"THE PREVALENCE OF HBV, HCV INFECTIONS AND ABNORMAL LIVER FUNCTION IN PATIENTS ON LONG-TERM HAART AT KENYATTA NATIONAL HOSPITAL."

A DISSERTATION SUBMITTED IN PART FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE (INTERNAL MEDICINE) OF THE UNIVERSITY OF NAIROBI.

BY

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DECLARATION

I declare that this is my original work and has not been presented for a degree elsewhere.

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DEDICATION

This work is dedicated to my wife Edith, my daughter Elaine and my mother Mary.

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ABBREVIATIONS

AIDS: Acquired immune deficiency syndrome

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

ALP: Alkaline phosphatase

CCC: Comprehensive care clinic

DNA: Deoxyribose Nucleic Acid

ELISA: Enzyme linked immunosorbent assay

HAART: Highly active antiretroviral therapy

HBV: Hepatitis B virus

HCV: Hepatitis C virus

HBeAg : Hepatitis B e antigen

HBcAg: Hepatitis B core antigen

HBsAg: Hepatitis B surface antigen

HIV: Human immunodeficiency virus

IgM anti HBc: Immunoglobulin M antibodies against hepatitis B core antigen.

IgG anti HBc: Immunoglobulin G antibodies against hepatitis B core antigen

IVDU: Intravenous drug users

Kd: Kilodalton

KDHS: Kenya Demographic and Health Survey

KEMRI: Kenya Medical Research Institute

KNH: Kenyatta National Hospital

NNRTI: Non nucleoside reverse transcriptase inhibitor

PCR: Polymerase chain reaction

RNA: Ribose Nucleic Acid

SPSS: Statistical package for social sciences

WHO: World Health Organization

YMDD: Tyrosine-methionine-aspartate-aspartate

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1. My supervisors Professor Lule, Dr Joshi M.D. Dr Okoth F.A and Dr Omu Anzala for guiding me through the study.
2. Dr Okoth F.A for the HbsAg assays that were done at the KEMRI hepatitis laboratory free of charge.
3. Mr Julius Tuei of KEMRI hepatitis laboratory for assisting me with the serological assays.
4. The staff KNH CCC laboratory for running the liver function tests.

ABSTRACT

Background

Hepatitis B and C viral infections are highly prevalent among HIV-infected persons as a result of shared transmission routes. Highly active antiretroviral therapy (HAART) has led to a reduction in HIV related opportunistic infections and death rates, however chronic viral hepatitis has become a major source of morbidity and mortality in HIV-infected populations. This is in part related to the effects of HIV on natural history of HBV/HCV with an increase in liver related complications and death rates, HAART associated hepatotoxicity and HBV/HCV immune reconstitution syndrome.

Objective

The aim of the study was to determine the prevalence of HBV, HCV infections and abnormal liver function in patients on long term HAART at Kenyatta National Hospital (KNH).

Study design and setting

The study design was a cross-sectional descriptive survey at the KNH CCC, Nairobi Kenya.

Methods

Patients aged 18 years and above who had been on HAART for at least one year were recruited. A history was obtained, physical examination done, and blood taken for HbsAg, HCV antibody and liver function tests.

Results

57 (60%) females and 38 (40%) males aged 22 years to 59 years with a median age of 38 years were studied. The prevalence of HBV and HCV were 4.2% and 3.2% respectively. No patient had dual infection with HBV and HCV. No patient reported history of IVDU or homosexuality.

The prevalence of abnormal liver function was 4.2% (95 % CI 3.2-5.2) in the entire group; however in the subgroups with HBV and HCV infections it increased to 28.6% (95% CI 4.9-61.6).

Conclusions

The prevalence of HBV in patients on long term HAART was similar to that reported in the general population. The prevalence of HCV was higher than that reported in blood donors. Majority of patients on long term HAART (95.8%) had normal liver function.

LITERATURE REVIEW

INTRODUCTION

Hepatitis B (HBV) and hepatitis C (HCV) viral infections are highly prevalent among HIV-infected persons, generally as a result of shared transmission routes (1). Improved survival due to the success of highly active antiretroviral therapy (HAART) has enabled conditions with long latency, such as chronic viral hepatitis, to become a major source of co-morbidity in HIV-infected populations (2).

HIV modifies the natural history of HBV, with higher rates of chronic HBV infection, replicative disease, and progression to advanced liver disease among persons with HIV/HBV co-infection (2). The impact of HBV on HIV natural history is less certain (1, 3). HIV also modifies the natural history of HCV infection, with clear evidence of higher HCV viral load and accelerated liver disease progression in persons with HIV/HCV co-infection (4).

As with HBV, there is contradictory evidence on the effects of HCV on HIV disease progression (5). Several studies before the era of HAART showed no impact of HCV on HIV disease progression (6, 7) while others suggested accelerated HIV disease progression (8, 9). A recent longitudinal cohort study demonstrated inhibited CD4 cell recovery following commencement of HAART in persons with HIV/HCV co-infection (10). Patients co-infected with HBV/HCV on HAART have a higher risk of HAART associated hepatotoxity that can interrupt HIV therapy and cause significant morbidity and mortality (11, 12).

Most studies done locally have looked at prevalence of hepatitis virus infections in mainly non –HIV cohorts e.g. in patients with primary hepatocellular carcinoma, Sickle cell disease, patients on maintenance haemodialysis, blood donors and patients with acute icteric hepatitis (13,14,15,16,17).

No study done locally has looked at the prevalence of HBV and HCV in HIV patients on HAART despite the known interactions between these diseases and the potential for life threatening hepatotoxicity related to HAART in this patient group. It is against this background that this study was intended.

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PREVIOUS STUDIES

E.O. Ogutu and colleagues studied the prevalence of HBsAg among patients with AIDS at KNH in 1990,and found the prevalence of HBsAg to be 12.2% which was similar to that reported in the Kenyan community without AIDS (18). It is important to note that the national prevalence of HIV then was 3% compared to 7% in 2003(19), the prevalence of HBV could also have changed over the past 16 years. The study did not look at hepatitis C prevalence in those patients.

Atina J.O et al in 2002 studied the prevalence of hepatitis A, B, C and HIV seropositivity among patients with acute icteric hepatitis at KNH and found acute hepatitis B (IgM anti-HBc) in 26.2%, HBsAg in 25% and hepatitis C antibodies in 7.1% of the patients.30.1% of the patients tested positive for HIV. The HIV/HBV coinfection rate was 22.7% while HIV/HCV was 0% (17). It is worth noting that this study looked at patients with acute icteric hepatitis while most HIV positive patients with hepatitis are asymptomatic and the hepatitis tends to be chronic. Majority of the patients in this study (69.9%) were HIV negative.

Karuru J.W et al in 2004 looked at the seroprevalence and genotypes of HCV infection and prevalence of HIV co-infection among various broad subgroups of the Kenyan population. She found a low prevalence of HCV/HIV co-infection at < 0.02% among blood donors. Among medical in patients the HCV/HIV co-infection rate was 3.7% (16). The study population comprised various subgroups of the Kenyan population some of which were not tested for HIV.

F.N Nakwagala et al in 2002 compared the frequency of exposure to HBV infection between HIV positive and HIV negative medical outpatients at Mulago Hospital and found the frequency of anti-HBc among HIV positive patients to be 65.1% compared to 41.9% in HIV negative patients (20).

H.Lodenyo et al in 2000, studied HBV and HCV infections in AIDS patients at Chris Hani Baragwanath Hospital and found evidence of previous and present HBV infection in 41%. HCV infection rate was 1 %(21). Studies from the west report HBV/HIV and HCV/HIV co-infection rates of 5-10% and 15-30% respectively. (22-26)

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HEPATITIS B AND HIV

Hepatitis B is common in HIV infected persons. In one study, over 90% of patients with HIV had HBV markers of current or past infection (27).

The most common risk factors for acquiring HBV, similar to HIV, are sexual intercourse, including both heterosexual and homosexual activity, blood transfusion, and injections with contaminated needles, tattooing and scarification. Horizontal transmission is responsible for most spread in Africa.

Vertical transmission occurs with 50% of children born to infected mothers getting infected and 90% of these becoming chronic carriers. Studies in Kenya have shown that vertical transmission is rare compared to South East Asia (28, 29).

Although the prevalence of past exposure is high (90-95%), active infection with HBV occurs in 10-15% with HIV infection (22-26).

VIROLOGY OF HBV

HBV is a member of the family Hepadnaviridae and the subgroup human hepatitis virus. It has a partially double stranded DNA genome and an envelope surrounding a spherical nucleocapsid.

The preS-S (presurface-surface) region of the genome encodes for the 24Kd protein HBsAg.The preC-C (precore-core) region encodes hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg), a 16kd fragment that is secreted into the blood.

HBeAg plays no role in viral replication or assembly and its function is not clear.

The X open reading frame region encodes the viral X protein (HBx), which modulates host cell signal transudation and can affect host and viral gene expression.

X-protein activity is absolutely required for the in vivo replication and spread of the virus.

During replication, which occurs in the liver, the HBcAg particles remain in the hepatocyte and only leave the cell after encapsulation with HBsAg. In contrast HBeAg is secreted into the circulation where it provides a convenient, readily detectable qualitative marker of HBV replication and relative infectivity.

Soon after infection HBsAg appears in the circulation preceding elevation of aminotransferases in serum and clinical symptoms. It disappears during convalescence at about the 24th week from the time of exposure when anti-HBs antibodies become detectable. HBsAg persists beyond 6 months if chronic disease or carriage follows acute infection.

Anti-HBc antibodies are also elaborated by the host, initially of IgM type but later replaced by IgG, which last indefinitely.

HBeAg appears concurrently with or soon after HBsAg and disappears when peak transaminase levels are reached and before HBsAg becomes undetectable. Anti-Hbe becomes detectable from then onward.(30)

EFFECT OF HIV ON HBV

HIV infected patients co-infected with HBV have higher rates of chronic hepatitis B infection. Reactivation of infection may occur despite seroconversion to anti-HBs antibody particularly if the CD4 count is low.

Rates of seroconversion from HBeAg to anti-HBe are lower in HIV infected patients.

HBV infected patients who acquire HIV infection demonstrate reduction in anti-HBs antibody and HBsAg may reappear.

Progression of liver disease to cirrhosis and decompensation is more common indicating accelerated fibrosis in HIV infected patients. This may be related to factors such as flares associated with immune restoration or increased direct pathogenicity in this patient group. (1, 23, 24, 31, 32)

IMPACT OF HBV ON HIV

In contrast to the negative effect of HIV on HBV disease, the impact of HBV on HIV disease is less clear (33, 34). However, co-infection with HBV or HCV has been associated with increased hepatotoxicity to highly active antiretroviral therapy (HAART) (35, 36). Consequently, its recognition and treatment has received increasing attention.

Use of interferon for HBV in HIV has been disappointing and a recent trial utilizing 5 MU of interferon alpha-2b three time a week for 24 weeks in chronic active HBV showed lower loss of HBV DNA (27% vs 56%), less HBV E antigen seroconversion (15% vs 52%), and higher reactivation rates following therapy (35% vs 9%) in HIV coinfected patients compared to HIV uninfected HBV controls (37).

Continued use of lamivudine results in a high rate (20%/year) of HBV resistance associated with the emergence of YMDD mutations in co-infected patients as well as immunocompetent HBV patients (38). Therefore, other agents for HBV are needed. Newer medications such as adefovir and tenofovir are effective against lamivudine resistant HBV and are associated with low rates of HBV viral resistance (39, 40).

HEPATITIS C VIRUS AND HIV

It is estimated that from 1% to over 30% of HCV patients may be co-infected with HIV (22, 41). The number is much higher in patients infected through IVDU or hemophiliacs who received contaminated blood products than in patients who acquired the infection through sexual practices (42).

Since the introduction of HAART and the dramatic improvement in the life expectancy of HIV infected individuals, the impact of HCV on mortality and the development of hepatocellular carcinoma have become more evident (43,44). Recent studies in HIV/HCV co-infected patients have demonstrated that HCV is the leading non –AIDS cause of death in these subjects, and end stage liver disease due to HCV infection accounts for up to 50% of all deaths (45,46).

HIV and HCV share common routes of transmission and there is increasing evidence that sexual and mother to child transmission of HCV is facilitated by HIV infection (47-49). HCV is approximately 10 times more infectious than HIV through percutaneous blood exposures (50). Sexual contact is a relatively inefficient mode of transmission. Vaccines are currently not available to prevent HIV or HCV infection.

VIROLOGY OF HCV

HCV is an enveloped, linear, single stranded RNA virus, which is distantly related to flaviviruses. It has a genome of about 94 nucleotides with an amino acid and a carboxyl group terminal, and multiple regions of the genome representing multiple viral protein antigens. This gives rise to the diverse nucleotide sequencing which is found among HCV isolates from different geographic areas (51)

There are six main genotypes designated 1 to 6 and multiple subtypes designated a, b,c etc and their global distribution varies widely. In the United States and Western Europe genotypes 1a and 1b are the most common, followed by genotypes 2 and 3.Genotype 4 is common in Egypt, South Africa genotype 5 and South East Asia genotype 6. (52). Knowledge of the genotype is important because it has predictive value in terms of response to antiviral therapy, with better responses associated with genotypes 2 and 3 than genotype 1.Genotypes 1b and 4 are associated with severe liver disease (51,52). There is a very high spontaneous nucleotide substitution rate of HCV at each nucleotide site each year, which explains the emergence of mutant strains of HCV and persistence of infection (53).

HCV infection is diagnosed by demonstrating anti HCV antibodies in serum.1st generation assays detect antibodies to the non-structural protein C100-3 between 1 and 3 months after the onset of acute hepatitis.2nd generation assays detect antibody to C100-3, C22 and C33 and become positive from the 4th week. 3rd generation assays also detect anti HCV from 4th week but lack specificity for genotypes other than type 1. 4th generation ELISA assays can detect types 2 and 3a as well (54). Thus 4th generation ELISA assays are better since they detect spectrum of antibodies, genotypes and do so earlier in the course of the illness.

Other tests such as the recombinant immunoblot assay (RIBA) and HCV RNA PCR are used to confirm the diagnosis. Detection of HCV RNA by PCR is the most sensitive method of detecting HCV infection. HCV RNA can be detected even before aminotransferase elevation and before the appearance of anti HCV in acute hepatitis. The 4th generation ELISA assay is the current gold standard in clinical practice and for population screening. HCV RNA by PCR is used to confirm diagnosis, determine HCV genotype and monitor treatment (54).

EFFECT OF HIV INFECTION ON HCV

Infection with HCV can be self-limited (viral clearance), can persist without causing clinical disease, or can lead to cirrhosis or hepatocellular carcinoma (55). Infection with HIV appears to adversely affect each outcome. Although approximately 20% of persons clear HCV RNA from their blood after acute infection, HCV clearance occurs in only 5% to 10% of HIV-infected persons, less frequently in those with lower $CD4^+$ cell counts (56, 57).

In persons with persistent HCV infection, the probability of cirrhosis after 20 years of infection is estimated to be 5% to 25%. After cirrhosis has developed, the annual rates of progression to liver failure and hepatocellular carcinoma are estimated to be approximately 2% to 4% and 1% to 7%, respectively (58-60). Infection with HIV has been associated with higher HCV RNA viral load and, in most studies, more rapid progression of cirrhosis, liver failure, and hepatocellular carcinoma (58-61)

As survival of HIV-infected person increase because of potent antiretroviral therapies and the prophylaxis of traditional opportunistic pathogens, hepatitis C–related morbidity and mortality is increasing. In some settings, HCV-related liver disease has already been reported to be a major cause of hospital admissions and death among HIV-infected persons (62, 63).

EFFECT OF HCV ON HIV PROGRESSION

There are conflicting reports on the effect of HCV infection on the natural history of HIV disease. In the Swiss cohort, the presence of HCV was independently associated with an increased risk of progression to AIDS and death together with blunting of immune recovery in HIV infected persons on HAART (10). Daar et al reported a detrimental effect of HCV viral load on HIV progression (64). Subsequent studies in other cohorts however did not find any difference in survival after correcting for the use of HAART, baseline viral loads, CD4+ cell counts, age, race and risk factor for transmission of HIV.

HCV CO-INFECTION AND HAART ASSOCIATED HEPATOTOXICITY

Antiretroviral drug use has been associated with hepatotoxicity that can interrupt HIV therapy and cause significant morbidity and mortality. In some cases, HAART-associated hepatotoxicity has been linked to liver failure and death. Some studies suggest that drug-induced hepatotoxicity may be more common among persons with HIV–HCV co-infection, particularly those taking HIV-1 protease inhibitors (ritonavir) and non-nucleoside reverse transcriptase inhibitor (NNRTI) Nevirapine. The use of didanosine, dideoxycytidine, stavudine leads to a higher rate of hepatic steatosis in the co-infected population (11,12,35). These drugs should therefore be avoided in HAART regimens for HIV/HCV coinfected patients. Hepatic steatosis has a role in the progression of hepatitis C to fibrosis.

There are currently no established guidelines for the management of antiretroviral-associated hepatotoxicity. Some studies have suggested that it is not necessary to discontinue antiretroviral therapy unless persons are symptomatic or develop significant elevations in liver enzyme levels (more than five times the upper limit of the reference range)(35). The mechanisms of enhanced drug-induced hepatotoxicity among patients with HIV–HCV co-infection are unknown but may include decreased drug metabolism, HCV-specific immune reconstitution, or increased susceptibility to mitochondrial dysfunction (65,66).

STUDY JUSTIFICATION

Improved survival due to the success of highly active antiretroviral therapy (HAART) has enabled conditions with long latency, such as chronic viral hepatitis to become a major source of co-morbidity in HIV-infected populations.

Endemic areas of HBV infection include Sub-Saharan Africa, an area also affected most by the HIV pandemic. Co-infection rate in this region is therefore expected to be high due to the shared routes of transmission.

HBV/ HCV infections in HIV patients are important because of the negative influence of HIV on the natural history of HBV or HCV infection. For this reason, the extent of co-infection with hepatitis B and C viruses needs to be determined locally.

Only one study done 16 years ago looked at the prevalence of hepatitis B serological markers in HIV cohort. New research is required to gather information on local trends not only on HBV/HIV co-infection but also on the more increasingly important HCV/HIV co-infection.

Due to the beneficial effect of HAART, its use is currently being escalated in Kenya with more patients initiating antiretroviral therapy. Reports indicate that co-infected patients are at increased risk of HAART associated hepatotoxicity that can be life threatening and can interrupt HIV therapy. It is therefore important to determine the prevalence of abnormal liver function in this patient group.

Data from this study will be useful in estimating the burden of HBV/HCV/HIV co-infection in our set up and thus facilitate the acquisition and access to drugs active against HBV/HCV, which are currently limited for the majority of infected Kenyans.

It will also help sensitize the health care providers to screen for HBV and HCV markers in HIV infected patients before initiating HAART and therefore be able to monitor for HAART associated hepatotoxicity which can be life threatening. This is currently not routinely done.

OBJECTIVES

MAIN OBJECTIVE

The main objective of the study was to determine the prevalence of HBV, HCV infections and abnormal liver function in patients on long term HAART at KNH.

SPECIFIC OBJECTIVES

The specific objectives of the study were:-

1. To determine the prevalence of HBV infection in patients on HAART for at least 1 year at KNH.
2. To determine the prevalence of HCV infection in patients on HAART for at least 1 year at KNH.
3. To determine the prevalence of abnormal liver function in patients on long term HAART and in subgroups of HBV/HCV co-infection.

CASE DEFINATION

Long term HAART was defined as having been on a combination of a minimum of three antiretroviral drugs continuously for duration of more than one year.

HBV infection was defined as positive HBsAg test.

HCV infection was defined as positive HCV antibody test.

PATIENTS AND METHODS

1. STUDY DESIGN

This was a hospital based descriptive cross sectional study conducted at the Kenyatta National Hospital Comprehensive Care Clinic (CCC).

2. STUDY POPULATION

Adult patients (>18years). who were HIV positive and on long term HAART.

3. INCLUSION CRITERIA

Patients of both sexes aged over 18 years diagnosed to be HIV positive by ELISA and on HAART for at least 1 year.

Patients with written and informed consent

4. EXCLUSION CRITERIA

Patients not on HAART for at least 1 year

History of poor/non-adherence to HAART as documented in the patient s file.

Patients who declined to give consent.

5. SAMPLE SIZE

The minimum sample size was 90 patients. This was calculated using the formula

$$N = \frac{[z(1-\alpha)]^2 . P.(1-P)}{D^2}$$

N = Minimum sample size

α = Level of significance = 5%

P = Prevalence of HBV in AIDS patients in a previous study (21).

D = Degree of precision ± 5%

$[z(1-\alpha)]^2$ = 1.96 (corresponds to 95% confidence interval)

2

$$\therefore \frac{1.96^2 \times 0.06 \times 0.94}{0.0025} = 90$$

SAMPLING METHOD

Consecutive patients satisfying the inclusion criteria were recruited into the study.

CLINICAL METHODS

STUDY PROCEDURE

The principal investigator visited the CCC from Monday to Friday at 9am and went through all the files of patients booked for the day. Files for patients meeting the inclusion criteria were identified. The principle investigator then approached the patients and explained to them the nature and purpose of the study. Those who gave written informed consent were recruited consecutively into the study. Thorough history and physical examination was then performed and blood drawn for laboratory tests as outlined below.

HISTORY

The principal investigator interviewed all recruited patients. Name, Age, Sex, Occupation, Marital Status and residence were recorded.

History of blood and blood product transfusion, illicit intravenous drug use with sharing of needles, tattooing, ear piercing and scarification with shared implements, sexual orientation (homosexual, heterosexual, bisexual) and the number of sexual partners were sought and recorded on an already prepared data sheet. (Appendix 1).

PHYSICAL EXAMINATION

The principal investigator examined all the recruited patients.

The general condition, presence of jaundice, liver span, ascites and other features of liver dysfunction were noted. The WHO clinical stage of HIV at initiation of HAART and the latest CD4+ cell count were also noted from the patients file.

LABORATORY TESTS

The principle investigator drew 5 milliliters of venous blood from each patient and put it in a clean biochemical bottle for the laboratory tests. HBsAg and HCV antibodies assays were done at KEMRI Centre for Virus Research Laboratory. Liver function tests were done at KNH CCC Laboratory.

Both laboratories have acceptable standards of internal and external quality control and assurance.

BIOCHEMICAL TESTS

CARO GmbH diagnostic kits were used. The serum AST level was determined using the Karmen technique (69) as modified by Bergmeyer (70). The serum ALT and ALP levels were determined using the Wroblewski technique (71) as modified by Bergmeyer(70).and ALP study group recommended method (72) respectively. The total serum bilirubin was determined using the method of Van den Bergh and Muller (73) as modified by Pearlman (74), the direct using the method of Keller (75). and the indirect by subtraction. An elevation of transaminases to more than twice the upper limit of normal was considered significantly abnormal.

SEROLOGICAL TESTS

Serological tests were done at the KEMRI Laboratory using standard ELISA based Kits (77). The principal investigator with the assistance of qualified technologists working in the laboratory ran the tests.

HBsAg was assayed using the Hepcell II kit, a reverse passive haemagglutinition test kit with a sensitivity of 98% and a specificity of 99 %(78).

HCV antibodies were assayed using the 4th generation kit HCV AB IV from Innogenetics, which has a sensitivity of 100% and a specificity of 99.8%.

DATA MANAGEMENT AND ANALYSIS

Data was recorded in a preformed questionnaire (appendix 1). It was verified and analyzed using the statistical package for Social Sciences (SPSS) version 10.

Frequencies, percentages, means, ranges and standard deviations were calculated.

The results were presented in tables, pie charts and bar charts.

The Pearson chi-square test was used to assess statistical significance.

ETHICAL CONSIDERATIONS

The study was undertaken after approval by the Department of Internal Medicine and Therapeutics, University of Nairobi and the Kenyatta National Hospital Ethics Review Committee.

Cases eligible to participate in the study were included after going through the consent process as outlined in Appendix II.

1. The cases were informed that the project involved research.
2. They were told the purpose of the research.
3. The procedures of the study were explained clearly with full details of all the tests to be done.
4. They were assured that participation would be voluntary and that no medical attention would be denied should they decline to participate.
5. The subjects were informed of the medical benefits and also physical and any psychological harms to their satisfaction prior to being included in this study.
6. The subjects were assured of full and free access to their results and that therapeutic interventions would be recommended where the need arose, according to accepted standards of practice.
7. It was asserted that confidentiality would be strictly maintained and all data securely stored and only revealed upon a need-to-know basis and that all costs regarding investigations in this study would be borne by the principal investigator.
8. Following the full explanation and acceptance by the patient, the patient was requested to sign the consent form.

RESULTS

1. Baseline demographic and clinical characteristics

95 patients were recruited for the study. The sex distribution and marital status of the patients are shown in figure 1 and 2 respectively.

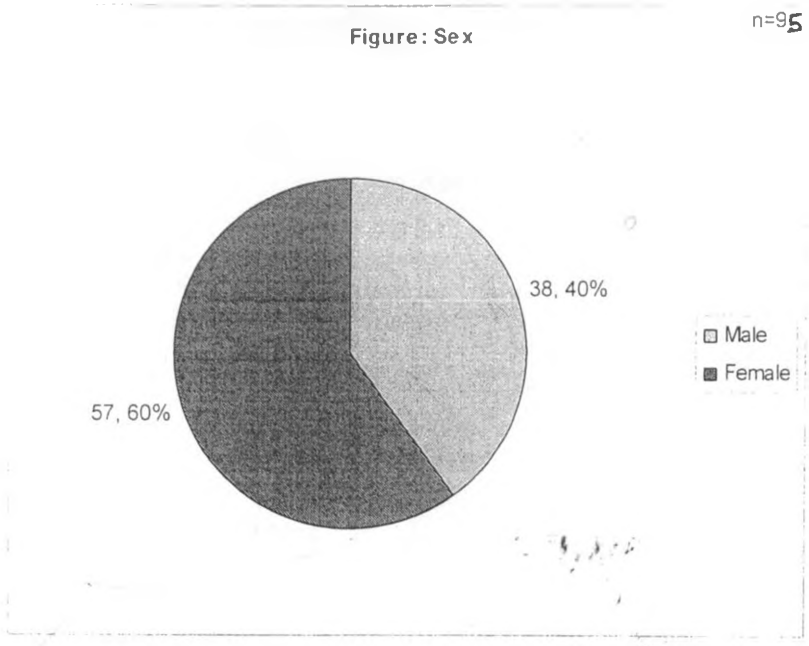


Figure 1: Distribution of patients by sex category

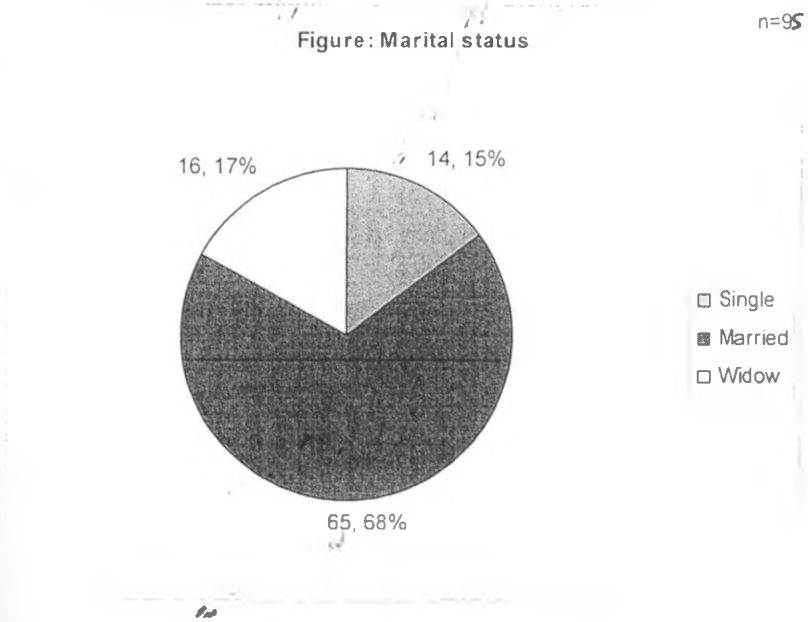
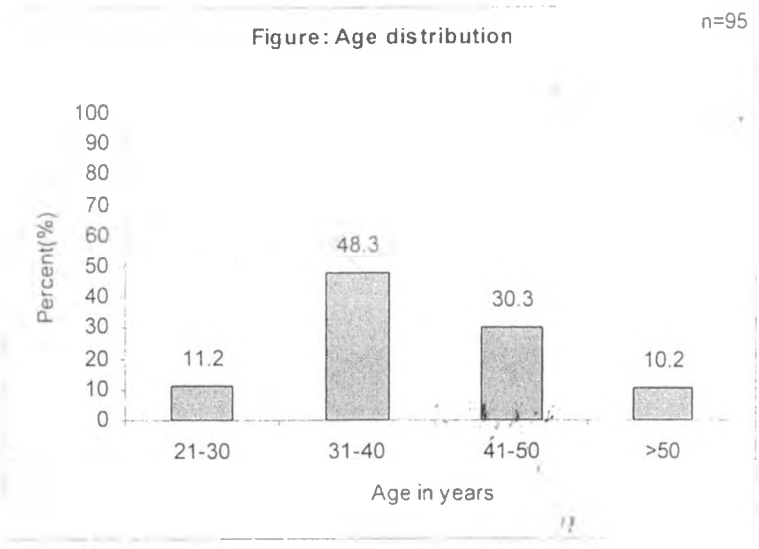


Figure 2: Distribution of patients by marital status

1.1 Age distribution

The mean age was 39.5 years with a median of 38 years and a standard deviation of 8.42 years. The age range was 22years to 59 years. The mean age for male patients was 41.79 years with a standard deviation of 7.67, whereas female patients had a mean age of 37.76 years with a standard deviation of 8.62. The mean ages between the males and females were statistically significant (p=0.025).



Mean age 39.5 median 38 SD 8.42 min 22 Max 59

Figure 3: Distribution of patients by age category

1.2 Age by Sex

Sex	n	Mean	Std. Deviation	*P Value*
Male	38	41.79	7.673	0.025*
Female	57	37.76	8.618	

Table 1: Age by Sex category

2.Risk factors for HIV infection

Majority of the patients (56.7%) had no risk factor.40% admitted to having had multiple heterosexual partners, 2.2% had blood transfusion. None of the patients admitted to ever being an intravenous drug user or homosexual. Of the two patients who had blood transfusion, none was reactive for HBV/HCV.

Table 2 illustrates the various risk factors for contracting HIV looked for in the study.

Risk factors	Number	Percent (%)
Intravenous drug use with sharing of needles	0	0
Homosexual	0	0
Blood or blood product transfusion	2	2.2
Tattooing/scarification/ear piercing	1	1.1
Multiple heterosexual partners	38	40
No risk factor	54	56.7

Table 2: Risk factors for HIV infection

3. WHO Clinical stage at initiation of HAART

Majority (70.5%) of the patients were at WHO clinical stage 3 at initiation of HAART. One patient was at WHO clinical stage 2 at initiation of HAART but satisfied the immunological criteria for initiating HAART.

Table 3 shows the WHO clinical stage of the patients at initiation of HAART.

WHO Clinical stage	Number	Percent (%)
2	1	1.1
3	59	70.5
4	20	28.4

Table 3: WHO clinical stage at initiation of HAART

4. Study population liver function tests and CD4+ cell count

Table 4 illustrates the study population mean, median, SD and range of CD4+ cell count, Total protein, Albumin, Bilirubin, AST, ALT, ALP.

	n	Mean	Median	SD	Minimum	Maximum
CD4 count (cells/µl)	95	259.6	219	188.86	4	1054
Total protein (g/l)	95	66	68	13.69	26	111
Albumin (g/l)	95	39.5	42	6.87	19	48
Total bilirubin(µmol/l)	95	20.5	5.1	101.43	2.3	960
Direct bilirubin(µmo/l)	95	15.5	2.2	96.75	0	927
AST (iu/l)	95	28.5	24	22.15	5	163
ALT (iu/l)	95	25.7	22	15.93	9	122
ALP (iu/l)	95	94.3	91	40.14	31	294

Table 4:Group mean, median, SD, range of CD4+ cell count, Total protein, Albumin, Bilirubin, AST, ALT, ALP.

5. Hepatitis prevalence

The prevalence of HBV and HCV was 4.2% and 3.2% respectively as shown in table 5. No patient was co-infected with both HBV and HCV.

Hepatitis type	Number	Prevalence (%)
HBV	4	4.2
HCV	3	3.2
HBV/HCV	0	0

Table 5: Prevalence of hepatitis

6. Prevalence of abnormal liver function in patients on long-term HAART

Abnormal liver function in this study was defined as elevation of AST/ALT to twice the upper limit of normal. The frequency of abnormal AST and ALT are shown in table 6 and 7 respectively.

AST	Frequency	Percent
Abnormal	3	3.2
Normal	92	96.8
Total	95	100

Table 6: Abnormal AST

ALT	Frequency	Percent
Abnormal	2	2.1
Normal	93	97.9
Total	95	100

Table 7: Abnormal ALT

The prevalence of abnormal liver function in patients on long-term HAART was 4.2% (95%CI 3.2-5.2).

Among the seven patients co-infected with hepatitis, two (one HBV, one HCV) had abnormal liver function giving a prevalence of 28.6% (95%CI 4.9-61.6).

One patient had both abnormal AST and ALT. This patient had HCV co-infection.

Two patients had abnormal AST with marginal elevation in ALT (68iu/l and 74iu/l). These patients had no co-infection.

The ALT levels were higher (115iu/l and 122iu/l) in the two patients with co-infection as compared to the two patients without co-infection (68iu/l and 74iu/l).

The two patients with abnormal liver function and co-infection also had lower CD4+ cell count (<100 cells/μl) as compared to those without co-infection (>200 cells/μl).

7. Liver function tests andCD4+ cell count by HBsAg status

	HBsAg	N	Mean	Median	SD	P Value
CD4 count(cells/μl)	Positive	4	274.5	250.5	72.73	0.87
	Negative	91	258.9	214.0	192.52	
Total protein(g/l)	Positive	4	67.5	66.5	5.07	0.82
	Negative	91	65.9	68.0	13.97	
Albumin(g/l)	Positive	4	42.3	42.5	0.97	0.41
	Negative	91	39.3	42.0	7.0	
Total bilirubin(μmol/l)	Positive	4	5.0	4.5	2.21	0.76
	Negative	91	21.1	5.1	103.6	
Direct bilirubin(μmol/l)	Positive	4	1.9	1.7	0.99	0.78
	Negative	91	16.1	2.2	98.3	
AST(iu/l)	Positive	4	16.8	17.5	7.97	0.28
	Negative	91	29.0	25.0	22.45	
ALT(iu/l)	Positive	4	17.8	18.0	7.14	0.31
	Negative	91	26.1	22.0	16.13	
ALP(iu/l)	Positive	4	73.3	68.5	29.19	0.29
	Negative	91	95.2	94.0	40.42	

Table 8: Liver function tests andCD4+ cell count by HBsAg status

There was no statistically significant difference in liver function tests and CD4+ cell count between those who were HBV positive as compared to HBV negative patients.

10. Liver function tests and CD4+ cell count by gender

	Gender	n	Mean	Median	SD	P Value
CD4 count (cells/μl)	Male	38	241.7	228.0	140.46	0.33
	Female	57	280.8	227.0	219.48	
Total protein (g/l)	Male	38	66.5	68.0	14.9	0.68
	Female	57	65.3	68.5	13.20	
Albumin (g/l)	Male	38	39.8	43.0	7.21	0.62
	Female	57	39.1	41.5	6.76	
Total bilirubin (μmol/l)	Male	38	11.4	5.2	34.90	0.45
	Female	57	27.9	4.2	132.57	
Direct bilirubin (μmol/l)	Male	38	6.4	2.3	22.42	0.42
	Female	57	22.9	1.9	128.15	
AST (iu/l)	Male	38	34.5	28.0	29.47	0.03
	Female	57	23.2	22.0	12.58	
ALT (iu/l)	Male	38	28.1	23.0	16.46	0.18
	Female	57	23.6	21.0	15.31	
ALP (iu/l)	Male	38	85.9	89.0	22.58	0.06
	Female	57	100.7	94.4	48.97	

Table10: Liver function tests and CD4+ cell count by gender

There was a statistically significant difference in AST levels between males and females (p= 0.03). The other parameters were statistically insignificant between the two groups.

8. Liver function tests and CD4+ cell count by anti-HCV status

	Anti-HCV	n	Mean	Median	SD	P Value
CD4 count (cells/μl)	Positive	3	198.3	254.0	165.67	0.57
	Negative	92	261.6	218.5	190.03	
Total protein (g/l)	Positive	3	59.3	55.0	10.2	0.40
	Negative	92	66.2	68.0	13.77	
Albumin (g/l)	Positive	3	41.0	42.0	3.61	0.69
	Negative	92	39.4	42.0	6.96	
Total bilirubin (μmol/l)	Positive	3	4.3	4.2	0.70	0.78
	Negative	92	21.0	5.2	103.0	
Direct bilirubin (μmol/l)	Positive	3	1.4	0.4	1.88	0.80
	Negative	92	15.9	2.2	98.3	
AST (iu/l)	Positive	3	34.3	28.0	10.97	0.65
	Negative	92	28.3	24.0	22.43	
ALT (iu/l)	Positive	3	27.7	29.0	4.16	0.83
	Negative	92	25.6	22.0	16.17	
ALP (iu/l)	Positive	3	86.7	95.0	16.20	0.74
	Negative	92	94.5	90.5	40.7	

Table 9: Liver function tests and CD4+ cell count by anti-HCV status

There was no statistically significant difference in liver function tests and CD4+ cell count between those who were HCV positive as compared to HCV negative patients.

DISCUSSION

The aim of this study was to determine the prevalence of HBV, HCV infections and abnormal liver function in patients on long term HAART. Ninety-five patients comprising 57(60%) females and 38(40%) males were studied. The ratio of female to male was 1.5 to 1; this is comparable to the ratio of 1.9:1 reported in the 2003 KDHS for HIV prevalence in Kenya (19). Majority of the patients (48.3%) were aged between 31-40 years. The males were significantly older than the females (p=0.025). This association between young females and older men with regard to HIV infection was also reported in the KDHS.

As regards risk factors for HIV infection and by extension HBV and HCV infection, majority of the patients (56.7%) reported no risk factor other than heterosexual sex with one partner, 40% admitted to having had multiple heterosexual partners, 2.2% had had prior blood transfusion and 1.1% reported tattooing or scarification with shared implements. None of the patients admitted to being homosexual or IVDU. This is in contrast to North America and Europe where HIV/HBV/HCV transmission is mainly through intravenous drug use and homosexual contact.

The prevalence of HBV and HCV were 4.2% and 3.2% respectively.

Studies that have looked at the prevalence of HBV and HCV infections in HIV/AIDS have given variable results depending on geographical region and the risk group involved.

E.O Ogutu and colleagues in 1990, found HBV prevalence of 12.2% in patients with AIDS at KNH (18) which is higher than that found in our study. This difference can be due to the small sample size in their study (41patients).

The reported prevalence of HBV carrier rate in Kenya is 3% in blood donors and 15% in central province with seroepidemiological surveys in other regions falling within this range (28). Our figure also falls within this range, however we would expect a higher prevalence in patients with HIV owing to the shared routes of transmission. A likely explanation for the low prevalence could be the fact that most patients in our set up

acquire HBV while they are immunocompetent and recover from the infection before later acquiring HIV.

H. Lodenyo in 2000 at Chris Hani Hospital in Johannesburg found the prevalence of HBV and HCV to be 6% and 1% respectively (21). This prevalence is comparable to that of our study.

Studies in N.America and Europe have reported the prevalence of HBV in HIV patients to be between 5 and 10% while that in the general population to be less than 1%. Most patients there acquire HBV during adolescence and adulthood through sexual contact or intravenous drug use at the same time they acquire HIV.

The prevalence of HCV in our study was 3.2%. This concurs to a prevalence of 3.7% found by Karuru et al in medical in-patients with HIV (16). According to the KDHS 2003, the mean prevalence of HCV among blood donors was 1%. This figure is much lower than that reported in our study but is expected, as blood donors are a highly selected low risk group. There is no data on the prevalence of HCV in the general Kenyan population to compare our results with, however, Studies in N.America and Europe report the prevalence of HCV/HIV co-infection to be 15-30% (26). The prevalence is much higher in IVDU and haemophiliacs. The low prevalence in our study as compared to that in N.America and Europe could be explained by the fact that the main mode of HIV transmission here is heterosexual sex while in N.America and Europe it is mainly through homosexual contact and intravenous drug use both of which are more efficient methods of HCV transmission.

The prevalence of abnormal liver function in the entire cohort was 4.2% (95% CI 3.2-5.2). In the subgroup with viral hepatitis the prevalence of abnormal liver function increased seven fold to 28.5% (95% CI 4.9-61.6). There are no local data to compare with, as this was the first study to look at the prevalence of abnormal liver function in patients on HAART. In N.America and Europe however, several studies have looked at HBV and HCV infections and the risk of hepatotoxicity in patients on HAART.

Our results concur with those of Den Brinker et al who found that patients with chronic viral hepatitis had higher risk of hepatotoxicity as compared with patients without co-infection (37% vs 12%) (11). The prevalence of HBV and HCV was 7% and 14%

respectively. Majority of the patients in their study were homosexuals (58%) and IVDU (9%). This could explain the higher prevalence of HBV and HCV as compared to our study.

Aceti et al also found that hepatotoxicity developed in a significantly higher percentage of co-infected patients compared to patients without co-infection regardless of the protease inhibitor used since some studies had indicated that use of protease inhibitors were associated with significant hepatotoxicity. The prevalence of hepatotoxicity in their cohort was 11.1% (67). This figure is higher than that found in our study. Possible explanations could be due to the higher prevalence of HCV (46.5%) and use of protease inhibitor in their cohort both of which increase the risk of hepatotoxity. Intravenous drug use in their study was high and IVDU may consume large amounts of alcohol that might result in ALT/AST elevations.

Martinez et al also found hepatotoxity in 12.5% of patients on nevirapine containing HAART regimen (68). HCV and higher baseline ALT levels were independent risk factors for hepatotoxicity. The higher prevalence of hepatotoxity as compared to our study could again be due to the high prevalence of HCV (46.2%) and HBV (8.9%) in their study.

In this study we only looked at the prevalence of abnormal liver function in patients on long term HAART, further work in elucidating the aetiologies of the abnormality in liver function needs to be done as the results herein cannot necessarily be attributed to HAART without exclusion of other causes.

STUDY LIMITATIONS

The abnormal liver functions noted in this study could have been due to other causes other than HAART hence there is need for another study to look into the individual contribution of the various aetiologies.

HBV DNA and HCV RNA copies were not measured due to financial constraints and limited availability of the tests. Occult HBV infection may have been misclassified as HBV negative.

Liver biopsy was not done to grade severity of liver disease in co-infected patients due to financial constraints and technicalities involved.

CONCLUSIONS

1. The prevalence of HBV infection in patients on long-term HAART at KNH was similar to that in the general population.
2. The prevalence of HCV in patients on long-term term HAART at KNH was higher than that reported in blood donors.
3. Majority of the patients on long-term HAART at KNH (95.8%) had normal liver function.

RECOMMENDATIONS

1. At initial evaluation, all patients with HIV should be tested for HbsAg and HCV antibodies before initiating HAART.
2. A study to look at the etiology of the abnormal liver function in patients on HAART and the histologic grade of hepatotoxicity in co-infected patients is recommended.

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APPENDIX 1

QUESTIONNAIRE

I.HISTORY

1. Name.....
2. Age.....IP No.....
3. Sex.....BP.....
4. Marital status.....
5. Residence.....
6. Occupation.....
7. Intravenous drug use with sharing of needles
   (Yes/No).....
8. Blood or blood product transfusion
   (Yes/No).....
9. Tattooing/Scarification/Ear piercing with shared implements
   (Yes/No).....
10. Sexual orientation(homosexual,heterosexual,bisexual).....
11. Multiple sexual partners
    (Yes/No).....

II. EXAMINATION

1. General condition.....
2. Jaundice (Yes/No).....
3. Hepatomegally (Yes/No).....Tender (Yes/No).....
   Span.....
4. Ascites (Yes/No).....
5. WHO Clinical stage.....

III.LABORATORY RESULTS

Liver function tests

Bilirubin Total (umol/l).....Direct (umol/l)..... Indirect (umol/l).....

AST (U/L)..... ALT (U/L).....ALP (U/L).....

HBsAg (Positive/Negative).....

Anti-HCV (Positive/Negative).....

CD4+ Count.....

APPENDIX 2

CONSENT EXPLANATION

My name is Dr. Chrispine Owuor Oduor. I am a postgraduate student pursuing a master's degree in internal medicine, University of Nairobi. In partial fulfillment of my degree course, I am required to conduct a research project. My research is on the prevalence of HBV, HCV infections and abnormal liver function in patients on long-term HAART at KNH.

To do this I will require a thorough history, physical examination and 5mls venous blood sample from each participant. The history will include filling a form with questions routinely asked by health care providers. Physical examination will be done in the examination room on a couch looking for features of chronic liver disease. This will not interfere with the participant's privacy. Taking venous blood for HBV/HCV serology and liver function tests has some level of minor discomfort. Use of sterilized needles and syringes with proper aseptic techniques during blood sampling will ensure no risk to the participants.

This study is intended to establish the prevalence of HBV, HCV infections and abnormal liver function in patients on long term HAART. Data from this study will help in estimating the burden of HBV/HCV/HIV co-infection in KNH, sensitize health care providers to screen for HBV and HCV in HIV infected patients before initiating HAART and subsequently closely monitor liver function considering that co-infected patients are at increased risk of HAART associated hepatotoxicity which can be life threatening. Participants found to have HBV/HCV and abnormal liver function will be informed and referred to their primary physician for further management and follow-up.

Participation in this study is purely voluntary. All information collected will be confidential. A written consent will be required. Participants have a right to withdraw from the study at any stage. They also have a right to know the results of all tests done. This study will not in any way jeopardize standard treatment participants are on during the study.

APPENDIX 3

CONSENT FORM

I.....of.....hereby agree to participate in the study entitled 'The prevalence of HBV, HCV infections and abnormal liver function in patients on long term HAART at KNH' as has been explained to me by Dr ..... I understand the nature of the study and my participation is on voluntary basis and I have willingly agreed to participate in it.
While the results remain the confidential property of the investigator, significant findings that may influence further management of my condition will be made available to me.

Signed..... Witness.....
Patient Date.....

I, Dr....., confirm that I have explained to the patient the nature of the study and the tests and procedures to be done.

Date..... Signed.....

APPENDIX 4: HBsAg ASSAY

PRINCIPLE:

The KEMRI Hepcell II test is a reverse passive haemagglutination method for detecting HBsAg.

Test serum is mixed with anti-HBs coated sheep erythrocytes in a microtitre plate. The cells will agglutinate if the test serum contains HBsAg. If the test serum does not contain HbsAg, the cells will settle and form a button like precipitate at the bottom of the plate.

PROCEDURE

1.Hepcell diluent is added to the microtitre wells

2.Test serum is added to the diluent producing a dilution of 1:16.

3.The reagent, which contains sheep erythrocytes is added, the contents mixed, and incubated at room temperatures for 1-2 hours.

4.Results are read by looking for the presence of agglutination.

5.Sera showing positive or indeterminate results are confirmed by repeating the test at greater dilutions of up to 1:64.

APPENDIX 5: HCV ANTIBODY ASSAY

PRINCIPLE

The INNOTEST HCV Ab IV test is an enzyme immunoassay for the detection of antibodies to HCV. Micro plate wells coated with a mixture of HCV antigens are utilized. Test sera are incubated in the wells. Viral specific antibodies to HCV, if present will bind to the solid phase antigens. Subsequently an affinity purified rabbit anti-human IgG labelled with the enzyme horseradish peroxidase is added. Upon a positive reaction this labelled antibody becomes bound to any solid-phase antigen-antibody complex previously formed. Incubation with enzyme substrate produces a blue colour, which turns yellow when the reaction is stopped with sulphuric acid.

PROCEDURE

1.A 1:10 dilution of test serum is made by adding diluent to serum in the well and mixing.

2.The wells are incubated at 37 ^{0}C for 60 minutes (to capture antibody).

3.Each well is washed 6 times with wash solution.

4.Conjugate (containing enzyme labelled anti-human IgG) is added to each well, mixed and incubated at 37^{0}C for 60minutes.

5.Each well is washed 6 times with wash solution.

6.Substrate is added and the wells incubated for 30 minutes in the dark at room temperature.

7.The reaction is stopped by adding stop solution and mixing thoroughly.

8.The absorbance of the solution in the wells is read at 450 nm with a micro plate reader.

9.The cut –off value is calculated by dividing the mean absorbance of positive controls by 2.75.

A test sample is positive or negative if its absorbance is greater or less than the 2.76 cut off value respectively.

APPENDIX 6

WHO staging system for HIV infection and disease in adults and adolescents

Clinical stage I

Asymptomatic
Primary HIV infection
Persistent generalized lymphadenopathy

Performance scale 1: asymptomatic, normal activity

Clinical stage II

Weight loss, <10% of body weight
Minor mucocutaneous manifestations (seborrheic dermatitis, papular pruritic eruption, fungal nail infections, recurrent oral ulcerations, angular cheilitis)
Herpes zoster within the last five years (uncomplicated)
Recurrent upper respiratory tract infections (i.e. bacterial sinusitis, otitis media) in past 1 months
Thrombocytopenia not responsive to steroids

And/or performance scale 2: symptomatic, normal activity

APPENDIX 6

WHO staging system for HIV infection and disease in adults and adolescents

Clinical stage I

Asymptomatic

Primary HIV infection

Persistent generalized lymphadenopathy

Performance scale 1: asymptomatic, normal activity

Clinical stage II

Weight loss, <10% of body weight

Minor mucocutaneous manifestations (seborrheic dermatitis, papular pruritic eruption, fungal nail infections, recurrent oral ulcerations, angular cheilitis)

Herpes zoster within the last five years (uncomplicated)

Recurrent upper respiratory tract infections (i.e. bacterial sinusitis, otitis media) in past 1 months

Thrombocytopenia not responsive to steroids

And/or performance scale 2: symptomatic, normal activity

Clinical stage III

Weight loss, >10% of body weight and/or BMI <18.5, unexplained.

Unexplained chronic diarrhoea, >1 month

Unexplained prolonged fever (intermittent or consant), >1 month

Oral candidiasis (thrush)

Oral hairy leukoplakia

Pulmonary tuberculosis within the past year

Severe bacterial infections (i.e. pneumonia, pyomyositis,bacterial meningitis, bacteraemia)

Bacillary angiomatosis

Herpes zoster: complicated (recurrent, disseminated, multidermatomal)

And/or performance scale 3: bedridden <50% of the day during the last month

Clinical stage IV

HIV wasting syndrome, as defined by the Centers for Disease Control and Prevention[3]

Pneumocystis carinii pneumonia

Toxoplasmosis of the brain

Cryptosporidiosis, Isosporiasis, Microsporidiosis with diarrhoea >1 month

Cryptococcosis, extrapulmonary

Cytomegalovirus disease of an organ other than liver, spleen or lymph nodes

Herpes simplex virus infection, mucocutaneous >1 month, or visceral any duration

Progressive multifocal leukoencephalopathy

Any disseminated endemic mycosis (i.e. histoplasmosis, coccidioidomycosis, Penicilliosis)

Candidiasis of the oesophagus, trachea, bronchi or lungs

Non-tuberculuos mycobacteriosis, disseminated

Non-typhoid *Salmonella* septicaemia

Extrapulmonary tuberculosis

Lymphoma

Kaposi's sarcoma

HIV encephalopathy, as defined by the Centers for Disease Control and Prevention.[b]

Invasive cervical carcinoma

American trypanosomiasis-reactivation

Major aphthous ulceration: ulcers of GI tract >5mm and for >1 month

Nephropathy

Cardiomyopathy, unexplained

Visceral leishmaniasis

Strongyloides hyperinfection syndrome

And/or performance scale 4: bedridden >50% of the day during the last month

Note: both definitive and presumptive diagnoses are acceptable.

a. HIV wasting syndrome: weight loss of >10% of body weight, plus either unexplained chronic diarrhoea
(>1 month) or chronic weakness and unexplained prolonged fever (>1 month).
b. HIV encephalopathy: clinical findings of disabling cognitive and/or motor dysfunction interfering with activities of daily living, progressing over weeks to months, in the absence of a concurrent illness or condition other than HIV infection which could explain the findings.

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Ref: KNH-ERC/ 01/ 3989

15th December 2006

Dr. Chrispine Owuor Oduor
Dept. of Internal Medicine
School of Medicine
University of Nairobi

Dear Dr. Oduor

RESEARCH PROPOSAL: "THE PREVALENCE OF HBV, HCV INFECTIONS AND ABNORMAL LIVER FUNCTION IN PATIENTS ON LONG TERM HAART AT K.N.H" (P250/10/2006)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** your revised research proposal for the period 15th December 2006 – 14th December 2007.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH-ERC

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