CHARACTERIZATION OF HEPATITIS B VIRAL GENOTYPES AND THEIR BIOMARKERS AMONG PATIENTS ATTENDING MOI TEACHING AND REFERRAL HOSPITAL LIVER CLINIC

BY

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DECLARATION

I declare that the work contained in this thesis report is my original work and that I have not previously submitted it, in its entirety or in part to Moi University or in any other institution for any degree.

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DEDICATION

I dedicate this thesis to my family for the immense support they have shown to me while pursuing my postgraduate studies, without them this dream would not have been met.

ABSTRACT

Background; Hepatitis B Virus (HBV) belongs to the genus orthohepadnavirus and is the smallest human deoxyribonucleic acid (DNA) virus with a genome of 3200bp in a partially double stranded circular DNA. Globally, about 2 billion people are infected with HBV. Of these, over 65 million who reside in Africa are chronically infected and are therefore at increased risks of HBV related complications including hepatocellular carcinoma (HCC). At least 10 hepatitis B virus genotypes (A to J) have been reported with distinct geographic distributions and are predictive of liver disease progression.

Problem statement: Kenya has one of the leading prevalence rates of HBV infection in the WHO African Region, thus there is an urgent need to conduct local studies on HBV genotyping to better understand its potential usefulness in clinical practice.

Objectives: To establish the prevalence and characteristics of the various HBV genotypes and their biomarkers among hepatitis B patients chronically infected with HBV attending Moi Teaching and Referral Hospital (MTRH) liver/ gastrointestinal (GI) clinic

Methods: A cross-sectional descriptive study with laboratory investigation was conducted. Purposive sampling was used to identify records of all chronic HBV positive adult patients (n=83) attending MTRH liver/ GI clinic during the study period who were then reviewed for potential inclusion into the study. A census was conducted to enroll all consenting adult patients with a history of >6 month of positive HBV status post-diagnosis (confirmed: HbsAg and Anti-HBc positive). Participants' demographic data (age/gender) was collected using the laboratory form and tabulated. Plasma samples were obtained for HBV genotyping and DNA viral load using Rotor gene Q PCR machine. Serum biochemical (alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT)) - marker levels were estimated using Hitachi c311 equipment whereas virological (surface antigen -HbsAg, envelop antigen - HbeAg and core antibody - Anti-HBc) markers were assayed on Cobas e411 platform.

Results: Out of the 83 HBV positive patients at the clinic, 43 (52%) met the full eligibility criteria. Males were 29 (67.4%) while females were 14 (32.6%) with no disparity in mean ages: (males = 35.1±10.8, females = 34.3±9.3). Characterization of HBV genotypes were A: n=34(79.1%), B: n=5(11.6%) and others uncharacterized (E-J): n= 9(20.9%). All cases of genotype B detection were associated with co-infection with genotype A. Genotypes C and D were not detected. ALT levels were normal in 31 (72.1%), abnormal 12 (27.8 %) while GGT levels were normal in 33 (76.7%) and abnormal in 10 (23.3%) cases with varied genotypes. Biochemical markers showed no statistical significance across the genotypes ($p>0.05$). A majority had HBeAg negative status and HBV DNA >10 IU/ml (81.4 % and 86.0 % respectively) with distribution among all the genotypes. HBeAg percentage negativity rate was 75.9, 80 and 88.9 for genotypes A, A/B and E-J respectively. Across genotypes, viral load mean percentage comparisons were: A vs. A/B = 2600 ($p=0.09$), A vs. E-J = 5260 ($p=0.09$) and A/B vs. E-J = 200 ($p=0.28$).

Conclusion: Genotype A was the most predominant with a higher proportion among the participants and with normalized biochemical markers and elevated viral loads. The study population in MTRH has mixed genotype (A and B). A sizable proportion had other genotypes (E-J) not characteristic of the African region needs characterization.

Recommendations: Adoption of HBV genotyping and the demonstrated markers to help improve on patient's outcome at MTRH to reduce the risk of HCC is recommended. Further characterization of the other genotypes (E – J) should be conducted in this population as the study has demonstrated its possible contribution to the current genotypes with further understanding of the new population with the co-infection in MTRH.

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LIST OF ABBREVIATIONS AND ACRONYMS

ALT	:	Alanine aminotransferase
Anti HBc	:	Hepatitis B core antibody
CHB	:	Chronic Hepatitis B
DNA	:	Deoxyribonucleic Acid
ELISA	:	Enzyme Linked ImmunoSorbent Assays
GGT	:	Gamma glutamyl transferase
HBeAg	:	Hepatitis B envelope antigen
HBsAg	:	Hepatitis B surface antigen
HBV	:	Hepatitis B Virus
HCC	:	Hepatocellular carcinoma
PCR	:	Polymerase Chain Reaction
RNA	:	Ribonucleic Acid
ASSLD	:	American Association for the Study of Liver Diseases
WHO	:	World Health Organization
ULN	:	Upper Limit of Normal
URL	:	Upper Reference Limit
ECL	:	Electrochemiluminescence

DEFINITION OF TERMS

Polymerase Chain Reaction (PCR) - It is a method widely used to rapidly make millions to billions of copies (complete or partial) of a specific DNA sample, allowing scientists to take a very small sample of DNA and amplify it (or a part of it) to a large enough amount to study in detail.

Genotype – It refers to the genetic makeup or genetic constitution of an organism. In a broad sense, the term "genotype" refers to the genetic makeup of an organism; in other words, it describes an organism's complete set of genes. In a narrower sense, the term can be used to refer to the alleles, or variant forms of a gene, that are carried by an organism.

Biomarker – a naturally occurring molecule, gene, or characteristic by which a particular pathological or physiological process, disease, etc. can be identified. It can also be refereed as a measurable indicator or characteristic that is used to evaluate a biological/physiological process, condition or response to treatment (molecule, gene, or protein). In this study it is either termed as a biochemical marker or parameter or a serological marker or test.

Serological marker - A laboratory test that checks for the presence of antibodies or other substances in a blood sample. Antibodies are proteins made by the body's immune system in response to a foreign substance or microorganism, such as a virus.

Biochemical marker - A laboratory test that checks for the presence of the molecules or other substances in a blood sample.

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It is by the grace of God that I was able to study. I wish to sincerely thank my family for their unrelenting support and encouragement throughout my academic journey.

I sincerely thank my supervisors; Prof. Arthur Kwena and Dr. Geoffrey Maiyoh for their continued support and guidance throughout this academic journey.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Hepatitis B Virus (HBV) is a human DNA virus that belongs to the genus Orthohepadnavirus of the Hepadnaviridae family. It is the smallest known human DNA virus, with a genome size of 3200 bp in a partially double-stranded circular DNA. Upon infection, this partially double-stranded DNA is converted into covalently closed circular DNA (cccDNA), which acts as a transcriptionally active template in the nucleus of hepatocytes. During the HBV life cycle, pregenomic RNA is transcribed from cccDNA and serves as the replication template for the negative-strand DNA through reverse transcription. Subsequently, fully double-stranded DNA is produced through DNA polymerase within the nucleocapsid, followed by the assembly of the envelope protein to form mature HBV virions (Lin & Kao, 2015).

HBV has been demonstrated to be the world's most widespread infectious agents which causes of millions of infections in the population each year. Approximately, around 500,000 - 700,000 people succumb each year from due to cirrhosis, hepatocellular carcinoma (HCC) due to the chronic infection of HBV or from acute hepatitis B. The Hepatitis B vaccine is known to provide protection against infection and its complications associated with liver cirrhosis and HCC. This however is the first vaccine against a cancer, protecting against a sexually transmitted infection, and the first ever vaccine against a chronic disease ever licensed. This has been a success as low incidences of new HBV infections including hepatocellular carcinoma has repeatedly demonstrated in East Asia countries including Taiwan and Gambia in Africa(Lavanchy, 2012).

Disease progression in hepatitis B virus (HBV) infection is influenced by a range of factors, including host characteristics (such as age at infection, gender, and immune status), viral factors (like viral load, genotype, and mutation), and external factors (including concurrent viral infections, alcohol consumption, and chemotherapy). The risk of chronic infection is also affected by variables such as age at infection, gender, ethnicity, and immune status. Currently, there are now five FDA-approved drugs for HBV treatment (interferon, lamivudine, adefovir, entecavir, and peginterferon alfa-2a), which have since been effective. Patients with decompensated cirrhosis or hepatocellular carcinoma (HCC) benefit from a successful liver transplant often the only chance for survival. Although novel anti-viral drugs have enhanced the management of cirrhosis, efforts to prevent and treat HCC still face challenges and limitations(Wright, 2006).

1.2 Epidemiology of hepatitis B infection

The epidemiology of hepatitis B is often categorized based on the prevalence of hepatitis B surface antigen (HBsAg) in a population. This classification broadly divides areas into three groups: high-prevalence areas (with over 8% HBsAg prevalence), intermediate-prevalence areas (with a prevalence ranging from 2% to 7%), and low-prevalence areas (with less than 2% HBsAg prevalence). These categories help us understand the primary patterns of transmission, infection outcomes, and the overall population impact of chronic hepatitis B, particularly in terms of its association with liver cancer(MacLachlan & Cowie, 2015).

According to Ambachew *et al.,(*2018*),* HBV contains four partially overlapping open reading frames that encode the surface protein, core protein, X protein (HBx), and polymerase/reverse transcriptase (RT). HBV replicates using a reverse-transcribed RNA

intermediate through the reverse transcriptase enzyme, which lacks proofreading capabilities, resulting in highly error-prone nucleotide synthesis during viral replication. HBV exhibits genetic variability with an estimated rate of 1.4–3.2 x 10^{-5} nucleotide substitution per site per year. This genetic variability has led to the emergence of ten HBV genotypes (A-J), which differ by more than 8% of the genome, and forty sub-genotypes that differ by at least 4% of the genome(Ambachew et al., 2018)Click or tap here to enter text..

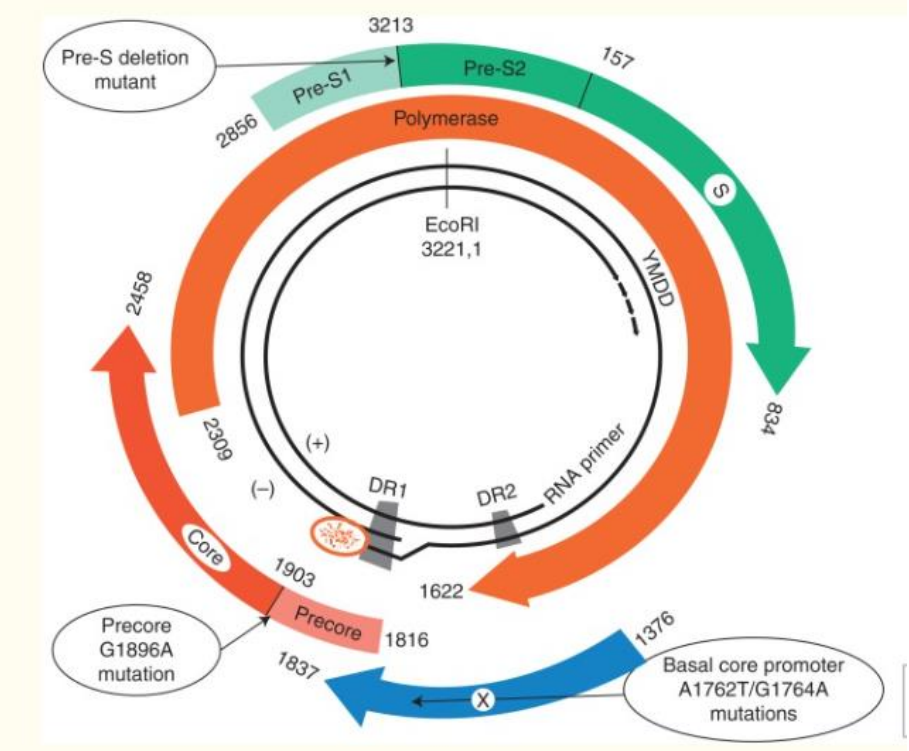

Figure 1: The partially double stranded circular DNA of hepatitis B virus

(**Source:** Lin & Kao, 2015)

Genotyping focuses on a partial sequence of hepatitis B virus (HBV) genome like the pre-S or S gene which codes for the surface antigen protein vital for viral entry and immune response. Numerous methods have been modified and used for HBV genotyping such as; direct sequencing, restriction fragment length polymorphism, line probe assay and enzyme-

linked immunoassay. A novel, rapid and economical genotyping method using PCR amplification assay with type-specific primers method revolutionized the molecular world D'Souza et al., 2004. Click or tap here to enter text.

At least 10 hepatitis B virus (HBV) genotypes (A-J) with discrete geographic distributions and some HBV mutants, including precore/core promoter mutations and pre-S/S deletion mutations, have been documented to be not only predictive of liver disease progression but also linked with response to antiviral therapy. HBV genotype–specific pathogenesis may greatly contribute to heterogeneous clinical outcomes in chronic hepatitis B patients across the world. According to the study, sequencing and genotyping of HBV isolates are not regularly done but also rarely reported in epidemiological studies. Hepatitis B virus genotypes have been demonstrated to vary in their clinical consequences, natural course of infection, disease progression and the treatment response (Velkov et al., 2018).

Most retrospective or case-control studies indicated that patients with genotype C infection develop more severe liver disease, cirrhosis and ultimately hepatocellular carcinoma (HCC), than those with genotype B (Lin & Kao, 2015). Clinical studies on HBV have shown that viral biomarkers can predict the prognosis of chronic hepatitis B patients, and the HCC risk calculator can be used to individualize the management of HBV carriers with different levels of HCC risk worldwide. Chronic HBV infection is a significant global burden, especially in developing countries. After infection, HBV goes through several stages, as illustrated in figure 2 below, until the patient becomes immunocompetent and clears the virus, or develops chronic HBV infection for life (Lin et al., 2015).

Hepatitis B virus (HBV) can be found in various bodily fluids of infected individuals, such as blood, saliva, semen, vaginal secretions, and menstrual blood. It is highly resistant to environmental breakdown, making it easily transmissible through contact with these fluids. The most common mode of transmission worldwide is perinatal vertical transmission, with the presence of HBV e antigen (HBeAg) in the mother's serum increasing the infectivity rate. (Lavanchy Danieland Kane, 2016)

The risk of perinatal HBV infection varies, with rates ranging from 10–40% in infants born to HBeAg-negative mothers and 70–90% in infants born to HBeAg-positive mothers. Even if infants of HBsAg-positive mothers are not infected perinatally, they still remain at a high risk of infection during early childhood in households with a chronically infected individual where the HBV can spread via person-to-person through nonsexual contact(Wright, 2006).

Below is a representation of the onset of infection and the ultimate development of CHB in patients infected with Hepatitis B Virus.

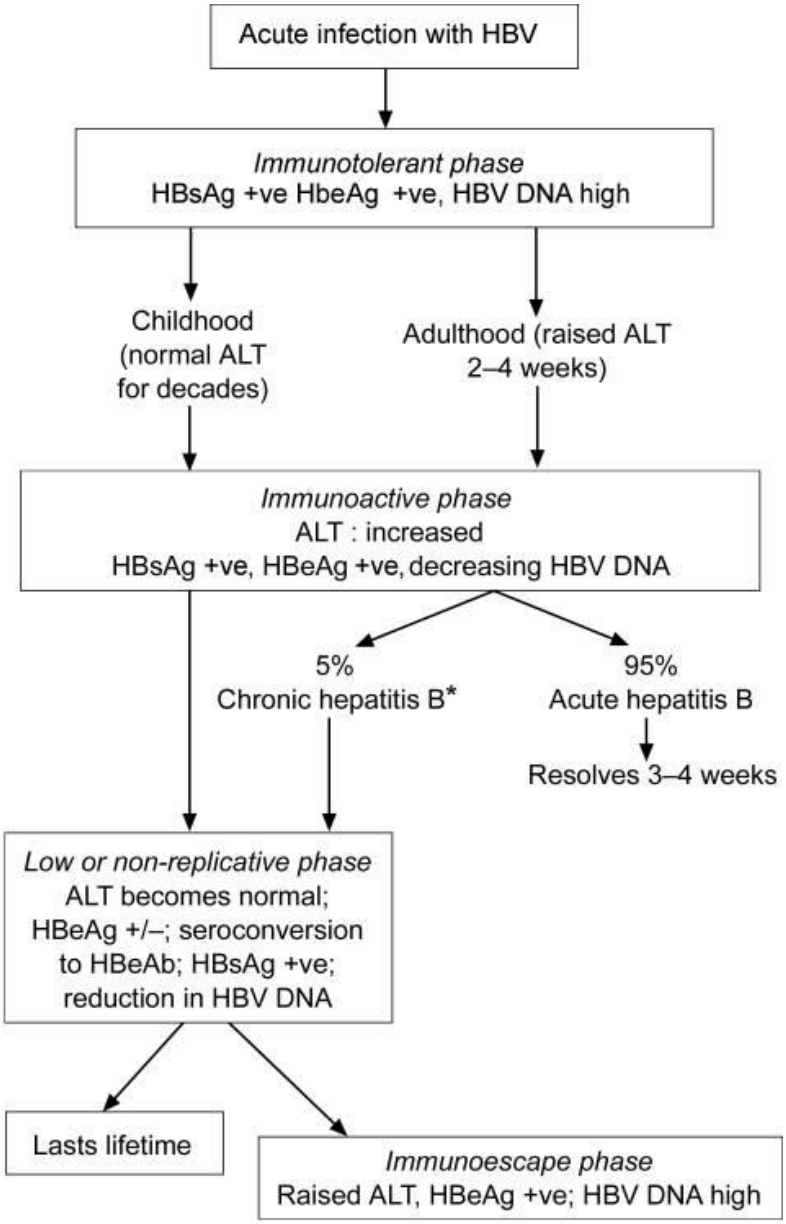

Figure 2: A diagrammatic flow showing onset and progression of the HBV disease (**Source:** Natural history of HBV infection, D'Souza *et al.*, 2004)

1.2 **Serological testing**

In the serological testing, HBV is interpreted using different immunological tests to categorize the disease as described below;

Table 1: Interpretation of Serological markers for HBV tests. Source: Division of Viral Hepatitis, National Center for HIV, Viral Hepatitis, STD, and TB Prevention

Test and Result	Interpretation	Action
HBsAg—Positive Total anti-HBc — Positive IgM anti-HBc — Positive Anti-HBs — Negative	Acute infection	Link to hepatitis B care
HBsAg — Positive Total anti-HBc — Positive IgM anti-HBc — Negative1 Anti-HBs — Negative	Chronic Infection	Link to hepatitis B care
HBsAg — Negative Total anti-HBc — Positive Anti-HBs — Positive	Resolved Infection	Counsel about HBV infection reactivation risk
HBsAg — Negative Total anti-HBc — Negative Anti-HBs — Positive2	Immune from receipt of prior vaccination (if documented complete series)	If no documentation of full vaccination, then complete vaccine series per ACIP recommendations.
HBsAg — Negative Total anti-HBc — Positive Anti-HBs — Negative	Resolved infection where anti-HBs levels have waned	Counsel about HBV infection reactivation risk
	Occult Infection	Link to hepatitis B care
	Passive transfer of anti-HBc to an infant born to an	No action

	HBsAg-positive gestational parent	
	A false positive, thus patient is susceptible	Offer HepB vaccine per Advisory Committee on Immunization Practices (ACIP)
	A mutant HBsAg strain that is not detectable by laboratory assay	Link to hepatitis B care
HBsAg — Negative Total anti-HBc — Negative Anti-HBs — Negative3	Susceptible, never infected (if no documentation of HepB vaccine series completion)	Offer HepB vaccine per ACIP recommendations

1.2.1 Hepatitis B surface antigen (HBsAg)

This is a protein on the surface of hepatitis B virus that can be detected in high levels in serum during acute or chronic hepatitis B virus infection. The presence of HBsAg indicates that the person is infected and infectious, except when it might be transiently positive within 30 days after a dose of hepatitis B vaccine (HepB). The body normally produces antibodies to HBsAg as part of the normal immune response to infection and this has been taken advantage to make HepB vaccine.

1.2.2 Hepatitis B surface antibody (anti-HBs)

The presence of anti-HBs is generally interpreted as indicating recovery and immunity from hepatitis B virus infection. Anti-HBs also develops in a person who has been successfully vaccinated against hepatitis B. Among vaccine responders that completed a vaccine series, anti-HBs levels can decline over time, though the majority still remain immune and will mount a response when exposed to HBV.

1.2.3 Total antibody to hepatitis B core antigen (anti-HBc)

Antibodies against hepatitis B core antigen appear at the onset of symptoms in acute hepatitis B and is a measure of both IgM and IgG, and persists for life. The presence of total anti-HBc indicates previous or ongoing infection with hepatitis B virus in an undefined time frame. People who have immunity to hepatitis B due to vaccination do not develop anti-HBc.

1.2.4 IgM antibody to hepatitis B core antigen (IgM anti-HBc)

Positivity indicates recent infection with hepatitis B virus (<6 months). Its presence indicates acute infection. IgM anti-HBc should be ordered only when acute HBV infection is a concern.

1.3 Global Trends and Distribution of HBV

According to Velkov, an estimated 250 million humans are chronically infected with HBV and an estimated 887,000 annual deaths occur due to liver cirrhosis and hepatocellular carcinoma (Velkov et al., 2018). According to the WHO newsletter global estimates dated 24th June 2022 (https://www.who.int/news-room/fact-sheets/detail/hepatitis-b), the burden of chronic hepatitis B infection is highest at >8% in the Western Pacific region (116 million) and African Regions (81 million) and South-East Asia Region (18 million).

Globally, HBV-related diseases are ranked ninth in mortality rate, fifth most important infectious agent causing about one million deaths annually mostly from cirrhosis and hepatocellular carcinoma(Guvenir & Arikan, 2020; Pourkarim et al., 2014).

In the 2022 theme during World Hepatitis Day 2022, Kenya the focus in Kenya was on "Bringing hepatitis care closer to you" and there were calls for simplified service delivery of viral hepatitis services by bringing care closer to communities.

As discussed by Sunbul, the transmission of the Hepatitis B Virus (HBV) is increasing globally, and urgent action is needed to control and manage it to prevent an epidemic among future generations. Sunbul's study reveals that HBV-related diseases, such as chronic hepatitis B, liver cirrhosis, and hepatocellular carcinoma, cause at least 600,000 deaths annually worldwide (Sunbul, 2014). A study by Pourkarim *et a.l*, 2004 reported that the World Health Organization (WHO) estimates that 2 billion people, comprising one-fourth to one-third of the world's population, have been infected with the Hepatitis B Virus (HBV) (Pourkarim et al., 2014).

Another study narrated the infection rate among gender of positive for HBV in Pakistan as 68.15% males and 31.85% females. Male were more frequently exposed to the risk factors as compared to female. Interestingly, the younger age group had high rate of infection as compared to the children's and the older age groups (Khan et al., 2011).

The discussion above highlights the urgent need to address the issue of HBV infection for the betterment of society and the world as a whole. Despite the availability of an effective vaccine against the hepatitis B virus, chronic hepatitis B remains a significant global public health issue. The World Health Organization (WHO) estimates that over 240 million people worldwide are chronic carriers of HBV, which puts them at an increased risk of developing liver complications such as cirrhosis and hepatocellular carcinoma. Therefore,

it is crucial to continue and upscale efforts to prevent and manage HBV infections worldwide.

1.3.1 Global distribution of HBV infection

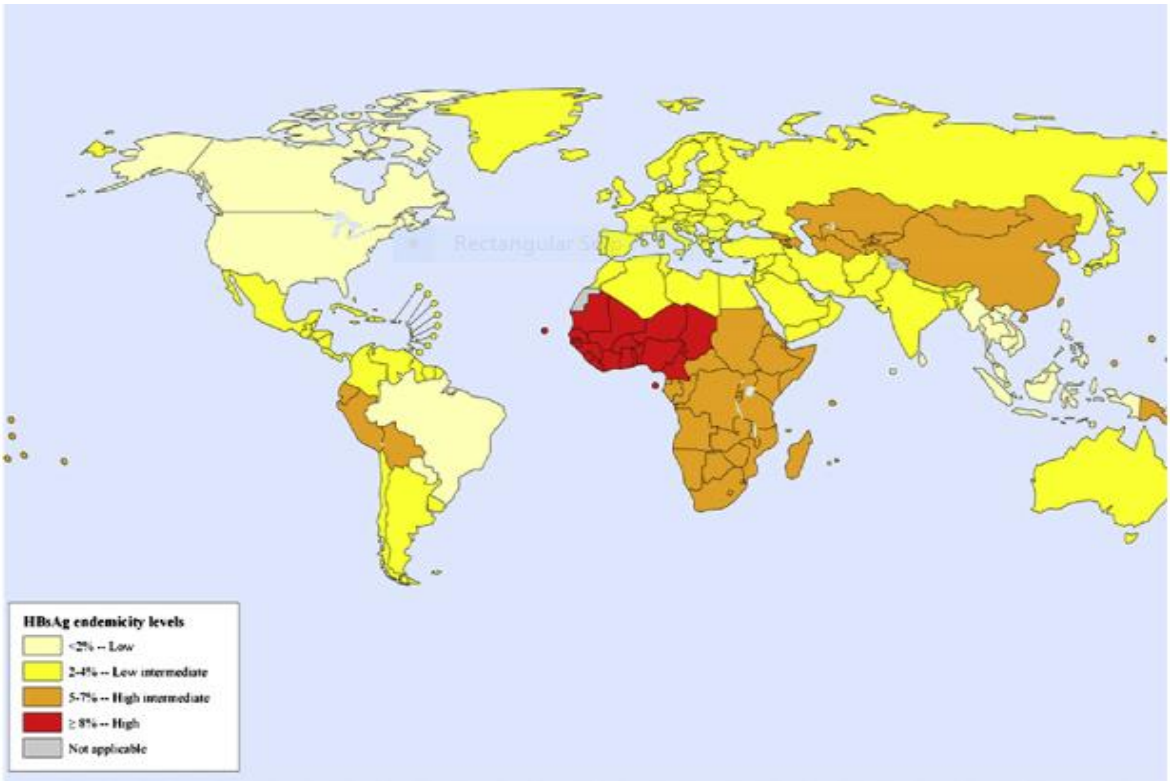

Figure 3: Global Prevalence of Chronic Hepatitis B Infection

(**Source:** Centers for Disease Control and Prevention, Atlanta, GA).

The areas with the highest prevalence of HBV infection, at over 8%, are in African, Western Pacific, and Asian countries. In these regions, the virus is primarily transmitted from mother to child during the perinatal period or acquired in early childhood(D' Souza et al., 2004)Click or tap here to enter text.

Globally, there was an increase in hepatitis B prevalence in both genders from the 1990s to 2005, shifting from a low to a low-intermediate endemicity level in young men. Notably, routine infant and childhood hepatitis B immunization programs have had a profound impact, significantly reducing the presence of HBsAg in immunized children and leading to substantial declines in HBV transmission, as well as the incidence of cirrhosis and liver cancer in various populations. However, the task is far from complete, and there is much work ahead to prevent new HBV infections and find effective treatments for individuals with chronic infections(MacLachlan & Cowie, 2015).

Viral hepatitis is a significant public health issue and highly endemic in many parts of the world. It is responsible for an estimated 1.4 million deaths each year, mostly from hepatitis-related liver cancer and cirrhosis. Unfortunately, many people with chronic viral hepatitis are unaware of their status, and traditional biomarkers such as HBeAg and biochemical markers may not accurately reflect their infectivity. (Di Bisceglie et al., 2017). While HBV DNA levels are a better predictor of liver complications, HBeAg is still considered in management decisions in some guidelines. Having HBV DNA levels greater than 2 times of 103 IU/ml is linked to a considerably higher chance of developing liver cirrhosis and hepatocellular carcinoma. The current recommendations suggest that treatment decisions should be based on the viral load level alongside other factors like liver enzymes, age at diagnosis, and liver histology. Nevertheless, the results of liver enzyme and histological tests may not always match up (Iregbu and Nwajiobi-Princewill: HBV DNA Viral Load, 2016).

1.4 HBV genotypes and their global distribution

HBV can be divided into 10 genotypes (HBV-A to HBV-J) based on differences of 7.5-15% at the nucleotide level of the complete genomes. Additionally, there are nearly 40 sub-genotypes named with the genotype letter followed by a digit, which differ by between 4 and 7.5% of the entire genomic sequence. These genotypes have distinct geographical distributions, with HBV-A being prevalent in Europe, North America, Southeast Africa, and India; HBV-B and HBV-C in Asia and Oceania; HBV-D being the most widespread in North America, North Africa, Europe, the Middle East, and Oceania; HBV-E in West Africa; HBV-F in South America; and HBV-G and HBV-H in Central and South America (Zuckerman, 2007).

Generally, the study concluded that there was no significant association noted between the various genotypes and some demographical factors, serological investigations, and liver markers. Prevalence of HBV genotypes was higher in male patients as compared to female patients and higher in non-Bahraini than in Bahraini (Janahi et al., 2019).

In another study, the results indicated that HBV genotype A is significantly associated with marked ALT increase, higher rate of HBeAg positivity and presence of liver cirrhosis in their population(Ashish Kumar et al., 2004).

In a review conducted by Karimi and others on the geographic distribution of HBV genotypes it was found that the distribution of genotypes is linked to the regional host population and endemicity. They reported that HBV/B and HBV/C are predominant in most parts of Asia, including China and Japan; Genotypes A, D and F are prevalent among the five geographic regions of Brazil; Genotype D is predominant in the Middle East,

including Iran; Genotype A is prevalent in the United States, while HBV genotype B is predominant in Canada. Furthermore, Genotypes E and F are confined to Africa and the Americas. HBV/G has a global distribution, and HBV/H was first revealed in Central America. HBV genotypes I and J have been sporadically reported from Asia and Japan, respectively (Karimi et al., 2015).

Further analysis of the global occurrence demonstrated that genotypes were distributed as follows; A (Worldwide), B (Worldwide), C (Asia, Africa, parts of Europe), D (Southeast Asia), E (Sub-Saharan Africa), F (Southeast Asia), G (Central Africa), H (Southeast Asia), I (South America) and J (Central Africa). Their frequencies were also tabulated as HBV genotype: C (26%), D (22%), E (18%), A (17%), B (14%), F-J (2%). Notably, 72% of genotype A is found in Sub-Saharan Africa (Velkov et al., 2018) with an elaborate occurrence of these genotypes identified in the East Africa occurrence in percentages as; A (50-90), D (20-50), E (5-20) and B (5) accordingly. It has also been demonstrated that the Kenyan genotypes already determined are: A, D and E across the country with genotype A with the highest frequency.

Patients infected with hepatitis B virus (HBV) genotypes C and D tend to experience lower rates and delayed onset of spontaneous HBeAg seroconversion compared to those with genotypes A and B. Genotype C is associated with a higher frequency of mutations in the basal core promoter (BCP) at A1762T/G1764A and preS deletions, as well as a higher viral load when compared to genotype B. Similarly, genotype D exhibits a higher prevalence of BCP A1762T/G1764A mutations than genotype A. These observations suggest that there are distinct pathogenic differences between various HBV genotypes (Liu & Kao, 2013).

According to the World Health Organization Africa Region, an estimated 19 million adults in the region suffer from chronic hepatitis C infection. Viral hepatitis is also becoming a significant cause of mortality among people living with HIV/AIDS, with 2.3 million coinfected with hepatitis C virus and 2.6 million with hepatitis B virus. Recent outbreaks of hepatitis E virus have been reported in Chad, Senegal, South Sudan, and Uganda, and high levels of endemicity have been reported in other areas (Organization, 2017).. To combat the spread of hepatitis B, all 47 countries in the WHO Africa Region have introduced hepatitis B vaccine into their routine infant immunization schedule. A total of 44 (94%) countries uses the pentavalent vaccine, while 33 (70%) countries follow a three-dose schedule at 6, 10, and 14 weeks of age (Breakwell et al., 2017).. This initiative was introduced in Africa to help prevent the spread of the disease, and in Kenya, the pentavalent vaccine was introduced in 2002.

Figure 4 below, is a representation of HBV genotypes worldwide.

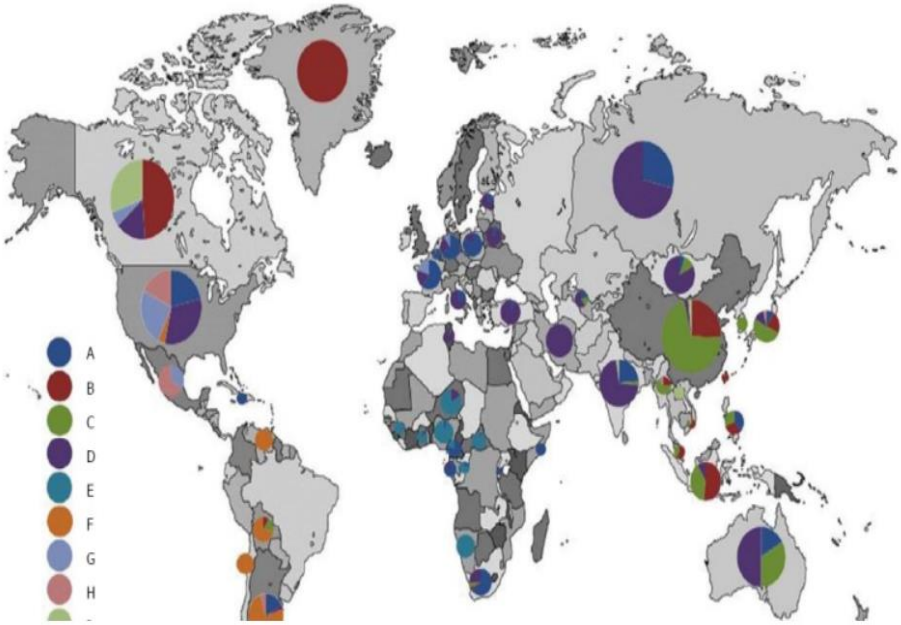

Figure 4: Showing geographical distribution of HBV genotypes worldwide. Source : (Sunbul, 2014).

1.5 Study findings of HBV in Kenya

The country of Kenya is situated on the eastern coast of Africa and has been categorized by the World Health Organization as part of the African Region (AFR-E). This region is known for its high child mortality rates, very high adult mortality rates, and a high prevalence of hepatitis B virus (HBV), with a median HBsAg prevalence of 14%. Although there were numerous studies conducted in the late 1970s and 1980s on the prevalence of HBV markers in Kenya, recent studies have been scarce and have focused on HBV in HIV-infected individuals. A study conducted by Ochwoto found that the presence of HBsAg, anti-HBc, and anti-by markers indicating exposure to HBV ranged from 10% in preschool children to 77% in adults in Nairobi. However, the frequency of HBsAg-positive individuals in a rural community was found to decrease with age, from 30% in children under 10 years old to 3% in those over 50 years old. A population study found that the prevalence of HBsAg was 3.2%, with a higher occurrence in males and a tendency towards familial clustering. This study confirmed the predisposition of males to HBsAg positivity, but there is a lack of information on HBV genotypes (Ochwoto et al., 2013).

As reported by a study done in 2018, carriers of subgenotype A1 and genotype E display very unique clinical features. These (A1-infected individuals) present with low viral loads, low frequency of HBeAg-positivity rate, horizontal transmission of the HBV, increased levels of liver damage and greater risk of developing hepatocellular carcinoma. These predominance of genotype A and subgenotype A1 in South Africa, Zimbabwe and Kenya was amongst blood donors, asymptomatic carriers of the Hepatitis B virus and patients

presenting with with cirrhosis and Hepatocellular Carcinoma. Outcome of these study poses the features subgenotype A1 and genotype E, with its unique molecular features of these African strains, which most likely influence the natural history and the subsequent clinical manifestations of chronic HBV infection in Africa. Genotype A predominates in SA, Zimbabwe and Kenya and genotype E is common in Namibia and Nigeria. However, Sudan presents with three genotypes, genotypes A, D and E, and the lack of a predominant genotype most likely reflects Sudan's unique geographical location and the flux of peoples across its borders (Kramvis, 2018).

Ten percent of outpatients patients at MTRH is unaware of HBV status and is a source of transmission in the community. HBV/A1 remains the most predominant genotype(Songok, 2020).

This study conducted at MTRH determined a general prevalence rate of HBV infection of 10% (20/200) where males accounted for 60% of infection as compared to the females. Additionally, HBV infection was noted higher in an age group of between 31-40 years and contributed to about 35% of the total infections. The likelihood of the infections showed no significant association between the infection with gender and age (p=0.149) and (p=0.070) respectively. Males were considered to be 6 times more likely to be infected (OR =1.286; p= 0.149)(Koech, 2023).

HBV genotypes in recent studies show genotype A as the predominant HBV genotype in Kenya with genotype D and E also being reported in the population, suggesting that HBV genetic diversity could be high in Kenya than the previous statistics (Afonso et al., n.d.)(Afonso et al., n.d.)(Afonso et al., n.d.

Genotype A variant have putative recombination with genotype E and/or D, as collected from coastal Kenya as observed within the study population, however, there seem to have very few reports of genotype A/E recombinants and therefore their impact needs to be assessed(Ochwoto et al., 2016).

Specifically, there is paucity of information on the prevalent and characteristics of genotypes in Kenya, particularly in the MTRH and Western regions of Kenya covered by the institution. This study aims to address this gap in knowledge.

1.6 Problems associated with HBV

Patients with persistent hepatitis B virus (HBV) infection are at risk of developing chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) in a sequential manner. HCC is ranked as the fifth most common cancer in the world, with over 75% of cases occurring in the Far East and Southeast Asia. The incidence of HCC varies significantly across geographical regions and ethnic groups, ranging from a low rate of 3.8 per 100,000 in Caucasian men in the USA to a high rate of over 25 per 100,000 in Asian men in Far East and Southeast Asia. The development of HCC is associated with a range of risk factors, including chronic infection with hepatitis B or C virus, cirrhosis, exposure to carcinogens like aflatoxin B1, smoking, alcohol consumption, obesity, aging, and male gender where amongst all the mentioned risk factors, chronic infection with HBV is the commonest particularly in Asian’s and Africa in continent (Lin et al., 2015).

Despite the campaign and the introduction of an effective vaccine, Hepatitis B virus remains a major global health concern among developing countries. The World Health Organization (WHO) on its campaign has emphasized on the important aspect for such

countries to critically estimate on their burden of viral infections. This would help predict on the global and regional economic impact that we are facing. It has however launched a global program which is aimed at curbing the hepatitis B and C infections with a target of reducing this burden by 2030 to 90% of new cases of viral infections of hepatitis related complications leading to deaths by 65, and treatment of around 80% of viral hepatitis infections. A published a systematic review and meta-analysis done on the global prevalence of HBV, estimates by country were pooled and analyzed for the prevalence and the African continent stood at 8.8% on hepatitis burden with a higher proportion of the prevalence demonstrated on HBV coinfected with HIV represented at 8.2%. Interesting a similar prevalence was also reported in Nigeria at 9.9%, Ghana at 8.9% a greater increase as compared to what the WHO global had estimated. This implied that the outcome of the study demonstrated a greater by half disease burden for the affected individuals with an increased risk with poor prognosis of development into hepatocellular carcinoma in the already immune-compromised state of the patients. (Afonso et al., n.d.) (Afonso et al., n.d.)(Afonso et al., n.d.

Over the past decade, increasing evidence has shown that the genotype of the hepatitis B virus (HBV) can impact both prognosis and treatment response, as well as the emergence of mutations in viral DNA(Malmstro et al., 2010). However, lack of proper awareness has contributed to the rising rates of HBV as many people are not proactive in getting vaccinated. The majority of people remain silent and unconcerned due to a lack of proper education, which could help save lives due to the unawareness of the disease as it poses no major signs that leads to sickness until at a late stage.

While medical practitioners are often vaccinated against HBV due to the increased risk of exposure, the larger group at risk has been ignored, leading to the rise of the disease. Every individual is at risk of exposure to HBV through various modes of contamination, and if vaccination is left only as a choice, then it becomes challenging to control the disease, especially for economically disadvantaged individuals who cannot afford HBV vaccines. Misconceptions about the target group at risk and who needs protection can also contribute to the spread of the disease. However, the inclusion of the hepatitis B vaccine in early childhood immunization programs, such as the Kenya Expanded Programme on Immunization (KEPI), is a significant milestone towards the total eradication of the disease. Despite the introduction of a safe and effective vaccine against HBV in 1982, hepatitis B remains a global public health burden, resulting in over 600,000 deaths worldwide each year (Al Baqlani et al., 2014). This is mainly due to the difficulty in predicting the progression of HBV and its response to treatment is due to the lack of proofreading ability during reverse transcription and the high replication rate of the virus. This results in genetic mutations and the emergence of new variations/variants of the virus, an important factors in predicting disease progression and treatment outcomes (Shen & Yan, 2014).

Although advances have resulted in several generations of HBV vaccines, as well as effective treatment options and a series of viral screening assays, HBV is still considered a dangerous, life-threatening illness and a serious public health problem (Pourkarim et al., 2014).

Apparently, data suggest that lower HBsAg levels at baseline are associated with a better treatment outcome. Interestingly, HBsAg levels at baseline purely is associated with the phase of HBV infection and the genotype itself where, HBeAg positive patients, who were non-responders to IFN therapy, showed notable differences in their HBsAg kinetics across the different genotypes A to D. This supports the above-mentioned idea that HBsAg levels need to be validated for each distinct genotype. Thus, attention needs to be focused on the specific HBV genotype, as highest levels of HBsAg are associated with genotype A infected patients, genotype A and D having overall increased values than genotype B and C. These facts need to be considered in HBsAg cut-offs in distinct patient populations Click or tap here to enter text.(Höner zu Siederdissen & Cornberg, 2014).

It is evident that factors such as viral load, genotype, and specific mutations also affect disease progression and management of the patients. Over the past decade, research has shown that the HBV genotype has a significant impact on both prognosis and treatment response. In particular, patients with genotype C are expected to experience a poorer response to treatment and a worse prognosis in comparison with those infected with genotype B, while genotype A patients more frequently experience normalization of alanine aminotransferase (ALT) levels, clearance of viral DNA, and clearance of HBsAg than genotype D patients (Velkov et al., 2018).. Due to the clinical importance of the infecting genotype, it is likely that genotyping of hepatitis B ought to be increasingly requested in the clinical appraisal and before treatment of infected individuals(Malmstro et al., 2010). In another study done, gamma-glutamyl transferase (GGT) to alanine aminotransferase (ALT) ratio has been shown to be an strong predictive indictor on the severity of hepatitis and HCC where its main objective was to determine the role of the

GGT/ALT ratio to predict the vascular invasion and survival outcomes (Z. Zhao et al., 2021).

HBV has been earlier classified into 9 genotypes (A-I) with a possible 10th genotype J, which are distributed in distinct geographical locations and have been linked to various outcomes. Genotype A is common in many African countries and has been associated with horizontal transmission, chronicity, early HBeAg seroconversion, cirrhosis, and HCC development. The effect of genotype on treatment response, including drug resistance, may impact clinical recommendations, but is not frequently utilized in clinical practice in most settings. Research on the impact of HBV genotype has mainly been conducted in Asia and Europe, with limited data available on circulating genotypes and subgenotypes in Africa, including Kenya(Downs et al., n.d.). (Downs et al., n.d.). (Downs et al., n.d.). The severity and influence of liver disease has primarily studied in genotypes A-D,(Kao et al., 2010). Genotype C is considered more oncogenic than genotypes A, B, and D (Chen et al., 2016).

Additionally, mutations in the basal core promoter (BCP) resulting in decreased expression of HBeAg but enhanced viral replication and deletions in the Pre-S region are associated with increased risk of HCC(Araujo et al., 2020).

Another major setback is the occurrence of Occult hepatitis B infection (OBI) a condition where hepatitis B virus (HBV) DNA is present in the liver or serum, but there is no detectable HBV surface antigen (HBsAg). OBI is concerning as it increases the risk of developing cirrhosis and hepatocellular carcinoma. In Kenya, the prevalence of OBI remains unknown in high-risk Kenyan populations for HBV infection (Jepkemei et al., 2020).

Chronic hepatitis B virus (HBV) infection is a major cause of liver cirrhosis and hepatocellular carcinoma (HCC), which is the primary form of liver cancer. Prevention of new infections through highly effective vaccines is possible, but there is currently no cure available for the 240 million patients worldwide who suffer from chronic hepatitis B infection. Chronic antiviral inflammatory response due to the persistence of HBV infection is a significant factor in the progression of HBV-associated diseases. In addition, integration of HBV DNA into the host cell genome has also been reported as a driver of HCC. This integration can cause chromosomal instability, cis activation of cellular genes, insertional mutagenesis into tumor suppressors, and persistent expression of mutant HBV proteins that drive cellular stress (Particles, 2018).

1.7 Efforts in combatting HBV so far.

Raising awareness about the importance of vaccination against Hepatitis B is crucial in eradicating the virus and reducing the infection rate. The government should also provide free testing and vaccination to ensure that more citizens can access these services, which would contribute to a significant reduction in HBV transmission rates and support the KEPI programme.

Given the significant public health risks posed by HBV, it is essential to gather comprehensive information on both viral and host properties to facilitate the reduction and eventual elimination of HBV infection in the near future(Pourkarim et al., 2014). As stated by Guirgis, at least ten different HBV genotyping methods available, each with varying sensitivity, specificity, turnaround time, and cost in regard to the disease (Guirgis et al., 2010). HBV genotyping and sero-virological markers can aid in determining the

prevalence of HBV genotypes in MTRH, thereby improving the management of patients' health. Therefore, genotype determination in chronic HBV infection is essential in estimating disease progression and planning optimal antiviral treatment (Sunbul, 2014).

Therapeutic endpoints for chronic hepatitis B treatment include sustained suppression of HBV replication to below the limit of detection of real-time PCR assays, biochemical remission, histological improvement, HBeAg loss, or HBeAg seroconversion for HBeAg-positive patients, and ideally, HBsAg loss or even HBsAg seroconversion. According to Lin and Kao, there are currently two recommended types of therapy for chronic hepatitis B: standard interferon or pegylated interferon and nucleos(t)ide analogs. These include lamivudine, telbivudine, entecavir, adefovirdipivoxil, and tenofovirdisoproxil. The impact of HBV genotype on therapeutic responses to both IFN-based and NUCs has been increasingly recognized (Lin & Kao, 2015). However, since patients infected with genotypes E-J are less common, their responses to antiviral therapy remain largely unknown. Therefore, monitoring HBV genotype-specific qHBsAg may improve response-guided treatment of HBeAg-negative chronic hepatitis B (Lin & Kao, 2015).

1.8 Problem Statement

According to the World Health Organization, the highest prevalence of Hepatitis B is observed in the Western Pacific Region and the African Region, where 6.2% and 6.1% of the adult population are infected, respectively. Specific HBV genotypes are known to be responsible for disease progression, and early identification of these genotypes can aid in the general management of patients with Hepatitis B. For instance, perinatally acquired HBV infection tends to be clinically silent and progresses to chronicity in 95% of cases.

(D'Souza, 2004).There is limited information about HBV genetic diversity, including genotype distribution and the prevalence of antiviral resistance, in Kenya. According to recent guidelines for the management of HBV infection, HBV genotyping is not recommended as part of the management for Chronic Hepatitis B. However, HBV genotypes and the variants have shown potential to be useful viral biomarkers for predicting disease progression and aiding clinicians in identifying patients who can benefit most from IFN-based therapy (Lin & Kao, 2015)..

Given that Kenya has one of the leading prevalence rates of HBV infection at 10% in the WHO African Region, there is an urgent need to conduct local studies on HBV genotyping to better understand its potential usefulness in clinical practice.

1.9 Study Justification

HBV genotypes, variants, including viral load, specific viral mutations, their associated biochemical and serological factors have shown potential to be useful biomarkers for the prediction of disease progression and in helping practicing clinicians identify patients who can benefit from specific regimen.

Since HBV genotypes and their subtypes have been shown to vary in different geographical regions, it is imperative to conduct region specific studies in order to ascertain the prevalent genotypes among the local population and the associated biomarkers in order to inform disease diagnosis, treatment and management strategies.

The pucity of such data at MTRH and the western region at large necessitated the present study which sought to characterize HBV genotypes and their biomarkers among hepatitis B patients chronically infected with HBV attending MTRH Liver (GI) clinic.

1.10 Research questions

i. What are the demographic characteristics related with the various HBV genotypes among hepatitis B patients chronically infected with HBV.

ii. What are the prevalence of the various HBV genotypes among hepatitis B patients chronically infected with HBV attending MTRH Liver/gastro-intestinal clinic?

iii. How does chronic infection with the various genotypes affect liver specific biochemical markers (ALT and GGT) among these patients?

iv. How does chronic infection with the various genotypes affect virological markers (Viral load, HBeAg and HBsAg) among these patients?

1.11 Study Objectives

1.11.1 Overall Objective

To establish the prevalence and characteristics of the various HBV genotypes and their biomarkers among hepatitis B patients chronically infected with HBV attending Moi Teaching and Referral Hospital (MTRH) liver/ gastrointestinal (GI) clinic.

1.11.2 Specific Objective

i. To determine the demographic characteristics related with the various HBV genotypes among hepatitis B patients chronically infected with HBV.

ii. To determine the prevalence rates of the various HBV genotypes among hepatitis B patients chronically infected with HBV.

iii. To assay the liver specific biochemical markers (ALT and GGT) among hepatitis B patients chronically infected with the various HBV genotypes.

iv. To profile selected virological markers (Viral load and HBeAg) among hepatitis B patients chronically infected with the various HBV genotypes.

1.12 Significance of the study

Overall demographic and genotypic characterization demonstrate the importance of genotyping as a guide on tailored treatment. Further, the important role of monitoring both biochemical and virological biomarkers among CHB patients will have an impact on understanding their occurrence and demonstrate effective care management and their preferred drug of choice

Consequently, improvement on the management will remarkably reduce the increasing number of liver diseases to help reduce the virulence of the disease that could lead to liver cirrhosis or worse hepatocellular carcinoma in targeting improvement of quality of life in CHB patients.

It also demonstrates the importance of surveillance on the prevailing genotypes around the region in the anticipation of good planning towards policies to combat and manage the HBV disease in the prevailing circumstances.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

The World Health Organization (WHO) estimates that more than 240 million people worldwide are chronic carriers of HBV, which represents an increasing risk of liver complications such as cirrhosis and hepatocellular carcinoma.

In other studies, as conducted by Bell et al., more than 786,000 people die annually due to these clinical manifestations of HBV infection (Bell & Kramvis, 2016). Epidemiological studies have identified chronic HBV infection of the liver as the primary risk factor for HCC development, despite the efforts made to reduce the burden of this infection (Lamontagne et al., 2016). Despite of a vaccine, an estimated 350-500 million people worldwide are chronically infected with HBV, and up to 25% of these individuals may go on to develop HBV-associated HCC, depending on their age and route of infection. This has become a major public health issue that requires special attention to reduce the number of people at risk and improve the treatment of affected individuals and report that chronic hepatitis B remains a significant global public health problem, despite the availability of an effective preventive vaccine against HBV (Lampe et al., 2017).

Hepatitis B virus (HBV) infection is known to cause liver diseases such as chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The infection can lead to a wide range of clinical outcomes, with the majority of cases being subclinical and transient. However, in 25% of cases, self-limited acute hepatitis can occur, and in 1% of these cases, acute liver failure may result. Approximately 90% of neonates and 5-10% of adults can develop

chronic infection, which can progress to chronic hepatitis or an asymptomatic carrier state, and ultimately to liver cancer or hepatocellular carcinoma (HCC), either with or without the intermediate cirrhotic stage (Mousavi et al., 2014).

Hepatitis B virus is the prototype member of a family of viruses called hepadnaviruses. It has a relaxed partially double-stranded circular DNA genome of approximately 3200 bases with four overlapping open reading frames (ORFs): pre-S/S (surface proteins), pre-C/C (pre-core/core), X (transcriptional co-activator) and P (DNA polymerase). The pre-S/S ORF is contained completely within the P ORF, but is translated in a different reading frame. ORFs C and X overlap the ORF P by 1/4 and 1/3 of lengths respectively in their sequence(Z. Zhang et al., 2016).

Hepatitis B virus is known to exhibits genetic variability with an approximately rate of 1.4-3.2 x10−5 nucleotide substitution per site per every year. This occurrence of genetic variability has ultimately resulted in the emergence of ten HBV genotypes (A-J) differing in approximately more than 8% of the genome with occurrence of about 40 sub genotypes.

HBV surface antigen (HBsAg) has been associated with mutations at a considerable effect. Mutations demonstrated within the HBsAg central region which is the Major Hydrophilic Region (MHR) experiencing amino acid (a.a.) substitutions at 99-169; has been strongly associated with the failure to detect HBsAg, antiviral resistance during therapy and vaccine escape is highly experienced amongst HBV patients (Songok, 2020). HBV infection can result in chronic infection or spontaneous clearance of the virus. Children are at the highest risk of developing chronic infections. It’s possible for a person to be infected with HBV for over 30 years without showing any symptoms, but inflammation can still occur in the

liver. Without testing and diagnosis, individuals may not be aware of their infection. It's important to seek medical attention and testing to prevent progression of the disease and potential serious health problems such as cirrhosis, liver failure, or liver cancer (World Health Organization, 2017).

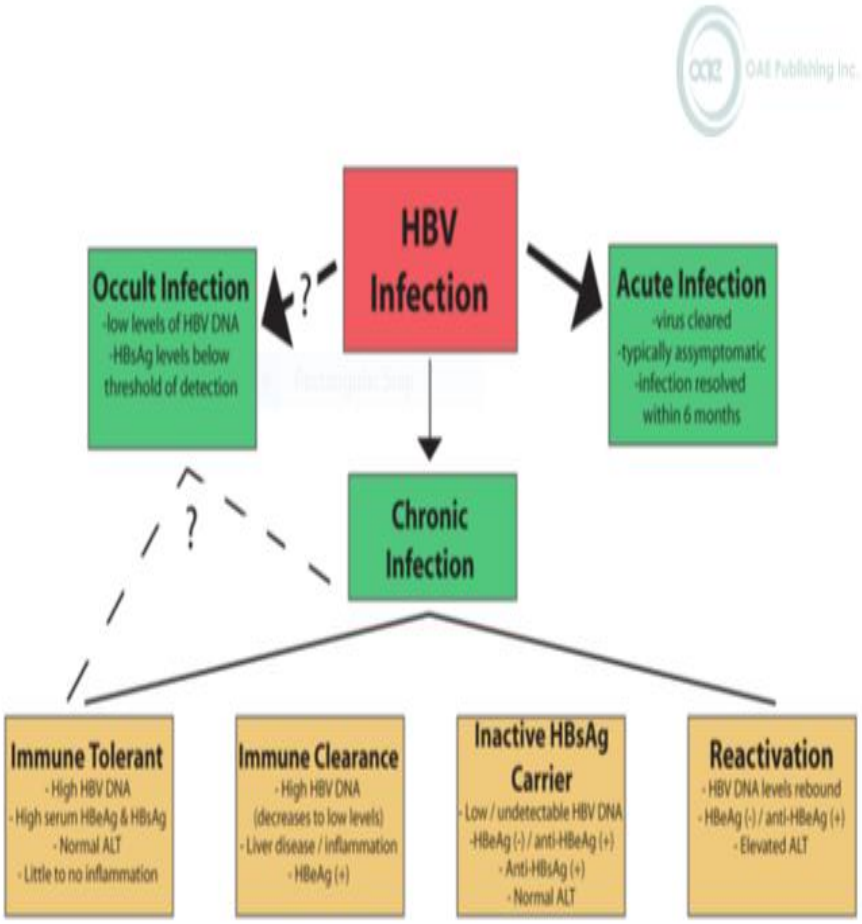

Figure 5: The natural history of hepatitis B virus (HBV) infection (Source : Jason Lamontagne et al., 2016).

HBV infection can lead to three possible outcomes – acute, self-clearing, or chronic HBV infection. Younger age is positively linked to the development of chronic HBV infection. In cases of chronic infection, the virus replicates at high levels for an extended period,

followed by immune-mediated control of viral replication, which leads to liver inflammation, whereas a favorable prognosis is indicated by seroconversion and maintenance of low levels of viral replication. However, if the disease persists long-term, it can result in cirrhosis and hepatocellular carcinoma (Jason Lamontagne et al., 2016).

In regard to the hepatitis B virus, DNA integration occurs in all HBV-susceptible cell types, including primary human hepatocytes (PHH), following in vitro infection. The integration junctions produced in vitro closely resemble the ones found in nontumor patient tissues concerning both HBV and cellular sequences, based on the sequence of integrations. This similarity suggests that the in vitro systems and true HBV infection use the same biological pathways. Using these findings, an optimal system based on hepatoma cell lines was developed, which can generate and detect hundreds of integration events relatively easily. This system enables the interrogation of the molecular mechanisms of HBV DNA integration (Particles, 2018).

Research has demonstrated that the genetic diversity of HBV plays a significant role in the pathogenesis of HBV infection. The HBeAg seroconversion rates, HbcAg seroconversion, viremia levels, immune escape, emergence of mutants, pathogenesis of liver disease, response and resistance to antiviral therapy, and vaccination against the virus can all be influenced by HBV genotypes, sub-genotypes, and mutations in certain regions of the HBV genome. Initially, 18 HBV strains were classified into four groups (genotypes A to D) with a divergence of over 8% between genotypes. Subsequently, at least 10 genotypes (A to J) have been identified. HBV genotypes can be categorized into sub-genotypes based on a 4% divergence. These sub-genotypes include A1-A7, B1-B9, C1-C16, D1-D8, and F1-F4,

and they have distinct geographical distributions, similar to HBV serological subtypes. Chronic HBV carriers with genotypes C and D infections have been proposed to have a lower rate of seroconversion to anti-Hbe antibodies, and faster disease progression to liver cirrhosis and HCC compared to other genotypes such as A and B. Additionally, HBV infection can provide insights into immunological function at different ages, and it is the immune response, not the virus, that plays a major role in liver damage in cases of fulminant liver failure with very low levels of HBV replication and carriers with high levels of HBV DNA but little evidence of liver damage, as suggested by (Dna, 1992). There is a general consensus on the course of chronic HBV infection where it comprises four distinct phases where the interpretation of the cut--offs for ALT and HBV DNA is based on the clinical presentation in regard to the actual phase the patient is experiencing as per the phase. The aim of the study stated was to describes the process involved in developing a computer algorithm for -assigning the participants into various phenotypes at the initial time / baseline and examine their relationships with demographic, biochemical, immunological characteristics which will aid in the identification of changes occurring over time and their long-term clinical implications in regard to different HBV phenotypes (Di Bisceglie et al., 2017).

Genotypes presents with geographical distribution globally and as shown, genotype A (Worldwide), genotype B (Worldwide), genotype C (Asia, Africa, parts of Europe), genotype D (Southeast Asia), genotype E (Sub-Saharan Africa), genotype F (Southeast Asia), genotype G (Central Africa), genotype H (Southeast Asia), genotype I (South America) and genotype J (Central Africa). They also present with different frequencies ranging from genotype C (26%), genotype D (22%), genotype E (18%), genotype A (17%),

genotype B (14%), genotype F-J (2%) with the majority of genotype A at 72% located in Sub-Saharan Africa of the 17% globally. (Stoyan Velkov et al., 2018). East Africa has a distribution of genotype A between 50-90%, genotype D between 20-50%, genotype E between 5-20% and genotype B at5% with the Kenyan distribution having genotype A, D and E by (Songok, 2020)(Koech, 2023)

2.2 Epidemiology

In epidemiological studies, sequencing and genotyping of HBV isolates are not routinely conducted and are infrequently reported. It is important to take note that the clinical consequences of hepatitis B virus genotypes vary, including the natural course of infection, disease progression, and treatment response(Afonso et al., 2023.). HBV genotypes B, C, and I are linked to more frequent vertical transmission from mother to child, whereas genotypes A, D, and G have a higher transmission rate during sexual contact or amongst drug users. A higher rate of chronification after infections with genotypes A and C, compared to genotypes B and D, has also been reported, but this may also be influenced by the transmission route. Among chronic HBV carriers, a lower rate of seroconversion to anti-Hbe antibodies has been observed in genotype C and D infections. Infections with genotypes C, D, and F are associated with faster disease progression to liver cirrhosis and HCC. While all genotypes respond similarly to treatment with reverse transcriptase inhibitors, genotypes A and B demonstrate an increased virological response and higher anti-Hbe seroconversion compared to other HBV genotypes under interferon-α treatment (Velkov et al., 2018).

In a study done by the Hepatitis B Research Network (HBRN) demonstrated that half of the participants in the HBRN study were men, with a median age of 42 years. The population were varied and demonstrates as; 72% Asian, 15% black, and 11% white and with 82% born outside of North America. Prevalent HBV genotype was B (39%) where 74% of the participants demonstrating HBeAg negativity. Additionally, HBV DNA was expressed with a median serum level at the onset of the study being 3.6 $\log_{10}$ IU/mL. The proportions of ALT distribution were 68% for male participants and 67% for female participants experiencing higher than the normal ranges (Ghany et al., 2015).

HBV genotypes, subgenotypes, and mutations can play a significant role in determining the clinical course of the disease, the response to antiviral therapy, and can be used to track transmission and human migrations. As a result, HBV genotyping is becoming increasingly relevant in clinical settings and could contribute to personalized treatment in the future, as well as being important in epidemiological and transmission studies, as discussed by (Bell & Kramvis, 2016). .

HBV genotypes can assist in determining disease burden and guiding management plans where according to Mousavi et al, there are differences in prognosis, clinical outcomes, and antiviral responses among HBV genotypes. Therefore, understanding the epidemiology and distribution of HBV genotypes is crucial (Mousavi et al., 2014)..

In another study, the results showed demonstrated showed no significant differences of the HBV genotype frequency in relation to the demonstrated demographic characteristics
As well as the hepatic biomarkers. The study screened 82 patients with 58.5% being male, and 41.5% females. It was however noted that there was a notably significant risk of HBV

infection in male in relation to females. About 53.7% of HBV positive patients were from the Bahraini nationality with the other population of about 46.3% belonging to the other eleven nationalities including Philippines, Syria Sudan, Yamen, Kuwait, Bangladesh, Ethiopia, Egypt, Indonesia India, and Pakistan. These areas are known to be highly Endemic regions for HBV. The relationship was also analyzed between genotype and the identified age-group indicating that the HBV disease prevails in 4.9% of the group below 21 years, 28% in 21–30 years, 25.6% in 31–40 years, 13.4% in 41–50 years, 15.9% in 51–60 years, and 12.2% in patients greater than 61 years (Janahi et al., 2019).

According to Guirgis et al., genotype C is associated with rapid progression to advanced liver fibrosis, higher rates of hepatocellular carcinoma (HCC) development, recurrence, and metastasis. It is also associated with higher HBeAg positivity and lower HBeAg clearance and seroconversion compared to genotype B. Genotype D is more associated with severe disease and liver cirrhosis compared to genotype A. Finally, genotype F was found to be more associated with higher mortality rates compared to other genotypes (Guirgis et al., 2010).

Untreated chronic viral hepatitis can lead to life-threatening complications, such as cirrhosis or hepatocellular carcinoma, and 20% or more of those with chronic infection develop end-stage chronic liver disease, depending on life expectancy. The progression towards end-stage liver disease can be accelerated by cofactors, such as alcohol or HIV infection. Cirrhosis and hepatocellular carcinoma are both life-threatening conditions (World Health Organization, 2017).

This study is focused on exploring the demographics, genotypic characterization and associated biochemical and virological markers of chronic HBV patients, with the aim of improving patient monitoring and care. By examining these markers, the study hopes to gain a knowledge of understanding the HBV disease and identify potential opportunities for personalized treatment and management strategies. Ultimately, the goal is to improve the care and outcomes for patients living with chronic HBV. It is well researched to show that HBV genotypes contributes greatly to both the course of infection and treatment approaches generally. The genotypes also exhibit structural and functional differences known to have an effect on how an HBV-infected person will respond to therapy against the virus influencing on the manifestation, disease severity and HBV vaccination.

2.3 Effect of genotypes /sub-genotypes on the natural history of HBV infection and response to antiviral therapy

Different HBV genotypes can contribute to variations in the natural progression of chronic HBV infection as discussed by (Kramvis, 2014) discussed how influencing the way the infection presents clinically and how it responds to antiviral treatment is helpful. Patients infected with genotype A, B, D, and F usually experience more frequent and earlier spontaneous HBeAg seroconversion compared to those infected with genotype C, regardless of their ethnicity (Yuen et al., 2009).. Genotype E patients are more likely to have higher HBeAg positivity rates and viral loads than those infected with genotype D (T. Zhao et al., 2014).

Individuals infected with subgenotype A1 typically lose HBeAg earlier than those infected with subgenotype A2 (Kramvis, 2014). Though HBeAg is not necessary for viral replication, it can act as an immune tolerogen and has a role in HBV transmission,

persistence, and the development of chronic hepatitis B. The expression of HBeAg is a crucial component for acute HBV infection to progress to chronic infection (Yuen et al., 2009)..

Perinatal transmission of hepatitis B virus (HBV) is uncommon in regions where HBeAg seroconversion occurs early, as few women are HBeAg-positive during their reproductive phase. However, in areas where HBV genotypes C and I are prevalent, perinatal transmission is common. Children born to mothers infected with genotype C have a higher risk of HBV transmission than those born to mothers infected with genotype B. Certain HBV genotypes/subgenotypes, including A1, C, B2-B4, F1, and possibly D, are associated with a greater risk of severe complications, such as cirrhosis and hepatocellular carcinoma (HCC) when compared to A2, B1, and B5. Genotype C infections also carry an increased risk of liver inflammation, fibrosis, cirrhosis, and HCC compared to other genotypes. Additionally, subgenotypes of B with genotype C recombination are associated with more severe liver disease compared to subgenotypes of B without recombination. Patients infected with subgenotype A1 in southern Africa and southern India have a 4.5-fold higher risk of developing hepatocellular carcinoma (HCC) and develop it about 6.5 years earlier than those infected with other genotypes. Subgenotype A2 is well adapted to sexual transmission and can establish chronic infection in adults whereas in India, subgenotype D1 is significantly associated with chronic liver disease, while D3 is associated with occult HBV infection (Kramvis, 2014)..

HBeAg which is commonly used as a clinical indicator of active viral replication and, along with viral load, has been linked to an increased risk of HCC. Individuals who are

asymptomatic carriers of hepatitis B surface antigen (HBsAg) and have a HBV DNA load higher than $1x10^4$ copies/mL are at an increased risk of developing hepatocellular carcinoma (HCC), regardless of their HBeAg status, alanine aminotransferase (ALT) levels, or presence of cirrhosis. Increased levels of HBV viral load is also associated with an elevated risk of cirrhosis in HBV-infected individuals who are negative for HBeAg. Among those with high HBV DNA levels, the time between diagnosis of cirrhosis and development of HCC is shorter compared to those with consistently low HBV DNA levels. These findings suggest that high viral load is a reliable marker of increased HCC risk in HBV-infected individuals and some carriers of HBV who have developed anti-HBe antibody also exhibit high viral load and active hepatitis (Zhang Q. & Cao, 2011). (Zhang et al., 2010.)

In a study by Baclig et al, 2020 it was found that 20% of cases did not have detectable HBV genotype, possibly because of low or undetectable HBV DNA levels. Among patients with undetectable genotype, 33% had HBV DNA levels of less than 6 IU/mL and a mean ALT of 53.5 U/L. A previous study also found that 24% of chronic hepatitis B (CHB) patients had non-detectable HBV genotype. Therefore, if clinically indicated, follow-up testing for HBV DNA detection and quantification should be performed in one to two months (Baclig et al., 2020).. Another study done by Yoosefi et al., 2016, suggested that any sample with a viral load of less than 10,000 copies/mL and negative for HBeAg should be excluded (Yoosefi et al., 2016).

Due to the fact that certain (sub)genotypes may have a higher occurence of HBV mutations than others has led to the differences in disease progression, response to antiviral therapy,

and clinical outcomes. However, because the distribution of HBV genotypes is different in Asian and Western countries, studies on HBV mutants have mainly focused on comparing genotypes B and C or A and D, with limited information available on other genotypes (Araujo et al., 2020).

It is worth noting that HBV genotyping is not routinely performed in clinical practice, but it can be useful in specific situations. Such as, in areas with high rates of vertical transmission where identifying the genotype of the mother and infant can help guide management decisions and reduce the potential risk of infection.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

Cross - sectional study design was employed to obtain demographic and laboratory data from participants at a single point in time without any future follow up.

3.2 Sample Size calculation

Cochran (1963) sample size determination formulae for populations >10,000

$$n_0 = \frac{Z^2 * p * (1 - p)}{e^2}$$

Z = Standard normal deviation set at 1.96, corresponding to 95% confidence interval.

p = proportion of the population presumed to have the characteristic of study (10% by Songok, 2020)

q = 1 – p.

e = desired level of precision (i.e., the margin of error), set at 0.05

n_0 = 138

$$n = \frac{n_0}{1 + \frac{(n_0 - 1)}{N}}$$

- n_0 = Cochran's sample size recommendation
- N = The population size for finite populations (Average of 88 patients/4 months who meets the eligibility criteria between Dec 2019 and March 2020)
- n = The new, modified sample size = 54 participants

3.3 Study site

This study was conducted at the outpatient liver (gastro - intestinal) clinic of the Moi Teaching and Referral Hospital, Eldoret, Uasin Gishu County, Kenya. MTRH is a teaching hospital for Moi University, College of Health Sciences and the main referral hospital in the North rift, Western Kenya, parts of Eastern Uganda and Southern Sudan with a catchment population of approximately 24 million. It has a bed capacity of about 1200 patients. The liver (gastro - intestinal) clinic attends to an estimated 200 patients every month (with approximately 12 adults chronically infected with HBV).

3.4 Population

3.4.1 Target population

The target population was patients diagnosed with CHB attending the outpatient liver (gastro - intestinal) clinic at Chandaria, MTRH.

3.4.2 Study Population

Patients diagnosed with CHB attending the outpatient liver (gastro - intestinal) clinic at Chandaria, MTRH between 1st Dec 2019 and 31st Mar 2020.

3.5 Sampling procedure

Census sampling method was used to recruit eligible participants willing to join the study within a period of 4 months. A total of 43 participants were enrolled between 1st Dec 2019 and 31st Mar 2020.

3.6 Eligibility

3.6.1 Inclusion criteria

i. All adult CHB patients (≥ 18yrs).

ii. History of not less than six months post-HBV diagnosis (Positive HBsAg).

3.6.2 Exclusion criteria

i. All pregnant women.

ii. Patients who declined to provide informed consent.

iii. Patients who returned a negative Anti-HBc-IgG test result.

3.7 Demographic data collection

Medical record of the chronic HBV patients attending Moi Teaching and Referral Hospital between 1st Dec 2019 and 31st Mar 2020 were reviewed for correlation for correct date of birth and gender. Age and gender information was then abstracted from patients records and entered into Microsoft Excel Spreadsheet (Microsoft 2016).

3.8 Laboratory data collection

3.8.1: Sample collection and preparation

4ml of whole blood was collected into a plain tube. Serum was separated after centrifugation at 3000 rpm for 5 minutes and aliquoted into two cryovials for analysis of biochemical and virological markers respectively. Another 4 mls of whole blood was collected into a EDTA tubes. Plasma was separated after centrifugation at 3000 rpm for 10 minutes and aliquoted into two cryovials each for HBV genotyping and Viral load determination respectively.

3.8.2 Laboratory assays procedures

3.8.2.1 Determination of HBV genotype and HBV DNA Viral Load

The RT Rotor gene Q equipment was the instrument used for the detection of HBV genotype and HBV DNA viral load where its principle entails the unique centrifugal design which ensures optimal thermal and optical uniformity between samples necessary for precise and reliable analysis by the illuminated and fluorescent signal rapidly collected in a single and short optical pathway during fast real-time PCR analysis.

Total DNA for HBV genotyping was extracted from plasma samples using Life River DNA/RNA Isolation Kit from Biotech technologies following the manufacturer's recommendation. The extracted DNA (10ul) was then added to the mastermix mixture of HBV-genotype-FRT Biotechnonogies® for qualitative amplification of genotype A, B, C and D as recommended by the protocol. RT Rotorgene Q 5 plex equipment, Qiagen was used for the amplification and the results were interpreted as per the manufacturer's instruction manual for the amplification kit.

HBV Real-TM Quant Dx Amplification kit was used for HBV viral load analysis after total DNA was extracted using Liferiver DNA/RNA Isolation Kit protocol from Biotech technologies. HBV DNA was then analyzed following the HBV Real-TM Quant Dx Amplification kit where 50 ul was added to the lyophilized master mix and loaded on the RT Rotor gene Q instrument for amplification. The results were then tabulated according to the Kit instruction.

3.8.2.2 Determination of ALT and GGT

Cobas c 311 analyzer was used whereby its principle operates on photometric assays and ion selective electrode measurements using serum samples. Assay of ALT and GGT tests were performed using the Hitachi c311 automated machine as per the manufacturer's instruction. The levels of the liver enzyme alanine aminotransferase were categorized into three groups as follows; normal (male: 0-41 UL, female: 0-32 UL); high (male: 42-123 UL, female: 33-96 UL); extremely high (male: >123 UL, female: 0>96 UL) whereas the gammaglutamyl transferase were also categorized into three groups i.e., normal (male: 10-66 UL, female: 5-39 UL); high (male: 67-198 UL, female: 40-117 UL); extremely high (male: >198 UL, female: >117 UL). .

3.8.2.3 Determination of HBsAg, HBeAg and Anti-HBc

Cobas e 411 instrument was used following the principle where competition for extremely small analytes, sandwich principle for larger analytes and a bridging principle to detect antibodies in the sample utilizing the electrochemiluminescence (ECL) method.

The serum sample was then analyzed for HBsAg, HBeAg, anti-HBc using the automatic Cobas e411 analyzer as per the equipment protocol.

3.9 Data Management

The study management of data involved the collection of demographic information through abstraction forms and laboratory blood specimens (serum and plasma) from the samples drawn from the participants. This robust data management process was essential for the successful execution of our research and the production of reliable results. The following data management steps were taken;

3.9.1 Data Collection Process

It involved compliance with all relevant ethical guidelines (IREC). An informed consent was obtained from all participants the study's protocols and objectives were communicated clearly the participants. Collection of demographic information was abstracted from the patients file which consisted of only age, gender and medical history of chronic hepatitis B. Blood specimens were collected with the utmost care and adherence to established laboratory standard operating procedures. Phlebotomists, trained in venipuncture, used sterile techniques to obtain blood samples from study participants. These specimens were collected in appropriate tubes, centrifuged at recommended speed, separated and stored at the recommended temperatures.

3.9.2 Data Entry and Recording:

The collected data was entered into an excel sheet ensuring accuracy of the data handling and security protocols. Regular data quality control checks were performed to identify and correct any errors or inconsistencies. This process involved cross-verification of data points. Access to the database was restricted to authorized personnel only, and stringent measures were in place to safeguard the privacy and confidentiality of the collected information.

3.9.3 Data Analysis and Reporting:

3.9.3.1 Statistical Analysis

The collected demographic and laboratory blood specimen data were subjected to statistical analysis. This analysis allowed us to derive meaningful insights, identify correlations, and draw conclusions. STATA version 16 software was used to analyze quantitative data using the two variables to determine the relationship between the dependent (HBV genotype) and the independent variable (age, gender, ALT, GGT, HBV DNA viral load and HBeAg). Descriptive analysis was used where mean was used as a measure of central tendency, standard deviation as a measure of dispersion, frequency distribution presented in tables, class intervals and percentages. Inferential statistics was also used where hypothesis testing was done using the t-test (p=value)..

3.9.3.2 Results Presentation

The results of the study were presented in a clear and comprehensible manner, with tables, charts, graphs and narratives.

3.9.4 Data Storage and Backup:

The data was stored in soft copies and the backup filed in hard copies.

3.10 Ethical Consideration

The study proposal was approved by IREC at MTRH/MOI University, Reference No.: IREC/2019/246 and Approval NO.: 0003476.

Study procedures were followed during patient preparation, sample collection and the goal of the research explained to the participants. Informed consent was sought from each participant before being enrolled and the participants benefited from the study through the provision of their results. Confidentiality maintained throughout the study where the participants data was kept under password restricted access.

3.11 Limitations of the study

The study did not get enough funding to analyze the some of the rare HBV genotypes and sub-genotypes.

Time factor in the master's programme did not allow follow up of the participants.

CHAPTER FOUR

4.0 FINDINGS OF THE STUDY

4.1 Confirmation of chronic HBV patients

Out of the 83 HBV positive patients at the clinic, 45 (54%) met the initial eligibility criteria by returning a positive test result for HBsAg (See table 2).

Table 2: Showing confirmatory screening results of HBV based on HBsAg

Variable	Category	Frequency	Percentage
HBsAg	Detectable	45	100
	Not detectable	0	0
	Totals	**45**	**100**

A total of forty-five (45) participants met full eligibility criteria after retesting for HBsAg was performed to ascertain their HBV status.

Of these, 43 (95.6%) of them returned a positive test result for anti-hepatitis B core Antibody (Anti-HBc Ig G) confirming chronic HBV infection. (table 3).

Table 3: Showing confirmatory screening results of HBV chronicity on anti-HBc (IgG)

Variable	Category	Frequency	Percentage
Anti-HBc (Ig G)	Detectable	43	95.6
	Not detectable	2	0.04
	Totals	**45**	**100**

43 out of 45 participants returned a positive anti- HBc and therefore met full eligibility to be further analyzed.

4.3 Demographic characteristics of study participants

4.3.1 Age

The age of the participants ranged between 21 years and 61 years with a mean age of 34.8+/- 10.2. Mean for males was 35.1±10.8 while the mean for females was 34.3±9.3 and a p-Value = 0.7999 with no significant difference (see figure 6).

4.3.2 Gender

As shown in figure below, there were more males 29 (67.4%) than females 14 (32.6%). The male to female ratio was 2:1 for the study participants (see figure 6)

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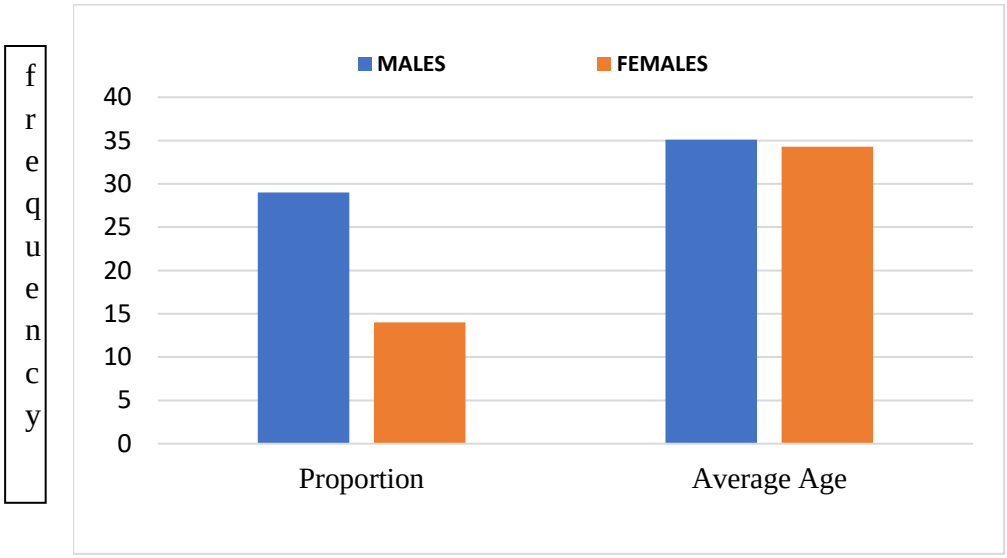

Figure 6: Showing demographic data of the age and gender in chronic HBV patients attending MTRH liver clinic.

4.3.3 Age frequency distribution (in years) amongst male and female participants

The distribution of gender across different age groups was observed more predominantly between the male gender where spikes were noted in ages between 21-25, 31-35 and 46-50 with the highest spike noted between the age group of 31- 35 years. Additionally, the female gender has a fairly well distributed curve amongst all the age groups. (see figure 7 below).

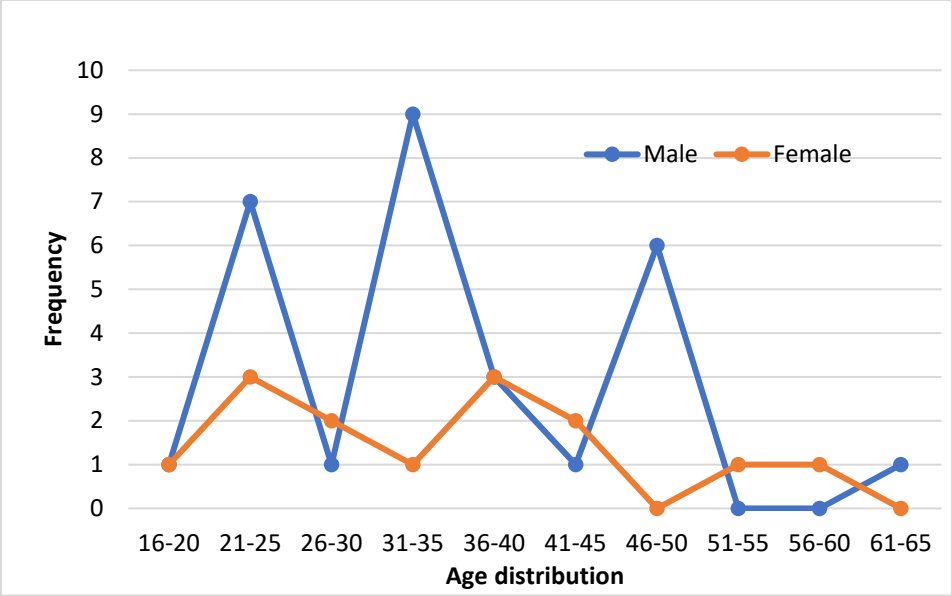

Figure 7: Age group frequency distribution (in years) amongst male and female participants

4.4 Prevalence of HBV Genotypes in among the participants

Out of the 43samples analyzed, 79.1% were diagnosed to have HBV genotype A, 11.6% to have co-infection of HBV genotype A and B, genotype C and D were undetected (0%) while 20.9% had the other un characterized genotypes E-J (see figure 8 below).

Note: The co-infection has been represented independently and as a single entity with the focus of narrating the circulating genotypes percentage and percentage of the co-infection thus the total percentage is above 100% (123.2%)

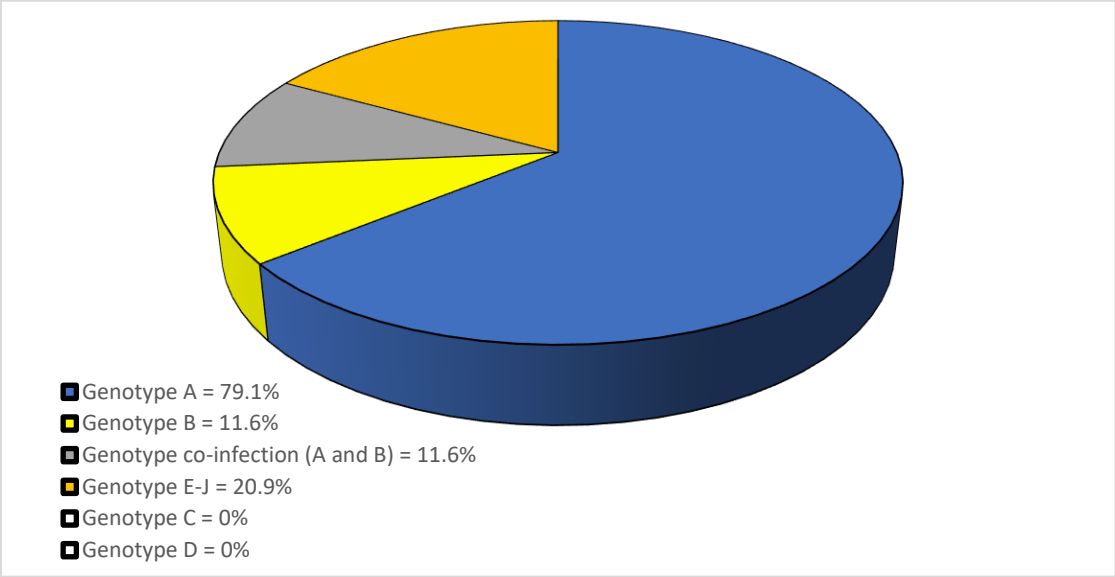

Figure 8: Showing chronic HBV genotypes and their prevalence among study participants

4.4.1 Gender versus genotypes among participants

The gender-based genotypic distribution were as follows: genotype A = 79% in both gender (male - M and female - F), genotype A/B = 10% (M); 14% (F), uncharacterized genotypes (E-J) = 21% in both M and F gender as shown in figure 9 below.

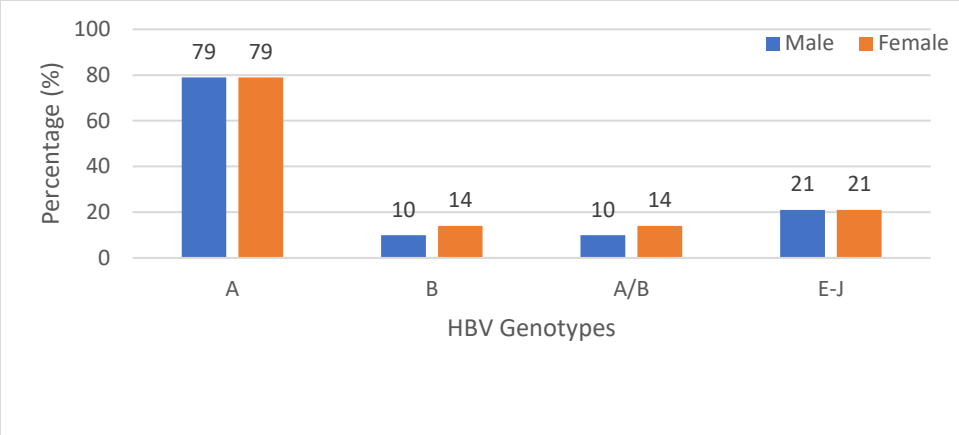

Figure 9: Showing frequency of various HBV Genotypes among male and female participants.

4.4.2 Age versus genotypes among participants

The spikes were noted among the age of all the at 31-35 and 46-50 years for male participants with genotype A, whereas the female gender had a fairly well distribution among all genotypes with a slightly higher distribution amongst genotype A/B between the age of 26-30 and 51-55 years. (see figure 10 below).

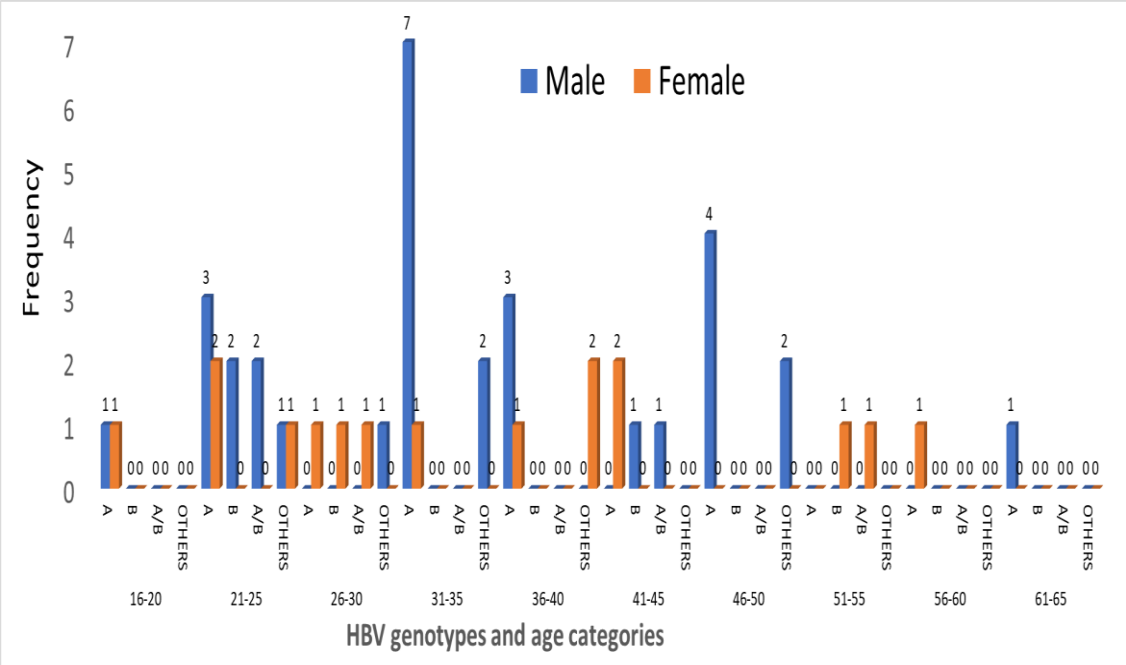

Figure 10: Frequency of various HBV Genotypes among male and female participants distributed based on various age categories

4.5 Relationship between biochemical markers (ALT and GGT) with the various HBV genotypes

4.5.1 Alanine aminotransferase (ALT) versus HBV genotypes

As shown in table 4 below, the frequencies of various levels of ALT among participants with various genotypes.

		Genotype			
Variable	**Category**	**A (n=29)**	**A/B (n=5)**	**Other (E-J) (n=9)**	**Total (n=43)**
ALT	Normal	20	4	7	31
	High	6	1	2	9
	Extremely high	3	0	0	3

Key **ALT Reference Ranges**	**Category**	**Male (U/L)**	**Female (U/L)**
	Normal	**0 -41**	**0 -32**
	High	**42-123**	**33 - 99**
	Extremely High (URL*3)	**>123**	**> 99**

Table 4: Confirmation of Biochemical markers (ALT) for chronic HBV patients attending MTRH liver clinic.

The results above shows that genotype A had a lower frequency of participants with normalize ALT levels at 68.9% as compared to the other genotypes A/B at 80% and genotype E-J at 77.8% with a slightly higher proportion of genotype A with an increased ALT levels at 31% being noted amongst this population.

4.5.2 Gamma glutamyl transferase (GGT) versus HBV genotypes

As shown in table 5 below, the frequencies of various levels of GGT among participants with various genotypes

		Genotype			
Variable	**Category**	**A (n=29)**	**A/B (n=5)**	**Other (E-J) (n=9)**	**Total (n=43)**
GGT	**Normal**	**21**	**4**	**8**	**33**
	High	**6**	**1**	**1**	**8**
	Extremely high	**2**	**0**	**0**	**2**

Key **GGT Reference Ranges**	**Category**	**Male (U/L)**	**Female (U/L)**
	Normal	**10-66**	**5-39**
	High	**67-198**	**6-117**
	Extremely High(URL*3)	**>198**	**>117**

Table 5: Confirmation of Biochemical markers (GGT) for chronic HBV patients attending MTRH liver clinic

The results above shows that genotype A had a lower frequency of participants with normalize GGT levels at 72.4% as compared to the other genotypes A/B at 80% and genotype E-J at 88.9% with a slightly higher proportion of genotype A with an increased ALT levels at 27.6% being noted amongst this population.

4.6 Relationship between virological markers (HBV viral load and HBeAg) with the various HBV genotypes

4.6.1 HBV viral load versus HBV genotypes

As represented in the table 6 below, virological markers (HBV viral load) were also compared amongst all the genotypes.

		Genotype			
Variable	**Category**	**A (n=29)**	**A/B (n=5)**	**Other (E-J) (n=9)**	**Total (n=43)**
HBV Viral Load	**Undetectable**	**1**	**1**	**4**	**6**
	Detectable	**16**	**1**	**4**	**21**
	Extremely high	**12**	**3**	**1**	**16**

Key **HBV Viral Load** **(Reference Ranges IU/ml or copies/ml)**	**Category**	**Male/Female (IU/mL)**
	Undetectable	**<10**
	Detectable	**10 - 2000**
	Extremely High	**>2000**

Table 6: Confirmation of Virological markers (HBV viral markers) for chronic HBV patients attending MTRH liver clinic

All genotypes presented with mostly detectable HBV DNA viral load (86%) with a higher proportion being genotype A (96.6%) followed by genotype A/B at 80% and finally genotype E-J at 55.6%. Genotype E-J also demonstrated with a higher proportion on undetectable HBV DNA levels at 44.4% higher than all the other genotypes.

4.6.2 HBeAg versus HBV genotypes

The virological markers (HBeAg) were also compared amongst all the genotypes. as shown in table 7 below.

	Genotype			
Variable	**Category**	**A (n=29)**	**A/B (n=5)**	**Other (E-J) (n=9)**	**Total (n=43)**
HBeAg	Negative	23	4	8	35
	Positive	4	1	0	5
	Extremely High	2	0	1	3

Key

HBeAg (OD Measurements)

Negative = < 1

Positive = 1-100

Extremely High

Table 7: Confirmation of Virological markers (HBeAg) for chronic HBV patients attending MTRH liver clinic

A majority of participants presented with HBeAg negativity with an overall of (81.4%) with the least being from genotype A at 79.3%, followed by genotype A/B at 80% and highest genotype E-J higher at 88.8%. Genotype A also presented with participants showing a slightly elevated HBeAg positivity at 21%, followed by genotype A/B at 20% and the least being from genotype E-J at 11.1%.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Characteristics of participants

The present study sought to establish the prevalence and characteristics of the various HBV genotypes and their biomarkers among hepatitis B patients chronically infected with HBV attending Moi Teaching and Referral Hospital (MTRH) liver / gastrointestinal (GI) clinic between 1st December 2019 to 31st March 2020. Participants were initially reviewed for eligibility. A total of 43 returned a positive test result for HBsAg and anti-Hepatitis B Core Antibody (Anti-HBc) and were thus included in the study.

5.2 Demographic characteristics related with the various HBV genotypes among the study participants

5.2.1 Age

The age group ranged between 18-61 years with a mean of 34.8 ± 10.2with mean of each gender represented by males at 35.1 ± 10.8 while the females at 34.3 ± 9.3 with a p-Value = 0.7999 without any significant difference..

It was also observed among women that there was a fairly even genotype distribution across all ages. On the other hand, males presented with variations of spikes with genotype A at 31-35 and 46-50 years.

It also compared with a Kenyan study where the mean age was 36.5 ± 11.2 with ages ranging from 16-64 years with a predominance of genotype A, Ochwoto et al., 2016.

This contrasted with a study done where the high level of infectionwas observed in the age group 25.0–38.0 years old across gender (P < 0.022) followed by age group 39.0–52.0 years (Kasera et al., 2021).

However**,** it contrasted with the Hepatitis B Research Network in the USA where the mean age was 42 years and without gender predominance in the overall population (Ghany et al., 2015).

5.2.2 Gender

The proportion of HBV in males was higher than that of females at 67.4% and 32.6% respectively the ratio being 2:1 between male and female gender

This compared well with a Kenyan study that concluded that males were significantly highly infected than females. As alluded in Kaseras et al., 2021 study, the observation pointed to the fact that more men tend to have multiple sexual partners with possibly unprotected sex, indulging in drugs and alcoholism could be the predisposing factors. (Kasera et al., 2021). This was however not investigated in this current study.

It also compared well with another Kenyan study by Ochwoto et al., 2016, where it also observerved a 2:1 male: female ratio.

There was no gender predominance across all genotypes except a slight increase in genotype AB where females predominated at 58%. Amongst all genotypes the most predominant genotype across each gender was genotype A.

5.2.3 Prevalence rates of the various HBV genotypes among the study participants

Genotype A was most predominant and compared with data across the globe where genotype A is the most predominant in Sub-Saharan Africa. A co-infection of genotype A/ B was also identified in amongst the population while a good proportion also identified fell under the category of genotypes E-J.

It compared well with a study done where genotype A was the most predominant at 93% by Songok et al, 2020 and Koech et al, 2023 among patients attending MTRH and another study done across Kenya where genotype A was 90.3% among blood donors, Ochwoto et al., 2016. Similarly, a comparison was with a Kenyan study conducted where genotype A was the most predominant genotype in Kenya and identifies occult HBV infections not previously reported in this population (Aluora et al., 2020)

Genotype A/B was also identified in the present study which is a new trend. Presence of genotype B has been associated with vertical transmission (Kafeero et al., 2023).

Studies across have also demonstrated the occurrence of recombination where there were possibilities of recombination between genotypes e.g. A with D or A with C or B with C and so on indicating a particular geographic distributional change and it is anticipated that with escalation of immigration worldwide may cause the former geographic distribution pattern may fluctuate (Aluora et al., 2020).

Hence, there, (Aluora et al., 2020) in regions where HBV is endemic, infection with more than one genotype frequently ends up to recombinant strain and possibility of co-infection or super-infection with other genotypes in a particular host. Furthermore, mixture of

different genotypes in patients suffering from chronic hepatitis B (CHB), in comparison with those who are infected with a single genotype, is related to higher viral load and acceleration of HBV replication in vitro. . Additionally, genotype mixture (mostly B and C) in comparison with C alone was correlated with more viral load and a more severe disease where according to above report co-infection and superinfection with various genotypes has poorer prognosis of HBV. However, a mixture of genotypes C/D in China has been documented. In the predominant genotype (D) which is in Pakistan, have described co-infection of genotypes B/D as well as C/D and as far as Iran is concerned, information on HBV genotype is limited nonetheless, with discrepancy of genotypes within a certain country being foreseeable, so HBV genotyping is necessary in different regions and multiplex PCR is suitable for diagnoses of co-infections with various genotypes (Yoosefi., et al., 2016).

In conclusion, the findings of our study confirm the circulation of genotype A is predominant. Mwangi and others, conducted a study where they found that out of 80 HBsAg seropositive samples, 52 were positive for nuclear acid testing (NAT). The remaining samples could not amplify specifically and were considered to be either false-positives or had a very low concentration of DNA that could not be detected by PCR (Mwangi et al., 2009). This implies that the actual concentration of HBV DNA plays a crucial role in identification of the genotypes and the patients should be followed up for genotyping after two months.

Another study done in Kenya, by Aluora et al., additionally states that there is an occurrence of a good proportion of genotype B and C coinfection (Aluora et al., 2020). These occurrences being reported in Kenya need to be thoroughly investigated and data

presented for proper patients care and management which includes the findings of these study where genotype E-J has also been recommended for further characterization in the region.

Further, unlike the common trend showing presence of genotypes D in Kenya (Ochwoto et al., 2016) and MTRH (Koech, 2023), genotype C and D were not detected in the present study.

5.3 Characterization of HBV genotypes based on liver specific biochemical markers among study participants

5.3.1 Alanine aminotransferase (ALT) levels

A predominantly higher proportion of participants presented with normal levels of liver biochemical markers across all the HBV genotypes identified in the study. The levels of the ALT levels are considered to be liver specific biomarkers are estimated to in hepatitis B patients as a tool to assess the status of the liver with the various genotypes. A general guideline according to the American College of Gastroenterology states that normal ALT levels are indicative of a healthy individual ALT limits as recommended by the clinical guideline, where patients with persistently normal ALT should be tested for ALT at 3-6-months intervals and for ALT/HBV DNA more often if ALT becomes elevated with high/ extremely high values of ALT among HBV patients correlate with the clinical symptoms and liver biopsy. This is important in this current study as a higher proportion of participants with genotype A presented with elevated liver markers unlike the other genotypes with approximately ALT and GGT respectively amongst its cohort. This could point to a more adverse prognosis among these participants and require closer monitoring.

Interestingly to note is that, genotype A/B participants represented with more frequently normalization of ALT levels than the rest of the genotypes.

Genotype E-J also presented with a good population having normalized ALT levels with a slightly higher group presenting with increased ALT levels that cannot be ignored as those of genotype A.

The results of this study correlated with a study where by, patients with genotype C are expected to experience a poorer response to treatment and a worse prognosis in comparison with those infected with genotype B, and A patients who more frequently experience a normalized alanine aminotransferase (ALT) levels than genotype D ((Malmström et al., 2010). This correlates well with this study where the majority of the biochemical markers presented with normalized levels across all genotypes and requires the patients to be monitored.

A predominantly higher proportion of patients presented with normal levels of liver biochemical markers (ALT: 72%) across all the HBV genotypes identified in the study.

Interestingly genotype A/B patients more frequently experienced normalization of ALT levels than the rest (80%).

Further, a higher proportion of patients with genotype A presented with elevated liver markers unlike the other genotypes with approximately ALT (31%) amongst its cohort.

This could point to a more severe prognosis among these patients and require closer monitoring.

5.3.2 Gamma glutamyl transferase (GGT) levels

In addition to the measurement of ALT levels, GGT serum levels were also estimated with the various genotypes. Both ALT and GGT are considered to be sensitive and specific markers of liver damage. Based on our study findings, the serum levels of GGT for genotype A had normalized levels I a majority of the study participants.

In a study conducted in the middle east, it was concluded that there was no significant difference between the HAI groups in terms of ALT, ALP, and HBV DNA levels but in the patient group with a high activity score, significant increases in age, AST, GGT. However, it was stated that these non-invasive markers cannot replace biopsy.

This contrasted with another study discussed in patients with chronic hepatitis C between ALT, AST, and GGT values and the overall HAI and the parameters making up this index, a relationship was found between HAI and these biochemical markers in patients with hepatitis B too (Eminler et al., 2014). However, this study findings of the GGT marker experienced a good proportion with normalized levels and suggested that in the event of an elevated GGT levels, then close monitoring is recommended.

5.4 Characterization of HBV genotypes based on virological markers among study participants

Serological markers for HBV infection consist of HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc IgM and IgG. The identification of serological markers allows: to identify patients with HBV infection; elucidate the natural course of chronic hepatitis B; assess the clinical phases of infection; and eventually monitor antiviral therapy (Song & Kim, 2016) All patients with HBV DNA level >20,000 U/ml, HBeAg positive, increased ALT twice higher than the normal upper limit and compensated liver cirrhosis are recommended to be

treated by all three regional guidelines of the particular country. However, there are other guidelines concerning these like AASLD (guideline) which recommends different upper limit of normal (ULN) of ALT in male (35) and female (25) respectively, EASL and APSL guidelines recommend 40 as ULN of ALT. HBeAg-negative patients with cirrhosis and HBV DNA > 2000 U/ml and the ALT twice higher than normal or family history of cirrhosis and HCC are also highly recommended for treatment. EASL additionally recommends treatment for patients with fibrosis (Yim et al., 2020).

5.4.1 HBV Viral load marker

All genotypes presented with mostly detectable HBV DNA viral load (86%) with a higher proportion being genotype A (96.6%). However, it was also noted that genotype of A/ B is also circulating in these population which needs to be investigated further with its relationship with the HBV viral load whereby it was observed that a high population of participants presented with HBV DNA levels of >20,000IU/ml. Genotype E-J presented with a lower percentage of participants with >20,000IU/ml than the rest of the genotypes as observed.

A comparison with this study on viral load with alarming levels (>20,000IU/ml) requires medical attention which is in agreement and suggested that the other portion showing that 24% of CHB patients had non-detectable levels be recommended for follow-up testing for HBV DNA detection and quantitation in one to two months (Baclig et al., 2020a). In this study, a slightly lower percentage of the undetectable HBV DNA was noted but agrees that follow up is also necessary for the confirmation of this group in proper health management of CHB.

High viral load levels and the HBeAg-positive stage have been identified as markers associated with the risk of liver disease progression as stated in a study ((Elizalde et al., 2021). This poses an increased risk of liver cirrhosis and is therefore important to keep monitoring these parameters as part of the routine checkup for the chronic HBV patients.

This is in comparison with a strong discussion made in a study tha suggested that Intrahepatic HBV DNA was found to be of clinical significance in several aspects. First, the intrahepatic HBV DNA concentration was found to correlate significantly with the degree of fibrosis. Second, in at least one patient, intrahepatic HBV DNA was still present even when serum HBV DNA was no longer detectable. It may be significant that the biopsy sample from this patient showed a mild degree of fibrosis, even though necroinflammation was minimal. This supports the previous findings that for Asian HBV carriers, there is no cutoff HBV DNA value below which disease progression would not occur was established. There is a strong correlation between serum and intrahepatic HBV DNA levels, suggesting that serum HBV DNA levels can be used as an indication of intrahepatic HBV DNA levels (Wong et al., 2004).

These findings strongly suggest the importance of the virological markers in patient management and thus effective management of HBV infection requires HBV DNA viral load assay in accordance with existing treatment guidelines.

A current guideline developed by the Society for Gastroenterology and Hepatology in Nigeria considers HBeAg status a major factor but discounts age (Iregbu and Nwajiobi-Princewill: HBV DNA Viral Load, 2016)

Further as in comparison with the findings and recommendation of the study it has been demonstrated that the detection of HBV DNA is a reliable marker of replication activity, and higher titers of HBV DNA are related to the more rapid disease progression and higher incidence of HCC. Furthermore, HBV DNA testing is useful in routine clinical setting to determine patients who need antiviral therapy and monitor them for suitable treatment (Song & Kim, 2016). These correlates well with the prior researches that stated that high viral load is recommended for efficient monitoring and care of these participants which is the aim towards this study.

5.4.2 HBeAg marker

A majority of participants in the study presented with HBeAg negativity at 81.4% with genotype E-J highest at 88.8%.

However, there is a slightly higher representation of HBeAg positivity across all genotypes that could be of importance stated that high viral load levels and the HBeAg-positive status have been identified as markers associated with the risk of liver disease progression. HBeAg status should be checked every 6-12 months with patients who remain HBeAg-positive with HBV DNA > 20 000 IU/mL after 3-6 months with elevated ALT levels of 1-2 ULN, or DNA levels > 20 000 IU/mL and aged < 40 years old, should be considered for liver biopsy (Elizalde et al., 2021).

Studies demonstrate the presence of HBV DNA and the development of HCC in HBeAg-negative HBsAg carriers (Yu et al., 2002)and as noted in this study where there was a portion of the patients who had elevated HBV viral load and a small population showing HBeAg positivity needs further follow up to monitor if its mutation causing the occurrence

of HBeAg negativity in these population. A study on mutation was conducted by (Chandra et al., 2007), looking at G1862T detected in 18 samples, 15 (83%) of them belonged to genotype A (sub-genotype HBV/A1), 3 (17%) to genotype D and stated that the mutation was more frequent in HBeAg-negative than in HBeAg-positive patients (21 vs. 9%), whereas in HBV/A1 it was as common in HBeAg-positive as in HBeAg-negative patients and significantly associated with T1762/A1764 mutation. Having a high population of HBeAg negativity across all genotypes it is therefore important to closely monitor and screen for these mutations in these patients. As a group, the most important predictor of elevated ALT and high HBV-DNA level was HBeAg status: HBeAg-positive patients were 1.5 times more likely to have elevated ALT and high HBV-DNA, compared with HBeAg-negative patients. The degree of observed difference in ALT and HBV-DNA levels between HBeAg-positive and HBeAg- negative patients is related to the age of the patients and the referral pattern (Tonetto et al., 2009).

Thus, the HBeAg plays a vital role in the monitoring of the disease progression and mutation in general to ensure proper medical care of the clients attending the MTRH clinic.

In regard to the coinfection, it is also noted that it causes more severe infection and resistance to treatment unlike the single infection. This new coinfection is a reason to study more with a larger population and confirm its distribution. The high prevalence of genotype A was similar to most of the previous studies, including those in Kenya. This result portrays that good clinical management with the interferons therapy (IFN/Peg-IFN) would be of great benefit to the patients attending MTRH as studied in other researches on treatment therapy of choice. Clinical trials and treatment regimens should be postulated individually based on the genotype to effectively manage chronic HBV patients.

A majority of patients presented with HBeAg negativity hence may pose lower infectability risk whereas genotype A patients experience better clearance HBeAg status (Malmstro et al., 2010). HBV genotype B is associated with earlier HBeAg seroconversion as presents a good prognosis in this population.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Among women, a fairly even genotype distribution was observed across all ages in the study population. There was a high variability in genotype representation across various age groups among the male gender especially with genotype A. The highest peak was observed in the age group of 31-35 years followed by 46-50 years. There was no gender predominance observed across other genotypes (A and A/B) except in the mixed genotype (A/B) where the females slightly predominated in these category. However, the most predominant genotype observed across each gender was genotype A which represented with the highest prevalence.

The prevalence rates of the various genotypes were 79.1%, 11.6%, 11.6% and 20.9% for genotypes A, B, AB and E-J respectively. This percentage was represented to categorically express genotype A and B independently and also as a combination of genotype A/B to allude to the fact that these participants presented with both genotypes for each individual. Genotypes C and D were undetected at 0%.

A higher proportion of participants presented with normal levels of liver biochemical markers across all the genotypes. In relative terms, a higher proportion of participants with genotype A presented with elevated liver markers amongst the study participants.

- HBV DNA was detectable in a majority of samples from all the genotypes with a higher detection rate observed in genotype A. Additionally, a majority of participants presented with HBeAg negativity with participants in genotype E-J

being highest. This alludes to the fact a majority of the participants in this study presented with detectable levels of HBV DNA levels and HBeAg negativity.

6.2 Recommendations

MTRH

I. Recommending that the HBV genotyping and HBV DNA viral load testing be adopted for management, monitoring, and gender specific therapies of CHB patients attending the facility.

Treatment

II. As observed in the study, most CHB participants at MTRH would benefit from interferon-alpha drugs as recommended for genotype A.

Gaps in the study

III. More studies be conducted for further characterization at sub-genotype level of HBV genotypes circulating in the region.

IV. Further characterization of genotype E-J due to the possibility of genotype diversity in the region.

V. Further research studies on the effects of co-infection / mixed genotype and the management criteria for management of these cohort.

6.3 Dissemination of Findings

i. **Published abstracts:**

Gikunyu C. W., Maiyoh, G. K., Kwena A. M. *Characterization of Hepatitis B Viral Genotypes and their Associated Sero-Virological Markers Among Patients Attending the Moi Teaching and Referral Hospital Liver Clinic.* Basic & Applied Sciences Conference held on 16TH – 18TH November 2022 at Kenyatta University.

Gikunyu C. W., Maiyoh, G. K., Kwena A. M. *Hepatitis B Viral Genotypes and their Associated Sero-Virological Markers Among Patients Attending the Moi Teaching and Referral Hospital Liver Clinic.* 27TH AKMLSO Scientific and Exhibition Conference held at Acacia Premier Hotel-Kisumu on 24TH – 27TH May 2022

ii. **Manuscript:** To be submitted to Journal of Viral Hepatitis (JVH).

iii. A bound thesis for Moi University library.

iv. Public thesis defense.

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APPENDICES

Appendix I: Consent Form

My name is Gikunyu C. Wangui, undertaking research on Characterization of Hepatitis B Viral Genotypes and the associated Sero-Virological Markers Among Patients Attending MTRH Liver Clinic. I am therefore inviting you to take part in this study.

The study will include drawing of blood for testing HBV genotypes and Sero-virological markers. Through this, I will be able to identify the specific HBV genotype and the presence or absence of the Sero-virological markers. Your results will be communicated back to you as soon as they are available. It is worth noting that during the blood draw procedure, some slight pain or discomfort will be experienced but no harm will be inflicted on you. Subsequently, there will be no monetary attraction of any form anticipated for this study. You will however benefit by knowing your HBV genotype and Sero-virological markers.

I welcome any questions or clarifications you may wish to ask at this point in time.

Do you therefore accept to participate in this study?

If the respondent accepts to participation, he/ she will append their signature or thumb print below.

Signature/ Thumb print of client: …….

Signature of Principal investigator: ……

Date……

Appendix II: Laboratory Request Form

STUDY HBV GSM LABORATORY DATA ENTRY FORM

Patients Details

Patient Op/Ip No: …….

Patient Initials: …….

Study No: …….

Date of Birth: …….

Gender: ……

Tel No: …….

Sample type

EDTA (ml of Blood): …….

Plain Tube (ml of Blood): ……

Date of collection: …….

Time of collection: ……

Results Interpretation

HBV Genotype: ……

Markers: HBsAg: Anti-HBc: HBeAg…….

ALT/ GGT: ……

Anti- Ig G: HBV DNA Levels ……...

Tested by: ……

Date: …….

Reviewed by: ……

Date: ……

Appendix III: MTRH Research Approval Letter

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O BOX 3
ELDORET
Tel:33471112/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O.BOX 4606
ELDORET
Tel:33471/2/3

Reference:IREC/2019/246 18th November,2019

Approval Number: 0003476

Ms.Gikunyu C. Wangui,

Moi University,18 NOV 2019

School of Medicine,

P.O.Box 4606-30100,

ELDORET-KENYA.

Dear Ms. Wangui,

CHARACTERIZATION OF HEPATITIS B VIRAL GENOTYPES AND THE ASSOCIATED SERO-VIROLOGICAL MARKERS AMONG PATIENTS ATTENDING MTRH LIVER CLINIC

This is to inform you that MU/MTRH-IREC has reviewed and approved your above research proposal.Your application approval number is FAN: 0003476 The approval period is 18th November, 2019-17th November, 2020.

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 MU/MTRH-IREC.

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 MU/MTRH-IREC 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 MU/MTRH-IREC 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 pior 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 MU/MTRH-IREC.

Appendix IV: IREC Approval

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)
MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3

ELDORET
Tel: 3347111213

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES

P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Nov 2020

Reference: IREC/2019/246
Approval Number: 0003476

Ms. Gikunyu C. Wangui,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Ms. Wangui,

RE: CONTINUING APPROVAL

The Institutional Research and Ethics Committee has reviewed your request for continuing approval to your study titled: -

"Characterization of Hepatitis B Viral Genotypes and the Associated Sero-Virological Markers among Patients Attending MTRH Liver Clinic",

Your proposal has been granted a Continuing Approval with effect from 18th November, 2020. You are therefore permitted to continue with your study.

Note that this approval is for 1 year; it will thus expire on 17th November, 2021. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

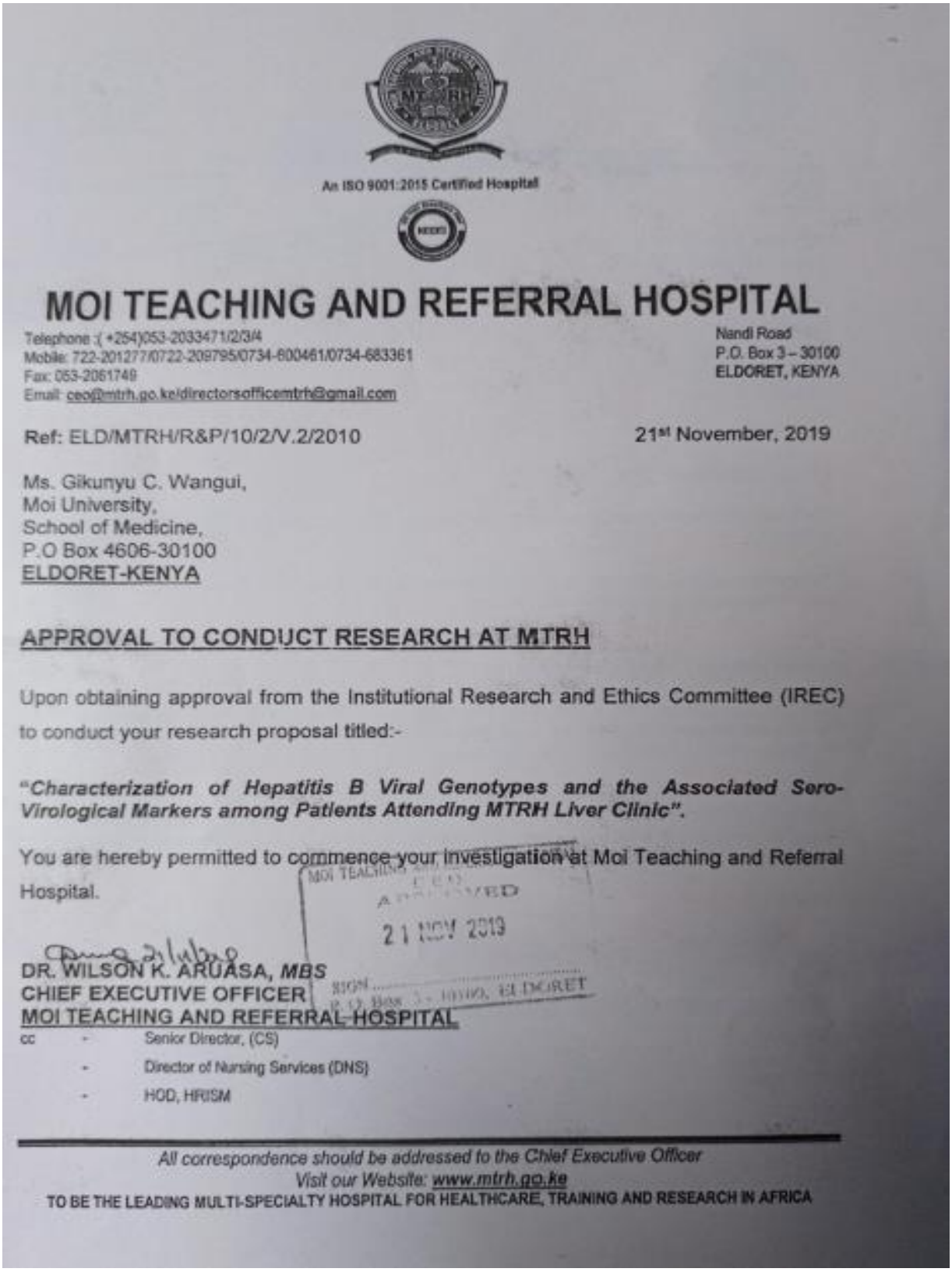
You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

DR. S. . NYABERA NY
DEPUTY-CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: CEO - MTRH Dean - SOD Principal - CHS
Dean - SPH Dean - SOM Dean - SON

Appendix V: Approval to Conduct Research at MTRH



An ISO 9001:2015 Certified Hospital

MOI TEACHING AND REFERRAL HOSPITAL

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Mobile: 722-201277/0722-209795/0734-600461/0734-683361
Fax: 053-2061749
Email: ceo@mtrh.go.ke/directorsofficemtrh@gmail.com

Nandi Road
P.O. Box 3 – 30100
ELDORET, KENYA

Ref: ELD/MTRH/R&P/10/2/V.2/2010

21st November, 2019

Ms. Gikunyu C. Wangui,
Moi University,
School of Medicine,
P.O Box 4606-30100
ELDORET-KENYA

APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Characterization of Hepatitis B Viral Genotypes and the Associated Sero-Virological Markers among Patients Attending MTRH Liver Clinic".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

MOI TEACHING AND REFERRAL HOSPITAL
APPROVED
21 NOV 2019
SIGN ..... P.O. Box 3 - 30100, ELDORET

DR. WILSON K. ARUASA, *MBS*
CHIEF EXECUTIVE OFFICER
MOI TEACHING AND REFERRAL HOSPITAL

cc - Senior Director, (CS)
- Director of Nursing Services (DNS)
- HOD, HRISM

All correspondence should be addressed to the Chief Executive Officer
Visit our Website: www.mtrh.go.ke
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