

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

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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 determine 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. 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.

Keywords: HBV; HIV; CCC; JOOTRH

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. Research shows that HIV to be in 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. 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 Virology Outcomes of Hepatitis B Virus Co-Infection in HIV-1 Positive Kenyan Women on Antiretroviral Therapy demonstrated a 7% prevalence of chronic HBV infection. There is therefore need for additional steps to combat hepatitis B now, including more extensive HBV screening and vaccination[1,2].

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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 Hepato Cellular Carcinoma (HCC) globally. Earlier research findings show that the virus causes acute hepatitis of varying severity and persists in 95% of children of adult patients. Leading to chronic liver disease, cirrhosis, hepatocellular carcinoma and even fulminant hepatitis[3].

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, which would modify the progression, manifestation or management of each of the infections [4,5].

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 mucous membrane[6,7].

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. 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. However, in Kenya, the few studies that have been conducted to determine the seroprevalence of HBV/HIV co-infections and HBV genome variability have targeted the blood donors. Hepatitis B virus genotypes are geographically grouped into 10 groups. 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.

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. 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 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 [8].

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?

Objectives

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

Specific objectives: To determine the seroprevalence of HBV infection among HIV infected patients in Nyanza. To determine the prevalence of occult HBV among HIV infected patients in Nyanza. To determine the prevalent HBV genotypes among HIV infected patients Nyanza [9].

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. It requires RNA intermediate hence the viral polymerase possesses reverse transcriptase activity as seen with the retroviruses. The

42 nm, surrounded by an envelope containing the surface antigen (HBsAg). The genome is about 3.2 kb.

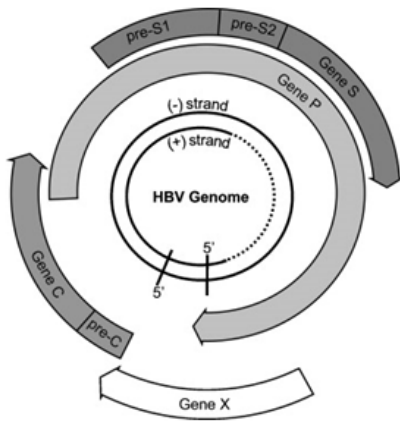


Figure 1: HBV genome.

Stages of HBV chronic infection

The chronic HBV infection progresses in four stages

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 [10].

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, 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, persistently 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 HBsAg.

The fourth phase of chronic HBV infection is characterized by lack of detectable HBeAg, the presence of anti-HBsAg 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 (Mahoney, 1999). 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.

HBV/HIV co-infections

Patients with HIV may have co-infection with one or more hepatitis viruses. 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. 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.

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.

MATERIALS AND METHODS

Collection of plasma

Venous blood will have been collected into commercially available anticoagulant 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. Upon collection laboratory.

HIV rapid screening test

A rapid HIV test will be performed using Determine kit. Briefly, 50 microliters 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 text for 2 hours or let to stand at any room temperature overnight.

PERIOD/	JAN-MAR 2012	Apr-12	MAY 2012-SEP 2013	OCT 2013-DEC 2013	JAN 2014-FEB 2014	MAR 2014-MAY 2014
Development & writing of proposal						
Proposal presentation to JKUAT						
Approval of proposal by SSC & ERC						
Fieldwork & Lab work in KEMRI						
Data analysis						

Figure2: Time frame.

CONCLUSION

Ethical clearance will be sought from KEMRI Scientific Steering Committee (SSC), Ethical Review Committee (ERC),

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 standard operating procedures as found in WHO guidelines.

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. Statistical analysis will be performed using SPSS 16. Data will be summarized in percentages and presented in frequency tables and graphs where necessary.

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