Immunogenetics: Open Access

OPEN ACCESS Freely available online

Research Article

Hepatitis B Virus Seroprevalence and Genetic Variants among HIV Infected Patients in Nyanza, Kenya

Raphael Lihana, Zipporah Nganga

Department of Immunology, Kenya Medical Research Institute, Nyaza, Kenya;2Department of Immunology, Jomo Kenyatta University of Agriculture and Technology, Nyaza, Kenya

ABSTRACT

Infections caused by hepatitis B virus (HBV) and human immunodeficiency virus (HIV) co-infection remain among the top ten most important health problems worldwide. HBV and HIV co-infection is common due to shared routes of transmission, which would modify the progression, manifestation or management of each of the infections. Although studies have been carried out among blood donors, and HBV genotypes established, this data on the seroprevalence of the co-infection remains insufficient in Kenya. Coupled with genetic diversity that drives disease outcome, there is need to monitor the diversity of HBV especially among HIV patients seeking medical intervention. This study intends to determine the seroprevalence and genetic diversity of HBV among HIV infected patients in Nyanza. Remnant plasma samples from Comprehensive Care Clinic (CCC) at Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH), Kisumu will be used in this study. HIV screening test will be performed on all the samples using Determine kit according to Kenya government guidelines. Hepanostika ELISA kit will be used to determime the HBsAg from the HIV positive plasma samples. HBV DNA will be extracted from those found to be HBsAg positive, and on the HBsAg negative plasma to determine the prevalence of occult hepatitis B infections (OBI) among HIV infected patients. PCR will be carried out on extracted DNA to amplify HBV preS1 region. PCR products will be directly sequenced using Big Dye chemistry on an automated ABI 310 sequencer. Molecular evolutionary genetic analysis will be done using Clustal W and Phylogenetic trees constructed using the neighbor-joining method. Statistical analysis will be performed using SPSS 16. Data generated will provide information on HBV genotypes among HIV infected patients and form a basis for future monitoring of HBV viral evolution and HBV infection in Kenya.

Abbreviations: Hepatitis B virus; Human immunodeficiency viruses

INTRODUCTION

Infections caused by hepatitis B virus (HBV) and human immunodeficiency viruses (HIV) remain the leading most important health problems worldwide. HBV is among the top 10 leading causes of infectious disease deaths worldwide. Globally, chronic infection with each of these viruses alone contributes to substantial morbidity. HIV infection accounts for an estimated 40 million persons and HBV infection accounts for an estimated 370 million chronic infections [1].

Research shows that HIV positive people with hepatitis B co-infection experience worse liver disease progression, but the effect of HBV on HIV disease progression is not well understood [2]. HBV co-infection has a significant impact on HIV outcomes; the hazard for an AIDS or death event is almost double for those with chronic hepatitis B compared, with HIV-mono-infected persons. A study carried out to determine the Prevalence, Clinical and Virologic Outcomes of Hepatitis B Virus Co-Infection in HIV-1 Positive Kenyan Women on Antiretroviral Therapy demonstrated a 7% prevalence of chronic HBV infection [3]. There is therefore need for additional steps to combat hepatitis B now, including more extensive HBV screening and vaccination [4].

The World Health Organization (WHO) currently estimates that 2 billion people have been infected with HBV and that 360 million are chronically infected. HBV is a significant contributor to morbidity worldwide. Current estimates suggest that it causes 30% of cirrhosis and approximately 50% of hepatocellular carcinoma (HCC) globally. Earlier research findings show that the virus causes acute hepatitis of varying severity [5] and persists in 95% of children and 2–10 % of adult patients [6] leading to chronic liver disease, cirrhosis, hepatocellular carcinoma and even fulminant hepatitis [7].

Correspondence to: Raphael Lihana, Department of Immunology, Kenya Medical Research Institute, Nyaza, Kenya, Email: rlihana@kemri.org

Received: January 11, 2021, **Accepted:** January 25, 2021, **Published:** February 02, 2021

Citation: Lihana R, Nganga Z (2021) Hepatitis B Virus Seroprevalence and Genetic Variants among HIV Infected Patients in Nyanza, Kenya. Immunogenet Open Access. 6:03.

Due to overlapping risk factors (including shared drug injection equipment and sexual transmission), co-infection with HIV and HBV is common; an estimated 5-10% of people with HIV have chronic hepatitis B, but as many as three-quarters or more show evidence of past infection that has since resolved. Infections caused by hepatitis B virus (HBV), is one of the leading important health problems worldwide. HBV and HIV share common routes of transmission in areas where they are endemic, but they differ in their prevalence by geographic region and the efficiency by which certain types of exposures transmit them, and therefore they occur as co-infections [8], which would modify the progression, manifestation or management of each of the infections. HBV is 50 to 100 times more infectious and unlike HIV, HBV can survive outside the body for at least 7 days.

Treatment can cost thousands of dollars per year and is not available to most patients in developing countries. Liver cancer is almost always fatal, and often develops in people at an age when they are most productive and have family responsibilities. In developing countries, most people with liver cancer die within months of diagnosis. In higher income countries, surgery and chemotherapy can prolong life for up to a few years in some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. The common modes of transmission include; sexual, percutaneous, blood transfusion, vertical and exposure to broken skin and mucous membrane.

The changing pattern of global epidemiology with characteristic geographical distribution has made molecular study of this virus an important scientific undertaking. Continual surveillance of co-infection with HBV/HIV is important since viral molecular diversity has implications for the diagnosis, treatment and prevention as well as epidemiological investigations. Surveillance for molecular variants is vital in assuring that blood screening and supplemental assays are sensitive to circulating strains of HBV genotypes. The study is intended to create baseline information for future reference to improve health service delivery in Kenya. Samples for the study had been collected from New Nyanza General Hospital, Kisumu.

Serological/immunological, molecular and genetic methods will be used to achieve the study goals.

Problem Statement

Hepatitis B virus and HIV infections are a global health problem. HBV shares modes of transmission with other known sexually transmitted viral infections such as HIV and HCV in areas where they are endemic, they occur as co-infections, which would modify the progression, manifestation or management of each of the infections.

There are few data regarding HBV and HIV co-infection in Africa [9]. The growing concern for the increase in co-infections of HBV and HIV in recent years necessitates a proper assessment and quantification of disease burden to foster appropriate intervention strategies [10]. However, in Kenya, the few studies that have been conducted to determine the seroprevalence of HBV/HIV co-infections [11] and HBV genome variability [12] have targeted the blood donors. Hepatitis B virus genotypes are geographically grouped into 10 groups, [13]. In Kenya, genotype A, D and E are prevalent among blood donors. There is need to determine the genetic variability of HBV in patients seeking medical attention and to reach an agreement if the findings conform to the available data. (Figure 1)

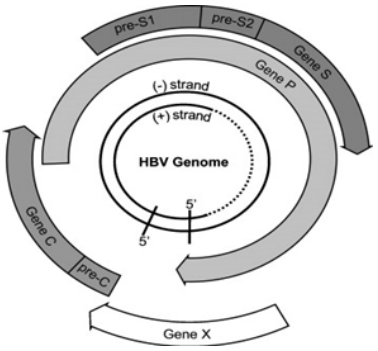

Figure 1: HBV genome

Justification of the study

HIV modifies the natural history of hepatitis B virus (HBV), with higher rates of chronic HBV infection, replicative disease, and progression to advanced liver disease among persons with HIV/HBV co-infection. The impact of HBV on HIV natural history is less certain [14]. The growing concern for the increase in co-infections of HBV and HIV in recent years necessitates a proper assessment and quantification of disease burden to foster appropriate intervention strategies. Data on seroprevalence of HBV among HIV co-infected patients and its molecular epidemiology in Kenya remains sparse despite the country being among the endemic regions for HBV infection. Studies carried out to determine the circulating genotypes in Kenya and do not clearly show any consensus on the HBV genotypes. It is hoped that the results of this study will provide more insight on the circulating HBV genotypes in Kenya and their significance in HIV co-infected patients. The findings will also form basis for future monitoring of the HBV/HIV co- infections.

Research Questions

What is the seroprevalence of HBV among HIV infected patients in Nyanza?

What is the prevalence of occult HBV in the study population?

Which is the prevalent HBV genotype in the study population?

General objective

To determine the seroprevalence and genotypes of HBV among HIV infected patients in Nyanza.

Specific objectives

1. To determine the seroprevalence of HBV infection among HIV infected patients in Nyanza.

2. To determine the prevalence of occult HBV among HIV infected patients in Nyanza.

3. To determine the prevalent HBV genotypes among HIV infected patients Nyanza.

LITERATURE REVIEW

Classification, morphology and genome of HBV

Hepatitis B virus belongs to the family Hepadnaviridae, which is a group of DNA viruses, with unique viral DNA replication [15]. It requires RNA intermediate hence the viral polymerase possesses reverse transcriptase activity as seen with the retroviruses[16]. The 42 nm, double stranded DNA virus, is composed of nucleocapsid

core (HBcAg), and surrounded by an envelope containing the surface antigen (HBsAg). The genome is about 3.2 kb long. The highly compact genome contains the four major open reading frames (ORFs) encoding the envelope preS1, preS2 and surface antigen (HBsAg), core (preCore protein, HBeAg and HBcAg), polymerase (HBPol) and X (HBX) proteins, respectively . HBV infects hepatocytes in vivo to produce an excess of noninfectious viral particles composed of envelope proteins. Persistent infections display pronounced hepatotropism [17].

Stages of HBV chronic infection

The chronic HBV infection progresses in four stages as follows:

Immune Tolerance Stage: This involves an incubation period of 120 days and duration of less than 6 months after infection. No host immune response is mounted at this initial stage despite high serum viral DNA levels in liver patients. The patients are considered to be at low risk of progressing to cirrhosis or hepatocellular carcinoma and no antiviral therapy is recommended for them.

Immune Clearance Stage: This stage involves clearance of HBV by host immune system. There is seroconversion of HbeAg to anti-HBe. Depending on the immune status of the infected individual, 90-95% is able to mount an immune response to HBV and this confers immunity to the person, which is confirmed by the presence of anti-HBe. Unfortunately, 5-10% of those infected who fail to develop this immunity progress to the third stage and are likely to develop HCC. In Africa, 70-80% of the general population has been previously exposed to HBV infection.

Inactive HbsAg carrier state: This phase is characterized by absence of HbeAg, persitently low transaminases, low or undetectable HBV DNA in serum. There is indefinite persistence, resolution of chronic infection (manifested by HBsAg clearance and appearance of anti-HBsAg antibody), or disease reactivation due to recrudescence of the original infection or the emergence of mutant viruses that fail to express HbeAg.

The fourth phase of chronic HBV infection is characterized by lack of detectable HBeAg, the presence of anti-HBeAg antibody, detectable HBV DNA, fluctuating liver enzymes, and active inflammation upon biopsy. Progression to this phase occurs spontaneously or as a result of immune suppression in inactive carriers.

Prevention and control of HBV

The prevention of chronic HBV infection has become a high priority in the global community. Immunization with hepatitis B vaccine is the most effective means of preventing HBV infection and its consequences. The following drugs are approved for therapy of chronic hepatitis B: IFN-α, pegylated IFN-α, lamivudine, adefovir dipivoxil, entecavir, and telbivudine. Tenofovir disoproxil fumarate though currently not approved for use in chronic hepatitis B is shown to be effective in HIV-1/HBV-coinfected patients. IFN-α (and pegylated formulations) is the only drug that eliminates cccDNA from hepatocytes and is therefore potentially curative. The highest rates of drug resistance are observed with lamivudine compared to other nucleoside and nucleotide reverse transcriptase inhibitors.

HBV/HIV co-infections

Patients with HIV may have co-infection with one or more hepatitis viruses (Colin and Laizizi, 1999). Among the estimated 37 million persons infected with HIV worldwide, an estimated 2–4 million are chronically infected with HBV. Several factors influence these co-infection estimates, including geographic differences in the prevalence of chronic infection by age, the efficiency of exposures that account for most transmission, and the prevalence of persons at high risk for infection. Reports reveal continuous increase of co-infection in many part of the world [18] with 41% in South Africa and South India, and 53% prevalence rate in Kenya, due to exposure of large population of persons to different sources of infection as the result of diverse lifestyles. This growing concern for the increase co-infections of HIV and HBV in recent years necessitates a proper assessment and quantification of disease burden to foster appropriate intervention strategies. HIV modifies the natural history of hepatitis B virus (HBV), with higher rates of chronic HBV infection, replicative disease, and progression to advanced liver disease among persons with HIV/HBV co-infection. The impact of HBV on HIV natural history is less certain. HIV co-infected adults who acquire acute HBV infection have a reduced likelihood of viral clearance and this is directly proportional to the level of CD4 cell count at the time of HBV acquisition, while their counterparts without HIV infection develop a vigorous immune response and clear acute HBV infection in the majority of cases (> 90%),. Effects of HIV on HBV have been depicted as increased HBV DNA, reduced ALT and increased prevalence of liver cirrhosis. The co-infection of HBV and HIV has been suspected to accelerate the progression of liver disease. Immunity impairment due to HIV infection is thought to be the cause of a higher rate of HBV replication with less intense liver damage and less effective immune response to HBV infection. Acute HBV infection in an HIV infected patient may have detrimental effects leading to the development of fulminant hepatic failure (FHF) in some cases, which worsens the morbidity and mortality in co-infected patients, compared to HBV mono-infection and associated accelerated loss of anti-HBs antibody. The dual infection by HBV and HIV leads to more aggressive liver disease with the two viruses interacting in poorly defined ways to increase the rate of hepatic fibrosis (Cropley and Main, 2000), chronic HBV infection, lower rate of spontaneous HbcAg seroconversion and re-activation of HBV disease. The co-infection leads to higher HBV replication, lower ALT levels and lower rates of seroconversion to anti-hepatitis B e (anti-HBe) and anti-hepatitis B (anti-HBs) antibodies.

Molecular diversity

Genetic variation among viruses can affect their detection by nucleic acid, antigen, and antibody-based methods, as well as impact the efficacy of vaccines and antiviral treatment. Molecular epidemiological studies of viral diversity can also reveal the likely origin of epidemic outbreaks and substantiate possible cases of virus transmission. Genetic diversity among viruses exists even within the same virus type resulting in genotypes and subtypes. HBV consist of readily recognizable lineages whose relative frequencies vary considerably in different regions of the world. The frequency of these distinct genetic lineages reflects viral trafficking into and within risk groups and geographic regions. By comparing the distribution of variants belonging to different genetic lineages, genetic analysis of incident viral strains allows the molecular epidemiology of these pathogens to be tracked over time and in different geographic areas.

Documentation of divergent viral strains can be used to accelerate development of serological and nucleic acid amplification test (NAT) assays for diagnostic applications. In addition, surveillance for viral variants among donors has implications for assessing the

prevalence of drug and vaccine escape mutants and for detecting and monitoring rare variants that may be newly introduced or increasing in a population.

HBV genetic diversity

The unique feature of HBV replication is associated with the genetic variability of HBV and persistent infection. HBV genotypes occur as a result of gene mutation. The viral polymerase lacks proofreading activity and sequence heterogeneity is therefore a feature of HBV. The nucleotide substitution rate per site, per year, for HBV is estimated to range between $1.4x10^{-5}$ to $5x10^{-5}$, closerto that of the retroviruses. HBV was formerly classified into four different subtypes that were afterward subdivided according to the antigenic determinants of HBsAg. These included: adw (adw2 and adw4), ayw (ayw1, ayw2, ayw3, and ayw4), adr (adrq+ adrq-), and ayr (Neurath, Thanavala, 1990). However, currently the virus has been classified into nine different genotypes, designated A to I (Yu et al., 2010), the most recent being described in North Western China, Vietnam and Laos. The JRB34 strain currently documented was found to be genetically and phylogenetically distinct from any of the previously published strains, including those of genotype I from Vietnam and Laos. To avoid possible misconceptions in thefuture, the strain is provisionally designated genotype J. Genotype A prevails in western Europe, North America and central Africa; genotype B is frequent in Japan, Taiwan, China, United States and Vietnam; genotype C is common in China, Korea, United States and Japan; genotype D is found mainly in Mediterranean region, India and United States; genotype E is restricted to Africa; genotype F in Polynesia, Central and South America; genotype G in UnitedStates and Europe while genotype H in Central and South America. In a recent study of chronic HBV carriers at 17 United States livercenters, genotypes A and C were found to be most common, although genotypes A-G were found among the study population. Data suggest that HBV genotypes may be associated with severity of liver disease and treatment response these genotypes have specific distribution worldwide.

Africa is one of the highly endemic regions of HBV, with 5 genotypes A-E identified. Genotypes A, D in Kenya and recently genotype E was found to circulate among blood donors, genotype D in Tunisia, genotype A-D in South Africa and genotype E in Nigeria were reported as predominant genotypes in these countries. Apart from these reports, however, there is little information of genotype distribution in Africa despite the importance of this infection in the region.

Detection of incident HBV infection during acute HBV infection, the first marker to appear in serum is HBV DNA followed by hepatitis B surface antigen (HBsAg). The first antibodies to appear are antibodies to the hepatitis B core antigen (anti-HBc IgG and anti-HBc IgM). Anti-HBc remains detectable after resolution of infection and its presence is indicative of either current acute, chronic, or past infection. High levels of anti-HBc IgM antibody are indicative of recent infection.

Genetic diversity evaluation of HBV genotypes among incident HBV infections will provide information on the dynamics of HBV transmission in risk population compared to populations with chronic HBV infection. The epidemiology of HBV infection in the United States may have changed over time as a result of immigration from highly endemic countries, and that differences in genotypes may account for differences in disease manifestations and antiviral therapy response among chronic hepatitis B patients. Structural and functional differences between genotypes can influence the severity, course and likelihood of complications, hepatitis B e antigen (HBeAg) seroconversion and response to treatment of HBV infection and possibly vaccination against the virus. There is mounting evidence that HBV genotype may influence the natural history of the disease and predicting the response to various therapies.

Distinguishing HBV genotypes

Several methods have been developed and used to analyze hepatitis B virus genotyping which include, direct sequencing, PCR (Polymerase Chain Reaction) based restriction fragment length polymorphism (RFLP), line probe assay and enzyme-linked immunoassay. Recently a new genotyping method, based on PCR amplification assay using type-specific primers has been developed. Although the most common method for HBV genotyping is by PCR-RFLP technique, it is reported that HBV genotyping by multiplex PCR is more sensitive than genotyping system using RFLP analysis. Studies have reported that nested PCR methodology for HBV genotyping is 1000-fold more sensitive than PCR-RFLP.

MATERIALS AND METHODS

Study Site

The study site will be Nyanza (Jaramogi Oginga Odinga Teaching and Referral Hospital, Kisumu).

Study design

This will be a cross-sectional study.

Study population

The study population will be HIV infected patients attending comprehensive care clinic (CCC) at Jaramogi Oginga Odinga Teaching and Referral Hospital, Kisumu.

Selection criteria

HIV positive samples from patients not attending CCC at Jaramogi Oginga Odinga Teaching and Referral Hospital.

Collection of plasma

Venous blood will have been collected into commercially available anticoagulant (EDTA) treated tubes. Samples will then have been processed into plasma for various diagnostic applications in the hospital laboratory. Remnant plasma samples after laboratory diagnosis will have been stored at -80oC. Upon collection, samples will be packaged and transported at –20°C or lower to KEMRI HIV laboratory.

HIV rapid screening test

A rapid HIV test will be performed using Determine kit. Briefly, 50 microlitres of the sample will be applied onto the sample pad and left to stand for 15 minutes. A red bar appearing in both the control window and the patient window of the strip will be considered positive while one bar appearing in the control window will be considered negative.

HBsAg detection from plasma

Serological testing to detect the HbsAg from HIV positive plasma will be carried out using Hepanostika ELISA kit at the laboratory in KEMRI. This will be in accordance with the kit manufacturer's instructions. Briefly, 0.1 ml of plasma sample will be added to a

Micro-Elisa plate, coated with anti-HBs antibody, and incubated at 37°C for 2 hours or let to stand at room temperature overnight. The wells will then be washed four times with 0.3 ml of wash fluid. Labeled antibody (0.1 ml) will then be added, incubated at 37°C for 2 hours and washed four hours times. Substrate solution (0.1 ml) will be added, and incubated (let to stand at room temperature for 50 minutes). Finally, 50 ul of H_2SO_4, 2mol/L, will then be added to stop the reaction. The plates will be read using a spectrophotometer.

DNA Extraction from plasma

Viral genome extraction from plasma will be carried out using an Extraction kit according to the manufacturers' instructions. The extracted nucleic acid will be resuspended in 20 ul of RNAse/ DNAse free water and stored at –80°C until use. Briefly, enzyme solution, precipitation solution and sample diluent will be put in a 1.5 ml tube.100 ul of plasma (virus) added into the same tube. This will be vortexed to mix and incubated at 55°C for 30 minutes by dry thermo unit. For nucleic acid purification, protein lyses buffer will be added, mixed gently and incubated at 55°C. 600ul Cold 2-Propanol will then be added and put in ice for 15minutes. After centrifugation (12,000g/40C/3mins), the supernatant will be discarded and pellet washed with 70% alcohol by centrifugation at 12,000g/4°C/3mins. The clear pellet obtained will be air dried at room temperature for 15 min, resuspended with RNAse/DNAse free water and kept at –80^{0}C until use.

Nested PCR

PCR will be performed on extracted DNA using a GeneAmp 9700 machine using specifically designed primers for the regions encoding thevarious viralgenes. Polymerase chainreaction (PCR) willbecarried out to amplify HBV preS1 region. HBV preS1 region corresponding to 2850-3246 nucleotides will be amplified with primer pairs, HBPr1 (GGGTCACCATATTCTTGGG) and HBPr135 (CA (A/G)
AGACAAAAGAAAATTGG) outer PCR. Inner PCR reaction primers will be HBPr2 (GAACAAGAGCTACAGCATGGG) and HBPr3 (CCACTGCATGGCCTGAGGATG) sense and antisense primers consecutively.

A master mix containing; water -9ul, 10x PCR buffer -2ul, dNTPs- 2ul, first round primer pair-0.4ul and Taq polymerase 0.2 ul will be used. Template DNA, (4.0ul) reaction will be used for amplification. HBV DNA, preS1 region, will be amplified using primers HBPr1 and HBPr135 (outer PCR) followed by a nested reaction using HBPr2 and HBPr3. Outer PCR will amplify the viral DNA over 40 cycles, with denaturation at 94°C for 30 s, annealing at 50°C for 30 s and elongation at 72°C for 30 s. Samples in first-round PCRwill further be amplified with nested PCR primers for 35 cycles with the same thermal profile. Products of the second PCR will be separated on 2% agarose gel, stained in ethidium bromide andvisualized under UV light.

Gel electrophoresis of PCR products

Amplified products will be detected by electrophoretic analysis on 2% agarose gel containing 0.005% ethidium bromide followed by examination under UV light.

HBV genotype analysis based on nucleotide sequencing

Sequencing is the gold standard to classify HBV genotypes and sub-genotypes. Upon successful PCR, samples will be sequenced directly using Big Dye chemistry. Briefly, proviral DNA (after being labeled using Big Dye chemistry in a PCR) will be precipitated in 2µl of 3M sodium acetate, 2ul of 125mmol EDTA and 50 µl of absolute ethanol, vortexed and incubated at room temperature for 15 minutes. Spinning at 15000 rpm for 30 minutes and washing in 70% ethanol for 10 minutes will follow after discarding the supernatant. DNA pellets will be air dried for 30 minutes followed by addition of 25 µl of TSR, in readiness for sequencing. Sequencing will be done on an automated ABI 3100.

Data management and analysis

Data storage

Identifiers will be eliminated from the samples to maintain confidentiality. Clinical and laboratory data will be entered into Microsoft Excel spreadsheet. The back- up data created will be stored in external compact disks and Flash disks. The soft copy files will be protected using password.

Data analysis

Computer software, MEGA (Molecular evolutionary genetic analysis), and Genetyx will be used on sequences. The sequences will further be aligned using Clustal W computer program. Phylogenetic trees will be constructed using the neighbor-joining method (PHYLIP), and trees viewed by Tree View version 1.6.5. Statistical analysis will be performed using SPSS 16. Data will be summarised in percentages and presented in frequency tables and graphs where necessary. (Figure 2)

PERIOD/ ACTIVITY	JAN-MAR 2012	APR 2012	MAY 2012-SEP 2013	OCT 2013-DEC 2013	JAN 2014-FEB 2014	MAR 2014-MAY 2014	JUN 2014-JUL 2014
Development & writing of proposal	■						
Proposal presentation to JKUAT		■					
Approval of proposal by SSC & ERC			■				
Fieldwork & Lab work in KEMRI				■			
Data analysis					■		
Thesis writing & publication						■	
Submission of thesis & defense							■

Figure 2: Time Frame

Ethical considerations

Ethical clearance will be sought from KEMRI Scientific Steering Committee (SSC), Ethical Review Committee (ERC), Jomo Kenyatta University of Agriculture and Technology. Since these are diagnostic remnant samples whose results will not be directly linked to the individuals, informed consent from the individuals will not be required. However, consent to collect and use the samples shall be sought from the hospital. To ensure confidentiality, code numbers will be used for the samples and no link shall be made with the sample source names (identifiers of the patients will be removed). The research will be conducted in accordance with KEMRI guidelines on human sample use and care and the internationally accepted principles for laboratory use and standardoperating procedures as found in WHO guidelines.
WHO Biosafety precautions shall be observed to prevent contact with blood and other potentially infectious materials. Laboratory

Activities involving hazardous materials and other human clinical specimens, body fluids, and untreated materials shall be handled using Biosafety Level 2 practices, containment equipment, and facilities. Further work may require use of the Biosafety level 3 laboratory at KEMRI. Staff carrying out the work will be updated on Post Exposure Prophylaxis protocols (PEP) before the work begins. Biological material will be stored at -80^{0}C before use. All personnel concerned with handling the clinical material will be screened for HBsAg and if negative must be vaccinated against hepatitis B. Disposable gloves and laboratory coat will be worn when working with the known Hepatitis B infectious materials. Work areas will be decontaminated with 0.5% sodium hypochlorite prepared fresh each month. Gloves, micro pipette tips, tubes, micro tubes and any other disposable materials and equipment used in the laboratory when handling Hepatitis B Virus will be autoclaved before being discarded.

REFERENCES

1. Perz JF, Farrington LA, Pecoraro C, Hutin YJF and Armstrong GL. Estimated global prevalence of hepatitis C virus infection. Infectious Diseases Society of America 42nd annual meeting, Boston, MA. 2004
2. Chun H M, Roediger MP and Hullsiek KH. Hepatitis B Virus Co-infection Negatively Impacts HIV Outcomes in HIV Seroconverters. J Infect Dis. 2011;205:185–193.
3. Day SL, Odem-Davis K, Mandaliya KN, Jerome KR, Cook L. Prevalence, Clinical and Virologic Outcomes of Hepatitis B Virus Co-Infection in HIV-1 Positive Kenyan Women on Antiretroviral Therapy. PLoS ONE. 2013;8:e59-e346.
4. Peters PJ and Marston BJ. Preventing Deaths in Persons with HIV/ Hepatitis B Virus Co-infection: A Call to Accelerate Prevention and Treatment Efforts. J Infect Dis. 2011;205:166-168.
5. Heerman KH, Gerlich WH, Michael C, Schaefer S and Thomssen R. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparartions. J Clin Microbiol. 1999;37:68–73.
6. Bowyer SM and Sim G. M. Relationship within and between the genotypes of Hepatitis B virus at point across the genome: footprints of recombination in certain isolates. J Gen Virol. 2000;1:379–392.
7. Mahoney FJ. Update on diagnosis, management, and prevention of hepatitis B virus infection. Clin Microbiol Rev. 1999;12:351–366.
8. Alter MJ. Epidemiology of Viral Hepatitis and HIV co-infection. J Hepatol. 2006;44:56-59.
9. Harania R, Karuru J, Nelson M and Stebbing J. HIV, hepatitis B and hepatitis C co-infection in Kenya. AIDS. 2008;22:1221-1222.
10. Bello H and Olabode HOK. Incidence and demographic distribution of Human Immunodeficiency Virus (HIV) in Biu, Borno State-Nig. Niger. J Parasitol Publ, 2011;32: 235-238.
11. Otedo AE, Mc'Ligeyo SO, Okoth FA and Kayima JK. Seroprevalence of hepatitis B and C in maintenance dialysis in a public hospital in a developing country. South Afric Med J. 2003;93:380-384.
12. Mwangi J, Nganga Z, Songok E. Molecular Genetic Diversity of Hepatitis B Virus in Kenya. Inter virol, 2008;51:417-421.
13. Mizokami M. A new subtype (subgenotype) Ac (A3) of hepatitis B virus and recombination between genotypes A and E in Cameroon. J Gen Virol; 2005;86:2047–2056.
14. Puoti M, Airoldi M and Bruno R. Hepatitis B virus co-infection in human immunodeficiency virus infected individuals. AIDS. 2000;4:27-35.
15. Valsamakis A. Molecular Testing in the Diagnosis and Management of Chronic Hepatitis B .J Clin Microbiol. 2007;20:426-439.
16. Kramvis A, Kew M and Guido F. Hepatitis B Virus genotypes. Journal of Viral Hepatitis, 2004;23:2409-23.
17. Hollinger FB and Liang TJ. Hepatitis B Virus. In Fields Virology. Knipe DM, Howley PM, eds. Philadelphia: Lippincott, Williams and Wilkins. 2001;58:2971-3036.
18. Thio C. Hepatitis B and Human Immunodeficiency Virus coinfection. W J Gastroentero. 2009;49:5138-5145.