DIAGNOSTIC VALUE OF HBsAg IN HBV INFECTION AMONG PATIENTS REFERRED TO PATHOLOGIST LANCET LABORATORY IN NAIROBI, KENYA

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Diagnostic value of HBsAg in HBV Infection among patients referred to Pathologist Lancet Laboratory in Nairobi, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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This thesis has been submitted for examination with our approval as the university supervisors**,**

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DEDICATION

This thesis is dedicated to my dear mother; Mrs. Susan Mathai

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I am grateful for the abundance of good health and the means through which I have been successful. Nobody has been more important to me in the pursuit of this project than the members of my family. I would like to thank my mother Mrs. Susan Mathai whose love and guidance are with me in whatever I pursue. She is the ultimate role model who provide unending inspiration.

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LIST OF ABBREVIATION/ACRONYMS

ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
CDC	Center for Disease Control
CHB	Chronic Hepatitis B
CI	Confidence Interval
COHES	College of Health Sciences
CRF	Case Report Form
CSC	Center Scientific Committee
CVR	Center for Virus Research
DIALY	Disability Adjusted Life Year
DICH -GGCP	Declaration of Helsinki and International Conference on Harmonization Guideline on Good Clinical Practice
DNA	Deoxyribonucleic Acid
EDTA	EthyleneDiamineTetraacetic Acid
ERC	Ethical Review Committee
GGT	Gamma-Glutamyl Transpeptidase
HbcAb	Hepatitis B Core Antibody
HbeAg	Hepatitis B e Antigen
HbsAb	Hepatitis B Surface Antibody
HbsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	HepatoCellular Carcinoma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IU	International Units
KEMRI	Kenya Medical Research Institute
MoH	Ministry of Health
OR	Odds Ratios
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid

SANAS	South African National Accreditation System
SID	Subject Identification Number
SSC	Scientific Steering Committee
SST	Serum Separator Tubes
STDs	Sexually Transmitted Diseases
WHO	World Health Organization

ABSTRACT

Quantification of Hepatitis B virus (HBV) Deoxyribonucleic acid (DNA) levels has been used to manage chronic hepatitis B (CHB) patients on treatment. However, HBV DNA quantification techniques are costly and rarely done. Measuring of hepatitis B surface antigen (HBsAg) levels by chemiluminescent micro-particle immunoassay has been suggested as an alternate marker. This study sought to determine the sero-prevalence of HBV, the chronicity of HBV and correlated the HBsAg with HBV DNA serum levels among patients attending Pathologist Lancet Laboratory in Nairobi, Kenya. Of all the patients routinely tested for HBsAg, those meeting the recruitment criteria were consented and recruited in this study. Blood samples were obtained from 2246 patients attending Pathologist Lancet Laboratory for HBV testing. A sociodemographic questionnaire was administered. The HBsAg levels were measured by Roche Cobas e 411 analyzer with Elecsys HBsAg II Quant reagent kits (Roche Diagnostics, South Africa, SA) while the HBV DNA viral load (IU/mL) was measured using Cobas AmpliPrep/CobasTaqMan HBV Test, version 2.0(Roche Molecular Systems, South Africa, SA). Overall, 346/2246 (15.4%) were HBsAg positive. The mean age (Standard deviation) was 37.5(10.8) years. The majority 129(37.3%) aged between 31 and 40 years, 221 (63.9%) were male, 111(32.1%) consumed alcohol for two or more years, 297(85.8%) did not use condom in their last two sexual encounters, 62(17.9%) shared personal effects with other family members, 78(22.5%) had received blood transfusion, 144(41.6%) had undergone invasive procedures before, 122(35.3%) had received HBV vaccination while 144(41.6%) had HBV related symptoms. Out of the 346 HBsAg positive 173 (50%) were also positive for either HCV or HDV and were excluded for further correlation analysis. For the 173 patients that were included for correlation analysis, the majority, 142 (82.1%) were hepatitis B e antigen (HBeAg) negative and were considered chronically infected. The overall mean (Log10) of HBsAg (SD) titter was 3.58 (0.39) IU/mL and an overall mean (Log10) of HBV-DNA (SD) of 2.89 (1.62) IU/mL. The HBV DNA levels was significantly different between HBeAg positive and negative patients (P = 0.001) similar to the HBsAg levels (P = 0.032). There were 22/31 (70.9%) HBeAg positive participants with HBV DNA $\geq$ 20,000 IU/ML and 14/142 (9.9%) HBeAg negative with HBV DNA $\geq$ 2000 IU/ML. There was a significant but weak correlation between HBV DNA and HBsAg (r = 0.171; P= 0.024). The prevalence of HBV was 15.4% and a significant proportion, 82.1% were chronically infected with HBV. Serum quantitation of HBsAg may not replace HBV DNA level estimation among patients with CHB in Nairobi Kenya. It is an estimate and not a gold standard, but it can definitely have a role in correlating disease especially in stable patients after an initial HBV DNA is performed to save expenses and minimize the cumbersome nature of HBV DNA testing. In additional larger prospective study, be conducted to re-evalute the correlation if any between HBV DNA and HBsAg levels in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background Informantion

Hepatitis is the inflammation of the liver mainly caused by infection with hepatitis A, B, C, D and E viruses (Hollinger and Liang, 2001). Of the many viral causes of human hepatitis, hepatitis B Virus (HBV) is of greater global importance (Ganem and Schneider, 2001). The disease causes up to 80% of all cases of hepatocellular carcinoma worldwide, second only to tobacco among known human carcinogens (Hollinger and Liang, 2001; WHO, 2002). The HBV is transmitted through percutaneous or parenteral contact with infected blood, body fluids, and by sexual intercourse (WHO, 2002; 2014). HBV is able to remain on any surface for about a week (e.g. table-tops, razor blades, blood stains) without losing infectivity (WHO, 2002; 2014). The severe pathological consequences of persistent HBV infections include the development of chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma (HCC). In addition, HBV carriers can transmit the disease for many years (WHO, 2002).

Chronic hepatitis B is a major global problem, affecting more than 400 million people worldwide (Lavanchy, 2004) and leading to 1 million deaths each year. The HBV infection occurs worldwide, the prevalence and transmission mode vary geographically, and the world can be classified into three endemic regions. Around 45% of the world's population lives in regions of high endemicity, defined as areas where 8% or more of the population are positive for HBsAg such as Southeast Asia, parts of the Middle East, the central Asian Republics, and some countries in Eastern Europe and Sub-Saharan Africa. The moderately endemic areas including Mediterranean countries and Japan are defined as those areas where 2–7% of the populations are HBsAg positive, and around 43% of the world's populations live in regions of moderate endemicity. Western Europe and North America are considered as areas with low endemicity (<2% of the population is HBsAg positive) and it constitutes 12% of the world's population

(Kowdley, 2004; Knipe and Howley, 2013; WHO, 2013). In Kenya, varied HBV prevalence rates have been reported; in 1989 Okoth *et al.,* (1989) reported a prevalence of 11.4% HBV infection among outpatients attending three distinct hospitals in Mombasa, Kilifi, and Malindi. Muruiki *et al.,* (2013) identified a prevalence of 6% HBV infection among HIV-1 infected individuals in Nairobi, Kenya. Among Kenyan health workers, 41% were positive for HBV (Suckling *et al.,* 2006). Among HIV sero-discordant couples in Thika, a prevalence of 8.4% was observed; 7.2% among those with HIV and 9.6% among those HIV negative. Work carried out by Mutuma *et al.,* (2011) indicates that a prevalence of 8.8% HBsAg in an asymptomatic rural normadic population in Kenya.

Report by Murray *et al.,* (2012) shows that the almost 1.2 million Disability-Adjusted Life Year (DALYs) were lost due to HBV globally in 2010, which corresponds to an overall rate of 134.54 DALYs per 100000 residents. Most DALYs (39.7%) were lost due to HBV-related cirrhosis. About 39.5% lost were due to HCC and 20.6% due to acute HBV infection. Hepatitis B virus infection early in life is associated with the highest risk of chronic infection. People with chronic infection risk progression to cirrhosis and primary liver cancer (WHO, 2009). The likelihood of progression to chronic infection is the same whether infection is symptomatic or asymptomatic. People with chronic hepatitis B virus infection have a 15% to 25% risk of dying prematurely from hepatitis B virus-related cirrhosis and liver cancer (WHO, 2009).

Currently HBV DNA levels, Alanine transaminase (ALT) levels, and histological findings are used as the basis for HBV treatment (Ganji, *et al.*, 2011). Active HBV infection can be detected by quantifying HBV DNA, but such assays are expensive. Considering the distribution of HBV, particularly in developing countries, a cheaper laboratory test that can be used for detection of HBV DNA would improve HBV management (Ganji, *et al.*,2011). The serological assays that detect HBsAg have guided the diagnosis of HBV infection. Studies have yielded various results when evaluating relationship between serum HBsAg concentrations and HBV DNA levels in HBV patients (Deguchi *et al.*, 2004; Lei

et al.,2006; Ozaras *et al.*,2008). On the other hand, Ozdil *et al*., (2009) showed a negative correlation between HBV DNA and HBsAg levels. The backdrop of these conflicting findings and the lack of equivocal data in Kenya prompted this study. This study seeks therefore to determine the usefulness of serum HBsAg concentration as a surrogate marker of HBV replication (HBV- DNA levels) in patients referred to the Pathologist Lancet Laboratory in Nairobi Kenya.

1.2 Statement of the Problem

Hepatitis B virus (HBV) causes transient and chronic infections of the liver. This may lead to serious illness, and approximately 0.5% leads to fatal, fulminant hepatitis. Chronic infections may also have serious consequences: nearly 25% lead to untreatable liver cancer (Seeger and Mason, 2000; WHO, 2013). Worldwide deaths from liver cancer caused by HBV infection exceed one million per year (Seeger and Mason, 2000; WHO, 2013). The global prevalence of HBV infection in HIV-infected persons is 7.4% (WHO, 2017), Infection with HIV increases rates of HBV chronicity, prolong the time the HBV stays in circulation and increase liver-related morbidity. A recent global burden of disease study estimated that most sub-Saharan African countries, including Kenya, have a prevalence of chronic hepatitis B infection (CHBI) in the higher intermediate (5–7%) to high range ≥ 8% (Ott *et al*., 2012). Current estimates put the prevalence of hepatitis B virus (HBV) infection in Kenya at 5-8% (ly *et al*., 2016). This calls for urgent prevalence and incidence surveys to identify carriers of chronic HBV in order to instigate management.

The HBsAg is a classical marker of infection with hepatitis B virus, and serological assays to detect HBsAg have guided its diagnosis. Upon HBV infection, closed circular HBV DNA genome forms within the hepatocytes nuclei which become enveloped and secreted into the blood (Arai *et al.*, 2012). HBV DNA, is the template for gene transcription and replication, correlating robustly with levels of total intracellular HBV DNA, serum HBV DNA, and HBsAg (Chan *et al.*, 2011). Thus, HBV DNA level is the most important and most direct etiological evidence for HBV (Zhou *et al.*,2015). However, in developing

countries endemic for HBV (Schweitzer *et al.,* 2015), robust, cheaper and easy-to-perform markers are essential in the management and monitoring of HBV patients (Maylin *et al.,*2015). HBV viral load measurement is rarely accessible and where available often done once – which may not reflect real viral activity (Maylin *et al.,* 2015). Further, usefulness of viral load in diseases monitoring, and medication in patients with undetectable HBV DNA levels is still missing (Zhu and Zhang, 2016). Such correlation studies in Kenya will be vital for finding an alternative cheaper method /tests to monitor HV infection.

1.3 Justification of the Study

The HBV infections of the liver leading to serious illness with some being fatal (WHO, 2013). Infection with HIV increases rates of HBV chronicity, prolong the time the HBV stays in circulation and increase liver-related morbidity. A recent global burden of disease study estimated that most sub-Saharan African countries, including Kenya, have a prevalence of chronic hepatitis B infection (CHBI) in the higher intermediate (5–7%) to high range ≥ 8% (Ott *et al*., 2012). This calls for urgent prevalence study to identify carriers of chronic HBV in order to instigate management.

The direct detection of HBV DNA in serum or plasma has become an important tool in the diagnosis of chronic HBV infection. Further, serum HBV DNA level may be an important prognostic indicator (Lok *et al.,* 2001; Castelnau *et al.,* 2002; Yu *et al.,* 2005) as well as an important marker for measuring therapeutic response and the development of resistance to antiviral agents (Puchhammer-Stockl*et al.,* 2000). Variety of commercial HBV DNA assays reporting in standardized units with improved sensitivity are currently available (Weiss, *et al.,* 2004; Jeffrey *et al.,* 2006). However, HBV DNA monitoring does not come without its limitations, such as cost, standardization, sensitivity, absolute cutoff levels, and being labor intensive (Sablon and Shapiro, 2005). Studies in various clinical settings have evaluated correlation of HBsAg titers and HBV DNA levels and whether the former could be used as a marker of disease progression and response to treatment. Varied outcomes have been realized in these correlations

(Brunetto *et al.*,2008; Kim *et al.*, 2011; Ganji *et al.*, 2011; Vigano *et al.*, 2012) while no equivocal data currently exist in Kenya.

1.4 Research Questions

1. What is the prevalence of HBV among patients referred to Pathologist Lancet Laboratory in Nairobi?
2. What is the chronicity of HBV among patients referred to Pathologist Lancet Laboratory in Nairobi?
3. Is there relationship between hepatitis B surface antigen (HBsAg) titers and hepatitis B virus (HBV) DNA levels in chronic HBV patients?

1.5 Objectives

1.5.1 General Objective

To determine the diagnostic value of HBsAg in HBV infection among patients referred to Pathologist Lancet Laboratory in Nairobi

1.5.2 Specific Objectives

1. To determine the prevalence of HBV among patients referred to Pathologist Lancet Laboratory in Nairobi.
2. To determine the chronicity of HBV among patients referred to Pathologist Lancet Laboratory in Nairobi.
3. To determine the relationship between HBsAg titers and HBV DNA levels in chronic HBV patients

CHAPTER TWO

LITERATURE REVIEW

2.1 Hepatitis B Virus

The HBV belongs to the genus *Orthohepadnavirus*. The genus contains three other species: The Ground squirrel hepatitis virus, Woodchuck hepatitis virus, and the Woolly monkey hepatitis B virus. The HBV belongs to the *Hepadnaviridae* family (King *et al.,* 2011). Generally, HBV is small (diameter of 42 nm) with three distinct morphological characteristics. The most abundant are small, spherical, noninfectious particles, containing HBsAg, that measure 17 to 25 nm in diameter and are composed of lipid particles (Hollinger andLiang, 2001). Tubular, filamentous forms of various lengths, but with a diameter comparable to that of the small particles. They also contain HBsAg polypeptides (Hollinger and Liang, 2001). The third morphological form; is the 42 nm hepatitis B virion, which is a complex, spherical, double shelled particle. This structure consists of an outer envelope containing host-derived lipids and all S gene polypeptides, the large (L), middle (M), and small (S) surface proteins, also known as pre-S1, pre-S2 and HBsAg. The nucleocapsid contains core proteins HBcAg, a 3.2 kb, circular, partially double stranded viral DNA genome, an endogenous DNA polymerase (reverse transcriptase) enzyme, and protein kinase activity (Ganem *et al.*,2001) (Figure 2.1).

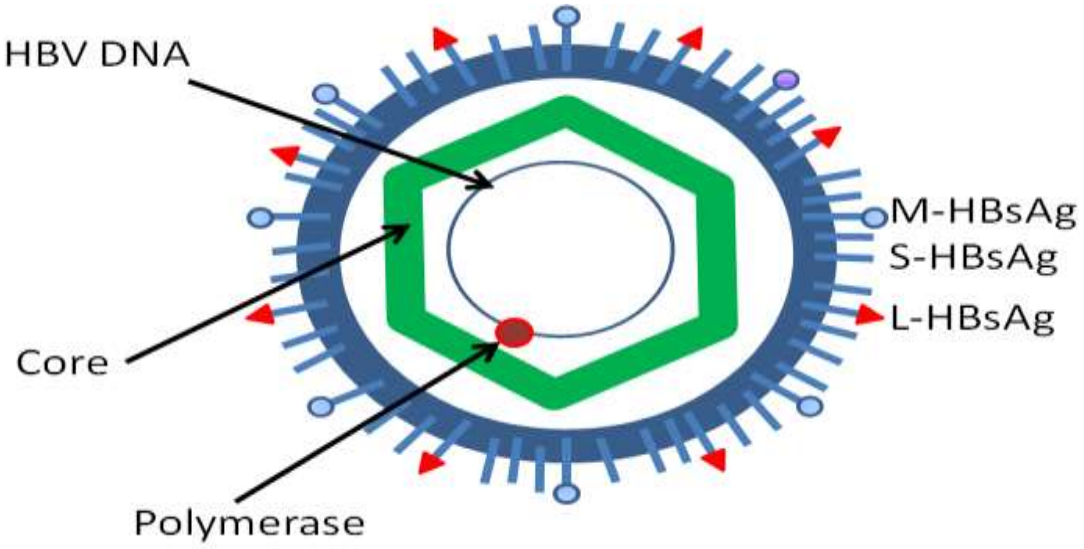

Figure 2.1: The Structure of Hepatitis B Virus (Roseman *et al.*, 2012).

The virus was originally divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope proteins, and presently into eight genotypes (A–H) according to overall nucleotide sequence variation of the genome (Schaefer, 2007). The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination (Kramvis *et al.*, 2005). Substantial genetic variations occur within distinct regions, globally facilitating classification of eight distinguishable genotypes (A through H), which have treatment implications (Schaefer, 2007).

2.2 The Life Cycle of Hepatitis B Virus

The virus enters the hepatocyte using a number of receptors, including the transferrin receptor, the asialoglycoprotein receptor molecule, and human liver endonexin. The mechanism of HBsAg binding to a specific receptor to enter cells has not been established yet (Ganem *et al.*, 2001). The partially double-stranded DNA passes into the nucleus, where it is turned into completely double-stranded circular DNA (Ganem *et al.*, 2001). Various messenger RNAs are then transcribed from the DNA and these go on to direct the synthesis of viral proteins

using the host cell machinery. Such as the viral nucleocapsid and precore antigen (C, pre-C), polymerase (P), envelope L (large), M (medium), S (small), and transcriptional transactivating proteins (X) (Ganem *et al.,* 2001; Schädler *et al.,* 2009). When the viral polymerase is translated from an RNA message that corresponds to the whole viral genome, it stays associated with this copy of the message, and then the core proteins, which have been separately synthesized, assemble around the complex to form an immature core particle. The new, mature, viral nucleocapsids can then follow two different intracellular pathways, one of which leads to the formation and secretion of new virions, whereas the other leads to amplification of the viral genome inside the cell nucleus (Ganem *et al.,* 2001). The viral polymerase then gets to work inside the core copying the RNA genome into the first strand of DNA, degrading the RNA as it goes. The second matching strand of DNA is then started, and by the time one-half to three-quarters of the second strand has been made, the core becomes mature. It can then interact with surface proteins, which have been separately synthesized and inserted into an inner cellular membrane, through which the core buds. The core thus gets enveloped and subsequently secreted from the cell to complete the viral cycle (Bruss, 2004) (Figure 2.2).

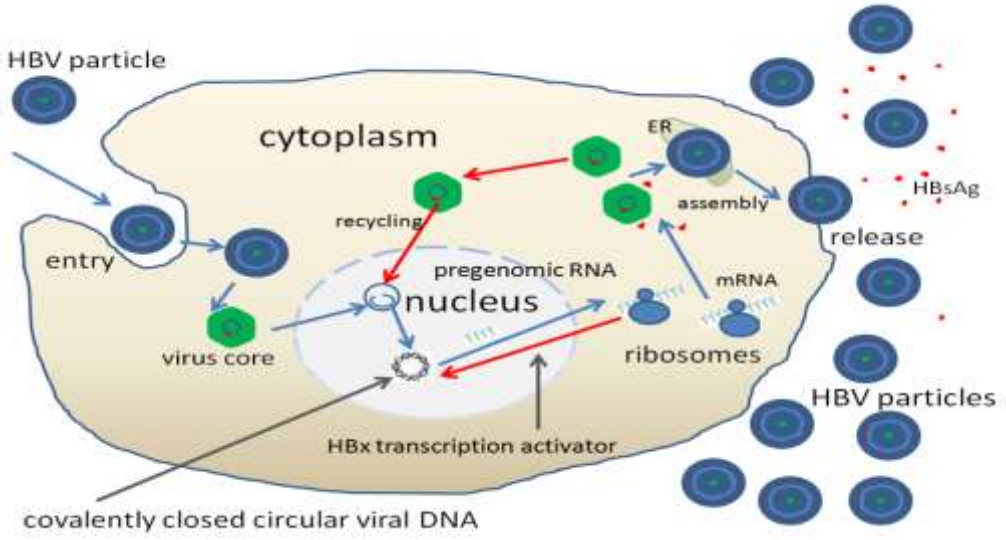

Figure 2.2: Replication of Hepatitis B Virus

(Schädler *et al.,* 2009).

2.3 Epidemiology of Hepatitis B Virus

The HBV infection occurs worldwide, with the prevalence and transmission mode varrying geographically, and it can be classified into three endemic patterns (Figure 2.3). Around 45% of the world's populations live in regions of high endemicity, defined as areas where 8% or more of the population are positive for HBsAg such as Southeast Asia and Sub-Saharan Africa. The moderately endemic areas, such as in Mediterranean countries and Japan, are defined as those areas where 2–7% of the population are HBsAg positive, and around 43% of the world's population live in regions of moderate endemicity. Western Europe and North America are considered as areas with low endemicity (<2% of the population is HBsAg positive) and it constitutes 12% of the world's population (Kowdley, 2004; Knipe and Howley, 2013; WHO, 2013). In Kenya varied prevalence rates have been reported ranging from 6% to 41% (Muruiki *et al.,* 2013; Suckling *et al.,*2006); The HBV prevalence was 6% reported in Nairobi (Muruiki *et al.,* 2013); about 8.8% in the rural normadic population (Mutuma *et al.,* 2011); about 11.4% in the coastal Kenya (Okoth *et al.,*1989) and 41% in Nairobi (Suckling *et al.,*2006).

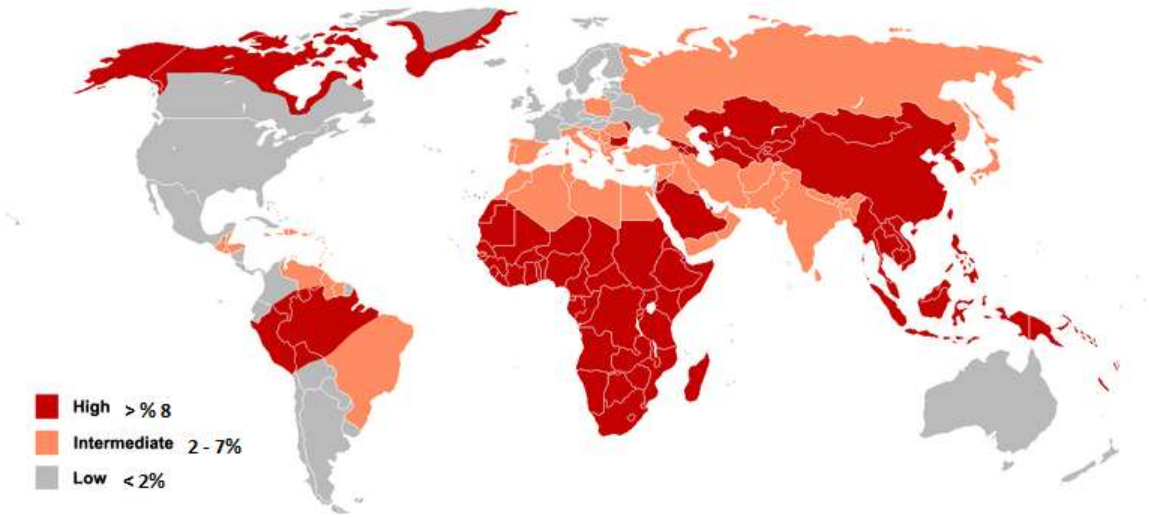

Figure 2.3: Epidemiology of Hepatitis B Virus

(Adopted from CDC, 2007)

2.4 Transmission of Hepatitis B Virus

The HBV is about 100 times more infectious than HIV (WHO, 2013). There are four recognized modes of transmission routes including: From mother to child at birth (perinatal), contact with an infected person (horizontal), sexual contact and parenteral (blood-to-blood) exposure to blood or other infected fluids (Hollinger and Liang, 2001). The HBV is stable on environmental surfaces for at least 7 days, and indirect inoculation can occur via inanimate objects like toothbrushes, baby bottles, toys, razors, eating utensils, hospital equipment and by contact with mucous membranes or open skin breaks (Hollinger and Liang, 2001). HBsAg has been found in all body secretions and excretions. However, only blood, vaginal and menstrual fluids, and semen have been shown to be infectious (Hollinger and Liang, 2001). Percutaneous exposures that have resulted in HBV transmission include transfusion of unscreened blood or blood products, sharing unsterilized injection needles for intravenous drug use, haemodialysis, acupuncture, tattooing and injuries from contaminated sharp instruments sustained by hospital personnel (Hollinger and Liang, 2001).

There is considerable variation between areas, countries and continents as to the age at which most transmission takes place. In Western Europe and the United States of America, HBV is mainly transmitted horizontally by blood products or mucosal contact. In highly endemic areas like Southeast Asia or Equatorial Africa, transmission is mainly vertical perinatally from an HBV-infected mother to the new-born child (Thomas *et al.*, 2005; Knipe and Howley, 2013). Certain types of behaviors increase the risk for contracting HBV such as : use of contaminated needle during acupuncture, intravenous drug abuse, ear piercing and tattooing, sexually active heterosexuals or homosexuals (having more than one sexual partner in the last 6 months), infants/children in highly endemic areas, infants born to infected mothers, health care workers, haemodialysis patients, blood receivers prior to 1975 (blood transfusion), haemophiliacs, prisoners with long term sentences as well as visitors to highly endemic regions (Thomas *et al.*, 2005).

2.5 Methods for diagnosis of HBV Infection

Tools used for diagnosis of HBV infection includes: serological markers such as HBV antigens and host antibodies (Table 2.1); molecular makers such HBV DNA quantification using PCR; biochemical markers such as alanine aminotransferase (ALT); Histological marker such the degree of hepatic fibrosis and inflammation (Danta, 2014).

Table 2.1: Interpretation of HBV Immunologic Markers

HBsAg*	HBcAb†	HBsAb‡	Interpretation
–	–	–	Susceptible to HBV infection (should be vaccinated)
–	–	+	Immune because of vaccination
–	+	+	Immune because of natural HBV infection
+	+	–	Acute or chronic HBV infection
–	+	–	Interpretation unclear; four possibilities: 1. Resolved HBV infection (most common) 2. False-positive HBcAb, thus susceptible 3. "Low-level" chronic HBV infection 4. Resolving acute HBV infection

*HBcAb - hepatitis B core antibody; HBsAb - hepatitis B surface antibody; HBsAg - hepatitis B surface antigen; + positive test result; – negative test result. * The presence of HBsAg indicates that the person is infectious. †HBcAb appears at the onset of acute HBV infection. Presence may also indicate chronic HBV infection or a false-positive test. ‡The presence of HBsAb indicates recovery and immunity from HBV infection or successful immunization against HBV (Mast et al., 2006).*

2.5.1 Biochemical Serology of HBV

2.5.1.1 Hepatitis B surface antigen (HBsAg)

HBsAg is an antigen on the three proteins that make up the envelope of the HBV virion. It is secreted as lipoprotein particles in excess of virions by a ratio of greater than 1000:1. HBsAg is the established serological marker used routinely for the diagnosis of acute or chronic HBV infection, the screening of blood or organ donors, and the surveillance of persons at risk of acquiring or transmitting HBV. The antigen is usually detectable between week 4 and week 10 in acute infection. Chronic HBV infection is defined by the persistence of HBsAg for more than six months (Chan *et al.,* 2011; Janssen *et al.,* 2012). HBsAg is the first marker that appears in the blood following infection with hepatitis B virus (HBV). The presence of HBsAg in human serum indicates an ongoing HBV

infection, either acute or chronic. Testing of additional HBV markers, such as the hepatitis B E antigen, is adopted to define the specific disease state. HBsAg immunoassays are used not only to diagnose HBV infections but also to monitor the course of the disease and the efficacy of antiviral therapy (Chan *et al.*,2011; Janssen *et al.*,2012).

2.5.1.2 Antibody to Core Antigen (anti-HBc)

Theanti-HBc is an antibody to a peptide of this core protein, which has been processed within an antigen presenting cell. It appears at the onset of symptoms in acute hepatitis B and persists for life. The presence of anti-HBc indicates previous or ongoing infection with hepatitis B virus in an undefined time frame (Janssen *et al.,* 2012). In acute infection, anti-HBc immunoglobulin M (IgM) is found in high concentrations which gradually decline, complementing the corresponding increase in anti-HBc IgG over a three to six-month period. Elevation of anti-HBc IgM usually signifies acute infection, but low elevations may also occur during the reactivation of chronic HBV (Chan *et al.,* 2011). Anti-HBc IgG remains positive for life following exposure to HBV, however, unlike anti-HBs, anti-HBc is not a protective antibody. Most serological assays do not directly measure anti-HBc IgG, but test for total anti-HBc antibody (Chan *et al.,* 2011; Janssen *et al.,* 2012).

2.5.1.3 Antibody to *e* antigen (anti-HBe)

The Hepatitis B e antigen (HBeAg) is aprotein from the pre-core region of the HBV genome, which is, produced during active viral replication and may act as an immunogen or a tolerogen, leading to persistent infection (Milich and Liang, 2003). The anti-HBe is not a protective antibody, its appearance usually coincides with a significant immune change associated with lower HBV DNA replication (<105 copies/mL or 20,000 IU/ml). The loss of HBeAg and the development of anti-HBe is termed HBeAg seroconversion, and has been used as an end-point for treatment in HBeAg-positive people, as it has been shown that

seroconversion is associated with a lower risk of disease progression (Milich and Liang, 2003).

2.6 Virological Markers for Hepatitis B Virus

2.6.1 Hepatitis B Virus DNA

With the availability of polymerase chain reaction (PCR) and other molecular amplification technology, it has become possible to directly quantify the level of HBV replication. PCR-based assays (target amplification assays) involve a process of lysing the virion and purifying the DNA, which is then amplified and quantified (Lindh and Hannoun, 2005). Alternatively, signal amplification assays can quantify the level of HBV DNA from serum. The present HBV DNA PCR assays have optimal quantification ranges. The introduction of real-time PCR has allowed for sensitivities ranging from 5–10 IU/mL up to 8–9 log10 IU/mL (Weiss *et al.*, 2004). The level of 20,000 IU/mL has been arbitrarily selected as the level below which there is a relatively low likelihood of hepatic damage, although this can still occur (Danta, 2014).

2.6.2 Genotypes of Hepatitis B Virus

The HBV genotyping involves sequencing the HBV genome. It is defined as a ≥ 4% divergence in the s antigen and ≥ 8% divergence in the entire nucleotide sequence. Currently about ten genotypes (A-J) have been identified (Mansourian *et al* 2013). These genotypes vary geographically, with the four most common genotypes being A–D. The most prominent genotypes in the Asia-Pacific region are B and C. Data now suggest that genotype may have an important influence on disease progression and treatment response (Fung and Lok, 2004). For unknown reasons, in Asian populations, genotype B has increased rates of HBeAg seroconversion, less aggressive liver disease and lower rates of HCC (Kao *et al.*, 2000). It is also observed that genotypes A and B have better response rates to interferon when compared to genotypes C and D (Wai *et al.*, 2002). Currently, genotyping is only a research tool; patients are not routinely genotyped in African

countries. However, it may become a relevant test in future clinical practice, to identify patients at greater risk for disease progression.

2.6.3 Biochemical Markers for assessing the serverity of liver disease

The biochemical markers assessing the severity of the liver disease include: aspartate aminotransferase (AST) and ALT, gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, bilirubin, and serum albumin and globulins, blood counts and prothrombin time, and hepatic ultrasound (European Association for the Study of the Liver- EASL, 2012). The ALT level is the main biochemical marker used in viral hepatitis which is used as a surrogate marker for necro-inflammation in the liver. An elevated ALT is also associated with better serological response to antiviral treatment. However, some studies have suggested that significant liver fibrosis can occur in the context of a normal ALT level (Danta, 2014). Usually, ALT levels are higher than those of AST. However, when the disease progresses to cirrhosis, the ratio may be reversed. A progressive decline in serum albumin concentrations and/or increase of (gamma) globulins and prolongation of the prothrombin time, often accompanied by declining in platelet counts, are observed after cirrhosis has developed. Studies show that between 12% and 43% of patients with chronic HBV and normal ALT levels have significant hepatic fibrosis (stage 2 fibrosis or greater) (Lai *et al.*, 2005; EASL, 2012).

2.6.4 Histological Markers for HBV

2.6.4.1 Liver Biopsy

The two histological features on liver biopsy used in the assessment of HBV are fibrosis (stage of disease) and necroinflammation (grade of disease). Liver fibrosis is usually graded from 0–4 (1 - limited portal fibrosis; 2 – periportal fibrosis; 3 - septal fibrosis linking portal tracts or central vein; and 4 - cirrhosis with development of nodules and thick fibrous septa). Liver biopsy, either performed percutaneously or transjugularly in those with ascites or significant coagulopathy, has been the gold-standard investigation for determining the stage

of HBV (EASL, 2012; Danta, 2014). Liver biopsy has several disadvantages including: invasiveness, uncomfortable, costly and laborious. The procedure carries a small, but significant risk of complications. It also suffers from sampling bias, as scarring and necroinflammation may be heterogeneously distributed in the liver.

2.7 Limitations of HBsAg and HBV DNA Assays

HBsAg can induce a strong immunogenic response by neutralizing antibodies. The main antigenic determinant ("a" determinant) is located between amino acids 124 and 147 within the major hydrophilic loop of HBsAg (amino acids 100 to 170) (Ly *et al.,* 2006). Antigenic variation of the "a" determinant occurs naturally due to HBV genetic heterogeneity. HBV has been classified into ten genotypes, designated A to J, based upon genetic divergence of 8% or more in the complete nucleotide sequence (Mansourian *et al* 2013). Additional divergence by S gene mutations has been reported in various population including vaccinated children (Hsu *et al.,* 2004), liver transplant recipients receiving anti-HBs immunoprophylaxis (Theamboonlers *et al.,* 2001), and in chronic carriers (Ly *et al.,* 2006). Various reports exist showing HBsAg-negative virus carriers (HBV DNA positive) with immunosilent infections (Koyanagi*et al.,* 2000). Natural variation and mutations can induce HBsAg conformational changes. Since many HBsAg immunoassays use monoclonal antibodies with epitopes directed against the major hydrophilic region, in particular against the "a" determinant, amino acid substitution in this region may account for false-negative results in immunoassays (Levicnic-Stezinar,2004; Louisirirotchanakul *et al.,*2004). The performance of various HBsAg assays show an increase in terms of specificity and sensitivity, allowing the detection of <0.15 ng/ml of HBsAg (Ly *et al.,* 2006). The prevalence of HBV mutations in the general population is assumed to be low, but selection pressures such as new antiviral drugs or large vaccination campaigns may change the situation (Biswas*et al.,* 2003).

DNA is the most reliable marker to monitor CHB patients on treatment (Andersson and Chung, 2009). However, serum HBV DNA measurement has several limitations. Despite being expensive and labor-intensive, HBV DNA assays lack uniformity and standardization. Many commercial kits are available and all have different linear ranges, lower limits of detection and conversion factors. Repeated assays done preferably on the same platform are needed to monitor a patient on antivirals.

2.8 Studies on HBsAg and HBV DNA Correlation

Relationship between serum HBsAg concentrations and HBV DNA levels has been evaluated with varied results. Among the studies that found a positive correlation includes one by Lei *et al.,* (2006) which showed that serum HBsAg concentration was related to HBV DNA replication level but indicated it was not feasible to use HBsAg concentration to monitor HBV replication levels. Park *et al.,* (2012) showed a positive HBsAg levels correlation with HBV DNA (r = 0.3373). The levels of HBsAg and HBV DNA have also been reported to correlate with each other with various correlation coefficients ranging from 0.121 to 0.862 in several previous studies (Deguchi *et al.,* 2004; Lee *et al.,* 2011; Kim *et al.,* 2011; Song *et al.,* 2011). One study however showed a lower correlation (r = 0.490) in 20 patients with CHB during lamivudine treatment and correlated better (r = 0.709) in 67 asymptomatic HBV carriers (Chen *et al.,* 2004). The degree of correlation was also different according to disease stage and HBeAg status (Chan *et al.,* 2011). There was also a trend toward decreased correlation according to the duration of treatment with interferon or nucleoside/nucleotide analogs observed in previous studies (Wursthorn *et al.,* 2010). Thus, variation in reported correlation coefficients could result from differences in disease stage, HBeAg status, HBV genotypes involved, and types/phases of treatment. These factors might also affect the correlation between the HBcAg and HBV DNA levels (Park *et al.,* 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Pathologists Lancet Kenya located in Nairobi upper hill area. Laboratories is one of the leading South African National Accreditation System (SANAS) accredited ultramodern pathology laboratories operating throughout Africa. In Kenya Pathologist Lancet was established in September 2009. Its main laboratory is located in Upper Hill, Nairobi. Pathologists Lancet Group of Laboratories (herein under referred to as 'Pathologist Lancet kenya') is a leading pathology laboratory service home-grown in Africa providing vital diagnostic, monitoring and screening testing from routine to specialized and esoteric tests. With our footprint throughout Anglophone Africa, Lancet Laboratories was founded in South Africa and now present in East Africa [Kenya, Tanzania, Rwanda & Uganda], Botswana, Ghana, Malawi, Mozambique, Nigeria, Swaziland, Zambia and Zimbabwe. Lancet operate primarily in the private healthcare environment and offer specialist pathology services to doctors and their patients directly or through clinics and hospitals, industrial sectors, corporates and insurances, it also serves clinical trials and research groups, and have engagement with governmental and NGO agencies through public private- partnerships. The Pathologists Lancet Kenya located in upper hill area of Nairobi county, where Nairobi county was founded in 1899 is the capital and the largest city of Kenya. The city proper had a population of 3,138,369 in the 2009 census, while the metropolitan area has a population of 6,547,547. Nairobi is the second-largest city by population in the African Great Lakesregion after Dar es Salaam, Tanzania. According to the 2009 census, in the administrative area of Nairobi, 3,138,295 inhabitants lived within 696 km2 (269 sq mi) (Kenya Central Bureau of Statistics, 2009).

3.2 Study Population

The participants in this study were patients who were referred from various hospitals/clinics to take HBV related tests in Pathologist Lancet Kenya.

3.3 Study Design

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

3.4 Sample Size Determination

Sample size was determined using formula described by Lemeshow *et al* (1990),

$$n = \left(\frac{z}{m}\right)^2 p(1-p)$$

Where,

z is the critical value based on the desired confidence level (e.g., z = 1.96 for 95% confidence level);

m is the margin of error or precision (0.05).

p is the prevalence of HBsAg positive cases in Nairobi (33.8 %) (Ochwoto *et al.*, 2016).

Substituting in the formula yielded = $(1.96^2 \text{ X } 0.34 \text{ X } 0.66) / 0.05^2 = 346$

In order to determine the prevalence of HBV, all the patients suspected of HBV infection and referred to the Pathologist Lancet Laboratory in Nairobi, Kenya HBsAg testing between 2014 and 2015 during the ethical approval period. A total of two thousand two hundred and fourty-six (2246) patients were consented and enrolled.

3.5 Inclusion Criteria

1. Patients with HBV referred to the Pathologist Lancet Laboratory in Nairobi, Kenya for HBsAg testing
2. The adults who were aged 18 years and above
3. Those who were willing and ready to consent to the study
4. Only patients infected with HBV

3.6 Exclusion Criteria

1. Patients unwilling to consent to the study
2. Patients on any previous antiretroviral therapy and/or chemotherapy
3. Patients infected with Hepatitis C Virus (HCV) and Hepatitis D Virus (HDV)
4. HBV vaccinated patients

3.7 Sampling Design

Two stage sampling was used. The first was convenience sampleing method where all the patients referred to the Pathologist Lancet Laboratory in Nairobi, Kenya for HBsAg testing between 2014 and 2015 during the ethical approval period were consented and enrolled. This was important to meet the first ojective. To determine HBV chronicity (Ojective two) and the relationship between HBsAg titers and HBV DNA levels in chronic HBV patients (ojective three), purposive sampling method was used to enrolled 346 patients who tested HBV positive. Further, purposive sampling method was used to select 172 out of the 346 patients who not co-infected with Hepatitis C Virus (HCV) and Hepatitis D Virus (HDV) inorder to meet objective three.

3.8 Data Collection Tools

Interviews using semi-structured questionnaires (Appendix I) was used to collect data regarding HBV infection including (socio-demographic and economic, practices associated with infection, e.g. sexual history, blood donation).

3.9 Laboratory Procedures

3.9.1 Sample Collection

This work was done in level three laboratory within Pathologist Lancet Laboratory in Nairobi. About 5 mL single draw whole blood samples were collected from each patient into EDTA and SST (clotting) tubes during their visit to Lancet laboratory in the phlebotomy room. Sample collection was done by trained phlebotomist. In level three iosafety laboratory in lancet, the EDTA and SST tubes containing blood were then centrifuged for 15 min at 1500 RCF. Carefully the supernatant (serum) was aspirated at room temperature and pool into a centrifuge tube, taking care not to disturb the cell layer or transfer any cells. A clean pipette was used for each tube. The serum was aliquot into pre-labelled cryovials and stored at –80°C until ready for use. Part of the serum was used for (i) serological marker - HBV antigens; and (ii) for virological marker HBV DNA level. If not used immediately the serum was stored at -80 °C until ready for analysis.

3.9.2 Detection of HCV

Detection of HCV was done using the BioMérieux (Lyon, France) enzyme linke assay according to the manufacture's instruction. Anti-HCV EIA was designed to detect anti-HCV core IgG using in the solid-phase only a recombinant C22 antigen produced by BioMérieux (Lyon, France). This antigen encodes the N-terminal 48 amino acids of the HCV core protein. All serum samples were stored at -20^{o}C. In brief, the wells of microtiter plates (Hemobagâ) were coated overnight at 4^{o}C with 100 µl volume of C22 antigen (5 µg/ml) in carbonate buffer (10 mM, pH 9.6). Thereafter, the wells were washed with phosphate buffer

saline (PBS), containing 0.05% Tween-20 (PBS/T) and blocked over-night at 4^{o}C with 100 µl of human albumin in PBS (1%). The plate was washed three times with PBS/T, and 100 µl of serum diluted (1/100) in PBS containing 1% albumin (pH 7.2) were added and incubated at 37^{o}for 60 min. The plate was washed a further three times with PBS/T, and 100 ml of horseradish peroxidase goat anti-human IgG (Sigmaâ), diluted as serum samples (1/100), were added to each well. The plate was incubated for 60 min at 37^{o}C and washed three more times with PBS/T. Then, 100 µl of o-phenylenediamine (in citrate buffer) was added to each well and incubated for 15 min at room temperature in the dark, with the reaction being stopped by the addition of 100 µl of H_2SO_4 (1N). The color was read in a micro-titer plate reader at a wavelength of 492 nm.

3.9.3 Detection of HDV

The HDV was detected using the Dia.Pro diagnostic (Milano, Italy) according to the manufacturer's instructions. Briefly, the required number of strips were placed in the microplate holder and stored into the bag in presence of the desiccant at +2.8°C, sealed. A total of 100 µl of Negative Control in triplicate, 100 µl of Calibrator in duplicate, 100 µl Positive Control in single and then 100µl of samples were added into the strips and incubated at +37°C for 60 min. 3. The microplate was then washed and in all the wells 100 µl Enzyme Conjugate added and incubate the microplate at +37°C for 60 min. the microplatres were then washed and 100 µl TMB/H2O2 mixture added in each well and then incubated at room temperature for 20 min. The reaction being stopped by the addition of 100 µl of H_2SO_4 (1N). The color was read in a micro-titer plate reader at a wavelength of 450 nm.

3.9.4 Quantification of HBsAg

The HBsAg quantification assay was done using Roche Cobas e 411 analyzers with Elecsys HBsAg II Quant reagent kits (Roche Diagnostics, South Africa, SA) as previously described (Deguchi *et al.*, 2004; Zacher*et al.*, 2011; O'Neill*et al.*, 2012). Briefly, this test uses the electrochemiluminescence immunoassay with a

sandwich complex formed from the 2 biotinylated monoclonal anti-HBsAg antibodies and a mixture of monoclonal and polyclonal anti-HBsAg antibodies labeled with a ruthenium complex as a chemiluminescence molecule (Zacher*et al.,* 2011; O'Neill*et al.,* 2012). The resulting chemiluminescence reactions was measured and converted to HBsAg concentrations in the specimens using the calibration curve generated by 2-point calibrators. The total assay duration is about 18 minutes. The analyzer provides an onboard dilution function, which prediluted the samples automatically after loading (Zacher *et al.,* 2011; O'Neill*et al.,* 2012). The analytic measurement range (AMR) suggested by the manufacturer is between 5 and 13,000 IU/mL when the samples are 100-fold diluted using the automated dilution function. Diluted samples with HBsAg levels less than 5 IU/mL was retested without predilution, and the specimens were manually diluted by 20-fold before being loaded on the analyzer (total dilution, 2,000-fold) when the HBsAg concentration was greater than 13,000 IU/mL (O'Neill*et al.,* 2012).

3.9.5 Quantitation of HBV DNA Viral Load

The HBV DNA viral load (IU/mL) was determined using Cobas AmpliPrep/CobasTaqMan HBV Test, version 2.0 (Roche Molecular Systems, South Africa, SA) according to the manufacturer's instructions (COBAS®AmpliPrep/COBAS®TaqMan®HBV Test, v2.0 Package Insert). CAP-CTM is an automated real-time PCR test based on a dual-labeled hybridization probe targeting the precore and core regions associated with an HBV DNA automated extraction based on the affinity of DNA for silica gel-covered magnetic beads (EASL clinical practice guidelines, 2012). The procedure processes 1,050 µl of plasma and consists of subsequent steps of lysis with chaotropic agents and proteinase K, DNA capture by use of glass particles, and purification. After DNA elution at high temperature (80°C), a robotic arm loads nucleic acids in microvials containing the PCR master mix prepared for each sample by the same robotic arm (Lindh and Hannoun, 2005). An internal quantitation standard (QS) is added to each sample during the processing step.

After HBV DNA extraction with the COBAS AmpliPrep instrument, a real-time PCR test is performed by the COBAS TaqMan 48 analyzer with a multiplex TaqMan assay. Two targets were amplified: HBV DNA and the internal QS. The QS is a noninfectious construct containing fragments of HBV sequences with primer binding regions identical to those of the HBV target sequence but with detection probe different from that for HBV. The results are expressed as international units per milliliter with a 5.82 copies per IU conversion factor. Prevention of carryover contamination and sample integrity is provided by the use of the Amperase system based on uracil-*N*-glycosylase and dUTP incorporation. According to COBAS®AmpliPrep/COBAS®TaqMan®HBV Test, v2.0 Package Insert the sensitivity of CAP-CTM is 12 IU/ml, with a dynamic range from about 54 to 1.1 × 108 IU/ml and is designed for the extraction of 24 plasma specimens in about 2hours.

3.10 Ethical Considerations

The study was conducted according to the Declaration of Helsinki and International Conference on Harmonization Guideline on Good Clinical Practice (ICH-GCP). The protocol and informed consent form were reviewed and approved by the Center for Virus Research Institute Center's Scientific Committee (CVR- CSC) and Kenyatta University Ethical Review Committee (KU-ERC: Ref: KU/R/COMM/51/405) (Appendix II), prior to any protocol-related procedures being conducted. Written informed consent was obtained from each participant prior to any protocol-specified procedures being conducted. To maintain confidentiality, initials and coded numbers were used to identify the participants' source documents, CRFs, and study reports. All study records were maintained in a secured location within Lancet Laboratories. Participation in this study was completely voluntary and the participants could have withdrawn even after accepting to participate.

3.11 Data Management and Analysis

All participants were assigned a subject identification number (SID). The SID contained the patient's initials and a serial number. All data entered into the study databases were de-identified and only associated with a SID in password protected files. The study maintained a double entry system for the data. All paper research records were kept in locked filing cabinet located in a restricted-access room at the Lancet Laboratory.

Descriptive statistics frequency (%), mean, standard deviation and medium (interquartile ranges) were used to express data to address objectives one and two. To address obbjectrive three included (i) testing for normality of the distribution for HBsAg and HBV DNA was achieved using Kolmogorov-Smimov test. (ii) Comparison of HBsAg and HBV DNA between HBeAg positive and negative patients was performed using the Mann-Whitney U test and (iii) Spearman correlation coefficient was used to correlate serum levels of HBsAg and HBV DNA levels. Data analysis was done using SPSS software for Windows, v.21 where $P \leq 0.05$ was considered significant.

CHAPTER FOUR

RESULTS

4.1 Socio Demographic Characteristics of Study Participants

Two thousand two hundred and fourty-six (2246) participants consented to participate in this study. The mean age (SD) of the enrolled participants was 34.84 (18.5) years. The 859 (38%) of the participants were aged between 31 to 40 years, 1215(53.7%) were males, 1769(78.3%) were Christians, 1316(58.1%) were married and 1119(49.4%) were unemployed (Table 4.1).

There were 728(32.2%) of the participants who reported consuming alcohol, 334(14.8%) who reported smoking cigarette. The majority of the participants 1295(57.2%) had their age of sexual debut below 18 years, 1930(85.2%) reported not using condom in their last two sexual encounters and 405(17.9%) reported sharing personal effects such as tooth brush, shaving razors with other family members (Table 4.1).

Table 4.1: Socio-Demographic Characteristics of Study Participants who visited Lancet Laboratory, Nairobi

Variable		Frequency	%	Chi Square	df	P
Gender	Male	1215	53.7	12.2	1	0.001
	Female	1049	46.3			
Age in Years	Mean (SD)	34.84 (18.5)				
Age group	20-30	859	38			
	31-40	652	28.8	298.1	3	0.001
	41-50	336	14.8			
	Above 51	417	18.5			
Religion	Muslim	495	21.9	716.9	1	0.001
	Christian	1769	78.1			
Marital status						
	Married	1316	58.1	894.3	2	0.001
	Single	792	35			
	Seperated	156	6.9			
Occupation	Employed	648	28.6	278	2	0.001
	Business	497	22			
	Unemployed	1119	49.4			
Alcohol consumption	Yes	728	32.2	288.4	1	0.001
	No	1536	67.8			
Duration of Alcohol consumption	≤1 year	281	12.4	1231.7	2	0.001
	> 1 year	447	19.7			
	Not applicable	1536	67.8			
Smoking	Yes	334	14.8	1125.1	1	0.001
	No	1930	85.2			
Age of sexual debut	≤ 18	1295	57.2	330.6	1	0.001
	> 18	969	42.8			
Condom use in last sexual act	Yes	334	14.8	1125.1	1	0.001
	No	1980	85.2			
Shared personal effects (shaving razor/toothbrush)	Yes	405	17.9	933.8	1	0.001
	No	1859	82.1			

%-Percentage, SD: Standard deviation; df = Degree of freedom, p- Level of significance

4.1.1 Clinical Characteristics of Study Participants

Five hundred and four (22.3%) of the participants reported having been hospitalized requiring blood transfusion, 389(17.2%) had body tattoos, 519(22.9%) had previously received acupuncture to treat illness and about 944(41.7%) of them reported having been done any invasive procedure. One thousand and fifty-six (46.6%) of the participants had heard about Hepatitis B virus/infection, 805(35.6%) of the participant had received HBV vaccination, (Table 4.2).

Table 4.2: Clinical Characteristics of Study Participants Who Visited Lancet Laboratory, Nairobi

Clinical Variable	Frequency	%	Chi Square	df	P
Been sick/hospitalised requiring blood transfusion					
Yes	504	22.3			
No	1760	77.7	696.8	1	0.001
Have any tatoo in your body					
Yes	389	17.2			
No	1875	82.8	975.4	1	0.001
Ever received acupuncture medical practice					
Yes	519	22.9			
No	1745	77.1	663.9	1	0.001
Undergone any invasive procedure					
Yes	944	41.7			
No	1320	58.3	62.4	1	0.001
Heard of Hepatitis B					
Yes	1056	46.6	10.2	1	0.001
No	1208	53.4			
Ever received Hepatitis B vaccination					
Yes	805	35.6	188.9	1	0.001
No	1459	64.4			
Doctor's advice taken a Hepatitis test					
Yes	944	41.7	62.4	1	0.001
No	1320	58.3			
Ever had these symptoms (Dark urine, dizziness, drowsiness, itchy skin, whites of eyes, tongue (jaundice) ?					
Yes	944	41.7	63.5	1	0.001
No	1320	58.3			

%- Percentage; Df- Degrees of Freedom, P- Level of Significance

4.2 Prevalence of HBV among Study Participants

Out of the 2246 patients attending Pathologist Lancet Laboratory during the study period, 346 (15.4%) were positive for HBsAg. Out of the 346 HBsAg positive participants, 114 (32.9%) were also co-infected with hepatitis C (HCV) while 59 (17.1%) were also co-infected with hepatitis D (HDV) (Figure 4.1)

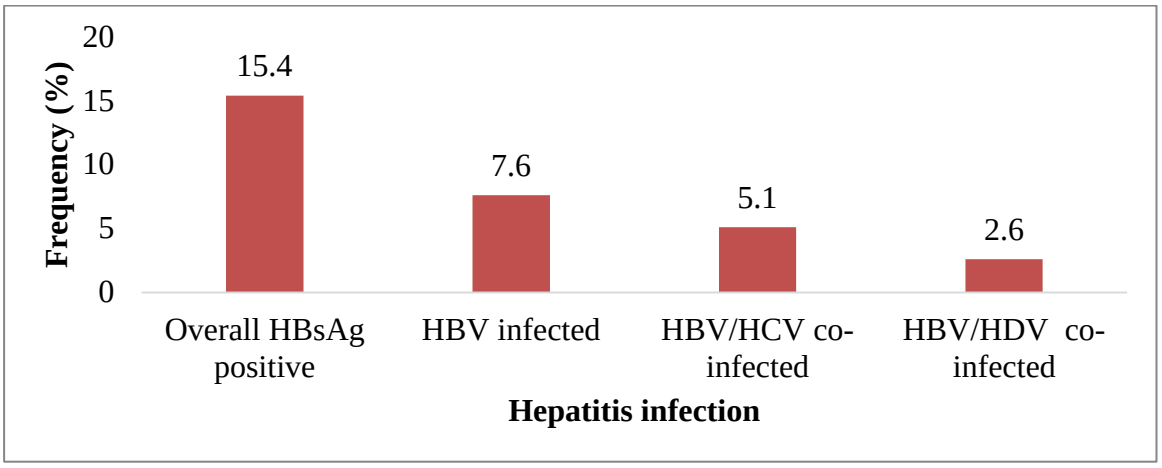

Figure 4.1: Distribution in the prevalence of Hepatitis among particicipants Infection at Lancet Laboratory Nairobi, Kenya

4.3 Chronicity of HBV among Study Participants

The HBeAg-negative participants (a form of the virus that does not cause infected cells to secret HBeAg) were considerd to be chronically infected with HBV. In this study, out of the 173 patients 142 (82.1%) were HBeAg-negative considered chronic compared to 31/173 (17.9%) HBeAg- positive (non-HBV chronic participants) (Figure 4.2)

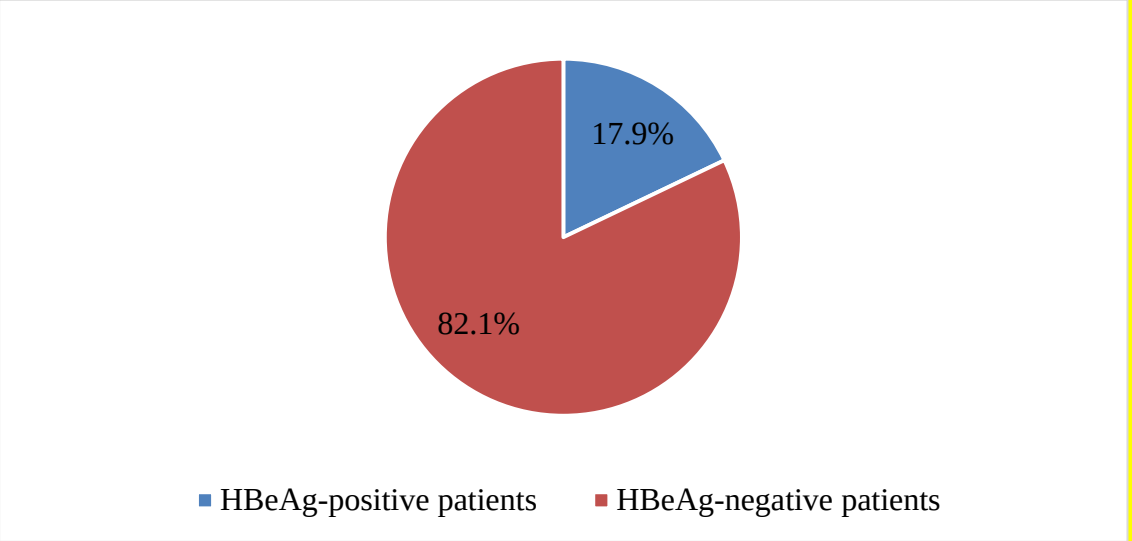

Figure 4.2: Distribution in the proportion of participants with Chronic Hepatitis B (HBeAg-negative) and non-HBV chronic participants (HBeAg-positive) Infection at Lancet Laboratory Nairobi, Kenya

4.3.1 Distribution of Chronicity HBV in Relation to Socio Demographic Characteristics at Lancet Laboratory, Nairobi

The distribution of HBV chronicity in relation to socio-demographic and clinical parameters is described in Table 4.3. There was no significant difference in the distribution of HBV chronicity by gender (85.2% female compared to 80.4 males) (P = 0.28); by age groups (79.2% - 20-30 years, 87.9% - 31-40 years; 78.6% - 41-50 years and 82.1% in those aged ≥51 years) (P = 0.475).

No significant difference in the distribution of HBV chronicity was reported among alcohol consumption period (78.3% one year; 90.6% two or more years; and 80.5% among non-alcohol consumers) (P = 0.365); by cigarette smoking pattern (89.7% smokers compared to 80.6% non-smokers) (P = 0.299); by sharing personal effects such as razor blades or tooth brush (83.9% yes and 81.7% no) (P = 0.774).

Further, no significant difference in the distribution of HBV chronicity was reported among the following variables; ever receiving blood transfusion (74.4%

yes versus 84.6% no) (P = 0.102); ever receiving blood transfusion (74.4% yes versus 84.6% no) (P = 0.102); undergoing body tattooing (84.4% yes versus 81.6% no) (P = 0.466); ever undergoing acupuncture (86.8% yes versus 80.7% no) (P = 0.272); having undergone an invasive procedures (79.1% yes versus 84% no) (P = 0.27); having received hepatitis B vaccination (82.8% yes versus 81.7% no) (P = 0.524) and lastly been presenting with HBV like symptoms (79.1% yes versus 84% no) (P = 0.27).

Table 4.3a: Distribution of Chronicity HBV in Relation to Socio Demographic at Lancet Laboratory, Nairobi

Variables		Total (n=173)	HBeAg-negative (n=142)	HBeAg-positive (n=31)	Chi square	d.f	p
Gender	Male	61	52 (85.2)	9 (14.8)	0.642	1	0.28
	Female	112	90 (80.4)	22 (19.6)			
Age (year)	20-30	53	42 (79.2)	11 (20.8)	2.503	3	0.475
	31-40	66	58(87.9)	8 (12.1)			
	41-50	26	20 (78.6)	6 (21.4)			
	>51	28	22(82.1)	6 (21.4)			
Duration of alcohol consumption	1 year	23	18(78.3)	5 (21.7)	2.015	2	0.365
	>1	32	29 (90.6)	3 (9.4)			
	NA	118	95 (80.5)	23 (19.5)			
Smoking	Yes	29	26 (89.7)	3 (10.3)	1.36	1	0.299
	No	144	116(80.6)	28 (19.4)			

%-Percentage, SD: Standard deviation; df = Degree of freedom, p- Level of significance

Table 4.3b: Distribution of Chronicity HBV in Relation to Socio Demographic at Lancet Laboratory, Nairobi

Variables		Total (n=173)	HBeAg-negative (n=142)	HBeAg-positive (n=31)	Chi square	d.f	p
Shared personal effects (shaving razor/toothbrush)	Yes	31	26 (83.9)	5 (16.1)	0.08	1	0.774
	No	142	116(81.7)	26 (18.3)			
Been sick/ hospitalized requiring blood transfusion	Yes	43	32 (74.4)	11 (25.6)	2.28	1	0.102
	No	130	110 (84.6)	20 (15.4)			
Have any tatoo in your body	Yes	32	27 (84.4)	5 (15.6)	0.14	1	0.466
	No	141	115(81.6)	26 (18.4)			
Ever received acupuncture medical practice	Yes	38	33(86.8)	5 (13.2)	0.75	1	0.272
	No	135	109 (80.7)	26 (19.3)			
Undergone any invasive procedure	Yes	67	53 (79.1)	14 (20.9)	0.66	1	0.27
	No	106	89 (84)	17 (16)			
Ever received Hepatitis B vaccination	Yes	58	48 (82.8)	10 (17.2)	0.027	1	0.524
	No	115	94 (81.7)	21 (18.3)			
Ever had these symptoms of jaundice?	Yes	67	53 (79.1)	14 (20.9)	0.659	1	0.27
	No	106	89 (84)	17 (16)			

%-Percentage, SD: Standard deviation; df = Degree of freedom, p- Level of significance

4.4 The HBsAg Titters and HBV DNA Levels

The overall mean (Log_{10}) of HBsAg (SD±) titer was 3.58 ± 0.39 IU/mL and an overall mean (Log_{10}) of HBV-DNA (SD±) of 2.89 ± 1.62 IU/mL. By Kolmogorov-Smimov test, the distribution of both the HBsAg and HBV DNA levels was irregular (Figure 4.3).

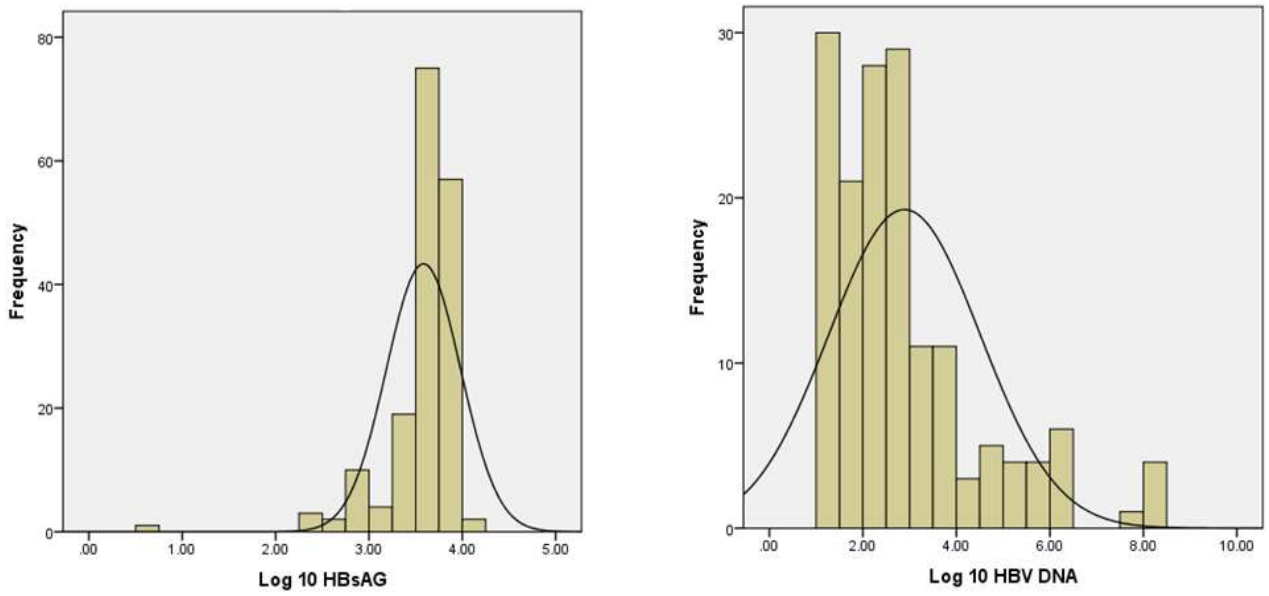

Figure 4.3: Log $_{10}$ Distribution of HBsAg and HBV DNA levels among Participants with Chronic Hepatitis B Infection at Lancet Laboratory, Nairobi

4.4.1 The HBV Levels above Threshold for Initiating Treatment

While a viral load greater than 300 copies/mL increases the risk of liver damage, researchers continue to use 20,000 IU/mL for HBeAg positive patients and 2,000 IU/mL for HBeAg-negative patients as the thresholds for initiating treatment. But many patients have fluctuating viral load levels, ranging from 2,000 to 20,000 IU/mL. When this occurs, experts recommend that treatment decisions be highly individualized and take into consideration the patient's age and other factors (Papatheodoridis *et al.*, 2012).

4.4.2 The HBeAg Positive Participants with HBV DNA ≥ 20,000 IU/ML

In this study out of the 31 HBeAg positive participants, there were 22 (70.9%) with HBV DNA ≥ 20,000 IU/ML (Table 4.4). The 22 participants had mean (SD) HBV DNA levels of 2.8 X 10^7 (6.1 X 10^7) IU/ML and a range of 1.7 X 108 (2.0 X 104 to 1.7 X 108) IU/ML.

4.4.3 The HBeAg Negative Participants with HBV DNA ≥ 20,000 IU/ML

Results of the study depicted that 14 HBeAg negative of the 142 (9.9%) of them with HBV DNA ≥ 2000 IU/ML (Table 4.4). These 14 participants had mean (SD) HBV DNA levels of 1.2 X 107 (4.5 X 107) IU/ML and a range of 1.7 X 10^8 (2.1 X 10^3 to 1.7 X 10^8) IU/ML.

4.4.4 Level of HBsAg and HBV DNA among Chronic HBV

The mean (Log_{10}) of HBsAg (±SD) titer in CHB who were HBeAg positive and negative patients were 3.67 ± 0.37 IU/mL and 3.56 ± 0.41 IU/mL, respectively (Figure 4.2). There was a significant difference in the HBsAg levels between HBeAg positive and negative patients (Mann-Whitney test, P = 0.032).

The mean (Log_{10}) of HBV-DNA (±SD) in HBeAg-positive patients and HBeAg-negative patients were 5.17 ± 1.58 and 2.07 ± 1.27 IU/mL, respectively. There was a significant difference in the HBV DNA levels between HBeAg positive and negative patients (Mann-Whitney test, P = 0.0001) (Figure 4.4).

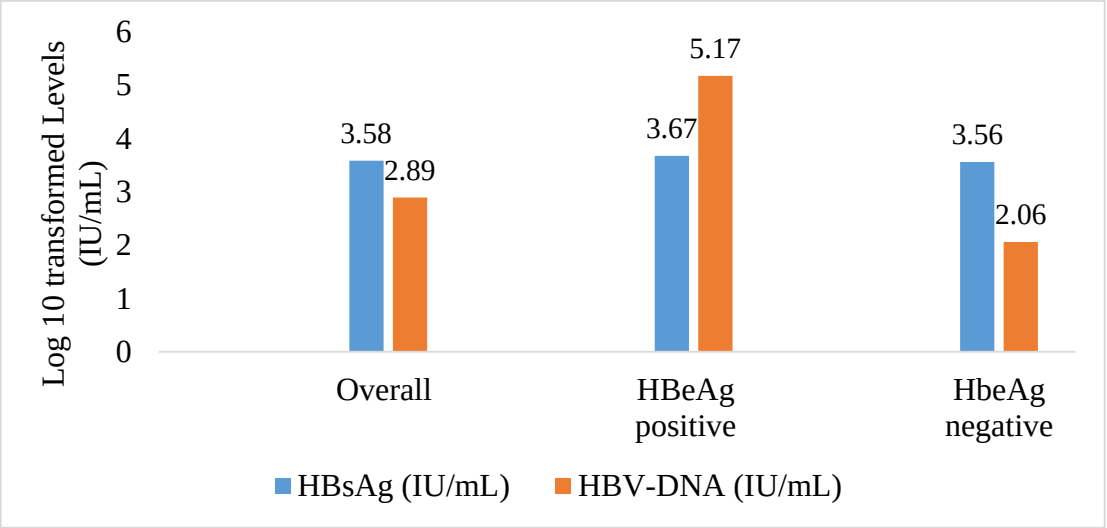

Figure 4.4: Distribution of HBsAg and HBV DNA Levels among Participants at Lancet Laboratory, Nairobi

4.4.5 Distribution of HBsAG and HBV DNA Levels among Chronic HBV

Overall, a weak but significant correlation was found between serum quantitative HBsAg and HBV DNA ($r = 0.171$; $p = 0.024$). Quantitative HBsAg was not significantly correlated with HBV-DNA among the HBeAg positive patients ($r = 0.309$; $p = 0.093$). Similarly, no significant correlation was found among the HBeAg negative patients ($r = 0.065$; $p = 0.443$) (Table 4.4).

Table 4.4: Correlation between HBsAg and HBV DNA Levels among Chronic HBV Patients at Lancet Laboratory, Nairobi

	Log 10 transformed			
Variable	**HBsAg (IU/mL)**	**HBV-DNA (IU/mL)**	**r**	**p**
All subject (n=173)				
			0.171	0.024
Mean (SD)	3.58 (0.39)	2.89 (1.62)		
Range	0.63-4.06	1.3-8.23		
HBeAg positive (n=31)				
			0.311	0.089
Mean (SD)	3.67 (0.37)	5.17 (1.58)		
Range	2.31-8.23	2.31-4.06		
HBeAg negative (n=142)				
			0.065	0.443
Mean (SD)	3.56 (0.41)	2.06 (1.22)		
Range	0.63-3.98	1.3-2.7		

SD: Standard Deviation, r=Correlation coefficient, p= p value

CHAPTER FIVE

DISCUSSION, LIMITATIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Despite the availability of an effective vaccine and potent antiviral treatments, Hepatitis B virus (HBV) infection is still a major global health problem (Buster and Janssen, 2006; Feld, 2013). Hepatitis B virus (HBV) and Hepatitis C virus (HCV) have received more attention due to their wide distribution and high prevalence, and also to the severe long-term sequelae of chronic infections with these agents, liver cirrhosis and hepatocellular carcinoma (Alter, *et al.,* 1999). By 2015, approximately two billion individuals had been exposed to HBV, with nearly 257 million estimated cases of chronic hepatitis B infection (Global Hepatitis Report, 2017). HBV and related conditions are also thought to be responsible for 500,000 to 700,000 deaths annually (Global Hepatitis Report, 2017). It is highly endemic in sub-Saharan Africa and East Asia, where approximately 5–10% of the adult population is chronically infected (WHO, 2015).

In the current study, the HBV prevalence rate of 15.4% was reported and with the majority, 142 (82.1%) testing negative for hepatitis B e antigen (HBeAg) meaning chronically infected. In Kenya, the seroprevalence of HBsAg positivity has been reported at about 5%–14% and the prevalence of HBV exposure at about 20%–70% (Muriuki *et al.,* 2013; Kerubo *et al.,* 2015). For this reason, treatment and follow-up of chronic HBV infection is crucial. In Kenya, studies have shown that there is a disparity in HBV infection prevalence by geographic area; one study found that the HBsAg prevalence to be 8.8% in Turkana county which is the largest and most north-western county in Kenya (Mutuma *et al.,* 2011), and another study was 11.2% in eastern Kenya (Atina, *et al.,* 2004). Study by Ngaira *et al.,* (2016) established that the prevalence of HBV infections among pregnant women attending antenatal clinic at Mbagathi District Hospital was

3.8%. Studies of HBV infection prevalence in health-care settings may yield higher estimates due to selection bias. For example, a study by Pettigrew and others found that 77% of Kenyan patients at the liver clinic at the Kenyatta National Hospital in Nairobi, Kenya, who had chronic aggressive hepatitis or cirrhosis also, had HBsAg positivity (Pettigrew*et al.*, 2011). Other previous studies in Kenya have reported carrier rate for HBsAg between 10 and 15 % (Greenfield *et al.*, 1991; Muchiri *et al.*, 2012). The prevalence of HBsAg in Nairobi and rural Kenya range between 3 to 30 % (Okoth *et al.*, 1991) and at the Kenyan coast, 11.4% of the population are positive for HBsAg (Okoth *et al.*, 1991). The wide range in the prevalence of HBV has been associated to differences in age at the time of infection, previous blood transfusion, history of induced abortion, HIV serostatus among others (Ezechi *et al.*, 2014; Ly *et al.*, 2016).

The study correlated the HBsAg levels with those of HBV DNA levels among HBV patients attending one of the largest private referral laboratories in Nairobi, Kenya. HBsAg is a classical marker of infection with hepatitis B virus, and serological assays to detect HBsAg have guided its diagnosis. This study asked the question if quantification of HBsAg can be used to manage and monitor HBV patients. Upon HBV infection, closed circular HBV DNA genome forms within the hepatocytes nuclei which become enveloped and secreted into the blood (Arai *et al.*, 2012). HBV DNA, is the template for gene transcription and replication, correlating robustly with levels of total intracellular HBV DNA, serum HBV DNA, and HBsAg (Chan *et al.*, 2011). Thus, HBV DNA level is the most important and most direct etiological evidence for HBV (Zhou *et al.*,2015). However, in developing countries endemic for HBV (Schweitzer *et al.*, 2015), robust, cheaper and easy-to-perform markers are essential in the management and monitoring of HBV patients (Maylin *et al.*,2015). HBV viral load measurement is rarely accessible and where available often done once – which may not reflect real viral activity (Maylin *et al.*, 2015). Further, usefulness of viral load in diseases monitoring, and medication in patients with undetectable HBV DNA levels is still missing (Zhu and Zhang, 2016). HBsAg quantification indirectly

reflects the number of infected hepatocytes (Seth, 2012) and is known to change over the natural course of chronic HBV infection. HBsAg, a membrane protein of HBV envelope, have been proposed as a marker for monitoring HBV infected patients (Günal *et al.,* 2014). During antiviral therapy HBsAg quantification can be used to differentiate true inactive carriers from patients in remission who are likely to progress to cirrhosis (Martinot-Peignoux *et al.*, 2013). Moreover, Tsai *et al.,* (2015) showed that HBV DNA positivity can be easily detected in patients with higher levels of serum HBsAg than those with lower levels**.**

This study mirrors the report by Ganji *et al.,* (2011) who found no significant correlation between HBsAg and HBV DNA levels among CHB including in HBeAg positive and negative patients. Similar results were also reported by Mahdavi *et al.,* (2015) in the overall population (r = 0.231, P = 0.656). Demiroren *et al.,* (2015) also showed an overall negative non-significant correlation. On the contrary, several reports have showed a positive correlation between HBsAg and HBV DNA level in the whole cohort, HBeAg-positive and -negative groups. Togo *et al.,* (2011) reported an overall positive correlation. Kim *et al.*, (2011) reported a slightly higher significant correlation and this was also observed in the HBeAg-positive and -negative patients. Teriaky and Al-Judaibi, (2013) showed a significant positive correlation in the whole cohort, HBeAg-positive and -negative groups. Zhu and Zhang, (2016) reported and overall positively correlated while Jaroszewicz *et al.,* (2010) showed even a stronger significant correlation between HBsAg and HBV-DNA in the overall population.

The variations in the correlation between HBsAg and HBV-DNA levels have been attributed to the differences in disease stage, HBeAg status, HBV genotypes involved, and types/phases of treatment (Par *et al.,*2012; Mahdavi *et al.,*2015) and should always be considered in HBV DNA and HBsAg levels correlation studies. Even though most of these factors were not established in our study, similar to report by Ganji *et al.,* (2011), our study highlights a significant insight into the differences in HBsAg levels between HBeAg positive and negative

patients, which appear to be affected by HBeAg status. HBV DNA and HBsAg levels were higher in HBeAg positive patients.

Although not a focus of this study, several biochemical parameters including aspartate aminotransferase (AST), Alanine aminotransferase (ALT) albumin, total bilirubin, gamma-glutamyl transferase (GGT), alpha-fetoprotein (AFP) and platelet count) have used for the assessment of HBV infection and treatment outcomes (Coskun *et al.*,2015). Variations in the correlation between HBV DNA levels and biochemical parameters have been reported; some showing positive correlations (Demiroren *et al.,* 2015) while others showing contrary outcomes (Ganji *et al.,* 2011). This should complement future HBsAg and HBV-DNA correlation studies in Kenya.

The WHO has set a target to treat 80% of eligible people with chronic HBV infection to eliminate HBV as a public health threat by 2030; globally, only 8% of this population was estimated to be under treatment in 2015 (WHO, 2017). This study demonstrated a higher seroprevalence of HBsAg in one of the high end/cost laboratories in Kenya meaning that the general access to HBV testing might be limited, and in people with a low soci-economic status who may be unable to pay for HBV testing and treatment. This clearly requires the need to decentralize the management of chronic HBV carriers in Kenya by using simple and low-cost diagnostic tools.

In this study, the fact that out of the 173 patients 142 (82.1%) were HBeAg-negative considered chronic requires the need to decentralize the management of chronic HBV carriers in Kenya. Contradictory reports on the correlation between HBsAg and HBV DNA levels exist in the literature. Because the variations in the correlation between HBsAg and HBV-DNA levels have been attributed to the differences in disease stage, HBeAg status, HBV genotypes involved, and types/phases of treatment (Par *et al.*, 2012; Mahdavi *et al.*, 2015), we recommend that additional larger prospective studies be conducted to re-evalute the correlation if any between HBV DNA and HBsAg levels in Kenya.

5.2 Limitations of the Study

This study had several limitations

1. The cross-sectional nature of this study; we were not able measure serially the HBsAg and HBV DNA levels, which have been shown to fluctuate especially among chronic HBeAg-negative HBV-infected patients
2. HBV DNA and not transcriptionally active covalently closed circular (ccc) HBV DNA were measured, which has been shown to correlate more appropriately with HBsAg (Wang *et al.,* 2013). The stage of disease, HBV genotypes and types/phases of treatment were not determined and we cannot rule out their role in the overall weak or the lack of correlation in the levels of HBV DNA and HBsAG in the entire patient population or either HBeAg-positive or HBeAg-negative patients respectively.

5.3 Conclusions

i. The prevalence of HBV was 15.4%.
ii. A significant proportion of these patients (82.1%) were chronically infected with HBV
iii. Serum quantitation of HBsAg may not replace the role of HBV DNA levels among patients with CHB in Nairobi Kenya. It is a rough estimate and not a gold standard, but it can definitely have a role in correlating disease especially in stable patients after an initial HBV DNA is performed to save expenses and minimize the cumbersome nature of HBV DNA testing

5.4 Recommendations

i. This study demonstrated a seroprevalence of HBV to be very high in one of the high end/cost laboratories in Kenya and recommend the need to create awareness through educating people on HBV and the introduction of simple and low-cost diagnostic tools.

ii. There is a need to decentralize the management of chronic HBV carriers in Kenya.

iii. Recommend that additional larger prospective studies be conducted to re-evalute the correlation if any between HBV DNA and HBsAg levels in Kenya.

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APPENDICES

Appendix I: Informed Consent Form

STUDY TITLE: DIAGNOSTIC VALUE OF HBsAg IN HBV INFECTION AMONG PATIENTS REFERRED TO PATHOLOGIST LANCET LABORATORY IN NAIROBI

INVESTIGATORS

Investigator	Title	Institution	Telephone
Faith Mathai	Principal Investigator	JKUAT	0726292969
Dr. Raphael Lihana	Co-Investigator	CVR KEMRI	0721879497
Prof.Simon Karanja	Co-Investigator	COHES, JKUAT	0726424669

My name is Faith Mathai. I am conducting a study entitled of study "Diagnostic value of HBsAg in HBV infection among patients referred to Pathologist Lancet Laboratory Nairobi Kenya"

Description: I am asking you to participate in a research study which seeks to find out the prevalence of HBV among patients referred Pathologist Lancet Laboratory. The chronicity of HBV, whether the level of hepatitis B infection can be quantified by its surface proteins (HBsAg) levels compared to its routinely used genetic (DNA) method. This information would help us validate a cheaper alternative method for assessing the quantity of HBV (hence disease progression) among infected patient. If you agree to participate we will consent you and conduct a face to face interviews to assess this issue as well as use the blood sample drawn from you for routine HBV testing.

Risks and benefits: One potential risk of being in the study is that you might feel a little discomfort at the time we will be taking your blood sample. There is no monetary benefit for your participation in this study. The benefit which may reasonably be expected to result from this study includes your contributions to efforts to accurately diagnosis and manage HBV infection. If you are found harbouring HBV levels the information will be communicated to you and your referring health care provider for better management. Your decision whether or not to participate in this study will not affect your current reference to Lancet Laboratories.

Time involvement: This interview will take about 30 minutes of your time.

Subject's rights: If you have read this form and have decided to participate in this project, please understand your participation is voluntary and you have the right to withdraw your consent or discontinue participating at any time without penalty. You have the right to refuse to answer particular questions. Your individual privacy will be maintained in all published and written data resulting from the study.

If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact - anonymously, if you wish – The secretary, KU Ethical Review Committee, PO Box 54840 – 00200 Nairobi, Kenya; Tel: 020-2722541, 0722205901, 0733400003; Email address: erc@ku.org.

I have read or have had the document read to me. I have discussed the information with study staff YES___ NO____

I agree to participate in this research study: YES___ NO____

I agree to have my blood collected/stored and analysed for study assays: YES___NO___

My questions have been answered. My decision whether or not to take part in the study is voluntary. If I decide to join the study, I may withdraw at any time. By signing this form, I do not give up any rights that I have as a research participant.

_____	_____	_____
Participant Name	Participant Signature/ Thumb print	Date
_____	_____	_____
Study Staff	Study Staff Signature	Date

Appendix II: Structured Questionnaires

STUDY TITLE: DIAGNOSTIC VALUE OF HBsAg IN HBV INFECTION AMONG PATIENTS REFERRED TO PATHOLOGIST LANCET LABORATORY IN NAIROBI

INVESTIGATORS

Investigator	Title	Institution	Telephone
Faith Mathai	Principal Investigator	JKUAT	0726292969
Raphael Lihana	Co-Investigator	CVR KEMRI	0721879497
Simon Karanja	Co-Investigator	COHES, JKUAT	0726424669

1. Patient identification number _____

2. Date of admission (in the hospital) (dd/mm/yr) ___/___/___

3. Gender Male ☐ Female ☐

4. Age ____ years

5. What is your marital status? a. Single b. Married c. Divorced/Separated

6. Religion a. Christian b. Muslim

8. Occupation of the respondent

a. Employed b. Business c. Unemployed

9. Do you drink alcohol?

a. Yes b. No

11. If yes for how long have you been consuming alcohol?

_____

12. Have you ever smoked cigarettes? _____

14. How long have you been smoking cigarettes?

_____

17. How old were you when you first had sexual intercourse?

_____

18. Have you ever used condoms? _____

19.Do you have personal effects such as tooth brush, shaving razors?

_____

Occasionally people share these personal effects with friends and family members, have you ever shared these personal effects with others?
a. Yes_____ b. No_____ c. Refuse_____

21. If yes, specify what you shared with household members / none-household members nd for how long?

Toothbrush No. of times shared (_____)

Shaving razor No. of times (_____)

23. Have you been sick/hospitalized requiring blood transfusion?

_____

24. If yes when did you or close family member receive blood transfusion?

_____

25. Tattoo are fashioned used by many, do you have any tattoo in your body? _____

26. If yes, when did you receive the tattoo? _____

27. Where did you receive the tattoo? _____

28. Acupuncture is a medical practice or procedure that treats illness or provides local anesthesia by the insertion of needles at specified sites of the body, have you ever been done this procedure? _____

29. If yes when and where: When _____ Where _____

30. Have you ever been done any invasive procedure? _____

31. If yes when and where: When _____ Where _____

32. Have you heard of hepatitis B? _____

33. Have you ever received vaccination for hepatitis B? _____

34. Have you been advised by your doctor to take a hepatitis test? _____

35. How about a member of your household? _____

36. Have you ever suffered from the following symptoms before (Dark urine, Dizziness, Drowsiness, enlarged spleen, Hives, Itchy skin, Light colored feces, the feces may contain pus, or yellow skin, whites of eyes, tongue (jaundice)?

a. Yes_____ b. No_____ c. Refuse_____

38. What was the reason why you are referred to pathologist for testing? _____

Appendix III: Ethical Approval Certificate/Authorization Form

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Email: chairman.kuerc@ku.ac.ke
secretary.kuerc@ku.ac.ke
cecku2006@gmail.com
Website: www.ku.ac.ke

P. O. Box 43844 - 00100 Nairobi
Tel: 8710901/12
Fax: 8711242/8711575

Our Ref: KU/R/COMM/51/405

Date: 25th February, 2015

Faith Mathai
Kenya Medical Research Institute, Nairobi

Dear Mathai,

APPLICATION NUMBER PKU/304/E280- "DIAGNOSTIC VALUE OF HAPATITIS B SURFACE ANTIGEN (HBsAg) IN HAPATITIS B VIRUS (HBV) INFECTION AMONG PATIENTS REFERRED TO PATHOLOGIST LANCET LABORATORY IN NAIROBI, KENYA."

1. IDENTIFICATION OF PROTOCOL
The application before the committee is with a research topic, **"Diagnostic Value Of Hapatitis B Surface Antigen (Hbsag) In Hapatitis B Virus (HBV) Infection Among Patients Referred To Pathologist Lancet Laboratory In Nairobi, Kenya."** Received on 20th January 2015 discussed on 20th February, 2015.

2. APPLICANT
Faith Mathai

3. SITE
Lancent Laboratory in Nairobi County, Kenya

4. DECISION
The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 25th February, 2015.**

5. ADVICE/CONDITIONS
i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

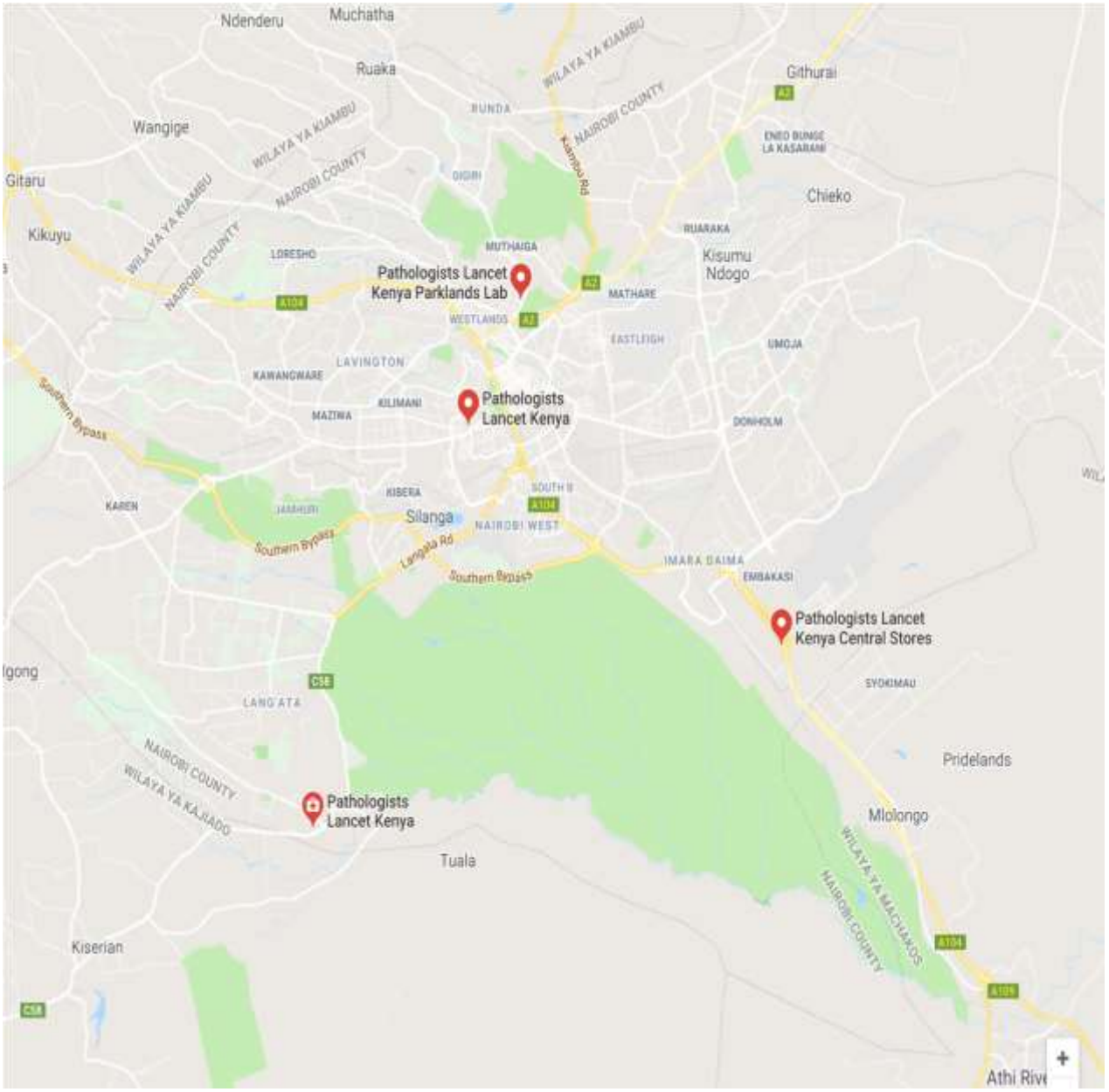
PROF. NICHOLAS K. GIKONYO
CHAIRMAN ETHICS REVIEW COMMITTEE

I ...Faith Mathai... accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of...25/2/15..... 2015.

cc. Vice-Chancellor

Appendix IV: The map showing the Locality of the Study



Adopted from google map

Appendix V: Publication

Arch Clin Infect Dis. 2017 October; 12(4):e13306. doi: 10.5812/archcid.13306.
Published online 2017 September 23. Research Article

Correlation of Quantitative Assay of HBsAg and Hepatitis B Virus DNA Levels Among Chronic HBV Patients Attending Pathologist Lancet Laboratory in Nairobi, Kenya

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Abstract

Background: Hepatitis B Virus (HBV) viral load measurements is recommended for diagnosis and monitoring of patients on treatment for Chronic Hepatitis B (CHB). However, these diagnostic techniques are molecular-based and expensive and unavailable to majority of the Kenyan population. Quantitation of hepatitis B surface antigen (HBsAg) by automated chemiluminescent microparticle immunoassay has been proposed as a surrogate marker.
Objectives: This study showed the correlation between HBsAg and HBV DNA levels among patients with CHB attending a referral Laboratory in Nairobi, Kenya.
Methods: One hundred and seventy-three (173) patients were enrolled while excluding Hepatitis C Virus (HCV) and Hepatitis D Virus (HDV) positive patients.
Results: The mean age of the study patients was 37.2 years, 112 (64.7%) were male, and 142 (82.1%) were hepatitis B e antigen (HBeAg) negative while 31/173 (17.9%) were HBeAg positive. The mean HBV DNA level was 2.89 log10 IU/mL and 41 (23.7%) had a baseline HBV DNA level of > 2000 IU/mL. By Mann-Whitney test, HBV DNA levels differed significantly between HBeAg positive and negative patients ($P = 0.001$), as did HBsAg titer ($P = 0.032$). Unfortunately, according to the Spearman test, there was a weak correlation between HBsAg and HBV DNA levels ($P = 0.024$ and $r = 0.171$).
Conclusions: Patients with HBeAg negative test results had lower levels of HBsAg and lower levels of HBV DNA. Serum quantitation of HBsAg may not replace serum HBV DNA levels among patients with CHB in Nairobi Kenya.

Keywords: Correlation, Quantification HBsAg, HBV DNA, Chronic Hepatitis B, Nairobi-Kenya

1. Background

Despite the availability of an effective vaccine and potent antiviral treatments, hepatitis B virus (HBV) infection is still a major global health problem (1, 2). By 2015, approximately 2 billion individuals had been exposed to HBV, with nearly 257 million estimated cases of chronic hepatitis B infection (3). Hepatitis B Virus and related conditions are also thought to be responsible for 500,000 to 700,000 deaths, annually (2). It is highly endemic in sub-Saharan Africa and East Asia, where approximately 5% to 10% of the adult population is chronically infected (4). In Kenya, the seroprevalence of HBsAg positivity is about 5% to 14% and the prevalence of HBV exposure is 20% to 70% (5, 6). For this reason, treatment and follow-up of chronic HBV infection is crucial.

Furthermore, HBV replication or detectable serum HBV DNA is closely related to the progression of liver disease, liver function decompensation, and the occurrence of cirrhosis and liver cancer in patients with chronic hepatitis B (CHB) infection (7).

Detection of serological markers is the backbone for the diagnosis of HBV infection, and quantification of HBV surface Antigen (HBsAg) in serum has been identified as the most reliable marker of HBV carriage (8). Hepatitis B Virus e antigen (HBeAg) is generally used as a secondary marker to indicate high levels of virus in the blood (8). The quantitative levels of HBV DNA, alanine aminotransferase (ALT), and histological outcomes constitute a major component in the management and treatment of HBV (9). The monitoring of HBV DNA in serum is as important as sero-