# PREVALENCE, GENETIC DIVERSITY AND RISK FACTORS ASSOCIATED WITH HEPATITIS B INFECTION AMONG WOMEN ATTENDING ANTENATAL CLINIC AT ST. ORSOLA HOSPITAL, THARAKA NITHI COUNTY, KENYA

**NJUGUNA JOSEPH KAMAU (BSC MEDICAL LABORATORY SCIENCE)**

**I56/33560/2015**

**A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (MEDICAL MICROBIOLOGY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY**

**JULY, 2025**

## DECLARATION

This is my original work and has not been submitted for a degree in any other University.

Signature _____ Date: 30/07/2025

Joseph Kamau Njuguna
Department of Biochemistry, Microbiology and Biotechnology

## APPROVAL BY SUPERVISORS

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

Prof. Anthony Kebira
Department of Biochemistry, Microbiology and Biotechnology
Kenyatta University

Signature..... Date: 01/09/2025

Dr. James Nonoh
Department of Biomedical Science and Technology
Maseno University

Signature_____ Date: 30/07/2025

## DEDICATION

I dedicate this project to my wife, Dorcas Kamau, my childrens, Abigael, Winny and Melvin, my parents, Mr. and Mrs. Simon Njuguna, as well as the rest of my family for their love, encouragement and support throughout this entire period.

**ACKNOWLEDGEMENTS**

My supervisors, Prof. Anthony Kebira and Dr. James Nonoh, deserve a special word of thanks due to their consistent & ongoing support of my M.Sc. research. The success of my thesis was greatly influenced by their advice, expertise, and support. The MCH clinic in charge at St. Orsola hospital and the laboratory officers are also deserving of my gratitude. The entire St. Orsola hospital health administration staff for approving and allowing me to conduct a study project there. Regards are also due to the participants in the study who agreed to take part and helped make it a success. Lastly, I would want to express my gratitude to my family, especially my dear wife Dorcas, my children Abigael, Winny and Melvin, for their tremendous support.

.

TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF APPENDICES

## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| **ANC** | Antenatal Clinic |
| **CccDNA** | Covalently Closed Circular DNA |
| **rcDNA** | Relaxed Circular DNA |
| **ER** | Endoplasmic Reticulum |
| **EN** | Enhancer |
| **FH** | Fulminant Hepatic |
| **HBV** | Hepatitis B Virus |
| **HBX** | Hepatitis B Virus X Gene |
| **HBsAg** | Hepatitis B Surface Antigen |
| **HBcAg** | Hepatitis B Core Antigen |
| **HBeAg** | Hepatitis B e Antigen |
| **HBIG** | Hepatitis B Immunoglobulin |
| **HCC** | Hepatocellular Carcinoma |
| **IFN** | Interferon |
| **IG** | Immunoglobulin |
| **KEPI** | Kenya Expanded Proramme on Immunization |
| **LEF** | Liver Enriched Factor |
| **LHP** | Large Envelop Protein |
| **MHR** | Major Hydrophilic Region |
| **OBI** | Occult Hepatitis |
| **ORF** | Open Reading Frame |
| **PCR** | Polymerase Chain Reaction |
| **PC** | Precore |
| **PRE-RNA** | Pre-Genomic RNA |
| **RB** | Retinoblastoma Gene |
| **RCDNA** | Resting Circular DNA |
| **RNA** | Ribonucleic Acid |
| **TMB** | Tetramethylbenzidine |
| **WHO** | World Health Organisation |

## ABSTRACT

There is a dearth of information on the impacts of hepatitis B virus (HBV) because it is rarely screened for in prenatal profiles in many facilities especially in Tharaka Nithi County. The transmission of Hepatitis B virus (HBV) occurs in the uterus through trans-placental, childbirth, and during newborn care. The likelihood of transmission is influenced by the presence of HBV markers in the mother where the risk is decreased if she is positive for HBsAg test result but rose if she has a positive HBsAg and HBeAg test result. Therefore, this study was carried out to determine the prevalence, genetic diversity and the associated risk factors for hepatitis B virus infection among expectant mothers seeking ANC at St. Orsola Hospital. A total of 385 pregnant mothers who were enrolled from September to December 2021 participated in a cross-sectional study. Blood samples were collected and examined for the presence of HBsAg and to determine the HBV risk of perinatal transmission, the positive sample for HBsAg were tested for HBsAg, HBsAb, HBeAg, HBeAb, HBcAb using five panel kit. The viral DNA was extracted from HBsAg positive samples, partial HBV-*pol* gene was amplified in nested PCR, directly sequenced using the Big Dye chain terminator method using ABI 3730xl DNA Analyzer. The obtained sequences were then scrutinized and assembled using BioEdit software v7.1.1 and subjected to multiple sequence alignment by MUltiple Sequence Comparison by Log- Expectation (MUSCLE). Phylogenetic relationships were estimated using Maximum likelihood-based inference of phylogenetic trees with Smart Model Selection (PhyML+SMS). From the analysis, the prevalence of HBV among pregnant womem was 25 (6.5%). Among these (25 pregnant women); 19 (76%) were inactive carrier, 4 (16%) chronically infected, 1 (4%) at recovery HBV and 1 (4%) at occult HBV. From the SPSS analysis, age ($\chi^2$= 13.55, $p$ = 0.019), education ($\chi^2$= 44.53, $p$ = 0.000), liver problems ($\chi^2$= 5.724, $p$ = 0.017) and HIV ($\chi^2$= 6.267, $p$ = 0.012) were associated with risk to HBV infection. From the phylogenetic analysis, the HBV genotype that was in circulation was genotype A, 11(100%). The detected prevalence of HBV in Tharaka Nithi region indicated possible increasing trends in HBV infection with age, education, liver problem and HIV being the associated risk factors, and HBV genotype A being the most predominate genotype in circulation. In order to control the spread of the virus there is need to identify cases both during antenatal as well as postnatal care, raise the risk awareness, track HBV genotypes and offer treatment for positive cases and HBV immunization for negative as well as all women of childbearing age.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background information

According to the World Health Organization (WHO), hepatitis refers to inflammation of the liver due to infectious agents like hepatitis viruses. Since HBV is highly infectious, the virus is easily transmitted across populations especially those on high-risk populations like intravenous drug user. Blood contact with any other polluted material could lead to infection resulting into serious liver pathological effect such as chronic hepatitis, cirrhosis, or liver cancer (Sunbul, 2014). According to estimates, 350 million out of the 2 billion HBV-infected persons globally go on to acquire chronic infection of which 65 million resides in Africa (Schweitzer *et al*., 2015). The WHO's 2012 study on the prevalence of HBV infection in the general population in European countries found that the prevalence ranged from low to intermediate 0.1% to 5.6% (Ahmad *et al*., 2018, Muller *et al*., 2015).

The research on the prevalence of HBV infection in several African nations revealed a growing range from low to moderate to high prevalence 2.4%–17.3% between 1999 and 2013 (Muriuki *et al*., 2013). In the sub-Saharan region, HBsAg carrier rates are 9–20%, while in Kenya, they are 5–30% (Mutuma *et al.,* 2011). Pregnant women's prevalence of HBV infection follows the similar trends as the general population at every level of infection (Kramvis and Kew, 2007). The prevalence of HBsAg was found to be 6.2% among expectant mothers from middle-class and upper-class backgrounds in Sierra Leone, which is consistent with the nation's high endemicity classification (Wurie *et al.,*

2005). HBV prevalence in Kenya was 8.8% among adult rural nomads (Mutuma *et al*., 2011), while pregnant women had a reported 9.3% nationwide and 7.7% in the Nairobi region (Okoth *et al.*, 2006). A Sudanese study (Elsheikh *et al.,* 2007) found the same pattern; with the prevalence being 5.6% compared to 6.9% in the general population.

Out of 360 million chronically infected individuals, about one million of them have viral-related illnesses such cirrhosis and hepatocellular carcinoma (HCC), which can be fatal, each year (WHO, 2002). With a prevalence of more than 8%, Africa is regarded as having the second-highest number of chronic carriers after Asia (WHO, 2018). Infection with HBV is thought to have affected between 56% and 95% of Africans, with seroprevalence (HBsAg) ranging from 6% to 20%. About 70% to 95% of adults in Asia and Africa exhibit signs of prior exposure to HBV infection, making these regions home to the majority of HBV chronic carriers (Hwang *et al*., 2011). The research shows that the Sub-Saharan Africa has a 9–20% HBsAg carrier rate, while Kenya has a 5–30% rate (Mutuma *et al.*, 2011). The HBV infection prevalence varies widely in Kenya by study population and region with a systemic review of the year 1990-2008 among HIV showing a prevalence between 6-12% (Barth *et al*., 2010) and 4% among the healthcare professionals respectively (Kisangau *et al*., 2017).

HBV is mainly transmitted vertically to newborns and horizontally to school-age children and adults. Due to lack of resources, financial issues, and illiteracy, many countries still have poor levels of awareness of HBV, screening information, treatment options, and prevention. This causes delays in the diagnosis of HBV and the related liver illness,

which accelerates the spread of infection. The risk factors for HBV infection differ according to the diversity of cultures in our communities, and they must be determined in each situation. The expectant mothers are susceptible to infection, and if they become infected, they can spread it during childbirth to partners, children, newborns, and medical personnel. In order to reduce the spread, it is crucial to prevent HBV vertical transmission, which can be done by screening all expectant moms and administering the vaccine (Onakewhor *et al*., 2001).

The epidemiology of HBV in expecting women in many African regions, however, is not well understood. The risk factors for acquiring HBV are largely influenced by cultural practices and beliefs, which differ depending on the context. International primary research has identified the following major risk factors for acquiring HBV: age, history of blood transfusions, low educational attainment, surgery, STDs, abortions, higher mean parity, early sexual activity, polygamy, maleness, rural birthplace, and having more than one partner (Breakwell *et al*., 2017). In order to design and implement the necessary interventions to reduce the spread of HBV and its effects on both expecting mothers and infants, detailed data about the situation in our country is necessary. Therefore, the purpose of this study was to examine a number of parameters related to this viral infection, as well as genetic diversity and prevalence among pregnant mother visiting ANC at St. Orsola hospital in Tharaka Nithi County.

### 1.2 Statement of the problem

The prevalence of HBV infection in pregnant women follows the similar trends as the general population at every level of infection (Kramvis and Kew, 2007). The prevalence

of HBV among pregnant women in Kenya had been reported at 9.3% nationwide and 7.7% in the Nairobi region (Okoth *et al.,* 2006). Antenatal attendees study population was selected as they are considered at high risk of contracting HBV infection due to increased exposure to risk factors. Furthermore, they have the potential to transmit to their infants hence the emergence of permanent carriers particularly if expectant mother is HBsAg and HBeAg positive (Lu et al., 2014).

Since HBV studies in Kenya have been limited to health workers, blood donors, high risk groups, those with liver disease and people living with HIV, HBV hasn't received as much attention as it should, and if the right interventions are not implemented, the prevalence of hepatitis B virus could increase. The high incidence of HBV in Kenya and the paucity of data on the disease in many health systems may be related to the lack of HBV screening as part of the ANC profile in many facilities especially in Tharaka region where there is no study that has been conducted to provide information on the burden of disease among this population. It is crucial to screen expecting mothers and their newborns to more effectively stop the HBV virus from spreading (WHO, 2000).

**1.3 Justification of the study**

The babies born to mothers who test positive for HBsAg experience perinatal transmission (10–20%) of HBV; however, that rate can reach 90% in cases where both HBsAg and HBeAg are present. Children born to mothers who are positive for HBsAg are carriers and at high chance of developing HBV consequences, including HCC and cirrhosis, while those born to mothers who are HBeAg positive are at risk of infection even if they do not get sick during birth (Wu & Chang, 2016). 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 genetic diversity. In our setting genotype A has been identified as predominant among blood donors, inpatient, and high- risk populations (Mwangi *et al*., 2008). The incidence of this viral infection, genetic diversity and the risk factors for it in pregnant mothers seeking ANC has not been done in Tharaka region. It is therefore necessary to conduct this study and the findings will provide additions in formulating policy guideline for the management of HBV infection.

## 1.4 Research Questions

(i) What is the prevalence of Hepatitis B virus infection among expectant mothers visiting antenatal care at St. Orsola Hospital?

(ii) What are the risk factors that could be predisposing pregnant women visiting antenatal care at St. Orsola Hospital?

(iii) What are the circulating Hepatitis B virus genotypes circulating in Tharaka Nithi region?

## 1.5 Objectives

### 1.5.1 General objective

To determine the genetic diversity, prevalence and risk factors associated with Hepatitis B virus infection among pregnant women visiting antenatal care at St. Orsola Hospital.

### 1.5.2 Specific objectives

(i) To determine the prevalence of Hepatitis B virus among pregnant mothers attending antenatal care at St. Orsola Hospital

(ii) To assess the risk factors associated with Hepatitis B virus infection among pregnant women visiting antenatal care at St. Orsola Hospital

(iii)To determine the genetic diversity of Hepatitis B virus among expectant women visiting antenatal care at St. Orsola Hospital.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Hepatitis B viral infection

Despite the long-standing readily available vaccines, HBV infection has become a serious public health issue across the globe. It is estimated that, 350 million chronically infected people worldwide (Amiri *et al*., 2016). It has been demonstrated that this virus has a potential to spread vertically from mother to her child and also through contact with contaminated fluids (Lu *et al*., 2014). The children born to HBV infected mother who are positive for surface and e-antigen, 70-90% has a possibility of developing HBV chronic infection. However, when a mother is positive for HBsAg and negative for HBeAg, the chance is reduced to 10% (Aggarwal *et al.,* 2004). Between late childhood and adulthood, cirrhosis or hepatic cancer will claim the lives of about 25% of the carrier newborns (Kew, 2010).

Although the HBV vaccine has been successfully introduced, new cases of infection are still increasing, especially among individuals with HIV (Thimm *et al*., 2005). Hepatitis B virus is one of the infectious viruses that cause acute and chronic hepatitis, accounting for approximately 35% to 40% of all new cases of infection worldwide during perinatal transmission (Zamani *et al*., 2001). According to estimates, the HBV virus still poses a serious threat to global health, infecting around 2 billion individuals per year and causing an additional 350 million chronic cases, which results in approximately 600,000 fatalities (Ranjbar *et al*., 2011). Liver cancer and cirrhosis fatalities are significantly increased by hepatitis B virus infection (Ott *et al*., 2012).

The prevalence rates of HBV in low, intermediate, and high endemicity globe regions are 0.5–2%, 2-7%, and ≥8%, respectively (Maclachlan and Cowie, 2015). According to a study, 70% to 95% of the population in Sub-Saharan Africa, Kenya included, exhibits current or previous HBV infection serological evidence, making the region a high endemic area (Franco *et al*., 2012). Prior research in Kenya has continuously shown varying rates of HBV prevalence throughout the nation, primarily in metropolitan areas. The reported prevalence is between 5% and 8%, and in certain instances, it can reach 50.6%. Numerous studies are carried out among high-risk groups, including injectable drug users, commercial sex workers, men who have sex with males, people with HIV, and those who have jaundice (Muriuki *et al*., 2013; Mabeya *et al*., 2016).

**2.2 Structure of HBV**

This virus has HBsAg on its surface and is a double-stranded circular DNA virus having a diameter of 42-nm. It is a member of the family Hepadnaviridae. HBcAg, one partly double-stranded DNA molecule, DNA-dependent DNA polymerase, and HBeAg are all found in the virus' inner core (Figure 2.1).

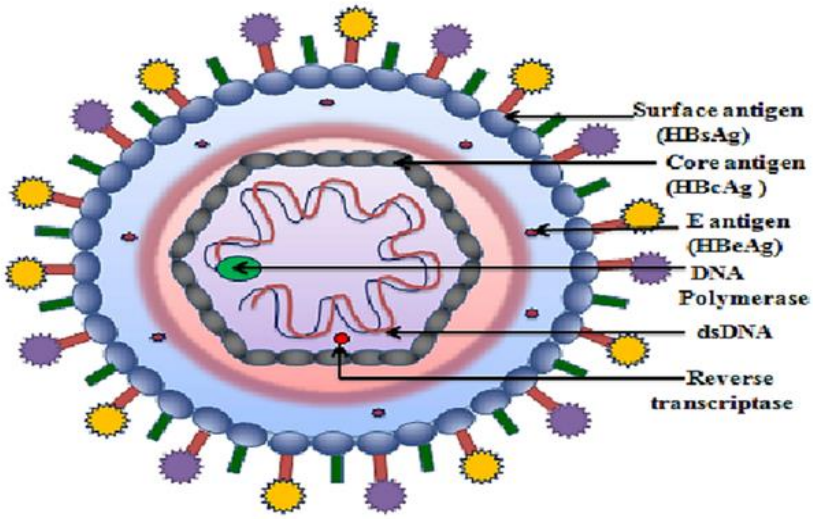



**Figure 2.1 HBV structural makeup (Lamontagne *et al*., 2016)**

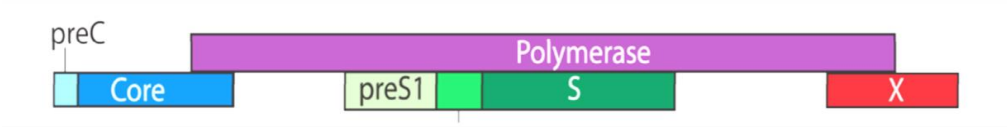



**Figure 2.2 HBV ORFs' overlapping structure (Lamontagne *et al*., 2016)**

The X proteins, polymerase, and structural proteins (pre-S, S, and C proteins) are all encoded by overlapping genes on the minus strand of DNA. The transcription and translation of HBV are mediated by four mRNA transcripts. The replication and production of polymerase proteins as well as pre-core and core are engaged in the longest (3.5 kB) (Mabeya *et al.*, 2016) (Figure 2.2). While a 2.1 kilobyte transcript only encodes pre-S2 and HBsAg and a 2.4 kilobyte transcript only encodes pre-S1 and pre-S2, 0.7 kilobytes encode the X protein. Open reading frame (C ORF) is used to encode core and v bE antigens, HBX by (X ORF), and DNA polymerase by (pol ORF) (Locarnini, 2004).

**2.3 The HBV Life Cycle**

HCC has been linked to HBV, which is a retrovirus. Reverse transcription, often known as pre-genomic (pg) RNA, is essential to life cycle. The interaction among viral protein envelopes and particular liver plasma membrane receptor cells often take place during attachment and entry. The N-terminal region of pre-S1 is involved in causing binding to receptor and starting an infection (Tai *et al*., 2002). A peptide in the pre-S1 region prevents HepaRG cells from becoming infected. This offers the potential for application of pharmacology. The virus gets into cell through the endocytosis and attaches to certain cells receptor. Low-protein and enveloped proteins bound to proteolytic enzymes enhance viral capsid release from the endosome into the cytoplasm (Ni *et al*., 2014).

The relaxed circular DNA (rcDNA) of the virus is transferred from the capsid into the nucleus via the pore (Kann *et al*., 2007) and undergoes a transformation into a covalently

closed circular (cccDNA) by synthesis of DNA plus-strand. The 3.5 kb pre-C mRNA and pg RNAs, big envelope mRNA protein, middle and major surface protein mRNA, and X protein mRNA are the four viral RNA systems that cccDNA transcribes in the nucleus (Schmidt *et al*., 2004). A "pre-C" protein is created by the conversion of pre-C mRNA. Later, this is proteolytically changed into HBeAg, which is released by infected cells and can be detected to indicate greater viral replication levels (Perlman *et al*., 2005). The new virion is encased, capsidized, and has convergent rcDNA in the nucleus. In the absence of cell re-infection, they undergo transformation into multiple molecules of cccDNA at this location when the virions are put together into HBsAg and pre-S proteins, and they bud from the endoplasmic reticulum (ER) membrane (Rabe *et al*., 2009).

**2.4 Epidemiology and transmission mode of HBV Infection**

Hepatitis B virus (HBV) is a disease that is spread around the world, and the infection rate varies according to the local population. Most notably in areas with low endemicities, like North America, hepatitis B infection is spread primarily through sex. According to Alter (2003), men who engage in sexual activity with other men are more prone to become infected (Figure 2.3).


**Figure 2.3 Prevalence of HBV infection worldwide (Gerlich, 2013)**

There are quite a few heterosexual explanations for how HBV is transmitted, including variables like the quantity of sexual companion, the length of the act, a history of STDs, and the existence of syphilis. Intravenous drug users, prostitutes, and their customers are additional groups that are more at risk of infection (Muriuki *et al.,* 2013).

Injecting drugs, receiving blood transfusions, undergoing renal dialysis, getting acupuncture, coming into contact with sick people, getting tattooed, and living with infected people are all examples of behaviors that might result in parenteral or percutaneous transmission. People who self-inject are more likely to contract HBV in industrialized nations like the U.S. and Western Europe (Wasmuth, 2009). The length of exposure determines the likelihood of contracting the infection. Even in the circumstances of a blood transfusion, asymptomatic carriers might still be a source of infection. Additionally, blood donors should be screened particularly those who participate in harmful activities (Anaedobe *et al*., 2015).

Potential sources of infection include contaminated blood, surgical instruments, and utensils. Similar operations include surgery, treatments for injuries caused by needles, intravenous drug injections, dental work, acupuncture, tattoos, ear piercings, dialysis, and circumcision. Workers in the law enforcement, hospital, and laundry industries are also in danger (Margolis *et al*., 1991). Even when provided immune-prophylaxis babies can occasionally contract HBV from infected mothers perinatally (Xu *et al*., 2002). Utero-transmission, perinatal transmission, and surface gene escape mutants are other causes of immune-prophylaxis failure (Zhu *et al*., 2003).

The risk of recurrence is decreased when larger dosages of this prophylactic are given to patients receiving liver transplants. Currently, it is coupled with a nucleoside analog in liver transplant situations where the viral load has been significantly reduced (Terrault and Vyas, 2003). The risk of contracting the virus after exposure can be decreased by using vaccination to prevent initial infection. Both the DNA recombinant vaccine and the inactive plasma-derived HBV vaccine were first made accessible in 1986. In 1991, the WHO declared these immunizations safe to administer and went on to encourage their use, especially in countries where carrier prevalence rates exceeded 8%. This was seen favorably, and by 2002, many nations had established hepatitis B infant routine vaccination (Lavanchy, 2004). The immunization campaign has decreased the prevalence of acute hepatitis in young people, particularly in Italy and the United States (Da Villa, 2000). Even when anti-HBs levels are undetectable after immunization, protection is still maintained. However, a shortage of funding has hampered the development and effectiveness of the hepatitis B vaccine, necessitating greater support (Ayerbe and Pe'rez, 2000). The behavioral Changes, such as condom use, blood screening, and the use of protective gear when handling infected patients, can cut down on transmissions.

**2.5 Perinatal Transmission and Prevention of HBV**

Vertical transmission frequently happens during delivery, contact with contaminated fluids in the uterus, or both. According to studies, there is a 70%–90% likelihood that a child born to a mother who tests positive for the e and s antigens will get HBV, and 80%–90% of those who do may go on to become chronic carriers. The likelihood of transmission is reduced to less than 10% if a mother tests positive and negative for the s antigen and e antigen respectively (Aggarwal and Ronjan, 2004).

Due to its smaller size and lack of agglutination compared to HBsAg, HBeAg is more easily transported through the placenta. Passive-active immunoprophylaxis using hepatitis B immunoglobulin (HBIG) and the hepatitis B vaccine has been shown to be very successful in avoiding perinatal transmission ever since the hepatitis B vaccine was first introduced. During immunization, the Haemophilus influenza type B vaccine is administered alongside the vaccines for Diphtheria, Pertussis, Tetanus, and HBV (Zanetti *et al*., 2008).

**2.6 The role of HBV serological markers**

Hepatitis B disease manifests in a variety of ways, including acute and chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma, due to vulnerable host-viral interactions (Liang, 2009). A variety of clinical, biochemical, serological, molecular, and histological findings are used to make the diagnosis. The host immune system is activated during exposure to HBV infection, leading to an increased cellular reaction against the virus. Virus-specific immunological markers (antigens and antibodies) are produced by susceptible hosts in response to viral contact, and these can be found in serum a few days after exposure. These indicators, which correspond to different phases of liver illness, include HBsAg, anti-HBs, HBeAg, DNA, anti-HBe, anti-HBc, IgM, and IgG (Song, 2016). The markers can be identified using molecular and serological means, which facilitates HBV infection screening, clarifies the natural progression of chronic hepatitis B, evaluates the infection's clinical phases, and tracks the effectiveness of antiviral medication (Ferreira, 2000). (It is crucial to interpret serological results in order to get an accurate diagnosis for any type of HBV infection) (Table 2.1).

**Table 2.1 Interpretation of HBV serological markers (Song & Kim, 2016).**

| HBV Serological Markers | Results interpretation |
|---|---|
| HbsAg | Acute and chronic HBV infection |
| HbeAg | High-level HBV infectivity and replication; a treatment response marker |
| HBV DNA | HBV replication level; a primary virological marker for treatment response |
| Anti-HBc (IgM) | Acute HBV infection or recovered |
| Anti- HBs | HBV vaccination or recovered marker; |
| 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) & /HBV DNA | Latent or occult HBV infection |

### 2.6.1 Acute phase

Following six months after infection, the acute stage of hepatitis B can be seen. Patients may experience fever, urticaria, arthralgia, and jaundice in addition to other clinical symptoms such as nausea, weight loss, and flu-like symptoms. Along with the elimination of DNA for HBV and seroconversion to anti-HBe from HBeAg, these symptoms typically go away within a few weeks. Many HBV patients who are in the acute phase of the disease are frequently asymptomatic, and the infection may go unnoticed. According to Fagan and Williams (1990), treatment with nucleoside analogues

that act as antivirals is necessary when acute hepatitis progresses to fulminant hepatitis, which can cause liver failure and a high death rate.

### 2.6.2 Chronic phase

Depending on the individual's immune system and age, a chronic infection can develop in 90% of infected infants but only in 5% of adults. There are four stages to a chronic hepatitis B infection. The first phase is immune-tolerant, lasting 20–30 years, with a 2-3% HBsAg yearly clearance rate and a 0.5% risk of developing HCC. The viral DNA in this instance is high, and e and s antigens can be found (McMahon *et al*., 1985). During the second phase (immune clearance), aminotransferase levels rise and the patient develops symptoms while the host immune system suppresses the amount of HBV DNA. Other individuals have HBeAg seroconversion, which is followed by a 10–20% yearly HBeAg clearance rate. In roughly 1–5% of patients, seroconversion is accompanied by the selection of HBV mutations, which results in HBeAg negative status (Chang, 2000). The third phase is an inactive carrier stage with negative HBeAg, positive anti-HBe, and undetectable viral DNA. Reactivation, the last stage, is marked by higher ALT, viral load level, and the potential for further damage of the liver that could result in fibrosis and cirrhosis in some patients (Yimet *et al*., 2006).

### 2.6.3 Occult hepatitis

Occult hepatitis B virus infection is defined by the continued presence of HBV DNA in the liver cells and, in certain cases, in the serum of people who are negative for HBsAg (Afyon and Artuk, 2016). This scenario is frequent with the introduction of viral genetic variations, which are responsible for surface antigen alteration that is not detectable using standard immunochemical assays. Occult hepatitis increases the likelihood of

reactivation, infection spread, and the advancement of liver conditions that can result in HCC (Raimondo *et al.,* 2006).

### 2.6.4 Hepatocellular carcinoma (HCC)

HBV is a hepatotrophic virus that is highly reported in HBV-endemic areas and can cause HCC. Numerous inflammatory cells become active during chronic inflammation and release nitric oxide (NO) (Nguyen *et al*., 2009). By damaging the retinoblastoma and p53 tumor suppressor genes, these free oxygen radicals may prevent them from activating the cell's death process. The loss of the p53 gene and genetic instability brought on by chromosomal deletions, disruptions, or translocations may also result from the integration of HBV DNA (Robinsons, 1999).

## 2.7 Antigenicity of HBV

The structural proteins of HBV activate particular T-lymphocytes with the capacity to eliminate HBV-infected cells, and HBV envelope protein contains immunogenic HBsAg, which triggers the release of antibody-mediated immunity during infection (WHO, 2015). The HBsAg has nine sub-genotypes that are antigenically diverse but have a standard antigen known as "a" as well as two pairs of alternate antigens, d and y and w and r. The HBcAg which is present as a HBV internal component rather than manifesting freely in blood, is found on the surface of the core molecule, and it triggers the production of anti-hepatitis B core antibodies (anti-HBc antibodies) is not a protective antibody and remains in the serum forever, hence an indicator of previous or ongoing HBV infection (WHO, 2015).

Although there hasn't been any research on the interaction between HBcAg and HBeAg, the two share sequences. The HBeAg is a soluble antigen that is used to diagnose

individuals with high viral loads, and its presence in blood indicates an acute active infection (Fessehaye *et al*., 2018). Anti-HBe, a particular antibody against HBeAg, is produced when HBeAg is present. Patients who test positive for HBeAg in blood samples and have both acute and chronic HBV infections are known to have the hepatitis Bx antigen. The HBx antigen is a transcriptional activator; it does not bind to DNA (Abbas *et al*., 2007), but it stimulates the formation of an anti-HBx antibody that only manifests when other viral antigens are undetectable.

**2.8 Genetic diversity of Hepatitis B Viruses**

Based on the HBV genomic sequence divergence of more than 8.0% or more than 4.0% in the S gene, the hepatitis B virus is divided into ten genotypes (Liu *et al*., 2016). The genomic sequence divergence between 4.0 and 8.0% is used to further divide hepatitis B virus genotypes into sub-genotypes (Liu *et al*., 2016). HBV genotypes A, B, C, and D were the first to be identified; later came E and F, then G, H, and I. According to Tallo (2008), genotype J, which was isolated from a single person, is the HBV genotype. Therefore, the ten genotypes (A–J) of the Hepatitis B virus are identified. According to Kumar and Singh (2011), genotypes A, C, and D each have five subgenotypes (A1–A5, C1–C5, and D1–D5), whereas genotypes B have six subgenotypes (B1–B6), and genotype F has four subtypes (F1–F4).

Different HBV genotypes demonstrate the varied geographic distribution pattern of HBV; for example, genotype A is more prevalent in Northwest Europe and North America (Kumar and Singh, 2011). Additionally, subgroups of genotype A have been found in the Philippines, Asia, Hong Kong, and Africa (Pourkarim *et al.*, 2014). According to reports,

sub-genotype A1 predominates in South Africa, Sub-Saharan Africa, Malawi, and the Philippines; A2 predominates in Europe, the United States, and Russia; A3 predominates in West and Central Africa; A4 predominates in Malawi and the Gambia; and A5 predominates in Nigeria (Tallo, 2008). While genotype D is the most widely dispersed worldwide, from Africa and India to Western Europe, genotypes B and C are more common in Indonesia, Asia, Japan, China, and the Pacific Islands (Kumar and Singh, 2011). Genotype E is mostly found in West Africa, genotype F is predominant in Central and South America, genotype G was initially isolated in the USA, Belgium, and France, genotype H was found in Japan and America, and genotypes I and J have been speculated to be present in South Asia (Pourkarim *et al*., 2014).

Hepatitis B virus has five genotypes: A, B, C, D, and E, which are all frequently found in Africa. However, genotype A predominates in Eastern and Southern Africa, genotype D predominates in Northern Africa, and genotype E predominates in a larger region of the Eastern Central African Republic and in Namibia, Senegal, Nigeria, Togo, Benin, and the Democratic Republic of the Congo (Zampino *et al*., 2015). In Africa, the majority of genotype A individuals belong to sub-genotype A1, which predominates in Somalia, Malawi, the Democratic Republic of Congo, Uganda, and Tanzania, in addition to Eastern and Central Africa (Sunbul, 2014). Sub-genotype A2 was isolated in South Africa. Additionally, Northern Africa has a high prevalence of sub-genotypes D1 and D7 (Zampino *et al*., 2015).

In Kenya, sub-genotypes A1 and D6 were found to be predominant in the previous study, and genotypes A, D, and D/E recombinant have all been identified among the population (Wylie *et al*., 2018). The circulating genotypes of intravenous drug users (IDUs) and blood donors have been investigated in two separate investigations in Mombasa, Kenya. According to the findings, the sub-genotype A1 of HBV is the most common genotype in circulation. The prevalence and genetic diversity of HBV among ANC patients have not been studied, despite the fact that research has been done. In this study, ANC mothers at St. Orsola Hospital were given information on the various circulating HBV genotypes and seroprofiles.

### 2.9 The HBV treatment

To prevent mother-to-child transmission of HBV, the World Health Organization advises pregnant women who test positive for HBV infection (HBsAg positive) and have an HBV DNA of 5.3 log10 IU/mL (200,000 IU/mL) to start tenofovir prophylaxis on the 28th week of pregnancy and continue until at least delivery. In addition, all infants receive a three-dose hepatitis B immunization, including a dose at the appropriate time of delivery (WHO, 2020).

#### 2.9.1 The HBV escape mutations

Estimated nucleotide substitutions per site/year for the hepatitis B virus range from 103 to 106 (Cao, 2009). According to Croagh and Lubel (2014), this is explained by the reverse transcriptase enzyme's error-prone activity in addition to the high daily virion generation (1012 viruses). It has been observed that vaccine escape is linked to HBsAg mutations. This causes an immunized individual to become infected with HBV irrespective of their vaccination either with pentavalent vaccination or by prophylactic

administration of Hepatitis B Immune Globulin (HBIG). Additionally, the variations have been connected to the spread of occult HBV infection due to diagnostic test failure to identify HBsAg (Coppola *et al*., 2015).

However, mutations in the basal core promoter (BCP) and precore (PC) have been associated with a decrease in HBeAg levels, which in turn has been connected to an increase in viral replication and the elimination of HBeAg production, leading to HBeAg-negative illness. Antiviral resistance is caused by drug-induced mutations that target the active site polymerase in the conserved region known as Tyr-Met-Asp-Asp (YMDD) (Archampong *et al*., 2017). These mutations have the ability to spread to a vulnerable, naive host and cause a persistent HBV infection (Horvat, 2011).

**2.9.2 Nucleic Acid Test**

Hepatitis B DNA testing measures viral load directly, this demonstrates virus replication. HBV DNA is discovered one month after blood inoculation, reaches its peak concentration three months later, and gradually decreases among chronically infected individuals during the healing process (Song, 2016). The presence of HBV DNA in blood accurately indicates HBV replication activity. Additionally, greater HBV DNA concentrations imply a link to faster disease development and an increase in the incidence of hepatocellular carcinoma (Fessehaye *et al*., 2018).

Target amplification, such as polymerase chain reaction and branched DNA technology, and signal amplification, such as the hybrid capture method, are the two major concepts of methodologies used in the detection and quantification of HBV DNA (Song, 2016). Quantitative PCR (q-PCR), real-time PCR (RT-PCR), and nested PCR are the results of

modifications made to the basic technique in the application of conventional PCR in order to increase the specificity and sensitivity of the test to be performed by the PCR (Fessehaye *et al*., 2018). RT-PCR measures the amounts of HBV DNA in blood in addition to detecting HBV DNA in blood. Sequencing the PCR results and building a phylogenetic tree after HBV DNA has been amplified allows for genotyping and sub-genotyping (Song, 2016).

### 2.9.3 Clinical Symptoms of HBV

While thirty-three percent of adults with acute infection with HBV have clinical symptoms ranging from nausea and fatigue to jaundice and uncommonly experience liver damage, about sixty-six percent of those diagnosed with acute HBV infection have an asymptomatic, mild, or sub-clinical illness that can't be detected (WHO, 2015). The incubation period for acute HBV infections typically lasts two to three months, although it can last up to six months after the virus has been injected into the body (WHO, 2015). A brief prodromal phase that is characterized by clinical symptoms such as body pains fever and a nausea issue after the incubation period has ended (WHO, 2017).

In this phase, ALT levels in serum rise, HBV DNA levels rise, and HBsAg levels climb until they are detectable in serum (Liang, 2009). Jaundice appears at the beginning of the prodromal phase of HBV, which lasts for 1 to 7 days. During the icteric phase, which lasts for 1-2 weeks, virus levels decline (WHO, 2015). The symptoms, such as pain fever and nausea, might persist for weeks or months despite the fact that jaundice clears up during the convalescence stage (WHO, 2017). Acute liver failure patients may develop fulminant hepatitis. Some individuals test negative for HBsAg while in a coma because

the progression of liver failure is likewise marked by a decline in blood levels of HBV DNA and HBsAg (Liang, 2009).

### 2.9.4 Influence of HBV Genotypes on Clinical Symptoms Progression

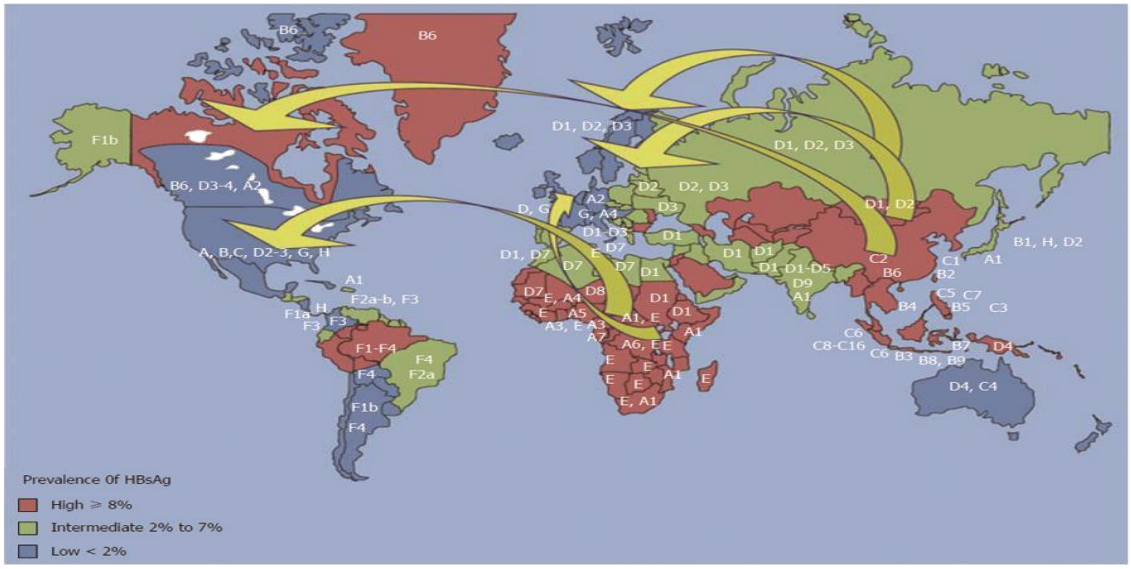



**Figure 2.4 HBV genotypes and sub genotypes distribution (Gerlich, 2013)**

The distribution of HBV genotypes varies from one region to another (Figure 2.4). The most patients with genotypes A and B have acute infections, whereas those with genotypes C or D are more likely to progress to liver cirrhosis (Croagh *et al*., 2015). While HBV genotypes B1, B6, and A2 are considered to have minimal risk consequences for hepatitis B virus illness, HBV genotypes B2–5, C, and F1 are connected to accelerating the progression of the disease to hepatocellular carcinoma (HCC). Additionally, because of the sub-genotype C2's slow seroconversion rate, which lengthens the viral replication process, individuals with genotype C infections are more likely to develop hepatocellular carcinoma (El-Serag *et al.*, 2012). Genotype B patients respond better to treatment and experience no recurrence of HCC for a considerable amount of time, contrary to genotype A patient, who are associated with severe liver

damage (Fessehaye *et al.*, 2018). According to Lamontagne *et al*. (2016), genotype C patients are more likely to develop severe liver illness due to their high HBV-DNA burden, whereas genotype D patients are more likely to develop HBV-related vasculitis than genotypes B6, A2, F1, and C2.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study Area

This study was carried among expectant women attending at St. Orsola Hospital, located in Tharaka Nithi County. This faith-based hospital facility is managed by the catholic diocese of Meru and is situated in the Tharaka South sub-county. It serves as catchment of approximately 100,000 with an average of 20,000 patients visits each month and bed capacity of 216. As a level 4 hospital, it provides services to three neighboring Counties of Embu, Meru and Kitui due to its location. The hospital features specialized out-patient clinics where an average of 250 pregnant women receive care each month. It is located along the Ishiara-Nkubu route in Matiri town within the Tharaka constituency (see Figure 3.1).

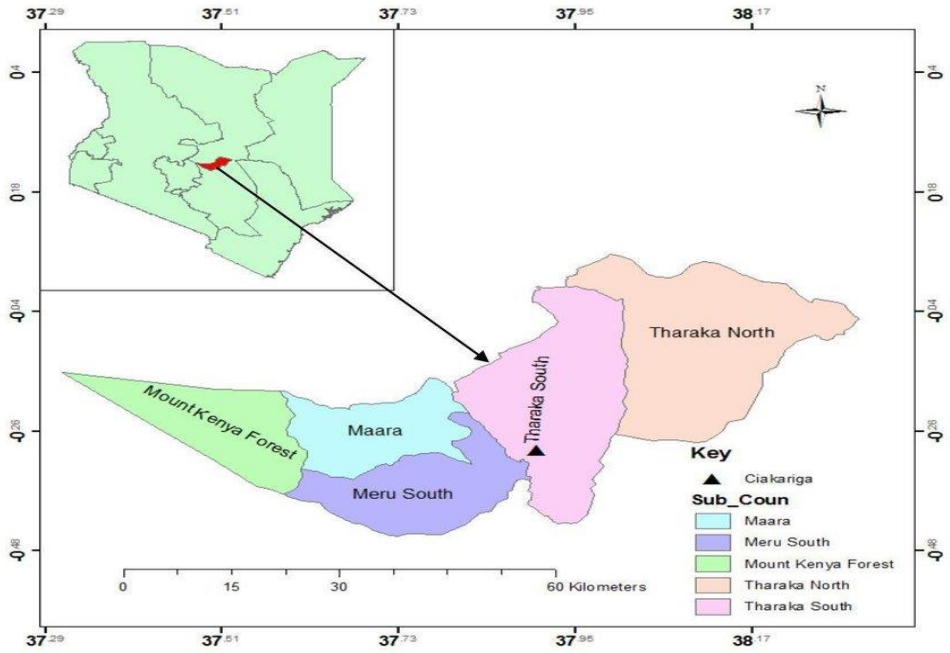



**Figure 3.1 A map of Kenya showing the study area**

### 3.2 Study Population

This study involved expectant mothers visiting St. Orsola Hospital seeking ANC services during the period of September and December 2021. Those who participated consented by signing the informed consent and demographical data collected (appendix E).

### 3.3 Study design

A cross-sectional study design was adopted and about 5 ml venous blood samples drawn from each consenting participant. Social-demographic data on; age, social behavior and information on exposure variables were collected from each participant by administering a structured questionnaire (appendix D).

### 3.4.0 Sampling design

A systematic random sampling technique was used and five out-of 10 eligible patients were recruited each day randomly as generated on daily basis, with every 2nd participant being selected. This was done until the expected sample was achieved.

### 3.4.1 Sample size determination

The Naing formula (2006), as shown below was used to arrive at the final sample size (Naing *et al*., 2006). The sample size was based on an estimated prevalence of 50% since this particular study has never been carried out within the County.

Minimum sample size: N

P = 50% estimated prevalence (Ngare *et al.,* 2024).

The inverse of the 95% confidence level is D = 0.05. Z = 1.96 standard deviation.

$N = Z^2 P (1-P)/D^2$.

$N = 1.962 \times 0.5(1-0.5)/0.052 = 385$

### 3.5 Selection criteria

#### 3.5.1 Inclusion criteria

i. Any consenting expectant woman who attended ANC at St. Orsola Hospital for ANC services was recruited into the study.

ii. Expectant women with unknown HBV status.

iii. Expectant women who have never been vaccinated against HBV vaccine.

#### 3.5.2 Exclusion criteria

i. Any expectant woman who is known to be HBV infected but recovered.

### 3.6 Ethical approval of study

This study was approved by the hospital management committees of St. Orsola Hospital (appendix I), approved by graduate and Ethical Review Committee of Kenyatta University (appendix F and G), and the National Commission for Science and Technology (NACOST) (appendix H). Through the use of a standard consent form created specifically for this investigation (appendix E), participants gave their informed consent to take part in the study. Participants who qualified for the study were given a questionnaire after an interview.

### 3.7 Blood samples collection

A skilled and certified medical laboratory technologist aseptically collected 5 ml of venous blood from patients at the antecubital fossa veins of the hand in lab. After being cleaned with a wipe of 70% isopropyl alcohol, the area was left to dry. Five (5) ml of blood were taken by a sterile needle and syringe from willing individuals and placed in an EDTA vacutainer (Biobank, 2011).

### 3.8 Hepatitis B surface antigen and HBV genotype detection

Using Onsite HBsAg Rapid Test Cassettes (Abbott, Ireland), presence of HBsAg in the blood plasma was determined. According to Chameera *et al*. (2013), the principle of test is lateral flow chromatographic immunoassay for the detection of HBsAg in serum or plasma samples. In brief, the blood samples were well mixed after thawing for the refrigerated samples and brought to room temperature together with the test cassette. The cassette was then taken out of the pouch on a spotless flat surface, and marked with the sample's identification number. Estimated 80 µl of the plasma sample was put in sample wells and reaction timed for fifteen minutes and reaction test to confirm the positive bands checked. HBsAg reactivity (positive) was revealed by having two distinct red bands in the Test Zone (T) and Control Zone (C). In contrast, for a negative test, red band was on control zone but not test zone. However, in case of a situation where there was absolutely no red band in the test and control wells, the test was considered invalid. In such sample, a repeat of the test with a new kit and fresh plasma was performed (Chameera *et al*., 2013). Those samples that were HBsAg positive were further analysed for other HBV markers using 5 panel test kit that also served as confirmation in case of false positive test before and finally tested for HBV genotyping.

#### 3.8.1 DNA extraction

The QIAamp DNA Blood Mini Viral Kit (Qiagen GmbH, Hilden, Germany) was used to extract hepatitis B viral DNA from the HBsAg-positive sera while adhering to the manufacturer's instructions. In order to prepare for gene amplification by PCR, the DNA was isolated, rinsed in Tris-EDTA, and then kept at -80 °C (Mabeya *et al*., 2017). About 1.5-ml micro-centrifuge tube was first filled with 20 µl of proteinase K and then with 200

µl of the sample. 200 µl of the buffer AL were added to the sample mixture, vortexed for fifteen seconds, and then incubated at 56 °C for 10 minutes while being briefly spun. 200 µl of ethanol was added to the sample mixture, vortexed for 15 seconds, and then centrifuged for 1 minute at 6000 x g. The QIAamp Mini spin column was filled with 500 µl of buffer AW1, closed, and centrifuged at 6000 x g for 1 minute, 500 µl of buffer AW2 was added, and it was centrifuged for 3 minutes at 20,000 x g. A 2 ml fresh collection tube with the QIAamp Mini spin column inside was centrifuged at 20,000 x g for one minute. The QIAamp Mini spin column was then put into a clean 1.5 ml micro centrifuge tube, and the collection tube containing the filtrate was thrown away. 200 µl of buffer AE was put into a mini-spin column, which was then incubated at 25 °C for 1 minute, then spun at 6000 x g for 1 minute (Mabeya *et al*., 2017).

### 3.8.2 Amplification of HBV-*pol* gene

The partial HBV-*pol* gene was amplified by a nested PCR using two specific forward and reverse primers by use of Kratec thermal cycler SC300G-R. The first cycle of PCR was carried out using the forward primer HBPF135 (position: 2850 –2868, 5'-GGGTCACCATATTCTTGGG-3') and reverse primer HBPR135 (position: 803-822, 5'-CAAAGACAAAAGAAAATTGG-3') while the second cycle of PCR was carried out using the forward primer HBPRF3 (position: 2868–2888, 5'-GAACAAGAGCTACAGCATGGG-3') and reverse primer HBPRR3 (position: 1547–1567, 5'- CCACTGCATGGCCTGAGGATG-3'). One Taq kit from England Bio-labs was used for PCR. Briefly, the first round PCR was performed in a 25 µl mixture containing 6.5 µl DNA, 5 µl nuclease-free water, 0.5 µl of each forward and reverse primers and 12.5 µl of master mix. For the seccond round Nested PCR, 3 µl of the first

round PCR product was used as DNA template, 8.5 µl nuclease-free water, 0.5 µl of forward primers, 0.5 µl of reverse primers and 12.5 µl of master mix making a final reaction volume of 25 µl. For both first and second round PCR, the thermocycling profile consisted of AmpliTaq acctivation at 94 °C for 5 min, followed by 35 cycles of PCR amplification using the following temperatures: denaturation at 94 °C for 30s, annealing at 50 °C for 30s and extension at 72 °C for 30s, with a final elongation at 72 °C for 7 min (Mwangi *et al.*, 2008).

### 3.8.3 Capillary Gel Electrophoresis

A PCR product evaluation was carried out using a QIAEXCEL (QIAGEN; Germany) equipped with a UV detector set to 254 nm. Separations were conducted in eCap Neutral Coated Capillaries with a 50 µm ID. By applying 50 psi pressure for one minute, the separation buffer was injected into the capillary. The 15 L amplicons inserted into the strip tubes were utilized in conjunction with a 15 bp loading marker. The alignment marker was covered with 20 microliters of oil, which was added to stop evaporation. A voltage of 4 kV was used to inject the sample combination into the tube, and a voltage of 6 kV was used to migrate it for 15 minutes. QIAXCEL software (QIAGEN; Germany) was used to collect and analyze the data (Zhao *et al*., 2014).

### 3.8.4 Purification of DNA amplicon

Following the instructions provided by the manufacturer, the amplified PCR samples were cleaned up by use of ExoSAP-ITTM PCR Product Clean-up Reagent Kit. Procedure: 240 ul of Buffer PB and 48 ul of PCR product were added to a 2 ml microcentrifuge tube, and the mixture was then poured in. The liquid was colored yellow by adding 10 microliters of (3 M sodium acetate, pH 5.0), then centrifuging for 30-60

seconds after adding the mixture to a QIAquick spin column inserted in collecting tube (2ml). The QIAquick column was re-entered into the same tube and flow-through was disposed of. A centrifuge was used to spin for 30 to 60 seconds after adding 0.75 cc of buffer PE for cleaning. After being thrown away, the QIAquick column was reinserted into the tube. Then, for a further minute, the column was centrifuged. A clean 1.5 ml microcentrifuge tube was then used to hold the QIAquick column. The QIAquick membrane was centrifuged for one minute after 50 microliters of Buffer EB (10 mM Tris•Cl) at pH 8.5 was added to its center in order to extract DNA. 50 ng of the purified nested PCR product were used for cycle sequencing, and the quantification of the purified DNA was done using Picogreen (Invitrogen).

**3.8.5 Sequencing of PCR Products**

Sequencing took place at University of Nairobi UNITID lab, employing 20 μL of the amplicon. Following purification with ExoSAP-IT™ PCR Product Clean-up Reagent Kit, the PCR product underwent sequencing using the Big Dye chain terminator method with ABI 3730xl DNA Analyzer with. A 20 microliters reaction mixture comprising 5X sequencing buffer, 3 microliters of DNA, and 2.0 microliters of Big Dye, 1.5 microliters of primer and 10.5 microliters of distilled water. Two reactions were made for each sample, using 50 ng of DNA and the primer set used for the second PCR product, followed by cycle sequencing in seconds. The primer HBPRF3 (5’-GAACAAGAGCTACAGCATGGG-3’) and HBPRR3 (5’-CCACTGCATGGCCTGAGGATG-3’) were used. The 25 cycles of amplification were carried out as follows: 5 minutes of denaturation at 96°C, followed by another 10 seconds at the same temperature; 5 seconds of annealing at 50°C; and 4 minutes of final extension at 60°C.

### 3.9.0 Sequence Analysis

The sequences were assembled, manually edited, and trimmed in BioEdit version 7.1.1 The cleaned sequences were subjected to the Basic Local Alignment Search Tool (BLAST) search (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed on 1 September 2024). The homologous sequences retrieved from BLAST and the sequences from this study were saved in fasta format and subjected to multiple sequence alignment by MUltiple Sequence Comparison by Log- Expectation (MUSCLE). The multiple sequence alignment was curated using Block Mapping and Gathering with Entropy (BMGE). Phylogenetic relationships were estimated using Maximum likelihood-based inference of phylogenetic trees with Smart Model Selection (PhyML+SMS). The phylogenetic tree was rendered as a newick file and visualized and annotated in FigTree version 1.4.4 (Mabeya *et al*., 2016).

### 3.9.1 Analysis of data

Tables were created that examined, summarized, and displayed the data. Data conversion and analysis were performed using IBM's SPSS version 20.0 (Chicago, IL). The link between age and sex among HBV infection was analyzed using the Chi Square to establish significant differences of positive HBV cases at 95% CI. It was deemed statistically significant if the p value was lower than 0.05.

# CHAPTER FOUR

# RESULTS

## 4.1 Socio Demographic characteristics of study participants

A total of 385 expectant women were recruited into the study. Their age ranged between 17 to 45 years and was categorized into six groups at five years Interval. Most of the respondents 151 (39.2%) were aged between 21 to 25 years, 134 (34.8%), between 26 to 30 years, 25 (6.5%) between 16 to 20 years while those aged between 41 to 45 years were 4 (1%) of the sample. On marital status, majority of the respondents 310 (80.5%) were monogamous, 51 (13.3%) single while 24 (6.2%) were polygamous. On occupational, most of the respondents 150 (39%) were in business, 109 (28%) casual workers, 66 (17%) professionals with the least being housewives 60 (16%). Depending on the levels of education, majority of the respondents 137 (35.6%) had secondary level of education, 122 (31.7%) primary, 70 (18.2%) had tertiary level of education with those without any education 56 (14.5%) being the least.

In this study, majority of the responds 367 (95.3%) have never had any blood transfusion with only 18 (4.7%) having been blood transfused. For dental procedure, 359 (93.2%) have never had a dental procedure with 26(6.8%) having had a dental procedure. Majority 375 (97.4%) had no tattoos while 10 (2.6%) had a tattoo in their body. Further, 366 (95.1%) had never had a caesarean section while 19 (4.9%) had had a caesarean section before. It was also found that 378 (98.2%) of the respondents didn’t have any liver problem while 7 (1.8%) had liver problems. Majority of the respondents 372 (96.6%) had never undergone an abortion while 13 (3.4%) had been through an abortion.

Finally, 383 (99.5%) of the respondents had never undergone FGM while only 2 (0.5%) had been through FGM.

### 4.2.0 Prevalence and sero-prevalence of Hepatitis B Virus

Of the total of 385 samples that were analysed, prevalence of 6.5% (25/385) was detected among pregnant women seeking antenatal at St. Orsola Hospital.

From of 25 patients that were confirmed to be HBV infected, five serological markers were used in categorizing them and to determine the various risk stage of HBV infection. From the study, 19/25 (76%) of them were at inactive carrier stage, 4/25 (16%) chronically infected, 1/25 (4%) at recovery HBV while 1/25 (4%) individual was found at occult HBV staging (Table 4.1).

**Table 4.1 Serological Marker of HBV present among pregnant women at St. Orsola Hospital, Tharaka Nithi County**

| Serological test | Chronic HBV | Inactive HBV | Recovery HBV | Occult HBV |
|---|---|---|---|---|
| HBsAg | + | + | + | - |
| HBsAb | - | - | + | - |
| HBeAg | - | - | - | - |
| HBeAb | - | + | + | + |
| HBcAb | + | + | + | + |
| n= (25) | 4 | 19 | 1 | 1 |
| Prevalence Rate (%) | 16 | 76 | 4 | 4 |


**Figure 4.1 Five panel kit with HBV showing serological reaction markers**

### 4.3 HIV Status and HBV status

In order to examine whether co-infection of HBV or HIV influenced any risk of infection, however this study indicate that there was a significant association between HIV status and HBV status ($p < .012$) (Table 4.2).

**Table 4.2 Associations between HIV and HBV**

| | | HIV status | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| HBV status | Positive | 4.0%(1) | 96.0% | 100.0% |
| | Negative | 0.3% | 99.7% | 100.0% |
| Total | | 0.5%(2) | 99.5%(383) | 100.0% |
| Chi square= 6.267 | | df = 1 | | p-value = .012 |

The Chi-Square results as shown on Table 4.2 indicate that there was a significant association between HIV status and HBV status ($p < .012$).

### 4.4 Risk factors associated with HBV infections among pregnant women of Tharaka Nithi

Socio-demographic information, including occupation, age, marital status, level of education, having tattoo, blood transfusion, dental procedure, liver problem, abortion or having had female genital mutilation were determined if they could have influenced any risk to HBV infection among the studied pregnant women. In this study, age ($p= 0.019$), education level ($p < 0.05$) was found to significantly influence HBV infection. However, marital status ($p= 0.119$) and occupation ($p= 0.442$) were not associated with the risk to HBV infection (Table 4.3)

**Table 4.3 Association between Social-demographic and HBV status among expectant women visiting St. Orsola Hospital, Tharaka Nithi County**

| | HBV Status | | | df | Chi-Square |
|---|---|---|---|---|---|
| **Age Groups** | Positive | Negative | Total | | |
| 16-20 | 1 (4%) | 24 (6.7%) | 25 (6.5%) | | |
| 21-25 | 9 (36.0%) | 142(39.4%) | 151(39.2%) | | |
| 26-30 | 8 (32.0%) | 126(35.0%) | 134(34.8%) | **5** | ***p*=0.019** |
| 31-35 | 3 (12.0%) | 50 (13.9%) | 53 (13.8%) | | |
| 36-40 | 2 (8.0%) | 16 (4.4%) | 18 (4.7%) | | |
| 41-45 | 2 (8.0%) | 2 (0.6%) | 4 (1%) | | |
| Total | 25 (100%) | 360 (100%) | 385 (100%) | | |
| **Marital Statu** | | | | | |
| Single | 3 (12.0%) | 48 (13.3%) | 51 (13.3)% | | |
| Monogamou | 18(72.0%) | 292(81.1%) | 310(80.5%) | | |
| Polygamous | 4 (16.0%) | 20 (5.6%) | 24 (6.2%) | **2** | ***p*=0.119** |
| Total | 25(100.0%) | 360(100.0%) | 385 (100.0%) | | |
| **Occupation** | | | | | |
| House wife | 3 (12.0%) | 57 (15.8%) | 60 (16.0%) | | |
| Business | 13 (52.0%) | 137 (38.1%) | 150(39.0%) | | |
| Casual | 7 (28.0%) | 102 (28.3%) | 109(28.0%) | **3** | ***p*=0.442** |
| Professional | 2 (8.0%) | 64 (17.8%) | 66 (17.0%) | | |
| Total | 25(100.0%) | 360(100.0%) | 385 (100.0%) | | |
| **Education** | | | | | |
| No educatio | 14 (56.0%) | 42 (11.7%) | 56 (14.5%) | | |
| Primary | 10 (40.0%) | 112 (31.1%) | 122(31.7%) | | |
| Secondary | 1 (4.0%) | 36 (10.0%) | 137(35.6%) | **3** | ***p*=0.000** |
| Tertiary | 0 (0.0%) | 70 (19.4%) | 70 (18.2%) | | |
| Total | 25(100.0%) | 360(100.0%) | 385 (100.0%) | | |

### 4.5 Influence of Risk Factors on HBV status

Selected risk factors were determined if they could influence any risk to HBV infection. From the analysis, liver problems, ($p < .05$) significantly posed a risk factor to HBV infection. However, in other risk factors; blood transfusion (p= 0.415); Dental procedure

($p$= 0.28); Tattoos ($p$= 0.398); Caesarean section ($p$= 0.092); Abortion ($p$ = 0.709) had no significant influence to HBV infection (Table 4.4).

**Table 4.4 Associations between risk factors and HBV status among expectant women visiting St. Orsola Hospital, Tharaka Nithi County**

| | | **Blood transfusion** | | | **Statistics** | | |
|---|---|---|---|---|---|---|---|
| | | **Yes** | **No** | **Total** | **Chi-square** | **df** | **P-value** |
| HBV status | Positive | 8.0%(2) | 92.0% | 100.0% | | | |
| | Negative | 4.4% | 95.6% | 100.0% | | | |
| Total | | 4.7%(18) | 95.3%(367) | 100.0% | 0.663 | 1 | 0.415 |
| | | **Dental procedure** | | | | | |
| HBV status | Positive | 12.0%(3) | 88.0% | 100.0% | | | |
| | Negative | 6.4% | 93.6% | 100.0% | | | |
| Total | | 6.8%(26) | 93.2%(359) | 100.0% | 1.169 | 1 | 0.28 |
| | | **Tattoo** | | | | | |
| HBV status | Positive | 0% | 100.0% | 100.0% | | | |
| | Negative | 2.8% | 97.2% | 100.0% | | | |
| Total | | 2.6%(10) | 97.4%(375) | 100.0% | 0.713 | 1 | 0.398 |
| | | **Caesarean section** | | | | | |
| HBV status | Positive | 12.0%(3) | 88.0% | 100.0% | | | |
| | Negative | 4.4% | 95.6% | 100.0% | | | |
| Total | | 4.9%(19) | 95.1%(366) | 100.0% | 2.844 | 1 | 0.092 |
| | | **Liver problem** | | | | | |
| HBV status | Positive | 8.0%(2) | 92.0% | 100.0% | | | |
| | Negative | 1.4% | 98.6% | 100.0% | | | |
| Total | | 1.8%(7) | 98.2%(378) | 100.0% | 5.724 | 1 | **0.017** |
| | | **Abortion** | | | | | |
| HBV status | Positive | 8.0%(2) | 92.0% | 100.0% | | | |
| | Negative | 3.1% | 96.9% | 100.0% | | | |
| Total | | 3.4%(13) | 96.6%(372) | 100.0% | 1.752 | 1 | 0.186 |
| | | **FGM** | | | | | |
| HBV status | Positive | 0% | 100.0% | 100.0% | | | |
| | Negative | .6% | 99.4% | 100.0% | | | |
| Total | | .5%(2) | 99.5%(383) | 100.0% | 0.14 | 1 | 0.709 |

### 4.6 Genetic diversity of HBV among pregnant women

In this study, 11 samples were successfully amplified and sequenced and the generated sequences phylogenetically analyzed. All the isolates were confirmed to be HBV based on the phylogenetic analysis of the sequenced partial P protein. All the Isolates were classified as HBV genotype A, this clade was supported by a bootstrap value of 0.99. All the isolates from this study clustered together into one clade with very strong bootstrap support of 0.92. This clade was paraphyletic to a sequences originating mainly from East Africa. Isolate 12 showed longer branch length than all other isolates which was an indication of more diversity, though it still clustered with these isolates (Figure 4.2).

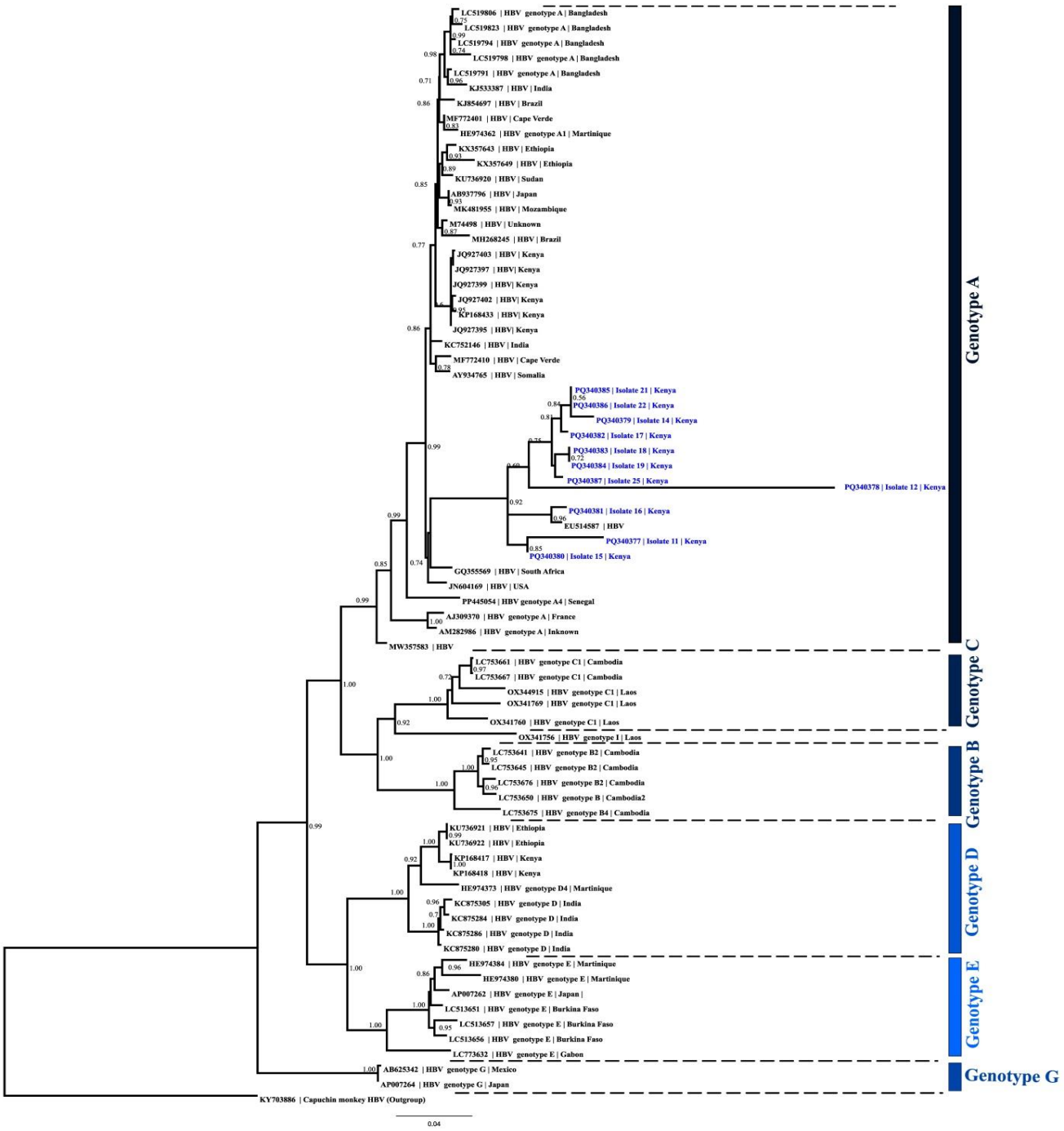



**Figure 4.2 Phylogenetic tree analyses 1**

The phylogenetic tree was created using the neighbor-joining method with a 1000-fold bootstrap resampling. The out group was Capuchin Monkey HBV (KY703886). Blue is used to denote HBV isolates from research participants

# CHAPTER FIVE

# DISCUSSION, CONLUSIONS AND RECOMMENDATIONS

## 5.1 Discussion

### 5.1.1 Prevalence of HBV among ANC mothers

The WHO cutoff level for HBV intermediate endemicity is 2-8%, in this study, a prevalence of 6.5% was detected among pregnant mothers hence confirming the intermediate endemicity of HBV infection in this region. The results of this study were in line with previous research on pregnant women conducted in Sudan (5.6%) (Elsheikh *et al*., 2007), Sierra Leone (6.2%) (Wurie *et al.,* 2005), Keffi Nigeria (6.67%) (Pennap *et al*., 2011), Niger Delta, Nigeria (5.3%) (Buseri *et al*., 2010). On the other hand, expectant mothers in the South West Ethiopia, Dawuro zone (3.5%) (Chernet *et al*., 2017) and Nigeria, Makurdi (3.3%) (Mbaawuaga *et al.,* 2008) had lower infection rates. The prevalence in this study also agrees with Nairobi's (7.7%) and the country's (9.4%) rates for comparable study respondents (Okoth *et al*., 2006).

Contrary, a research conducted in outpatients presenting with jaundice in Mombasa, Nairobi, Kisumu and Eldoret showed a prevalence of 50.6% (Ochwoto *et al*., 2016). In coastal Kenya, research conducted among injected drug user and non-drug user infected and non-infected with HIV, showed a prevalence of (16.8%) among HIV negative injecting drug users and 14.6% among HIV non-drug users (Webale *et al*., 2015). This suggests that in high-risk populations, HBV infection is well characterized. Since high-risk behaviors have the potential to spread the virus to the wider population, controlling HBV infection in these groups is crucial. Although the HBV prevalence in this study is low than national prevalence (9.3%), there 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 comparison with the general population in the country, this study finding concurs with those conducted in other parts of the country where Ly *et al*. (2016) reported 3.19%, in Nairobi, Ngaira *et al.* (2016) reported 3.8%, and Muriuki *et al*. (2013) reported 6.0%, 7.25% (Mabeya *et al*., 2017) in Nairobi and Wambani *et al*. (2015) 5.7% in Eldoret. These findings also agree with those conducted in sub-Saharan countries; Opaleye *et al*. (2014) Nigeria 5.7%, Ramírez *et al.* (2016) Tanzania 7.3%, Wandeler *et al.* (2016) Zambia 7.3%, Mutagoma *et al*. (2017) Rwanda 3.7%. Although the sample size and the study populations could influence the HBV diseases burden, this study varied with some studies that have been conducted in the country. The findings of this study concurred with those of an earlier investigation carried out in Maragua, Kenya (Hyams *et al.,* 1989) that revealed intermediate-to-high HBV infection prevalence, with carrier HBsAg frequencies between five to thirty percent. The finding of this analysis, however, are consistent with studies on expectant mothers reported by Nabulsi *et al*. (1997) in Lebanon, Turkey (Erdem *et al.,* 1994), and India (Juszozyk, 2000), that revealed prevalent of 3.9%, 5.3%, and 6.6%, respectively. High prevalence (8–20%) has been revealed in most of region in Africa.

In this study, the exposure of HBV infection varied significantly by age with the high exposure occurring in the age groups of 21–30 years. These results reported 21-30 years as most vulnerable HBV infections age group, which have been reported in studies from

Ethiopia (Zenebe *et al.*, 2014) and Togo (Kolou *et al.*, 2015). These studies also support data from Yaounde, Cameroon, which indicated a greater positive rate among women between the ages of 21 and 29 years (Fomulu *et al*., 2013). This may be because of the age group's strong correlation with sexual activity, sex engagement frequency, and sexual experience. 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 (Pennap *et al*., 2011). The increased incidence of HBV among married individuals could possibly be related to their sexual activeness, which puts them at risk of contracting the virus and serves as a sign of horizontal transmission.

**5.1.2 The risk factors associated with HBV among pregnant women**

During the study's duration, a number of hepatitis risk variables were assessed and only four variables were discovered to be HBV infection risk factors among the study`s participants. These four variables included education, age, a history of liver problems and HIV. In this study, there was a strong correlation between low educational attainment and HBV infection which was in line with study conducted in the Africa region, where it was found that as education levels increased, HBsAg positive decreased (Pennap *et al*., 2011). Participants who have some schooling comprehend the means of hepatitis B infection and will therefore be more inclined to take preventative measures. Moreover, education can also facilitate access to healthcare services, including vaccination. Individuals with increased level of education may have better access to healthcare facilities, resources, and information, which can increase their likelihood of receiving the hepatitis B vaccine and

other preventive interventions. The dearth of community information and advocacy materials specific to HBV infection may potentially be the cause of this discrepancy.

These study results were in line with those found in Thailand, where a history of jaundice (a liver issue) was a significant HBV risk factor among expectant mothers (Kuszewski, 2001). This could be associated with the fact that if an individual already has an existing liver condition, such as liver cirrhosis or chronic liver disease, their liver may already be compromised and more vulnerable to the effects of HBV infection. In these cases, HBV can further aggravate liver damage and worsen the existing liver disease. Also, Liver problems can result in impaired liver function, such as decreased production of clotting factors or impaired detoxification capabilities. When a person has impaired liver function, their ability to fight off infections, including hepatitis B, may be compromised. This can lead to a higher risk of chronic HBV infection or difficulty in clearing the virus from the body.

A high HBV infection was observed in age group 21-30 years 17/25 (68%) followed by 31-40 years 5/25 (20%) while the least infected were below 20 years 1/25 (4%). The observed results were consistent with other findings from research conducted in Ethiopia (Zenebe *et al*., 2014), China (Zu *et al*., 2017) and Togo (Kolou *et al*., 2015), confirming the age range as the most susceptible to HBV infections. This might be a result of the substantial correlation between this age group and frequency sexual engagement On the other hand, this outcome differed with the findings reported from research studies conducted in Kenya 2.36% (Ly *et al*., 2016 and Nagu *et al*., 2008).

HIV was considered as HBV infection a risk factor. This could have been attributed to the fact that both can be transmitted through similar routes, such as unprotected sexual contact, sharing of contaminated needles and syringes and from an infected mother to child during delivery or lactation. Engaging in high-risk behaviors, such as having many sexual partners or injecting drug use, can increase the likelihood of acquiring both infections. The populations that are already infected with HIV are more susceptible to contracting HBV if they are exposed to it. This is because HIV can weaken the immune system, impairing the body's ability to fight off infections effectively. Thus, HIV-infected individuals may be more prone to acquiring HBV if they are exposed to the virus (Ranjbar *et al.,* 2011).

The findings of this study did not agree with those from Italy study (Stroffolini *et al*., 2000) that identified blood transfusion as an HBV infection risk factor. Although positive identified cases were spread throughout, there was no statistically significant difference in HBV positivity between married and unmarried pregnant women. This suggests that being married is not a risk factor for HBV infection. Similar outcomes were seen in the Nigerian state of Abia, in the Niger Delta (Buseri *et al*., 2010). In contrast to a study conducted in Mexico which suggested tattoo as HBV infection risk factor, tattooing was not linked to HBV in this current study, which also concurs with previous report in Japan (Tanaka, 2000). Dental operation was not found to be a risk factor for acquiring HBV in this research contrary to previous study which linked dental procedure to HBV infection (Mendez, 1999). Other risk factors like FGM and abortion did not show any association with HVB infection in this current study.

### 5.1.3 Genetic Diversity of HBV among study participants

The finding of phylogenetic tree analysis revealed that all the sequences were of genotype A origin that clustered with reference sequences from East Africa (Sunbul, 2014). This study demonstrated that HBV genotype A was the most predominant genotype among the studied women population. These findings concurs with the previous studies that have been conducted in the country among blood donors (Mwangi *et al*., 2008; Kwange *et al.,* 2013), those with liver disease (Ochwoto *et al*., 2016), and high-risk groups (Webale *et al*., 2015). In comparison to the global HBV genetic diversity, these findings collaborate with previous studies that have shown that the HBV genotype A1 not only that predominant in Kenya but the entire Africa (Kramvis & Kew, 2007). In East Africa region, genotype A and D forms major contributors to HBV infection (Velkov *et al.,* 2018). Genotype HBV/A that were reported in this study is often associated with horizontal mode of transmission with a high risk of progression to chronicity as compared with genotype B and D (Araujo *et al*, 2020). Also, this genotype A is is associated with low HBeAg positivity, hence low replication rate compared with genotype B and D (Lin &Kao, 2015).

Just like the previous studies Mabeya *et al*. (2017), that have shown a repeated confirm of HBV genotype A, this finding confirm of in country transmission of HBV infection a challenge which could be easily controlled. The phylogenetic analysis revealed that it is highly likely that the research individuals had a typical method of conveyance given the genotype`s resemblance sequences used in this investigation. The low HBV transmission rates may be the reason for genotype A's dominance in this study. This observation raises questions about the probable dynamics of transmission or the importance of closely

monitoring HBV genotype surveillance internationally. The confirmation of this genotype in this and its predominance in the country often demands for close monitoring of genetic diversity in this (Sunbul, 2014).

Additionally, the results of interferon and pegylated interferon therapy are also influenced by genotype where genotype A and B respond well to treatment as compared with genotype C and D (Cooksley, 2010). Previously considered a research tool, HBV genotyping has been proposed by a number of researchers and national professional groups as a crucial component to help guide the choice of treatment (Cooksley, 2010). HBV genotype was a powerful predictor of prolonged virological response in patients treated with peg-interferon, according to recent results from multivariate analysis (Chan *et al*., 2003). The ability to identify patients at risk of disease development and to choose the best antiviral treatment is made possible by genotype information of chronic HBV infections (Tanwar & Dusheiko, 2012). While the HBV viremia was not quantified in this investigation, a recurrent HBV flare-related consequence could lead to acute liver failure and severe hepatitis in prenatal and postpartum periods (Rafat, 2016). Considering that majority of study populations were infected with genotype A, treating them with nucleoside analogs throughout the third trimester may reduce the risk of viremia and maternal transmission because these drugs have a greater response to the genotype (Archampong *et al*., 2017). The identification of genotype A raises the possibility that Tharaka Nithi County might harbor HBV genotypes that have been documented in studies.

## 5.2 Conclusions

(i) The study revealed a prevalence of 6.5% (HBV) among pregnant mothers visiting ANC at St. Orsola Hospital in Tharaka Nithi County.

(ii) The risk factors liver problem, HIV, age and education were significantly associated with the risk to HBV infection. However, caesarian section, tattoo, abortion, blood transfusion, occupation, gender, marital status and FGM were not associated with any risk to HBV infection in the study area.

(iii) According to the phylogenetic tree analysis, hepatitis genotype A was the most common genotype causing HBV infection in the study region.

## 5.3 Recommendations

1. The study recommends a need in making prenatal testing for Hepatitis B mandatory requirement to all pregnant women.

2. The study recommends that a target-specific oversight and HBV control procedures to be put into practice on target group with liver disease, HIV, age group 21-30 years and low education level to lessen the disease's impact and stop more infections from spreading.

3. The study recommends for further study of a similar nature to determine the HBV co-infection, the circulating genotypes, and drug resistant strains in the region.

# REFERENCES

Abbas, N., Laboratories, P., and Lahore, C. (2007). Hepatitis B Virus: X Gene. *Research Gate*, 27(84): 127–136.

Afyon, M., & Artuk, C. (2016). Is occult Hepatitis B virus infection with detectable anti-HBs infectious or not? *Middle East Journal of Digestive Diseases*, *8*(2), 147.

Aggarwal, R., & Ranjan, P. (2004). Preventing and treating hepatitis B infection. *Bmj*, *329*(7474), 1080-1086.

Ahmad, A. A., Falla, A. M., Duffell, E., Noori, T., Bechini, A., Reintjes, R., & Veldhuijzen, I. K. (2018). Estimating the scale of chronic hepatitis B virus infection among migrants in EU/EEA countries. *BMC infectious diseases*, *18*, 1-14.

Alter, M. and Mast, E. (1994). The epidemiology of viral hepatitis in the United States. *Gastroenterology Clinics North America*, 23 (**3**): 437–440.

Alter, M. J., Kruszon-Moran, D., Nainan, O. V., McQuillan, G. M., Gao, F., Moyer, L. A. and Margolis, H. S. (1999). The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *New England Journal of Medicine*, 341(**8**), 556-562.

Alter M. (2003). Epidemiology of hepatitis B in Europe and worldwide. *Journal of Hepatology*, 39(**1**):64 - 69.

Anaedobe, C. G., Fowotade, A., Omoruyi, C. E., & Bakare, R. A. (2015). Prevalence, socio-demographic features and risk factors of Hepatitis B virus infection among pregnant women in Southwestern Nigeria. *The Pan African Medical Journal*, *20*, 10-16.

Archampong, T. N., Boyce, C. L., Lartey, M., Sagoe, K. W., Obo-Akwa, A., Kenu, E., ... & Kwara, A. (2017). HBV genotypes and drug resistance mutations in antiretroviral treatment-naive and treatment-experienced HBV–HIV-coinfected patients. *Antiviral therapy*, *22*(1), 13-20.

Archampong, T., Ojewale, O., Bears, K., Chen, Y., Lartey, M., Sagoe, K. W., ... & Kwara, A. (2019). Brief report: relationship between ABCC4 SNPs and hepatitis B virus suppression during tenofovir-containing antiretroviral therapy in patients with HIV/HBV coinfection. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *82*(4), 421-425.

Ayerbe, M.C. and Pe´rez-Rivilla, A. (2000). (ICOVAHB Group) Assessment of long-term efficacy of hepatitis B vaccine. *European Journal of Epidemiology*, 17(**2**): 151–156.

Bagheri Amiri, F., Mostafavi, E., & Mirzazadeh, A. (2016). HIV, HBV and HCV coinfection prevalence in Iran-a systematic review and meta-analysis. *PloS one*, *11*(3), e0151946.

Barth, R. E., Huijgen, Q., Taljaard, J., & Hoepelman, A. I. (2010). Hepatitis B/C and HIV in sub-Saharan Africa: an association between highly prevalent infectious diseases. A systematic review and meta-analysis. *International Journal of Infectious Diseases*, *14*(12), e1024-e1031.

Biobank, U. (2011). Blood Sample Collection, Processing and Transport. *UK Biobank Blood*, 1(1): 1-60.

Bittaye, M., Idoko, P., Ekele, B. A., Obed, S. A., & Nyan, O. (2019). Hepatitis B virus sero-prevalence amongst pregnant women in the Gambia. *BMC infectious diseases*, *19*, 1-8.

Breakwell, L., Tevi-Benissan, C., Childs, L., Mihigo, R., & Tohme, R. (2017). The status of hepatitis B control in the African region. *The Pan African Medical Journal*, *27*(Suppl 3).

Buseri, F. I., Seiyaboh, E., & Jeremiah, Z. A. (2010). Surveying infections among pregnant women in the Niger Delta, Nigeria. *Journal of Global infectious diseases*, *2*(3), 203-211.

Bwogi, J., Braka, F., Makumbi, I., Mishra, V., Bakamutumaho, B., Nanyunja, M., ... & Lewis, R. F. (2009). Hepatitis B infection is highly endemic in Uganda: findings from a national serosurvey. *African health sciences*, *9*(2): 56-63.

Cao, G. W. (2009). Clinical relevance and public health significance of hepatitis B virus genomic variations. *World journal of gastroenterology: WJG*, *15*(46), 5761.

Chambal, L. M., Samo Gudo, E., Carimo, A., Corte Real, R., Mabunda, N., Maueia, C., ... & Antunes, F. (2017). HBV infection in untreated HIV-infected adults in Maputo, Mozambique. *PloS one*, *12*(7), e0181836.

Chameera, E. W. S., Noordeen, F., Pandithasundara, H., & Abeykoon, A. M. S. B. (2013). Diagnostic efficacy of rapid assays used for the detection of hepatitis B virus surface antigen. *Sri Lankan Journal of Infectious Diseases*, *3*(2): 12-45.

Chang, J. and Lewin, S. (2000). Immunopathogenesis of hepatitis B virus infection. *Immunology and Cell Biology*, 85(**1**):16–23.

Chernet, A., Yesuf, A., & Alagaw, A. (2017). Seroprevalence of Hepatitis B virus surface antigen and factors associated among pregnant women in Dawuro zone, SNNPR, Southwest Ethiopia: a cross sectional study. *BMC research notes*, *10*, 1-5.

Coppola, N., Corvino, A. R., De Pascalis, S., Signoriello, G., Di Fiore, E., Nienhaus, A., ... & Lamberti, M. (2015). The long-term immunogenicity of recombinant hepatitis B virus (HBV) vaccine: contribution of universal HBV vaccination in Italy. *BMC infectious diseases*, *15*, 1-7.

Croagh, C. M., & Lubel, J. S. (2014). Natural history of chronic hepatitis B: phases in a complex relationship. *World journal of gastroenterology: WJG*, *20*(30), 10-35.

Croagh, C. M., Desmond, P. V., & Bell, S. J. (2015). Genotypes and viral variants in chronic hepatitis B: A review of epidemiology and clinical relevance. *World journal of hepatology*, *7*(3), 289-290.

Da Villa, G. (2000). Rationale for the infant and adolescent vaccination programmes in Italy. *Vaccine*, *18*, S31-S34.

El-Serag, H. B. (2012). Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology*, *142*(6), 1264-1273.

Elsheikh, R. M., Daak, A. A., Elsheikh, M. A., Karsany, M. S., & Adam, I. (2007). Hepatitis B virus and hepatitis C virus in pregnant Sudanese women. *Virology journal*, *4*, 1-3.

Erdem, M. E. H. M. E. T., Sahin, I., Erdem, A., Gürsoy, R., Yildiz, A., & Güner, H. (1994). Prevalence of hepatitis B surface antigen among pregnant women in a low-risk population. *International Journal of Gynecology & Obstetrics*, *44*(2), 125-128.

Fagan, E. A., Portmann, B., & Williams, R. (1990). HBV-DNA sequences in tumor and nontumor tissue in a patient with the fibrolamellar variant of hepatocellular carcinoma. *Hepatology*, *12*(4), 676-679.

Ferreira, C., Bertoletti, A., Penna, A., Cavalli, A., Valli, A. and Missale, G. (2000). Identification of immunodominant T cell epitopes of the hepatitis B virus nucleocapsid antigen. *Journal of Clinical Investigation*, 88(**1**): 214–222.

Fessehaye, N., Berhane, A., Ahmed, H., Mohamed, S., Tecle, F., Gikunju, J. and Odari, E. (2018). Prevalence of Hepatitis B Virus Infection and Associated Seromarkers among Pregnant Women in Eritrea. *Journal of Human Virology & Retrovirology*, 6(1): 30–38.

Fomulu, N. J., Morfaw, F. L., Torimiro, J. N., Nana, P., Koh, M. V., & William, T. (2013). Prevalence, correlates and pattern of Hepatitis B among antenatal clinic attenders in Yaounde-Cameroon: is perinatal transmission of HBV neglected in Cameroon? *BMC pregnancy and childbirth*, *13*, 1-10.

Franco, E., Bagnato, B., Marino, M. G., Meleleo, C., Serino, L., & Zaratti, L. (2012). Hepatitis B: Epidemiology and prevention in developing countries. *World journal of hepatology*, *4*(3), 74-78.

Guo, H., Jiang, D., Zhou, T., Cuconati, A., Block, T. M., & Guo, J. T. (2007). Characterization of the intracellular deproteinized relaxed circular DNA of hepatitis B

virus: an intermediate of covalently closed circular DNA formation. *Journal of virology*, *81*(22), 12472-12484.

Horvat, R. T. (2011). Diagnostic and clinical relevance of HBV mutations. *Laboratory Medicine*, *42*(8), 488-496.

Hwang, E. W., & Cheung, R. (2011). Global epidemiology of hepatitis B virus (HBV) infection. *North American Journal of Medicine and Science*, *4*(1): 56-54.

Juszczyk, J. (2000). Clinical course and consequences of hepatitis B infection. *Vaccine*, *18*, S23-S25.

Kann, M., Schmitz, A. and Rabe, B. (2007). Intracellular transport of hepatitis B virus. *Journal of Gastroenterology*, 13(**1**):39–47.

Kerubo, G., Khamadi, S., Okoth, V., Madise, N., and Ezeh, A. (2015). Hepatitis B , Hepatitis C and HIV-1 Coinfection in Two Informal Urban Settlements in Nairobi , Kenya. *PLOS ONE Journal*, 268(10): 1–9.

Kew, M. C. (2010). Epidemiology of chronic hepatitis B virus infection, hepatocellular carcinoma, and hepatitis B virus-induced hepatocellular carcinoma. *Pathologie Biologie*, *58*(4), 273-277.

Kolou, M., Katawa, G., Salou, M. and Gozo-akakpo, K. S. (2015). High Prevalence of Hepatitis B Virus Infection in the Age Range of 20-39 Years Old Individuals in Lome. *The Open Virology Journal*, 11(7): 1–7.

Kisangau, E. N., Awour, A., Juma, B., Odhiambo, D., Muasya, T., Kiio, S. N., ... & Lowther, S. A. (2019). Prevalence of hepatitis B virus infection and uptake of hepatitis B vaccine among healthcare workers, Makueni County, Kenya 2017. *Journal of public health*, *41*(4), 765-771.

Kramvis, Anna, and Michael C. Kew. (2007). "Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes." *Hepatology Research* 37: S9-S19.

Kumar, R. and Singh, J. (2011). Genetic Diversity of HBV Genotypes / Subgenotypes and Their Correlation with Disease Progression. *Euroasian Journal of Hepato-Gastroenterology*, 1(2): 53–67.

Kuszewski, K., & Czarkowski, M. P. (2003). Hepatitis B in Poland in 2001. *Przeglad Epidemiologiczny*, *57*(1), 117-123.

Kwange, S. O., Budambula, N. L., Kiptoo, M. K., Okoth, F., Ochwoto, M., Oduor, M., & Kimotho, J. H. (2013). Hepatitis B virus subgenotype A1, occurrence of subgenotype D4,

and S gene mutations among voluntary blood donors in Kenya. *Virus Genes*, *47*, 448-455.

Lamontagne, R. J., Bagga, S., & Bouchard, M. J. (2016). Hepatitis B virus molecular biology and pathogenesis. *Hepatoma research*, *2*, 163-167.

Lavanchy, D. (2004). Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *Journal of viral hepatitis*, 11(**2**):97-107.

Liang, T. J. (2009). Hepatitis B: The Virus and Disease. *Journal of Hepatology*, 49(5): 13–21.

Liu, M., Cao, B. and Zhang, H. (2016). Association of interferon-gamma gene haplotype in the Chinese population with hepatitis B virus infection. *Immunogenetics*, 58 (**11**):859–864.

Locarnini, S. (2004). Relevance of HBV mutations in severity and progression of chronic hepatitis B. *Journal of Gastroenterology and Hepatology*, *19*, S108-S112.

Ly, K. N., Kim, A. A., Umuro, M., Drobenuic, J., Williamson, J. M., Montgomery, J. M., Teshale, E. H. (2016). Prevalence of Hepatitis B Virus Infection in Kenya , 2007. *American Society of Tropical Medicine and Hygiene*, 95(2): 348–353.

Mabeya, S. N., Ngugi, C., Nyamache, A. K. and Lihana, R. (2016). Prevalence of hepatitis B virus infections among HIV infected individuals in Nairobi, Kenya. *East African Medical Journal*, 93(**6**): 41-45.

Mabeya, S. N., Ngugi, C., Lihana, R. W., Khamadi, S. A., & Nyamache, A. K. (2017). Predominance of hepatitis B virus genotype A among treated HIV infected patients experiencing high hepatitis B virus drug resistance in Nairobi, Kenya. *AIDS research and human retroviruses*, *33*(9), 966-969.

MacLachlan, J. H., & Cowie, B. C. (2015). Hepatitis B virus epidemiology. *Cold Spring Harbor perspectives in medicine*, *5*(5), a021410.

Margolis, H. S., Alter, M. J., & Hadler, S. C. (1991, May). Hepatitis B: evolving epidemiology and implications for control. In *Seminars in liver disease* (Vol. 11, No. 02, pp. 84-92).

Mbaawuaga, E.M., Ebenebeaku, MNO., Okopi, J.A. and Damen, J.G. (2010). Hepatitis B virus (HBV) infection among pregnant women in Makurdi, Nigeria. *Africa Journal of Biomedical Research*, 11(**1**):155-159.

Mbaawuaga, E. M., Enenebeaku, M., & Okopi, J. (2008). Hepatitis b virus (hbv) infection among pregnant women in makurdi, nigeria. *African Journal of Biomedical Research*, *11*(2): 22-24.

McMahon, B. J., Alward, W. L., Hall, D. B., Heyward, W. L., Bender, T. R., Francis, D. P., & Maynard, J. E. (1985). Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. *Journal of infectious diseases*, *151*(4), 599-603.

Menendez, C., Sanchez-Tapias, J. M., Kahigwa, E., Mshinda, H., Costa, J., Vidal, J., ... & Alonso, P. L. (1999). Prevalence and mother-to-infant transmission of hepatitis viruses B, C, and E in Southern Tanzania. *Journal of medical virology*, *58*(3), 215-220.

Milich, D.R, and Liang, T.J. (2003). Exploring the biological basis of hepatitis B e antigen in hepatitis B virus infection. *Hepatology*, 38(**5**):1075–1086.

Muriuki, B. M., Gicheru, M. M., Wachira, D., Nyamache, A. K. and Khamadi, S. A. (2013). Prevalence of hepatitis B and C viral co-infections among HIV-1 infected individuals in Nairobi, Kenya. *Biomedical research notes,* 6(**1**): 363-367.

Mutagoma, M., Balisanga, H., Malamba, S. S., Sebuhoro, D., Remera, E., Riedel, D. J., ... & Nsanzimana, S. (2017). Hepatitis B virus and HIV co-infection among pregnant women in Rwanda. *BMC infectious diseases*, *17*, 1-7.

Mutuma, G. Z., Mbuchi, M. W., Zeyhle, E., Fasana, R., Okoth, F. A., Kabanga, J. M., ... & Kaiguri, P. (2011). Prevalence of Hepatitis B Virus (HBV) surface antigen and HBVassociated hepatocellular carcinoma in Kenyans of various ages.

Mwangi, J., Nganga, Z., Songok, E., Kinyua, J., Lagat, N., Muriuki, J., ...& Ichimura, H. (2008). Molecular genetic diversity of hepatitis B virus in Kenya. *Intervirol*, *51*(6), 417-21.

Nabulsi, M. M., Araj, G. F., Nuwayhid, I., Ramadan, M., & Ariss, M. (1997). Prevalence of hepatitis B infection markers in Lebanese children: the need for an expanded programme on immunization. *Epidemiology & Infection*, *126*(2), 285-289.

Nagu T, Bakari M. and Matee M. (2008). Hepatitis A, B and C viral co-infections among HIV-infected adults presenting for care and treatment at Muhimbili National Hospital in Dar es Salaam, Tanzania. *BMC Public Health*, 8(**1**): 416-418.

Naing, L., Winn, T. B. N. R., & Rusli, B. N. (2006). Practical issues in calculating the sample size for prevalence studies. *Archives of orofacial Sciences*, *1*, 9-14.

Ngaira, J., Kimotho, J. and Mirigi, I. (2016). Prevalence, awareness and risk factors associated with Hepatitis B infection among pregnant women attending the antenatal

clinic at Mbagathi District Hospital in Nairobi, Kenya. *Pan African Medical Journal*, 8688(315): 1–7.

Ochwoto, M., Chauhan, R., Gopalakrishnan, D., Chen, C. Y., Okoth, F., Kioko, H., ... & Kramvis, A. (2013). Genotyping and molecular characterization of hepatitis B virus in liver disease patients in Kenya. *Infection, genetics and evolution*, *20*, 103-110.

Ochwoto, M., Kimotho, J. H., Oyugi, J., Okoth, F., Kioko, H., Mining, S., ... & Osiowy, C. (2016). Hepatitis B infection is highly prevalent among patients presenting with jaundice in Kenya. *BMC infectious diseases*, *16*, 1-14.

Okoth, F., Mbuthia, J., Gatheru, Z., Murila, F., Kanyingi, F., Mugo, F., &Wanjuki, N. (2006). Seroprevalence of hepatitis B markers in pregnant women in Kenya.*East African medical journal*, *83*(9), 47-78.

Onakewhor, E. Offor, FE Okonofua, J. (2001). Maternal and neonatal seroprevalence of hepatitis B surface antigen (HBsAg) in Benin City, Nigeria. *Journal of Obstetrics and Gynaecology*, *21*(6), 583-586.

Opaleye, O. O., Oluremi, A. S., Atiba, A. B., Adewumi, M. O., Mabayoje, O. V., Donbraye, E., ... & Olowe, O. A. (2014). Occult hepatitis B virus infection among HIV positive patients in Nigeria. *Journal of tropical medicine*, *2014*(1), 79-121.

Osiowy, C., Kaita, K., Solar, K., & Mendoza, K. (2010). Molecular characterization of hepatitis B virus and a 9-year clinical profile in a patient infected with genotype I. *Journal of medical virology*, *82*(6), 942-948.

Ott, J. J., Stevens, G. A., Groeger, J., & Wiersma, S. T. (2012). Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine*, *30*(12), 2212-2219.

Pennap, G. R., Osanga, E. T., & Ubam, A. (2011). Seroprevalence of hepatitis B surface antigen among pregnant women attending antenatal clinic in federal medical center Keffi, Nigeria.

Perlman, D.H., Berg, E.A., O'Connor, P.B., Costello, C.E. and Hu, J. (2005). Reverse transcription-associated dephosphorylation of hepadnavirus. *Proceedings of the National Academy of Science of the United States of America*, 102(**25**): 9020– 9025.

Pourkarim, M. R., Amini - Bavil - Olyaee, S., Kurbanov, F., Van Ranst, M. and Tacke, F. (2014). Molecular identification of hepatitis B virus genotypes/sub-genotypes: *World Journal of Gastroenterology*, 20(**23**): 7152-7168.

Rabe, B., Delaleau, M., Bischof, A., Foss, M., Sominskaya, I., Pumpens, P., ... & Kann, M. (2009). Nuclear entry of hepatitis B virus capsids involves disintegration to protein dimers followed by nuclear reassociation to capsids. *PLoS pathogens*, *5*(8), e1000563.

Raimondo, G, Pollicino, T, Cacciola, I, & Squadrito, G. (2006). Occult hepatitis B virus infection. *J Hepatol*,*46*,60–170.

Rafat, D., & Hakim, S. Hepatitis B in Pregnancy: Challenges and Solutions.
Ranjbar, R., Davari, A., Izadi, M., Jonaidi, N., & Alavian, S. M. (2011). HIV/HBV co-infections: epidemiology, natural history, and treatment: a review article. *Iranian Red Crescent Medical Journal*, *13*(12), 855-860.

Reesink, H. W., Reesink-Brongers, E. E., Lafeber-Schut, B. J., Kalshoven-Benschop, J., & Brummelhuis, H. G. (1980). Prevention of chronic HBsAg carriership in infants of HBsAg-positive mothers by hepatitis B immunoglobulin. *Acta haematologica Polonica*, *11*(2), 79-81.

Robinson, W. S. (1999). Hepatitis B viruses. General features (human). *Encyclopedia of virology. London: Academic Press Ltd*, 554-69.

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

Song, J. E. (2016). Diagnosis of hepatitis B. *Annals of translational medicine*, *4*(18): 34-54.

Stroffolini, T., Mele, A., Tosti, M. E., Gallo, G., Balocchini, E., Ragni, P., ... & Moiraghi, A. (2000). The impact of the hepatitis B mass immunisation campaign on the incidence and risk factors of acute hepatitis B in Italy. *Journal of hepatology*, *33*(6), 980-985.

Sung, P. S., Park, D. J., Kim, J. H., Han, J. W., Lee, E. B., Lee, G. W., ... & Yoon, S. K. (2019). Ex vivo detection and characterization of hepatitis B virus-specific CD8+ T cells in patients considered immune tolerant. *Frontiers in Immunology*, *10*, 13-19.

Sunbul, M. (2014). Hepatitis B virus genotypes : Global distribution and clinical importance. *World Journal of Gastroenterology*, *2*0(18): 5427–5434.

Tai, P.C., Suk, F.M., Gerlich, W.H., Neurath, A.R. and Shih, C. (2002). Hypermodification and immune escape of an internally deleted middle-envelope (M) protein of frequent and predominant hepatitis B virus variants. *Virology*, 292(**1**):44–58.

Tallo, T. (2008). Molecular Epidemiology of Hepatitis A,B and C in Estonia. *Swedish Institute for Infectious Disease and Control*, 6(16): 1–85.

Tanaka, Y., Hasegawa, I., Kato, T., Orito, E., Hirashima, N., Acharya, S. K., ... & Mizokami, M. (2004). A case-control study for differences among hepatitis B virus infections of genotypes A (subtypes Aa and Ae) and D. *Hepatology*, *40*(3), 747-755.

Terrault, N.A. and Vyas, G. (2003). Hepatitis B immune globulin preparations and use in liver transplantation.*Clinical Liver Disease*, 7(**3**):537–550.

Thimme, R., Spangenberg, H. C., & Blum, H. E. (2005). Hepatitis B or hepatitis C and human immunodeficiency virus infection. *Journal of hepatology*, *42*(1), S37-S44.

Thio, C. L. (2009). Hepatitis B and human immunodeficiency virus coinfection. *Hepatology*, 49(**5**):138–145.

Velkov, S., Ott, J. J., Protzer, U., & Michler, T. (2018). The global hepatitis B virus genotype distribution approximated from available genotyping data. *Genes*, *9*(10), 495-498.

Wambani, R. J., Ogola, P. E., Makori, A. W., Nyamai, D. W., Lihana, R., & Burugu, M. W. (2015). Hepatitis B and C Co-Infections among HIV-1 Infected Patients Attending the Academic Model Providing Access to Healthcare Clinic, Kenya, 2014. *J Infect Dis Diagn*, *1*(102), 20-30.

Wandeler, G., Mulenga, L., Vinikoor, M. J., Kovari, H., Battegay, M., Calmy, A., ... & Rauch, A. (2016). Liver fibrosis in treatment-naive HIV-infected and HIV/HBV co-infected patients: Zambia and Switzerland compared. *International journal of infectious diseases*, *51*, 97-102.

Webale, M. K., Budambula, V., Lihana, R., Musumba, F. O., Nyamache, A. K., Budambula, N. L., ... & Were, T. (2015). Hepatitis B virus sero-profiles and genotypes in HIV-1 infected and uninfected injection and Non-injection drug users from coastal Kenya. *BMC infectious diseases*, *15*, 1-8.

WHO. (2000). *Hepatitis A. Department of Communicable Disease Surveillance and Response* (Vol. 7). Geneva.

World Health Organization. (2002). Hepatitis B vaccines. *Weekly Epidemiological Record*, 40 (**84**):405- 420.

WHO. (2009). The Global Prevalence of Hepatitis A Virus Infection and Susceptibility: A Systematic Review. *World Health Organization*, 10(1): 7–431.

World Health Organization (WHO) (2010). Hepatitis B virus Fact sheet N° 204. Retrieved from http://www.who.int/en/news-room/fact-sheets/detail/hepatitis-b

WHO. (2015). Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. *World Health Organization*, 1(8): 1–127.

WHO. (2017). Hepatitis B and C in the Spotlight. *Pan African Health Organisation, 715(1): 1–78.*

World Health Organization. (2018). *Global hepatitis report 2018*. World Health Organization.

WHO. (2020). *Global hepatitis report, 2020*. Geneva.

Wu, J. F., Chiu, Y. C., Chang, K. C., Chen, H. L., Ni, Y. H., Hsu, H. Y., & Chang, M. H. (2016). Predictors of hepatitis B e antigen-negative hepatitis in chronic hepatitis B virus-infected patients from childhood to adulthood. *Hepatology*, *63*(1), 74-82.

Wu, T., Chang, L., Wu, L., & Yuan, Y. F. (2015). IL-6 plays a crucial role in HBV infection. *Journal of clinical and translational hepatology*, *3*(4), 27-40.

Wurie, I. M., Wurie, A. T., & Gevao, S. M. (2005). Sero-prevalence of hepatitis B virus among middle to high socio-economic antenatal population in Sierra Leone. *West African Journal of Medicine*, *24*(1), 18-20.

Wylie, K. M., Wylie, T. N., Buller, R., Herter, B., Cannella, M. T., & Storch, G. A. (2018). Detection of viruses in clinical samples by use of metagenomic sequencing and targeted sequence capture. *Journal of clinical microbiology*, *56*(12), 10-1128.

Xu, D., Yan, Y., Choi, B.C., Xu, J., Men, K. and Zhang, J. (2002). Risk factors and mechanism of transplacental transmission of hepatitis B virus: a case-control study. *Journal of Medical Virology*, 67(**1**):20–26.

Yimet, H. J., & Lok, A. S. F. (2006). Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. *Hepatology*, *43*(S1), S173-S181.

Zamani, A., Shajari, H., & Sedighy, I. (2001). Study on the ef ficacy of recombinant hepatitis B vaccine in Iranian infants. *Medical Journal of The Islamic Republic of Iran (MJIRI)*, *14*(4), 347-349.

Zampino, R., Boemio, A., Sagnelli, C., Alessio, L., Adinolfi, L. E., Sagnelli, E., Adinolfi, L. E. (2015). 2015 Advances in Hepatitis B virus hepatitis B virus burden in developing countries. *World Journal of Gastroenterology*, 21(42):11941–11953.

Zanetti, A. R., Van Damme, P., & Shouval, D. (2008). The global impact of vaccination against hepatitis B: a historical overview. *Vaccine*, *26*(49), 6266-6273.

Zenebe, Y., Mulu, W., Yimer, M. and Abera, B. (2014). Sero-prevalence and risk factors of hepatitis B virus and human immunodeficiency virus infection among pregnant women in Bahir Dar city , Northwest Ethiopia : a cross sectional study. *BMC Infectious Diseases*, 14(118): 1–7.

Zhao, J., Zhang, Z., Luan, Y., Zou, Z., Sun, Y., Li, Y., ... & Wang, F. S. (2014). Pathological functions of interleukin-22 in chronic liver inflammation and fibrosis with

hepatitis B virus infection by promoting T helper 17 cell recruitment. *Hepatology*, *59*(4), 1331-1342.

Zhu, Q., Yu, G., Yu, H., Lu, Q., Gu, X. and Dong, Z. (2003). A randomized control trial on interruption of HBV transmission in uterus. *Chinese Medical Journal (Engl)*, 116(**5**):685–687.

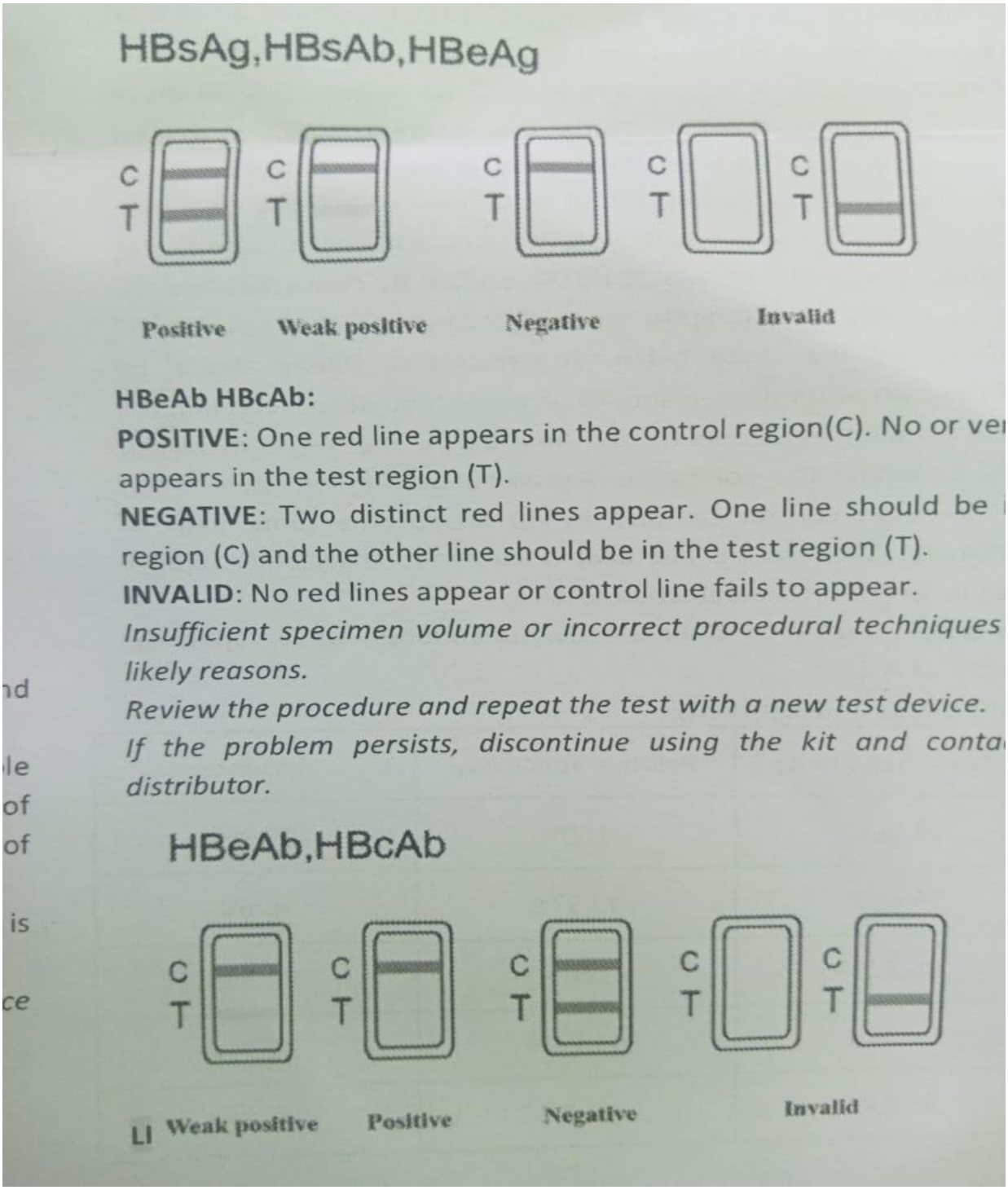
Zu, J., Zhuang, G., Liang, P., Cui, F., Wang, F., Zheng, H., & Liang, X. (2017). Estimating age-related incidence of HBsAg seroclearance in chronic hepatitis B virus infections of China by using a dynamic compartmental model. *Scientific Reports*, *7*(1): 291-298.

# LIST OF APPENDICES

## Appendix A -Hepatitis B markers

## Appendix B- Samples Results

**Appendix C- Markers Interpretation Chart**



## Appendix D- Study Questionnaire

**TITLE: GENETIC DIVERSITY, PREVALENCE AND RISK FACTORS ASSOCIATED WITH HBV INFECTION AMONG WOMEN SEEKING ANC IN ST. ORSOLA CATHOLIC MISSION HOSPITAL**

**Questionnaire code**…….

**1. Your age.....**

**2. Your status**☐ Married ☐ Single

**3. Your occupations** Professional ☐ Casual ☐ Business☐

**4. Level of Education** No Education-Primary ☐ Secondary-Tertiary☐

**5. Risk Factors Information**

a) Do you have any blood transfusion history? ☐ Yes ☐ No

b) Do you have any history of dental or dialysis procedure? ☐ Yes ☐ No

c) Do you have any tattoo on your body? ☐ Yes ☐ No

d) Do you have any history of Caesarean section? ☐ Yes ☐ No

e) Do you have a history of liver problem or jaundice? ☐ Yes ☐ No

f) Do you have any history of Abortion or Miscarriage? ☐ Yes ☐ No

g) Do you have any history of Female circumcision? ☐ Yes ☐ No

**6. Screening Results: HBV**, Positive ☐ Negative ☐

**7. HIV status** R☐ NR☐

## Appendix E-Consent Form

**Form Consent Form for Hospital Based Study**

**Consent to Participate in a Research Study**

**RESEARCH TITLE: GENETIC DIVERSITY, PREVALENCE AND RISK FACTORS ASSOCIATED WITH HBV INFECTION AMONG WOMEN SEEKING ANC IN ST. ORSOLA CATHOLIC MISSION HOSPITAL**

Health Facility: _____

Study Number: _____ Date of Hospital Visit: _____

**Purpose**

The objective of the study is to investigate the genetic diversity, prevalence and risk factors associated with HBV infection among women seeking ANC in Njabini hospital. I kindly request you to volunteer and participate in the research study. A total of 130 patients will be included in this study.

**Procedures**

If you consent to participate in the study, you will be requested for a small sample of blood. A blood sample of 5ml will be taken from the arm (venipuncture). The ANC profile will be conducted, and the remaining blood sample will be screened for the presence of HBsAg.

**Risk to Participant**

A sample of blood will be obtained from your arm using a needle and syringe by a trained and qualified medical laboratory technologist. There is a minimal risk of you being injured at the time of blood sample collection; however, there is possibility of mild pain and discomfort during removal of blood from the arm but this feeling will subside and pass faster.

**Potential Benefits**

You may benefit in participating in this study by receiving information on the presence or absence of HBV infections from the screened blood samples. For those who will be found to be infected, a referral will be made for further management. However, in identification of actual HBV genotypes, the person will indirectly benefit since the information will be used in redesigning of prevention and screening of the community, in order to control further spread of this infection within the community.

**Care and protection of research participant`s**

The skin on the puncture site (vein) will be disinfected using sterile 70% alcohol pads. You will feel minimal pain; needle and syringes used in the collection of blood will be safe and have never been used by anybody else.

**Cost and Compensation**

Participants do not incur any cost during the study, however, medical care will be offered to you in case of injury during the study.

**Protection of research participants Confidentiality**

During the interview, high level of confidentiality will be maintained. All participants will be coded and no name will be used for references. The interviews will be conducted in an excluded room while administering the questionnaire including specimen collection.

**Voluntary Participation**

A participant can decide not to take part in this study without any negative consequence.

In the event of a change in decision, and you would want to withdraw from the study, you can contact Joseph Kamau (PI) Tel. 0719288233, Dr. Anthony Kebira (Lead Supervisor) Tel. 0735757560 or ethical review committee secretariat Kenyatta university through, secretariat.kuer@ku.ac.ke, chairman.kuerc@ku.ac.ke

Your participation rights enquiries should be addressed to; the secretary, institute of research science and technology P.O.Box 43844 GPO Nairobi, Kenya. Tel 254-20-8710901

**Consent**

The purpose of the study has been interpreted to me in a simple language and to the level of my understanding. I have been inspirited to enquire more about the research study. My signature below will signify my voluntary consent to participate in this research study.

Name _____Age: _____Sex: _____ Signature: _____

Date_____

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE
P.O BOX 43844-00100,
Nairobi.

## Appendix F- Graduate School Approval Letter

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Internal Memo

FROM: Dean, Graduate School

DATE: 4th October, 2018

TO: Mr. Njuguna Joseph Kamau
C/o Department of Microbiology

REF: I56/33560/2015

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

We acknowledge receipt of your Research Proposal after fulfilling recommendations raised by the Graduate School Board of 22nd August, 2018.

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking Forms per semester. The form has been developed to replace the Progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

JOHN M. ODONGI
FOR: DEAN, GRADUATE SCHOOL

CC. Chairman, Department of Microbiology

Supervisors:

1. Dr. Anthony Kebira
   C/o Department of Microbiology
   Kenyatta University

2. Dr. James Nonoh
   C/o Department of Microbiology
   Kenyatta University

JMO/ewv

# Appendix G- Ethical Approval Letter

**KENYATTA UNIVERSITY**
**CENTRE FOR RESEARCH ETHICS AND SAFETY**

**Fax: 8711242/8711575**
**Email: chairman.kuerc@ku.ac.ke**
**Nairobi, 00100**

**P. O. Box 43844,**

Website: www.ku.ac.ke
Our Ref: **KU/ERC/APPROVAL/VOL.1**

Tel: 8710901/12

Date: 17th /01/2022

Njuguna Joseph Kamau
P.O Box 43844, 00100
Nairobi.

Dear Mr. Kamau,

**APPLICATION NUMBER: PKU/2238/I1382 GENETIC DIVERSITY PREVALENCE AND RISK FACTORS ASSOCIATED WITH HEPATITIS B INFECTION AMONG ANTENATAL WOMEN IN ST. ORSOLA HOSPITAL, THARAKA COUNTY KENYA**

This is to inform you that ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** has reviewed and approved your above research proposal. Your application approval number is **PKU/2238/I1382.** The approval period is **17th *01/2022***

***to 12th/01/2023.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.

# Appendix H- NACOSTI Approval Letter

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **679541**

Date of Issue: **22/February/2022**

## RESEARCH LICENSE

**This is to Certify that Mr.. joseph kamau njuguna of Kenyatta University, has been licensed to conduct research in Tharaka-Nithi on the topic: GENETIC DIVERSITY, PREVALENCE AND RISK FACTORS ASSOCIATED WITH HEPATITIS B INFECTION AMONG ANTENATAL WOMEN IN ST.ORSOLA HOSPITAL, THARAKA COUNTY KENYA for the period ending : 22/February/2023.**

License No: **NACOSTI/P/22/15946**

**679541**

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

## Appendix I- Hospital Approval Letter

Orsola Catholic Hospital-Matiri
Catholic Diocese of Meru
P.o Box 41 (60200) Matiri Meru-TharakaNithi county, Kenya, E.Africa
Tel. +254,703983686,+ 254 733 404 161 +254700433220

**Ref. ERC-ST.OCMH/MSc/VOL.1/12** **Date: 10TH MAY, 2022**

NJUGUNA JOSEPH KAMAU

**RE: GENETIC DIVERSITY, PREVALENCE AND RISK FACTORS ASSOCIATED WITH HEPATITIS B INFECTION AMONG WOMEN ATTENDING ANTENATAL CARE AT ST. ORSOLA CATHOLIC MISSION HOSPITAL, THARAKA NITHI COUNTY, KENYA**

This is to inform you that the Ethics Review Committee reviewed the document submitted and is satisfied that the issues raised at the meeting of Ethics Review Committee on 10th May, 2022 have been adequately addressed.

The study is granted approval for implementation effective from the date of this letter. Please note that authorization to conduct this study will automatically expire on 10th May, 2023. If you plan to continue with data collection and analysis beyond this date, please submit an application for continuing approval to the ethical Review Committee-ST. Orsola Catholic Mission Hospital in appropriate time.

Any unanticipated problem resulting from the implementation of this protocol should be brought to the attention of ERC-ST.OCMH.

The ERC-ST.OCMH looks forward to receiving a summary of the research finding upon completion of the study to be part of the data base to be consulted when processing related researches to minimize duplication.

ST. ORSOLA HOSPITAL - MATIRI
10 MAY 2022
P.O. BOX 41 - 60200, MERU

**Rev. FR Gerrard Njeru Emilio**

Hospital C.E.O & Administrator

## Appendix J: Isolate Sequences with accession number

**PQ340377 | Isolate 11 HBPRF3| Kenya**

GWWYYWWAAASCKTCSMAAARGCATGGGGAGAATCTTTCTGTTCCAAACCC
TCTGGGATTCTTTCCCGATCATCAGTTGGACCCTGCATTCGGAGCCAATTCAA
ACAATCCAGATTGGGACTTCAACCCCGTCAAGGATCACTGGCCACAAGCCAG
GCAGGTAGGAGTGGGAGCCTTCGGGCCAGGGTTCACTCCCCCACACGGAGGT
GTTTTGGGGTGGAGCCCTCAGGCTCAGGCATAATGGCTACAGTGCCAGCACT
TCCTCCTCCTGCCTCCACCAATCGGCAGKCAGGAAGGCAGSCTACTCCCATCT
TCCACCTCTAAAGACAGCCATCCTCRGCCATGCAGGGG

**PQ340378 | Isolate 12 HBPRF3| Kenya**

TTAMCMGTCATCCTCTGGGATCTTTCCCGACCACCAGTTGGATCCAGCCTTCA
GAGCAAACACCACAAATCCAGATTGGGACTTCAATCCCAACAAGGACACCTG
GCCAGACGCCAACAAGGTAGGAGCYGGAGCATTCGGGCTGGGATTCACCCC
ACCGCACGGAGGCCTTTTGGGGTGGAGCCCTCAGGCTCAGGGCATAATACAA
ACTTTGCCAGCAAATCCGCCTCCTGCCTCTACCAATCGCCAGTCRGGAAGGC
AGCCTACTCCGCTGTCTCCACCTCTGAGAAACACTCATCCTCAGCCATGCAGT
GGA

**PQ340379 | Isolate 14 HBPRF3| Kenya**

GAATCAAGCTCGCAAGGCATGGGGAGAACCTTTCTGTTCCCAACCCTCTGGG
ATTCTTTCCCGATCATCAGTTGGACCCTGCATTCGGAGCCAATTCAAACAATC
CAGATTGGGACTTCAACCCCATCAAAGACCATTGGCCACAAGCCAACCAGGT
AGGAGTGGGAGCATTCGGGCCAGGGTTCACTCCCCCACACGGAGGTGTTTTG
GGGTGGAGCCCTCAGGCTCAGGGCATATTGGCTACAGTGCCAGCAGTTCCTC
CTCCTGCCTCCTCCATCGGCAGTCAGGAAGGCGCCTACTCCCATCTCTCCCCT
CTAGGGAAGCTCTCAGMATGAKGGAACACGTGCKCAATTTGAGCCAATKGG
GCTGATTTCGGGCGCACKTTGGGAAACACCGGTCATATGTCCAAAACYCACC
CCGGGTGGAACTGTCCAAAYAAAAWCTTSGGTCCAAGKKTTGGGTAAGGGG
GTTTTTTGAASSTTTAGGTAGAAAACRAATTCGGAGSCTTAATTSGAGGGGGG
CCGGGTGTTGGCAAAATCAWCACAACTTAAKAAYCCRCCTGGGTGCCTTTSC
CGGGAATCCGGGGGCKCCCGGTTTTCTTTCCTTTTCCCCGGSAWACAGAGGA
ATT

**PQ340380 | Isolate 15 HBPRF3| Kenya**

GGMTCAACCTCGCAAGGCATGGGGAGAATCTTTCTGTTCCCAACCCTCTGGG
ATTCTTTCCCGATCATCAGTTGGACCCTGCATTCGGAGCCAATTCAAACAATC
CAGATTGGGACTTCAACCCCATCAAGGACCACTGGCCACAAGCCAATCAGGT
AGGAGTGGGAGCCTTCGGGCCAGGGTTCMCTCCCCMMCGGAGGTGTTTTGG
GGKGGRGCCCTCAGGCTCAGGCATAATGGSTACMGTGCCACACTTCYYCYYC
TKSCTCMCCAATCRGRRKCAGRARGMRCCMCTYCMTYTYTYCMCYYAAARC
WGKCATSCTCAGGCMWGMRTGGR

**PQ340381 | Isolate 16 HBPRF3| Kenya**

TACTCAGCTCGCAAGGCATGGGGAGAATCTTTCTGTTCCCAACCCTCTGGGAT
TCTTTCCCGATCATCAGTTGGACCCTGCATTCGGAGCCAATTCAAACAATCCA
GACTGGGACTTCAACCCCATCAAGGATCACTGGCCACAAGCCAATCAGGTAG
GAGTGGGAGCATTCGGCCCAGGGTTCACTCCCCCACACGGAGGTGTTTTGGG
GTGGAGCCCTCAGGCTCAGGGCATATTGGCTACATTGCCAACAGTTCCTCCTC
CTGCCTCCACCAAACGGSAGTCAGGAAGGCAGCCTACTCCCATCTCTCCCCTC
TAAGAGAAGTCTCCTCAGCCATGCATGGA

**PQ340382 | Isolate 17 HBPRF3| Kenya**

TKMMTCAACTCGCAAGGCATGGGGAGAACCTTTCTGTTCCCAACCCTCTGGG
ATTCTTTCCCGATCATCAGTTGGACCCTGCATTCGGAGCCAATTCAAACAATC
CAGATTGGGACTTCAACCCCATCAAGGACCATTGGCCACAAGCCAACCAGGT
AGGAGTGGGAGCATTCGGGCCAGGGTTCACTCCCCCACACGGAGGTGTTTTG
GGGTGGAGCCCTCAGGCTCAGGGCATATTGGCTACAGTGCCAGCAGTTCCTC
CTCCTGCCTCATCCAATCGGCAGTCAGGAAGGCAGCCTACTCCCATCTCTCCA
CCTCTAAAGACAGTCATCCTCAGGMCATGCATGGA

**PQ340383 | Isolate 18 HBPRF3| Kenya**

CMTWAACTCGCAARGCATGGGGAGAACCTTTCTGTTCCCAACCCTCTGGGAT
TCTTTCCCGATCATCAGTTGGACCCTGCATTCGGAGCCAATTCAAACAATCCA
GATTGGGACTTCAACCCCATCAAGGACCACTGGCCACAAGCCAACCAGGTAG
GAGTGGGAGCATTCGGGCCAGGGTTCACTCCCCCACACGGAGGTGTTTTGGG
GTGGAGCCCTCAGGCTCAGGGCATATTGGCTACAGTGCCAGCAGTTCCTCCT
CCTGCCTCCTCCATCGGARWCAGGARGSAGCCTACTCCCATCTCTCCACTCTA
CGGGACATCATCCTCAGGCWTGCAKGGA

**PQ340384 | Isolate 19 HBPRF3| Kenya**

TRWWCAAACTCGCAGGCATGGGGAGAACCTTTCTGTTCCCAACCCTCTGGGA
TTCTTTCCCGATCATCAGTTGGACCCTGCATTCGGAGCCAATTCAAACAATCC

AGATTGGGACTTCAACCCCATCAAGGACCACTGGCCACAAGCCAACCAGGTA
GGAGTGGGAGCATTCGGGCCAGGGTTCACTCCCCCACACGGAGGTGTTTTGG
GGTGGAGCCCTCAGGCTCAGGGCATATTGGCTACAGTGCCAGCAGTTCCTCC
TCCTGCCTCCTCCAATCGGCAGTCAGGAAGGCAGCCTACTCCCATCTCTCCAC
CTCTACGGGACAGTCATCCTCAGCCATGCAGTGGA

**PQ340385 | Isolate 21 HBPRF3| Kenya**

TKTYCAAACTCGCAAGGCATGGGGAGAACCTTTCTGTTCCCAACCCTCTGGG
ATTCTTTCCCGATCATCAGTTGGACCCTGCATTCGGAGCCAATTCAAACAATC
CAGATTGGGACTTCAACCCCATCAAGGACCATTGGCCACWAGCCAACCAGGT
AGGAGTGGGAGCATTCGGGCCAGGGTTCACTCCCCCACACGGAGGTGTTTTG
GGGTGGAGCCCTCAGGCTCAGGGCATATTGGCTRCAGTGCCAGCAGTTCCTC
CTCCTGCCTCCTCCAATCGGCAGTCAGGAAGGCAGCCTACTCCCATCTCTCCA
CCTCTAAGAGACAGCCATCCTCAGCCATGCAGTGGA

**PQ340386 | Isolate 22 HBPRF3| Kenya**

TKAYCAACTCGCAAGGCATGGGGAGAACCTTTCTGTTCCCAACCCTCTGGGA
TTCTTTCCCGATCATCAGTTGGACCCTGCATTCGGAGCCAATTCAAACAATCC
AGATTGGGACTTCAACCCCATCAAGGACCATTGGCCACAAGCCAACCAGGTA
GGAGTGGGAGCATTCGGGCCAGGGTTCACTCCCCCACACGGAGGTGTTTTGG
GGTGGAGCCCTCAGGCTCAGGGCATATTGGCTACAGTGCCAGCAGTTCCTCC
TCCTGCCTCCTCCAATCGGCAGTCAGGAAGGCAGCCTACTCCCATCTCTCCAC
CTCTAAGAGACAGCCATCCTCRGSCATGCAGTGGA

**PQ340387 | Isolate 25 HBPRF3| Kenya**

TGAYCAACTCGCAAGGCATGGGGAGAACCTTTCTGTTCCCAACCCTCTGGGA
TTCTTTCCCGATCATCAGTTGGACCCTGCATTCGGAGCCAATTCAAACAATCC
AGATTGGGACTTCAACCCCATCAAGGACCACTGGCCACAAGCCAACCAGGTA
GGAGTGGGAGCATTCGGGCCAGGGTTCACTCCCCCACACGGAGGTGTTTTGG
GGGGGAGCCCTCAGGCTCAGGGCATATTGGCKACAGTGCCAGCAGTTCCTCC
TCCTGCCTCCTCCAATCGGCAGTCAGGAAGGCAGCCTACTCCCATCTCTCCAC
CTCTAAAGAAGTCATCCTCAGCATGCAGTGGAACCATTTTTGTTTGATTAATT
TCTTTTTGTGTTTTTTTTCTTTTGGGATCATKTAACCCAAAACCGGCCGATTGC
TTCAAACCAMGA