SEROPREVALENCE, GENETIC DIVERSITY, AND DRUG RESISTANCE OF HEPATITIS B VIRUS AMONG GRAVID WOMEN ATTENDING ANTENATAL CLINIC IN SARETHO HEALTH CENTRE IN DADAAB - GARISSA, KENYA

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REGISTRATION NO. 156/CE/37755/2016

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

OCTOBER, 2025

DECLARATION

I declare that the work presented in this thesis is my original work and has not been submitted for a degree or examination in any other University or for the obtention of any other award.

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DEDICATION

I dedicate this thesis to my wife Emily Rama and son my son Patterson for moral support, prayers and endless love throughout the study period.

ACKNOWLEDGEMENT

I wish to acknowledge my supervisors; Prof. Anthony Kebira and Dr. Regina Ntabo for their support, guidance, encouragement and patience. They sacrificed their valuable time to ensure this work was a success.I am also immensely grateful for the management of Saretho H/C and Dadaab Subcounty at large for granting me permission to conduct this study. Finally, I would like to express my deep appreciation to Celestine Kemunto from CMB Genomics Lab, Gideon Mutemi from InqabaBiotec and all my friends who contributed awesome ideas towards completion of this work.

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ACRONYMS AND ABBREVIATIONS

ADV	AdefovirDipivoxil
ANC	Antenatal Clinic
Anti HBc	Hepatitis B core Antibody
Anti HBe	Hepatitis B pre-core Antibody
Anti HBs	Hepatitis B Surface Antibody
ccc DNA	Circular Closed DNA
CD4	Clusters of differentiation 4
CTL	Cytotoxic T Lymphocyte
CD4	Clusters of differentiation 8
DNA	Deoxyribonucleic acid
ds DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
ETV	Entecavir
FGM	Female Genital Mutation
GCRH	Garissa County Referral Hospital
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B e antigen
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HIV	Human Immunodeficiency Virus
IFN	Interrferon
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LdT	Telbivudine
MEGA	Molecular Evolution Genetics Analysis
mRNA	Messenger Rebonucleic Acid
NACOSTI	National Commission for Science, Technology and Innovation
NAS	Nucleoside/Nucleotide analogues
NTCP	liver-specific receptor protein
ORFs	Overlapping Open Reading Frames
PCR	Polymerase Chain Reaction
Peg-IFN	Peglylated Interferon
Pg RNA	PregenomicRebonucleic Acid
rc DNA	Relax Circular DNA
RPM	Revolution Per Minute
RT	Reverse Transcriptase
rtA181T	Shared Pathway
rtL180M	Entecavir resistance Pathway
rtM204V/I	L -Nucleoside Pathway
rtN236T	Acyclic Phosphate Pathway
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
TDF	TenofovirDisoproxl Fumarate
TLRs	Toll-like receptors

WHO	World Health Organisation
YMDD	Lamivudine Resistant Mutations
3TC	Lamivudine

ABSTRACT

The Hepatitis B Virus remains one of the global infectious diseases of public health concern. It is estimated that 2 billion people are infected worldwide with about 1.2 million mortality being reported yearly. Gravid women are considered a vulnerable group due to their immune suppression hence posing a risk for vertical transmission to their newborns. However, HBV disease burden and circulating HBV strains causing this endemic infections among gravid women remains elusive, in Garissa. Therefore, this study was aimed at determining seroprevalence, of HBV, genetic diversity, and drug resistance among gravid women attending Saretho Health Centre at Dadaab in Garissa.A cross-sectional study was conducted. A total of 186 consenting gravid women were recruited using systematic random sampling design. The study participants were recruited consecutively during the period between August to October 2023. A structured questionnaire was administered and demographic data; that includes age, level of education, marital status, ear piercing, tattooing, female genital mutilation (FGM), history of undergoing a caesarean section, history of blood transfusion, and dental procedure collected.About, 5ml venous blood was collected in EDTA tubes and screened for AntiHBs, AntiHBc, AntiHBe, HBsAg, and HBe-sero using Lumiquick HBV 5 panel kit according to the manufacturers instructions. The HBV positive samples viral DNA was extracted from separated plasma using the GeneProof pathogen free DNA isolation kit (Gene Proof, Czech Republic) as per the manufacturer's instructions. The partial HBV *pol* gene was amplified and directly sequenced using Sanger sequencer platform. The genarated HBV sequences were phylogenetically analysed using MEGAx software.In addition, the HBV sequences were also analysed for drug resistance mutations using Insilico HBV drug resistance database. The demographic data was analyzed using SPSS version 19 and frequency tables generated. The sero-prevalence markers for HBV was presented in percentage and tabulated. Two tailed chi-square tests with significance set at *p value* of < 0.05 was used to compare the association between socio-demographic factors, serological markers and HBV infection stages across the study participants. Overall 8.6% prevalence was detected. Marital Status, level of education, history of undergoing FGM, having Ear piercing were risk factors associated with HBV infection ($p < 0.05$) while Age, history of; blood transfusion, caesarean section, dental procedures, tattoo, having liver disease or having family history of liver diseases ($p > 0.05$), were not risk factors associated with HBV infection. From the analysis, 19(10.2%) were immune to HBV infection, 6(3.2%) acute infection, 11(5.9%) on HBV recovery, 8(4.3%) were on chronic stage and 5(2.7%) had occult HBV. After the confirmation of HBV DNA by Gel electrophoresis, 9 samples were successful amplified, purified and sequenced. The phylogenetic analysis of HBV sequences revealed that seven (7) isolates were classified as HBV genotype A while two (2) isolates belonged to genotype D. The study found high overall drug resistance prevalence of 77.8%. Five participants harbored cross-resistance mutations to Lamivudine, Entacavir, and Telbivudine. Two had additional resistance to Adefovir and Entacavir, while no drug resistance mutations associated with tenofovir. Two participants had no detectable resistance mutations.This study suggests need for utilisation of HBV -5 panel seromarkers in HBV testing for early detection, infection staging and effective treatment. HBV genotype A predominates among pregnant women in Dadaab, with a high prevalence of drug resistance mutations. Continuous genotype surveillance is essential to monitor viral evolution and resistance dynamics. Based on observed cross-resistance, combined therapy with Tenofovir and Lamivudine is recommended for effective HBV management.

CHAPTER ONE

INTRODUCTION

1.1 Background

Hepatitis B virus infection is among the major contributors to morbidity and death across the globe. This infectious disease continues to pose a serious public health concern especially in Kenya where HBV is endemic (Kilongosi *et al*., 2015), (Hou *et al.,* 2020). Despite the availability of effective vaccines and potent effective antiviral drugs, at least 2 billion people are HBV infected globally. This result into atleast 0.6 million deaths and 300 million chronic HBV infections (Kilongosi *et al.,* 2015). Common comorbidity diseases like HIV, contributes to at least three million co-infections globally (Sorianoa *et al.,* 2005).

The hepatitis B virus's prevalence in Sub-Saharan Africa is 9–20%. In Kenya, reported prevalence varies between 5% and 30%, with an estimated 9.4% among pregnant women (Malungu *et al*.,2016). The Hepatitis B virus prevalence in expectant mothers in Garissa was reported to be high 14.1% (Mohamed, 2008). It is therefore important to establish the current HBV infection.

HBV infection in pregnancy is of particular concern due to the risk of mother to child transmission during childbirth and breastfeeding (Barth *et al.,* 2010). Infants infected by their mothers are far more likely to develop chronic HBV infection, which has long-term health consequences. Prevention of mother-to-child transmission is crucial for HBV control (Eyong *et al.,* 2019). Nevertheless, having several sexual partners, using unscreened blood, getting tattoos,

and using non-sterile devices during surgery are also other risk factors for HBV infection (Maepa *et al*., 2015).

Continuous monitoring of HBV infection across the nation is essential for controlling and preventing these infections. This screening could confirm HBV status in an individual either active or chronic disease stage (HBsAg) (Abbas *et al*., 2006), vaccinated (HBsAb) (Krajden *et al*., 2005), replication and infective stage (HBeAb) and pre-core antigen (HBeAg) for hepatitis B for like-hood of a protective stage against repeated HBV exposure or Hepatitis B core antibody (HBcAb) either IgM or IgG to indicate either acute or past HBV infection (Kilongosi *et al*., 2015). The use of these seromarkers is essential for sero-epidemiologic surveillance and for starting and tracking treatment response (Kilongosi *et al.*, 2015). In Saretho, HBV screening during antenatal care is not routine. This means many pregnant women with HBV are undiagnosed and hence not counselled or managed to reduce risk of passing infection to newborns.

The WHO recommends administration of HBV vaccination to newborns as soon as possible after birth, within 24 hours to prevent vertical transmission. Kenya's immunisation schedule gives the HBV-containing vaccine starting at six weeks via the pentavalent vaccine rather than giving a birth dose. This leaves a window during which infants born to HBV-positive mothers are vulnerable (Ngare *et al.*, 2024).

Many pregnant women at Saretho are illiterate and have low awareness of HBV infection, its modes of transmission, risk factors, consequences, and preventive measures. This predispose these women to HBV infection and transmission particularly through traditional practices that involves surgical procedures like ear piercing, FGM and tattooing (Maepa *et al.*, 2015). .

In Kenya, genotypes A, D, and E have been described, with genotype A being most predominant. Sub-genotypes A1 and A2 circulate at varying frequencies (Mwangi *et al.,* 2008). However, genotype distribution and drug resistance patterns in north eastern Kenya, particularly in Dadaab and Garissa, remain largely uncharacterized. No studies have specifically examined HBV resistance mutations in this setting, despite their implications for patient management, antiviral therapy, and diagnostic accuracy.

Saretho is near Dadaad which host large refugee populations, especially from Somalia. The refugee populations often have limited access to healthcare. HBV screening during antenatal care is not routinely done hence missing early detection and posing a risk of infected pregnant mother infecting their children through verical route or through breast feading. The disruptions in health service delivery at the refugee camps, possibly lowers the coverage of preventive services like immunization agaist HBV infection. It is close to Somali border where cross border economic activities like trade and intermarriage is at play, further promoting the spread of this infection. The pregnant women participate in cultural practices like FGM, tattooing and ear piercing. These practices are done by those with low awareness of HBV infection making them vulnerable to contracting the disease.

It was therefore important to coduct this study to establish the current sroprevalence of HBV infection, seromarkers, infection stages and risk factors associated with HBV infection and characterize the genetic diversity of HBV and identified resistance-associated mutations among pregnant women attending Saretho Health Centre in Dadaab, Garissa County, Kenya.

1.2 Statement of the problem

HBV is a serious infectious disease endemic in Kenya (Hou *et al.,* 2005), with high prevalece of 14.1% reported in Garissa (Mohamed, 2008). The diease has significant consequences for both mother and child. Its infection increases the risk of adverse pregnancy outcomes among pregnant women such as risk of vertical transmission or through breastfeeding. The infected children may develop liver diseass, liver carcinoma and may die. (Afraie *et al.,* 2023). Despite this danger that this disease poses to pregnant women and their children, there is lack of awareness among pregnant women at saretho about HBV risk, transmission and prevention (Bahati *et al.,* 2021). There is absence routine screening of pregnant women for HBV in antenatal care clinic to identify mothers at highest risk of transmitting HBV to their infants. Preventive measure like vaccination against HBV is unavailable at this locality where pregnant women participate in cultural practices like FGM, ear piercing and tatoing further compounding the spread of HBV infection (Ngaira *et al.,* 2016).

The presence of Somali refugees around this locality, their interaction both in trade, intermarriage and cross border activies around the Somali border which is near, has promoted the spread of the disease. Given the high risk for HBV infection, the vulnerabilities of refugee populations, the existing gaps in screening and prevention, and the established dangers of HBV in pregnancy both for the mother and child, there was a pressing need to conduct this study to establish the current seroprevalence, the actual risk factors associated with these infections, circulating HBV genotypes and drug resistance mutations at Saretho in Dadaab, Garissa County in order to institute proprer interventions to curb the disease.

1.3 Justification

The prevalence of HBV infection at Saretho in Dadaab is unclear with previous studies (Mohamed, 2008) and (Bahati *et al.*, 2021) being14.1% and 5-7% at Garissa. Continuous screening and monitoring of HBV seroprofiles is essential for sero-epidemiologic surveillance, starting and tracking treatment response (Kilongosi *et al*., 2015). The seroprofiles at Saretho in Dadaab are unknown. Out of the three known HBV genotypes in Kenya: A, D and E (Mwangi *et al*., 2008), it is not known which genotypes are circulating at Saretho in Dadaab, Garissa County. Little is known about drug resistance mutations that influence patient management and therapy. It was therefore necessary to conduct this study, to establish the current HBV seroprevalence, its seromarkers, the risk factors for HBV infection, genetic diversity and drug resistance among gravid women at Saretho in Dadaab, in order to help in formulating policy guidelines for prevention and managent of HBV infection.

1.4 Research Questions

1. What is the sero-prevalence of Hepatitis B virus among gravid women attending antenatal clinic at Saretho Health Centre in Dadaab?
2. What are the risk factors associated with HBV infection among gravid women attending antenatal clinic at Saretho Health Centre in Dadaab?
3. What are the sero-profiles of HBV among gravid women attending antenatal clinic at Saretho Health Centre in Dadaab?
4. What is the HBV genetic diversity circulating among gravid women attending antenatal clinic at Saretho Health Centre in Dadaab?
5. What are the drug-resistance mutations of HBV among gravid women attending antenatal clinic at Saretho Health Centre in Dadaab?

1.5 Hypotheses

1.5.1 Null Hypothesis

1. The sero-prevalence of Hepatitis B virus among gravid women attending antenatal clinic at Saretho Health Centre in Dadaab is same as the national of 8%**.**
2. There were no risk factors associated with HBV infections among gravid women attending antenatal clinic at Saretho Health Centre in Dadaab.
3. There were no sero-profiles of HBV among gravid women attending antenatal clinic at Saretho Health Centre in Dadaab.
4. There was no HBV genetic diversity among the gravid women attending antenatal clinic at Saretho Health Centre in Dadaab.
5. There was no HBV drug resistance mutations among the gravid women attending antenatal clinic at Saretho Health Centre in Dadaab.

1.6 Objectives

1.6.1 Broad Objective

To determine HBV sero-prevalence, sero-profiles, drug resistance and genetic diversity among the gravid women attending antenatal clinic at Saretho Health Centre in Dadaab.

1.6.2 Specific Objectives

1. To determine the sero-prevalence of Hepatitis B virus among gravid women attending antenatal clinic at Saretho Health Centre in Dadaab.
2. To determine the risk factors of HBV Infections among gravid women attending antenatal clinic at Saretho Health Centre in Dadaab.
3. To determine the sero-profiles of HBV among gravid women attending antenatal clinic at Saretho Health Centre in Dadaab.

4. To determine the HBV genetic diversity among the gravid women attending antenatal clinic at Saretho Health Centre in Dadaab.
5. To determine HBV drug resistance mutations among the gravid women attending antenatal clinic at Saretho Health Centre in Dadaab.

CHAPTER TWO

LITERATURE REVIEW

2.1 Hepatitis B VirusNomenclature

This virus is double-stranded belonging to the family Hepadnaviridae and the genus Orthohepadnavirus (Hunt, 2007; Ryu, 2017).

2.2 Structure and Replication of Hepatitis B Virus

2.2.1 Structure of Hepatitis B Virus

Structurally, the HBV genome has circular DNA. It is approximately 3200 base pairs (**Figure2.1**) (Abbas *et al.*,2006).

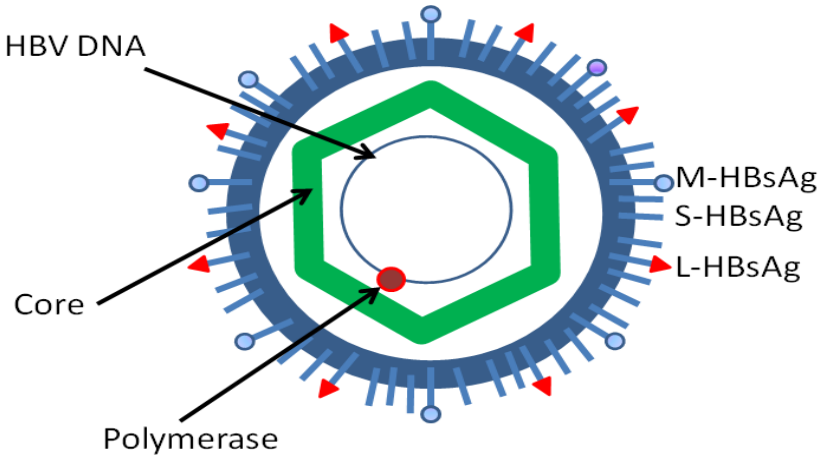

Figure 2.1: HBV Structure (Howard, 1986).

The HBV has DNA genome double-stranded with an incomplete positive strand. The strand is negatively chargedwith four genes that overlaps the open reading frames (ORFs); S, P, X, and C (Tiollais *et al.*,1985). The enveloped proteins are coded by *S* gene. They are large, middle, and small HBsAg and HBeAg. The polymerase and reverse transcriptase for DNA are coded by the *P* gene. The X proteins are coded by the X gene for transcription, initiating and maintaining the replication of HBV. The core proteins around the capsid are encoded by the C gene (Lucifora *et al.*, 2011) (Figure 2.2).

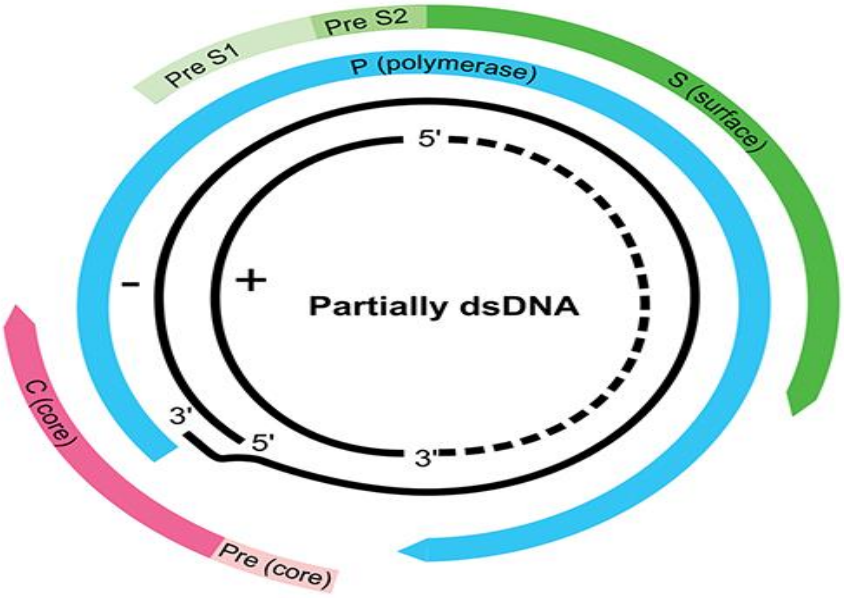

Figure 2.2: The HBV genome (Bell and Kramvis, 2016).

2.2.2 Replication of Hepatitis B Virus

The HBV replication starts when its receptors attach to heparansulfate proteoglycans. These are specific glycoproteins found in the basement membrane. They are also found around the secretory vesicles. The attachment is made to be of high affinity when a transit protein NTCP exists on the liver cell membrane(Maepa *et al*., 2017). The HBV internalizes its proteins by endocytosis. The HBVuncoats its envelope. This helps the capsid totranslocate with relaxed DNA (rcDNA) which is circular in the infected human hostnucleus. They repair in such a way that the DNA (cccDNA) which is circular gets closed (Parija, 2012). The transcription of mRNA is facilitated by the action of cccDNA on the template. The mRNA is then translated to the viral protein. It is at the cytoplasm of the infected cells where the viral proteins are assembled. Using the RT enzyme in the capsid, the pg-RNA is converted to DNA. In some cases, it may be back to the nucleus for a repeat of the replication process. Thecompletely developed viral particles will bereleased from the infected host cell (Figure 2.3) (Lucifora *et al*., 2011).

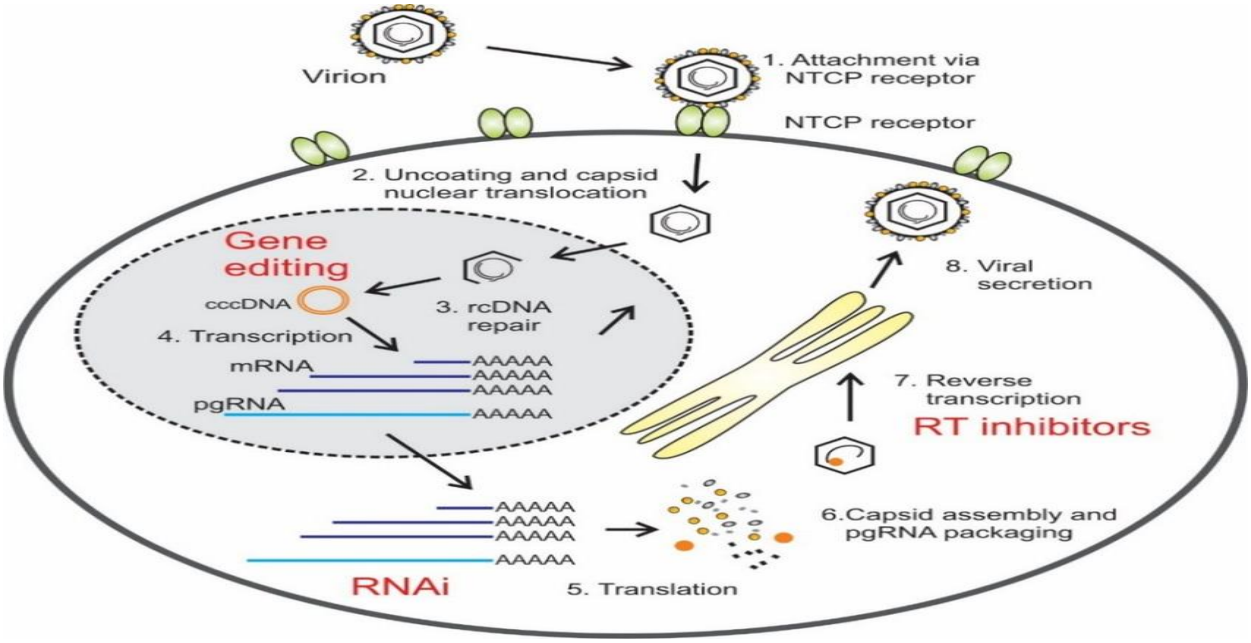

Figure 2.3: Replication of HBV (Maepa *et al*., 2015).

2.3 HBV Infection and Disease Burden

2.3.1 HBV Infection and Disease Burden among general population

HBV infection is still a major public health concern, particularly in Kenya where HBV is endemic (Kilongosi *et al.*, 2015), (Hou *et al.*, 2020). This problem is further compounded by existing risk factors to HBV infections including common comorbidity diseases like HIV resulting in a minimum of three million co-infections globally (Sorianoa *et al*., 2005). Parenteral, perinatal, and sexual pathways are common points of entry for HBV infection, which has been found in these populations (Barth *et al*., 2010). Nevertheless, having several sexual partners, using unscreened blood, getting tattoos, and using non-sterile devices during surgery are also other risk factors for HBV infection (Maepa *et al*., 2015).

Despite the availability of effective vaccines and potent effective antiviral drugs, at least 2 billion people are HBV infected globally resulting into 0.6 million mortality and at least 300

million chronic HBV infections (Kilongosi *et al.*, 2015). Approximately 75% of the individuals who are chronic carriers live in West Pacific and others in Asia. Among the HBV-infected patients, 15-40% develop liver complications ranging from Liver failure to Liver cancer (Shepard. *et al.,* 2006). Between half a million to more than 1.2 million HBV-related fatalities are reported yearly. The disease burden of Hepatitis B is said to be substantial because of the high HBV-related mortality and morbidity (Hou *et al*., 2005).

Chronic HBV infection has a prevalence of either high, intermediate, or low endemicity (Mohamed, 2008). The low endemicity is characterized by a 2% or less carrier rate. It generally includes Europe, America, and Australia. The intermediate endemic areas are characterized by a 2-7% carrier rate. It includes Sub-Saharan Africa, the Middle East, Europe, and South America. The high endemic areas are Sub-Saharan, South Asia, and , China (Hou *et al.,* 2005).

The HBV chronic carriers in Africa are estimated to be 50 million. They have a mortality risk of 25% (Kiire, 1996). Both, Africa and Asia have an HBV prevalence of >8%. Approximately more than 2 billion people have HBV markers of either active or resolve infection (Eyong *et al.,* 2019). In Kenya, the prevalenve of chronic infection in the intermediate range is between 5-7%. The higher levels are >8% with regional variation. Howerver,the current HBV disease burden is unknown at Garissa.

2.3.2 HBV Infection among Gravid women

HBV infection in pregnancy is of particular concern due to the risk of mother to child transmission during childbirth and breastfeeding (Barth *et al.,* 2010). Infants infected by their mothers are far more likely to develop chronic HBV infection, which has long-term health consequences. Prevention of mother-to-child transmission is crucial for HBV control (Eyong *et*

al., 2019). The WHO recommends administration of HBV vaccination to newborns as soon as possible after birth, within 24 hours to prevent vertical transmission. Kenya's immunisation schedule gives the HBV-containing vaccine starting at six weeks via the pentavalent vaccine rather than giving a birth dose. This leaves a window during which infants born to HBV-positive mothers are vulnerable (Ngare *et al.,* 2024).

HBV screening during antenatal care is paramount in preventing HBV infections. In some communities like the one in this study, HBV screening during ANC visit is not routine. This means many pregnant women with HBV are undiagnosed and hence not counselled or managed to reduce risk of passing infection to newborns. Many pregnant women like the ones in the community where the current study was undertaken, are illiterate and have low awareness of HBV infection, its modes of transmission, risk factors, consequences, and preventive measures. This predispose these women to HBV infection and transmission particularly through traditional practices that involves surgical procedures like ear piercing, FGM and tattooing (Maepa *et al.,* 2015).

2.4 Pathogenesis of Hepatitis B Virus

Hepatitis B virus infection can either be chronic or acute. The acute hepatitis B virus infection presents with anorexia, malaise, vomiting, jaundice, and liver tenderness with an increase in HBV titer (Ryu, 2017). Chronic HBV infections are mostly without any symptoms. In case of any symptoms, they resemble acute hepatitis. These clinical manifestations symbolize damage to the liver. It may progress to liver cancer and final death. Chronic infections may take years before it manifests (Hunt, 2007).

HBsAg, a surface antigen of hepatitis B also called hepatitis-associated antigen or Australia antigen.Generally, HBsAgoccurs before sign and symptoms are observed and peaks when the disease is full blown.Within four to six months of infection, HBsAg is undetectable in patients who recover from an HBV infection without progressing to the chronic carrier stage. When HBsAg is present after six months, it indicates chronic infection (Abbas *et al.*, 2006).

HBeAg is a protein soluble in nature found in the core of HBV. It is a marker for HBV infectivity and replication.HBeAg increases during incubation and is present during acute and chronic stages of the disease. When HBeAg is present in chronic stage itindicates chances of active transmissibility. The absence of HBeAg indicates low chances of transmission (Krajden *et al.*, 2005).

Since Hepatitis B core antigen (HBcAg) is an intracellular antigen generated andexpressed by infected liver cells only, test for HBcAg is not readily available. The producedHBcAg is assembled into the viral core. The free HBcAg does not circulate in larger amount in the blood but circulateasHBV virion therefore is undetected in serum tests (Maepa *et al*., 2015).

IgM anti-HBc is the first antibody to show up against the hepatitis B core antigen. Acute HBV infection is indicated by the presence of IgM anti-HBc antibodies. IgM antiHBc can be found prior to the onset of symptoms. IgG antiHBc is a sign of a previous HBV infection. While IgG anti-HBc is found and may last a lifetime, IgM anti-HBc goes away in weeks (Parija, 2012). The presence of antiHBs suggests protection against HBV exposure and may last a lifetime. The HBeAg vanishes and antiHBe may be found when viral infectivity drops and replication stops. According to Abbas *et al*. (2006), antiHBe can last for years (Abbas *et al.*, 2006) (Figure 2.4).

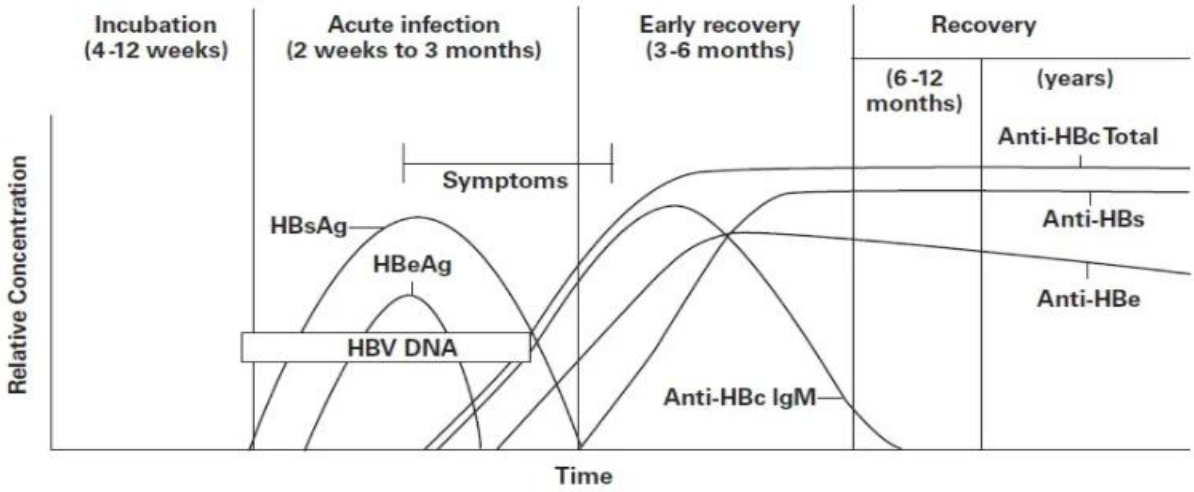

Figure 2.4: Timeline for Acute HBV Infection (Krajden*et al.,* 2005).

2.5 HBV Genotpes and it's distribution

The HBV genotypes are heterogeneous. This is due to the viral-encoded polymerase not having a proofreading ability. It uses the phylogenetic analysis of the complete HBV genome.The intergroup divergence is more than 7.5%. HBV has nine genotypes. These genotypes are classified from A to I (Glebe and Bremer, 2013). HBV genome has intergroup nucleotide differences between 4-8%. There are 35 subgenotypes grouped from genotypes A, B, C, D, F, and H. (Kramvis, 2014). The length of the genome and size of ORFS and translated proteins differ (Hunt, 2007).

The way people migrate has an impact on the distribution and pattern of HBV genotypes. There is a distinct regional distribution of the HBV genotypes and sub-genotypes. In addition to the rest of the world, Europe, North America, India, and Africa are home to genotype A. According to Santos *et al*. (2010), Southeast Asia is home to the majority of genotypes B and C. Within HBV genotype A, there are seven subgenotypes (A1-A7). Subtypes A1 and A2 are primarily found in Africa and Europe, respectively. There has been evidence of the spread of genotype A3 throughout West and Central Africa. Subgenotypes A4 and A5 have been shown to be prevalent in the Gambia, Haiti, and Nigeria, respectively, while subgenotype A6 has been found in

African patients from Rwanda and Congo in Belgium, and subgenotype A7 hasbeen reported in Cameroon and Rwanda (Zhang *et al*., 2016).

HBV subgenotype B1 is more prevalent in Japan, while subgenotype B2 is more prevalent in C hina and Vietnam. While subgenotype B4 is prevalent in Vietnam, subgenotype B3 has been do cumented in Indonesia. Subgenotypes B7, B8, and B9 have been found on an island in Southeas t Asia. HBV Southeast Asia has reported subgenotype C1, East Asia has reported subgenotype C2, Oceania has reported subgenotype C3, Australia has reported subgenotype C4, the Philippin es is the only location for subgenotypes C5 and C7, and Indonesia has reported subgenotypes C 6 and C8 (Lusida*et al.,* 2008).

HBV Genotype D has four sub genotypes (D1, D2, D3 and D4). These genotypes are predominant in Asia and Middle East. The same sub genotypes are found in Europe and Africa. Sub genotypes D5, D6 and D7 has been reported in Indonesia and India(Meldal *et al.,* 2009). In the western region of subSaharan Africa, HBV genotype E is prevalent. Other than people of African descent, it is uncommon outside of Africa. However, reports of this genotype have com e from India and Colombia (Alvarado *et al.,* 2010). In France, the United States, and Mexico, H BV Genotype G has been identified. It coinfects people with other HBV genotype, particularly A. HBVgenotype F has foursubgenotypes.: F1, F2, F3, and F4. South and Central America are dominated by the HBV genotypes F and H. Recently, the genotypes I and J were found in China, Vietnam, and Japan (Arankalle *et al*., 2010).

Furthermore, intergenotype recombination has a major impact on HBV evolution (Zhang *et al*., 2016). For example, recombinants of genotype B/C have been found in East and Southeast Asia Intergenotypes A/D, A/E, C/D, and G/C recombinants have been found in the rgeographic areas (Yang *et al.,* 2006).

HBV genotypes A, D, and E have all been detected in the bloodstream in Kenya, with genotype A being the most common.The same genotypes have been found in most sub- Saharan Africa countries.Kenya is considered to be among theHBV endemic countries (Mwangi *et al.,* 2008). However, data on current HBV genotypes in circulation remains elusive. Previous studies like Kilongi*et al*., 2015) found out that only sub-genotype A1 of HBV genotype A waswas exclusive present among the participants.

Globally, there is increase in viral genetic diversity due to increase in migration of human. This has resulted in increasing HBV epidemic. The HBV epidemic management is made more complex by the intermixing of HBV subtypes resulting to mutations by the virus leading to drug resistance (Modi *et al*., 2007). In Garissa, the information on circulating HBV genotypes is unknown. This study determined HBV genotypes among gravid women at Saretho, in Dadaab Sub county, Garissa County, Kenya.

2.6 Diagnosis of HBV

It is majorly serological and molecular. Serology diagnosis detects serological markersHBcAb-IgM, HBeAg, HBeAb, HBsAb, and HBsAg (Maepa *et al*., 2015). Molecular diagnosis detects the genome of HBV. It's amplified by PCR.Molecular diagnosis is mostly relied on when treating a patient who is in chronic stages. It is also helpful in diagnosing the causes of liver failure (Ryu, 2017).

2.7 HBV Treatment

2.7.1 HBV Drugs and Mode of Action

Treatment is given to a HBV infected patient to help decrease the disease's progression to a chronic stage and lower the viral load.The danger is patient developing liver cirrhosis and cancer of the liver.The drugs which include interferon-α (IFN) and the nucleoside approved for treatment of HBV patients work by targeting the viral polymerase.There are five approved medication for HBV infection. These are

adefovirdipivoxil (ADV), lamivudine (3TC),entecavir (ETV), telbivudine (LdT) and tenofovirdisoproxil fumarate (TDF) (Zhu *et al.,* 2009).

The IFNs modulates the immune system rendering it's antiviral effects. NAs disrupts the hepatitis B virus reverse transcription therefore it has a strong antiviral effect. IFN are advantageous over NAs because the NAs develop resistance mutations during treatment therefore reducing its antiviral effects.WHO has recommended use of Lamivudine for treatment of HBV chronic stages in order to relieve inflammation in the liver as well as lowering the viral load. The set back of this treatment, is the existence of lamivudine-resistant mutant (Kramvis and Kew, 2005).

The continuous utilisation of Lamivudine as recommended by WHO has increased Lamivudine-resistant mutations. WHO (2015) recommendtreatment to start right away, regardless of the patient's CD4 count.The patients are subjected to pegylated interferon for a period of 48 weeks under close monitoring.Pegylated interferon help reduce chances of the patient progressing to chronic liver disease and developing hepatocellular carcinoma which can lead to death.Entecavir and tenofovir can later be initiated based on viral load and liver function tests (WHO, 2015).

2.7.2 Future prospects of HBV Drugs and Treatment

The challenge of HBV treatment even for the patients who heal from acute stages of HBV infection with HBsAg to anti-HBs, is persistent of HBV in the liver as cccDNA. This form of HBV can become full-blown in immunosuppression therapy. There is need to have a permanent cure where HBV DNA many completely be eliminated from the patient. To attain this,there is need to develop a mix of antiviral medications that target distinct stages of the life cycle of HBV (Chan *et al*., 2014).

New advancements have been made treatments of chronic hepatitis B. There are innovation of molecules which exists as combination or individual as future treatment strategies. Studies on the molecules have shown higher HBV loss. Several drugs are in second phase of clinical trial evaluation. The molecules are grouped into two based on mode of action; those acting as immunomodulators and those focusing on several stages of the HBV life cycle (Broquetas and Carrion, 2023).

Entry inhibitors is an example of drugs targeting the steps in HBV lifecycle. They work by blocking liver specific bile acid transporter (Bogomolov *et al*., 2016).Other drugs are, capsid assembley modulators which work interfering with formation of capsid (Taverniti *et al.,* 2022) and post transcriptional control inhibitors which work by silencing post transcriptional genes therefore inhibiting translation of viral proteins (Hui *et al.*, 2022).

Examples of Immunomodulators drugs are Innate immune activators which work by stimulating Innate immune system through TLRs and RIG-1 (Langford *et al.*, 2013) and Adaptive immune activator by blocking pathways to reverse T cell to generate CD4 and CD8 HBV – specific T cells (Yau *et al*., 2019).

2.8 HBV Drug Resistance

2.8.1 Drug Resistance Pathways

HBV treatment failure is caused by drug resistance.It is mainly experienced when nucleoside analogues therapy are used.Drug resistance as a result of HBV polymerase involves complex eight codons linked with initial resistance and leads to to five major pathways.The firstpathway is the L-nucleoside pathway (rtM204V/I). Treatment with lamivudine,emtricitabine, telbivudine, and clevudine excludes the rtM204V/I.

In patients with a history of lamivudine, the pathway involves entecavir. The second pathway is the acyclic phosphonate pathway (rtN236T). Treatment with adefovir and tenofovir eliminates a nd/or combines the rtN236T HBV quasispecies (Angus *et al.*, 2003).

The third pathway is the shared pathway (rtA181T/V). HBV quasispecies with rtA181T/V are selected by L-nucleosides or acyclic phosphonates. About 40% of adefovir failure and 5% of lamivudine failure are caused by this route. Of the rtT184, S202, or M250 codon alterations, the fourth pathway is the naive entecavir resistance pathway (rtL180M + rtM204V). The extremely low resistance profile of entecavir is explained by the simultaneous appearance of three mutations in this pathway (Suzuki *et al.*, 2007).

The fifth route is multidrug resistance. Clusters of HBV polymerase mutations linked to multidr ug failure are among them. These mutations include, for example, rtA181T + rtI233V + rtN236 T + rtM250L.Although rtI233V and M250L substitutions alone do not result in appreciable drug resistance, they seem to make up for the replication flaws linked to the development of multidr ug resistance (Locarnini, 2008). Nevertheless, some of these mutations are undermining potenti al advancements in treatment strategies, particularly those obtained after lamivudine therapy (Zhang *et al.*, 2016).

However, deletion mutations in the PreS gene region indicate that the primary hydrophilic region of the S gene is mutated, which can lead to occult HBV infection (Zhang *et al.*, 2016). Mutations in the CTL epitope of the HBV core gene result in decreased T cell immune escape. (Zhang *et al*., 2016).

2.8.2 HBVDrug Resistance Strains

There is variedinformation on HBV drug resistance worldwide.In Europe, (Lucas *et al*., 2016) had majority of the participants (73.8%)having Lamivudine as a single drug. 52.7% of the participants had drug resistance strains while most common mutation was M204V/I (48.7%),A181T/V (3.8%) and N236T (2.6%). The participants who had encountered entecavir (n = 102), 35.3% had full blown resistance.

A study in Brazil (Homes *et al.*, 2015) found that HBV strains which cause mutations associated with NA resistance in 16% (11/702) of the participants. In addition, compensatory mutations were detected in the HBV strains 0.4% (3/702) of the patients. These compensatory mutations were (rtL180M/rtV173L+rtL180M/rtV207I). There were other isolates which had mutations related to ADV resistance 7.7% (54/702) of the patients.. These mutation were (rtS85A, rtL217R, rtI233V, rtN238T, rtN238D, rtN248H, rtV214A, rtQ215S).

In China, (Yan *et al*., 2021) hepatitis B virus drug resistance strainswas detected in 0.67% (190/28,236) of chronic HBV-infected paticipantswho were on NAs treatment in clinical practice.Out of the sixteen HBV drug resistance strains eight were LAMr + ADVr and other eight were ETVr + ADVr). Eleven novel strains were also identified.

A review study in subsahara Africa by (Mokaya*et al*., 2018) revealedthat most information were of HBV/HIV coinfected patients.The exclusive mutations were rtM204I/V in combination or alone and were reported both in participants who were on treatment or not.

In Kenya, there is little information about HBV resistant strains (Trevino *et al*., 2009). Astudy in Mombasa (Kasera*et al*., 2021)detected drug resistance in 12 (26.4%)out of 45 samples which were successfully sequenced.

Six patients (13.3%) had mutations in rtV173L, rtL180M, and rtM204V. Five participants (11.1 %) had rtL180M and rtM204V mutations, whereas one patient (2.2%) had rtM204V mutations. All subjects were found to have cross-resistance to entecavirand lamivudine. In Nairobi, (Mabeya*et al.*,2017) revealed that six out of the 13 participants had HBV strains with primary or secondary mutations. NAs that were detected were at position 204 (rtM204), 180 (rtL180M) and 173 (rtV173L). A sequence had mutations at position 204 (rtM204V) and 180 (rtL180M).

Another study in Nairobi (Aluora *et al*., 2020) on voluntary blood donors in Nairobi revealed that mutations especially rt181T in the P gene were associated with resistance against Lamivudine. In Garissa, there is no data on HBV Drug resistance. This study, therefore, determined the HBV Drug resistance among gravid women at Saretho, in Dadaab Sub county, Garissa County, Kenya.

2.9 Prevention and control

HBV is controlled and prevented by the use of vaccines and immunoglobulin (Parija, 2012). The immunoglobulins confers passive immunization before and after an individual gets exposed to HBV. The available vaccines are plasma-derived and recombinant DNA HBV and generate anti-HBs that confer immunity to both adults and children (Glebe and Bremer, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted at Saretho Health Centre, a level three facility located in Dadaab Sub-County, Garissa County, northeastern Kenya. The facility provides outpatient, maternal, and child health services, including antenatal, maternity, and family planning care to both Kenyans and the refugees. It is staffed by three nurses, a medical laboratory officer, a public health officer, and a nutritionist. The catchment population is approximately 30,000 residents of Abakaile Ward. Saretho Health Centre is situated less than 20 km from Dadaab refugee camps, which host more than 260,000 Somali refugees. The refugee populations often have limited access to healthcare services like immunization agaist HBV infection. In addition, HBV screening during antenatal care is not routinely done hence missing early detection and posing a risk of infected pregnant mother infecting their children.. The close to Somali makes this community engage in cross border economic activities like trade and intermarriage, further promoting the spread of HVB infection. The pregnant women participate in cultural practices like FGM, tattooing and ear piercing. These practices are done by those with low awareness of HBV infection making them vulnerable to contracting the disease.

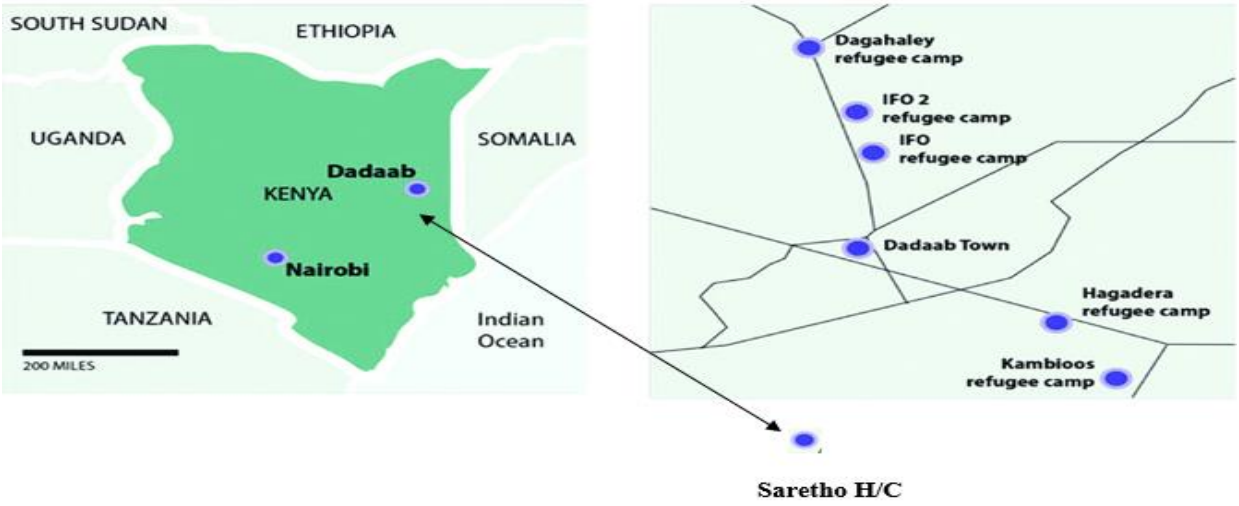

Figure 3.1 A map of Kenya showing study area

3.2 Study population

The study targeted only consented gravid women who had come for first ANC visit at Saretho Health Centre.

3.3 Study design and sampling design

A cross-sectional study was conducted. The choice of this study design was because there was no manipulation of the subjucts. Only observation of the subject was required. A total of 186 consenting gravid women were recruited into this study using systematic random sampling design. During the First antenatal clinic, every third eligible gravid woman visiting the facility were sampled during the period between August to October 2023. Three to four gravid women were selected after every three consecutive gravid women among the nine to twelve gravid women who came for first ANC visit each day at the facility.

3.4 Sample Size and Determination

The Fisher *et al.*(1998) formulae was used in determining the sample size.

General formulae: $\mathbf{n = \frac{Z^2 Pq}{d^2}}$

n = Sample size

Z = Statistic of a level of confidence of 95%, 1.96 (Lwanga and Lemesho, 1991)

P = Expected prevalence or proportion, 0.141 (14.1%) (Mohamed, 2008).

q = (1 – p) therefore P = 1 – 0.0141 = 0.859

d = degree of accuracy desired, set or desired 0.05 (5%) (Naing *et al.*, 2006)

Therefore: $\mathbf{\frac{(1.96^2)\ (0.141)\ (0.859)}{(0.05^2)} = 186\ samples}$

3.5 Exclusion and Inclusion criteria

3.5.1 Inclusion criteria

Consented gravid women visiting Saretho Health Centre for the first ANC visit during the study period.

3.5.2 Exclusion criteria

i. Women visiting Saretho Health Centre for the first ANC visit who declined to Gravid participate in the current study.
ii. Gravid women who had come for 2^{nd} to 4^{th} ANC visit.
iii. Non pregnant women.
iv. Men.

3.6 Data collection

The demographic data; that includes age, level of education, marital status, ear piercing, tattooing, female genital mutilation (FGM), history of undergoing a caesarean section, history of blood transfusion, and dental procedure was captured through administered structured questionnaire (Appendix iv).

3.7 Data analysis

The data was analyzed using SPSS version 19 and frequency tables generated. The sero-prevalence markers for HBV was presented in percentage and tabulated. Two tailed chi-square tests with significance set at p value of < 0.05 was used to compare the association between socio-demographic factors, serological markers and HBV infection stages across thestudy participants.

3.8 Ethical considerations

Ethical approval was obtained from the Kenyatta University Ethical Review Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki (Parsa-parsi *et al.,* 2014) where pain was minimised by following the SOPS on safe blood withdrawal. Written informed consent was obtained from all participants, and confidentiality was strictly.

3.9 Laboratory Analysis

3.9.1 Blood sample collection

Sterile 5cc syringes and 23G needles were used to collect blood samples. 5millilitres of the blood sample was collected in EDTA tubes. Plasma was harvested after centrifugation at 3000 rpm for 5 minutes. The viral DNA extraction and the serological tests were performed using the plasma with known HBV positive sample and known HBV negative as internal Quality control to gurantee accuracy of the results (Mabeya*et al*., 2016).

3.9.2 Serological analysis

Using the one-step Lumiquick HBV-5 panel kit (LumiQuick Diagnostics, Inc. California, USA), the serum reactivity of samples for the five serological markers—hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B pre-core antigen (HBeAg), hepatitis B pre-core antibody (HBeAb), and hepatitis B core antibody (HBcAb-IgM)—was determined according to the manufacturer's instructions. Briefly, approximately 2-3 drops (80-120) µL of the plasma was placed on each of the five sample wells corresponding to specific markers. Two to three drops of buffer were added to each well and HBV seromarkersreactivities were recorded after 20 minutes incubation at room temperature (Figure 3.1).

Figure 3.2: Five serological markers HBV testing

3.9.3 Molecular Analysis

3.9.3.1 HBV DNA Extraction

The GeneProof pathogen free DNA isolation kit (Gene Proof, Czech Republic) was used to extract HBV positive samples viral DNA as per the manufacturer's instructions (GeneProof, 2022). In order to prepare for gene amplification by PCR, the DNA was isolated, rinsed in Tris-EDTA, and then kept at -80 °C. 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.

3.9.3.2 HBV-pol Gene Amplification

The partial HBV pol gene was amplified by a nested PCR using two specific forward and reverse primers.The sequences of the forward PCR primers pairs were HBPr1 (position: 2850–2868,5'-GGGTCACCATATTCTTGGG-3') and HBPr135 (position: 803-822, 5'-CAAAGACAAAAGAAAATTGG-3'). The sequences of the reverse pairs were

HBPr2 (position: 2867–2888, 5'-GAACAAGAGCTACAGCATGGG-3') and HBPr3 (position: 1547–1569, 5'- CCACTGCATGGCCTGAGGATG-3') (Stuyver *et al.*, 2000).

One Taq kit from England Biolabs was used for PCR. Briefly, the first round PCR was performed in a 25µl mixture containing 6.5µl DNA,5µlnuclease-free water, 0.5µl of forward primers, 0.5 µlof reverse primersand 12.5µl of master mix.For the seccond round PCR or Nested PCR,3µl of the first round PCR product was used as DNA template, 8.5µlnuclease-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 AmpliTaqacctivation 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 for7 min (Angounda *et al.*, 2016). All PCR amplification were performed in a Kratec thermal cycler SC300G-R.

3.9.3.3 Analysis of PCR Products by Gel electrophoresis

The PCR products were analyzedon a 2 % agarose gel and visualized by UV Transilluminator after staining with 0.05% ethidium bromide.

3.9.3.4 Sequencing of PCR products

The successful PCR products which formed the bands were direct sequenced using Sanger sequencer platform3730xl GA according to sanger sequencing protocol (Merck, 2024)

3.9.3.5 Analysis of DNA sequence

For each of the 11 PCR products, the forward and reverse primer sequences were produced. The generated sequences were phylogenetically analysed using MEGA X. Briefly, the forward and reverse sequences were edited, trimmed, and assembled using BioEdit v7.1.1. Multiple sequence alignment was performed, and phylogenetic trees were constructed using the neighbor-joining method with 1,000 bootstrap replicates in MEGA X (version 5.1). Woolly monkey HBV (AY226578-WMHBV) served as the outgroup. Phylogenetic trees were visualized with TreeView 1.4.4 (Angounda *et al.*, 2016).

3.9.3.6 HBV Drug Resistance

The obtained sequences, were analysed for drug resistance mutations using Insilico using the Geno2pheno HBV drug resistance database (https://hbv.geno2pheno.org/Resistance). The isolated HBV sequences were aligned with reference RT sequences and computed. The algorithm searches for mutations in the HBV isolated sequences that define resistance to Lamivudine, Adefovir, entecavir, tenofovir and telbivudine drugs. The detected mutations were reported with the associated drugs, resistance status and mutation position in the query sequence (Merck, 2024).

CHAPTER FOUR

RESULTS

4.1 Socio-demographic Characteristic and their association to HBV infection.

The socio-demographic characteristic of the participants was determined (Table 1). The study participants consisted of women aged between 16 and 45 years old with mean age of 28years standard deviation (1SD) (8.0). Most participants were aged between (20-39) years (78.5%, n=146) with those age below 19 years being the least (8%, n=15). No gravid woman under age (below 18years) participated in the study. Among the participants who were HBV seropositive, most affected population were those aged between 20-39 years (68.75%, n=11). However, age was not significantly associated with HBV infection (p = 0.57).

On marital status, most participants were in monogamous marriage (73.1%, n=136) and the least were single (2.2%, n=4). Those in polygamous marriage were most affected with HBV infection (50%, n=8) and those who had low proportion of HBV infections were detected among single women (6.25%, n=1). Marital status was associated with HBV infection (p = 0.01).

Most of the study participants were illiterate (71.5%, n=133) with those who had attained tertiary education being the least (3.2%, n=6). There was no HBV infection among gravid women who had attained tertiary education. The HBV seropositive among the participants, those who were illiterate were most infected (75%, n=12). This data showed that level of education was significantly association with HBV infection (p = 0.03).

The HBV sero-prevalence among gravid women in Garissa was 8.6% (16/186) (Table 4.1).

Table 4.1: Socio-demographic Characteristic and their association to HBV infection.

	Categories	Frequency		Negative		Positive		p value
Variable		n = 186	%	n = 170	%	n = 16	%	
Age Groups	Under 19	15	8.0	12	7.0	3	18.75	0.57
	20 – 39	146	78.5	135	79.4	11	68.75	
	Above 40	25	13.5	23	13.6	2	12.5	
Marital Status	Single	04	2.2	03	1.8	1	6.25	0.01
	Polygamous	46	24.7	38	22.2	8	50.00	
	Monogamous	136	73.1	129	76.0	7	43.75	
Education	Illiterate	133	71.5	121	71.2	12	75.00	0.03
	Primary	35	18.8	32	18.8	3	18.75	
	Secondary	12	6.5	11	6.5	1	6.25	
	Tertiary	6	3.2	6	3.5	0	0.00	

4.2 Risk Factors for Hepatitis B virus infection among Gravid Women

A total of 186 participants were interviewed, with majority of them having history of undergone FGM (86.6%, n=161), ear piercing (83.9%, n=156), and blood transfusion (12.3%, n=23). Furthermore, less than five individuals per group had a history of liver diseases, tattoo, caesarean sections, blood transfusions, dental procedures, ear piercings or female genital mutilation. Having history of blood transfusion (p = 0.43), caesarean section (p = 0.34), dental procedure (p = 0.51), liver disease (p = 0.23) or family member (p = 0.49) were not associated with HBV sero-positivity neither association with HBV infection among the study participant. However, there was a significant association with HBV infection among those who had history of undergoing FGM (p= 0.02) and Ear piercing (p = 0.01) (Table 4.2).

Table 4.2: Risk Factors for Hepatitis B Virus infection among Gravid Women

Variables	Option	Frequency		HBV status				
				Positive		Negative		p value
		n = 186	%	n = 12	%	n = 174.	%	
Blood Transfusion	Yes	23	12.3	2	12.5	21	12.3	0.43
	No	163	87.7	14	87.5	149	87.7	
Caesarian Section	Yes	8	4.3	3	18.75	7	4.1.	0.34
	No	178	95.7	13	81.25	163	95.9	
Dental Procedure	Yes	34	18.3	1	6.25	33	19.4.	0.51
	No	152	81.7	15	93.75	137	80.6	
FGM	Yes	161	86.6	16	100	145	85.3	0.02
	No	25	13.4	0	0	25	14.7	
Ear Piercing	Yes	156	83.9	14	87.5	142	83.5	0.01
	No	30	16.1	2	12.5	28	16.5	
Tattoo	Yes	6	3.2	1	6.5	5	2.9	0.65
	No	180	96.8	15	93.5	165	97.1	
Liver Diseases	Yes	2	1.1	1	6.5	1	0.6.	0.23
	No	184	98.9	15	93.5	169	99.4	
Family History	Yes	11	5.9	2	12.5	9	5.3	0.49
Of Liver Diseases	No	175	94.1	14	87.5	161	94.7	

4.3 Seroprofile of HBV infection stages among gravid women

A total of 186 gravid women enrolled in this study, their venous blood samples were drawn and screened for HBV sero-profile using 5 panel seromarkers. From the disease staging categorization, (10.2%, n=19) were HBV immunized, (3.2%, n=6) were on acute infection, (5.9%, n=11) were on HBV recovery phase, (4.3%, n=8) were on HBV chronic infection stage, (2.7%, n=5) were occult HBV but (73.7, n=137) HBV non-respondents (Table 4.3).

Table 4.3: Hepatitis B Virus Serological markers

HBV Sero-marker	HBV Immunization	Acute HBV	Recovery status	Chronic HBV	Occult HBV	Non Response
HBsAg	-	+	-	+	-	-
HBsAb	+	-	+	-	-	-
HBeAg	-	+	-	-	-	-
HBeAb	-	-	+	-	-	-
HBcAb-IgM	-	+	+	+	+	-
N (186)	19	6	11	8	5	137
% (Prevalence)	10.2	3.2	5.9	4.3	2.7	73.7

4.4. Agarose gel analysis of PCR products

The sixteen samples that were serologically positive were analysed on gel electrophoresis. The positions of bands on the gel were observed and a photograph of the bands taken. Ten out of the sixteen samples successful yielded clearer bands. The bands appeared as shown in Figure 4.1.

Figure 4.1: Agarose gel analysis of PCR products

4.5 Nucleotide Sequence Accession Numbers

The nucleotide sequence data were submitted to the GenBank database and allocated the following Accession number PQ421417 to PQ4214125 (able 4.4 below).

Table 4.4: GenBank databases Accession numbers

Samplenuber	Isolate number	GenBank Accession number
GCRH 11	Banklt2868430 Isolate_11	PQ421417
GCRH 79	Banklt2868430 Isolate_79	PQ421418
GCRH 84	Banklt2868430 Isolate_84	PQ421419
GCRH 85	Banklt2868430 Isolate_85	PQ421420
GCRH 92	Banklt2868430 Isolate_92	PQ421421
GCRH 138	Banklt2868430 Isolate_138	PQ421422
GCRH 174	Banklt2868430 Isolate_174	PQ421423
GCRH 185	Banklt2868430 Isolate_185	PQ421424
GCRH PC	Banklt2868430 Isolate_PC	PQ421425

4.6 HBV genetic diversity

All the nine (9)generated sequences were confirmed to be of HBV based on the phylogenetic analysis of the amplified HBV partial *pol* gene. The seven (7) generated isolates 84, 85, 92, 138, 174,185 and PC were classified as HBV genotype A, this clade was supported by a bootstrap value of 1.00. Isolates 84, 138 and 174 clustered with known isolates from East Africa with strong bootstrap support of 0.98 suggesting a possible regional transmissions route, supporting the hypothesisi of localized HBV strains in Garissa, Kenya. The clade that housed isolates 85, PC, 185 and 92, was paraphyletic to a clade with sequences originating from Asia and the Americas. Phylogenetic analysis also revealed that two (2) isolates 11 and 79 belonged to genotype D, this clade was also supported by a strong bootstrap value of 1.00. The isolates 11 and 79 clustered in a clade that included sequences isolated from Ethiopia and Central African Republic, the clade was moderately supported with a bootstrap value of 0.69 (figure 4.1).It was noted that the sequences for isolates 68 and 111 were not clear for any downstream analysis.

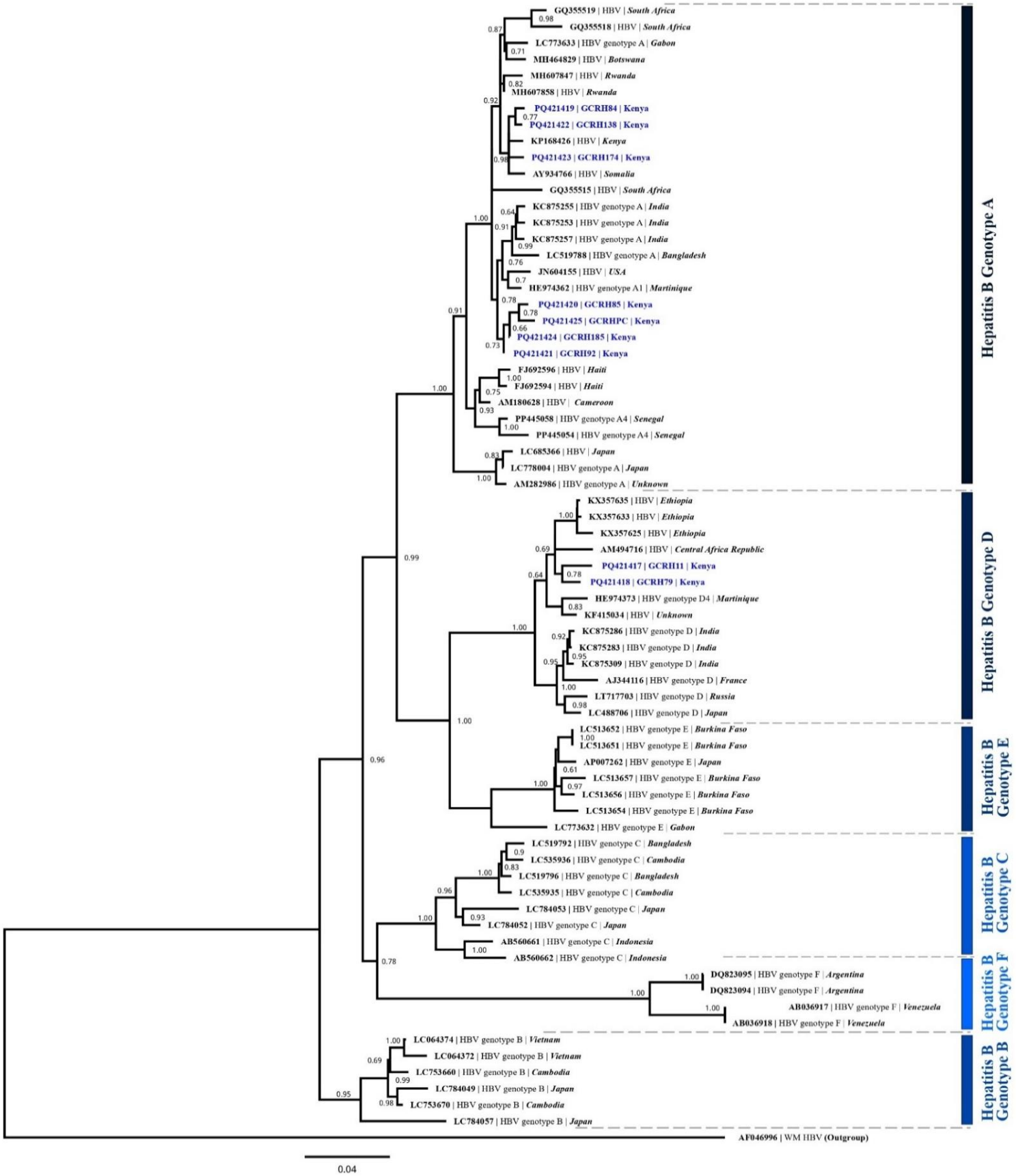

Figure 4.2: Phylogenetic tree of HBV-pol gene sequences from Saretho in Dadaab, Garissa, Kenya. Neighbor-joining method based on 1000 bootstrap replicates was used. Wooly monkey HBV (AY226578-WMHBV) was used as the out group.The phylogenetic trees were observed using Tree view version 1.4.4. Bootstrap value> 68% are shown and HBV isolates from participants are specified in blue.

4.5 HBV Drug Resistance

HBV drug resistance was analysed from sequences isolated from 9 participants. Five participants exhibited hepatitis B virus drug resistance mutations, which resulted in resistance to Lamivudine, Entecavir, and Telbivudine. The mutations linked to nucleoside analogues primarily occurred at position 181 (rt181T), with compensatory mutations present at positions 204 (rt204F), 180 (rt180T), and 173 (rt173). One participant demonstrated HBV drug resistance mutations at positions 236 (rt236) and 181 (rt181T) which resulted in Adefovir resistance. Furthermore, one participant had HBV drug resistance mutations at positions 250 (rt250A), 202 (rt202E), 184 (rt184K), and 169 (rt169T), leading to Entecavir resistance. Two participants did not have any major mutations, indicating susceptibility to all HBV drugs. It was also found that the five participants with HBV drug resistance showed cross-resistance to Lamivudine and Telbivudine (Table 4.5).

Table 4.5: Patterns of HBV Drugs Resistance Mutations among Gravid women

Combined mutations patterns	n (9)	Frequency (%)	Drug associated
rt173G, rt180T, rt204F, rt181T	5	55.6	3TC, ETV and LdT
rt181T and rt236L	1	11.1	ADV
rt169T, rt184K, rt202E, rt250A	1	11.1	ETV
No major mutations	2	22.2	Susceptible

Key

3TC: Lamivudine, ETV: Entecavir, LdT: Telbivudine, ADV: Adefovir

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Seroprevalence

Socio-demographic characteristic (age, marital status and level of education) were assessed to ascertain their association with HBV infection. The HBV infection was unevenly distributed across the age groups. However, this difference was not significant across ages ($p = 0.57$). Despite all ages being equally affected, those aged between twenty and thirty-nine years, were most affected, Contrary to previous studies (Gedefaw *et al*., 2019), individuals of this age groups, most of them were married. Based on the local's nomadic lifestyle, women in this age range are married off at young ages.

While considering marriage fact in the present study, the gravid women who were single had lowest HBV infection rate (6.2%, n=1) compared to those in marriages. The low levels of HBV infection among the single women was associated with likelihood of low or none existent multiple sexual partners. Those married were most affected due to polygamous nature of the marriages in the studied community hence increased risk of infection (62.3%, n=10). Having multiple sexual partners increases the risk of HBV infection (Zhang *et al*., 2016). However, in the studied population, most of the partners were of monogamous nature of marriages hence low rate of HBV infection. It could be argued then that, marital status significantly influenced the risk of HBV infection ($p = 0.01$).

The probability of contracting HBV is highly influenced by education level ($p = 0.03$). This result is consistent with previous studies that demonstrated that highly educated people are less

likely to contract HBV (Muriuki *et al*., 2013). These individuals it could be argued that based on his/her behavior, greater awareness of the risk of contracting HBV and the precautions to avoid HBV infection was the key (Muriuki *et al*., 2013). Furthermore, blood transfusion was associated with the risk of HBV infection. However, with the improvement in screening of blood donors for HBV infection, the level of HBV infection has been low or non-existent (Zhang *et al*., 2016). Studies have also confirmed that blood transfusions could increase the risk of contracting HBV (Tiollais *et al*., 1985). Despite, having history of undergoing through Caesarean section ($p = 0.34$), Dental procedures ($p = 0.51$), having tattoo ($p = 0.65$), liver disease ($p = 0.23$) or family history of having liver diseases ($p = 0.49$) which could pose high risk to HBV infection. In this study these risk factors were not associated with HBV infection, findings that concurs with previous studies (Eyong et al., 2019). These findings were contrary to studies conducted in Ethiopia, Antioch Turkey (Cetin *et al*., 2018) and China (Zhang *et al*., 2013) which tattoo were found to be risk to HBV infection (Umer *et al*., 2023).

In this region which practices female genital mutilation (FGM), among the studied gravid women, those who had undergone through FGM ($p = 0.02$) and Ear piercing ($p = 0.01$) were significantly associated with HBV infection. These findings concur with previous studies (Muriuki *et al*., 2013) which have shown that, these cultural practice like FGM and ear piercing are conducted at home without consideration of septic conditions not forgetting their ignorance on HBV transmission either through haematogenous route or use of contaminated equipment that risk acquisition of HBV.

The sero-prevalence of Hepatitis B Virus among the sampled gravid women at Saretho, in Dadaab, Garissa County was 8.6% (HBsAg) which was found to be considerably highly endemic based on WHO categorization, (where low (<2%), intermediate (2-7%) and high

(>8%) than expected (Meldal *et al*., 2009); (Mabeya*et al*., 2016); (Hou *et al*., 2020).The detected HBV infection burden was slightly higher than expected intermediate endemicity levels in the general population of 8.0% (Downs *et al*., 2023).

This finding was consisted to previous studies conducted in the country from selected hospitals from all regions in the country 9.3 % (Okoth*et al*., 2006), 9.4% (Gatheru *et al*., 2018). Findings from this study confirms the presence of high disease carrier state corresponding to low levels (5.6%) of HBeAg and suggesting the high risk of horizontal but low vertical transmission (Okoth *et al*., 2006). Though the HBV infection burden has been conducted before in the country across different populations, however, most these studies have been conducted among other population rather than among gravid women. From these studies, the country's HBV infections falling within intermediate HBV infection levels, with most of these studies confirming HBV prevalence ranging between 2% and 5% (Downs *et al*., 2023).

Compared to previous studies conducted within the regions and elsewhere, these findings concur with suggested HBV endemicity with those previously detected in the country among pregnant women, in Ethiopia 8% (Umer *et al*., 2023).), 9.51% Chinese women (Zhang *et al*., 2013), Cote d' Ivoire 9% (Route *et al*., 2004) and Gambia (9.2%) (Bittaye *et al*., 2019).

However, the detected levels of HBV infections were relatively higher than studies conducted at Mbagathi, Nairobi 3.8% (Malungu*et al*., 2016) also elsewhere in, Haiti (Tohme *et al*., 2016), 4.3% South Ethiopia (Yang *et al*., 2006), 5.7%, 6.5% DR Congo and Zambia (Gedefaw *et al*., 2018), (Mohammed and Solomon, 2005) and 7.5% in Limbe and Muyuka Health Districts of South West of Cameroon (Eyong *et al*., 2019). Much lower rates of HBV infection among

pregnant women from Ethiopia 1.35% (Mohammed and Solomon, 2005), Mexico 3.7% (Jose *et al*., 2003), Rwanda 2.4% (Pirillo *et al*., 2007) and Israel 0.88% (Bogomolski *et al*., 1991).

There have been prior reports of elevated rates of HBV infection in contrast to this study. The higher rates of HBV infection have been reported elsewhere in Nigeria 11.6%, Hong Kong China 10%, Papua New Guinea 11%, Yemen (10.2%) (Murad *et al*., 2013) and those on extreme high like Cameroon 25.3% (Mohammed and Solomon, 2005).

These variations in HBV Sero-prevalence could be associated with discrepancies attributed to socioeconomic status of diverse populations, geographical and cultural differences, sexual behaviours across studies their adopted HBV preventive measures or the sample sizes of the population sampled (Bahati *et al*., 2021).

According to (Mabeya *et al*., 2016), the HBV 5 panel (HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb) has been utilized to guide correct diagnosis of HBV, including infectious illness stage. However, due to cost, more often HbsAg markers is only been used hence risking detection of occult hepatitis (Kilongosi *et al*., 2015). More often with the single screening of HbsAg as a marker for HBV infection has often led to an oversight occult hepatitis hence risking gravid women in transmitting this virus. Findings on 2.7% occult hepatitis concur with previous studies (Mabeya *et al*., 2016). Similar rates of individuals on acute stage of infection were detected that collaborates with previous studies.

However, in this population, low proportions of women had been vaccinated showing the vulnerability of these populations to HBV infection. With such low levels of HBV vaccination coverage, this study shows a serious requirement to increase HBV vaccination coverage.

5.1.2 The HBV diversity

The evolutionary clusters of isolates from the phylogenetic analysis of HBV revels that the analysed HBVin the study were of Sub Saharan origin.The phylogenetic analysis of HBV sequences revealed that seven (7) isolates were classified as HBV genotype A while two (2) isolates belonged to genotype D.

This shows possible multiple sexual partners or networks activities in the population resulting into the observed viral circulation of the two genotypes in the region as the community practice polygamy.This study showed the predominance of the genotype A. The findings like the previous studies conducted in the most regions in the country (Downs *et al.*, 2023);Kericho and Nairobi (Ochwoto *et al*., 2016; Webale *et al*.,2015;Kibaya *et al.*, 2015; Mwangi *et al*., 2008).

The predominance of HBV genotype A in the region points out to low rates of HBV transmission dynamics and recombination (Webale*et al.*, 2015). The phylogenetic analysis of the study sequences, were clustered with reference sequences of HBV genotype A with those of Kenya and other East African origin. This clustering indicated a likelihood of possible East African origin as well as local circulation of these strains among Kenyan residents. Garissa County is close proximity to the Somali border and it holds high number of refugee community around Dadaab, the detected strains in this region concurs to those that have been detected in Somalia and other parts of Africa like Gabon, South Africa and elsewhere in Asia, where

economic activities of citizens could be also be playing role on the spread of these infections (Wylie *et al.,* 2018).

In contrast, genotype D was not found in Gravid women, but it was found in high-risk groups such as intravenous drug users, blood donors, and sex workers, according to earlier research (Mwangi *et al.*, 2009; Webale *et al.*, 2015; Kibaya *et al.*, 2015) (Figure 4.1). Reference sequences from Ethiopia, Central Africa, India, and Japan were found to be clustered with genotype D (Sunbul, 2014).

The identified viral genotypes attest to the stability and persistence of these strains of the virus that are circulating in the area. It is impossible to completely rule out the possibility of additional genotypes being in the area, particularly if a greater percentage of people are involved. Since the cross-border effect, the presence of refugees, and the selective presence of other genotypes may have an impact on viral evolution, transmission, and disease management, it is necessary to continue monitoring the diversity of HBV in the area.

5.1.3 HBV drug resistance

The emergence of HBV drug resistance is major clinical challenge in the management of chronic HBV infection. In this study, of the sequences analysed, high overall drug resistance prevalence of 77.8% was detected (Table 4.4). The exceptionally high prevalence of HBV drug resistance mutations (77.8%) detected in this study, including cross-resistance to Lamivudine, Entecavir, and Telbivudine, reflects the long-standing reliance to low genetic barrier antivirals, particularly Lamivudine, in HIV treatment programs in the region. This prevalence was higher compared to the global and even previous local previous studies (Kasera *et al.,* 2021; Mabeya *et al.,* 2017; Gomes *et al.,* 2015).

The widespread use of lamivudine in HIV programs and lamivudine monotherapy in HBV/HIV co-infected individuals have led to emergence of drug resistance. Nevertheless, the detected high levels of drug resistance in this study concurs previous studies that have indicated Lamivudine due to its low genetic barrier could results into 70-80% levels of drug resistance in patients within 5 years of treatment or in patients delayed on transition to Tenofovir-based regimens (Mabeya *et al.,* 2017).

The detected cross-resistance in Lamivudine, Entecavir, and Telbivudine confirms the prolonged use of lamivudine monotherapy in HBV/HIV coinfected patients likely provided selective pressure for resistant variant, a problem compounded by limited access to HBV DNA monitoring and the high co-infections burden in Garissa. Furthermore, migration and cross-border interactions with Somalia, where similar treatment practices prevail, may have contributed to the spread of resistant strains..

Drug resistance mutations to Lamivudine, Telbivudine, Adefovir and Entecavir were detected but no drug resistance mutations associated with tenofovir were found. This finding concurs with previous studies that have demonstrated the high efficacy of Tenofovir based regimens (Magoro *et al.,* 2016; Lim(2017)).

Importantly, the absence of resistance to Tenofovir in the analysed sequences reinforces its positions its position as the cornerstone of HBV therapy due to its genetic barrier and robust antiviral activity. These findings emphasize the necessity to enhance routine HBV resistance surveillance, integrate HBV management into HIV care, and ensure broad access to tenofovir-

based regimens in Garissa and similar areas with high HBV disease burden (Kasera *et al*., 2021).

5.2 Conclusion

i. The study revealed a seroprevalence of 8.6% (HBsAg) among gravid women in Garissa.

ii. Marital status, level of education, history of undergoing FGM and ear piercing were risk factors associated with HBV infection in this region.

iii. The seroprofile screening of participants were categorised according to their HBV stages asfollows: 19(10.2%) were HBV immunized, 6(3.2%) were on acute infection, 11(5.9%) were on HBV recovery phase, 8(4.3%) were on HBV chronic infection stage, 5(2.7%) were occult HBV but 137(73.7) HBV non-respondents.

iv. Genotype A and D were the circulating genotypes with Genotype A being the most predominant genotype.

v. This study found high overall drug resistance prevalence of 77.8%. of the analysed sequences. Cross-resistance to Lamivudine, Entecavir, Telbivudine and resistance to Adefovir and Entecavir was observed from analysed sequences. However, no drug resistance associated with Tenofovir was observed.

5.3 Recommendation

i. The endemicity of HBV infections shows the need for a continuous surveillance for HBV using HBV -5 panel rapid diagnostic cassette for conclusive diagnosis and effective treatment for early detection and management.

ii. The study revealed low proportionsof HBV vaccination coverage hence need to implement effective universal HBV immunization programme with focus on women of childbearing age to prevent HBV infection during pregnancy.

iii. The HBV screening among the gravid women should be scheduled in routine ANC profile testing at first contact in the hospital for early detection and treatment interventions.

iv. There should be continuous sensitization of gravid women on the risk factors predisposing them to HBV infection in this region during ANC visits at the hospital to help prevent HBV infection.

v. Due to the predominance of HBV genotypes A and D in Garissa, coupled with a strikingly high prevalence of drug resistance mutations, this study recommend routine HBV drug resistance monitoring and the prioritization of tenofovir-based regimens for effective treatment of HBV infection.

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APPENDIX

Appendix i: License from NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 746915

Date of Issue: 22/July/2024

RESEARCH LICENSE

This is to Certify that Mr.. LAWI EMMANUEL MWANGOME of Kenyatta University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Garissa on the topic: SEROPREVALENCE, GENETIC DIVERSITY, AND DRUG RESISTANCE OF HEPATITIS B VIRUS AMONG GRAVID WOMEN IN DADAAB - GARISSA, KENYA. for the period ending : 22/July/2025.

License No: NACOSTI/P/24/38180

746915

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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See overleaf for conditions

Appendix ii: Ethical approval

KENYATTA UNIVERSITY
CENTRE FOR RESEARCH ETHICS AND SAFETY

Fax: 8711242/8711575 **P. O. Box 43844,**
Email: chairman.kuerc@ku.ac.ke
Nairobi, 00100

Tel: 8710901/12

Website: www.ku.ac.ke
Our Ref: **KU /APPROVAL/VOL.1/15** Date: 9th July, 2024

Lawi Emmanuel Mwangombe,
P.O Box 43844, 00100
Nairobi.

Dear Emmanuel,

APPLICATION NUMBER: PKU/2950/I12973- "SEROPREVALENCE, GENETIC DIVERSITY, AND DRUG RESISTANCE OF HEPATITIS B VIRUS AMONG GRAVID WOMEN IN DADAAB – GARISSA, 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/2950/I12973.** The approval period is **9th /7 /2024 to 9th / 7 /2025.**

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.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.

vii. Submission of an executive summary report within 90 days upon completion of the study to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

To serve you better, researchers are kindly requested to access and complete a customer feedback form and sent it back online as you continue with research and upon completion of data collection found on the following website link; ;(https://docs.google.com/forms/d/1ytWefDwvyz5h1oz_VIn0xbxg3uGdlDzMXFWNDsMrRPQ/edit?usp=sharing

Yours sincerely

Prof. Judith Kimiywe

Director: Centre for Research Ethics and Safety

Appendix iii: Informed Consent Form

My Name is Lawi Mwangome. I am a MASTER student from Kenyatta University. I am conducting a study on Seroprevalence, Genetic diversity, and Drug Resistance of the Hepatitis B virus among gravid women in Dadaab - Garissa, Kenya. The information will help to determine the disease burden and levels of drug resistance in response to treatment.

Procedures to be followed

Participation in this study will require that I ask you some questions and also examine you to screen you for HBsAg some specimens will be taken from you for further Molecular tests. I will record the information from you in a questionnaire.

You have the right to refuse participation in this study. You will get the same care and medical treatment whether you agree to join the study or not and your decision will not change the care you will receive from the clinic today or that you will get from any other clinic at any other time

Please remember that participation in the study is voluntary. You may ask questions related to the study at any time.

You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to the services you receive from this clinic or any other organizations now or in the future.

Discomforts and risks

Some of the questions you will be asked are on the intimate subject and may be embarrassing or make you uncomfortable. If this happens, you may refuse to answer these questions if you choose so. You may also stop the interview at any time. The interview may add approximately half an hour to the time you wait before you receive your routine services

Benefits

If you participate in this study you will help us to determine the disease burden and levels of drug resistance in response to treatment.

Reward

If you agree to participate in this study, lunch will be provided and transport expenses will be reimbursed.

Confidentiality

The interviews and examinations will be conducted in a private setting within the clinic. Your name will not be recorded on the questionnaire. The questionnaire will be kept in a locked cabinet for safekeeping at Kenyatta University. Everything will be kept private.

Contact information

If you have any questions you may contact Prof. Anthony Kebira, Supervisor 1. On 0715032643 or Dr. Regina Ntabo, Supervisor 2. On 0712308322 or the Kenyatta University Ethical Review Committee Secretariat at chairman.kuerc@ku.ac.ke,

Participant's statement

The above information regarding my participants in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is entirely voluntary. I understand that I will still get the same care and medical treatment whether I decide to leave the study or not and my decision will not change the care I will receive from the clinic today or that I will get from any other clinic at any other time.

Code of participant……..... ….……

Signature or thumbprint **Date**

Investigator`s statement

I, the undersigned, have explained to the volunteer in a language she/he understands, the procedures to be followed in the study, and the risks and benefits involved.

Name of interviewer……

....

Interviewer signature **Date**

Appendix iv: Questionnaire

A research study Questionnaire on Seroprevalence, Genetic diversity, and Drug Resistance of Hepatitis B virus among Gravid women in Dadaab - Garissa, Kenya.

Investigators:

i. Mr. LawiMwangome
ii. Prof. Anthony Kebira
iii. Dr. Regina Ntabo

Serial number…….....Date…….

Instructions

Please mark appropriately against the choices for all the Questions.

(1) What is your age?

(a)Under19 (b) 20 – 39 (c) 40 – 59

(2)What is your highest level of education?

(a) Illiterate (b) Primary (c) Secondary (d) College

(3) What is your marital status?

(a) Single (b) Married (Monogamous) (c) Married (Polygamous)

(4) Have you ever been transfused?

(a) Yes (b) No

(5) Do you have any history of undergoing a cesarean section?

(a) Yes (b) No

(6) Do you have any history of undergoing dental procedures?

(a) Yes (b) No

(7) Do you have any history of undergoing FGM?

(a) Yes (b) No

(8) Have you been pierced for a tattoo?

(a) Yes (b) No

(9) Do you have an Ear piercing?

(a) Yes. (b) No

(10) Do you have any history of liver disease?

(a) Yes (b) No

(11) Do any of your family members have any history of liver disease?

(a) Yes (b) No