CO-INFECTION OF HEPATITIS (A, B & C) AMONG PATIENTS WITH HIV: MAGNITUDE AND CHARACTERISTICS OF PATIENTS ATTENDING MUHIMBILI NATIONAL HOSPITAL

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for submission a dissertation entitled: **"Co-infection of Hepatitis (A, B & C) Among Patients with HIV: Magnitude and Characteristics of Patients Attending Muhimbili National Hospital"**, in partial fulfillment of the requirements for the degree of Master of Medicine (Internal Medicine) of the Muhimbili University of Health and Allied Sciences.

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Date 27/11/2007

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I, Tumaini Joseph Nagu, hereby solemnly declare that this is my original work and that it has not been presented nor will it be presented to any other University for a similar or any other degree award.

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Dr Tumaini J Nagu

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DEDICATION

To Davidson our son; for adding more meaning to my life.

To Lawrence my husband, for his love, constant support and endurance.

ABSTRACT

Background: In Tanzania very little is published regarding HIV and Hepatitis A, B and C viral co-infection.

Objective: To determine the prevalence and describe the clinical and demographic characteristics of HIV positive individuals co-infected with single or multiple Hepatitis (A, B, C) viruses.

Methodology: A cross-sectional study was conducted between April and September 2006. A total of 260 HIV-infected HAART naïve patients aged ≥18 years at the Muhimbili National Hospital Care and treatment clinic were enrolled. Interview, physical examination and search for markers of Hepatitis A, B and C infections by ELISA were done.

Results: The prevalence of Anti HAV IgM antibodies, HBsAg, HBc IgM antibodies and anti HCV antibodies were 3.1%, 17.3%, 2.3% and 18.1% respectively. The prevalence of HBV by either HBsAg or HBcAb marker was 18.1%. None of the patients had all the three hepatitis viruses. Notably, most (81.1%) of the patients with hepatitis co-infection had neither specific clinical features related to the infection nor raised serum transaminases.

Conclusion and recommendations: The high prevalence of Hepatitis B and C viral co-infections among HIV patients underscores the need for routine screening of these viruses prior to initiation of Highly Active Anti Retroviral Therapy (HAART).

TABLE OF CONTENTS

	Page
TITLE	i
CERTIFICATION.....	ii
DECLARATION	iii
COPYRIGHT.....	iii
ACKNOWLEDGEMENT.....	iv
DEDICATION.....	vi
ABSTRACT.....	vii
TABLE OF CONTENTS.....	viii
LIST OF TABLES.....	x
ABBREVIATIONS/ACRONYMS.....	xi
CHAPTER ONE	
INTRODUCTION AND LITERATURE REVIEW.....	1
PROBLEM STATEMENT.....	29
RATIONALE	31
CHAPTER TWO	
OBJECTIVES.....	32
CHAPTER THREE	
METHODOLOGY.....	33
CHAPTER FOUR	
RESULTS.....	43

	Page
CHAPTER FIVE	
DISCUSSION.....	62
CHAPTER SIX	
CONCLUSIONS & RECOMMENDATIONS.....	68
CHAPTER SEVEN	
REFERENCES.....	69
CHAPTER EIGHT	
APPENDICES	
QUESTIONNAIRE.....	84
LABOLATORY FORM.....	94
CONSENT FORM (ENGLISH)	95
CONSENT FORM (KISWAHILI)	99
WHO HIV STAGING	103

LIST OF TABLES

Table 1: Distribution of Socio-demographic Characteristics of HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=260) 44

Table 2: Seroprevalence of Viral Hepatitis Markers among HIV patients recruited at Muhimbili National Hospital-Care & Treatment Centre (n=260) 47

Table 3: Prevalence of Hepatitis (A, B, C) Co-infection according to socio-demographic characteristics of HIV patients at Muhimbili National Hospital- Care & Treatment Centre (n=260)..... .50

Table 4: HAV co-infection and clinical characteristics among HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=260).....53

Table 5: Presence of HBsAg and clinical characteristics among HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=260) 54

Table 6: Presence of HBc antibodies and clinical characteristics among HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=260) 55

Table 7: Presence of anti HCV antibodies clinical characteristics among HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=260) 56

Table 8: Presence of 'Any hepatitis' marker and clinical features among HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=260) 58

Table 9: Summary of independent predictors of markers of viral hepatitis (A,B,C) among HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=243) 60

Table 10: Comparison of Geometric mean CD4+ T lymphocytes and Hepatitis infection among HIV patients at Muhimbili National Hospital - CTC (n=260)..... 61

ABBREVIATIONS/ACRONYMS

AIDS = Acquired Immunodeficiency syndrome

ALAT = Alanine aminotransferase

ASAT = Aspartate aminotransferase

ART = Anti retro viral therapy

CTC= Care and Treatment Clinic

ELISA = Enzyme linked Immunosorbent assay

HAART = Highly Active Anti Retroviral Therapy

HAV = Hepatitis A Virus

HBV = Hepatitis B Virus

HBsAg = Hepatitis B Surface Antigen

HBcAb = Hepatitis B core Antibody

HCV = Hepatitis C Virus

HIV = Human Immunodeficiency Virus

IgM = Immunoglobulin M

IgG = Immunoglobin G

IVDU = Intravenous Drug Users

MNH = Muhimbili National Hospital

MUHAS = Muhimbili University of Health and Allied Sciences

MSM = Men having Sex with Men

NACP= National AIDS Control Program

NIMR=National Institute for Medical Research

TACAIDS = Tanzania Commission for AIDS

UNDP = United Nations Development Programme

VCT = Voluntary counselling and testing

WHO = World Health Organization

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Background

1.1.1 HIV/AIDS in Tanzania

HIV/AIDS is a major global health problem. About 40 million people were living with HIV and AIDS in the world in 2004, with two third of this global burden in Sub-Saharan Africa.[1]

Tanzania is one of the Sub-Saharan African countries hard hit by the HIV/AIDS pandemic. The first cases of AIDS in Tanzania were reported in 1983.[2] It became a notifiable disease in late 1980's. Since then until December 2004 a total of 192,532 cases were reported in the country.[2] It is estimated that 1 out of 14 cases of AIDS are reported, and therefore by 2004 a total of 1,840,000 people were living with HIV in Tanzania.[2] Heterosexual intercourse, has been reported to be the main mode of HIV transmission is accounting for 78.1% of all reported cases in 2004.[2] Mother-to-child transmission and blood transfusion contributed 4.6% and 0.5% respectively of the reported cases.[2] Moreover, majority of the reported cases are between 20-49 years indicating that infection occurs at the age of maximum sexual activity. A recent household survey estimated the prevalence of HIV among adults to be 7% with a wide regional variation.[1] The prevalence of HIV in blood donors was estimated to be 7.7%[2] while among Antenatal Clinics Attendees was 8.7%[3] in 2004. Prevalence is highest in the areas along the main roads[3] with prevalence of 16% of the antenatal clinic attendees.[3] The regions in Tanzania most affected are Mbeya, Dar es Salaam and Tanga.[2]

The impact of HIV and AIDS in Tanzania is significant. HIV/AIDS and TB were the leading causes of mortality in Hai, Temeke and Morogoro rural districts.[1] It therefore causes a significant burden on the health sector. Ill health and death due to HIV/AIDS have had economic and social impacts. In Kagera, one of the regions mostly affected by HIV and AIDS, it was indicated that Gross Domestic Product (GDP) declined from US$ 268 to US$91 between 1983 and 1994 respectively.[1] AIDS was believed to be a major cause.[1] Socially, AIDS has left orphans subjected to material, emotional and social deprivations. Furthermore, it claimed lives of families' income generators prematurely hence children are rendered with lack of opportunities for education and health care.[1]

1.1.2 Hepatitis

Hepatitis describes the inflammation of the liver. It may be caused by alcohol, drugs, autoimmune diseases, metabolic diseases, and viruses.[4] Viral infections account for more than half the cases of acute hepatitis in the United States[4] and some places it is the commonest cause of fulminant hepatic failure.[5] Almost all cases of viral hepatitis are caused by one of the five viral agents; Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis E virus Hepatitis and Hepatitis D virus which, occurs in association with Hepatitis B virus.[6] Although these viruses can be distinguished by their molecular and antigenic properties, they produce clinically similar illnesses ranging from asymptomatic infection to fulminant and fatal diseases.[6] Histopathological appearances in hepatitis can be classified into acute or chronic, however they are not readily separable.[7]

Acute viral Hepatitis following infection with the viruses may occur after an incubation period, which varies according to the responsible virus.[6] Clinically, there are a number of stages of the acute hepatitis namely prodromal, clinical jaundice and recovery phases.[6] Recovery depends on the type of the infecting virus, it is almost a rule following hepatitis A or E and may not occur with Hepatitis B and C in which patients proceed to chronic infections.[6,7] Prodromal phase mainly consist of constitutional feature e.g. anorexia, nausea, vomiting, fatigue, malaise arthralgia, myalgia and headache,[4] low grade fever is often present in hepatitis A and E than in hepatitis B or C.[6] During this phase too, biochemical profile, e.g. serum transaminase enzymes namely, ALAT and ASAT may begin to rise.[4] When jaundice becomes apparent prodromal symptoms usually diminish[7] at this point patients may have tender hepatomegally, abdominal discomfort and spleenomegaly may be present in up to 10% of patients.[7]

Chronic infection leading to liver cirrhosis with or without hepatocellular carcinoma may occur with HBV and HCV.[6] Other infrequent causes of viral hepatitis include, adenovirus, cytomegalovirus, Epstein-Barr virus, Human Immunodeficiency virus (HIV) and, rarely, herpes simplex virus infection.[6]

1.2 HEPATITIS A VIRUS

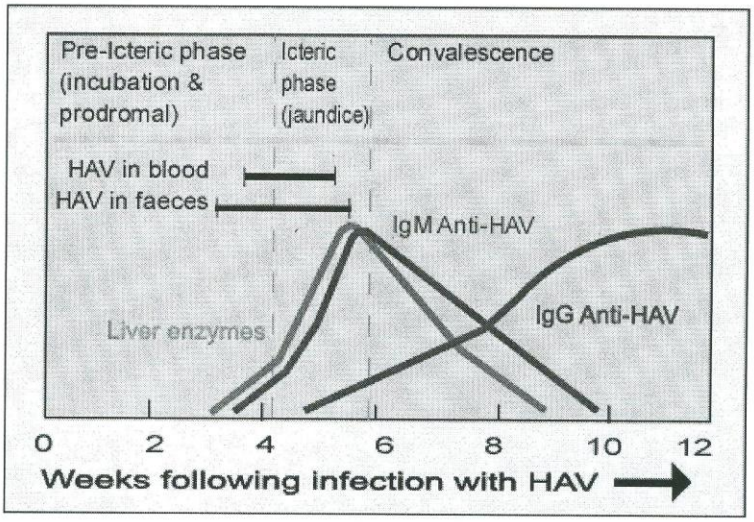
1.2.1 Microbiology

HAV is a picornavirus consisting of a non-enveloped 7.5-kb RNA; it has a diameter of 27 nm and has one serotype but multiple genotypes.[4]

1.2.2 Mode of transmission and natural history

Transmission of HAV is mainly via the faecal-oral route.[4] The incubation period of HAV is 15-45 days with an average of approximately four weeks,[4] as shown in figure 1. Its replication is limited to the liver but the virus may also be present in bile, stool and blood during late incubation and early icteric periods.[4] While viral shedding in faeces continues, viremia diminishes once jaundice becomes apparent[4]. As shown in figure 1, liver enzymes return to normal and IgM anti-HAV are almost undetectable showing recovery at about ten weeks after infection.

Figure 1: Natural History of HAV infection



Reproduced from::http://www.safetyline.wa.gov.au/institute/level2/course20/lecture64/images /l64_04.gif

Hepatitis A causes an acute usually self-limiting infection of the liver and is one of the commonest vaccine-preventable infectious diseases in the world.[1] While children infected with HAV usually remain asymptomatic,[6] the disease is severer and has higher mortality in adults, the explanation for which is unknown.[6]

1.2.3 Pathophysiology of hepatocytes injury

Like other Hepatitis viruses, HAV does not cause direct cytopathic effect on the hepatocyte. Evidence suggests that clinical manifestations are a result of immunological reaction by the host.[4] Classic findings of acute HAV infection include a mononuclear cell infiltrate, interface hepatitis, focal hepatocyte dropout, ballooning degeneration, and acidophilic (Councilman-like) bodies.[4]

1.2.4 Diagnosis

Diagnosis of the infection is by the presence of HAV specific IgM antibodies, which rises from day 25 after infection[6] and disappears 6 – 12 months after the initial infection.[6] The presence of immunoglobulin G (IgG) anti-HAV merely demonstrates that an individual has been infected with HAV at some point in the past, from 2 months to decades ago.[6] Once present, IgG anti-HAV appears to offer patients lifelong immunity against recurrent HAV infection.[6]

1.2.5 Epidemiology

Hepatitis A virus infection is transmitted primarily by oral route and hence of concern in developing countries where there are difficulties in maintenance of hygiene due to problems associated with scarcity of water and lack of health education.[6] In Tanzania prevalence of past exposure to HAV was documented to be 99% among general population[8] while in Kenya, Hepatitis A was the leading cause of acute icteric Hepatitis.[9] It accounted for 41.7% of the causes and was commoner in younger age groups compared to older ones.[9]

1.2.6 Hepatitis A virus and HIV interaction

Not much has been done on HAV within the context of HIV neither in Africa nor in Tanzania. Since HAV infection is not transmitted predominantly by parenteral routes its prevalence might be similar in HIV infected and non-infected individuals however, it is not uncommon to find HAV in HIV infected individuals.[10] In Argentina the prevalence of past exposure to HAV was documented in 84% of the HIV positive patients.[10] In HIV infected patients co-infected with HCV, a super-infection with HAV can progress to fulminant hepatic failure and death.[11, 12]

Moreover, anti retroviral therapy (e.g. Nevirapine) can cause significant hepatotoxicity in the setting of Hepatitis.[13, 14] For these reasons, even a generally self-limited, acute hepatotropic virus such as hepatitis A (HAV) is of particular concern when co-infected with HCV, HIV or Both. Therefore guidelines suggest a move towards offering hepatitis A vaccination in these patients.[15]

1.2.7 Treatment

There is no specific treatment for hepatitis A infection. Spontaneous recovery occurs 2 - 4 weeks following clinical jaundice **(figure 1).** During the acute phase, severe cases warrant hospitalization where supportive care is given in terms avoiding further damage to the Liver.[7] Restriction of protein and hepatotoxic drugs is recommended during this period.[7] HAV infection produces lifelong immunity that protects against future hepatitis A virus infection.[4]

1.2.8 Prevention

The first line prevention method is by observation of good sanitation and hygienic practices in handling food. Improving social conditions in terms of water availability and avoiding overcrowding together with health education may prevent infection in the community.[7]

Primary prevention is also possible by passive immunization with Immunoglobulin and active immunization with killed vaccines especially among the risk groups e.g. post exposure prophylaxis for intimate contacts, travellers to endemic areas, military personnel and patients with chronic liver disease especially those with chronic HCV.[4] The United States Public Health Service and the Infectious Diseases of Society of America (USPHS/IDSA) guidelines for preventing opportunistic infections among HIV-Infected Persons of 2002 recommends HAV vaccine for all persons with HIV and are at increased risk for HAV.[15]

Safety studies indicate no major concerns for the inactivated HAV vaccine among HIV infected individuals. Rates of adverse events are comparable to those in HIV-negative patients, with no demonstrable effect on viral loads, progression to AIDS, or CD4+ counts.[16] While immunocompetent individuals achieve 90–100% seroconversion rates after HAV vaccination,[17, 18] HIV infected individuals show less immunogenic response to the vaccine.[19] In a recent study various factors have been shown to vary with the response rate in HIV infected individuals namely; female gender, levels of CD 4+ and viral load, however the latter has been found to be a single modifiable independent predictor to the response.[19]

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1.3 HEPATITIS B VIRUS

1.3.1 Microbiology

HBV is a double-stranded DNA virus in the *Hepadnaviridae* family.[6] It causes hepatitis in humans and a related virus in this family causes hepatitis in ducks, ground squirrels and woodchucks.[6, 20] The HBV genome has four genes: pol, env, pre-core and X that respectively encode the viral DNA-polymerase, envelope protein, pre-core protein (which is processed to viral capsid) and protein X.[20] The function of protein X is not clear but it may be involved in the activation of host cell genes and the development of cancer.[20]

1.3.2 Modes of infection transmission

Horizontal transmission of HBV occurs through blood, blood products and sexual transmission.[20] In Sub-Saharan Africa, where the disease is highly endemic, horizontal transmission through skin contact is said to be the most important, however the mechanism is unclear.[21] Vertical transmission from mother to infant in the perinatal period is a major mode of transmission in regions where hepatitis B is endemic.[20] The vast majority of HBV cases around the world result from perinatal transmission.[6] Perinatal transmission appears to be due to contact with a mother's infected blood at the time of delivery, as opposed to transplacental passage of the virus.[4, 6] HBV is readily detected in serum; it is seen at very low levels in semen, vaginal mucus, saliva, and tears.[20] The virus is not detected in urine, stool, or sweat. Although breast milk can contain HBV virions, the role of breastfeeding in

transmission is unclear.[4] The risk of acquiring HBV after a needle stick from an infected patient is estimated to be as high as 5%.[20]

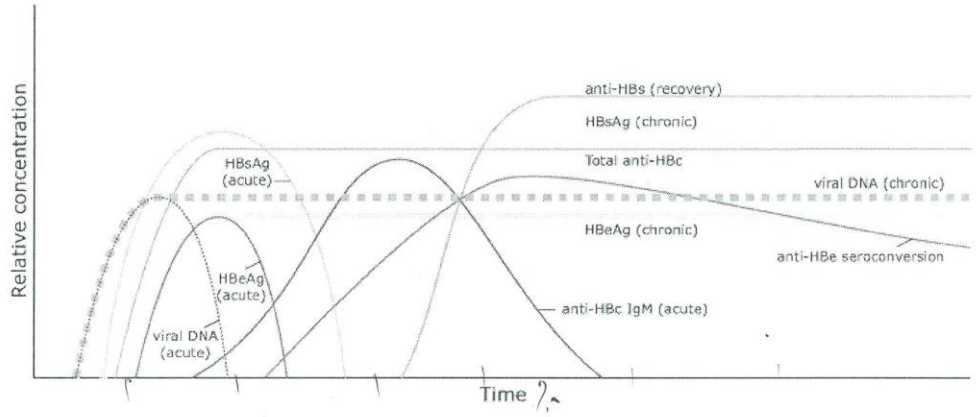
HBV can survive storage at -20° C (-4° F) and heating at 60° C (140° F) for 4 hours. It is inactivated by heating at 100 °C for 10 min or by washing with sodium hypochlorite – bleach.[20]

1.3.3 Natural history

The incubation period of HBV following infection is 40-150 days, with an average of approximately 12 weeks.[4] Following infection, the individual will go through the incubation, prodromal and convalescence periods with various viral markers appearing in blood as shown in figure 2.

Approximately 90-95% of neonates with acute infection and 5% of adults with acute infection develop chronic HBV infection, while the remainder clear their infection and develop a life-long immunity against repeated infections.[6, 20] However, neonates infected via perinatal infection are usually asymptomatic.[4] Of cases of chronic HBV infection 20% progress to cirrhosis or hepatocellular carcinoma (HCC)[4] resulting in more than 1 million global deaths each year.[4] This makes hepatitis B the ninth leading cause of death in the world.[4]

Figure 2: Natural History of HBV infection



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1.3.4 Diagnosis

Diagnosis of HBV infection is generally based on serology, looking for antibodies and antigens. Infection with HBV is defined by the presence HBsAg.[6] Virtually all individuals infected with HBV, either acutely or chronically, will have detectable serum hepatitis B surface antigen (HBsAg).[20] In acute infection, HBsAg is detectable several weeks after infection, it is the first serological marker and its appearance precedes elevation of liver enzymes and onset of clinical symptoms.[20] Persistence of HBsAg for more than six months implies chronic infection, which is associated with the presence of viremia as measured by HBV DNA.[20]

IgM antibodies against core antigen are detectable in serum 1 – 2 weeks after the appearance of HBsAg preceding anti-HBs by weeks to months and may serve as a marker of acute or recent infection during "window period" i.e. after disappearance

of HBsAg before Anti – HBs antibodies appear.[4, 6] IgM Antibodies predominate in the first 6 months whereas after six month IgG type predominates.[4]

HBeAg is also detectable in acute infection, either concurrently or shortly after appearance of HBsAg. Its occurrence coincides with high levels of virus replication and reflects presence of circulating intact virions and detectable HBV DNA.[4, 20]

Subjects who develop an immune response against HBV develop antibodies against Hepatitis B surface Antigen i.e. anti HBs.[20] Such antibodies are also produced by vaccination[6, 20] Most people who have had acute infection that resolves, continue to have IgG antibodies against core antigen for life.[6] Some remain immune with antibodies against HBsAg but some lose these antibodies and may be susceptible to future infection.[6, 20]

Acutely infected individuals who do not clear HBV continue to have serum HBsAg. In most cases, the chronic infection becomes "non-replicative" and the subjects lose serum HBeAg and develop antibodies against HBeAg. In some cases, "replicative" infection persists along with detectable serum HBeAg.[20] In chronically infected individuals, infection can switch from "non-replicative" to "replicative" and vice-versa.[20]

1.3.5 Pathophysiology of liver damage

The pathogenesis of hepatitis due to HBV is mainly immune-mediated and not due to cytopathic effects of the virus. Patients who spontaneously recover from HBV

infection mount vigorous CD4+ and CD8+ T-cell responses to various HBV epitopes.[22, 23] Some evidence suggest that it may result from destruction by MHC-restricted cytotoxic T lymphocytes targeted to the expressed hepatitis B core antigen on the surface of hepatocytes.[24] By contrast, patients who develop chronic hepatitis B infection have narrowly focused T-cell responses.[22, 23] Trace viremia can be detected with polymerase chain reaction (PCR) in individuals who are immune to HBV. This may suggest the effect of cellular and humoral immune in controlling residual virus and that viremia can re-emerge in the setting of immunosuppression. For example, patients undergoing chemotherapy or HIV-infected patients with advanced immunosuppression can experience reactivation of HBV replication and hepatitis flares.[25] In a liver allograft recipient, potent immunosuppression needed to prevent rejection interferes with normal mechanisms of viral clearing and contributes to the progression to chronic disease in most recipients.[26] On the other hand, lower maintenance doses of corticosteroids may modify the course of post-transplant HBV infection by leading to less viral replication, milder HBV-related liver disease and better overall patient survival.[26]

1.3.6 Epidemiology

Hepatitis B is the most common cause of chronic liver disease worldwide, affecting an estimated 400 million people and responsible for 500,000 to 1 million deaths per year.[27] Approximately 5% of the world's population (300 million people) is chronically infected with Hepatitis B virus (HBV)[6] and 10% of these are in the sub-Saharan Africa and East Asia[4] and thus represents a viral pandemic, being nearly twice as widespread as infection with hepatitis C virus (HCV).[4] Maintenance of high

HBsAg carrier rate in these parts of the world is partially explained by the high prevalence of perinatal transmission and by low rate of HBV clearance by neonates.[4,7]

Hepatitis B is highly endemic in areas such as China, Southeast Asia, and Sub-Saharan Africa and perinatal transmission predominates.[28] In these areas, about 70 to 90% of the population becomes HBV-infected before the age of 40, and 8 to 20% of people are HBV carriers.[28] On the contrary, North America, Western and Northern Europe, Australia, and parts of South America prevalence is very Low. The carrier rate here is less than 2%, and evidence of exposure is present in less than 20%.[28, 29]

In Tanzania, the prevalence of HBsAg among blood donors is within the range of 8.0% - 11%[30, 31] and among HIV infected patients presenting with dermatological conditions prevalence was 8%.[32] In Kenya, the prevalence of HBV was reported to be 26.2% among those presenting with jaundice in the hospital.[9]

Shared epidemiological risks for transmission have resulted in HIV infected populations having a high prevalence of HBV co-infection.[33] Prevalence of Co-infection with hepatitis B virus (HBV) and HIV is common, with 70-90% of HIV-infected individuals having evidence of past or active infection with HBV.[34, 35] Earlier studies reported prevalence of hepatitis B surface antigen (HBsAg) chronic carriage among HIV-infected individuals as low as 1.9-10%.[36] However, other studies reported much higher prevalence later on. In Argentina, the prevalence of HBsAg among HIV infected individuals was reported as 58.8%.[10] Furthermore,

Among IVDU, 90% of HIV-infected individuals have evidence of exposure to hepatitis B (hepatitis B core antibody [anti-HBc] positivity) and 60% also have evidence of past infection with presence of hepatitis B surface antibody (anti-HBs).[36] In Tanzania no study has compared the prevalence of Hepatitis among HIV infected and HIV uninfected populations. However, prevalence of HBV infection in a study done among HIV infected patients with dermatological conditions was similar to the one obtained in another study that involved voluntary blood donors.[30, 32]

1.3.7 Hepatitis B Virus and HIV Interaction

The management of HIV/ Hepatitis co-infected individuals is very challenging due to the increased risk for hepatotoxicity from Anti retroviral treatment as well as the fact that both hepatitis and HIV viruses influences the natural history of the other as elaborated below.

(a) Natural History

(i) Influence of HIV on the natural history of HBV infection

It has been documented that, the presence of HIV infection may modify the natural history of hepatitis by a number of ways. Firstly, co-infection increases the probability of becoming a chronic carrier; and secondly there are higher markers of replication e.g. HBV DNA and DNA polymerase and lower rate of viral clearance.[37] Unlike in the immunocompetent individuals, HIV patients co-infected with HBV show a higher prevalence of chronic HBV infection.[38] Moreover their, HBV replication is higher; alanine transaminase levels are lower, and there are lower rates of seroconversion to anti-hepatitis B e (anti-HBe Ab) and anti HBs antibodies (anti-

HBs Ab).[38] The impact on the outcome of HBV infection is still controversial, although evidence from recent studies suggests that there is an accelerated progression towards cirrhosis and liver failure in HIV co-infected subjects.[39]

The course of acute hepatitis B may be modified in the presence of HIV with lower incidence of icteric illness and a higher HBV carriage rate of about 25% compared with about 5% in those uninfected with HIV.[40, 41]

In chronic infection, markers of HBV replication appear to be influenced by HIV infection. There is a trend towards lower rate of clearance of the hepatitis B "e" antigen (HBeAg) and HBV DNA as well as a significant increase in the serum HBV DNA viral load.[42] HIV-induced immunosuppression is thought to be responsible for the lower serum transaminases, possibly due to a reduction in the severity of liver disease.[43] However, immunosuppression also may be associated with reactivation of HBV infection in persons who have lost detectable HBsAg or HBeAg.[44]

Although symptomatic reactivation and loss of anti-HBs is uncommon in HIV-infected individuals,[35, 40] asymptomatic reactivation or re-infection occurs frequently in patients who develop AIDS, leading to a significantly higher prevalence of HBsAg.[40]

(ii) Influence of HBV on the natural history of HIV Disease

There are conflicting data with respect to the impact of HBV on the course of HIV infection. While some studies have shown an increased rate of HIV progression to AIDS among individuals with markers of exposure to HBV,[45, 46] others have not

shown any change in the progression of HIV disease or survival.[34] In a prospective cohort of 3,040 HIV-seronegative MSM, 296 underwent HIV seroconversion during the 4-year follow-up period. The likelihood of HIV infection was associated with serologic markers of HBV (RR = 2.03 for HBV carriers and 2.22 for HBV-immune subjects), an association that remained even after adjustment for sexual behaviour or sexually transmitted disease over time.[45] However, there was no significant increase in the rate of progression to AIDS in these individuals over a 2.5-year follow-up.[47] Therefore, HBV does not appear to significantly influence the progression of HIV infection but may correlate with risk for HIV infection in some populations. Mean CD4+ T lymphocytes has shown to be lower in HBV co-infected HIV individuals.[48]

(b) Anti Retroviral Treatment for HIV and Hepatotoxicity: Role of HBV

It is not uncommon for HIV patients with HBV co-infection to have hepatotoxicity due to HAART.[49, 50, 51] HBV co-infected HIV patients were more likely to develop HAART toxicity than HIV monoinfected.[50] Two out of 8 persons with chronic HBV infection had severe hepatotoxicity; both received indinavir and one was co- infected with HCV as well.[49] In this study, 13 (9.4%) of 138 patients on non-ritonavir PI and nucleoside analogs having chronic HCV or HBVco-infection developed severe hepatotoxicity compared to 3 (2.7%) of 110 HBV or HCV uninfected patients (RR, 3.7; 95% CI, 1.0-11.8).[48] Increased risk for hepatotoxicity has also been described in HBV co-infected HIV patients receiving Nevirapine.[51]

1.3.8 Treatment of Hepatitis B virus

Among previously healthy adults who present with clinically apparent acute hepatitis recovery occurs in 99%, therefore anti viral therapy may not alter the outcome and hence may not be required.[6] However, on rare occasions of severe acute Hepatitis B occur which requires treatment. Several agents like Interferon alpha, Lamivudine, Adefovir Dipivoxil and Tenofovir Disoproxil Fumarate have been studied.

Interferon α

Interferon α is useful in the treatment of Hepatitis B infection and was the first approved therapy for the treatment of chronic HBV.[6] Response to Interferon α in HBV/HIV co-infected individuals may be poor due to HIV-induced T-cell immunosuppression [52], however some patients have shown good response.[53]

Lamivudine

This drug is used in the treatment of HIV as well HBV infection by its ability to inhibit RNA- and DNA-dependent DNA polymerase activities of the viruses respectively.[6]

In HBV-infected individuals, a 1-year course of lamivudine resulted in a 16-18% rate of HBeAg seroconversion, [54] an efficacy that is similar to that of a 4-month course of interferon.[54] However, prolonged lamivudine therapy can result in drug-resistant HBV mutants (tyrosine-methionine-aspartate-aspartate [YMDD] motif mutation) leading to an increase in serum ALT and HBV DNA levels in about 15-30% of cases after 1 year of therapy,[6, 54] 49% after 3 years [54] and reaching about 70% after 5 years of treatment.[6] In the HIV co-infected population, the development of lamivudine resistance may be more frequent and can be associated with hepatitis flares.[55, 56]

Adefovir Dipivoxil

This is a pro drug of Adefovir, a nucleotide analogue and potent inhibitor of HBV infection, used in the treatment of chronic HBV.[6] Adverse effects associated with use of adefovir include renal failure and acute worsening of hepatitis B after discontinuation of therapy.[6] Adefovir may be effective for the treatment of lamivudine-resistant HBV in individuals co-infected with HIV. Benhamou treated 35 HIV/HBV co infected patients receiving lamivudine as part of their antiretroviral regimen with adefovir at the dose of 10 mg/day.[57] In this study, adefovir therapy decreased HBV DNA levels, and 2 of 31 patients who completed 48 weeks of therapy underwent HBeAg seroconversion.[57] Adefovir was well tolerated but resulted in a transient increase in ALT levels in 15 patients.[57]

Tenofovir Disoproxil Fumarate

Tenofovir disoproxil fumarate (TDF) is an acyclic nucleotide reverse transcriptase inhibitor that is effective against HIV as well as HBV.[6] More evidence suggests better treatment outcome in the combination of Lamivudine and Tenofovir in the treatment of HBV/HIV co-infected individuals.[58]

1.4 HEPATITIS C VIRUS

1.4.1 Microbiology

HCV was discovered in 1989 by investigators at Chiron, Inc.[4] Portions of the HCV genome were isolated by screening cDNA expression libraries made from RNA and DNA from chimpanzees infected with serum from a patient with post-transfusion

non-A, non-B hepatitis.[4] HCV is a positive, single-stranded RNA virus in the *Flaviviridae* family. The genome is approximately 10,000 nucleotides and encodes a single polyprotein of about 3,000 amino acids.[59] Given its high rate of mutation, at least 6 distinct clades of HCV, and more than 100 subtypes, have been identified by nucleotide sequencing. Clades 1-3 have a worldwide distribution; clades 4 and 5 are found largely in Africa; and clade 6 is confined largely to Asia.[60] Differences in pathogenicity, as well as responsiveness to treatment have been reported among the different clades.[60]

1.4.2 Modes of transmission

Primarily blood and blood products transmit the virus.[59] Intravenous drug use and the sharing of paraphernalia used in the intranasal snorting of cocaine and heroin account for approximately 60% of new cases of HCV infection.[4] More than 90% of patients with a history of intravenous drug use have been exposed to HCV.[10]

Occupational exposure to HCV accounts for approximately 4% of new infections.[59] On average, the chance of acquiring HCV after a needle stick injury involving an infected patient is 1.8% (range, 0-7%).[59] Of importance, reports of HCV transmission from healthcare workers to patients are extremely uncommon. Approximately 20% of cases of hepatitis C appear to be due to sexual contact.[59]

In contrast to hepatitis B, approximately 5% of the sexual partners of those infected with HCV contract hepatitis C.[61] Vertical transmission appears to be uncommon. It has been observed in less than 5% unless the mother is co-infected with HIV where the risk increases to 18%, in children born to mothers co-infected with HIV and

HCV.[61] There has been no association between HCV transmission and breast-feeding. From a public health perspective, no specific recommendations for preventing the vertical transmission of HCV have been made.[61]

1.4.3 Natural History

Primary exposure to the hepatitis C virus leads to an acute infection that is usually relatively mild after an incubation period of 2-8 weeks.[4] Only 20-30% of infected individuals develop clinically evident acute Hepatitis C in their attempt to resolve the infection.[4] Symptoms include jaundice, fatigue, abdominal pain, and intermittent nausea.[4] Following acute HCV infection, 70-80% result in persistent viral infections.[59, 62] The sequel of chronic HCV infections includes cirrhosis of the liver as well as hepatocellular carcinoma, both of which carry significant socio-economic costs and public health consequences. In developed countries, HCV-related liver disease is currently the leading cause of liver transplantation.[59, 62]

1.4.4 Pathophysiology of liver damage

The mechanism of liver injury in acute and chronic hepatitis C is unknown, but since there is little evidence as to the cytopathogenicity of HCV, it is thought that liver damage may be mediated by the host's cellular immune response to the infection.[61, 63] Given the lack of definitive symptoms, liver histology and the circulating levels of liver enzymes are currently considered the most reliable predictors of progression to cirrhosis.[61,63] Studies show that severe necrotising inflammatory activity, as well as severe fibrosis in hepatic tissue both due to elevated levels of liver enzymes; can be correlated with progression to cirrhosis within 10 years.[61] In cases where

inflammation and fibrosis is mild, progression is slow and even limited.[63] Thus, individuals with normal levels of enzymes progress more slowly towards disease than those with elevated levels of the same enzymes.[61]

Factors influencing the rate of progression of chronic hepatitis C to cirrhosis and liver cancer include alcohol abuse, the duration of the infection, and, possibly, viral titres.[61] In addition, viral genotype, co-infection with another type of hepatitis virus, co-infection with the human immunodeficiency virus (HIV), and gender, have all been thought to play a role in the progression of disease.[61] Unfortunately, conclusive evidence about the exact role and impact of these factors is lacking.

1.4.5 Diagnosis

The diagnosis of hepatitis C is made through history taking, serological testing.[59] Suspected individuals should be tested for the presence of serum antibodies against HCV. The presence of anti-HCV antibodies in a person with a risk factor or evidence of liver disease strongly suggests the diagnosis of chronic hepatitis C.[59] The absence of anti-HCV antibodies generally rules out the diagnosis. Tests for HCV RNA in blood should be done in those individuals with anti-HCV antibodies to confirm the diagnosis and in the rare patient who does not have anti-HCV antibodies but in whom the diagnosis is still strongly suspected on clinical grounds.[4]

Enzyme immunoassay (EIA) is the main screening assay for the detection of HCV antibodies. EIA-2 (second generation) has a sensitivity of 92-95% and a specificity of 88-95% in high-prevalence populations.[64] Hepatitis C viremia is detectable by

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polymerase chain reaction (PCR) testing in 60-90% of HIV co-infected HCV antibody-positive patients.[65,66] However, a single negative antibody assay may not sufficiently exclude HCV in HIV-infected individuals. The first generation EIA tests (sensitivity 70-80%) can result in a significant rate of false negativity in HIV-infected individuals, underreporting the incidence of HCV infection by 10-30%.[67] This increased rate of false-negative antibody testing in the presence of HIV has been attributed to several factors, including a possible lack of HCV antibody production with immunosuppression, more rapid decline in HCV antibody titre, possible interaction between the two viruses, and an anti-HCV seroreversion into a negative state.[68] Also, the hypergammaglobulinemia reported in HIV infection can lead to false-positive anti-HCV EIA results.[68] Additionally, there are inconsistencies in the results of confirmatory recombinant immunoblot assay (RIBA) for HCV. Specifically, 10-23% of RIBA results can be indeterminate in the setting of co-infection.[65, 69] Therefore, with these finding although patients should be screened by EIA, measurement of serum HCV RNA by highly sensitive reverse transcriptase PCR may be required for detection of HCV in individuals with undetectable antibodies and other evidence of chronic liver disease.

1.4.6 Epidemiology

There are about 150 million chronic hepatitis C virus (HCV) carriers throughout the world, with an estimated global prevalence of 3% (range 0.1-5%).[70] In Africa the prevalence is estimated to be 5.3%.[71] Symptomatic acute infection occurs in an estimated 1-3 cases per 100,000 persons annually but the actual incidence of new HCV infection is higher as the majority of cases are asymptomatic.[62] According to

recent population based studies, approximately 40% of the cases of chronic liver disease are HCV-related, resulting in 8,000-10,000 deaths per annum.[62] Chronic liver disease is the tenth leading cause of deaths among adults in the United States, and accounts for around 25,000 deaths annually.[62]

Hepatitis C virus (HCV)/ HIV co-infection has become a major contributor to morbidity and mortality.[33] Factors contributing to the emergence of HCV infection as a significant medical issue in the HIV-positive population are shared modes of transmission and the success of highly active antiretroviral therapy (HAART) in prolonging the survival of HIV-infected patients.[72] Longer survival of HIV patients allows for potential decompensation of previously undiagnosed cirrhosis in co-infected patients, so that in the United States end-stage liver disease has become the leading cause of death in the HIV-infected population.[72]

The prevalence of HCV/HIV co-infection depends on the geographical location and epidemiological risk factor. It is estimated that 30% to 60% of patients with HIV are co infected with HCV.[10, 33] Almost all HIV-positive haemophilia patients were also HCV-positive.[73] Hepatitis C virus and HIV co-infection is commonly observed in injection drug users, with an estimated prevalence as high as 52% to 90%.[74, 75] Clinically, hepatitis C is a more severe disease in HIV-infected individuals, characterized by rapid progression toward end-stage liver disease.[33]

The U.S. Public Health Service (USPHS) and the Infectious Diseases Society of America (IDSA) recommend screening with EIA for all HIV-infected individuals to detect anti-HCV antibodies.[15]

1.4.7 HIV and HCV Interaction

(a) Natural History

(i) Influence of HIV on the natural history of HCV

HCV-induced liver disease can be progressive with cirrhosis developing in 20-30% of individuals over a 10-20 year follow-up.[76] HIV co-infection has been associated with a more rapid progression of liver disease as well as a higher prevalence of cirrhosis.[77] In individuals with parenterally acquired HCV infection (blood transfusion recipients or IVDU), there was a higher incidence of cirrhosis within the first 15 years of follow-up (15-25% vs. 2.6-6.5%, respectively, $p < .05$) in HIV-positive individuals compared with those who are HIV negative.[78, 79] The estimated interval from HCV infection to cirrhosis may be significantly shorter in the HIV-infected individuals (7 vs. 23 years, $p < 0.001$).[78] Another study compared a cohort of 122 HIV/HCV co-infected individuals with 122 HIV-negative, HCV-infected patients. HIV seropositivity, alcohol consumption of >50 grams/day, age at infection >25 years, and CD4 lymphocyte counts <200 cells/μL were significantly associated with an increased rate of liver fibrosis progression.[80] Among the co-infected individuals, alcohol consumption, low CD4 counts, and higher age at HCV infection were independent predictors of fibrosis progression.[80] Although HIV/HCV co-infection results in a more rapid progression of liver disease, its effect on mortality requires further assessment. Some studies have found a higher rate of mortality from

liver-related diseases in the co-infected patients,[81] yet others have not shown any effect on survival.[82]

HCV replication is enhanced in the presence of HIV, resulting in higher serum and liver HCV RNA levels.[79, 83] While some studies have shown a further increase in HCV RNA levels as immunodeficiency progresses,[84] others have failed to find a correlation between CD4 count and hepatitis C viral load.[85] Hence, there are discrepancies in the literature regarding the influence of immunodeficiency on HCV replication.

HIV co-infection may also affect hepatitis C viral heterogeneity.[86] Cameron showed that, patients with CD4 counts <50 cells/μL demonstrated a marked decrease in HCV nucleotide diversity, despite the lack of a decrease in the number of the HCV quasi-species clones.[86]

(ii) Influence of HCV on the natural history of HIV

The influence of HCV infection on the natural history of HIV disease is controversial. Some studies refute the association between HCV and CD4+ count or CD4+ percentages.[87] Many studies favour the impaired immune reconstitution after HAART by demonstrating an association between HCV infection and faster HIV-1 disease progression and poorer CD4 cell response in co-infected individuals receiving HAART.[83, 88] Hershow found no difference in CD4+ at baseline between HCV infected and un-infected, however, during follow up HCV was predictive of slightly higher but significant mean CD4+ lymphocyte percentage over time.[89] Patients with HCV genotype 1 experienced a more rapid progression to AIDS[90] another study did not reveal this HCV genotype effect.[91]

(b) Anti Retroviral Treatment for HIV and Hepatotoxicity: Role of HCV

Treatment of HIV with HAART may result in severe hepatotoxicity in a proportion of co- infected patients.[86,92] This risk is higher in HCV co-infected individuals than in non-HCV co-infected HIV.[50] In a cohort of 141 HIV-positive patients, use of ritonavir and saquinavir resulted in >5-fold increase in serum transaminases in 10% of cases.[86] The risk of hepatotoxicity was associated with pre-treatment abnormal liver enzymes as well as co-infection with chronic HCV or HBV (relative risk [RR] 5.0, confidence interval [CI] 1.5-16.9).[86] Similarly, in a prospective cohort of 298 patients, the incidence of severe hepatotoxicity was approximately 10%, but this toxicity occurred more frequently with ritonavir compared with other protease inhibitors such as indinavir and nelfinavir.[49] Therefore, ART should be administered cautiously and liver function tests must be followed in all patients, especially those co-infected with HCV or HBV.

The mechanism of drug-related toxicity in patients with chronic viral hepatitis is not known. Some studies have suggested that hepatic injury may be due to enhanced HCV replication and CD8 cell activity during highly active antiretroviral therapy–associated immune reconstitution, although other studies have failed to confirm this.[93, 94, 95]

1.4.8 Treatment of Hepatitis C

In typical cases of HCV infection, recovery is rare and progression to chronic liver disease may occur in up to 90% of those infected.[6] However, the following agents have been investigated and have been used in the treatment of HCV infection;

Interferon α, Interferon α and Ribavirin, Peg-interferon, Peg-interferon alfa-2a and Ribavirin.

Interferon α

Treatment with Interferon α brings about similar response rates in both HIV and non-HIV infected individuals.[96] Some studies have also shown that response is more likely with higher CD4 count, lower HCV viral load and genotype3a.[83, 96]

Interferon α and Ribavirin

This combination has shown higher rates of sustained response (40%) in chronic HCV infection.[97] In HIV co-infection 28 – 31%, showed end-of-treatment and only 19% attained sustained virological response rate.[98, 99]

Peg-interferon

Conjugation of interferon with polyethylene glycol ("pegylation") delays the clearance of the drug, permitting once-weekly dosing.[96] A higher rate 39% of sustained loss of HCV RNA at 6 months following discontinuation of therapy was observed in of once weekly therapy with pegylated interferon compared with 19% with interferon (6 million units thrice weekly) after 12 months of therapy.[100]

Peg-interferon alfa-2a and Ribavirin

In patients with HCV infection alone, the combination of peginterferon and ribavirin has resulted in a higher overall sustained virologic response (defined as loss of HCV RNA at 24 weeks following cessation of therapy) of 54-56%.[101, 102] Furthermore, co-infected patients with genotype 1 had sustained virologic responses as follows: 29%

with peginterferon alfa-2a plus ribavirin, 14% with peginterferon alfa-2a plus placebo, and 7% with interferon alfa-2a plus ribavirin.[103] Therefore, peginterferon and ribavirin combination therapy is superior to other forms of treatments in the treatment of HCV among HIV co-infected patient as well as among HCV monoinfected patients. However, the same regimen shows higher response among HCV-monoinfected individuals than in HCV/HIV co-infection.

1.5 Current Clinical Care of HIV/AIDS in Tanzania.

Since October 2004, the government of Tanzania started its HIV/AIDS care and treatment services offering HAART free of charge. HIV/AIDS care and treatment services are now available throughout the country at-least to the District level.[104] Currently, there are 17 facilities offering HAART in Dar es Salaam including public and private health facilities as well as faith based organisations.[104] The available HAART regimens are categorised into first and second line drugs. Not all the HAART available in the world market are provided by the National AIDS Control Programme. First line drugs offered are Stavudine, Zidovudine, Lamivudine, Nevirapine and Efavirens.[1] Second line drugs provided include, Didanosine, Abacavir, Saquinavir/ritonavir, and Lopinavir/ritonavir used in various combinations according to the presentation of the patient.[1] Unfortunately, out of all HAART drugs available in Tanzania, only Lamivudine may as well be effective for HBV. Currently there is no specific treatment for HCV infection in Tanzania.[1]

Initial work-up of the patients for HAART initiation include complete blood count, Liver enzymes (ALAT, ASAT) and Bilirubin, renal function tests (Blood urea and serum creatinine), baseline absolute CD4+ count and chest X-ray. In Tanzania, few

centres, including the centre where the study was done can perform HIV viral load testing.[1] Markers for hepatitis viruses are usually done when liver enzymes are raised to about five times the upper limit of normal, and in such circumstances, the use of Nevirapine is avoided.[1]

The Muhimbili National Hospital care and treatment centre (MNH-CTC) up to March 2007 had a total of 3928 patients' aged 15 years or more; females were about 57%. Out of all the patients, 2932 were on HAART. This clinic receives on average seven (range of 2-12) new patients each day (unpublished data from MNH-CTC database). These new patients would have either been transferred or referred from other care and treatment clinics or are newly HIV diagnosed patients from nearby VCT.

1.6 PROBLEM STATEMENT

Hepatitis/HIV co-infection has become a major contributor to morbidity and mortality.[33] Factors contributing to the emergence of HCV infection as a significant medical issue in the HIV-positive population are shared modes of transmission and the success of highly active antiretroviral therapy (HAART) in prolonging the survival of HIV-infected patients. Longer survival of HIV patients allows for potential decompensation of previously undiagnosed cirrhosis in co-infected patients, so that end-stage liver disease has become the leading cause of death in the HIV-infected population in the United States of America.[72]

Studies suggest that the liver disease may now pose the greater threat to the morbidity and mortality of HIV infected individuals.[105, 106] Rates of Hepatitis B and

C infection are consistently higher among HIV infected compared to non-HIV infected persons,[55] this fact may be related to the shared modes of transmission. Moreover, it has been shown that HIV infection alters the natural history of these Hepatitides.[33, 39, 72] Hepatitis C in HIV persons causes a severer and faster progression to liver cirrhosis.[24, 107] Eyster documented that progression to cirrhosis in HIV person can be as low as three years[107] while others have shown a significant difference in the median interval period to cirrhosis in HIV persons 6.9 years compared to 23.2 years in non-HIV infected.[78] Meanwhile, hepatitis A infection which is very common in developing countries,[10] usually which pass unnoticed or with a milder form of disease, however, it can cause fulminant hepatic failure in hepatitis B or C carriers.[11, 12, 108] Furthermore, hepatitis A vaccine has proven to be safe but less immunogenic when administered in HIV infected individuals,[19] therefore increasing the challenge in the prevention of HAV among this population.

In Tanzania little is published on hepatitis. A study done in 1999 among blood donors showed that the prevalence of HBsAg and Ant HCV Antibodies were 11.0% and 8 respectively.[30] In another study conducted in the same hospital there was a slight reduction in the prevalence of the markers among blood donors.[31] When they analyzed a sub- sample of HIV infected persons the prevalence of HBsAg and HCV was 8.8% and 0% respectively.[27] Small sample size of HIV infected persons and fact that they studied healthy individuals (blood donors) limits extrapolating these results in HIV infected population in our environment. Furthermore, they did not study hepatitis A infection. Despite, a growing body of knowledge that hepatitis co-infection among HIV patients is higher than in non HIV infected population, no

difference in the prevalence was suggested in Tanzania.[32] HCV and HAV co-infection among HIV infected patients has not been fully investigated in this country.

Paucity of information on the magnitude of this problem in our set-up may halt the effort to give better care to our patients, and on the other hand, may worsen patients condition in view of HAART hepatotoxicity associated with hepatitis co-infection.[50,51]

1.6 RATIONALE

Chronic viral hepatitis in HIV-infected patients has emerged as a significant public health problem worldwide. Little is known about this problem in Tanzania. To the best knowledge of the investigator, this will be the first study on viral hepatitis co-infection among HIV patients in a HIV/AIDS care and treatment centre and therefore it will provide information necessary for planning in matters related to HIV care and treatment. Moreover, it will assist practitioners on how to give better care to the patients who are co-infected with the viruses. Knowledge of the predictors of hepatitis/HIV co-infection will facilitate its diagnosis and treatment whenever possible so as to avoid its complications. Furthermore, it will guide practitioners' choice of HAART that may cater for hepatitis viruses whenever possible, as well as avoid regimes with increased risk of toxicity in the presence of hepatitis co-infection.

CHAPTER TWO

OBJECTIVES

General Objective

To determine the prevalence and describe characteristics of HIV positive individuals co-infected with single or multiple Hepatitis (A, B, and C) viruses at Muhimbili National Hospital.

Specific Objectives

1. To determine the prevalence of Hepatitis A, B, and C infections among HIV infected individuals.
2. To determine the prevalence of active Hepatitis B virus hepatitis among HIV infected individuals
3. To determine prevalence of Hepatitis (A, B and C) and HIV co-infection according to socio-demographic characteristics.
4. To compare the clinical characteristics of HIV positive subjects with and without Hepatitis (A, B & C viruses) co-infection.
5. To explore the association between CD 4+ level and Hepatitis A, B and C

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study area

This study was conducted at Muhimbili National Hospital's (MNH) HIV care and treatment centre (CTC). MNH is a public a tertiary hospital located in the largest commercial city of Tanzania namely, Dar es Salaam.

Dar es Salaam city located 6° 48' S and 39° 17 E is the largest city in Tanzania. It has a population of 2.5 million people with the annual growth rate of 4.3% (third fastest in the world) outgrowing the available infrastructure and availability of good housing and sanitation.[109] These conditions increase the risk for disease transmission. Dar es Salaam is also an important city for business having an important harbour and an International airport.

3.2 Study design

This study was a hospital based descriptive cross-sectional study.

3.3 Study population

The study population consisted of HIV positive individuals who attended at MNH Care and treatment clinic (CTC). These individuals were recently HIV diagnosed and referred from voluntary counselling and testing (VCT) centres including those located within the same hospital. They were referred to the CTC for initial work-up and possibly treatment with highly active antiretroviral therapy (HAART).

Consecutive recruitment of newly referred patients was done to get the study participants.

3.4 Study Period

The study was conducted between April 2006 and September 2006. Patients were recruited between 24th April 2006 and 21st August 2006. Analyses were done between August and September 2006

3.5 Inclusion criteria and Exclusion criteria

This study included HIV positive patients at the MNH-CTC, who had no history of HAART use (HAART naive by history). They were adults aged 18 years or more who gave consent to participate. All patients who refused to participate in the study were excluded.

3.6 Sample size and sampling technique

Sample size for this study was calculated using the formula for cross-sectional study; $n= z^2 p (1-p)/ \varepsilon^2$

Where n =expected minimum sample

z = standard, corresponding to 95% confidence; 1.96

P= prevalence of Hepatitis 58.5%[10]

ε = maximum likely error taken as 6%

Hence, minimum sample size calculated was 259.

This study enrolled a total of 260 subjects. Patients who attended MNH CTC were consecutively recruited to the required sample size after an informed consent. The process of recruitment was done just before or soon after their first counselling

session at the CTC. All the new patients at the CTC were asked as to whether they had used HAART for any period before. Only those who gave no history of prior HAART use were enrolled in the study

3.7 Data collection methods

Subjects were interviewed through a face-to-face interviewer assisted questionnaire after which clinical evaluation and HIV staging according to WHO HIV staging criteria were performed. Finally, venopuncture was done to draw about eight (8) mls of blood that was sent to the laboratory for haematological, biochemical and immunological analysis.

The investigator herself performed the first part including, history taking, and examination and clinical staging. One of the phlebotomist at the clinic assisted with the venopuncture for the study, which served as well for the initial work-up of the patient for anti retroviral therapy. Blood was kept in respective vacutainer bottles for haematological, biochemical and immunological analyses. Haematological, biochemical and CD4+ count assays were immediately at the MNH central Pathology laboratory, while samples for Hepatitis viral markers were stored at -20° C in the MUHAS immunology laboratory until the time for analysis. The ELISA test for all the viral hepatitis markers were done between August and September 2006. The details of the laboratory methods are as shown in section 3.7.2 below.

3.7.1 Definition of Terms

1. Prevalence of Hepatitis A infection was taken as the proportion of subjects positive for anti HAV IgM antibody.[6]

2. Prevalence of Hepatitis B infection was taken as the proportion of subjects positive for Hepatitis B surface antigen (HBsAg).[6]
3. Prevalence of Hepatitis C infection was taken as the proportion of subjects positive for anti HCV antibody.[6]
4. Prevalence of Acute Hepatitis B infection was taken as the proportion of subjects positive for anti HBc IgM antibody.[6]
5. Any Hepatitis was considered as the presence of any marker of Hepatitis tested.
6. Hepatomegally was considered as liver span greater than 15 cm.[110]
7. Spleenomegally was considered as palpable spleen below the right costal margin.[110]

3.7. 2 Laboratory Methods

1. Serum Transaminases (ALAT & ASAT)

These were determined using direct spectrophotometric measurements at MNH central Laboratory. The reference values of the laboratory were used for categorisation into high or normal. High levels were considered as $\geq$ 55 IU and $\geq$ 34 IU for ALAT and ASAT respectively. Normal levels were considered below the respective high values.

2. T-Lymphocyte CD4+

CD4+ cell count was determined by flow cytometry using Becton Dickson Facs Count machine.

3. Anti Hepatitis A virus Antibodies (anti HAV IgM) detection

An 'IgM capture' ELISA assay was used in which, IgM class antibodies in the sample are first captured, during the first incubation, by the solid phase (polystyrene microwell strips) pre-coated with anti human immunoglobulin M (hIgM) antibodies.

After washing out all the other components of the sample and in particular IgG antibodies, the specific HAV IgM captured on the solid phase are detected by the addition of a purified preparation of inactivated HAV antigens labelled with an antibody conjugated to horseradish peroxidase (HRP-Conjugate).

During the second incubation, the HRP-conjugated antigens will specifically react only with the HAV IgM antibodies and following second wash to remove unbound HRP-conjugate, Chromogen (substrate) solution is added to the wells.

In presence of the (anti-**μ**)-(HAV-IgM)-(antigen-HRP) immunocomplex, the colourless Chromogens is hydrolysed by the bound HRP conjugate to a coloured end product whose optical density may be measured and is proportional to the amount of HAV-antibodies captured in the wells, and to the sample respectively. Wells containing samples negative for HAV-IgM remain colourless. ELISA reader was used and results interpreted as per instruction from the manufacturer. (Adaltis – EIAgen Anti HAV IgM kit)

4. Hepatitis B Surface Antigen (HBsAg) detection

A mouse monoclonal antibody specific for HBsAg is fixed to the surface of microwells. Patient's serum/plasma is added to the microwells together with a

second mouse monoclonal antibody, conjugated with Horseradish Peroxidase (HRP) and directed against a different epitope.

The specific Immunocomplex, formed in the presence of HBsAg in the sample, is captured by the solid phase. At the end of one-step incubation, microwells are washed to remove unbound serum proteins and HRP conjugate.

The Chromogen/substrate is then added and, in the presence of captured HBsAg immunocomplex, the colourless substrate is hydrolysed by the bound HRP conjugate to a coloured end product. After blocking the enzymatic reaction, its optical density is measured by an ELISA reader.

The colour intensity is proportional to the amount of HBsAg present in the sample. ELISA reader was used and results interpreted as per instruction from the manufacturer (Adaltis – EIAgen HBsAg kit)

5. Anti Hepatitis B Core IgM Antibody (anti HBcAb) detection

An 'IgM capture' ELISA assay was used in which, IgM class antibodies in the sample are first captured, during the first incubation, by the solid phase (polystyrene microwell strips) pre-coated with anti human immunoglobulin M (hIgM) antibodies.

After washing out all the other components of the sample and in particular IgG antibodies, the specific HBc IgM captured on the solid phase are detected by the addition of a purified preparation of a recombinant HBcAg labelled with a monoclonal antibody conjugated to horseradish peroxidase (HRP-Conjugate).

During the second incubation, the HRP-conjugated antigens will specifically react only with the HBc IgM antibodies and following second wash to remove unbound HRP-conjugate, Chromogen (substrate) solution is added to the wells.

In presence of the (anti-**μ**)-(HBc-IgM)-(antigen-HRP) immunocomplex, the colourless Chromogens is hydrolysed by the bound HRP conjugate to a coloured end product whose optical density may be measured and is proportional to the amount of HBc-antibodies captured in the wells, and to the sample respectively. Wells containing samples negative for HBcAb-IgM remain colourless. ELISA reader was used and results interpreted as per instruction from the manufacturer. (Adaltis – EIAgen Anti Core IgM kit)

6. Anti Hepatitis C Virus Antibody (anti HCV – Antibodies) detection

Antibodies to HCV were determined using ELISA technique. This involves inoculation of diluted sample on micro-well coated with highly purified HCV-specific antigens, derived from core and "ns" regions encoding for conservative and immunodominant antigenic determinants (core peptide, recombinant NS3, NS4 and NS5 peptides). The solid phase is the first treated with the diluted sample and HCV Ab are captured, if present, by the antigens. After washing out all other components of the sample in the second incubation bound HCV antibodies, IgG and IgM as well are detected by the addition of polyclonal specific anti hIgG&M antibodies, labelled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HCV antibodies

present in the sample. A cut-off value let optical densities can be interpreted into HCV antibody negative and positive results. ELISA reader was used to measure the optical densities and results were interpreted as per instruction from the manufacturer. (Adaltis – EIAgen Anti HCV Ab kit).

3.8 Data Quality Control

The investigator performed the interview, physical examination and clinical staging of the subjects. One phlebotomist assisted in the collection of blood samples. Three different laboratory technicians performed haematological, biochemical and immunological analysis of the serum. To assure blinding, confidentiality and concordance between questionnaire and blood sample, only questionnaire identification number (QIN) appeared on the Questionnaire, laboratory form and on the specimen containers of the samples for Hepatitis markers. Samples for haematological, CD4+ counts and biochemical assays had names because they were done in the MNH laboratory. Internal quality control for the hepatitis markers, (given by the producer guidelines) was ascertained before result interpretation.

3.9 Data Processing and Analysis

We entered data into the computer using Epi-info version 6. In order to enhance data cleanliness, data entrants used double entry. Every record was uniquely verified to make sure of the coverage and consistency. Then, data were exported to SPSS version 10 for analysis. We used univariate analyses (frequency tables) when running descriptive procedures to characterize study participants and bivariate analyses for

initial investigations for the associations of different Hepatitis (A, B and C). Unless otherwise stated, Pearson's χ^2 was used to determine the association between hepatitis infections with demography and clinical features. Where Pearson's χ^2 was not applicable due to small numbers in the cells, fisher's exact test was used.

Since the distribution of baseline, absolute CD4+ cell count was skewed to the right baseline absolute CD4+ counts were log transformed before analysis using parametric test could be used. Students' t - test was used to examine the differences in CD4+ counts and age between male and female patients. In addition, t-test was performed to determine the association between CD4+ (after transformation) and hepatitis infections. We also performed multivariate analysis by which multiple logistic regression was used to determine the independent predictors for each hepatitis/HIV co-infection while controlling for co-infections with other hepatitis viruses and a set of pre-determined independent factors. The outcomes were best estimates for best predictors of different Hepatitis (A, B and C). Odds ratios (OR) and associated 95% confidence intervals (CI) were used to quantify weights of predictors and their strengths respectively. Finally, all the hepatitis markers were combined and predictors for any viral (A, B and C) hepatitis/HIV co-infection were determined. $P < 0.05$ was taken to be significant.

3.10 Ethical issues

Ethical clearance was sought from ethical clearance board of the Muhimbili University of Health and Allied sciences (MUHAS). Permission was sought from MNH management to use MNH HIV clinic for the study. Information about the

study and consent form was given to the subjects prior to interview. Informed written consent was required from the individuals who agreed to participate in the study. Post test counselling was done to the patients. Subjects with HIV/Hepatitis co-infection were kept on non-nevirapine containing HAART regimen.

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CHAPTER FOUR

4.0 RESULTS

A total of two hundred and sixty (260) HIV infected patients were recruited during the study period out of whom 70% were females. The overall mean (SD) age of the subjects was 37.3 (± 9.5) with a range of 19 to 81 years. Almost half of the subjects were in the age group 30 – 39 years. Males (mean age of 41.4 years) were significantly older than females (mean age of 35.6 years), $p<0.0001$. The sociodemographic characteristics of the study population are presented in table 1.

As compared to females, a higher proportion of males were married (60.3%) or divorced (12.8%), while a higher proportion of females were either single (28.0%) or widowed (16.5%) $p = 0.04$.

The majority 169 (65.0%) of the study subjects had primary or no education at all while only 10.8% had post secondary education. Almost half 38 (48.7%) of the male subjects were private sector employees. Females were more likely to be unemployed ($p <0.01$) and had lower levels of education compared to male subjects ($p = 0.04$). (Table 1)

Table 1: Distribution of Socio-demographic Characteristics of HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=260)

Characteristic	Male (%) n=78	Female (%) N=182	Total n=260	p-value
Age group				
18 – 29	3 (3.8)	48 (26.3)	51 (19.6)	
30 – 39	38 (48.7)	87 (47.8)	125 (48.1)	
40 – 49	28 (35.9)	31 (17.0)	59 (22.7)	
50+	9 (11.5)	16 (8.8)	25 (9.6)	<0.01
Marital Status				
Single	16 (20.5)	51 (28.0)	67 (25.8)	
Married	47 (60.3)	83 (45.6)	130 (50.0)	
Divorced	10 (12.8)	18 (9.9)	28 (10.8)	
Widowed	5 (6.4)	30 (16.5)	35 (13.5)	0.04
Education				
Primary & below	44 (56.4)	125 (68.7)	169 (65.0)	
Secondary	22 (28.2)	41 (22.5)	63 (24.2)	
Post Secondary	12 (15.4)	16 (8.8)	28 (10.8)	0.04
Occupation				
Government	9 (11.5)	16 (8.8)	25 (9.6)	
Private	38 (48.7)	29 (15.9)	67 (25.8)	
pettytrader/peasant	24 (30.8)	68 (37.4)	92 (35.4)	
Unemployed	1 (1.3)	66 (36.3)	67 (25.8)	
Other	6 (7.7)	3 (1.6)	9 (3.5)	<0.01
Baseline CD4+ count (cells/µl)				
< 200	43 (56.6)	77 (46.1)	120 (49.4)	
200 – 350	19 (25.0)	27 (16.2)	46 (18.9)	
>350	14 (18.4)	63 (37.7)	77 (31.7)	<0.01
WHO-HIV stage				
I	15 (19.2)	45 (24.7)	60 (23.1)	
II	20 (25.6)	47 (25.8)	67 (25.8)	
III	32 (41.0)	72 (39.6)	104 (40.0)	0.66
IV	11 (14.1)	18 (9.9)	29 (11.2)	

Overall, most of the patients 104 (40.0%) were in the WHO-HIV clinical stage III. The proportion of females in WHO-HIV clinical stage I was more than that of males in the same clinical stage (24.7% vs. 19.2%). In contrast, the proportion of females in WHO-HIV stage IV was less compared to that of males in the same clinical stage (9.9% vs. 14.1%). However, the differences were not statistically significant.

The distribution of baseline CD4+ T lymphocyte count was skewed to the right with a median of 202 cells/μl (range 1 - 1665 cells/μl). The median baseline absolute CD4+ T lymphocyte count of 133 cells/μl among males was significantly lower than that of 177 cells/μl among females ($p= 0.01$). The distribution of CD4 count between the sexes is as shown in table 1. When the relationship between WHO-HIV stage and baseline absolute CD4+ T lymphocyte count was examined, there was a significant negative association ($\beta= - 0.003$, $p< 0.0001$). CD4+ results for 17 (6.5%) subjects could not be traced from the MNH Central Laboratory were these tests were performed .However, the group whose CD4+ T lymphocyte count results missed did not differ significantly with those whose results were available in terms of sex, age, and WHO-clinical stage.

Prevalence of Hepatitis

The seroprevalence of anti HAV-IgM, HBsAg, HBcAb and anti HCV were 3.1%, 17.3%, 2.3% and 18.1% respectively among the study population. Acute Hepatitis B (defined as presence of HBc antibodies) was found in 6 patients i.e. (2.3%) out of whom two were negative for HBsAg. Therefore, HBsAg carrier state was considered present in 41 patients i.e. (15.8 %), and the prevalence of HBV by any of the two markers was 18.1%. Co-infection of HBV and HCV occurred in 10 individuals (3.9%) while that of HAV and HBV was detected in two subjects (0.8%). There was no patient who was co-infected with both hepatitides A & C or with all the three viruses together (Table 2). Subjects with active Hepatitis B infection had neither HAV nor HCV co-infections. Besides, there was no association in the occurrence of any pair of the hepatitis viruses.

Table 2: Seroprevalence of Viral Hepatitis Markers among HIV patients recruited at Muhimbili National Hospital-Care & Treatment Centre (n=260)

Marker	Patients with the marker n (%)	95% CI
Anti HAV– IgM antibodies	8 (3.1)	1.3 – 6.0
Hepatitis B surface antigen (HBsAg)	45 (17.3)	12.9 – 22.5
Anti HBc IgM antibodies (HBcAb)	6 (2.3)	0.9 – 5.0
Anti HCV antibodies	47 (18.1)	13.6 – 23.3
HBsAg and HbcAb	4 (1.6)	0.4 – 3.9
HBsAg or HBcAb	47 (18.1)	13.6 – 23.3
HBsAg & anti HCV Abs	10 (3.9)	1.9 – 7.0
Anti HAV antibodies & HBsAg	2 (0.8)	0.1 – 2.8
Antibodies against HAV & HCV	0	
Anti HAV Abs & HBsAg & anti HCV abs	0	
ANY (ABC) Hepatitis marker	90 (34.6)	28.9 – 40.7

Prevalence of Hepatitis (A, B, C) and HIV co-infection according to socio-demographic characteristics

The highest prevalence for Hepatitis A and B were 5.1% and 25.4% respectively, which occurred in the age group 40 – 49. The highest prevalence for HCV and any Hepatitis were 36.0% and 44.0% respectively and occurred in the age group 50+. The prevalence in the different age groups was not statistically significant, table 3. However, when trend test was performed, it showed that the prevalence of antibodies against HCV increases with age ($\chi_{trend} = 4$; $p= 0.04$) and that the oldest age group (50+) had about 4 times increased risk for having HCV compared to the youngest age group of 18 – 29 years.

The prevalence of HBV infection among males (21.8%) was non significantly higher than that among females (15.4%). However, when all the viruses were combined, "any of Hepatitis" prevalence were equal between males (34.6%) and females (34.6%), table 3.

There was no significant association between the educational status and the prevalence of viral hepatitis infections. However, highest proportions of subjects with HCV (20.7%) as well as any hepatitis (36.7%) infections occurred among subjects with primary school education or below. The respective prevalence were 13.2% and 30.8% among subjects with secondary education or above. Similarly among subjects with HAV infection, there was a higher prevalence of 4.4% in those with secondary education or above compared to 2.4% among those with lower level

of education. The prevalence of HBV were similar between the different levels of education for Hepatitis B virus infection (Table 3).

Similarly, the prevalence of viral hepatitis co-infection were not associated with marital status. However, when compared to married and never married subjects, widowed and divorced subjects had the highest prevalence (39.7%) for HCV and any of the Hepatitis co-infection, (39.7% and 27.0%) respectively. Those who were never married had the highest prevalence (20.9%) for HBV infection. These differences were not statistically different table 3.

There was no association between each of the hepatitis viruses and the following risk factors; blood transfusion, illicit drug use, uvulectomy and traditional or cosmetic scarification.

Generally there was an elevated risk for HCV with advancing age of patients.

Table 3: Prevalence of Hepatitis (A, B, C) Co-infection according to socio-demographic characteristics of HIV patients at Muhimbili National Hospital- Care & Treatment Centre (n=260)

	Anti HAV-IgM (%)	HBsAg (%)	Anti HCV (%)	Any Hepatitis (%)	Total number tested n
Age					
18 – 29	2.0	23.5	13.7	31.4	51
30 – 39	2.4	12.8	7.6	30.4	125
40 – 49	5.1	25.4	15.3	42.4	59
50+	4.0	8.0	36.0	44.0	25
p-value	**	0.06	0.09	0.28	
Sex					
Male	1.3	21.8	12.8	34.6	78
Female	3.8	15.4	20.3	34.6	182
p-value	0.442*	0.211	0.149	1.0	
Education					
Primary& Below	2.4	17.2	20.7	36.7	169
Secondary& Above	4.4	17.6	13.2	30.8	91
p-value	0.456*	0.932	0.133	0.339	
Marital status					
Never Married	3.0	20.9	17.9	35.8	67
Currently Married	3.8	17.7	3.8	31.5	130
Divorced/ widowed	1.6	12.7	27.0	39.7	63
p-value	**	0.460	0.084	0.522	

Key: * = fishers' exact test ** = no test done due to small numbers. "**Any hepatitis**" = positivity of any of the viral markers tested antibodies.

Hepatitis co-infections and clinical characteristics

The prevalence of various markers of hepatitis were not significantly different across the different WHO-HIV clinical stages even when stratified by sex. However, the values were too small to make the stratified analysis for hepatitis A. The highest prevalence of HCV infection occurred among those with WHO HIV clinical stage one (25%), whereas the highest prevalence of HBV (28%) occurred among those with stage IV disease. The proportions of HAV infected subjects among the four clinical stages were similar.

Few signs of chronic liver disease were observed among the study subjects. These included finger clubbing in 15/260 (5.8%) and parotid enlargement in 5/260 subjects (1.9%). Out the five subjects with parotid enlargement, two (2) subjects had only HCV infection (p=0.20), while the remaining had neither HBV nor HAV infections. A higher proportion of subjects with finger clubbing (53%) had any of the viral hepatitis markers compared to those without finger clubbing (34%), however; the difference was non significant (p = 0.12). No subjects had leukonychia, palmar erythema, dupuytrens contracture, caput medusae, or spider naevi. Three subjects had tattoos and none of these had any of the viral hepatitis infection.

Clinical features of Hepatitis A co-infected patients

As shown in table 4, a significantly higher proportion of patients co-infected with HAV presented with nausea or vomiting when compared with those not having HAV co-infection. It was found that the risk of having HAV infection was 6 and 4 times more in those with nausea and vomiting respectively ($p<0.01$ and $p=0.04$) respectively. Similarly, a higher proportion of HAV co-infected patients presented with fever, but the difference was not statistically significant. However, the proportion of patients with anorexia was 12.5% among the HAV co-infected, as compared to 23.4% among the non-HAV co-infected ($p=0.69$). Similarly, none of the 8 subjects co-infected with HAV gave a history of jaundice or were found to have jaundice on examination and all had normal liver spans (table 4).

Table 4: HAV co-infection and clinical characteristics among HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=260)

Clinical feature	HAV +ve No (%)	HAV -ve No (%)	RR	p*
Fever	3 (37.5)	88 (34.9)	1.1	1.00
Diarrhoea	1 (12.5)	29 (11.5)	1.1	1.00
Nausea	**4 (50.0)**	**21 (8.3)**	**6.0**	**< 0.01**
Vomiting	**3 (37.5)**	**23 (9.1)**	**4.1**	**0.04**
Anorexia	1 (12.5)	59 (23.4)	0.5	0.69
Jaundice by History	0	8 (3.2)	N.D	0.78
Jaundice by Examination	0	16 (6.3)	N.D	1.00
Abdominal Pain	1 (12.5)	16.3 (41)	0.8	1.00
Hepatomegally	0	12.3 (31)	ND	0.60

* Fishers exact test; ND= not determined

Clinical features of Hepatitis B co-infected patients

It was generally, more likely for subjects with HBV co-infection to report various symptoms compared to those without the co-infection despite the fact that the association was non significant. However, anorexia and hepatomegaly were more frequent among those who were negative for HBsAg than those who were infected. These differences however, were not significant. (Table 5)

Table 5: Presence of HBsAg and clinical characteristics among HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=260)

Clinical feature	HBsAg +ve no (%)	HBsAg -ve no (%)	RR	p-value
Fever	17 (37.8)	74 (34.4)	1.1	0.67
Nausea	5 (11.1)	20 (9.3)	1.2	0.71
Vomiting	6 (13.3)	20 (9.3)	1.4	0.41
Anorexia	6 (13.3)	54 (25.1)	0.5	0.09
Jaundice by History	2 (4.4)	6 (2.8)	1.6	0.63
Jaundice by Examination	5 (11.1)	11 (5.1)	2.2	0.17
Abdominal pain	7 (15.6)	35 (16.3)	1.0	0.91
Hepatomegally	5 (11.1)	26 (12.1)	0.9	0.85
Spleenomegally	2 (4.4)	5 (2.3)	1.9	0.35

Six (6) subjects had acute hepatitis, these subjects were more likely to be symptomatic than those without active hepatitis. Jaundice by history (33.3%), jaundice on examination (66.7%) and hepatomegally (66.7%) were reported with significantly higher frequencies in those with acute hepatitis B infection than those without the infection (Table 6). Four subjects out of those with active hepatitis (66.7%) had both jaundice on examination and hepatomegally. They all had a baseline CD4+ count of 200(cells/μl) or below. The remaining two (33.3%) of those with Active Hepatitis but with no jaundice had baseline CD4+ counts of 678 and 715 cells/μl.

Table 6: Presence of HBc antibodies and clinical characteristics among HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=260)

Clinical feature	HBcAb +ve no (%)	HBcAb -ve no (%)	RR	p-value*
Fever	2 (33.3)	89 (35.0)	1.0	1
Nausea	1 (16.7)	24 (9.4)	1.8	0.46
Vomiting	1 (16.7)	25 (9.8)	1.7	0.47
Anorexia	3 (50.0)	57 (22.4)	2.2	0.14
Jaundice by History	**2 (33.3)**	**6 (2.4)**	**13.9**	**0.02**
Jaundice by Examination	**4 (66.7)**	**12 (4.7)**	**14.2**	**< 0.01**
Abdominal pain	3 (50.0)	39 (15.4)	3.7	0.06
Hepatomegally	**4 (66.7)**	**27 (10.6)**	**6.3**	**< 0.01**
Spleenomegally	0	7 (2.8)	ND	1.00

Key: p-value* = fisher's exact test

Clinical features of Hepatitis C co-infected patients

HCV co-infection was not significantly associated with any of the clinical characteristics (Table 7). However, the proportion of symptomatic subjects was higher in the HCV infected than in those non-infected. Among HCV infected, jaundice by history (4.3%), jaundice on examination (8.5%), and abdominal pain (19.1%) were more frequent in HCV infected than in those non-infected (Table 7). Out of all with HCV infection, one subject (2.1%) had a reduced liver span of 6.4cm.

Table 7: Presence of anti HCV antibodies clinical characteristics among HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=260)

Clinical feature	HCV +ve no (%)	HCV -ve no (%)	RR	P-value
Fever	18 (38.3)	73 (34.3)	1.1	0.60
Nausea	7 (14.9)	18 (8.5)	1.8	0.18*
Vomiting	4 (8.5)	22 (10.3)	0.8	1.00*
Anorexia	11 (23.4)	49 (23.0)	1.0	0.95
Jaundice by History	2 (4.3)	6 (2.8)	1.5	0.64*
Jaundice by Examination	4 (8.5)	12 (5.6)	1.5	0.50*
Abdominal pain	9 (19.1)	33 (15.5)	1.2	0.54
Hepatomegally	3 (6.4)	28 (13.1)	0.5	0.19
Spleenomegally	1 (2.1)	6 (2.8)	0.8	1.00*

Key:* = Fisher's exact test

Clinical features of 'Any hepatitis' co-infected patients

When all hepatitis viral markers checked were combined as one variable (any hepatitis), only nausea remained to be significantly associated to any viral hepatitides infection (Table 8). Nausea was reported by 16% of those with any Hepatitis, and these subjects had twice an increased risk of having any hepatitis (p=0.02). For the other symptoms, association with any hepatitis infection was non-significant. The general trend with exception of anorexia and hepatomegally was an increased proportion of symptomatic subjects among those with any hepatitis co-infection than those without any of the co-infection. A higher proportion of subjects without any viral hepatitis infection had hepatomegally (12.9%) compared to those with any viral hepatitis (10.0%) infection; however, the difference was non-significant (p=0.49) (Table 8)

Table 8: Presence of 'Any hepatitis' marker and clinical features among HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=260)

Clinical feature	Any hepatitis +ve no (%)	Any hepatitis -ve no (%)	RR	P-value
Fever	32 (35.6)	59 (34.7)	1.0	0.89
Nausea	**14 (15.6)**	**11 (6.5)**	**2.4**	**0.02**
Vomiting	11 (12.2)	15 (8.8)	1.4	0.39
Anorexia	19 (21.1)	41 (24.1)	0.9	0.58
Diarrhoea	11 (12.2)	19 (11.2)	1.1	0.80
Jaundice by History	5 (5.6)	3 (1.8)	3.1	0.13
Jaundice by Examination	9 (10.0)	7 (4.1)	2.4	0.06
Abdominal pain	17 (18.9)	25 (14.7)	1.3	0.38
Hepatomegally	9 (10.0)	22 (12.9)	0.7	0.49
Spleenomegally	4 (4.4)	3 (1.8)	2.4	0.24

Biochemical derangements

It was found that despite the high prevalence of markers for the viral hepatitis (A, B, C) among the study patients, with the exception of acute hepatitis, there were no significant concomitant derangements in serum levels of serum transaminases; namely ALAT and ASAT. Only 1 (12.5%) of anti HAV-IgM antibody, 4 (8.9%) of HBsAg 2 (4.3%) of anti HCV antibody and 9(10%) of any Hepatitis viral marker positive patients had raised ALAT. When these proportions were compared to non-hepatitis infected subjects, the differences were non-significant. A similar pattern was observed when all the hepatitis viruses were considered together as 'any

hepatitis'. However, acute HBV (HBcAb positive) infection was significantly associated with a raise in ALAT ($p<0.0001$) but not ASAT.

When clinical features and biochemical markers were considered together, only 17/90 (18.9%) of patients with any of the markers for hepatitis had jaundice (history or examination), hepatomegally or high ALAT.

In multivariate analysis, nausea remained to be an independent predictor for occurrence of hepatitis giving a three fold increased risk for any hepatitis ($p=0.02$) and even higher (4 times) for HAV infection $p<0.01$ (Table 9). Blood transfusion was significantly associated with HBsAg positivity; however, this variable did not show any association at univariate analysis. Active hepatitis can be predicted with a finding of jaundice on clinical examination only and the risk was tremendously high, 41 times higher in those with active hepatitis than those without active hepatitis, however the confidence interval was very wide indicating lack of precision due to the small numbers with the active HBV.

Table 9: Summary of independent predictors of markers of viral hepatitis (A,B,C) among HIV patients at Muhimbili National Hospital-Care & Treatment Centre (n=243)

Outcome	Predictor	OR	(95% CI)	p-value
HAV	Nausea	4.18	(1.46 – 11.96)	**<0.01**
HBsAg	Blood transfusion	2.59	(1.09 – 6.15	**0.03**
HBcAb	Jaundice on examination	41.07	(6.78 – 248.89)	**<0.01**
Any hepatitis	Nausea	3.03	(1.20 – 7.21)	**0.02**

Summing up, most of the hepatitis infections were not significantly associated with specific clinical features related to hepatocellular damage e.g. jaundice and hepatomegally. Nevertheless, non-specific symptoms like nausea, and history of blood transfusion emerged as important predictors and should raise an alarm on the possibility of the infection.

/iral hepatitis co-infection and Immunological status

ıs shown in table 10 below, the mean baseline absolute CD4+ counts did not vary onsistently across the different hepatitis viral markers. However, no marker of epatitis used was significantly associated with baseline absolute CD4+ counts. levertheless, among patients in whom HBsAg or anti HCV antibodies was positive, ıeir mean baseline CD4+ count was slightly higher than that among the un-infected roups. On the contrary, the opposite was observed with anti HBcAb and anti HAV-gM antibodies. However, the differences were not significant (table 10). Similarly, t Multivariate analysis CD4+ T-lymphocytes was not found to be a predictor of any f the three viral hepatitis studied.

'able 10: Comparison of Geometric mean CD4+ T lymphocytes and Hepatitis ıfection among HIV patients at Muhimbili National Hospital- CTC (n=260)

Variable	Geometric mean CD4+ cells/µl	t-statistic	P-values
Anti HAV Ab			
+ve	113	0.32	0.57
-ve	152		
HBsAg			
+ve	198	1.85	0.18
-ve	143		
Anti HBcAb			
+ve	134	0.04	0.84
-ve	152		
Anti HCV Ab			
+ve	222	3.81	0.05
-ve	139		
Any Hepatitis			
+ve	188	2.99	0.09
-ve	135		

N.B: n=247, since CD4+ counts results for 17 patients were lost

CHAPTER FIVE

5.0 DISCUSSION

This study was conducted among HAART naïve HIV infected persons who were seeking care and treatment at MNH-CTC. The aim was to determine the prevalence of hepatitis A, B & C and to describe clinical and demographic characteristics of HIV/Hepatitis co-infected patients.

In this study, hepatitis C virus was the commonest of the three hepatitis viruses, with a seroprevalence of 18.1%. The seroprevalence of hepatitis C found in this study was higher than that reported in previous studies (0– 1.5%) among blood donors in the same hospital.[31, 111] Both the previous studies dealt with blood donors, a population with much less risk for HCV as compared to the current study population, all of whom were HIV infected. Moreover, we used a serological method (ELISA) in detecting HCV infection while Waddell co-workers detected HCV RNA, [111] that is a more stringent assay. It has been documented that HCV viremia is detectable in 60 – 90% of HIV co-infected HCV antibody positive patients.[65, 66] Thus, the methodological differences may contribute, at least in part, to the observed difference. A study conducted in North Western part of Tanzania involving a general population in Tanzania, reported a HCV prevalence of 9.0%.[112] The higher prevalence found among the HIV infected individuals in the present study, suggest an increased risk for HCV among our study population. Other developing countries have also reported higher prevalence of HCV among HIV infected compared to non-HIV infected individuals ranging between 53 to 59%.[10, 37] This difference could partly be explained by variations in the risk for HCV infection through the different

modes of transmission in the studied populations.[10,73-74] For example, the predominant mode of HCV transmission in our population is probably heterosexual, which is a less efficient mode of transmission of HCV as compared with intravenous drug abuse.[10] Indeed, in our study population there was only one intravenous drug user.

The seroprevalence of HBsAg found in this study (17.3%) is much higher than that observed in the same institution among blood donors of 11.0% in 1999 and 8.8% in 2000.[30, 31] This is to be expected since all of our study patients were HIV infected and are therefore more likely to have HBsAg.[35,37,38,40] The two subjects who were HBcAb positive but HBsAg negative could possibly be in the transition between disappearance of HBsAg and seroconversion to Anti – HBs.[6] This implies that a proportion of patients with HBV infection can be missed when HBsAg is used for the diagnosis of HBV. The patients who were HBsAg positive but HBcAb negative are likely to be those with persistent Hepatitis B infection[6] or having an acute hepatitis B infection, before the appearance of IgM antibodies to HBc antigen.[6]

In this study the magnitude of HBV/HCV co-infection was low (4%), and as with previous studies done in the country the occurrence of the two viruses was not linked.[30, 31] Collectively, this seems to suggest that in our setting the two viruses do have different epidemiology.

The prevalence of HAV in this study (3%) was much lower than that reported by Miller and colleagues who found a prevalence of 99.0% among the general population using anti HAV – IgG antibodies.[8] This observed difference in the results

between Miller et al and our study is due to the differences in methods of detecting evidence of HAV infection. In this study, we detected IgM antibodies to HAV, which are the markers of acute HAV infection[6], while Miller's study checked for the presence of IgG antibodies, which are indicative of past exposure.[6] A recent study conducted in Kenya reported a higher prevalence of 41.7% of IgM antibodies to HAV among hospitalized jaundiced patients.[9] The relatively higher prevalence of IgM antibodies to HAV in the Kenyan study is probably because all participants had clinical hepatitis as manifested by a history of jaundice.

In this study, except for Age and HCV infection, demographic characteristics did not influence the occurrence Hepatitis infections. Our results shows that HCV co-infection was associated with increasing age, which may reflect a cumulative risk over time as previously reported by Kondili.[113] The increase in HCV prevalence with age seem to suggest that factors other than vertical transmission play a role in acquisition of HCV in this population. HBV infection was not associated with age, sex or marital status. On the contrary, other studies had reported a significant association between HBV infection with increasing age, [114, 115] being widowed or divorced, and having no education.[114] Unlike these two studies, ours was an HIV infected population, and since HIV and Hepatitis B share modes of transmission this may confound the difference.

Generally, subjects co-infected with the hepatitis viruses showed minimum clinical features, most of which were non-specific. For specific clinical features, the only

significant association was with acute HBV infection and jaundice, abdominal pain and hepatomegally, features that have been previously documented.[6]

On the other hand, nausea or vomiting were features that were significantly associated with HAV and 'any hepatitis' marker checked (anti HAV IgM antibody, Hepatitis B surface antigen, anti HBc IgM antibody, anti HCV antibody). Although non-specific, these symptoms have been documented in individuals with hepatitis[6] and therefore should prompt the attending physician to think of the possibility of hepatitis co-infection. The paucity of specific clinical features has been a common observation among HIV infected populations [40, 41] and this could probably be due to a decreased hepatocellular injury as a result of HIV induced immunodeficiency.[43]

The proportion of our study subjects with hepatitis virus A, B or C co-infection and an elevated serum ALAT was as low as 10%. Only acute HBV co-infection (HBsAg) was significantly associated with raised ALAT. The relatively low proportion of subjects with raised ALAT among the hepatitis co-infected group could to be due HIV-induced immunosuppression that reduces the severity of liver disease.[43] For example, in a study that was conducted in Beijing, China, 58.5% of HIV/HCV co-infected had abnormal liver function tests compared to 85.5% among those HCV infected but with no HIV.[88] Based on our study, 73 (81.1%) HIV-infected subjects with viral hepatitis co-infection had neither raised ALAT nor specific clinical features (jaundice, hepatomegally) and would be certainly missed if more rigorous investigation are not done. Apparent from these findings, is the fact that it is prudent

to screen for presence of hepatitis viruses using the various markers, as we cannot rely on the serum transaminases only as a guide to the choice of HAART regimen.

Some agents effective in the treatment of HIV are as well effective against viral Hepatitis.[54, 57, 58] Screening for the hepatitis viruses before initiation of HAART will therefore make sure that the choice of HAART will include agents effective against the Hepatitis infection whenever available. Furthermore, HAART regimen with increased risk for toxicity in the presence of hepatitis co-infection e.g. Nevirapine[51] will be avoided.

Additional observations in this study were that hepatitis viral co-infection with HIV was not associated with baseline absolute CD4+ counts. As previously stated, the relationship between hepatitis viral co-infection and CD4+ T lymphocytes is controversial.[82, 84, 87] Similar to our study, some studies, did not find any relationship between HCV and either absolute CD4+ or CD4+ percentages.[48, 87] Nevertheless, hepatitis co-infection with HIV was reported to be significantly associated with low CD4+ count in some studies.[48, 92] It has also been reported that HCV was predictive of a slightly higher increase in mean CD4+ lymphocyte percentage over time following initiation of HAART.[89] These differences may be explained by the fact that in hepatitis/HIV co-infection the loss of immunological control of the infection is due to the reduction in CD4+ specific response i.e. IFN γ which may be unrelated to the actual number of CD4+ T-lymphocytes.[116]

5.1 STUDY LIMITATIONS

In this study, primarily due to financial constraints, it was not possible to confirm the serological tests either with a repeated ELISA, Immunoblot or viral detection. We may have had individuals with HCV for example whom have cleared the viremia. Similarly, genotyping was not possible. In addition, only a few markers of hepatitis infection were included. It would indeed have been interesting to find the proportion of patients with HBsAg having HBeAg inorder as to determine risk of infectivity of these patients.[33]

Another limitation was the fact that baseline absolute CD4+ count results for 17 subjects were unavailable, this meant exclusion of these subjects from analyses that required use of the variable and in multivariate analysis. However, this group did not differ significantly with those whose results were available in terms of sex, age, and WHO-clinical stage.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

This study has revealed important facts in the management of HIV- infected individuals. The prevalence of hepatitis A, B and C co-infections are high among the HIV infected subjects, and they do not necessarily present themselves with specific clinical features. Moreover, majority of these infections have accompanying normal serum transaminases.

The finding of high proportion (81.1%) of patients with hepatitis co-infections without specific clinical features or deranged biochemical markers, demonstrates the need to investigate HIV-positive patients for occult hepatitis infection prior to initiating HAART. In view of this, there is a need to design a cost effective testing mechanism for the diagnosis of these infections.

Further studies to determine the prevailing genotypes of HCV will be useful for therapeutic decisions, prognostication and designing appropriate vaccines in the future. There should also be studies to determine the natural history of hepatitis co-infection among the HIV infected in our settings

CHAPTER 7

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