitis B virus does not cause hepatic dysfunction unless the host immunity is intact. The stronger the immunity, the more severe the liver enzyme elevation is such that in immunocompromised HIV infected patients, severe liver enzyme elevation is not likely to be common[24].

Of the antiretroviral drugs, nevirapine and ritonavir were seen to be associated with most of the hepatic impairment and especially so in patients co- infected with HCV and HBV.[11-14] Another way in which HIV/HCV or HBV co-infected patients develop hepatic dysfunction is through immune reconstitution inflammatory syndrome (IRIS). In IRIS, as the body's immunity improves, so does the inflammatory response against the antigens of HBV and HCV.[62-63] In this situation however, continuation of ARVs is essential.

### 2.6. **Treatment of HBV in HIV co-infected patients**

Seven drugs have been licensed for the treatment of hepatitis B. The drugs are lamivudine, tenofovir disoproxil fumarate, telbivudine, adefovir dipivoxil, entecavir, interferon alfa and peginterferon alfa-2a.[18, 64-68] Of these drugs, lamivudine and tenofovir have dual effects against HIV and HBV while adefovir, telbivudine and entecavir has only shown activity against HBV at the normal safe doses.[66] One downside of lamivudine is the development of HBV drug resistance with a resultant flare-up of HBV infection that may lead to further liver injury.[69-71] Adefovir, tenofovir and entecavir have been found to be effective against the lamivudine resistant HBV mutants and can be effectively be used

in such cases to avoid further deterioration of the patient.[72-73] In co-infected patients, optimal use of drugs that target both HIV and HBV can lead to treatment of both conditions without increasing the pill burden, side effects and drug interactions that occurs when treating more than one condition in a patient.

### 2.7 **Treatment of HCV co-infected patients.**

Unlike HBV, drugs that target HIV have no effect on HCV. HCV should be treated with ribavirin and pegylated interferon as the pegylated form of interferon has shown to be better than the standard form.[74-75] The response to treatment of HCV with those drugs is low, with the worst response being for HCV genotype 1 and in patients in whom the HCV viral load is greater than 800,000. [74-75] In the APRICOT study, no effect of HCV therapy on HIV progression was noted.[74] It is noteworthy that ribavirin, a nucleoside analogue, interacts with didanosine and the combined use of these two drugs should be discouraged as it leads to mitochondrial toxicity. This effect though, has not been observed for other nucleoside analogues such as zidovudine and stavudine.[77]

# CHAPTER 3

## 3.0 Methodology.

### 3.1 Site:

The University Teaching Hospital (UTH), Lusaka, Zambia, is the biggest referral hospital in Zambia. It has a total of 1200 in-patient beds and specialized out-patient clinics. The UTH department of Internal Medicine runs an out-patient Adult Antiretroviral Clinic (ARV clinic) which handles HIV patients referred from the various adult in-patient facilities as well as referrals from outside the hospital. The ARV clinic was thus an ideal setting for this study.

Two types of HIV infected patients seen at the ARV clinic include those who have just been diagnosed with HIV and are not yet commenced on ARVs as well as patients who are already on ARVs but requiring specialist treatment of complications due to treatment or opportunistic infections or malignancies. Both kinds of patients are reviewed at the ARV clinic.

Consultations in the ARV clinic are done daily from Monday to Friday between 08:00 hours and 13:00 hours. On a monthly average, 118 HIV infected patients that are not yet commenced on antiretroviral drugs are seen by doctors in the ARV clinic.

### 3.2 Data collection

Demographic data, data pertaining to risk factors of HCV and HBV co-infection and data on possible causes of hepatic dysfunction was collected by means of an interviewer administered questionnaire which took about five minutes to complete (See Appendix).

4 milliliters of blood was then sampled from the patient and sent to the Zambia National Blood Bank laboratory to test for hepatitis B surface antigen (HBsAg) and anti-hepatitis C (anti-HCV) antibody (details in Chapter 4).

For patients without liver function tests, another 4 milliliters of blood was drawn for these tests according to current practices. Patient identifiers were a randomly assigned study number and a patient number that is provided by the University Teaching Hospital and used by the SmartCare/ Patient Tracking System (PTS). The two numbers were kept separately in order to maintain confidentiality and for easy correlation of laboratory results for each patient, a book that was always under lock and key had both numbers (study number and UTH number). Study subjects were not identified by name.

### 3.3 Ethics

Ethical approval for this study was granted by the University of Zambia Research Ethics Committee (REC) and the University of Alabama Institutional Review Board.

All participants were enrolled on a voluntary basis following detailed informed consent explaining all the aspects of the study. A patient information sheet in English or either one of two of the predominant local languages, Bemba or Nyanja was given to the patient. Subjects were free to withdraw from the study at any stage and this did not affect their access to standard antiretroviral care. Subjects were also not coerced into participating in this study as there were no payments made for participating. Strict confidentiality was maintained at all times during the study and subjects' rights were respected at all times.

Laboratory findings were made available to the patient and treating physicians for appropriate management of the patients according to the prevailing standard of care at the UTH.

### 3.4 Selection of Subjects

The first of the 329 study participants was enrolled on 12th December, 2007 and the last one was enrolled on 13th June, 2008.

Study assistants identified potential subjects by perusal through patient files a day before the patient presented themselves for scheduled reviews at the adult ARV clinic or on the actual day of presentation for the unscheduled subjects referred from outside the clinic.

All HIV positive patients meeting the following characteristics were enrolled in the study:

- Patients who were documented HIV positive according to the national protocol.
- Attendance at the outpatient clinic of UTH.
- Patients qualifying for ART by the Zambian National Guidelines but not yet on antiretroviral therapy other than for postexposure prophylaxis and prevention of mother to child transmission programs.
- Willing to participate in the study.
- Aged 16 years and above.

### 3.5 Sample size calculation

We estimated prevalence of hepatitis B virus to be 31% based on previous work.[7] For a population survey using random sampling, this effect with a precision of 5% of the prevalence estimate and a confidence interval of 95% required a sample size of n=329 patients. This calculation was performed using EpiInfo StatCalc, version 6.

### 3.6 Data Analysis

Patient information was transcribed from the data forms to an access dataset. The data were then analyzed using statistical analysis program, SAS, version 9.1.3. Prevalence of hepatitis B and C viruses and of liver dysfunction was calculated with 95% confidence intervals. Predictors of Hepatitis B or Hepatitis C were then analyzed by means of two by two tables, using Chi-square tests to determine association. A p-value of $< 0.05$ was considered statistically significant.

# CHAPTER 4

## 4.0 Laboratory

### 4.1 Introduction.

Testing of blood samples was outsourced. The Zambia National Blood Transfusion Service (ZNBTS) was chosen as a site for testing for HBsAg and anti-HCV antibodies. Consideration was given to the credibility of the testing laboratory. The considered factor was the laboratory standing at international level. The External Quality Assessment (EQA) performance was used as the yardstick. ZNBTS laboratory is enrolled on the EQA programme provided by the Royal College of Pathologists of Australia (RCPA). Records of performance since enrolment were provided.

### 4.2 Sample delivery.

4mls of whole blood was collected from a subject in a plain container. Samples together with a unique subject identifier forms were delivered to the ZNBTS laboratory on the same day. An aggregated manifest form upon which receipt of samples was acknowledged by laboratory staff was maintained by study assistants.

### 4.3 Sample receiving procedure.

- Blood bank laboratory staff counter-checked information on the sample and the unique subject identifier form to ensure that prior to processing, information tallied. In the event that a discrepancy was discovered, the Principal Investigator was immediately contacted and sample quarantined pending clarification.
- Other quality control measures included checking for volumes of samples as well as sample integrity.
- Samples were allocated laboratory accession numbers.

### 4.4 Sample processing.

Procedure employed was as outlined in the ZNBTS Process Description for Transfusion Transmitted Infections (TTI) testing.

Whole blood samples were centrifuged at 3000 rpm for five minutes using bench top centrifuge. 1-2ml of supernatant was collected as serum for testing.

### 4.5 Testing for HBV and HCV viral markers.

Procedures for testing was as outlined in the Standard Operating Procedures (SoPs) for each assay.

1. HBsAg:

Technology: Enzyme Immunoassay (EIA).

Platform: Axsym automated immunoanalysers.

Assay: Abbott Axsym HBsAg (V2).

Back up system for HBsAg;

Assay used: Abbott Murex HBsAg Version 3

Platform: Tecan Reader (washer: Tecan Columbus plus)

Incubator: Stuart Forced Dry Air Incubator.

2. Anti-HCV antibodies:

Technology: Enzyme Immunoassay (EIA).

Platform: Axsym automated immunoanalysers.

Assay: Abbott Axsym HCV Version 3.

Back up system for anti-HCV antibodies;

Assay used: Abbott Murex anti-HCV Version 4

Platform: Tecan Reader (washer: Tecan Columbus plus)

Incubator: Stuart Forced Dry Air Incubator.

### 4.6 Scoring of Global Results.

The ZNBTS testing algorithm was used in scoring results.

The summary of result interpretation was as follows;

- An initially non-reactive result was scored as such.
- An initially reactive sample was repeated in duplicate and a score was arrived at depending on the combination of three outcomes. For a sample to be considered reactive, there should have been at least two out of three outcomes being reactive.

**4.7 Result documentation.**

Results on worksheets were kept on ZNBTS files. Compiled results were recorded on Excel worksheets and signed out by the ZNBTS Medical Director. Hard copies of compiled results were delivered by ZNBTS to the Principal Investigator.

**4.8 Disposal of blood samples.**

Tested blood samples were kept for a period of two (2) weeks and thereafter discarded as per ZNBTS procedure.

# CHAPTER 5

## 5.0 Results.

### 5.1 General description of results.

Between 12th December, 2007 and 13th June, 2008, 896 patients were enrolled into the anti-retroviral treatment program at the UTH Adult Anti-retroviral clinic. These subjects included patients who were in the wards and found in need of anti-retroviral drugs, out-patients already on HAART but referred for advanced care and patients referred for initiation of HAART from either Lusaka Urban District clinics or newly discharged patients from the UTH adult wards.

329 subjects of the 896 seen at the adult ART clinic met the inclusion criteria, though only 323 were included in the data analysis. Of the remaining six out of 329, one withdrew from study, two were double enrolled, and two had been on HAART for over a month before enrolment while one did not meet clinical and immunological criteria for enrolment. Of the 896 subjects seen, 567 could not be enrolled as they did not meet the enrolment criteria. The population was representative of the city of Lusaka demographically in terms of sex ratio, education and income.[78]

Of the 323 patients included in the study, 174 (53.9%) were female. The age range was 17 years to 64 years and the median age was 37 years. Some laboratory results were not available for some subjects with regards to CD 4 counts, ALT and AST results. The CD4 count was below 200 cells/μl in 74.9% of the subjects while 70.2% of the subjects were WHO stage III or IV. The median CD4 count was 118.0 cells/μl with an inter-quartile range of 59.0 to 199.0 cells/μl. Only three of the subjects had started though not completed injections for hepatitis B vaccination. Previous or current consumption of alcohol or anti-tuberculous drugs was evaluated as those factors would also independently affect liver function tests. It was found that most respondents (75.2%) had consumed alcohol at some point while a large minority (34.0%) had taken anti-tuberculous drugs (Table 1).

**5.2 Hepatitis B and Hepatitis C Prevalence in HIV infected patients.**

Hepatitis B surface antigens (HBsAg) and anti-Hepatitis C antibodies were found in 9.9% and 1.2% of the subjects respectively.

**5.3 Risk factors for Hepatitis B or C infection.**

Examination of age showed that people younger than forty years were more likely to test positive for HBsAg than those older than forty years ( p= 0.0105). Other factors assessed and expected to be associated with risks of contracting hepatitis B or C were not significantly associated with a positive HBsAg test. Those factors included blood transfusion, multiple sexual partners, unsafe sex such as none use of condoms or condom breakage during sexual intercourse as well as past history of sexually transmitted infections. None of the subjects abused illicit intravenous drugs (Table 2).

The number of patients with a positive hepatitis C was too small to assess significance for risk factors or hepatotoxic effects.

**5.4. Liver enzyme elevation.**

Liver enzyme elevation was assessed by looking at AST and ALT. Mild to moderate liver enzyme elevation was common in subjects who were HBsAg positive and those that had taken anti-tuberculous therapy (p=0.003 and <0.0001 respectively). Other factors such as alcohol and the use of herbal remedies were not significantly associated with elevation of liver enzymes. Severe liver enzyme elevation was not common including in HIV and HBV co-infected patients (Tables 3a and 3b).

*Table 1: Characteristics of ART naïve adults eligible for treatment at UTH*

| Characteristic | N | Value |
|---|---|---|
| Viral Hepatitis | 323 | |
| HBsAg + | 32 | 9.9% |
| Anti-HCV antibodies+ | 4 | 1.2% |
| Age, median years (IQR) | 323 | 37.0(32.0-44.0) |
| Sex | 323 | |
| Men | 149 | 46.0% |
| Women | 174 | 54.0% |
| Education | 323 | |
| No formal | 4 | 1.2% |
| Formal | 319 | 98.8% |
| Income/month | 323 | |
| ZMK <500,000 | 170 | 52.6% |
| ZMK>500,000 | 153 | 47.4% |
| WHO stage | 309 | |
| I or II | 92 | 29.8% |
| III or IV | 217 | 70.2% |
| CD4+ lymphocyte count, median cells/ μL (IQR) | 301 | 118.0(59.0-199.0) |
| <200 cells/ μL | 226 | 75.1% |
| ≥200 cells/ μL | 75 | 24.9% |
| AST, median U/L (IQR) | 274 | 37.0(25.0-53.0) |
| <200 U/L | 269 | 98.2% |
| ≥200 U/L | 5 | 1.8% |
| ALT, median U/L (IQR) | 293 | 22.0(14.0-34.0) |
| <200 U/L | 290 | 99.0% |
| ≥200 U/L | 3 | 1.0% |
| Ever taken Anti – TB therapy | 323 | |
| No | 213 | 66.0% |
| Yes | 110 | 34.0% |
| Consumes alcohol | 323 | |
| Never | 80 | 24.8% |
| Ever | 243 | 75.2% |
| Recalls Hepatitis B vaccination | 323 | |
| Started | 3 | 1% |
| Completed | 0 | 0% |

*Table 2: Risk factors for Hepatitis B infection*

| | HBsAg+ (N=32) | HBsAg-(N=285) | P-value |
|---|---|---|---|
| Age | | | |
| <40 years, n(%) | 27(84.4%) | 175(61.4%) | 0.0105 |
| ≥40 years, n(%) | 5(15.6%) | 110(38.6%) | |
| Sex, n | | | |
| Men, n (%) | 16(50.0%) | 130(45.6%) | 0.6375 |
| Women, n (%) | 16(50.0%) | 155(54.4%) | |
| Education, n | | | |
| No formal, n (%) | 1(3.1%) | 3(1.1%) | 0.3481 |
| Formal, n (%) | 31(96.9%) | 282(99.0%) | |
| Income/month, n | | | |
| ZMK <500,000, n (%) | 19(59.4%) | 149(52.3%) | 0.4465 |
| ZMK>500,000, n (%) | 13(40.6%) | 136(47.7%) | |
| WHO stage, n | | | |
| I or II, n (%) | 7(22.6%) | 84(30.6%) | 0.3585 |
| III or IV, n (%) | 24(77.4%) | 191(69.4%) | |
| CD4+ count, median cells/ μL (IQR) | 151.5(82.0-224.0) | 115.0(56.5-198.5) | 0.2480 |
| <200 cells/ μL, n (%) | 22(79.3%) | 202(75.4%) | 0.8066 |
| ≥200 cells/ μL, n (%) | 8(26.7%) | 66(24.6%) | |
| Recalls Hepatitis B vaccination | | | |
| Started, not completed, n (%) | 0(0%) | 3(1.1%) | 0.5590 |
| Never got any, n (%) | 32(100%) | 280(98.9%) | |
| Sexual partners | | | |
| 1 partner, n (%) | 20(76.9%) | 194(87.0%) | 0.2266 |
| > 1partner, n (%) | 6(23.1%) | 29(13.0%) | |
| Never used condoms, n (%) | 26(81.2%) | 189(67.5%) | 0.1120 |
| History of STD, n (%) | 11(34.4%) | 121(42.5%) | 0.3800 |
| History of blood transfusion | | | |
| Yes, n (%) | 2(6.2%) | 43(15.1%) | 0.2824 |
| No, n (%) | 30(93.8%) | 241(84.9%) | |
| Tattooing, n (%) | 6(60.0%) | 54(78.3%) | 0.2416 |

*Table 3a: Derangement of liver enzymes*

| | AST /ALT ≥200 (severe) N=7 | AST/ALT<200 (non-severe) n=291 | p-Value |
|---|---|---|---|
| Consumes alcohol | | | |
| Never, n (%) | 3 (42.9%) | 69 (23.7%) | 0.3655 |
| Ever, n (%) | 4 (57.1%) | 222 (76.3%) | |
| Ever taken anti-TB therapy | | | |
| No, n (%) | 5 (71.4%) | 191 (65.6%) | 0.7500 |
| Yes, n (%) | 2 (28.6%) | 100 (34.4%) | |
| CD4+ count, median cells/ μL (IQR) | 60.0(47.0-92.0) | 121.0(61.0-200.0) | 0.0991 |
| <200 cells/ μL, n(%) | 7 (100%) | 211 (74.0%) | 0.1974 |
| ≥200 cells/ μL, n(%) | 0 (0%) | 74 (26.0%) | |
| Hepatitis B | | | |
| HBsAg+, n (%) | 1 (14.3%) | 28 (9.7%) | 0.5192 |
| HbsAg-, n (%) | 6 (85.7%) | 260 (90.3%) | |

*Table 3b: Derangement of liver enzymes*

| | AST /ALT≥40 N=128 | AST/ALT<40 n=170 | p-Value |
|---|---|---|---|
| Consumes alcohol | | | |
| Never, n (%) | 30 (23.4%) | 42 (24.7%) | 0.8004 |
| Ever, n (%) | 98 (76.6%) | 128 (75.3%) | |
| Ever taken anti-TB therapy | | | |
| No, n (%) | 66 (51.6%) | 130 (76.5%) | <0.0001 |
| Yes, n (%) | 62 (48.4%) | 40 (23.5%) | |
| CD4+ count, median cells/ μL (IQR) | 108.0 (56.5-192.0) | 132.5(67.5-204.0) | 0.3277 |
| <200 cells/ μL, n(%) | 98 (79.0%) | 120(71.4%) | 0.1405 |
| ≥200 cells/ μL, n(%) | 26 (21.0%) | 48(28.6%) | |
| Hepatitis B | | | |
| HBsAg+, n (%) | 20 (15.8%) | 9 (5.4%) | 0.0030 |
| HBsAg-, n (%) | 107 (84.2%) | 159 (94.6%) | |
| Ingested Herbals, n (%) | 20 (90.9%) | 47(90.4%) | 0.9442 |

# CHAPTER 6

## 6.0 Discussion

Co-infection of HIV and HBV was 9.9% and HIV and HCV was 1.2%. None of the subjects had all triple infection. Young age at presentation was a predictor of co-infection of HIV and HBV. Mild to moderate liver enzyme elevation (>40 to 199IU/l) was common in co-infected subjects.

## 6.1 HIV and HBV co-infection

That 9.9% of subjects with HIV were positive for HBsAg is not surprising. This is in line with world-wide trends that put Zambia in the high prevalence region for hepatitis B. Sub-Sahara Africa, the region where Zambia lies has a hepatitis B virus prevalence that is greater than 5% (as measured by positive HBsAg) and this is taken as high prevalence.[25,43] Zambia also has high prevalence of HIV with about 16% of the adult population affected.[79] The hepatitis B prevalence rate of 9.9% compares favorably with statistics from the Zambia National Blood Transfusion Service (ZNBTS) which routinely tests for hepatitis B in blood donors and is the largest database in Zambia for this kind of information. The prevalence rate of 9.9% is however, in contrast to the 31.3% prevalence found in an earlier study.[6] The difference here could be attributed to the time frame between the two studies as well as in the choice of patients. The study was done in the era before anti-retroviral drugs became freely and readily available to eligible patients. The easy availability of anti-retroviral drugs which mostly contain lamivudine and tenofovir could have inadvertently led to the treatment of HBV in co-infected patients and thus lowering the HBV disease burden in circulation in the community in whom this current study was done. It should also be noted that the chronicity of HBsAg depends on the immune system as well as the age at which one contracted the virus, information which was not availed in the earlier study. In this study, we did not look at serological factors that could point to the timing of HBV infection though the immunity of the patients was assessed by looking at the immunological (CD4 count) and WHO clinical staging. However, the immunity of the subjects did not significantly affect the possibility of being infected with HBV (p= 0.8066). Assessing the subjects for the presence of antibodies against the HBV would help to categorize patients that tested negative for HBsAg as previously exposed and immune or not previously exposed. The aspect of chronicity of hepatitis infection was not evaluated.

The only factor that was associated with being positive for HBsAg was age younger than forty years (p=0.0105). Previous studies have noted that HBsAg is more common in younger patients.[25] This finding is in line with the likely mode and timing of HBV transmission in the Zambian set-up, which as already noted lies in the high HBV prevalence region of sub-Sahara Africa were HBV is transmitted mainly horizontally in children under five years of age in whom poor immunity leads to failure of clearance of the virus.[25,43]

Knowing the prevalence of HBsAg in our setting where the roll out of the HAART program is in progress cannot be overemphasized.[78] This is because such information can guide policy makers into making HBV testing a routine test for all HIV positive patients prior to initiation of HAART. This is a widely practiced activity in developed countries such as USA and many European countries. Factors in favour of routine testing for HBV in HIV patients include the need to optimally choose drugs that favour the clearance of HIV and HBV as well as avoiding the use of drugs that worsen the hepatotoxicity in HIV and HBV co-infected patients.[8-14, 18, 64] It is well documented that drugs like lamivudine and tenofovir,[18,62,65-66] while effective against HIV, also work against HBV where as drugs like nevirapine or ritonavir, which are widely used as first and second line drugs in Zambia, promote hepatotoxicity in HIV and HBV co-infected patients.[8-14]

### 6.2 HIV and HCV co-infection

The prevalence of HCV in HIV infected subjects in this study was found to be 1.2%. This is a low prevalence and tallies well with the nature in which HIV is contracted in Zambia, which is mainly through heterosexual means. It is also well known that HCV is not efficiently transmitted sexually and the means through which it is efficiently transmitted were not commonly practiced by the study subjects. For instance, intravenous drug abuse was not practiced by any of the subjects. Furthermore, blood transfusion, though a common practice, could not contribute to transmission of HCV as blood in Zambia is carefully screened for the presence of HCV before it can be transfused. Another noteworthy possibility for the low prevalence of HCV in HIV patients is the adherence by health practitioners to the policy of non- reuse of syringes. In Zambia, once a syringe is used on one patient, it is disposed of immediately and safely. This is in contrast to the situation in Egypt where the prevalence of HCV is very high due to the re-use of syringes

that resulted in iatrogenic transmission of HCV during the period of mass treatment of schistosomiasis during the 1920s to 1980s.[80] It was not possible to work out the means through which the four subjects who tested positive for HCV contracted the virus, as was noted in a previous study in Zambia.[1]

### 6.3 Liver enzyme elevation in co-infected patients

Liver enzyme elevation, albeit not severe, was common in the subjects that tested positive for HBsAg. Mild liver enzyme elevation as measured by either ALT or AST was common in HBsAg positive subjects ($p=0.003$). Only one HBsAg positive patient had severe liver enzyme elevation (ALT or AST > 200 U/L). The population of subjects with a positive HCV was small and thus tests of significance were not possible.

That severe liver enzyme elevation was not common is not surprising as the subjects studied had poor immunity with most of them (74.9%) having a CD4 count of less than 200 cells/μl or WHO clinical staging of III or IV. It is well known that poor immunity leads to failure of the body to mount an inflammatory reaction against the hepatitis B or C viruses and this failure of the immune response spares the body from serious hepatotoxicity that would manifest as severe liver enzyme elevation had the immunity been stronger. It is thus not surprising that the liver enzymes were not severely deranged in most of the subjects with HIV and HBV co-infection.

Exposure to anti-tuberculous therapy was also associated with mild to moderate elevation of liver enzymes ($p<0.0001$). Alcohol consumption and use of herbal medication was not associated with liver enzyme elevation and this could be attributed to under-reporting of such practices.

# CHAPTER 7

## 7.0 Conclusion

HIV and hepatitis B viral co-infection is common among patients seeking treatment at the UTH adult ARV clinic while hepatitis C virus is not very common in HIV co-infected patients. Severe liver enzyme elevation, unlike mild to moderate liver enzyme elevation, is not common among HIV/HBV co-infected patients pre-HAART. Due to the high prevalence of HIV and HBV co-infection, it would be worthwhile to routinely test HIV patients who are about to initiate HAART for hepatitis B virus co-infection in order to optimize HAART.

## 7.1 Limitations

Other causes of elevated liver enzymes such as auto-immune disorders, metabolic conditions like haemochromatosis and Wilson's disease were not evaluated as they were beyond the scope of this study. These conditions are also presumably rare in Zambia even though no prevalence study has been conducted for any of those conditions in Zambia.

There was also no way of telling whether the hepatitis B infection was acute or chronic as this would have required the application of further tests. This was not practical for this prevalence study.

## 7.2 Recommendations

A follow up study should be done to look at the long term outcome of HIV/HBV co-infected patients who commence on HAART. This should be with respect to HBV serostatus and hepatotoxicity following commencement of HAART.

## REFERENCES.


1. Oshitani H; Kasolo F; Luo N P; Mpabalwani M; Mizuta K; Numata N; Suzuki H; Numazamki Y **Low prevalence of hepatitis C virus infection in Lusaka, Zambia.** Transactions of the Royal Society of Tropical Medicine and Hygiene 1995; 89(4):380.

2. Kalota V, **Prevalence of Hepatitis C in Blood Donors,** 2005 University of Zambia

3. Alter M J. **Epidemiology of viral hepatitis and HIV co-infection.** J Hepatol 2006; 44: Suppl: S6-S9

4. Oshitani H, Kasolo F, Tembo M, Mpabalwani M, Mizuta K, Luo N, Suzuki H, Numazaki Y. **Hepatitis B virus infection among pregnant women in Zambia.** East Afr Med J, 1995. 72(12):813-5.

5. Oshitani H; Kasolo F C; Mpabalwani M; Mizuta K; Luo N P; Suzuki H; Numazaki Y **Prevalence of hepatitis B antigens in human immunodeficiency virus type 1 seropositive and sero-negative pregnant women in Zambia.** Transactions of the Royal Society of Tropical Medicine and Hygiene 1996;90(3):235-6.

6. Kasolo F C, Sakala I G, Baboo K S. **Hepatitis B virus infection in human immunodeficiency virus seropositive patients at the University Teaching Hospital, Lusaka, Zambia**: interrelationship. Second IAS Conference, Paris, 2003, abstract 963: Antiviral Therapy 2003; 8(Suppl. 1):S456.

7. Becker, S. **Liver Toxicity in Epidemiological Cohorts**. Clin Infect Dis, 2004. 38(Suppl 2):S49-55.

8. Sulkowski M S, Thomas D L, Chaisson R E, Moore R D. **Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection.** JAMA, 2000. 283(1):74-80.

9. Law W P, Duncombe C J, Mahanontharit A, Boyd M A, Kiat Ruxrungtham K, Lange J M, Phanuphak P, Cooper D A, Dore G J. **Impact of viral hepatitis co-infection on response to antiretroviral therapy and HIV disease progression in the HIV-NAT cohort.** AIDS, 2004. 18(8):1169-77.

10. Carr A, Miller J, Law M, Cooper D A. **A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor-related lipodystrophy syndrome.** AIDS 2000. 14(3):F25-32.

11. Martinez E, Blanco J L, Arnaiz J A. **Hepatotoxicity in HIV-1 infected patients receiving nevirapine containing antiretroviral therapy.** AIDS 2001; 15: 1261-8.

12. Aceti A, Pasquazzi C, Zechini B, De Bac C. **THE LIVERHAART Group. Hepatotoxicity development during antiretroviral therapy containing protease inhibitors in patients with HIV: the role of hepatitis B and C virus infection.** J AIDS 2002; 29:41-8.

13. Wit F W, Weverling G J, Weel J, Jurriaans S, Lange J M. **Incidence of and risk factors of severe hepatotoxicity associated with antiretroviral combination therapy.** J Infect Dis 2002; 186: 23-31.

14. Arribas J R, Ibanez C, Ruiz-Antoran B. **Acute hepatitis in HIV-infected patients during ritonavir treatment.** AIDS 1998; 12: 1722-4

15. de Matt M M, ter Heine R, van Gorp E C, Mulder J W, Mairuhu A T, Beijnen J H. **Case series of acute hepatitis in a non-selected group of HIV-infected patients on nevirapine-containing antiretroviral treatment.** AIDS, 2003. 17(15):2209-14.

16. Farrell G C, Liddle C: **Hepatotoxicity in the twenty-first century.** Semin Liver Dis 22: 109, 2002

17. Sulkowski MS, Thomas DL, Mehta SH, Chaisson RE, Moore RD. **Hepatotoxicity associated with nevirapine or efavirenz-containing antiretroviral therapy: role of hepatitis C and B infections.** Hepatology 2002, 35:182-9.

18. Chang T T, Gish R G, de Man R, Gadano A, Sollano J, Chao Y C, Lok A S, Han K H, Goodman Z, Zhu J, Cross A, DeHertogh D, Wilber R, Colonno R, Apelian D, **A Comparison of Entecavir and Lamivudine for HBeAg-positive Chronic Hepatitis B**, N Engl J Med 2006: 354: 1001-10.

19. Ministry of Health (Zambia) September, 2007 **Antiretroviral therapy Protocols.** Pocket Guide

20. Ganem D, Prince A M, **Hepatitis B Virus Infection- Natural History and Clinical Consequences,** N Engl J Med, 2004; 350:1118-29

21. Graham C S, Baden L R, Yu E, **Influence of Human Immunodeficiency Virus Infection on the Course of Hepatitis C Virus Infection: A meta-analysis.** Clin Infect. Dis., 2001: 33:562-569

22. Koziel MJ, Peters MG, **Viral Hepatitis in HIV infection,** N ENGL J MED, 2007

23. Teo EK, Lok SFA. **Epidemiology, transmission and prevention of Hepatitis B virus,** UpToDate, 2008

24. Lee MW, **Hepatitis B virus Infection,** N ENGL J MED 1997: 337: 1733-45

25. Burnett R J, Francois G, Kew M C, Leroux-Roels G, Meheus A, Hoosen A A, Mphahlele M J. **Hepatitis B virus and human immunodeficiency virus co-infection in sub-Saharan Africa: a call for further investigation.** Liver International 2005: 25: 201-213

26. Tabor E, Bayley AC, Cairns J, Pelleu L, Gerety RJ, **Horizontal transmission of hepatitis B virus among children and adults in five rural villages in Zambia.** J Med Virol 1985; 15:113-20.

27. Ayoola E A. **Viral hepatitis in Africa. In: ZUCKERMAN A J, ed. Viral Hepatitis and Liver disease.** New York: Alan R Liss, 1988; 161-9

28. Kew M C. **Progress towards the comprehensive control of hepatitis B in Africa: a view from South Africa.** Gut 1996; 38 (Suppl. 2): S31-6

29. Gill GV, Fimls AE , **Hepatitis B surface antigenaemia: incidence and significance in Zambian blood donors.** 1983; Med J Zambia; 13:52-4.

30. Arends JE, Boucher CAB, Hoepelman AIM, **Hepatitis C Virus and Human Immunodeficiency Virus co-infection: where do we stand?** The Netherlands Journal of Medicine, 2005

31. Lauer GM, Walker BD, **Hepatitis C Virus infection,** New England Journal Of Medicine, 2001: 345(1): 45-52

32. Soriano V, Rodriguez-Rosado R, Garcia-Samaniego J. **Management of Chronic Hepatitis C in HIV infected patients.** AIDS 1999:13(5):539-46

33. Staples CT Jr, Rimland D, Dudas D. **Hepatitis in the HIV (Human immunodeficiency virus) Atlanta V.A (Veterans Affairs Medical Centre) Cohort Study (HAVACS): the effects of co-infection on survival.** Clin Infect Dis 1999:29(1):150-4

34. Waldrep TW, Summers KK, Chiliade PA. **Co-infection with HIV and HCV: more questions than answers?** Pharmacotherapy 2000; 20(12):1499-507.

35. Greub G, Ledergerber B, Battegay M. **Clinical progression, survival and immune recovery during antiretroviral therapy in patients with HIV-1 and Hepatitis C virus co-infection: the Swiss HIV Cohort Study.** Lancet 2000; 356 (9244): 1800-5.

36. Sherman KE, Rouster SD, Chung RT, Rajicic N. **Hepatitis C virus prevalence among patients infected with Human Immunodeficiency Virus: a cross-sectional analysis of US adults AIDS Clinical Trial Group.** Clinical Infectious Disease 2002: 34 (6): 831-7.

37. Sulkowski MS, Moore RD, Mehta SH, Chaissson RE, Thomas DL. **Hepatitis C and progression of HIV disease.** JAMA 2002; 288 (2): 199-206.

38. Ruys T A, Reesink H W, Lange J M. **(Co-infection with Hepatitis C virus and HIV)** Ned Tijdschr Geneeskd 2003; 147 (42):2056-60.

39. Hepatitis B and **Hepatitis C in the EuroSIDA Cohort: Prevalence and Effect on Mortality, AIDS Progression and Response to HAART.** 11th Conference on retroviruses and opportunistic infections: San Francisco, CA: 2004

40. Terrault NA. **Sexual activity as a risk factor for Hepatitis C.** Hepatology 2002: 36 (5 suppl 1): S99-1005.

41. Richter SS, **Laboratory Assays for Diagnosis and Management of Hepatitis C Virus Infection**, Journal of Clinical Microbiology 2002; 40 (12) 4407-12.

42. Hoofnagle J H, Di Bisceglie A M. **Serological diagnosis of acute and chronic hepatitis.** Semin Liver Dis 1991; 11: 73-83.

43. Zuckerman J N, Zuckerman A J. **Viral Hepatitis. In: COOK G C, ZUMLA A, eds. Manson's Tropical Diseases** 2003 Ch 40, 710- 718.

44. Hadler S C, Judson F N, O'Malley P M. **Outcome of hepatitis B virus infection in homosexual men and its relation to prior human immunodeficiency virus infection.** J Infect Dis 1991: 163:454-9.

45. Thio CL. **Hepatitis B in the human immunodeficiency virus-infected patient: epidemiology, natural history, and treatment.** Semin Liver Dis 2003; 23: 125-36

46. Thio C L, Seaberg E C, Skolasky R, **HIV-1, hepatitis B virus, and the risk of liver-related mortality in the Multicenter Cohort Study (MACS).** Lancet 2002; 360: 1921-6.

47. Horvath J, Raffanti S P, **Clinical aspects of the interactions between human immunodeficiency virus and the hepatotropic viruses.** Clin Infect Dis 1994; 18(3): 339-47.

48. Martin- Carbonero L, Benhamou Y, Puoti M. **Incidence and predictors of severe liver fibrosis in HIV infected patients with chronic hepatitis C: a European collaborative study.** Clin Infect Dis 2004; 38 (1): 128-33.

49. Puoti M, Bonacini M, Spinetti A. **Liver fibrosis progression is related to CD 4 cell depletion in patients coinfected with hepatitis C virus and human immunodeficiency virus.** J Infect Dis 2001; 183 (1): 134-7.

50. Benhamou Y, Bochet M, Di M V. **Liver fibrosis progression in human immunodeficiency virus in hepatitis C virus coinfected patients. The Multiviric Group.** Hepatology 1999; 30 (4): 1054-8.

51. Di Martino V, Rufat P, Boyer N. **The influence of human immunodeficiency virus coinfection on chronic hepatitis C in injection drug users: a long term retrospective cohort study.** Hepatology 2001; 34 (6): 1193-9.

52. Garcia- Samaniego J, Rodriguez M, Berenguer J. **Hepatocellullar carcinoma in HIV infected patients with chronic hepatitis C.** Am J Gastroenterol 2001; 96 (1): 179-83

53. Freeman A J, Marinos G, French R A, Lloyd A R. **Immunopathogenesis of hepatitis C virus infection.** Immunol Cell Biol 2001; 79(6): 515-36.

54. Fauci A S, Schnittman S M, Poli G, Koenig S, Pantaleo G. **NIH conference. Immunopathogenic mechanisms in human immunodeficiency virus (HIV) infection.** Ann Intern Med 1991; 114 (8); 678-93.

55. Detels R, Tarwater P, Phair J P, Margolick J, Riddler S A, Munon A. **Effectiveness of potent antiretroviral therapies on the incidence of opportunistic infections before and after AIDS diagnosis.** AIDS 2001; 15(3): 347-55.

56. Pelella F J, Delaney K M, Moorman A C. **Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV outpatient study investigators.** N Engl J Med 1998; 338(13): 853-60.

57. Monga H K, Rodriguez- Barradas M C, Breaux K. **Hepatitis C virus infection related morbidity and mortality among patients with human immunodeficiency virus infection.** Clin Infect Dis 2001; 33(2): 240-7.

58. Bica I, McGovern B, Dhar R. **Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection.** Clin Infect Dis 2001; 32(3): 492-7.

59. Pratt D S, Kaplan M M. **Laboratory tests. In: Schiff E R, Sorrell M F, Maddrey W C, eds. Schiff's diseases of the liver.** 8th ed. Vol.1. Philadelphia: Lippincott-Raven, 1999: 205-44.

60. Goldberg D M, Martin J V. **Role of gamma-glutamyl transpeptidase activity in the diagnosis of hepatobiliary disease.** Digestion 1975; 12: 232-46.

61. AIDS Clinical Trials Group Criteria, Division of AIDS. Table for grading severity of adult adverse experiences, August 1992.

62. Dore G J, Coopr D A, Barrett C, Goh L E, Thakrar B, Atkins M. **Dual efficacy of lamivudine treatment in HIV/hepatitis B virus coinfected persons in randomized, controlled study (CAESAR). The CAESAR Coordinating Committee.** J Infect Dis 1999; 30: 1320-6.

63. John M, Flexman J, French M A. **Hepatitis C virus-associated hepatitis following treatment of HIV-infected patients with HIV protease inhibitors: an immune restoration disease?** AIDS 1998; 12: 2289-93.

64. Lok A S, McMahon B J. **Chronic hepatitis B: update of recommendations.** Hepatology 2004; 39: 857-61.

65. Nelson M, Portsmouth J, Stebbing J. **An open label study of tenofovir in HIV-1 and hepatitis B virus coinfected individuals.** AIDS 2003; 17: F7-F10.

66. Ristig M B, Crippin J, Aberg J A. **Tenofovir disoproxil fumarate therapy for chronic hepatitis B in human immunodeficiency virus/hepatitis B virus coinfected individuals for who interferon-alpha and lamivudine therapy have failed.** J Infect Dis 2002; 186: 1844-77.

67. Marcellin P, Chang T-T, Lim S G. **Adefovir dipivoxil for the treatment of hepatitis Be antigen-positive chronic hepatitis B.** N Engl J Med 2003; 348: 808-16.

68. Lai C L, Gane E, Liaw Y F. **Telbivudine versus lamivudine in patients with chronic hepatitis B.** N Engl J Med 2007; 357:2576-88

69. Melegari M, Scaglioni P P, Wands J R. **Hepatitis B virus mutants associated with lamivudine and famciclovir administration are replicative defective.** Hepatology 1998; 27: 628-33.

70. Liaw Y F, Cheung N W, Chang T T, **Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B.** Gastroenterology 2000; 119: 172-80.

71. Hookoop P, de Man R A, Niesters H G, Zondervan P E, Schalm S W. **Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy.** Hepatology 2000; 32: 635-9.

72. Perrillo R, Schiff E, Yoshida E. **Adefovir dipivoxil for the treatment of lamivudine resistant hepatitis B mutants.** Hepatology 2000; 32: 129-34.

73. Ying C, De Clercq, Nicholson W, Furman P, Neyts J. **Inhibition of replication of the DNA polymerase M550V mutation variant of human hepatitis B by adefovir, tenofovir, L-FMAU, DAPD, penciclovir and lobucavir.** J Viral Hepatitis 2000; 7: 161-5.

74. Torrian F J, Rodriguez-Torres M, Rockstroh J K. **Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV infected patients.** N Engl J Med 2004; 351 (5): 451-9.

75. Carrat F, Bani- Sadr F, Pol S. **Pegylated interferon alfa-2b vs. standard interferon alfa-2b, plus ribavirin, for chronic hepatitis C in HIV infected patients: a randomized controlled trial.** JAMA 2004; 292 (23): 2839-48.

76. Chung R T, Andersen J, Volberding P. **Pegylated interferon alfa-2a plus ribavirin vs. interferon alfa-2a plus ribavirin, for chronic hepatitis C in HIV coinfected persons.** N Engl J Med 2004: 351 (5): 451-9.

77. Fleischer R, Boxwell D, Sherman K E. **Nucleoside analogues and mitochondrial toxicity.** Clin Infect Dis 2004; 38(8): e79-80.

78. Central Statistical Office (Zambia), Central Board of Health (Zambia) and ORC Macro 2003. Zambia Demographic and Health Survey 2001-2002. Calverton, Maryland, USA; Central Statistical Office, Central Board of Health, and ORC Macro.

79. Stringer JSA, Zulu I, Levy J, Stringer EM, Mwango A, Chi BH, Mtonga V, Reid S, Cantrell RA, Bulterys M, Saag MS, Marlink RG, Mwinga A, Ellerbrock TV, Sinkala M. **Rapid Scale-up of Antiretroviral Therapy at Primary Care Sites in Zambia-Feasibility and Early Outcomes.** JAMA 2006; 296:782-793

80. Pybus O G, Drummond A J, Nakano T, Robertson B H, Rambaut A. **The epidemiology and iatrogenic Transmission of Hepatitis C Virus in Egypt: A Bayesian Coalescent Approach.** Mol. Biol. Evol. 20(3): 381-387.2003

# APPENDIX

## APPENDIX 1.

**Patient Information Sheet/ Consent Form**

**Title: Hepatitis B and C viral co-infection in HIV infected patients enrolled in the antiretroviral treatment program at the University Teaching Hospital in Lusaka, Zambia.**

**Investigator: Dr. Kenneth Kapembwa**

**Sponsor: The University Teaching Hospital, Lusaka, Zambia**
**The Centre for Infectious Disease Research in Zambia (CIRDZ)**
**The University of Alabama at Birmingham (UAB), United States**

**Introduction:**

You are being asked to take part in a research study to find out how often the viruses which cause hepatitis (hepatitis B and C viruses) and HIV (human immunodeficiency virus) occur in the same person.

This form gives you information about the study. We want you to know the purpose of the tests, the possible risks and benefits, and what will be expected of you if you decide to join. You may ask questions about this study at any time. If you decide to take part in this study after it has been fully explained to you, we will ask you to sign this consent form or make your thumbprint in front of a witness. You may keep a copy of the consent form if you like.

Please note that:

1. Your participation in this research is entirely voluntary.
2. You may decide not to take part in the study, or to withdraw from the study at any time without losing the benefits of your standard care.

**Initials/Thumbprint:** _____

**Purpose of the study:**

This study is being conducted to determine how frequently Hepatitis B and Hepatitis C viruses and HIV occur together in the same patient. Hepatitis B and Hepatitis C viruses affect the liver and can cause liver damage. You have been picked for this study because you are HIV positive.

Hepatitis B and Hepatitis C viruses have similar modes of transmission with HIV, namely through unprotected sexual intercourse with an infected partner, contact with contaminated blood and blood products and mother to child transmission.

The purpose of this study is to find out how commonly Hepatitis B and Hepatitis C viruses occur in people with HIV in Zambia. This information is currently not available. It is important to know the rates of co-infection with HIV and Hepatitis B and Hepatitis C viruses as these diseases may interact negatively and worsen the patient's clinical outcome. Knowing the co- infection rates may also help in the choice of drugs to optimize the treatment of the infections.

**Study procedures:**

This study is taking place at the University Teaching Hospital's Adult Antiretroviral Therapy (ART) Clinic, the medical clinic where many HIV positive patients receive care. All study participants will receive the same treatment for HIV whether or not they participate in the study. Blood will be drawn (4ml) to see if Hepatitis B and Hepatitis C viruses are in the blood. If you have not previously had blood drawn for liver function tests, an additional 4ml will be drawn for these tests. A questionnaire will be administered with questions aimed at determining if you have risk factors for contracting these hepatitis viruses. The questionnaire will also try to determine other risk factors for liver damage other than those due to hepatitis viral infections.

This study is cross-sectional, meaning that you will meet with the researchers at least twice, at initial contact and to get your results. However, you are free to contact the person mentioned below at any time. You will not be required to come to any additional appointments for this research study. The laboratory tests results will be made available to you and your treating physicians.

**Initials/Thumbprint:** _____

If you are found to have any of the viruses in your blood, your physicians will be informed (after obtaining your permission) in order to optimize your treatment schedule. In case the laboratory tests reveal that your liver is damaged, your physician will be informed in order to adjust your medication.

**Possible Risks:**

There is minimal risk of injury, infection or discomfort for participating in this study. The only risk is the drawing of extra 4ml blood on top of the usual safety labs that are collected during your routine assessment clinical visits. In addition, an extra 4ml blood will be drawn for liver function tests if you have not previously done the liver function tests. The testing for Hepatitis B and C is actually recommended in most international ARV guidelines while liver function tests are standard tests before one commences on antiretroviral therapy.

**Potential Benefits:**

You and your attending physician will know your hepatitis B and C status so that your level of care can be improved i.e. optimum choice of antiretroviral drugs that will target both HIV and Hepatitis B viruses. In addition knowledge gained from this study may in the future help other Zambians who suffer from dual HIV infection and hepatitis B and C co-infection.

**Alternatives to this study:**

You may choose not to participate in this study. If you choose not to participate you will still receive the same level care for your HIV disease at UTH ART Clinic without any prejudice.

**Costs to You:**

There will be no cost to you for any of the tests that you have as part of the study.

**Confidentiality:**

Your research records will be confidential to the extent permitted by law. You will be identified by a code, and personal information from your records will not be released without your written permission including disclosure of your results to your attending physician. You will not be personally identified in any publication about this study.

**Initials/Thumbprint:** _____

However, your records may be reviewed, by the Centre for Infectious Disease Research in Zambia (CIDRZ), the Zambian Research Ethics Committee (REC), the University of Alabama at Birmingham (UAB) Institutional Review Board (IRB), Vanderbilt Institutional Review Board (IRB), National Institutes of Health (NIH), and study staff.

**Persons to contact for problems or questions:**

If you have any problems or questions about the study there are people you can contact. The study counsellors at the clinic and other staff can help you contact the right person to answer any questions you have.

For study questions, contact:
Dr. Kenneth Kapembwa
Department of Medicine
University Teaching Hospital
Private Bag RWIX, Lusaka, Zambia
*Mobile: 095-5-209015*

For questions about your rights as a research subject:
Dr. James Munthali
Secretary, Research Ethics Committee
Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia
*Tel: 01- 256067/ Mobile: 096-6-765-422*

**Legal rights:**

You are not giving up any of your legal rights by signing this informed consent document.

**Initials/Thumbprint:** _____

**Statement of consent:**

*You will receive a copy of this signed informed consent.*

If you have read the informed consent or had it read and explained to you and you voluntarily agree to join this study, please sign your name or make your mark below. If there is any part of this form that is unclear to you, be sure to ask questions about it. Do not sign until you get answers to all your questions. By signing this consent form you agree to join the study.

I agree to join this study.

| _____ | _____ | _____ |
|---|---|---|
| Participant's Name (print) | Participant's Signature/thumbprint | Date |

Participant has stated that he/she is:

____ Literate and can sign her name

____ Illiterate and cannot sign her name

I have observed the participant sign or make his /her mark above and I believe he/she has understood and knowingly gives consent for participation.

| _____ | _____ | _____ |
|---|---|---|
| Witness' Name (print) | Witness' Signature | Date |

I have explained the purpose of this study to the participant. He/She had the form read to him/her, was given the chance to ask questions, accepted the answers, and signed to enroll in the study.

| _____ | _____ | _____ |
|---|---|---|
| Study Staff's Name (print) | Study Staff's Signature | Date |

Note: This consent form with original signatures must be retained on file by the principal investigator. A copy must be offered to the volunteer.

## APPENDIX 2.

**Icipepa ca chakusuminisha**

**UMUTWE: Ubulwele Bwa Hepatitis B na C Mubulwele Bwa HIV Pabaingila Mu Anti-Retroviral Treatment Programu Pa University Teaching Hospital , Mu Lusaka, Zambia.**

**Abalefwailisha: Dr. Kenneth Kapembwa.**

**Ifyakubala**

Mwaipushiwa ukuti mwinga senda ulubali muli uku ukusoma ukwakufwaya ukwishiba ifyo fyaseka ukusanga utushishi utonaula ilibu (Hepatitis B na C) kabili na kashishi ka HIV (Human Immunodeficiency Virus) mumuntu umo wine.

Ici icipepa cilepela amashiwi pali uku kusambilila. Tulefwaya ukuti mwishibe ichinto ya aya mapimo, ama sanso nefisuma, nefyo tulechetekela kuli imwe nga mwaingila muli aka akabungwe. Kuti mwaipusha amepusho yoonse eyo mukwete inshita ili yoonse. Nga mwasala ukwingila muli aka akabungwe ukokonka ukulondolola kwesu, tukamipusha uku saina ici icipepa nangula ukufwatika ichikumo chenu pali ici icipepa chakusuminisha patanshi yaba kambone. Ngamulefwaya, kuti mwasunga cimo icipepa chakusuminisha icho mwa saina.

**Twapapata mwishibe ukutila:**

1. Ukusenda lubali muli uku kufwailisha chilikuli imwe mwebene.
2. Kuti mwasala ukana ukubamo muli uku kusambilila, nangula kuti mwafumamo inshita ili yoonse ukwaabula ukupanya ifisuma fyakumisunga.

**Nchinto Yesambililo.**

Uku kufwailisha kulecitiwa pakuti chishishibwe ati nimunshita shinga ilyo utu tushishi twa Hepatits B na Hepatitis C na HIV tusangwa pamo mumuntu umo. Utushishi twa Hepatitis B na Hepatitis C tuleekata ilibu elyo kuti twa lyonaula. Mwasalwa mu kufwailisha uku pantu namukwata akashishi ka HIV.

**Participant's initials/ Thumbprint**_____

Utushishi twa Hepatitis B na C twambukila fimo fine nefyambukila HIV, ukupitila muli bukalalelale ukwabula ukuyicingilila nomuntu wakwata aka akashishi, ukukumya umulopa nefipangwa kumulopa wakowela, no kufuma kuli banyina kuya kumwana.

Inchinto yaili sambililo kwishiba ifyo fyaseka ukukwata Hepatitis B na Hepatitis C mubantu abali na HIV nomba tabalayamba ukunwa ama ARV mu Zambia. Ubu bwishibisho pali ino nshita tapaba. Chikankala ukwishiba ukupela kwamalwele aya HIV pamo naya Hepatitis B na Hepatitis C pantu kuti ayamalwele yayangalila pamo ububi nokulwalikishamo sana umulwele. Ayameshibisho kuti yayafwilisha mukusala umuti uwakundapa ayamalwele bwino.

**Ukuchitilwa kwe Sambililo.**

Uku kusambilila kulechitikila pa University Teaching Hospital ku Adult Antiretroviral Therapy (ART) Clinic kulya ukundapilwa abalwele ba HIV. Nga mwasenda lubali nagula tamusendele lubali, mukalaundapwa cimo cine. Umulopa ukafumishiwa (4mls) pakwishiba nga akashishi ka Hepatitis B na C kali mumulopa. Ngatamwasendewa umulopa wakipima ukubomba kwelibu, naimbi 4mls ikasendwa pakuti yakapimwe. Tukamipela ne cipepa chamepusho ichikepusha nga mwalikwata ifigalenga ukuti mwambule utushishi twa hepatitis. Aya amepusho yakesha ukufwaya nafimbi ifingalenga ati ilibu lyonaike nga twafumyako utushishi twa hepatitis.

Mukakumanya abalechita ayamasambilo imiku yibili, pakutampa elo na pakusenda ifyo basanga. Lelo kuti mwamona uyu muntu weshina lilembelwe panshi pepepala ili inshita ili yoonse. Tamwakafwaikwe ukwisa mukutumona nakabili kumulandu wa ili sambililo. Ifyo tukasanga panuma yakumipima, tukamyeeba, kabili tukeeba na ba shing'anga benu. Nga twasanga utuli tonse utushishi mumulopa wenu, ba shing'anga benu bakebwa (nga twapoka insambu kuli imwe) pakuti baka chinje ukudapwa kwenu. Nga twasanga ukutila ilibu lyenu lyalyonaika, ba shing'anga benu bakebwa pakuti bakachinje umuti.

**Ifibi Fingasangwa.**

Ukupitila naku kuyicena, kukwata akashishi kali koonse, nokukana umfwa bwino kunono saana kumulandu wekweba ati namusenda lubali mulyuku kufwailisha. Lelo icibi chingasanwampo chakusenda naumbi 4mls umulopa uucilile po pali ulya basenda lyoonse nga mwaya ku clinic mukupimwa nga lyoonse. Ukulundapo, naumbi umulopa 4mls ukasendewa nga tamwapimapo imibombele ye libu lyenu.

**Participant's initials/ Thumbprint**_____

Uku kupima kwa Hepatitis B na C kwalisuminishiwa mufipope fya ARV muchende sha fyalo fimo, elyo ukupima imibombele lye libu fintu bachita lyoonse ilyo tabalayamba umuntu pama ARVs.

**Ifya Kunonkamo.**

Imwe naba shing'anga benu mukeshiba nga namukwata ubulwele bwa Hepatitis B na C pakuti ukumimona kuka wamepo pantu cikalenga ukusala kwamuti wama-antiretroviral yakucefya akashisi ka HIV nakashishi ka Hepatitis B panshita imo. Ukulundapo, ici chishibo kuntanshi cikafwilisha ama Zambians bambi abakwata yonse amalwele ya HIV na Hepatitis B na C panshinta imo.

**Ifyakusala.**

Kuti mwasala ukusenda lubali nangula iyo. Ngamwakana ukusenda lubali, mukatwalilila ukundapwa kubulwele bwa HIV kulya ku ART Clinic kwaabula kutitikishiwa.

**Ifyakulipila.**

Tamwakakwate amalipilo nelyo yamo ku mapimo eyo mukakwata pakusenda lubali muli uku kufwailisha.

**Ifya Nkama.**

Fyoonse fikalembwa pali imwe, tafyakasokolokwe ku uli oonse, ukulinga nefyo ifunde lyasuminishya. Tukamishibila kwi nambala, na fyoonse ifya pabumi bwenu ifikafuma recordi lyenu tatwakasekeshe ku uli onse ukubikapo naba shing'anga benu ukwabula insambu iyo mwalemba. Ishina lyenu talyakabikwe palwaalala muli uku kufwailisha. Lelo, ama recordi yenu kuti yamonwa kuliba Centre for Infectious Disease Research in Zambia (CIDRZ), the Zambian Research Ethics Committee (REC), the UAB Institutional Review Board (IRB), Vanderbilt Institutional Review Board (IRB), National Institutes of Health (NIH), na balebomba mukufwailisha uku.

**Participant's initials/ Thumbprint**_____

**Abakwipusha Nga Mwakwata Amafya Nangula Mepusho.**

Nga namukwata ubwafya nangula amepusho pali uku kufwailisha, kuli abantu mwinga mona. Ama counsellor muli uku kufwailisha ku clinic nababomfyi bambi ukuti bamitungulule kubantu abengaasuka amepusho yenu bwino bwino.

Pali uku kufwailisha/kusambilila moneni ba:

Dr. Kenneth Kapembwa

Dept of Medicine

University Teaching Hospital

P/B RWIX

Lusaka

Mobile: 0955209015

Nga mulefwaya ukwishiba insambu shenu muli uku ku fwailisha, moneni ba:

Dr. James Munthali

Secretary, Research Ethics Committee

Ridgeway Campus

P.O Box 50110

Lusaka,

Zambia

Tel: 01-252-641; Mobile: 096-6-765-422

**Insambu shenu.**

Tamuleshitisha insambu shenu nga mwasaina ici cipepa.

**Participant's initials/ Thumbprint**_____

## UKUSUMINISHA KWENU

Tukamupeela ici cipepa cakusuminisha.

Nga mwabelenga ici cipepa cakusuminisha, kabili nga mwasumina nokusala ukusenda lubali muli uku kusambilila lembeni ishina lyenu nangula fwatikeni icikumo apa panshi. Nga namukwata amepusho pali ici cipepa ipusheni ilyo tamulasaina pantu nga mwasaina ekweba ati namusumina ukusenda lubali muli uku kufwailisha.

Nasumina ukusenda ulubali muli uku kufwailisha.

| _____ | _____ | _____ |
|---|---|---|
| Ishina lya uyo ulesenda lubali | Ishina/umunwe wa uyo ulesenda lubali | Ubushiku |

Ulesenda lubali alanda atila:

_____ Alisambilila kuti alemba

_____ Tasambilila teti alembe

Ninebo kamboni kuli uyu muntu kabili nimona ilyo acila lemba ishina lyakwe kabili ndecetekela ukutila naumfwa nokusumina asumina ukusenda ulubali.

| _____ | _____ | _____ |
|---|---|---|
| Ishina lya kamboni | Ishina/umunwe wa kwa kamboni | Ubushiku |

Nalondolola eco tulecitila ili sambililo kuli uyu muntu. Icipepa nacibelgwa kuli yena, napelwa ne nshita yakwipusha amepusho, naasumina kumasuko eyo twamwasuka no kusaina asaina ici cipepa cakusenda lubali muli uku kufwailisha.

| _____ | _____ | _____ |
|---|---|---|
| Ishina lya kasambilila | Ishina/umunwe wakwa kasambilila | Ubushiku |

Lubeni ukutila ici cipepa eco mwasaina, cifwile ukushaala kuli ba kafwailisha nokubaula kelo cimo cikapelwa kuli kasambilila.

## APPENDIX 3.

**Pepala Lovomelezana.**

**Mutu Wake:** Tulombo Twa Hepatitis B Ndi Hepatitis C Mu Matenda A HIV Mu Anthu Amene Athengako Mbali Ya Antiretroviral Treatment Programu Pa University Teaching Hospital Ku Lusaka, Zambia.

**Ofuza**: Dr. Kenneth Kapembwa.

**Chiziwiso.**

Muli opemphedwa kutengako mbali pamaphunzilo yo fufuza kuti nicha pafupi pafupi pomwe tulombo twamene tumapangisa hepatitis (hepatitis B ndi C) tungapezeke ndi kalombo ka HIV (Human Immunodeficiency virus) mu munthu umodzi. Iyi pepala ikupasani nkani pamaphuzilo aya.

Tifuna kuti muziwe nchito yake yamapimidwe aya, choipa ndi chabwino, kapena kukoma kwake ndi chamene chizafunidwa kwa inu mukavomela kutengako mbali mumaphunzilo awa. Mungafunse mafunso pakufufuza uku panthawi ili yonse. Ngati mwafuna kuthengako mbali mukufufuza uku pamene akulogoloselani tuzakufunsani kusaina pepala iyi kapena kufwatika chikumo chanu pamanso pa mboni. Mungasungenso china chi pepala chovomeleza ngati mufuna.

**Pepani: Ziwani Kuti.**

1. Mulinasankho muchigwilidzano cha kufufudza uku.
2. Mungasakhe kusatengako mbali kukufufuzaku kapena kuleka kutengako mbali pamaphunzilo aya pa nthawi ili onse kupanda kutaya chabwino chilichonse cha kasungidwe.

**Nchito Yake Yakufufuza**

Uku kufufuza kuli pakufuna kuziwa kuti ndi mwapafupi pafupi otani mwamene tulombo twa Hepatitis B ndi C na HIV tumapezeka mu munthu wodwala pa nthawi imozi.

Aya matenda ya tulombo twa Hepatitis B ndi C tunzakunze muchibindi kapena kuti mu ndiponso tukonza ku ononga chibindi. Mwasakhidwa inu mu maphunzilo awa chifukwa munapezeka na kalombo ka HIV. Tulombo twa Hepatitis B ndi C tumatengedwa chimozi mozi monga kalombo ka HIV.

**Participant's initials/ Thumbprint**_____

Uku ndi kugonana ndi amene ali ndi magazi yamatenda awa, kapena kugwila magazi alina tulombo kapena zisulozi zilina magazi alina tulombo, na kuchokela kwa mai wache kupitila kwa mawana.

Tifuna kuziwa kuti ndi anthu angati amene ali ndi matenda otele muno mu Zambia, chifukwa pa nthawi ino tilibe umboni weni weni. Ndi chinthu chachikulu kudziwa kangati matenda ya HIV ndi Hepatitis B ndi Hepatitis C viruses ya ma dwazika banthu chifukwa aya matenda siyamagwelizana bwino ndiponso odwala matenda yama pitilila patsongolo. Kudziba kafafa nizidwe kamatenda aya chimathandiza kuziba makwala otandidza kupaya matende aya.

**Munandanda Wamapuzitsidwe.**

Maphunzilo awa aku chitikila ku University Teaching Hospital ku Adult Antiretroviral Therapy (ART) Clinic kumene ambili odwala matenda a HIV amapita. Ngati mwavomela kutengako mbali kapena simunavomele muzalandila thandizo chimodzimodzi monga mwamene mumalandilila nthawi zontse. Magazi (4mls) adzatengedwa kuti tidziwe ngati kalombo ka Hepatitis B, kapena C kalimo mugadzi. Ngati mukalibe kupimiwa mwamene chibindi chisebenzela, tuzatenge nayambili magazi (4ml) kuti tumupimani tizibe momene chisebenzala.

Buku lofufuza lidzapelekedwa kwa inu lomwe cholinga chake ndikufuna kupeza ngati muli ndi zizindikilo zoonetsa kuti mungatenge tizilombo tomwe tubweletsa matenda ya Hepatitis B ndi Hepatitis C. Bukuli loofufuza lidzayesetsa kupeza zina mwa zizinkilo zosonyeza kuti mphafa zanu ndizoonongeka ndipo apa ndipambuyo pogwilitsa nchito zizindikilo za tizilombo tuja tomwe tubweletsa matenda ya hepatitis.

Muzaonana ndi ofunsa mafuso kawili, pomufusani mafuso ndi potenga ma results. Koma ndimwe omasuka kufusa omwe alebedwa pansi pachipepa itawi ili oonse. Sitiza kufunsani kubwelanso kuli aya maphuzilo, koma tizakuuzani zone zimene tizapeza pambuyo yokupimani nakuti tizauza a dotholo amene anali kukuonani ngati (mwativomeleza) kuti aone mwamene zilili, nakuti achinje makwala ngati ndi lotheka pambuyo yopimidwa.

**Zoipa Zingapezeke.**

Palibe zodetsa nkhawa kweni-kweni zomwe zingapweteke munthu aliyense monga upitila munjila yakuti mungatenge matenda pakutengako mbali mkufufuza kwathu pamatenda yatchulidwa pamwambapa.

**Participant's initials/ thumbprint**_____

Chomwe chingakhale chopweteka kapena kuti chobvuta nchakuti mudzapemphedwa kupeleka mwazi ofika pa muyeso wa 4mls apa nkuti mudzakhala mutapeleka kale mwazi wina omwe uja mumapeleka monga mwanthawi zonse ngati mwapemphendwa kupimitsa mwazi wanu. Moonjezelapo mwazi wina okwanila pa muyeso wa 4mls udzakhetsendwanso ngati mukalibe upimitsapo mphafa zanu, kuti zipimidwe tsopano ndikudziwa ngati zigwila bwino nchito yace. Uku kupima kwa hepatitis B ndi C kuma vomelezewa ndi muvolemba pa ARV chalo chonse.

**Pindu Yake.**

Pambuyo yopimidwa magazi imwe ndi a dotholo anu muzaziwa ngati muli nako kalombo ka hepatitis B ndi C nakuti chizawama chifukwa chizapangisa a dotholo anu kukuyambisani makwala wochepesako kalombo ka Hepatitis B ndi ka HIV pa thawi imozi.

**Zosakhamo Kuli Aya Maphunzilo.**

Ngati mwakana kutengako mabali ku fufuzaku mungapitilize kulandila tandizo matenda a HIV kuja ku UTH ART Clinic kulibe kupatulula.

**Zolipila.**

Simuza lipila kanthu kali konse mukapimidwa mumaphuzhilo awa.

**Za Chisinsi.**

Zonse zimene tizalemba pali imwe zizakhala chisinsi.

Muzaziwika ndi nambala imene muzapasidwa. Zonse zizapezeka pali imwe, sitizauza munthu aliyense kufakilapo a dotholo anu koma kuli ololedwa naimwe. Koma zalembedwa pali imwe zinga onedwa ndi Centre for Infectious Diseases Research in Zambia (CIDRZ), ndi Zambian Research Ethics Committee (REC), ndi University of Alabama (UAB) Institutional Review Board (IRB), Vanderbilt Institutional Review Board (IRB), National Institute of Health (NIH), na anchito kuli aya maphunzilo.

**Anthu Ofunsa.**

Ngati muli ndi mafunso kapena mavuto pa kufufuza uku kapena pa kuphunzila, funsani anchito a ku clinic kuti akulangize anthu amene munga funse.

**Participant's initials/ Thumbprint**_____

**Pakuphuzila/Kufufuza**

1. Dr. Kenneth Kapembwa
   Department of Medicine (UTH)
   P/B RW 1X
   LUSAKA
   Mobile: 095-520-9015

2. Pa Mafunso Ya Umunthu

   Dr. James Munthali
   Secretary, Research Ethics Committee
   Ridgeway Campus
   P.O. Box 50110
   LUSAKA
   Tel: 01-256067/096-6-765-422

**Umunthu Wanu.**

Simugulisa umunthu wanu pamene mu saina pepala iyi

**Participant's initials/ Thumbprint**_____

**KUVOMELEZA KWANU**

Tizakupasani yosainiwa pepala lovomelezana. Ngati mwawelenga pepala iyi kapena aku masulilani, nakuti mwavomeleza kutengako mbali mukufufuza uku lembani zina lanu apa pansi. Ngati muli ndi mafunso funsani pamene mukalibe ku saina, chifukwa ngati mwa saina ndiye kuti mwavomela kutengako mbali.

Navomela Kutenga Mbali Mukufufuza Uku.

| _____ | _____ | _____ |
|---|---|---|
| Zina ya wotengakombali | Chikumo/kusaina cha Wotengako mbali | Siku lo saina |

Wotengako mbali akamba kuti:

____ Ndi wophunzila angalembe

____ Ndi wosaphunzila sanga lembe

Ndine mboni kuli uyu munthu pakusaina pepala iyi nakuti wakhutila zonse zolembedwa nakuti afunisisa kutengako mbali.

| _____ | _____ | _____ |
|---|---|---|
| Zina ya mboni | Chikumo cha mboni | Siku lo saina |

Natokoza zonse zimene tichita muli ili phunzilo kuli uyu muthu na pepala labelengedwa, nakuti akala na mpata yakufunsa mafunso nakuti wakhutila nayo mayanko nakusaina kutengako mbali muku fufuza uku.

| _____ | _____ | _____ |
|---|---|---|
| Zina ya wophuzila | Zina/chikumo cha wophuzila | Siku lo saina |

Ziwani kuti iyi pepala mwa saina imozi iyenela kukhala ndi wophuzisa kapena ina kwa wotengako mbali.

## APPENDIX 4.

**Questionnaire**

**Hepatitis B and C viral co-infection in HIV infected patients enrolled in the Zambian Antiretroviral therapy program at the University Teaching Hospital in Lusaka.**

Investigator: Dr. K. Kapembwa.

Patient Id. Number: _____

Administered by: _____

Date: ____/_____/_____

1. Age: _____years.
2. Sex:
   a= Male,
   b=Female
3. Highest Educational Status:
   a = Tertiary level (college/ university)
   b = Secondary level
   c = Primary level
   d = No formal education
4. Marital status:
   a = never married before
   b =married
   c = divorced
   d = widowed
   e = separated from spouse
5. Monthly Income (personal):
   a= <K150, 000.00
   b= K150, 000.00 to K300, 000.00
   c= K301, 000.00 to K500, 000.00
   d =K500, 000.00+

6. Residential Area: _____

7. Number of sexual partners in the last twelve months?

a = nil

b = 1

c = 2

d = >2

e= declines to answer.

8. Do you or your partner use a condom?

a = Yes

b = No

c = Declines to answer.

9. If answer is "Yes" to previous question, how often do you use the condom?

a = Always

b = frequently (>50%)

c = occasionally (=<50%)

10. Has there ever been any condom breakage during a sexual act?

a= Yes

b= No

c= cannot recall.

11. Have you ever been diagnosed with a sexually transmitted infection?

a= Yes

b= No

c= Declines to answer

12. If answer to '11' above is yes, was the infection associated with:

**i) = Genital sores**

a = Yes

b = No

c= cannot recall

**ii) = Genital discharge**

a = Yes

b = No

c= cannot recall

**iii) = Inguinal growth(s)**

a = Yes

b = No

c= cannot recall

13. Any history of blood transfusion?

a = Yes

b = No

c= cannot recall

14. When was the latest blood transfusion?

a= within the last three months

b= within the last six months

c= within the last twelve months

d= more than twelve months ago.

15. Any history of consulting a traditional healer?

a =Yes

b= No

c= declines to answer

16. If answer is "Yes" to '15' above, did you ingest any herbal medication prescribed from that consultation?

a =Yes

b=No

c = cannot recall

d = none were prescribed

17. If answer is 'Yes' to '15' above, any history of tattooing at that visit?

a = Yes

b = No

c= declines to answer

18. If answer to '17' is 'Yes" was an unused razor blade used on you?

a= Yes

b= No

c= cannot recall.

19. Have you ever shared intravenous needles?

a =Yes

b =No

c= Declines to answer

20. If answer to '19' is 'Yes', how often did you share the needles?

a= rarely

b= occasionally

c=frequently

d=always

e=declines to answer

21. If answer to questions '19' is 'Yes' when was the latest use?

a= within the last three months

b= within the last six months

c= within the last twelve months

d= more than twelve months ago.

22. Do you take Alcohol?

a = Yes.

b = Never.

c = Stopped drinking.

23. Have you ever taken Anti- TB therapy?

a = No

b = Yes

24. Have you ever suffered from yellowing of eyes that was attended to by medical personnel before?

a = Yes

b = No

c = Cannot recall.

25. Have you ever been diagnosed with the following conditions before?

**a= Hepatitis B**

i) Yes

ii) No

**b= Hepatitis C**

i) Yes

ii) No

**c= Hepatitis A**

i) Yes

ii) No

**d= cannot recall.**

26. Has any member of your household ever suffered from yellowing of eyes that was attended to by the medical personnel before?

a = Yes

b = No

c= Declines to answer

27. Has any member of your household been diagnosed with the following conditions...

**a= Hepatitis B**

i) Yes

ii) No

**b= Hepatitis C**

i) Yes

ii) No

**c= Hepatitis A**

i) Yes

ii) No

**d= cannot recall.**

28. Have you ever received immunizations (a series of three injections) for Hepatitis B?

a = Finished series.

b = Started but did not complete series.

c = Never got any.

d = Cannot recall.



Thank you for your time.

## APPENDIX 5.

**Testing Algorithm For Transfusion Transmitted Infections (TTI's)**

1. Changes compared to previous version

   N/A

2. **Area of Application**

   This SOP will be applied by Medical Laboratory Staff with specific training and competence in performing the task in the Laboratory

3. **Aim of this process description**

   The aim of this process is to describe the testing algorithm

   For Transfusion Transmitted Infections (TTI's) carried out on donor blood samples.

4. **Definitions and acronyms**

   TTI --- Transfusion Transmitted Infections

5. **Introduction**

   The mandatory Laboratory tests carried out on donated units of blood and donor samples are Antigen/Antibody test for HIV 1&2, HBsAg, HCV antibody test and RPR for Syphilis.

   Repeat testing in duplicate of initially reactive samples is necessary in order to rule out false positive results that may have been encountered in the initial test. Conclusion of sero-status of initially reactive sample is arrived at only after repeat testing in duplicate.

6. **Materials**

6.1 Initially reactive samples for HIV Ag/Abs, HBsAg, anti- HCV and RPR

6.2 Refer to Specific SOPs for HIV Ag/Abs, HBsAg, anti- HCV and RPR

6.3 Daily Log forms, Reactivity forms, Release and Discard forms

7. **Procedure**

7.1 Compile unit numbers which are initially reactive

7.2 Units of Blood are kept in quarantine (special quarantine refrigerator) awaiting testing to be concluded.

7.3 Retest all initially reactive (positive) samples in duplicate.

7.4 Fill data in the "daily Log form", "reactivity form" and "Initial and repeat reactivity form"

7.5 Enter "end" results (conclusion) onto" Release / Discard form"

7.6 Label Units of blood for use according to SOP.

7.7 Put repeatedly reactive units in a biohazard waste bag and dispose according to SOP NO ZNBTS/GP/10.005/06

7.8 At the end of the month compile data onto "TTI Reactivity Form"

8 **Safety and precautions**

8.1 Follow recommended safety precautions when performing the assays

8.2 Waste must be disposed of according to recommended procedure

9. **Additional information**

| **Initial Result** | **Repeat Test 1** | **Repeat Test 2** | **Conclusion** |
|---|---|---|---|
| R | NR | NR | Non Reactive |
| R | NR | R | Reactive |
| R | R | NR | Reactive |
| R | R | R | Reactive |

Labeling of blood must be done in **"batches"** after repeat testing has been concluded.

**10. Forms and procedures**

10.1 Daily Log form

10.2 Reactivity form

10.3 Initial and repeat reactivity form

10.4 SOP on Murex HIV Ag/Abs

10.5 SOP on Murex HBsAg

10.6 SOP on Murex HCV

10.7 SOP on Disposal of Biohazard Waste