PREVALENCE OF HEPATITIS B VIRUS, SOCIAL DEMOGRAPHIC FACTORS AND CIRCULATING GENOTYPES AMONG ANTENATAL CLINIC ATTENDEES AT MBAGATHI COUNTY REFERRAL HOSPITAL

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Prevalence of Hepatitis B Virus, Social Demographic Factors and Circulating Genotypes among Antenatal Clinic Attendees at Mbagathi Country Referral Hospital

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DECLARATION

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

Signature……..……Date …….

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

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DEDICATION

This work is dedicated to my late parents Mr. and Mrs. Malungu for laying down a solid education foundation for me.To my siblings, my sons Emmanuel and Matthew for moral support and encouragement during my study.

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ABBREVIATIONS AND ACRONYMS

A**g**	Antigen
Anti-Hbs	Antibodies against hepatitis B surface antigen
Anti-Hbe	Antibodies against hepatitis e s antigen
Anti-Hbc	Antibodies against hepatitis core antigen
ANC	Antenatal clinic
CLIA	Chemi-luminescent Immunoassay
cccDNA	covalent closed circular Deoxyribonucleic acid
CHB	Chronic Hepatitis B
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EPI	Expanded program on immunization
ELISA	Enzyme-Linked Immunosorbent Assay
HbeAg	Hepatitis B e Antigen
HBsAg	Hepatitis B surface Antigen

HBV	Hepatitis B Virus
HDV	Hepatitis D Virus
HBIG	Hepatitis B immune Globulin
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IgM	Immunoglobin M
IgG	Immunoglobin G
IFN-	Interferon
MEGA 6	Molecular, Evolutionary Genetic Analysis software version 6
MTCT	Mother to child transmission
RNA	Ribonucleic acid
KEMRI-	Kenya Medical Research Institute
WHO-	World Health Organization

DEFINATION OF TERMS

Hepatitis It is the inflammation of the liver, which is characterized by jaundice.

Hepatitis Endemic areas Regions with prevalence of hepatitis B surface antigen above 8%.

Horizontal transmission Acquisition of virus through sex contacts or injecting of drugs or household contacts.

Perinatal transmission Transmission of virus from infected mother to unborn fetus in utero.

Vertical transmission Transmission of hepatitis B virus (HBV) from infected mother to in during birth.

ABSTRACT

The World Health Organization approximates that 257 million people are living with hepatitis B virus (HBV) infection with 887,000 deaths related to its complication. This is fuelled by the occurrence of varied risk factors in populations across continents. Majority of HBV chronic carriers reside in Asia and Africa. There are nine genotypes of HBV (A-I) and their variants exist worldwide. Genotypes A, D and E are found in Sub-Saharan Africa. In Kenya genotypes A is dominant. This cross-sectional study was aimed at identifying the prevalence of HBV , associated social demographic risk factors and circulating genotypes, among 287 antenatal attendees at Mbagathi county referral hospital Nairobi.A structured questionnaire capturing social, demographic, explanatory variables was administered. Blood samples were also drawn from the participants and tested for HBV using the ELISA system. The viral DNA was extracted, purified and sequenced. Phylogenetic and mutation analysis was conducted on the generated sequences. The study established the prevalence of HBV infections in the study subjects to be 3.8% with highest infection rate being among the 35-39 years age group(9.5%).The risk factors associated with HBV positivity were; type of family (χ^2 =19.753 df2 p=0.01), parity (χ^2=7.128 df2 p=0.01), History of abortions (χ^2=9.094 df1 p=0.01), early age (11 – 15 years) at first sexual encounter (χ^2 =8.185 df1 p=0.01). The prevalent HBV genotype was genotype A. Hence HBV was transmitted horizontally in this study population. In order to control the spread of the virus there is need to identify cases both during antenatal as well as postnatal care, offer treatment for positive cases and HBV immunization for negative s as well as all women of childbearing age.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Hepatitis B virus (HBV) is a major causative agent for liver diseases which encampuses chronic hepatitis, liver cirrhosis, and liver cancer (Sunbul, 2014).It is listed the second commonest human carcinogen after tobacco (Kew, 2010). The virus is highly contagious and is 50 to 100 times more infectious than the human immunodeficiency virus (HIV). (Samwuel, 2004). It is also characterized by resilience enabling it to survive for more than a week on dry surfaces, hence complicating its epidemiology and increasing the risk of horizontal intra-familial transmission (MacLachlan & Cowie, 2015). According to the World Health Organization (WHO), countries of Africa, Asia, and South America exhibit carrier rates as high as 8%, (WHO;2018).The report further indicates high mortality rates with an estimated 887,000 deaths arising due complication of the virus annually. Majority of HBV chronic carriers reside in Asia and Africa with 70%-95% of the adult population showing evidence of past exposure to HBV infection (Hwang *et al.,* 2011).Epidiemiological reviews further identifies sub saharan Africa as endemic (Kew, 1996; Kiire, 1996)The HBsAg carrier rates for sub-Saharan region stands at 9-20% with Kenya at 5-30%(Mutuma *et al.,* 2011). Humans are the only known reservoir for hepatitis B virus. The virus is transmitted to susceptible host in contact with blood and contaminated body fluids. Route of transmission varies and are linked to HBV endemicity most common routes are parenteral ,sexual contact and vertical transmission . In Kenya the peak of HBV infection occurs during early childhood and at child bearing age suggesting both horizontal and sexual transmission occuring within the population (Mutuma et al., 2011). Pregnancy does not alter the natural history of hepatitis B infection. About 90% of pregnant women will clear the infection like other adults (Vallet-Pichard & Pol, 2014).

Hence only pregnant women with a serological evidence of HBsAg and HbeAg without interventions have 85% risk of transmitting of HBV to their infants.HBV infection acquired at infancy has a 90% chance of developing to chronic hepatitis .About 10% of transmission occurs in utero, vertical transmission occurs intrapartum and neonatal transmission mainly occurs at birth through the mixing of maternal blood and genital secretions. Approximately 25% of the carrier neonate will die from cirrhosis or hepatic carcinoma between late childhood and adulthood (Kew, 2010). Africa and Asia region are highly endemic but the modes of transmission are not similar. The later exihibit predominantly vertical versa ve horizontal transmission This is linked to low rate HbeAg positivity among HbsAg positive pregnant women identified in Africa (Hwang et al., 2011).The risk factors of HBV acquisition is dependent largely on beliefs and cultural practices, both of which vary per setting .Primary studies carried out globally have highlighted the key risk factors associated with HBV acquisition including age, history of blood transfusion, low level of education, surgery, sexually transmitted infections, abortions, higher mean parity, engaging in early sexual activities, polygamy, being male, having a rural birthplace, and engaging in sex with multiple partners (Breakwell et al., 2017) .Based on 8% divergence, genotypes A-J have been reported globally. In Kenya, genotypes A, C, D, E have been identified in blood donors, populations at increased risk of HBV infection as well as patients with liver disease (Mwangi et al., 2008).

Although hepatitis pentavalent vaccine is known to reduce the risk of developing hepatitis B infection among infants of hepatitis B positive mothers by 3.5 times; the timing of the vaccination is crucial.To infer the intented protection the vaccine is recommeded to be offered 3 days after birth for infants of HBV exposed infants. This can only be implemented if HbsAg positive pregnant women are identified promptly during their antenatal scheduled clinics.This is not so in Kenya as access to HBV screening services is limited. In addition although a highly effective HBV vaccine is available, immunization among adult populations in sub-Saharan African countries is neither free nor universal. Potent antivirals formulations for treatment of virus are available but not readily accessible due to high cost (Simmonds & Midgley, 2005).

Based on this there need to engage new strategies in the control and management of HBV infection considering its asymptomatic nature thus those infected are unaware resulting to uncontrolled tranmission hence a silent epidemic in Africa.

1.2 Justification

The study was conducted at Mbagathi county referral hospital is a public health facility located about 8 Km radius from the Nairobi Central Business District with Kenyatta National referral hospital in close vicinity. The hospital was selected based on its location and its ability to offer affordable health care services to everyone regardless of social economic status.The facility is accessible and offers both inpatient and outpatients specialized health care service at a sub-subsidized cost that is affordable to the majority of Nairobians and extended to neighbouring towns. The facility offers antenatal clinic services on scheduled days that is Monday and Wednesday from 8.00am to 5.00pm. Antenatal attendees study population was selected as they are considered at high risk of contracting HBV infection due to increased exposure to risk factors .Futhermore they have the potential to transmit to their infants hence the emergence of permanent carriers (Lu et al., 2014).

Despite their key role, their is scarcity of information on the burden of disease among this population. Since HIV studies in Kenya have been limited to health workers , blood donors, high risk groups, those with liver disease and people living with HIV.

1.3 Problem statement

In Kenya ,maternal and horizontal are major transmission routes of HBV to infants and children. Hence infected mothers and household contacts are major reservoir of HBV transmission to children in their early years of life. In absence of immunoprophylaxis, 90% of infected children remain chronically infected HBV hence maintaining the HBV infection in the population (Ho & Ho, 2012).This is occuring despite of the availability of highly effective vaccines and treatment regimen. Identification of those with disease

is the main pillar in the linkage to care, prevention control of HBV infection among adult population. This is a drawback due limited access to diagnostic services. These services are accessed out of pocket and clinician initiated.

Although the virus is 50 times more infectious that HIV, in Kenya, government advocacy resource mobilization and goodwill is limited as compared to HIV. In regards to vaccination the intervention targets children under 5 year of age and extended to health workers due to financial and logistic challenges (Maina et al., 2013).Based on this majority of those infected by HBV Kenya are identified at later stage (liver disease) and liver cancer resulting to high morbidity and mortality rate.

Hepatitis B virus genotypes are associated with determining the clinical outcomes, natural course, modes of transmission and control of HBV infection. Hence necessitating frequent monitoring of the HBV genotype diversity. In our setting genotype A has been identified as predominant among blood donors, inpatient, and high-risk populations (Mwangi et al., 2008) .Study findings will provide additions in formulating policy guideline for the management of HBV infection.

1.4 Research question

i. What is the prevalence of hepatitis B virus among antenal care attendees?
ii. What are the social demographic risk factors that are associated with HBV infection among antenatal care attendees ?
iii. What are the type of HBV genotypes are circulating among antenatal care attendees ?

1.4 Objectives

1.4.1 General Objectives

To determine prevalence ,associated social demographic risk factors and circulating genotypes of HBV infection among antenatal care attendees at the Mbagathi County Referral hospital.

1.4.2 Specific Objectives

i. To determine the prevalence of Hepatitis B among antenatal care attendees at the Mbagathi County referral hospital .
ii. To determine the social demographic risk factors associated with Hepatitis B infection among HBsAg positive antenatal care attendees at the Mbagathi Country Referral hospital.
iii. To determine the circulating HBV genotypes among HBsAg positive antenatal care attendees at Mbagathi County referral hospital

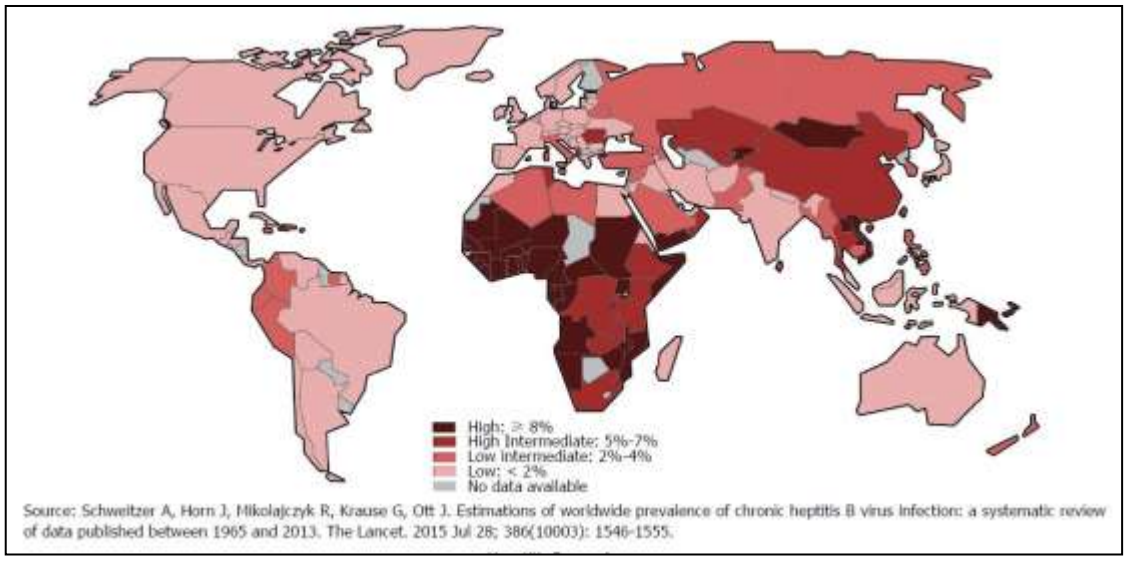
CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology of Hepatitis B virus infection

Globally Hepatitis B virus is a major public health concern. It is causative agent of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Approximately 78,0000 HBV-related deaths are documented yearly worldwide (Kew, 2010).Prevalence of this HBV chronic infection varies widely across the continents leading to categorising endemicity into low, intermediate and high (

Figure 2.1).



Source: Schweitzer A, Horn J, Mikolajczyk R, Krause G, Ott J. Estimations of worldwide prevalence of chronic heptitis B virus infection: a systematic review of data published between 1965 and 2013. The Lancet. 2015 Jul 28; 386(10003): 1546-1555.

Figure 2.1: Global categorisation of chronic hepatitis B virus endemicity

Characteristics of HBV infection vary per each category of endemicity where by in high endemic continents with at least 8% prevalence such as sub saharan Africa, and Asian countries , 70–95% of the population shows past or present serological evidence of HBV

infection (Hou et al., 2005).According to the authors ,most infections occur during infancy or childhood.

This is characterized by little evidence of acute disease related to HBV and high prevalence rates of chronic liver disease and liver cancer in adults. On the other hand moderate endemic regions (Eastern and Southern Europe, the Middle East, Japan, and part of South America) with 2-7% prevalence rates are characterized by 10–60% of the population having evidence of chronic infection, acute disease with most of infection acquired during adolescent and adulthood. In comparison with high endemic regions where chronic infection is maintained by infants and children , here mixed pattern of transmission exist, including infant, early childhood and adult transmission (Hou et al., 2005).Low endemicity is observed in developed countries such as North America, Northern and Western Europe and Australia. In these regions, HBV infect 5–7% of the population, and only 0.5–2% of the population are chronic carriers . In these areas, most HBV infections occur in adolescents and young adults and relatively well-defined high-risk groups, including injection drug users, homosexual males, health care workers, patients who require regular blood transfusion or hemodialysis.

2.2 Burden of Hepatitis Virus in Kenya

The prevalence of HBV infection in Kenya varies widely by region and study population. Systematic reviews of HBV studies carried out among HIV infected population between the years 1990-2008 reported a prevalence to be 6-12% (Barth et al., 2010). A study among patients presenting with jaundice at four referral hospital that is; Kenyatta National Hospital (Nairobi), Moi Teaching and Referral Hospital (Eldoret), New Nyanza Provincial General Hospital (Kisumu), and Coast General Hospital (Mombasa) described 6.3% prevalence of HBV infection (Ochwoto et al., 2016).

Another study carried among intravenous drug users at the coastal region reported a prevalence of 9.6% among those infected with HIV and 2.3% of those not infected

(Webale et al., 2015). Among the youth aged 13-18 years attending public day schools in Machakos and Nairobi counties the prevalence was 3.0% (Ng'ang'a, n.d.) In addition among voluntary blood donor from Siaya, Homabay and Kisumu a prevalence of 3.5% was reported (Onyango et al., 2018) .

The prevalence of 4% was observed in study carried out among health workers in Makueni county (Kisangau et al., 2018).In regard to the MSM population a study carried out at the coastal region, a prevalence of 6.2% was recorded (Chisari et al., 2010).

2.3 Natural course of hepatitis B infection

Infection with HBV can result in either acute or chronic HBV infection (Figure 2.2).Development of chronic infection correlate positively with younger age.Chronic infection is long term and is characterized by high level multiplication heightened immune response to control the virus resulting to inflammation of the liver of where the virus replicate (Croagh & Lubel, 2014) Seroconversion and maintenance of undetectable levels of viral replication are markers of a favourable prognosis, but long-term disease can lead to the development of cirrhosis and hepatocellular carcinoma (Lamontagne et al., 2016).

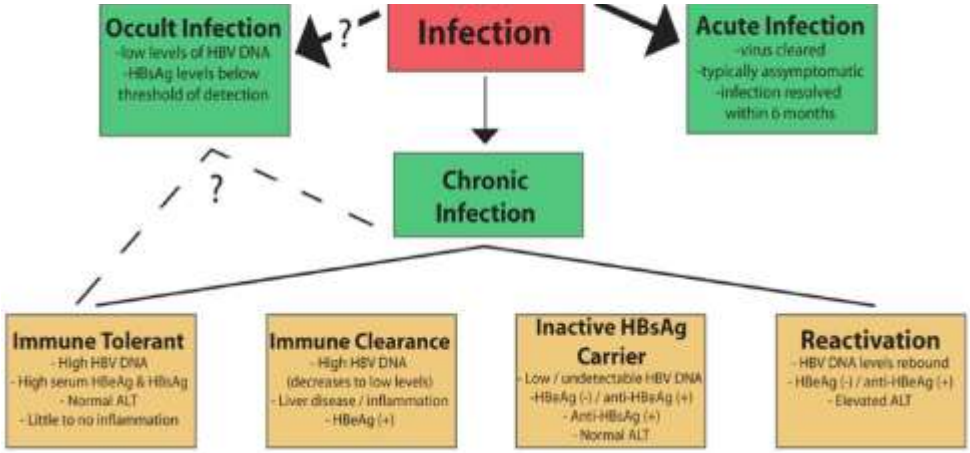


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Figure 2.2: Natural course of HBV infection

(Lamontagne et al., 2016)

2.4 Acute HBV infection

Hepatitis B virus infection in adults results in 'acute hepatitis' in 90-95% of them (Lamontagne et al., 2016).Majority of infected persons remain asymptomatic.

On the other hand symptomatic HBV-infected individuals present with inflammation of the liver,nausea,jaundice,abdominal pain, and vomiting. (MacLachlan & Cowie, 2015)

This is resolved within 6 months of infection with evidence undetectable viral load and presence of antibodies against HBsAg (**Error! Reference source not found.**). There is no specified treatment for acute HBV infection unless patients are suffering from fulminant hepatitis B or protracted severe acute hepatitis B (Lok & McMahon, 2009)

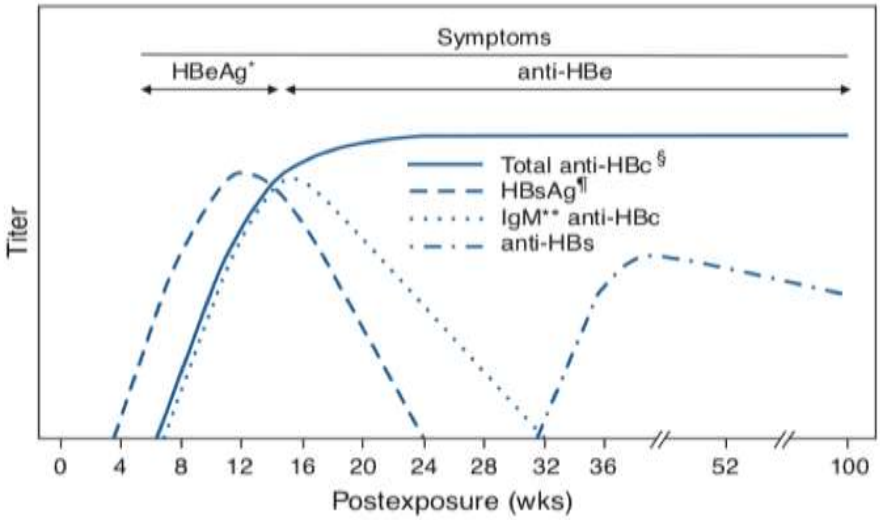

Figure 2.3: Serological course of acute HBV infection with progression to immune cleareance (Weinbaum,Mast, & Ward, 2009

2.5 Chronic HBV infection

Age at infection is an important risk factor for development of HBV chronicity. In absence of immunoprophylaxis, 90% of infants infected at the age of 6 months remain chronically infected (Ho & Ho, 2012) .

This due to immature infants' immune systems (Hadziyannis, 2011).While acquiring infection in adulthood has a less than 5% risk in developing chronicity (Lok & McMahon, 2009).

Chronic HBV infections occur in four phases that vary in duration and outcome of the underlying liver disease. The phases are linked to the level of HBV replication as well as the host immune response against the virus.

The phases are namely immune tolerant phase, immune clearance phase, inactive HBsAg carrier phase, and reactivation. Many factors are postulated to influence the occurrence of these stages, these include age, sex, immunosuppression, and co-infection

with other viruses. Not all patients exhibit the transition from one phase to the next of chronicity as highlighted above (M. Liang et al., 2011).

2.5.1 Immune tolerant phase

The immune tolerant phase is observed among Asian children infected perinatally with HBV and can last for several decades. The phase is also seen in childhood acquired, horizontal infection but is thought to be of much shorter duration While in order children and adults the infection. is transient (Gunardi et al., 2017).The phase is characterized by high viral load (>20 000 IU/ml), HBeAg ,HbsAg and normal or mildly elevated levels of liver enzymes (Lok & McMahon, 2009).

2.5.2 Immune clearance phase

At this phase Immune tolerance to HBV is lost and the virus is cleared by the immune system. The phase can last from several weeks to years (Pan & Zhang, 2005).Its main feature is the occurrence of spontaneous flares which represent an intensification of the immune response to HBV. This results in an increase in the HBV DNA level, elevated levels of aminotransferases. Seroconversion from HBeAg to anti-HBe occurs (Lamontagne et al., 2016).This is an important clinical outcome of the immune clearance phase as it marks the transition to the next phase .

The timing of HBeAg sero- conversion, is impacted by age, genotype and age at acquisition of virus.The HBeAg seroconversion is associated with a favorable long-term outcome and with decreased risk of developing cirrhosis or HCC (Shi, 2012).

2.5.3 Inactive HBsAg carrier phase

The phase is also called immune control phase; it is characterized by multiple changes to the disease state. This includes loss of HBeAg expression, seroconversion from HBsAg to anti-HBs and low to undetectable levels of serum HBV DNA. In addition,

aminotransferase levels are normal, mild hepatitis and fibrosis may be observed. This is a favorable clinical outcome (Moradpour & Wands, 1995).Some patients at this stage may enter reactivation/HBeAg-negative chronic hepatitis B" phase due to immunosuppression resulting to increased necroinflammation. The end result of chronic HBV infection is the development of HBV-associated HCC (Lamontagne et al., 2016).

2.6 Factors that influencing the natural history of Hepatitis B infection

The natural history of HBV virus evolves around the complex interactions between the host, viral and environment factors (

Figure 2.3).

2.6.1 Viral factors that influence the natural history of Hepatitis B infection

During infection HBV virion as high as 1010 particles /ml circulate in the blood of an infected person. Hence making it the main mechanism of HBV transmission but other body fluids have also been implicated (Lamontagne et al., 2016). Hepatitis B virus is not cytopathic hence manifestations of syndromes/ flares are a result of the cellular immune response towards the viral antigen (Pan & Zhang, 2005) .Hence an intact immune response determines the clinical outcome of HBV infection.

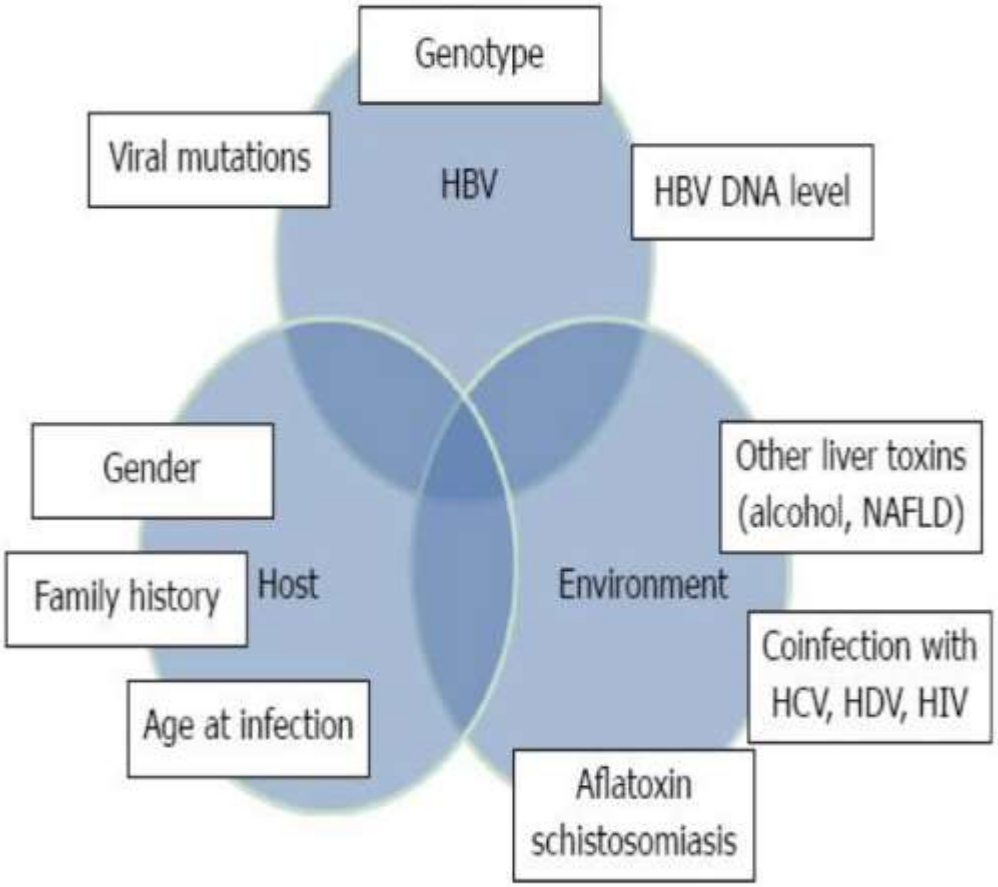

Figure 2.3: Interacting Factors to affect the natural history of chronic hepatitis B

(Croagh & Lubel, 2014)

2.6.2 Viral mutations and enviromental factors that influence on the natural history of HBV infection

Hepatitis B virus hasa high mutation rate estimated at 103 to 106 nucleotide substitutions/site/year (Cao, 2009) .This is attributed to error-prone activity of reverse transcriptase enzyme as well as high virion production per day (1012 viruses) (Croagh & Lubel, 2014).HBsAg mutants have been associated with occurrence of vaccine escape. This leads to HBV infection among immunize persons either by pentavalent vaccination or by prophylactic administration of Hepatitis B Immune Globulin (HBIG)

.The variants have also been linked to diagnostic assay failure to detect HBsAg resulting in the propagation of occult HBV infection (Coppola et al., 2015).

On the other hand, basal core promoter (BCP) and precore (PC) mutations have been linked to a reduction in HBeAg levels with a consequent increase in viral replication and eliminating HBeAg production, resulting in HBeAg-negative disease respectively. Drug-induced mutations target the active site polymerase within the conserved region known as Tyr-Met-Asp-Asp (YMDD) resulting in antiviral resistance (Archampong et al., 2017). These mutants can be transmitted to a naïve susceptible host and establish a stable HBV infection (Horvat, 2011).

Overlap between conserved regions in the HBV polymerase gene and the "a" determinant of the HBsAg gene, mutations within the shared DNA sequence results to a virus with a variant HBsAg and antiviral resistance hence the possibility of virus escape (Dos Santos et al., 2017).Environment factors that have been identified to influences HBV infection are co-infection with HCV, HDV, HIV, liver injury (alcoholic or fatty liver disease), exposure to infectious agents (schistosomiasis). This have been linked to accelerating the progression towards end-stage liver disease (Croagh & Lubel, 2014).

2.6.3 Genotypes influence on the natural history of HBV infection

Hepatitis B virus genotypes play a role in the virus-host relationship as they are associated with differences in clinical and virological characteristics (Norder et al., 2004) All HBV genotypes have the potential to infect individuals leading to chronic infection with various stages of progressive liver disease. Seroconversion of hepatitis B e antigen (HBeAg) and seroclearance of HBsAg are events that are critical to the control of HBV infection. Studies have established that genotype C patients experience delayed HBeAg seroconversion and, thus, have a longer duration of high HBV replication than genotype B patients (Kramvis, 2016). On the hand patients infected with genotypes A and B have a higher rate of spontaneous HBsAg seroclearance (Wu & Chang, 2015).

Thus genotypes C and D patients, compared with genotypes A and B patients, have late or absent HBeAg seroconversion after multiple hepatitis flares that may accelerate the progression of chronic hepatitis, thereby conferring a worse clinical outcome (Hadziyannis & Vassilopoulos, 2001).

2.7 Molecular biology of hepatitis B virus

2.7.1 Structure and components of Hepatitis B virus

Hepatitis B virus is a double-stranded DNA virus of the Hepadnaviridae family.Its genome is approx. 3200 bp encased within viral specific proteins. The genome encodes for four partial or completely overlapping open reading frames (ORFs) that is S, C, P, and X as illustrated in The S encodes for the viral surface envelope (HBsAg) that is subdivided into pre S1, preS2 and S regions (Figure 2.4). The C gene is broken down to precore and core regions. It encodes for HBeAg or HBcAg depending on the initiation of translation either from the precore or core region.X genes for transcriptional transactivator protein required for HBV replication, P gene encoding for polymerase reverse transcriptase (T. J. Liang, 2009).HBV replicates via an RNA intermediate. The DNA genome is copied by virus-specific reverse transcriptase which lacks proof reading capacity. Nucleotide substitution rate for HBV is higher than in other viruses. Hence persistent long-term infection and under different selective pressures results to emergencence of variants .This extraordinary genomic diversity in addition to high viral replication rate and ability to survive outside the body for a prolonged period (7 days) allows HBV to adapt to several host's (Coppola et al., 2015) .

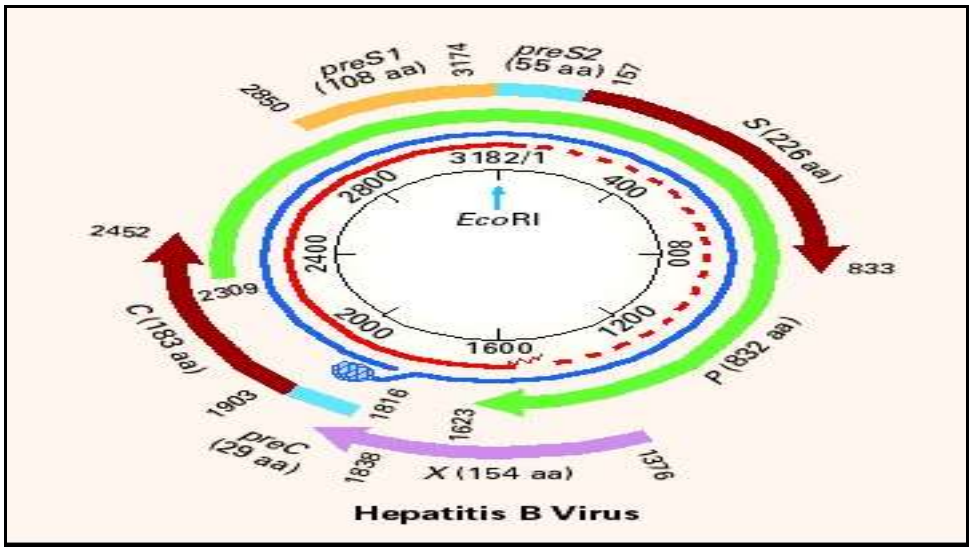

Figure 2.4: Structure of Hepatitis B virus

2.8 Hepatitis B genotypes

The HBsAg is a peptide with 226 amino acids with a single major antigenic determinant called the "a" determinant , which located between amino acid positions 100 and 160. Within this protein, there are highly conserved areas defining the genotype of the virus (Coppola et al., 2015)(Coppola et al., 2015).The"a" determinant region is common in all HBV genotypes. It is the main neutralizing epitope.Genotypes evolve in the presence of selective pressure exerted by the host immune system or during certain therapeutic measures (Araujo et al., 2009). Based on this, HBV genotypes are thought to occur as a result of neutral evolutionary drift of the virus genome, recombination or a consequence of long term adaptations of HBV to genetic determinants of the specific host population(Bartholomeusz & Schaefer, 2004). HBV phylogenetic analysis of nucleotide sequence of the whole HBV genome remains a gold standard for genotyping and subgenotyping of the virus.(Cao, 2009).Based on nucleotide divergence of at least 8% and 4% over the entire HBV genome, 9 genotypes (A-I) have been identified and several subgenotypes (Cao, 2009).HBV genotypes are distinct and differ in geographical distribution and mode of transmission (Sunbul, 2014).

2.9 Modes of transmission of HBV

Hepatitis B virus is 50-100 times more infectious than HIV and 10 times more infectious than HCV HBV is spread by contact with infected body fluid. Blood is the most important vehicle for transmission but other body fluids have also been implicated (Chisari et al., 2010). Persons with chronic HBV infection are the major reservoirs for transmission, although any person testing positive for HBsAg is potentially infectious to both household and sexual contacts. Perinatal transmission is the predominant route of HBV transmission in high endemic areas particularly China and South Asia.This accounts for 35% to 50% HBV carriers (Wu & Chang, 2015).The risk of perinatal transmission is dependant on the presence of hepatitis B e antigen (HBeAg) in the blood of infected pregnant women.

This increases the risk of acquiring chronic HBV infection by 70-90% as compared to 5-20% from those who are HBeAg-negative (Wu & Chang, 2015). The risk of chronic HBV infection is 70-90% from mothers who are HBeAg-positive and 5-20% from those who are HBeAg-negative (T. J. Liang, 2009). Hepatitis B is considered a sexually transmitted disease. Heterosexual transmission accounts for an increasing proportion of HBV infection.

Factors associated with increased risk in heterosexuals include the duration of sexual activity, number of sexual partners and history of STI's. Intravenous drug users and their sexual partners, prostitutes and their clients are at increased risk of infection (Anaedobe et al., 2015b).The infection is also transmitted through percutaneous route. This includes intravenous drug use, household contacts, tattooing, and ear piercing. In the hospital setting, patients and health care workers are exposed to HBV infection through surgery, contaminated surgical instruments, a need stick and by nosocomial infection during dialysis and dental surgery**.**

2.10 Clinical manifestations of hepatitis B infection in pregnancy women

Women of childbearing age with serological evidence of HBsAg and HBeAg have 85% risk of transmission of HBV to their infants as compared to women who have serological evidence of HBsAg (10%) only (Bittaye et al., 2019).Hepatitis B virus infection in pregnant women manifests as either acute or chronic (Jhaveri & Bryson, 2006). The infection does not have a special predilection in pregnancy and its severity is similar among pregnant and non-pregnant females (Khuroo & Kamili, 2003) . Although some infected pregnant women experience higher incidences of hyperemesis gravidarum and low birth weight. Incidence of prematurity observed is 31.6% higher than that observed in the general delivery population 10-11% (Bohidar, 2004).

Those with severe liver abnormal functions are prone to postpartum hemorrhage, puerperal infection fetal distress, death, and neonatal asphyxia.During pregnancy metabolic needs of the mother as well the growing fetus increases. This simultaneously increases the detoxification load on the liver that further aggravates pre-existing liver disease as well as exacerbating liver damage (Saravolatz et al., 2004).

Severe modification on the maternal immune system also occurs leading to a depressed immune system against HBV infection (Tran, 2016).

Perinatal hepatic flare reactions occur at a prevalence of 6% to 14% during pregnancy and 10% and 50% in post postpartum period leading to an increase in viral DNA, severe hepatitis and hepatic failure (Kushner & Sarkar, 2018) .For those pregnant women who have serological evidence of both HBsAg and HBeAg, the risk of their infant developing HBV infection by the age of 6 months, in the absence of immunoprophylaxis, is 70–90%, with approximately 90% of these children remaining chronically infected.(Ho & Ho, 2012).

2.11 Prevalence studies of Hepatitis B infection among pregnant women

The prevalence of HBV infection among pregnant women reflects the same pattern as that observed in the general population under each level of HBV infection (Kramvis & Kew, 2007). Seroprevalence study of HBsAg among pregnant women of middle and high-class social status in Sierra Leone reported a prevalence of 6.2% which coincides with the high endemicity classification of the country (Wurie et al., 2005). In Kenya HBV prevalence among rural nomadic adult population was 8.8% (Mutuma et al., 2011), while that among pregnant women was reported at 9.3% country wide and 7.7% in Nairobi region (Okoth et al., 2006) The same pattern is observed by a Sudanese study where the prevalence was (5.6%) while 6.9% in the general population (Elsheikh et al., 2007). The high prevalence of HBsAg observed in these studies among pregnant women could be an indication that pregnant women serve as a very important reservoir fuelling the HBV epidemic in the general population. Contrary to what was reported in Mali and Gambia where the HBsAg prevalence is lower than the general population at 8% and 9.2 % versus15 % respectively (MacLean et al., 2011; Bittaye et al., 2019). The authors attributed the high carrier rates to challenges in adapting routine HBV screening for pregnant women as well as in accessibility of and low HBV vaccine coverage.

Breakwell et al further expounds that in Africa timely administration of HepB-BD is hampered by inadequate knowledge of or lack of vaccine implementation guidelines, unreliable vaccine supply as well as high prevalence of home births (2017).

2.12 High risk groups and factors of acquiring Hepatitis B viral infection

The hepatitis B virus can infect infants, children, teens and adults. It is not a genetic disease thus everyone may be at risk for a hepatitis B infection during their lifetime. There are groups of people who are at higher risk because of where they were born, their occupation or life choices. Globally high risk groups for HBV infection identified include intravenous (IV) drug users, persons born in endemic areas, and men who have

sex with men. In addition healthcare workers exposed to infected blood or bodily fluids, recipients of multiple blood transfusions, patients undergoing hemodialysis, heterosexual persons with multiple partners or a history of sexually transmitted disease, institutionalized persons (eg, prisoners), and household contacts or sexual partners of HBV carriers have been enlisted (World Health Organization & Global Hepatitis Programme, 2015) .

The age of acquiring the infection is one of the major determinants of the prevalence rates of HBsAg.The chronic infection will occur among 90% of infants infected at birth,25-50% of children infected 1-5 years of age and 1-5% of persons infected as older children or adults.

HBsAg positivity is found in all age groups among pregnant women. The highest seroprevalence observed is among the age group 20-24 years as reported by studies carried out among this population in Niger Delta, Nigeria 8.6% 1.15% for India (Buseri et al., 2010; Dwivedi et al., 2011).Therefore, following acute HBV infection, the risk of developing chronic infection varies inversely with age.A similar finding was noted in Mexico by Vazquez-Martinez who observed that the average age of women infected with the Hepatitis B virus was 26. The high prevalence of HBsAg in age group 20-24 is attributed to early sexual activeness and the age where most females have their first pregnancy. Education is presumed a risk factor in acquiring HBV infection. Higher levels of education attained improves hygiene standards (Buseri et al., 2010).

In addition, HBsAg positivity decreased with ascending order of education. It was observed that pregnant women with no formal education had a prevalence of 18.9%, primary level 5.8%, Secondary 3.7% and tertiary at 1.6%.

Findings in a study carried out in Keffi, Nigeria reported that illiterate women had a higher risk of infection (10.4%) than the literate women (3.0%). This was related to the ability of the literate woman to read about the viral infection and methods of preventing

it (Pennap et al., 2011).Hepatitis B virus is sexually transmitted.Hence those married have increased risk of contracting the virus due to constant exposure to infected partner. Vazquez-Martinez study findings from Mexican study showed that , marital status influenced the risk HBV infection in single and divorced women (OR1.04, 95% CL 1.003-1.08) (2003).

Eyong further eluded that there was significant association between the prevalence of HBsAg and marital status (p = 0.000) among 287 pregnant women in Cameroon (2019).

A study carried out among youth aged 15-17 years showed that those who had ≥2 partners had a 2.66 times (95%CI = 1.96–3.87) greater chance of acquiring HBV infection than those with no partner.(Apidechkul, 2019) . Hence early sexual debut and multiple sexual partners is major risk factor of acquiring. infection. Other factors reported to be associated with acquisition of HBV include, male gender and history of previous surgery, HIV infection, and non-use of condoms (Bwogi et al., 2009). A Nigerian study among pregnant women enlisted age at sexual debut and multiple sexual partners as significant risk factors for HBV infection in their setting (Anaedobe et al., 2015a).

2.13 Diagnosis. and role of hepatitis B virus serological and virological markers.

Based on susceptible host-viral interactions, hepatitis B disease presents with a wide spectrum from acute to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (Liang, 2009; Wu & Chang, 2015).Its diagnosis includes a constellation of clinical, biochemical, serological, molecular and histological findings. Exposure to HBV infection triggers the host immune system resulting in heightened cellular response against the virus. Susceptible host and viral interaction brings forth virus-specific immune products/markers (antigens and antibodies) that are detected in serum during the post-exposure period. These markers include; HBsAg, anti-HBs, HBeAg, HBV

DNA,anti-HBe, anti-HBc IgM and IgG and are associated with specific stages of liver disease (Song & Kim, 2016).

Serological and molecular identification of the makers enables screening of HBV infection, elucidation of the natural course of chronic hepatitis B, assesses the clinical phases of infection and monitors antiviral therapy (Ferreira, 2000). (Interpretation of serological results is essential to correct diagnosis for various form of HBV infection (Table 2.1).

Table 2.1: Interpretation of the hepatitis B virus serological and virological markers

Hepatitis B Virus Serological and Virological Markers	Results interpretation
HbsAg	HBV infection, both acute and chronic
HbeAg	High-level HBV replication and infectivity; a marker for treatment response
HBV DNA	Level of HBV replication; a primary virologic marker for treatment response
Anti-HBc (IgM)	Recovered or chronic HBV infection
Anti- HBs	Recovered HBV infection or marker of HBV vaccination; immunity to HBV infection (titer can be measured to assess vaccine efficacy)
Anti- Hbe	Low-level HBV replication and infectivity; a marker for treatment response
Anti-HBc (IgG) and Anti- HBs	Past HBV infection; could lose anti-HBs
Anti-HBc (IgG) and HbsAg	Chronic HBV infection
Anti-HBc (IgG) and /or Anti- HBs and HBV DNA	Latent or occult HBV infection

2.14 Management of hepatitis B infection

In patients with acute HBV infection the disease is self-limiting within 6months of exposure. Management of infection during this phase is supportive therapy. However, in some infected persons their repeated attempts by the host immune system to control the infection causes hepatic injury resulting hepatocellular inflammation hence chronic infection (Tang et al., 2014) . At this phase antiviral agent are used. lamivudine, adefovir dipivoxil, telbivudine entecavir, tenofovir and PEGylatedinterferon are drugs of choice that are utilized by most countries worldwide for this purpose (M. Liang et al., 2011).Chronic hepatitis B treatment is aimed at suppression of HBV replication and re-mission of liver disease. Hence preventing cirrhosis, hepatic failure and hepatocellular carcinoma (Caligiuri et al., 2016).

Parameters used to assess treatment response include normalization of serum ALT, decrease in serum HBV DNA level, loss of HBeAg with or without detection of anti-HBe, and improvement in liver histology (Lok & McMahon, 2007).Chronic infection is life long thus antiviral drugs utilized in treatment cannot stop virus replication ,thus minimizing liver damage, but viral clearance is not achieved. Based on these prevention strategies used include; screening of family members and sexual partners for HBV infection and vaccination of those who are sero-negative. In addition for those chronically infected enrollment in follow up clinic and continuous patient education is utilized. Application of this strategies vary per geographical location , setting and target population (Brown et al., 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted at Mbagathi County Referral hospital a public health facility located about 8 Km radius from the Nairobi Central Business District with Kenyatta National referral hospital in close vicinity. The hospital was selected based on its location and its ability to offer affordable health care services to everyone regardless of social economic status.The facility is accessible and offers both inpatient and outpatients specialized health care service at a sub-subsidized cost that is affordable to the majority of Nairobians and extended to neighbouring towns. The facility offers antenatal clinic services on scheduled days that is Monday and Wednesday from 8.00am to 5.00pm.

3.2 Study Design and population

The cross-sectional study involved pregnant women attending antenatal clinic at Mbagathi district hospital in Nairobi for a period three-month (September and December 2014).

3.3 Inclusion Criteria

Pregnant women attending antenatal clinic at Mbagathi County Referral hospital, aged 13-49 years were eligible to participate in the study voluntarily. However, for antenatal clinic attendees with age below 18 years, a “guardian” (or parent, or husband) assent was required to enable enrollment. Each participant was enrolled in the study only once.

3.4 Exclusion criteria

Pregnant women already recruited in the study with repeat visits at antenatal clinics during the study period and those not consenting or assenting to the study were excluded.

3.5 Ethical consideration

3.5.1 Informed consent process

Eligible pregnant women were offered participant information sheet (Appendix) in a version they were able to read and understand.

For those not able to read they were assisted. Participants were allowed to ask questions for clarification and decision to participate or not in the study was participant dependant(Appendix). The choice taken by an eligible participant was respected.

3.5.2 Benefits

Enrolling to participate in the study was at no cost. Volunteering participants were offered fee HBV screening with no monetary benefit. Participants who tested positive for hepatitis B virus had their results relayed to antenatal clinic for follow up and referral to care.

3.6 Confidentiality

Personal identifiers were not used in the questionnaire and blood sample. A unique code was allocated to all participants that linked the questionnaire and blood sample.

3.7 IRB approval

Approval to conduct the study was granted by KEMRI Scientific Steering committee SSC No. 2724 (

Appendix) and Ethical Review committee SSC No. 2724

KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1 **September 03, 2014**

TO: **JACQUELINE A. MALUNGU,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. GEORGE NAKITARE,**
ACTING DIRECTOR, CVR,
NAIROBI

Dear Madam,

RE: **SSC PROTOCOL NO. 2724 (*RESUBMISSION*): PREVALENCE OF HEPATITIS B VIRUS GENOTYPES AMONG ANTENATAL CLINIC ATTENDEES AT MBAGATHI DISTRICT HOSPITAL**

Reference is made to your letter dated 28th August, 2014 and received at the KEMRI ERC office on 1st September, 2014.

This is to inform you that the Ethics Review Committee (ERC) reviewed the documents submitted and is satisfied that the issues raised at the 229th meeting of the KEMRI ERC on 22nd July, 2014 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this **3rd September 2014** for a period of one year. Please note that authorization to conduct this study will automatically expire on **September 2, 2015.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to the ERC Secretariat by **July 22, 2015**. The regulations require continuing review even though the research activity may not have begun until sometime after the ERC approval.

You are required to submit any proposed changes to this study to the SSC and ERC for review and the changes should not be initiated until written approval from the ERC is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of the ERC and you should advise the ERC when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

PROF. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE

CENTRE FOR VIRUS RESEARCH 10 SEP 2014

In Search of Better Health

( )

Approval was also sought from the of Mbagathi district hospital.

3.8 Sample size determination

The study sample size was determined by using Fisher et al,1998 formula for cross-sectional study based on the HBsAg prevalence of 7.7% among pregnant women in Nairobi region (Okoth *et al.,* 2006). The formula is $n= z^2 p (1-p)/ \varepsilon^2$ where

n =expected minimum sample

z = standard, corresponding to 95% confidence; 1.96

P= prevalence of Hepatitis B for Nairobi region 7.7%

ε = maximum likely error is taken as 5%

The minimum sample size calculated for the study was 109 but 287 pregnant women were recruited.

3.9 The Sampling of pregnant women

Recruitment of pregnant women within the study was integrated within the existing day to day clinic procedures. Mass HBV health education was provided by the nurses before the start of the clinic.

Simple random sampling was used to select 15 participants for the study per day using random numbers generated on a daily basis. Those meeting the inclusion criteria and providing consent were recruited until the sample size was attained.

3.10 Administering of study questionnaire

A pre-labeled validated structured questionnaire was administered by face to face interview. Participant demographic, past medical history and lifestyle data were captured (Appendix 1).

3.11 Blood sample collection, shipment, detection, and storage

Blood samples were collected by venipuncture by a trained phlebotomist. The arm of the participant was tied with a tourniquet and the position of the veins disinfected using cotton wool soaked in methylated spirit. Using a disposable sterile needle a blood sample was collected into 5 ml pre-labeled plain vacutainer tube and sealed with parafilm to avoid sample leakage. The sealed blood samples were placed on sample rack and absorbent material wrapped around it and placed in a cool box containing ice packs and packing list. The samples were then transported to the Kenya Medical research

institute, Centre for virus research laboratories by courier services. In the laboratory, the blood sample was centrifuged to obtain serum. Two serum aliquots of 2ml were made in pre-labeled screw-capped vials. This were then packed in cryoboxes of 100 samples each and stored at -80^{0}C until use in serological screening and genotyping of HBV.During analysis biohazard safety measures and standard operating procedure as stipulated by laboratory applied. Hepatitis genotyping was done at KEMRI production unit.

3.12 Detection of HBsAg markers

All blood samples collected from enrolled pregnant women were screened for the presence of HBsAg using Hepanostika® HBsAg Ultra kit (Biomerieux, Netherlands). The kit contains; microelisa removable strips in a 96 wells plate, each pre-coated with anti-HBs, a negative control (human serum non-reactive for HBsAg), positive control (bovine serum containing HBsAg), 100 ml of phosphate buffer concentrate (washing buffer), 11 ml of Tetramethylbenzine (TMB) in citric acid and 11ml urea peroxidase solution.

The test procedure followed the manufacturer instructions which included pipetting of 25µl of diluent was added into pre-coated wells with HBsAb.This was followed by addition of 100µl of undiluted sample and the controls to respective well.

After 1 hour incubation at 37^{0}C, 50µl of the conjugate was added and then incubated at 37^{0}C for another 1 hour. The wells were washed six times with washing buffer, using Elisa washer before adding 100µl TMB for a colour generation. The plates were then incubated at room temperature in dark for 30 minutes after which 100µl stop solution (M sulphuric acid) was added and the optical densities(OD) read on ELISA plate reader set at wavelength 450 nm within 15 minutes of addition of stop solution. This was then analyzed using manufacturers instructions and results obtained.

3.13 Extraction of viral DNA

HBV DNA from HBsAg positive samples was extracted using QiAmp® DNA blood mini kit (USA). The extraction followed the manufacturers procedural steps as follows: into a 1.5 ml microcentrifuge tube, 20 µl of proteinase-K (Qiagen) was aliquoted into the bottom of the tube, 200 µl serum, buffer AL were added respectively.Pulse-vortexing for 15 seconds for homogeneity followed. The mixture was then incubated at 56ºC for 10 min followed by a short spin down, 200 µl ethanol (96-100 %) was added to the sample, vortexed briefly spun down The lysate was then transferred into to the QIAamp Mini spin column (in a 2ml collection tube) without wetting the rim.The cap was closed and the QIAamp Mini spin column placed in the collection tube, centrifuged at 8000 revolutions per minute (RPM) for 1 min. Thereafter, the QIAamp Mini spin column was placed in a clean 2ml collection tube, and the tube containing the filtrate discarded. Five hundred (500) µl Buffer AW1 was added to the QIAamp Mini spin column without wetting the rim and centrifuged at 8000 rpm for 1 min. The filtrate was discarded. The QIAamp Mini spin column was transferred into another clean 2 ml collection tube and 500 µl Buffer AW2 was added. The filtrate was discarded, the centrifuging step was repeated.

Finally, the QIAamp Mini spin column was placed in a clean microcentrifuge tube and 60µl nucleases free water (Ambion® RNA company–USA) . to the column and centrifuged at 14000 RPM for 3 min to eluate the extract. DNA was then stored at -80°C

3.14 Nested polymerase chain reaction

Amplification of the HBV S gene by nested PCR was done using an ABI Thermal Cycler 9700 system (Applied Biosystems, USA) Five microliters (5µl) of the extract was amplified in a nested PCR. Two different sets of primers were used to target the HBsAg (S1 and S2) (Table 3.1) in a total volume of 50µL per tube. Each tube had 5µL

of 10X PCR buffer, 5µL of Magnesium chloride (Applied bio-systems), 20µM of forwarding and reverse primers, 0.5µL of amplitaq Gold (Applied bio-systems) and 1.25mM of dNTPs. The PCR reaction conditions for HBsAg were 94^{o}C for 10min, 40 cycles of 94^{o}C for 30 sec, 55^{o}C for 30 sec and 72^{o}C for 40 sec and final extension of 72^{o}C for 5min (Osiowy et al., 2010) The amplicons were viewed on 2% agarose electrophoresis gel.

Five microliters of the 1st PCR amplicons were utilized in the second amplification using S2 primer set for HBsAg.The master mix and the PCR profile were similar to the first round. The amplified PCR product fragment after nested PCR was 530 base pairs.

Table 3.1::Sequences of primers utilized in nested PCR

Primer Name (orientation	**Sequence (5'-3')**	**Nucleotide position**	Expected Fragment size region
S1 (LLr)	CGTTGACATACTTTCCAATCAA	995-974	940
S1 (LLf)	TCCTGCTGGTGGCTCCAG	55-72	
S2 (nLLr)	CAACTCCCAATTACATARCCCA	835-812	680
S2 (nLLf)	ACCCTGYRCCGAACATGGA	155-173	

3.15 Detection of amplified HBV S gene PCR products

3.15.1 The casting of 2% agarose gel

Amplified PCR products were detected using 2.0% agarose (Seakem LE® agarose; FMC BioProducts, Rockland, Marine, USA) gel in 1X TAE buffer (0.04Tris acetate, 0.001 M EDTA). The casting platform was assembled including the U.V. Transmissible (UVT) gel trays. To prepare molten 2% agarose gel, 2g of the gel powder was weighed

and dissolved into 100 mL of 1X TAE buffer by heating in a microwave for approximately 90 seconds. The molten gel was allowed to cool for 4-8 minutes with occasional stirring.DNA gel safe stain 5 µl in volume was added and the mixture stirred by swirling. The molten agarose was poured into the assembled UVT tray and allowed to set, for 30 minutes.

Once the gel has solidified the gel casting dams and combs were remove and sufficient 1X TAE buffer(1X Tris-acetate-EDTA (TAE) buffer (Qiagen, Hilden, Germany) poured into the electrophoresis tank to submerge the gel to a depth of 2 mm.

3.15.2 Loading PCR product on to gel

Molecular weight marker was loaded at extreme left-hand side lane. This was followed by a mixture of 2µl; of PCR product per sample and blue 5x loading dye (bromophenol blue, xylene cynol, and orange G) (Promega, Madison, USA) into their respective wells. The loaded agarose gel was then subjected to 100 Volts of a unidirectional (from the anode to the cathode) electrical current for a duration of 30 minutes.

3.15.3 Visualizing of PCR product bands

Migrated sample DNA bands and 100bp DNA ladder (Promega, Madison, USA) in the agarose gel were visualized using ultraviolet transillumination.A. A digital image of the gel was taken using a gel documentation system. Sequencing the Isolates

3.15.4 Purification of the Isolates

All PCR positive samples were purified using the Qiagen Gel purification kit according to the manufacturer's protocol. Procedural steps were as follows; into 2 ml microcentrifuge tube,240ul of Buffer PB was added to 48 ul of PCR product, and then mixed.. 10 µl of 3 M sodium acetate, pH 5.0 was added until the mixture turn to a yellow color. QIAquick spin column was placed in a provided 2 ml collection tube and

the mixture added and centrifuged for 30–60 seconds. The flow-through was discarded and the QIAquick column placed back into the same tube.To clean, Buffer PE. 0.75ml was added and spun in a centrifuge for 30–60 seconds. The flow-through was discarded and QIAquick column placed back into the same tube.

The column was then centrifuged for an additional 1 min. This was followed by the transfer of QIAquick column into a clean 1.5 ml microcentrifuge tube.

To elute DNA, 50 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) was added to the center of the QIAquick membrane and column centrifuged for 1 min. Purified DNA was quantified with Picogreen (Invitrogen) Purified nested PCR product with 50 ng of DNA proceeded for cycle sequencing.

3.15.5 Cycle sequencing

The sequencing PCR for purified nested PCR carried out with a total reaction mixture of 20 µl containing 3 µl of DNA, 5X sequencing buffer, 2.0 µl Big Dye, 10.5 µl of distilled water, and 1.5 µl of primer. For each sample, two reactions were prepared based on the set of primers utilized for the second PCR.

That is S2 (nLLr) 5'-CAACTCCCAATTACATARCCCA-3'and S2 (nLLf)5'-ACCCTGYRCCGAACATGGA -3'. (Osiowy et al 2011)The amplification was performed as follows; denaturation for 5 minutes at 96°C, and again for 10 seconds at 96°C, annealing at 50℃ for 5 seconds and final extension 60℃ for 4 minutes for 25 cycles.

3.15.6 Purification of pre sequencing products

Cycle sequencing components which include salt ions, unincorporated dye terminators,dNTPS were sequestered to prevent their co-injection with dye-labeled extension products. Big Dye X-terminator purification kit (Applied Biosystems) was

utilized. Procedural step included mixing of the 20ul Big Dye X-terminator and 90 ul Sam solution as shown in **Error! Reference source not found.**

Table 3.2: Preparation of Big Dye X terminator working solution

Number of reactions	n=1	n=20
Volume Big Dye X-terminator	20ul	400ul
Sam Solution	90ul	1800ul
Total	**100ul**	**2200ul**

This was followed by the addition of 110 ul Big Dye X-terminator working solution to each plate well containing the cycle sequenced products. The plate was sealed and vertically vortexed at 2500 rpm for 30 minutes. The plate was then centrifuged at 3000 rpm for 3 min. The 20ul of supernatant was aliquoted and transferred to a new plate. This proceeded to sequence detection on automated ABI 3730 genetic analyzer (Applied Biosystems, Foster City, CA).

3.16 Sequence Analysis

Sequencing chromatogram obtained for 9 samples out of 10 were assembled into contig sequences using Molecular Evolutionary Genetics Analysis (MEGA) software version 6. The assembled sequences were then uploaded for cleaning and editing where necessary using Bio-Edit software (Hall 1999).

The assembled sequences inclusive of 32 downloaded reference strains from Africa Asia, South America, Europe origin from the GenBank were exported to MEGA6 software where pairwise and multiple alignment were computed using multiple sequence comparison by log-expectation.Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).Study sequences were assigned to specific genotypes and subgenotype in comparison with HBV references sequences

3.17 Data Management

3.17.1 Study Variables

Hepatitis infection was used as the dependent variable during analysis where the presence of and its mutations in the blood samples of enrolled pregnant women and independent variables include age, education, marital status, parity, sexual activity, history of blood transfusion, surgery and other possible risk factors.

3.17.2 Data and analysis

The data collected were subjected to descriptive and inferential statistical analysis using SPSS V.20 software (SPSS Inc.Illinois, USA). The Mean, Standard Deviation and test of comparison where categorical variables were summarized as proportion and further analyzed using Chi-square and Fisher´s Exact Test to assess the association between the variables. Means were compared to determine the difference in HBV awareness among subjects.

Test of association using Logistic Regression was done to describe the relationship between the predictor variables (risk factors for HBV infection found to be statistically significant) and the outcome variable (HBsAg). The P-value < 0.05 was considered significant.

CHAPTER FOUR

RESULTS

4.1 Description of the study population

4.1.1 Participants distribution by county of residence

Two hundred and twenty eight (79%) enrolled participants were the residence of Nairobi county. The neighbouring country of Machakos and Busia had the least participants.

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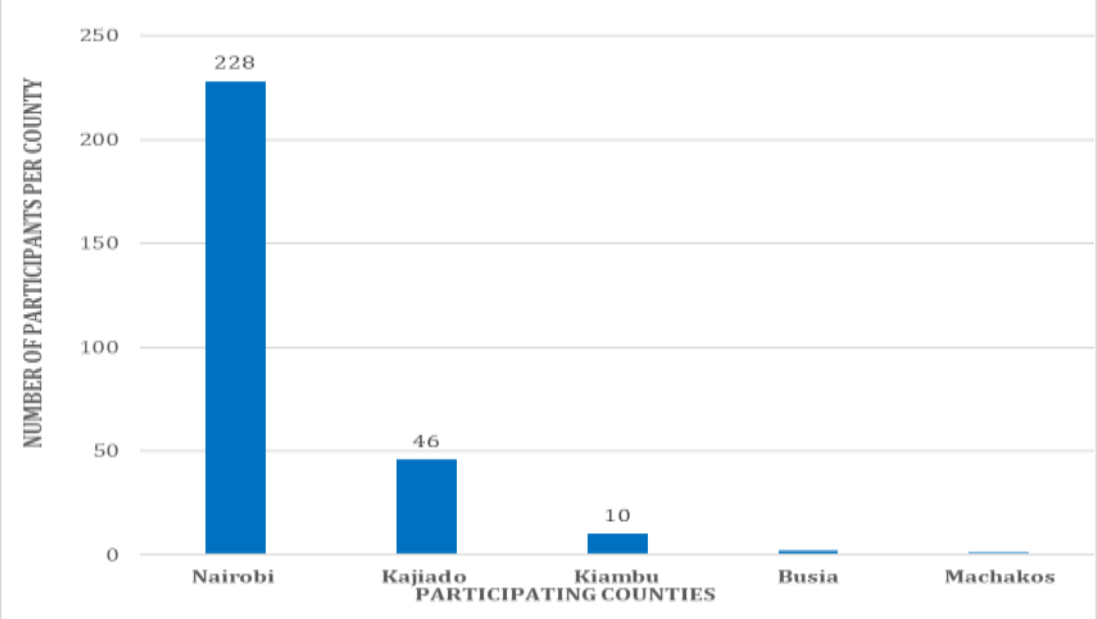


Figure 4.1 :Distribution of enrolled participants per county of residence

4.1.2 Participant distribution in relation to residence

Analysis of the participants distribution according to sub county of residence; indicates Dagorretti, Kibra sub-counties of Nairobi county contributing the highest number of participants that 25%(56) and 22%(51) to the study.

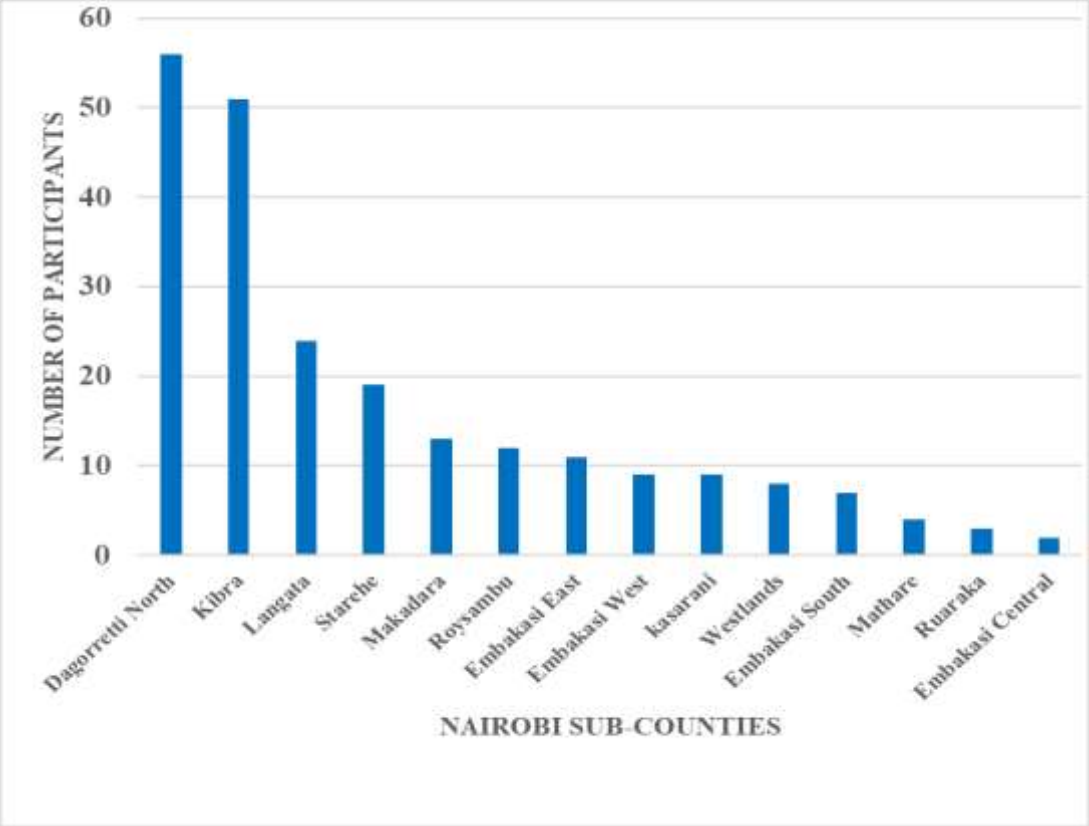


Figure 4.2: Distribution of participants per Sub country

4.2 Social demographic characteristics of respondents

During the study period, 287 pregnant women were recruited. The study participant's ages ranged from 15 to 49 years with the mean age of 26.7± 5.5 years. Majority 35.5% (102) of them were age group 25-29 years. In regards to marital status, 243 (84.7%) of

the women were married, 9 (3.1%) were cohabiting while 35 (12.2%) were single. Most of them had classroom-based education 284 (98.1%). Their distribution at each level of education is enlisted with the secondary school category having more participants as compared to the primary and post-secondary levels.More than half of the women were employed 166 (57.8%) while 121 (42.2%) were unemployed. Of those employed 46.7% (134) were employed informally. The mean gestation period was 23.9 weeks SD = ± 9. A majority (53.3%) of the participants were in their third trimesters The 1st trimester had the least number of participants (41) out of the 287 recruited. Gravidity was almost equally distributed among the participants with primigravida at 46.7% (134) and Multi gravida at 53.3% (153) (Table 4.1)

Table 4.1: Social demographic factors of respondents

Variable	Frequency=287	Percentage
Age category		
15-19	17	5.9
20-24	91	31.7
25-29	102	35.5
30-34	50	17.4
35-39	21	7.3
40-44	5	1.7
45-49	1	0.3
***Mean age group=26.7 SD=±5.5**		
Education level		
No formol Eduaction	3	1
Primary	81	28.2
Secondary	128	44.6
Post secondary	75	26.1
Occupation		
Employed formal sector	32	11.1
Employed in formal sector	134	46.7
Not employed	121	42.2
Marital status		
Married	243	84.7
Cohabiting	9	3.1
Single	35	12.2
Gravidity		

Primagravida	134	46.6
Multigravida	153	53.3
Pregnancy stage		
1st Trimester	41	14.3
2nd Trimester	93	32.4
3rdTrimester	153	53.3
***Mean gestation period = 23.9 weeks SD=± 9**		

4.3 Clinical characteristics of study respondents

Forty-eight (16.8%) participants reported having had an abortion and 4.5 % had been treated for sexually transmitted infections during their life time. During the study period, 13 (4.5%) participants reported to have ever been transfused blood and 3.1% (9) immunized for hepatitis B.

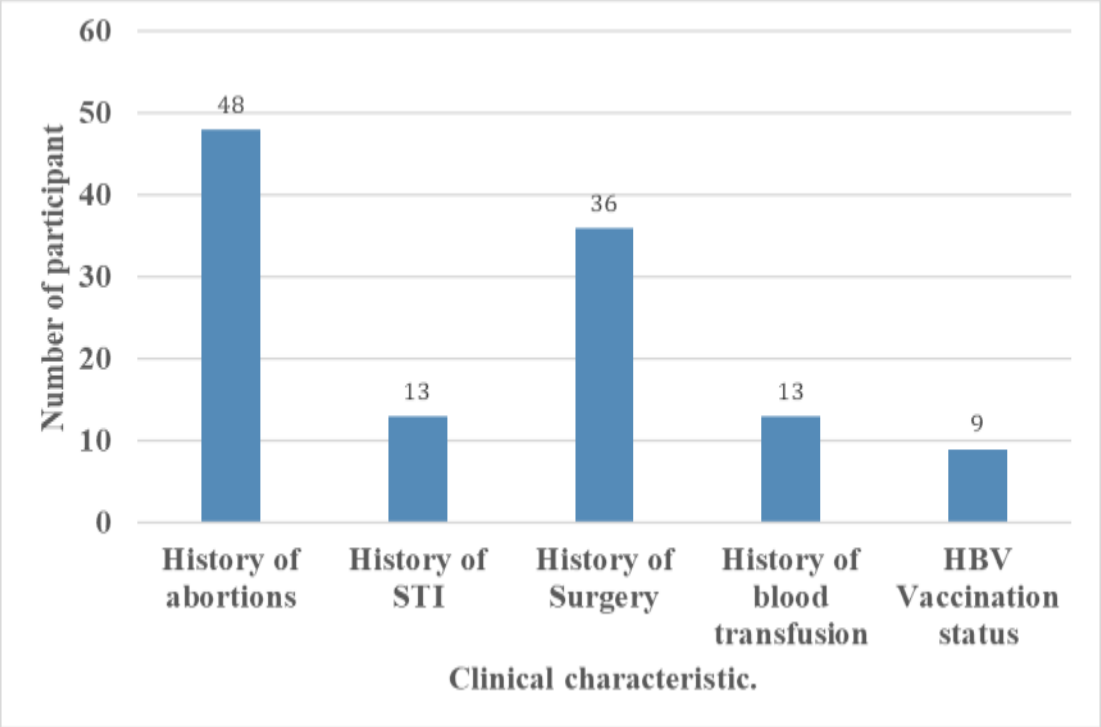


Figure 4.3: Participants clinical characteristics

4.4 Hepatitis B virus prevalence among study subjects

Out of the two hundred eighty seven enrolled subjects , eleven tested positive for HBsAg 11(3.8%) .Two hundred and seventy six (96.2) of them were negative.The prevalence was highest in the age groups 35-39 years (9.5%) and 20-24 years, (4.4%) repectively (Table 4.2).All the detected cases were from the married at 4.5% . The highest prevalence was obtained in multigravid 8(5.5%) while housewives registered a higher

prevalence of 7 (5.5%). In regards to education those who had attained only primary eduaction had a higher prevalence of 5 (6.2%).

Table 4.2: Prevalence of hepatitis B surface antigen among study subjects

Age category	Frequency=287	Frequency=11	Percentage
15-19	17	0	
20-24	91	4	4.4
25-29	102	2	2.1
30-34	50	2	4
35-39	21	2	9.5
40-44	5	1	20
45-49	1		
Education level			
No formol Eduaction	3		
Primary	81	5	6.2
Secondary	128	5	3.9
Post-secondary	75	1	1.3
Occupation			
Employed formal sector	32		
Employed in formal sector	134	4	2.9
Not employed	121	7	5.5
Marital status			
Married	243	11	4.5
Cohabiting	9	0	
Single	35	0	
Type of family			
Monogamous	235	7	3
Polygamous	17	4	24
Single	35	0	
Parity			
0	134	3	2.24
<3	142	6	4.2
>3	11	2	18.2
Pregnancy stage			
1st Trimester	41	3	7.3
2nd Trimester	93	4	4.3

3rdTrimester	153	4	2.6

4.5 Association of Hepatitis B infection and Socio-demographic characteristics

The chi-square test of association was used in bivariate analysis to find association between having Hepatitis B infection and socio-demographic characteristics. Type of family ($\chi^{2} = 19.735$; $P > 0.01$, df= 2) and parity ($\chi^{2} = 7.128$; $P < 0.01$, df=2) and =0.004) were significantly associated with Hepatitis B infection (P = 0.05). Age group ($\chi^{2} = 4.828$; $P < 0.03$, df=3) education level (Fishers Exact =0.359; $P > 0.05$, was not statistically significant with Hepatitis B infection. The Socio-demographic factors associated with exposure to HBsAg were also determined by comparing the proportion of HBsAg detection for study participants by their socio-demographic characteristics. Highest prevalence of 9.5% was found among, those aged 35-39. In regard to marital status, prevalence of 4.5% was recorded. In respect to their family type those in polygamous family set up had a higher prevalence of 24%. In relation to parity , pregnant women with more than three children had a prevalence of 18.2%(

Table 4.3: Association of Hepatitis B infection and Socio-demographic characteristics. .

Table 4.3: Association of Hepatitis B infection and Socio-demographic characteristics.

Social demographic factors	HBsAg (+) (%) N=11	HBsAg (-) N=276	Chi Square X^2	P value
Age group			4.828	0.003
15-19	0	17		
20-24	4	91		
25-29	2	102		
30-34	2	50		
35-39	2	21		
40-44	1	5		
45-49	0	1		
Education level				0.359
No formol education	0	3		
Primary	5	76		
Secondary	5	123		
Post secondary	1	74		
Type of family			19.735	0.01*
Monogamous	7	228		
Polygamous	4	13		
Single	0	35		
Parity			7.128	0.01 *
0	3	131		
<3	6	136		
>3	2	9		

***Level of significance = 0.05**

4.6 Association of Hepatitis B infection and clinical characteristics

History of abortions (χ^2=9.094; p <0.03, df=1) ,age at sex debut (χ^2=8.185; p <0.001, df=1were found to have a significant association HBV infection. History of blood transfusion (χ^2=0.331; p >0.05, df=1) and having been immunized (χ^2=1.802; p >0.05 df=1) against HBV had no significant association with Hepatitis B infection (

Table 4.4) .One hundred and forty-four (50.2%) participants provided the age at sexual debut whose mean was 19 years SD ± 3 Majority of the respondents 31.7% (91) had their sexual debut between 16 and 20 years of age. Out of eleven cases detected with hepatitis B infection, nine provided age at sexual debut. They were equally distributed between age group 11<15 and 16<20 each having 4 cases (1.4%) respectively. Hepatitis B virus immunization rate was 3.1% (9/286) among the enrolled subjects.

Table 4.4: Association of Hepatitis B infection and clinical characteristics

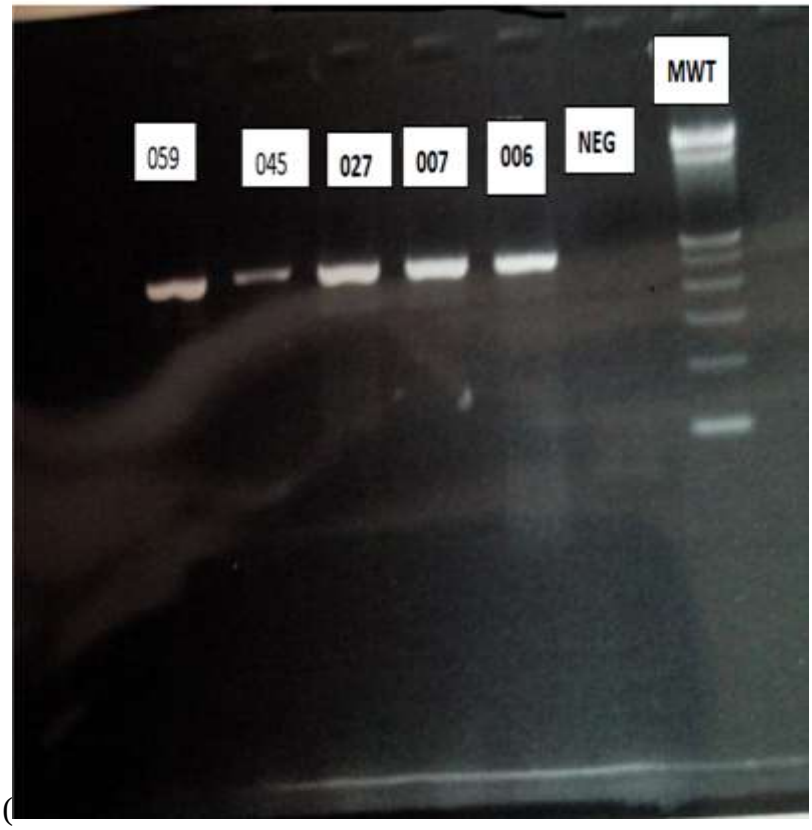
Clinical manifestation	HBsAg (+) N=11	HBsAg (-) N=276	Chi Square X2	P value
History of abortions			9.094	0.003
Yes	6	42		
No	5	234		
History of STI			0.55	0.458
Yes	1	12		
No	10	264		
History of Surgery			0.667	0.414
Yes	0	36		
No	11	240		
History of blood transfusion			0.543	0.461
Yes	0	13		
No	11	263		
Age at sexual debut	N=9	N=144	8.185	0.001
11<15	4	18		
16<20	4	91		
21<25	0	29		
26<30	0	3		
21<35	1	3		

***Level of significance = 0.05**

4.7 HBV DNA detection

HBV DNA was successfully extracted and amplified from 11 HBsAg detected samples in reference to the molecular weight 100 base pairs marker used in gel electrophoresis

PCR product obtained was 530 base pairs



(Plate 4.1).

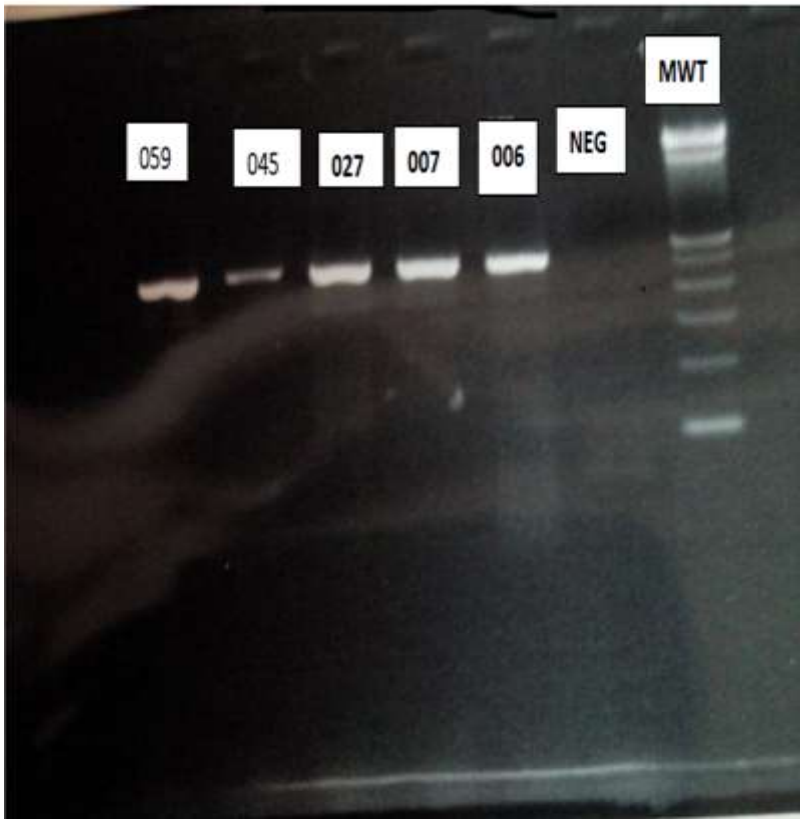


Plate 4.1: Representatitive electrophoretic gel photograph obtained from DNA Extraction from 10 samples

4.8 BV phylogenetic analysis

HBV S coding region sequences were sucessfully amplified from the 10 DNA positive samples.The sequences were subjected to a BLAST similarity search throughout GenBank, and 32 most similar strains were obtained and used for phylogenetic analysis together with the reference sequences of human HBV genotypes The analysis involved 42 nucleotide sequences .Codon positions included were1st+2nd+3rd+Noncoding. There were a total of 661 positions in the final dataset.

4.8.1 Study gene sequences and corresponding gene bank accession numbers

MK484595.1 Hepatitis B virus isolate Hepan007_nLL S protein (S) gene, partial cds

GGAGAACATCACATCAGGATTCCTAGGACCCCTGCTCGGGTTACAGGCGGGGTTTTTCTTGTTGACAAGA

ATCCTCACAATACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGCTCACCCGTGT

GTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTACTGTCCTCCAATTTGTCCTGG

TTATCGCTGGACGTGTCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTA

TTGGTTCTTCTGGATTACCAGGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCACAGCAACCAGTA

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CCACTGTTTGGCTTTCAGTTATATGGATGATATGGTGTTGGGGGCCAAGTCTGTACAACATCTTGACTCC

CTTTATACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACC

MK484596.1 Hepatitis B virus isolate Hepan045_nLL S protein (S) gene, partial cds

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CCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAACAYCTTGAGTCC

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MK484597.1 Hepatitis B virus isolate Hepan059_nLL S protein (S) gene, partial cds

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CCACTGTTTGGCTTTCAGCTATATGGATGATGTGGTACTGGGGGCCAAGTCTGTACAACATCTTGAGTCC

CTTTATACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACC

MK484598.1 Hepatitis B virus isolate Hepan102_nLL S protein (S) gene, partial cds

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CCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAACACCTTGAGTCC

CTTTATACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACC

MK484599.1 Hepatitis B virus isolate Hepan298_nLL S protein (S) gene, partial cds

GGAGAACATCACATCAGGATTCCTAGGACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGA

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CCACTGTTTGGCTTTCAGCTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCC

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MK484600.1 Hepatitis B virus isolate Hepn027_nLL S protein (S) gene,

partial cds

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CCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCC

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MK484601.1 Hepatitis B virus isolate Hepn070_nLL S protein (S) gene,

partial cds

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CCACTGTTTGGCTTTCAGCTATATGGATGATGTGGTACTGGGGGCCAAGTCTGTACAACATCTTGAGTCC

CTTTATACCGCTGTTACCAATTTTCTTCTGTCTTTGGGTATACATTTAAACC

MK484602.1 Hepatitis B virus isolate Hepn115_nLL S protein (S) gene, partial cds

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CCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGGGCCAAATCTGTACAACAYCTTGAGTCC

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MK484603.1 Hepatitis B virus isolate Hepn275_nLL S protein (S) gene, partial cds

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CCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCC

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4.8.2 Genebank blast results for sequence similarity search

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D-E_FN594771_Niger

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D-E_FN594769_Niger

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D-E_FN594770_Niger

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E_AB091255_Cote d'Ivoire

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H_AY090454_Nicaragua

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F2a_X69798_Brazil

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TTATCTGTGGGTATCCATTTAA

4.8.3 Phylogenetic tree

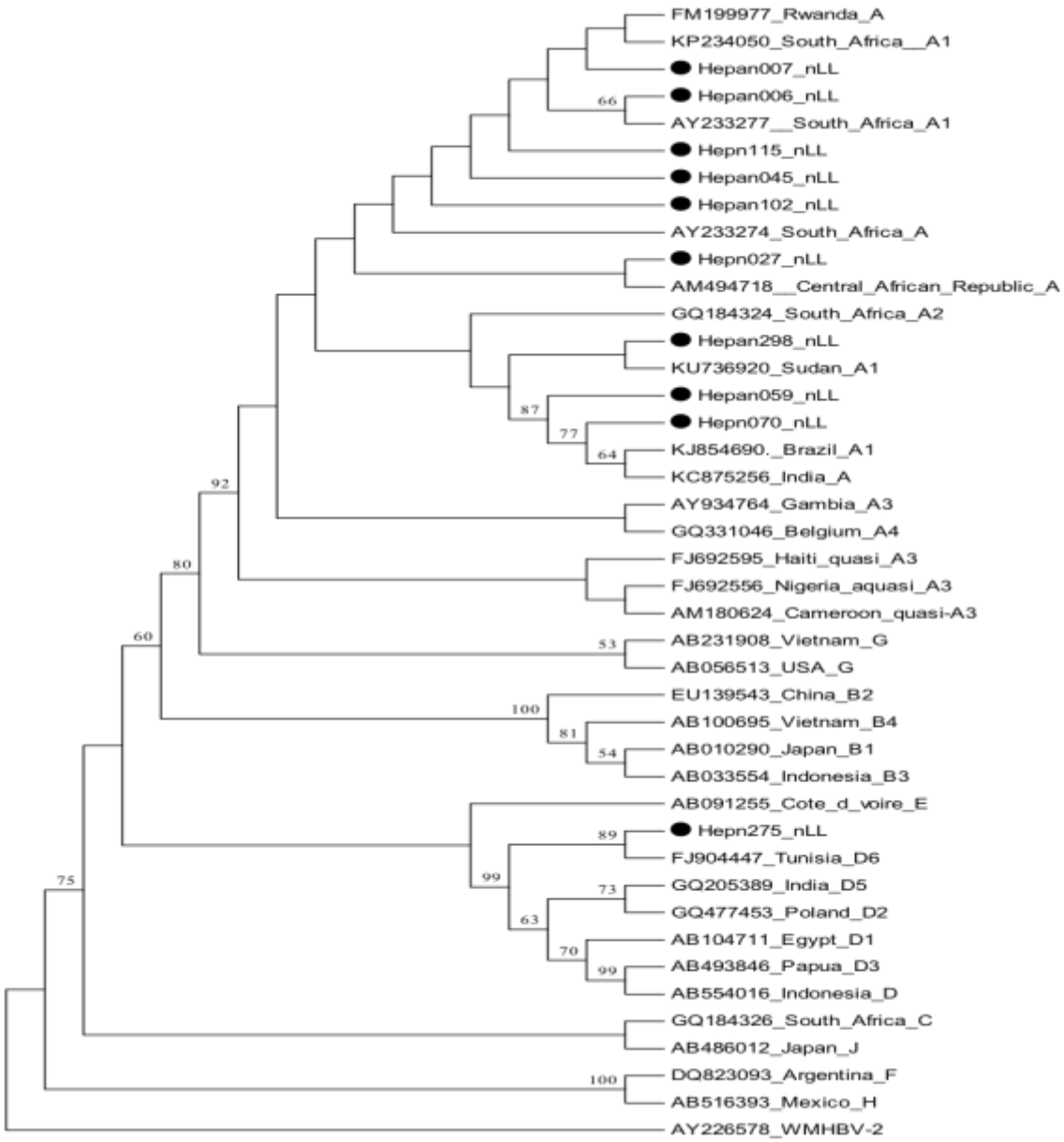

Figure 4.4: Phylogenetic tree 10 HBV study isolates

Study obtained HBV sequences (Hepn007,006,115,045,102,27,298,059,070 &275) were aligned with HBV known reference genotypes(A-H) , similarity blast results sequences as well as Wolly monkey HBV-2 for phylogenic analysis. The evolutionary

history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987) The were computed using the p-distance method (Nei & Kumar, 2000)

CHAPER FIVE

DISCUSSION ,CONCLUSIONS AND RECOMMEDATIONS

5.1 Discussion

5.1.1 Prevalence of Hepatitis B virus among antenatal care attendees

The HBsAg prevalence rate of 3.8% was found among enrolled participants indicating intermediate endemicity (2-7%) according to WHO HBV classification criteria. This study finding are in agreement with previous reports of HBsAg infection rate among pregnant women in Dawuro zone ,South West Ethiopia (3.5%), Makurdi Nigeria (3.3%) (Chernet et al., 2017; Mbaawuaga et al., 2010).In contrast higher infection rates were observed in Nekemle town Ethiopia(5.6%), Sudanese(5.6%) Sierra Leone (6.2%), South West (8.3%),Keffi (6.6%)Nigeria, Mali (8.9%) and Gambia (9.2%)(Elsheikh et al., 2007; Bwogi et al., 2009; Anaedobe et al., 2015a; Bittaye et al., 2019; Buseri et al., 2010; Wurie et al., 2005; Pennap et al., 2011).Prevalence in this study is also lower than the national prevalence among similar study subjects of 9.4% and that of Nairobi (7.7%) (Okoth et al., 2006) Contrary, higher rates of HBV (6 -27.6%) have been reported from studies carried out in among HIV infected (13.3%),outpatient population presenting with jaundice (50.6%),and among persons who inject drug non infected with HIV (14.6) while non drug users HIV negative had prevalence of 16.8% (Kerubo et al., 2015; Ochwoto et al., 2016; Webale et al., 2015) This indicates HBV infection is well defined in high risk groups. Control of HBV infection in high-risk groups is paramount as they may bridge the infection to the general population where herd immunity is limited. Although the HBV prevalence in this study is low ,they is need to monitor the disease among the antenatal population as they are a major reservoir for infection with the capability of transmitting both vertically and horizontally to the community. In this study higher prevalence of 9.5% was observed among olde ager group. 35-39 ,20-24 years which is the second peak of HBV infection (Mutuma et al., 2011) . Higher HBV

rates was observed among the married (4.5%) and multiparous (5.5%).This may be linked to sexual activeness predisposing them to risk of acquiring HBV infection and an indicator of horizontal transmission (Pennap et al., 2011). These studies agrees with findings obtained from Yaounde-Cameroon which reported higher positivity rate among women of child bearing age 25-29 years (Fomulu et al., 2013). Although HBsAg positivity was found in all age groups in this study , the finding supports the role of sexual transmission in HBV infection since none of the HBsAg detected cases had been exposed to blood transfusion and surgical procedures. In addition, the 8/11 cases detected had their sexual debut in adolescence stage (11-20) years suggesting infection may have not been acquired during infancy.

5.1.2 Risk factors associated with Hepatitis B infection among HBsAg positive antenatal care attendees

Risk factors associated with HBV infection assessed in this study were classified into, social demographic and clinical factors. In regard to bivariate analysis of social demographic factors with HbsAg positivity; study finding indicate equally distribution of positivity across the level of education attainment. This suggest that education does not influence HBV prevalence in our setting as compare to studies carried out in Africa region where HBsAg positivity was reported to decrease with increase in education level (Pennap et al., 2011). This variation may also be due to the limited /scarcity of focused HBV infection advocacy material and awareness within the community. In relation to marital status, there was no statistical difference between married and single pregnant women in relation to HBV positivity although all positive detected cases were married suggesting that pregnancy is not a risk factor to HBV infection. Similar results were reported from Abia state, Niger Delta Nigeria (Buseri et al., 2010). Income is noted to be inversely associated with HBV infection. A similar trend was found in this study where higher positivity (2.4 %) was detected among unemployed pregnant women. This is similar to the study in South East Nigeria which indicated that unemployed pregnant women formed the bulk of positive cases (Ikeako et al., 2014).

This may be explained by the low economic status initiating women to multiple sexual partnerships and unprotected sex thus making them vulnerable to STIs . In respect to their family type those monogamous family set up had a higher frequency as compared to polygamous family settings. This is not similar with findings reported from studies carried out in west Nigeria where pregnant women in polygamous setting were four times at risk of acquiring HBV infection due to husband having multiple sexual partners. Likewise those with less than three children had higher frequency of positive cases compared with multigravida women (Ajayi et al., 2013) This finding may suggest occurrence of high risky behaviour among those in monogamous family setting as well as those with less than three children.

This is because none of the positive cases had a history of blood transfusion nor underwent surgical procedures. The study also accessed clinical factors that have been document by various studies predisposing persons in the acquisition of HBV.The were history of abortion, blood transfusions, surgical procedures, HBV immunization and age sexual debut. In regard to abortion 48 of the participants self-reported to have had an abortion. Out of which 12.5% were positive for HBsAg. On bivariate analysis. this was significant at ($\chi 2=9.094$; $p <0.03$, $df=1$), This finding conquers with study carried out in Ethiopia where history of abortion increased the risk of HBV acquisition by 4 times .This findings may be linked to poor practice of infection prevention control during abortion. Suggesting use of contaminated instruments during the procedure, and related activities increasing the probability of acquiring HBV infection. With regard to be having been hospitalized and receiving blood transfusion, 4.5 %(13) reported to have accessed the services. None of them were among the detected cases of HBsAg.The was no significant association with HBV acquisition.

This may be explained by screening of transmissible blood borne pathogens being implemented by blood banks in Kenya. With respect to HBV vaccination 3.1% (9) participants reported to be immunized for the virus. This may be linked to HBV

immunization implementation strategy adapted by the ministry of health, Kenya which prioritizes the less than 5 years and health workers.

These services are offered free for named population while the untargeted population accesses it out of pocket. This study finding suggests infections are acquired horizontally through sexual contact .Hence immunization of infants alone cannot be utilized as the final strategy for controlling the spread of HBV infections in our setting. Further more, gaps still exist in attaining full immunization coverage in our setting among the target population (<5 years).Thus the need of expanding immunization services to general population.

5.1.3 Circulating HBV genotypes among HBsAg positive antenatal care attendees

HBV genotypes and subgenotypes differ with respect to geographical distribution, transmission routes, disease progression, response to antiviral therapy, vaccination and clinical outcome. In this study, eleven serum samples obtained from study participants were DNA positive. Out of which 10 were sucessfully sequenced. Nine (90%) of them were.Genotype A, sub-type A1 while 1(10%) was Genotype D, sub-type D6. This shows that. HBV genotype A prevails among HBV infected pregnant women.

The findings are similar to those obtained from Kenyan studies carried out among blood donor population (Kwange et al., 2013; Mwangi et al., 2008) those with liver disease (Ochwoto et al., 2016) and high risk groups (Webale et al., 2015).

In comparison with worldwide. HBV genotypes distribution, the finding aligns with Kramvis documetation that genotype A1 dorminates in Africa(2007).In East Africa region, genotype A and D are major contributers to HBV infections with the former prevailing (Velkov et al., 2018). Genotypes HBV/A and HBV/D reported in this study as well as previous studies carried out in-country has been associated with horizontal modes of transmission as well as associated with increased risk to progression to chronicity and liver failure hence close monitoring genotype diversity in this population

is paramount. (Sunbul, 2014).The mean gestation period of study participants was 23.9 weeks thus the majority of them were in their 2nd trimester where antiviral could not be administered.

Although this study didn't quantify HBV viremia, a complication arising due to frequent HBV flare may result in severe hepatitis and acute liver failure during the prepartum and postpartum phase.(Rafat, 2016). Majority of them have been infected with genotype A strain, treatment with nucleoside analogs (third trimester) which have a better response rate to the genotype may reduce viremia and risk of maternal transmission (Archampong et al., 2017).

5.2 Study limitations

1. Mbagathi County Referral hospital is a public health facility that provides services to the middle and lower low-income earners. Information gathered from participants enrolled willing in the study did not capture data from pregnant women from high-income category representative of all pregnant women in Nairobi county.
2. During the study period unavailability of HBV diagnostic assays was a major drawback in accessing HBV clinical management services. Only those consenting to participate in the study were offered HBV screening services by the study. Hence a larger population of pregnant women were readily available but not reached. Based on this the finding cannot be generalized to all pregnant women. Risk factors to HBV acquisition were self reported hence are subjected to recall bias.

5.3 Conclusion

1. Hepatitis B virus is low prevalent (3.8%) among pregnant women

2. Social demographic and clinical factors associated with HBV acquisition among pregnant women were; type of family (monogamous), parity (less than 3 children), and history of abortion and age sexual debut respectively.
3. Hepatitis B virus is transmitted horizontally that is sexually and by use contaminated equipment during abortions.
4. Hepatitis B genotype A was found to prevailed among antenatal care attendees

5.4 Recommendation

Based on the study finding the following recommendation are made to the Ministry of health to

1. Establish routine screening of HBV as a mandatory test for antenatal care attendees
2. Expand list of priority population targeted for free HBV immunization to include women of child bearing age.

REFERENCES

APPENDICES

Appendix I: Questionnaire

SECTION 1: DEMOGRAPHIC INFORMATION

Do you reside in Nairobi county or elsewhere? Yes No

Elsewhere: indicate the name of town

1. Which part of Nairobi County do you reside?

2. In what month and year were you born? Month

Don't know month

Year

Don't know year

3. How old were you at your last birth day? Age in completed years

Compare and correct 4 and 5 if inconsistent.

4. Have you ever attended school? Yes ☐ No ☐

5. What is the highest level of school you attended?

   Primary, secondary, or higher? Primary ☐

   Secondary ☐

   Higher ☐

6. What do you do to earn a living? Employed formal sector

   Employed in- formal sector ☐

SECTION 2: PARITY

1. Now I would like to ask about all the births you have had during your life. Have you ever given birth? ☐

   Yes

No ☐

2. How many sons have you given birth to and are alive? ☐

   And how many daughters have you given birth to and are alive? ☐

3. Have you ever given birth to a boy or girl who was born alive but later died? Yes ☐ No

   How many boys have died? ☐

   And how many girls have died? ☐

IF NONE, RECORD '00'.

SUM ANSWERS TO 2 AND 3, AND ENTER TOTAL

IF NONE, RECORD '00'.

Just to make sure that I have this right: you have had in TOTAL _____ births during your

life. Is that correct?

SECTION 3: MARRIAGE

1. Are you currently married or living together with a man as if married?

Yes, currently married ☐

Yes, living with a man ☐

No, not in union ☐

If not in union ask question 2

2. Have you ever been married or lived together with a man as if married?

Yes, formerly married ☐

Yes, lived with a man ☐

No ☐

3. What is your marital status now: are you widowed, divorced, or separated?

Widowed ☐

Separated

Divorced ☐

4. Is your (husband/partner) living with you now or is he staying elsewhere?

Living with her ☐

Staying elsewhere ☐

5. Does your (husband/partner) have other wives or does he live with other women as if married?

Yes ☐

No ☐

Don't know ☐

6. Including yourself, in total, how many wives or

Total number of wives ☐

live- in partners does he have? ☐

Don't know ☐

7. Are you the first, second, wife? Rank

SECTION 4: SEXUAL ACTIVITY

1. In what month and year did you start living with

   your (husband/partner)?

Month ☐

Don't know the month ☐

Year ☐

Don't know the year ☐

2. How old were you when you first started living with him?

check for the presence of others. before continuing, make every effort to ensure privacy

3. How old were you when you had sexual intercourse for the very first time? ☐

4. When was the <u>last</u> time you had sexual intercourse? ☐

If less than 12 months, the answer must be recorded in days, weeks or months.

5. What was your relationship to this person with whom you had sexual intercourse?

Husband ☐

Live-in partner ☐

Boyfriend ☐

Casual acquaintance ☐

Client/prostitute

Other:_____

IF BOYFRIEND: Were you living together as if married? Yes No

.

SECTION 5: HEALTH CARE

1. Have you ever been admitted in Hospital? Yes

   No

2. In what month and year were you admitted?

   Month Don't know the month

   Year Don't know the year

3. While in the hospital did you undergo any surgery? Yes No

4. Have you ever been transfused blood? Yes No

5. How many times have you been transfused blood?

6. When was the first time you were ever transfused blood?

Month Don't know the mont

Year Don't know the year

7. When was the last time you were transfused blood?

8. Have you ever been treated for an STI? Yes No

Appendix II: Participant information sheet

Prevalence of Hepatitis B virus genotypes among antenatal clinic attendees at Mbagathi County Referral hospital

Dear participant,

Before you decide to take part in this study it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. A member of the team can be contacted if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Purpose of the study

Hepatitis B is a virus infects the liver causing swelling of and liver disease. It transmitted by contact to infected person's blood, semen, or other body fluid. Once the liver is infected it cannot perform its functions which include: removing harmful chemicals from your blood, fighting infection, helping to digest food and nutrients, vitamin and energy.

Hepatitis B virus (HBV) genotyping refers to the process of determining the difference in the genetic makeup of the HBV by examining its DNA(Deoxyribonucleic acid) Sequence using biological assays. The purpose of this study is to determine the prevalence of Hepatitis B virus genotypes among women attending the antenatal clinic. The information gathered during this study will be utilized in the management of Hepatitis B virus infection. The study will be carried for a period of 3 months.

Do I have to take part?

Please note that your participation in this research is entirely voluntary and you can withdraw from the study any time you feel like without any penalty nor affecting your antenatal subsequent visits.

What will happen to me if I take part?

The study will be conducted at the Mbagathi district hospital antenatal clinic. A total of 400 pregnant women are expected to participate in the study. Once you have agreed to enroll in the study, you will be asked some questions concerning the study about your personal life.

You can refuse to answer if you feel uncomfortable. 5ml of blood will be drawn aseptically by phlebotomist using sterile blood collection set for each participant.

Blood sample collected will be shipped to Kenya Medical Research Institute, Centre of Virus Research laboratories for Hepatitis B virus screening and coded processed samples sent to the International Livestock Research Institute for genotyping respectively. Remnants of the collected blood sample will be store at – 80^{o}c and utilized only in further research on HBV. Bio-data and blood sample collection will be carried only once per each participant during the study period.

Are there possible disadvantages and/or risks in taking part?

During the drawing of blood discomfort and slight pain will be felt. Except for the slight pain, there is no other risk in participating in the study.

What are the possible benefits of taking part?

Enrolling to participate in the study will be at no cost. The monetary benefit will not be offered to those volunteering to participate in the study but free HBV screening will be offered.

Will my taking part in this project be kept confidential?

All bio-data and blood samples collected during the study will be identified only by a unique code. No personal identifiers will be used in the study.

What will happen to the results of the research project?

Results will be presented at conferences and written up in journals. Results are normally presented in terms of groups of individuals. If any individual data are presented, the data will be totally anonymous, without any means of identifying the individuals involved.

Contact for further information

If you ever have questions about this study you should contact Ms. Jacqueline Malungu, telephone number +254716355496 email address: jaymalasu@yahoo.com or to the Secretary, Kenya Medical Research Institute Ethical committee P.O BOX 548400-00200 Nairobi, telephone number +254733400003, email address: ercadmin@kemri.org.

Appendix III: Habari Kuhusu Mhusika

Kichwa: “Kuwepo kwa virusi vinavyosababisha Ugonjwa wa maini aina B katika kundi la kina mama wanaohudhuria kituo cha malezi ya watoto katika hospitali kuu ya wilaya ya Mbagathi”

Mhusika mpendwa,

Kabla ya kuhusika na utafiti huu ni muhimu kuelewa ni kwa nini utafiti hii unafanywa na kama utaiti huu utahisisha nini nanini. Tafadhali chukua mmuda wako mfupi ujisomee taarifa hii kwa umakini na unaweza kujadiliana na wenzako kuhusu utafiti huu.

Unaweza kuuliza mutafiti yeyote aliye karibu nawe kuhusu utafiti huu kuhusu swala leloteutakalotaa kufahamishwa zaidi. Chukua muda wako uamue kama ungelipenda kuhusika na utafiti huu.

Madhumuni ya Utafiti huu

Virusi vya homa ya maini B huambukiza maini na kusababisha kufura kwa maini na ugonjwa wa maini kwa jumla. Virus vya homa ya maini husambazwa ntu anapogusa damu, ukojo na aina zingine za maji maji ya mwilini yaliyoambukizwa. Mara tu maini yanapoambukizwa virusi vya homa la maini B, maini hushindwa kufanya kazi yake kama vile kutoa uchafu kwa damu, kugigana na maambukizi mbali mbali, kusaga chakula mwilini kama madini na viungo vya chakula viletavyo nguvu mwilini.Kutofautisha aina mbali mbali za virusi vinavyosababisha ugonjwa wa maini aina B ni hatua ya kudhibiti tofauti za kijinsia za virusi vya kusababisha ugonjwa wa maini aina B kwa kuchunguza chembe za kiasilia za virusi yaani “Deoxyribonucleic acid” kwa kutumia mbinu za kibayolojia. Madhumuni ya utafiti huu ni kuchunguza kuwepo kwa virusi vinavyosababisha Ugonjwa wa maini aina B katika kundi la kina mama wanaohudhuria kituo cha malezi ya watoto katika hospitali kuu ya wilaya ya Mbagathi.

Matokeo ya utafiti huu itakuwa ni taarifa itakayosaidia kukabiliana na ugonjwa wa maini aina B katika hospitali za kimataifa. Utafiti huu utafanyika kwa muda wa miezi mitatu.

Je, ni lazima nihusike katika utafiti huu?

Tafadhali tambua kwamba kuhusika katika utafiti huu ni kwa hiyari na chaguo lako na kwamba unaweza kujitoa wakati wowote unapojihisi kufanya hivo bila garama yeyote ama bila kuadhibiwa kwa njia yeyote na kujitoa kwako kwa utafiti huu hakutatatiza kupewa huduma za kiafya.

Je, ni nini kitafanyika nikikubali kuhusika na utafiti huu?

Utafiti huu utafanyika katika hospitali ya wilaya ya Mbagathi katika kitengo cha kina mama waliojifungua. Takriban kina mama waja wazito mia nne watatarajiwa kukubali kuhusika na utafiti huu.Punde tu unapokubali kuhusika na utafiti huu, utaulizwa maswali machache ya ubinafsi kuhusu maisha yako ambayo itatumika kawa utafiti huu.Iwapo hutajisikia wasiwasi kuyajibu maswahili haya uko huru.kutoyajibu. Baadaye utatolewa damu kiasi cha mililita tano na mtaalamu na kwa njia ya usalama. Kitengo cha damu kitakachotolewa kutoka kwa mwili wa mhusika kitasafirishwa hadi taasisi ya utafiti ya "Kenya Medical Research Institute" katika mahabara ya kituo cha utafitiwa wa virusi kwa matayarisho. Damu iliyo tayarishwa na kupewa nambari ya kisiri itapelekwa katika taasisi ya utafiti "International Livestock Research Institute". Damu zote zitahakikishwa kwamba kweli zimeambukiwa na virusi vya maradhi ya maini aini ya B. Mabaki ya damu itakayo chukuliwa yatahifadhiwa katika jumba la barafu nyuzi – 80^{o}C na zitatumika kwa utafiti wa hbv wa baadaye. Taarifa ya kijinsia ya wahusika na taarifa kuhusu damu zao zitakusanywa katika utafiti huu.

Je kuna hatari kujihusisha na utafiti huu?

Wakati mhusika anapotolewa damu, hakuna madhara yanayotarajiwa ila maumivu kidogo yatakayosababishwa na kudungwa kwa sindano ya kutoa damu.

Kuna faida gani ukihusika na utafiti huu?

Hutatarajiwa kutoa pesa zozote kwa utafiti huu. Wahusika hawatapatiwa pesa. Wahusika watafanyiwa ukaguzi wa ugonjwa wa maini ya B bila malipo.

Je taarifa ya kuhusika kwangu katika utafiti huu itawekwa faragha?

Taarifa yote ya kijinsia ya wahusika pamoja na damu zitakazotolewa zitapatiwa nambari ya kipekee ya utafiti. Hakuna taarifa ya kibinafsi itakayotolewa kwa hadhara.

Je, matokeo ya utafiti huu yatafanyiwa nini?

Matokeo ya utafiti huu yatatangazwa kwenye kwangamano za kisayansi na utafiti kuhusu ugonjwa wa maini aina B, na pia taarifa za utafiti huu zitachapishwa katika jarida mbalimbali za kimataifa. Matokeo yatahusiha makundi na pia wahusika binafsi. Kama taarifa itakayopeperushwa itakuwa ya kibinafsi, taarifa hiyo itawekwa faragha bila jina wala aina ya kutambulika kwa mhusika.

Anwani kwa mawasiliano ya ziada

Kama uko na maswali yeyote kuhsu utafiti huu tafadhali wasiliana na Jacqueline Malungu, nambari ya simu; +254716355496 barua pepe : jaymalasu@yahoo.com au kwa karani, Kamati ya maadili ya utafiti katika kituo cha utafiti wa kimatibabu cha Kenya: sanduku la barua 548400 - 00200 Nairobi, nambari ya simu +254733400003, barua pepe: ercadmin@kemri.or

Appendix IV: Consent Form

Study code:.....

Prevalence of Hepatitis B virus genotypes among antenatal clinic attendees at Mbagathi district hospital

- I confirm that I have read and understood the Participant Information Sheet:

  YES NO

- I have had the opportunity to ask questions and had them answered: **YES NO**
- I understand that all personal information will remain confidential and that all efforts will be made to ensure I cannot be identified: **YES NO**
- I agree that data gathered and a blood sample collected for this study will be stored anonymously and securely, and may be used for future research: **YES NO**
- I agree that my sample be shipped from Mbagathi district hospital to KEMRI:

  YES NO

- I agree that my sample is shipped stored at -80^0c for further studies in HBV.

  YES NO

- I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason: **YES NO**
- I agree to take part in this study*:* **YES NO**

Participant Name: .....

Signature.....

Date:.....

Name of participant below 18years :.....

Guardian‘s Name:.....

Signature::.....

Date.:.....

Appendix V: Consent Form Translated to Kiswahili

Nambari/Usajili ya/wa utafiti:.....

Kichwa: "Kuwepo kwa virusi vinavyosababisha Ugonjwa wa maini aina B katika kundi la kina mama wanaohudhuria kituo cha malezi ya watoto katika hospitali kuu ya wilaya ya Mbagathi"

Ninakubali kwama nimesoma na kuelewa maandishi yaliyomo katika jani lenye habari kwa mhusika: **NDIO LA**

- Niko na fursa ya kuuliza na kujibiwa maswali yote niliyo nayo: **NDIO LA**
- Ninaamini kwamba matokeo ya utafiti huu utakaonihusu mimi yatakuwaya faragha na kwamba juhudi zote zitawekwa na kuzingatiwa ili kwamba majina yangu halisi yasijulikane: **NDIO LA**
- Ninakubali kwamba matokeo ya utafiti wa damu yangu yatawekwa kwa hali ya usalama na faragha, na kwamba damu yangu inaweza ikatumiwa kwa utafiti mwingine kama huu siku zijazo: **NDIO LA**
- Ninakubali kwamba sehemu ya damu yangu itatolewa katika kituo cha afya ya Mbagathi na kusafirishwa kwa kituo cha utafiti cha KEMRI: **NDIO LA**
- Ninakubali kwamba kitengo cha damu yangu kitakachotumiwa katika utafiti huu kitahifadhiwa katika kiwango cha baridi cha -80^0C kwa utafiti zaidi wa baadae kuhusu HBV. **NDIO LA**
- Ninaelewa kwamba kuhusika kwangu katika utafiti huu ni kwa hiyari yangu na kwamba niko huru kujitoa wakati wowte hata bila ya kujieleza: **NDIO LA**
- Ninakubali kuhusika katika utafiti huu*:* **NDIO LA**

Jina la muhusika:.....

Sahihi Tarehe ya makubaliano:.....

Jina la muhusika chini ya miaka 18: .....

Jina la musimamizi:.....

Sahihi ya musimamizi.:.....

Sahihi Tarehe ya makubaliano:.....

Appendix VI: SSC

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/SSC/102844 11th June, 2014

Jacqueline Malungu

Thro'
Director, CVR
NAIROBI

forwarded June 12th 2014

FOR DIRECTOR
CENTRE FOR VIRUS RESEARCH
P. O. Box 54628
NAIROBI

REF:SSC No. 2724 (Revised) – Prevalence of Hepatitis B Virus Genotypes Among Antenatal Clinic Attendees at Mbagathi District Hospital

Thank you for your letter dated 9th June, 2014 responding to the comments raised by the KEMRI SSC.

I am pleased to inform you that your protocol now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval.

Sammy Njenga, PhD
SECRETARY, SSC

CENTRE FOR VIRUS RESEARCH
11 JUN 2014
P.O. Box 54628 - NAIROBI

Appendix VII: ERC Approval

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-206901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **September 03, 2014**

TO: **JACQUELINE A. MALUNGU,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. GEORGE NAKITARE,**
ACTING DIRECTOR, CVR,
NAIROBI

Dear Madam,

RE: **SSC PROTOCOL NO. 2724 (*RESUBMISSION*): PREVALENCE OF HEPATITIS B VIRUS GENOTYPES AMONG ANTENATAL CLINIC ATTENDEES AT MBAGATHI DISTRICT HOSPITAL**

Reference is made to your letter dated 28th August, 2014 and received at the KEMRI ERC office on 1st September, 2014.

This is to inform you that the Ethics Review Committee (ERC) reviewed the documents submitted and is satisfied that the issues raised at the 229th meeting of the KEMRI ERC on 22nd July, 2014 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this **3rd September 2014** for a period of one year. Please note that authorization to conduct this study will automatically expire on **September 2, 2015.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to the ERC Secretariat by **July 22, 2015**. The regulations require continuing review even though the research activity may not have begun until sometime after the ERC approval.

You are required to submit any proposed changes to this study to the SSC and ERC for review and the changes should not be initiated until written approval from the ERC is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of the ERC and you should advise the ERC when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

PROF. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE

CENTRE FOR VIRUS RESEARCH
10 SEP 2014

In Search of Better Health