PREVALENCE AND GENOTYPIC CHARACTERIZATION OF HEPATITIS B VIRUS AMONG OUTPATIENT ATTENDEES AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET

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Prevalence and Genotypic Characterization of Hepatitis B Virus among Outpatient Attendees at Moi Teaching and Referral Hospital, Eldoret

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DECLARATION

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

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This thesis has been submitted with our approval as the University supervisors.

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DEDICATION

I dedicate this work to my dear husband Mr. Nelson Maiyo, our lovely child Kyllan, my parents Mr. Stephen Koech and Mrs. Salina Koech and my siblings Silas, Geoffrey and Gloria who have always been my pillars of moral support.

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LIST OF ABREVIATIONS AND ACRONYMS

A	Adenine
Aa	Amino acid
ALT	Alanine transaminase
Anti -HBS	Antibodies to the HBV surface antigen
Anti-HBC	Antibodies to the HBV core antigen
ART	Antiviral therapy
C	Cytosine
CVR	Centre for Virus Research
DNA	Deoxyribonucleic acid
CDC	Centres of Disease Control
cDNA	Complimentary DNA
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
G	Guanine
HBcAg	Hepatitis B core Antigen
HBeAg	Hepatitis B envelope Antigen
HBIG	Hepatitis B immune globulin
HBsAg	Hepatitis B surface antigen
HIV	Human Immunodeficiency Virus

HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
KEMRI	Kenya Medical Research Institute
MHR	Major Hydrophilic Region
MSM	Men who have sex with men
MTRH	Moi Teaching and Referral Hospital
mRNA	Messenger ribonuclecc acid
nM	Nano metres
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
SERU	Scientific Ethical and Review Unit
µl	Microlitre
WHO	World Health Organization

ABBREVIATION AND AMBIGUITY CODES FOR AMINO ACIDS

Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamic Acid	Glu	E
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Glycine	Gly	G
Glutamine	Gln	Q

ABSTRACT

Hepatitis B virus (HBV) infection remains one of the major worldwide public health burdens and a major cause of hepatocellular carcinoma. There is growing evidence that HBV genotypes influence not only clinical outcomes of HBV infection, but response to therapy and vaccination. Although HBV prevalence among the rural populations in Kenya is increasing, there is limited information on the circulating genotypes. This study aimed to evaluate the prevalence and genetic diversity of HBV among outpatient clinic attendees at Moi Teaching and Referal Hospital in Eldoret. A cross-sectional laboratory based study was conducted, where blood samples were collected from a total of 200 study participants. Enzyme-Linked Immunosorbent Assay (ELISA) screening was done for the presence of HBV surface antigen (HBsAg). From the positive samples, molecular analysis was done by first extracting the viral DNA followed by amplification, to yield enough materials that were then sequenced and the sequences aligned and compared with the reference sequences retrieved from the GeneBank. The study determined a general prevalence of HBV infection of 10% (20/200). Demographically males accounted for 60% of infection compared to the females. Further HBV infection was higher in those aged 31-40 years who accounted for 35% of the total infections. On the likelihood of infections, there was no significant association of infection with gender and age (p=0.149) and (p=0.070) respectively, where males were 6 times more likely to be infected (OR = 1.286; p= 0.149) and those aged between 31–40, showing a higher likelihood of infection (OR = 1.447, p=0.070). Of the 14 HBsAg positive samples sequenced, HBV subgenotype A1 (HBV/A1) accounted for 92.9% (13/14) of all infections with the remaining 1 sample belonging to subgenotype D1. Analysis on mutations revealed occurance of the mutations within the Major Hydrophilic Region (MHR), with the main mutation being S114T. Phylogenetic analysis revealed that 14 isolate sequences with reference sequences obtained from GenBank; 13 (92.9%) of the isolates classified into subgenotype A1 (HBV/A1), while 1 (7.1%) of isolates categorized into subgenotype D1 (HBV/D1). In conclusion, the general prevalence observed in this study shows a potential for a higher rate of transmission of HBV in this population. The genetic diversity analysis however reveals that the circulating genotype is still restricted and could be possible source of HBV transmission in the community. The study recommends that campaigns for VCT in HBV should be enhanced among the public and that HBV Diversity in the country should be considered when designing interventions for HBV.

CHAPTER ONE

INTRODUCTION

1.1. Background

Hepatitis B virus (HBV) infection remains one of the major worldwide public health burdens. Global estimates suggest that more than 2 billion people have been infected with HBV, and 250 million of these people are chronically infected, of which 65 million live in Africa (Schweitzer *et al*., 2015; Ganem *et al*., 2004; Kramvis & Kew, 2007). The virus is associated with 30% of liver cirrhosis and 53% of liver cancers globally (Perz *et al*., 2006), with approximately 15-40% of patients with chronic HBV developing cirrhosis, end-stage liver failure or hepatocellular carcinoma (HCC) in their lifetime (Ferlay *et al*., 2010).

Hepatitis B virus prevalence is highest in Sub-Saharan Africa and East Asia, where between 5-10% of the adult population is chronically infected. It accounts for 500,000-1.2 million deaths per year and is the tenth leading cause of mortality worldwide (WHO, 2015; Blum, 2016). These numbers far exceed the number of people living with HIV which is estimated at 34 million (WHO, 2011).

The Prevalence of HBV among the rural population in Kenya stands at 8.8% (Mutuma *et al*., 2011) while limited studies in Eldoret, Kenya showed 9.3% of infections among pregnant women attending antenatal clinic at Moi Teaching and Referral Hospital (MTRH).

Hepatitis B virus exhibits genetic variability with an estimated rate of 1.4-3.2 x 10^{-5} nucleotide substitution per site per year (Fares *et al*., 2002). Hepatitis B virus genotypes play a role in both course of infection and treatment management (Kao *et al*., 2002). It is established that genotype based structural and functional differences can influence the severity, course and likelihood of complications, and response to treatment of HBV infection and possibly vaccination against the virus (Kramvis *et al*., 2005; Kramvis & Kew, 2005). Mutations within the HBsAg Major Hydrophilic Region (MHR) associated with failure of HBsAg detection, antiviral resistance and

vaccine escape (Coleman *et al*., 2006; Wu *et al*., 2010). In Kenya there are limited publications on the HBV genotypes in the country. (Hannoun *et al*., 2005; Bell *et al*., 2012; Makondo *et al*., 2012). Previous studies have shown a high prevalence of HBV among populations in North Rift Kenya (Ochwoto *et al*., 2016).

1.2. Statement of the problem

Hepatitis B is a liver condition caused by a viral infection (Makokha *et al*., 2023). Hepatitis infection has become a health problem in Kenya, posing a threat on the disease management, treatment regime of an individual and also the progress of the disease. HBV is currently being detected more in rural communities in Kenya. Previous studies have shown a high prevalence of HBV among populations in North Rift Kenya (Ochwoto *et al*., 2016). Hepatitis B virus genotypes play a role in both course of infection and treatment management (Kao *et al*., 2002). It is established that genotype based structural and functional differences can influence the severity, course and likelihood of complications, and response to treatment of HBV infection and possibly vaccination against the virus (Kramvis *et al*., 2005; Kramvis & Kew, 2005). HBV genotypic patterns help in the designing of better diagnostic tests, response to vaccines and predicting clinical outcomes. Mutations within the HBsAg Major Hydrophilic Region (MHR) are associated with failure of HBsAg detection, antiviral resistance and vaccine escape (Coleman *et al*., 2006; Wu *et al*., 2010). In Kenya there are very limited data on HBV which has been published with regards to rural communities and genetic diversity of the virus yet there is a lot of migration. (Hannoun *et al*., 2005; Bell *et al*., 2012; Makondo *et al*., 2012).

1.3. Justification of the study

Kenya being a middle income country is still using genotype specific regiments for the treatment and management of HBV patients. This requires the need to continuously document circulating genotypes to guide on effective management of the disease (Hannoun *et al*., 2005). The population at MTRH and in the North Rift has been documented as highly infected with HBV, yet they keep interacting with the

general population unnoticeably (Ochwoto *et al*., 2016). They therefore act as a bridge of infection of HBV to the general population and thus documenting the burden of infection within this population for appropriate intervention. This has an indirect effect in protecting the general population against the disease. Eldoret, being a cosmopolitan city stands as a hub where various HBV genotypes could silently enter and circulate in the country, hence the need for this study.

1.4 Research Questions

1. What is the prevalence of hepatitis B virus among outpatient attendees at Moi Teaching and Referral Hospital in Eldoret?

2. What are the demographic characteristics associated with hepatitis B virus infection among outpatient attendees at Moi Teaching and Referral Hospital in Eldoret?

3. What are the common genotypes of HBV among HBV infected outpatient attendees at Moi Teaching and Referral Hospital in Eldoret?

1.5. Objectives

1.5.1. General objective

To determine the prevalence, genotypes and demographic characteristics of HBV among outpatient attendees at Moi Teaching and Referral Hospital in Eldoret

1.5.2. Specific objectives

1. To determine the prevalence of HBV among outpatient attendees at Moi Teaching and Referral Hospital in Eldoret.
2. To determine the demographic characteristics associated with HBV infection among outpatients attendees at Moi Teaching and Referral Hospital in Eldoret.
3. To determine the common genotypes of HBV among HBV infected outpatient attendees at Moi Teaching and Referral Hospital in Eldoret.

1.6. Significance of the study

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). It is a major global health problem. It can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer (WHO, 2022). Genotyping of HBV is an important diagnostic tool in predicting the prognosis and response of therapy in hepatitis B patients. Therefore this study contributes substantially to solutions of global burden on healthcare.

CHAPTER TWO

LITERATURE REVIEW

2.1. Hepatitis B structure and genome

Hepatitis B virus belongs to the genus *Orthohepadnaviridae* of the *Hepadnaviridae* family (Schaefer, 2007). The virus virion consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. These virions are 30-42 nm in diameter. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity (Locarnini, 2004). The outer envelope contains embedded proteins that are involved in viral binding of, and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses with 42 nM virions which are capable of infecting hepatocytes and are referred to as Dane particles (Harrison, 2009). Figure 2.1 shows the structure of hepatitis B virus.

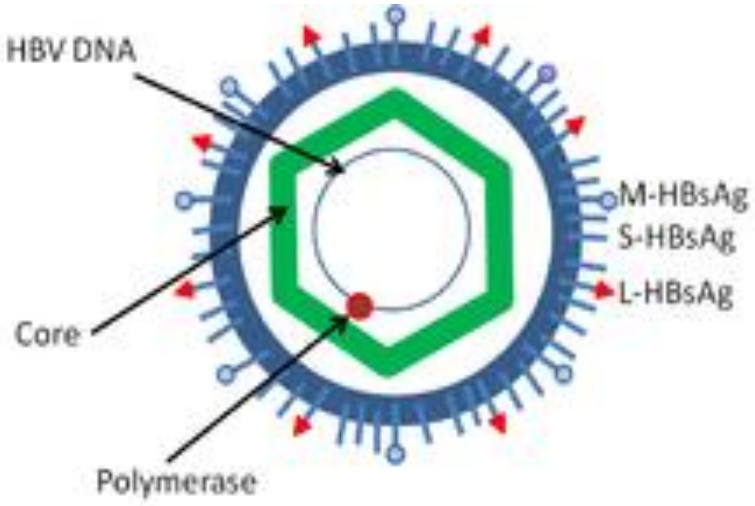

Figure 2.1: Structure of Hepatitis B Virus (Graham, 2013)

The genome of HBV is made of circular DNA which is not fully double stranded. One end of the full length strand is linked to the viral DNA polymerase. The genome is 3020–3320 nucleotides long for the full-length strand and 1700–2800 nucleotides long for the short length-strand (Kay & Zoulim, 2007). The negative-sense (non-

coding) is complementary to the viral mRNA. The viral DNA is found in the nucleus soon after infection of the cell. The partially double-stranded DNA is rendered fully double-stranded by completion of the (+) sense strand and removal of a protein molecule from the (-) sense strand and a short sequence of RNA from the (+) sense strand. Non-coding bases are removed from the ends of the (-) sense strand and the ends are rejoined. There are four genes encoded by the genome, called C, X, P, and S. The core protein is coded for by gene C (HBcAg), and its start codon is preceded by an upstream in-frame AUG start codon from which the pre-core protein is produced. HBeAg is produced by proteolytic processing of the pre-core protein. The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg). The HBsAg gene is one long open reading frame but contains three in frames "start" (ATG) codons that divide the gene into three sections, pre-S1, pre-S2, and S. Because of the multiple start codons, polypeptides of three different sizes called large, middle, and small (pre-S1 + pre-S2 + S, pre-S2 + S, or S) are produced (Beck, & Nassal, 2007). The function of the protein coded for by gene X is not fully understood but it is associated with the development of liver cancer. It stimulates genes that promote cell growth and inactivates growth regulating molecules (Li *et al*. 2010). Figure 2.2 shows the genome organization of hepatitis B virus.

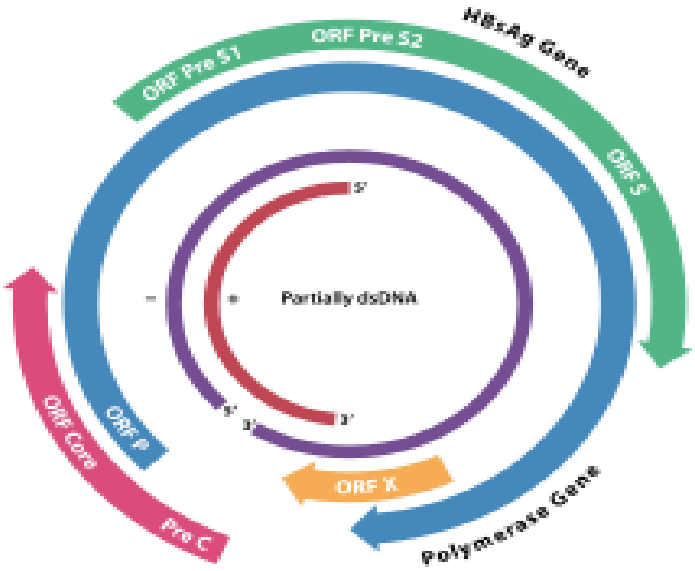

Figure 2.2: Genome Organisation of HBV (GrahamColm, 2007)

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes presented on its envelope proteins (Kramvis *et al*., 2005). Currently, there are 10 genotypes of HBV, A through J, each with a distinctive geographical distribution. The HBV genotypes, except for genotype E and G, can be further divided into sub-genotypes (Schaefer, 2007).

Epidemiological studies have shown that each genotype is characterized by a distinct geographic and ethnic distribution. In brief, genotype A is prevalent in northwestern Europe, North America and Africa. Genotypes B and C are commonly found in Asia, whereas genotype D shows a worldwide distribution but predominates in the Mediterranean region, including the Middle East and central Asia (Cooksley, 2010). Genotype E is found in the Western region of Africa, genotype F is present in the aboriginal population of South America, and genotype H is confined to Central America (Schaefer, 2007) Genotype G was initially isolated from HBV carriers in France and Georgia (United States) and was later detected in the United Kingdom, Italy and Germany. Recently, HBV genotype I was described in northwestern China, Vietnam and Laos (Yu *et al*., 2010; Olinger *et al*., 2007). Finally, a recently identified tenth genotype provisionally assigned as genotype *J* was proposed for a HBV isolate from a Japanese patient with HCC (Tatematsu *et al*., 2009).

2.2. Types of hepatitis B markers and their expression

Hepatitis B serologic testing involves measurement of several hepatitis B viruses (HBV)-specific antigens and antibodies. Different serologic "markers" or combinations of markers are used to identify different phases of HBV infection and to determine whether a patient has acute or chronic HBV infection, is immune to HBV as a result of prior infection or vaccination, or is susceptible to infection.

2.2.1. Hepatitis B surface antigen (HBsAg)

This is a protein on the surface of hepatitis B virus. It is detected in high levels in serum during acute or chronic hepatitis B virus infection. The presence of HBsAg indicates that the person is infectious. The body normally produces antibodies to HBsAg as part of the normal immune response to infection. HBsAg is the antigen used to make hepatitis B vaccine (CDC, 2023).

2.2.2. 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 (CDC, 2023).

2.2.3. Total hepatitis B core antibody (anti-HBc)

Appears at the onset of symptoms in acute hepatitis B and persists for life. The presence of anti-HBc indicates previous or ongoing infection with hepatitis B virus in an undefined time frame (CDC, 2023).

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

Positivity indicates recent infection with hepatitis B virus. Its presence indicates acute infection (CDC, 2023).

2.3. Signs and symptoms of hepatitis B virus infection

Acute infection with hepatitis B virus is associated with acute viral hepatitis which is an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, and dark urine, and then progresses to development of jaundice. Itchy skin has been an indication as a possible symptom of all hepatitis virus types. The illness lasts for a few weeks and then gradually improves in most affected people. A few people may have more severe liver disease (fulminant hepatic failure), and may die as a result. The infection may be entirely asymptomatic and may go unrecognized (Terrault, 2005).

Chronic infection with hepatitis B virus either may be asymptomatic or may be associated with a chronic inflammation of the liver, leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of hepatocellular carcinoma (El-Serag & Rudolph, 2007; El-Serag, 2011).

2.4. Mode of HBV transmission

Hepatitis B virus is reported to be 100 times more infectious than the human immunodeficiency virus (HIV) and 10 times more infectious than HCV, making it highly transmissible (Gupta *et al*., 2008; Pande *et al*., 2011). HBV can be transmitted through contacts with body fluids from infected HBV patients. Blood is the most important route of HBV transmission, but other body fluids such as semen and saliva have been reported to be the source of transmission (Seeger *et al.*, 2007). It is a highly stable virus, resistant to breakdown, can survive outside the body for at least 7 days (Lavanchy, 2004; Seeger *et al.*, 2007). Three major modes of transmission are documented, namely; the horizontal, sexual and vertical routes of transmission. Horizontal transmission of HBV may occur through direct contact with infected blood and blood products, dialysis, sharing of needles among injection drug users, needle stick accident among healthcare professionals and intravenous drug abuse (Lavanchy, 2005; Wasmuth, 2009). Having multiple sexual partners and continuous unprotected sexual intercourse with HBsAg positive partners is the main cause of HBV infection in the adolescent and adult-age populations (Wasmuth, 2009). Chronically infected individuals do not immediately present with symptoms and as such are not aware of the HBV infection (Lavanchy, 2005). In the case of men who have sex with men (MSM), receptive anal intercourse is also a major determinant of acquisition of HBV infection (Freitas *et al*., 2014).

Vertical transmission or mother-to-child transmission (MTCT) occurs when a mother has an active infection during pregnancy or at birth. The risk of transmission is directly proportional to the viral replication rate in the mother. Pregnant women with no detectable HBV DNA are thus less likely to pass the infection on to their babies while the risk in mothers with high levels of viremia may be up to 90% (Wasmuth, 2009).

2.5. Prevention of HBV infection

Prevention strategies include primary prevention of new infections by use of vaccines and post-exposure prophylaxis, secondary prevention of HBV transmission by appropriate sexual and sanitary practices, and tertiary prevention of the pathological consequences of chronic HBV by anti-viral treatment (WHO, 2009). Kenya is one of the 31 countries in Sub-Saharan Africa that is supported for hepatitis B vaccination by the GAVI Alliance. Safe and effective HBV vaccines have been available since the 1980s. Recombinant DNA vaccines were licensed in 1986 and 1989 (Zanetti *et al*., 2008) After the introduction of recombinant vaccines and the subsequent drop in cost of the plasma-derived vaccines, the WHO set a goal in 1992 for all countries to introduce the HBV vaccine into their national routine infant immunization programs by 1997. By 2006, 162 of 193 countries had introduced the vaccine into their national infant immunization schedules whereby all newborns receive all 3 doses of the HBV vaccine (CDC, 2008)

2.6. Clinical relevance of the HBV genotypes

The different HBV genotypes have been associated with some clinical features of HBV associated to liver disease, Hepatocellular Carcinoma (HCC), viral mutations and response to treatment (Kramvis & Kew, 2007). Genotype A is associated with a greater risk of progression to chronicity in adult acquired HBV infections (Kew *et al.*, 2005). In sub-Saharan Africa, subgenotype A1 is the predominant and has been associated with high rates of HCC, patients infected with subgenotype A1 have a higher rate of HBeAg negativity, low HBV DNA levels and develop HCC at a younger age compared to patients infected with other genotypes (Kew *et al.*, 2005). Data has indicated that genotype C is associated with faster liver damage and higher risk of HCC compared to genotype B (El-Serag *et al.*, 2012). Genotype D has been reported to be associated with severe liver disease compared to genotype A in some 21 studies, whereas genotypes A and F are more associated with HCC development than genotype D (Toan *et al.*, 2006). The influence of HBV genotypes on response to antiviral therapy has been also studied, genotypes A and B have been associated with

a better response to interferon-based therapies compared to genotypes C and D (Raimondi *et al*., 2010).

Hepatitis B Genotype C has also been associated with a more severe liver disease and with a lower rate of response to interferon therapy (Croagh *et al*., 2015). The emergence of resistance to antiviral therapy with lamivudine seems to be independent of the genotype although it is related to the presence of HBeAg. HBV carriers infected with genotype C are more frequently positive for HBeAg (Echevarría *et al.,* 2006).

2.7. Hepatitis B virus mutations

A mutation is defined as a random error occurring in the genome, which may lead to genetic variation in the organism (Paraskevis *et al.,* 2013) Because of the lack of proof reading activity by the HBV reverse transcriptase, error frequencies occur during replication. These result in HBV mixture of mutants and wild type in the entire genome (Datta, 2008). The mutations can occur either naturally or may be evoked during antiviral therapy where the mutation rate of HBV is relatively high with the nucleotide substitution rate per site per year estimated at 1.5×10^{-6} to 3.0×10^{-6} (Paraskevis *et al.,* 2013; Bouckaert *et al.,* 2013). The HBV genome encompasses four overlapping reading frames preS/S, preC/C, P and X, with the polymerase gene overlapping the envelope gene. As a result, mutations in the catalytic domain of the polymerase gene can affect the amino-acid sequence of the envelope protein (HBsAg) and vice versa (Pollicino *et al.,* 2014). Mutations in the HBsAg have several clinical effects, including: (i) reduced sensitivity to available diagnostic tests, (ii) a lack of immunity following vaccination with non-mutant HBV 22 variants (vaccine escaped mutant) and (iii) a failure of passive immunisation with HBV IgG (El Chaar *et al*., 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study site

This study was carried out at Moi Teaching and Referral Hospital (MTRH) in Eldoret, Uasin-Gishu County. Uasin-Gishu County is one of the forty-seven Counties in Kenya located in the former Rift Valley Province. Eldoret has the county's largest population center as well as its administrative and commercial center (Uasin Gishu County overview, 2020). It lies between longitudes 34 degrees 50’ east and 35 degrees 37’ West and latitudes 0 degrees 03’ South and 0 degrees 55’ North. It is a highland plateau with altitudes falling gently from 2,700 meters above sea level to about 1,500 meters above sea level. The topography is higher to the east and declines gently towards the western borde (KIPPRA PPR Home, 2022). Uasin Gishu is located on a plateau and has a cool and temperate climate. The county borders Trans-Nzoia County to the north, Elgeyo-Marakwet and Baringo counties to the east, Kericho county to the south, Nandi county to the south, south-west and Kakamega county to the west.

3.2. Study design

This was a cross-sectional study. It was carried out at the center for virus research (CVR) in Kenya Medical Research Institute [KEMRI].

3.3 Study population

This study was conducted among outpatient attendees both male and female of Moi Teaching and Referral Hospital in Eldoret during the period of

3.3.1. Inclusion criteria

1. The patient must be attending the outpatient of MTRH.

2. The patient must be unaware of his/her HBV status.

3. The patient must consent to the study.

4. The patient must be aged 18 years or above.

3.3.2. Subject exclusion criteria

1. Any patient on Hepatitis B medication.

3.4. Sampling

3.4.1. Sample size determination

Sample size was determined using Fisher's 1998 standard statistical formula: $n=Z^2pq/d^2$

where;

$$N = \frac{Z^2 \times p \times q}{d^2}$$

N = Minimum sample size required

Z = Z-score for normal standard deviation for a 95% confidence interval (1.96)

p = Prevalence of HBV by Mutuma, *et al*., 2011- 9%)

q = (1-P)

d = Significance level at 95% confidence interval (0.05)

$$N = \frac{(1.96)^2 \times 0.09 \times 0.91}{(0.05)^2} = 125.851 = 126$$

The minimum sample size for the study was 126 samples.

3.4.2. Ethical considerations

Approval to conduct the study was granted by Scientific and Ethical Review Unit (SERU) of KEMRI (SERU No. KEMRI/SERU/CVR/004/3125). The participants of this research project participated after being clearly explained the study aspects and after voluntarily accepting to consent. Those who agreed to consent were required to sign an informed consent. All data obtained was kept confidential by having the data accessed strictly by authorized personnel only and this was done by storing data in excel worksheets only accessible via passwords. Patient information was protected by using a study code to identify them rather than their names.

There were minimal risks relating to the study population because blood samples from patients were only collected by qualified and experienced phlebotomists to prevent harm and unnecessary pain to the patients.

The results of this study were returned to the study participants. This benefited the patients who now managed to know their HBV status. At the same time those who were positive for HBV benefited more with proper therapeutic measures being put into place by the hospital to prevent progression to more fatal courses of co-infection such as progression to liver disease, hepatocellular carcinoma, and toxicity to ART or even death.

3.4.3. Sampling procedure

Systematic sampling of every third person that met the inclusion criteria was applied until the required sample size was attained. Recruitment of the patients in the study was integrated within the day-to-day procedures of the outpatient department in the hospital. The patients were approached before seeking the health service they came for by the principal investigator in a private room or being distant from other patients on the queue to maintain privacy and to avoid inadvertent disclosure of potential study participants to other patients on the queue. A consent form was presented and explained before they signed. After consenting five millilitres of blood was drawn by venipuncture from the participants by an experienced phlebotomist using accepted venipuncture standard operating procedures at the hospital. The blood samples were

transported to the laboratory in cool boxes where they were centrifuged at 2500 rpm to obtain sera which was stored in the refrigerator at -70 ^{o}C until use.

3.4.4. Data collection

Demographic factors data of the participants were collected using a structured questioner, the information obtained from the participants were; Age, gender, marital status, occupation, level of education and area of residence.

3.5. Laboratory procedures

3.5.1. Screening of the sample for HBV

All samples collected were screened for HBsAg using Hepanostika® ultra II HBsAg (Netherlands) kit as per the protocol described by the manufacturer. Briefly, the test included pipetting of 25µl of specimen diluent into the wells, addition of 100µl of the patient's sample and the controls, incubation at 37^{0}C for 1 hr, addition of 50µl conjugate, incubation at 37^{0}C for 1hr, washing six times with phosphate Buffered solution (BPS), addition of 100µl tetramethylbenzidine (TMB), incubation for 30 minutes in dark at room temperature, addition of 100µl of 1M sulphuric acid and reading the results (OD values) on ELISA plate reader set at 450 nanometers (nM) within 15 minutes and the Cut off Value (COV) was calculated by adding the mean value of the negative controls.

3.4.2. DNA extraction

Hepatitis B virus DNA was extracted from the plasma of the twenty ELISA positive samples collected using the QIAamp DNA MiniKit (Qiagen, Hilden, Germany). The extraction steps were performed according to the manufacturer's instruction as follows: twenty (20) µl Qiagen Proteinase K were pipetted into the bottom of a 1.5 ml microcentrifuge tube, 200 µl plasma was added to the microcentrifuge tube and 200 µl buffer AL were added to the sample, mixed by pulse-vortexing for 15 seconds. The mixture was incubated at 56ºC for 10 min followed by a short spin down, 200 µl ethanol (96-100 %) was added to the sample, mixed by vortexing and

briefly centrifuged to remove drops from the inside of the tube lid. The mixture was transferred carefully to the QIAamp Mini spin column (in a 2ml collection tube) without wetting the rim. The cap was closed and the column in the collection tube was centrifuged at 8000 revolutions per minute (RPM) for 1 min. Thereafter, the QIAamp Mini spin column was placed in a clean 2ml collection tube, and the tube containing the filtrate discarded. Five hundred (500) µl Buffer AW1 were added to the QIAamp Mini spin 32 column without wetting the rim and centrifuged at 8000 rpm for 1 min. The filtrate was discarded. The QIAamp Mini spin column was replaced in a clean 2 ml collection tube, 500 µl Buffer AW2 was added. Filtrate was discarded and centrifuging step was repeated. Finally, the QIAamp Mini spin column was placed in a clean microcentrifuge tube, 60 µl elution buffer was added to the column and centrifuged at 14000 RPM for 3 min. The filtrate in this step contained the extracted DNA. The DNA was stored at -80°C (Tajik *et al.,* 2015). HBV from all the serologically positive samples for HBsAg were sequenced by amplifying to determine the viral genotype followed by phylogenetic analysis. HBV genomic DNA was extracted from plasma of seropositive individuals using the Qiagen® DNA extraction kit (QIAGEN) following the manufacturer's instructions. The concentration of the eluted DNA was determined spectrophotometrically.

3.4.3. Amplification of HBV surface region and genotyping

DNA extracted was used as template to amplify the Pre S1 region by nested PCR using a set of primers. The first PCR was done using primers S1F (5'-TCC TGC TGG TGG CTC CAG-3') and S1R (5'-CGT TGA CAT ACT TTC CAA TCA A-3'). Second PCR was carried out with primers S2F (5'-ACC CTG YRC CGA ACA TGG A-3') and S2R (5'-CAA CTC CCA ATT ACA TAR CCC A-3')- (Macrogen). Amplification conditions were initial denaturation at 95^0C for 7min, followed by 35 cycles of 45sec at 94^0C denaturation, 30sec at 46^0C annealing, and 2min at 72^0C extension, followed by a final extension of 7min at 72^0C; using Applied Biosystems Thermal Cycler (Bio-Rad Lab, Singapore). Cycling parameters for the second PCR remained the same as in the first one except that the annealing temperature was increased to 50^0C. In order to prevent PCR carryover contamination strict care and procedures was implemented as previously mentioned (Aslanzadeh, 2004).

A master mix was prepared depending on the number of samples as shown in Table 3.1

Table 3.1: PCR master mix preparation

Reagent	Stock concentration	Final Concentration	Volume per tube (µL)
PCR Buffer with $MgCl_2$	10X	1	2.5
dNTPS	2mM	1	0.5
$MgCl_2$	25 mM	3	2
Primer Forward	10 µM	0.8	0.5
Primer Reverse	10 µM	0.8	0.5
Taq Polymerase	2.5U	2.5	0.1
PCR water	-		16.9
DNA	-		2
Total	-		25

3.4.4. Detection of amplified HBV S gene PCR products

The samples were subjected to nested PCR to amplify the HBV S gene. Detection of the amplified PCR products was performed using 2.0% agarose (SEakem LE® agarose; FMC BioProducts, Rockland, Marine, USA) gel in 1X TAE buffer (0.04M 34 Tris acetate, 0.001 M EDTA). The gel was prepared by dissolving 2 g of agarose gel into 100 mL of 1X TAE buffer by heating. This was cooled to about 50ºC. Briefly 2µl of the PCR product mixed with 2µl of blue 5x loading dye containing bromophenol blue, xylene cynol, and orange G (Promega, Madison, USA) were loaded into the wells on the gel. The loaded agarose gel was then subjected to 100 Volts of a unidirectional (from the anode to the cathode) electrical current for a duration of 30 minutes, while submerged in a 1X Tris-acetate-EDTA (TAE) buffer (Qiagen, Hilden, Germany).

The agarose gel was stained for 20 minutes with ethidium bromide (1µl in 100ml), Sigma-Aldrich® and the migrated sample DNA bands and 200bp DNA ladder (Promega, Madison, USA) in the agarose gel were visualized using ultraviolet transillumination and corresponded to positive controls. The expected bands was read against 100bp DNA ladder (Promega, Madison, USA). The size of the bands was 1063 bps. A digital image of the gel was taken using a gel documentation system. The purified products were purified and sequencing was sourced out at Macrogen.

3.4.5. Sequence analysis

Nucleotide BLAST was performed on our HBV sequences to acquire a homologous sequence from NCBI. Multiple sequence alignment was conducted using ClustalW implemented in MEGA-7 (Kumar, *et al*., 2016). HBV genotypes were determined with phylogenetic analysis of aligned sequences in comparison to HBV reference sequences retrieved from NCBI. Phylogenetic tree was constructed based on Neighbor-Joining method with Kimura-2 parameter model in conjunction with estimation of the tree reliability by bootstrap method of 1000 replicates; all of these processes were done using MEGA-7 (Kumar, *et al*., 2016). Visualization of the tree was done using Tree View program. The sequences were deposited to Genbank as MK177878-MK177891.

3.4.6. Mutation analysis

Amino-acid sequences obtained from translation of DNA sequences were compared with known wild type gene bank sequences from GenBank, to analyze presence of mutations within the MHR. The mutation pattern was assessed based on published reports (Echevarria, *et al*., 2006; Ma, *et al*., 2012) using GENETYX *ver*.9.

3.6. Data management and analysis.

Data was typed into a Microsoft Excel spread sheet. After data entry into Microsoft Excel, the data was double-checked against the original paper copy and soft copy for quality control and assurance. In order to ensure privacy for the study subjects, serial

numbers were assigned during data entry. Names and any other personal identifiers were then deleted. All subsequent presentations and publications used these serial numbers. The proofed Microsoft Excel worksheet was password-protected and backed-up on a flash disk. The flash disk was then stored under lock and key at the Moi Teaching and Refferal Hospital laboratory in Eldoret.

The data generated was analyzed using statistical package STATA version 14.0. Descriptive statistics of socio-demographic variables and other characteristics of the sampled population were computed. Multiple logistic regressions were used for multivariate analysis to determine association between the socio-demographic characteristics and the presence of anti-HCV in the selected population.

CHAPTER FOUR

RESULTS

4.1. HBV prevalence

4.1.1. Demographic characteristics

A total of 200 participants were recruited to the study (96 males and 104 females). The unequal number of males and females happened due to the fact that the study was not biased. This did not affect the study in any way since there was no significant association between HBV infection and all the variables in the study. An overall prevalence rate of 10% (20/200) was established for this population, with a high proportion of those positive being male (12 of 20) (Figure 4.1). The average age among those infected was 44.05 years; (male 44.3 female 43.6 years) as shown in Table 4.1.

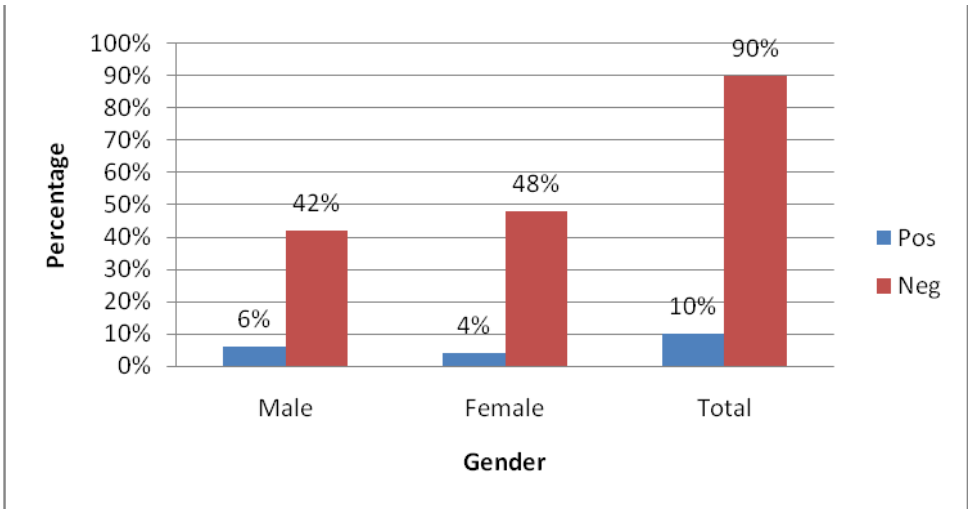

Figure 4.1: Showing overall prevalence of hepatitis B virus infections per gender among outpatient attendees at MTRH in Eldoret.

Table 4.1: Demographic data of outpatients seeking medical services at MTRH in Eldoret Kenya

Variable	Variable group	Frequency	Percentages
Age	<20 Years	29	15
	21-30 Years	62	31
	31-40 Years	57	29
	41-50 Years	23	12
	51-60 Years	14	7
	61-70 Years	8	4
	71-80 Years	5	3
	>81 Years	2	1
Gender	Male	96	48
	Female	104	52
Marital status	Married	125	62.5
	Single	72	36
	Separated	3	1.5
Occupation	Employed	73	36.5
	Business	19	9.5
	Farmer	25	12.5
	Unemployed	18	9
	Student	65	32.5
Level of Education	Tertiary	108	54
	Secondary	80	40
	Primary	12	6
Residence	North Rift	173	86.5
	Nyanza	9	4.5
	South Rift	5	2.5
	Western	13	6.5

Within the marital status, majority (125, 62.5%) were married, (72, 36%) were singles and (3, 1.5%) were separated or divorced. Of those infected, the average age among the males was 44.3 years, while the female had an average of 43.6 years. The association between HBV positivity and; age, gender, marital status, occupation, level of education and residence variables showed varied results (Table 4.2).

There was no significant association between HBV infection and all the variables whereby; age (P<0.07; OR=1.447; CI=0.97-2.157), gender (P<0.149; OR-0.475; CI=0.172-1.307), marital status (P<0.668; OR=0.718; CI=0.158-3.260), occupation (P<0.054; OR=1.503; CI-0.994-2.273), level of education (P<0.342; OR= 1.487; CI= 0.655-3.376) and residence (P<0.769; OR=0.904; CI=0.463-1.765) (Table 4.2).

Table 4.2: Demographic association analyzed by binary logistic regression correlation among outpatient attendees at MTRH in Eldoret, Kenya (n=200)

Variable	Variable group	No./% Total Pos	% Pos within group	% Pos within HBV	P- Value	Odds ratio	95%CI for Odds
Age	<20 Years	1 (0.5%)	3.45%	5%	0.070	1.447	0.97-2.157
	21-30 Years	4 (2%)	6.45%	20%			
	31-40 Years	7 (3.5%)	12.3%	35%			
	41-50 Years	1 (0.5%)	4.35%	5%			
	51-60 Years	3 (1.5%)	21.4%	15%			
	61-70 Years	2 (1%)	25%	10%			
	71-80 Years	1 (0.5%)	20%	5%			
	>81 Years	1 (0.5%)	50%	5%			
Gender	*Male*	12 (6%)	12.5%	60%	0.149	0.475	0.172-1.307
	Female	8 (4%)	7.7%	40%			
Marital status	Married	15 (7.5%)	12%	75%	0.668	0.718	0.158-3.260
	Single	4 (2%)	5.6%	20%			
	Separated	1 (0.5%)	33.3%	5%			
Occupation	Employed	0 (0%)	0%	0%	0.054	1.503	0.994-2.273
	Business	4 (2%)	21.1%	20%			
	Farmer	8 (4%)	32%	40%			
	Unemployed	5 (2.5%)	27.8%	25%			
	Student	3 (1.5%)	4.6%	15%			
Level of education	Tertiary	7 (3.5%)	6.5%	35%	0.342	1.487	0.655-3.376
	Secondary	9 (4.5%)	11.25%	45%			
	Primary	4 (2%)	33.3%	20%			
Residence	North Rift	17 (8.5%)	9.8%	85%	0.769	0.904	0.463-1.765
	Nyanza	2 (1%)	22.2%	10%			
	South Rift	0 (0%)	0%	0%			
	Western	1 (0.5%)	7.7%	5%			

4.1.2 Confirmation of HBsAg using Gel electrophoresis

All the 20 samples that were positive on ELISA were confirmed using PCR before proceeding for sequencing. Of the 20 ELISA antibody positive samples, 14 amplified to the expected band size of 1063 bp which was confirmed by gel electrophoresis as shown by figure 4.2.

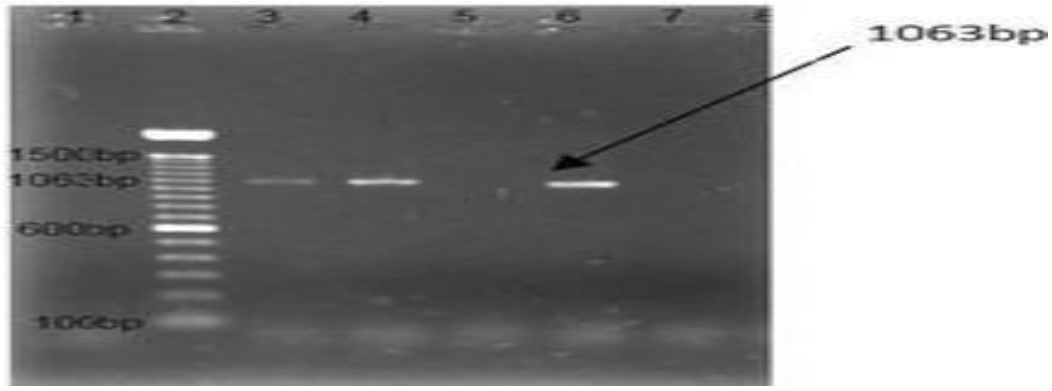

Figure 4.2: Agarose gel electrophoresis of selected samples. The samples in wells; 3,4, and 6 represents HBV positive samples, well 5 and 7 were negative samples, and well 2 represents the molecular weight marker.

The obtained sequences from successfully sequenced samples were aligned with selected reference from Hepatitis B data base center and presented in graphic form (appendix (iv) and appendix (v). The aligned sequences were used to construct a phylogenetic tree in order to confirm the actual HBV genotypes (Figure 4.3).

HBV partial S-gene was successfully amplified and sequenced from 14 of 20 HBsAg positive samples. The remaining 6 specimens had insufficient DNA for sequencing analysis. Based on phylogenetic analysis of the 14 isolate sequences with reference sequences obtained from GenBank (Figure 4.3), 13 (92.9%) of the isolates classified into subgenotype A1 (HBV/A1), while 1 (7.1%) of isolates categorized into subgenotype D1 (HBV/D1). None of the sequence isolates from this study classified with the recombinant strains sequences.

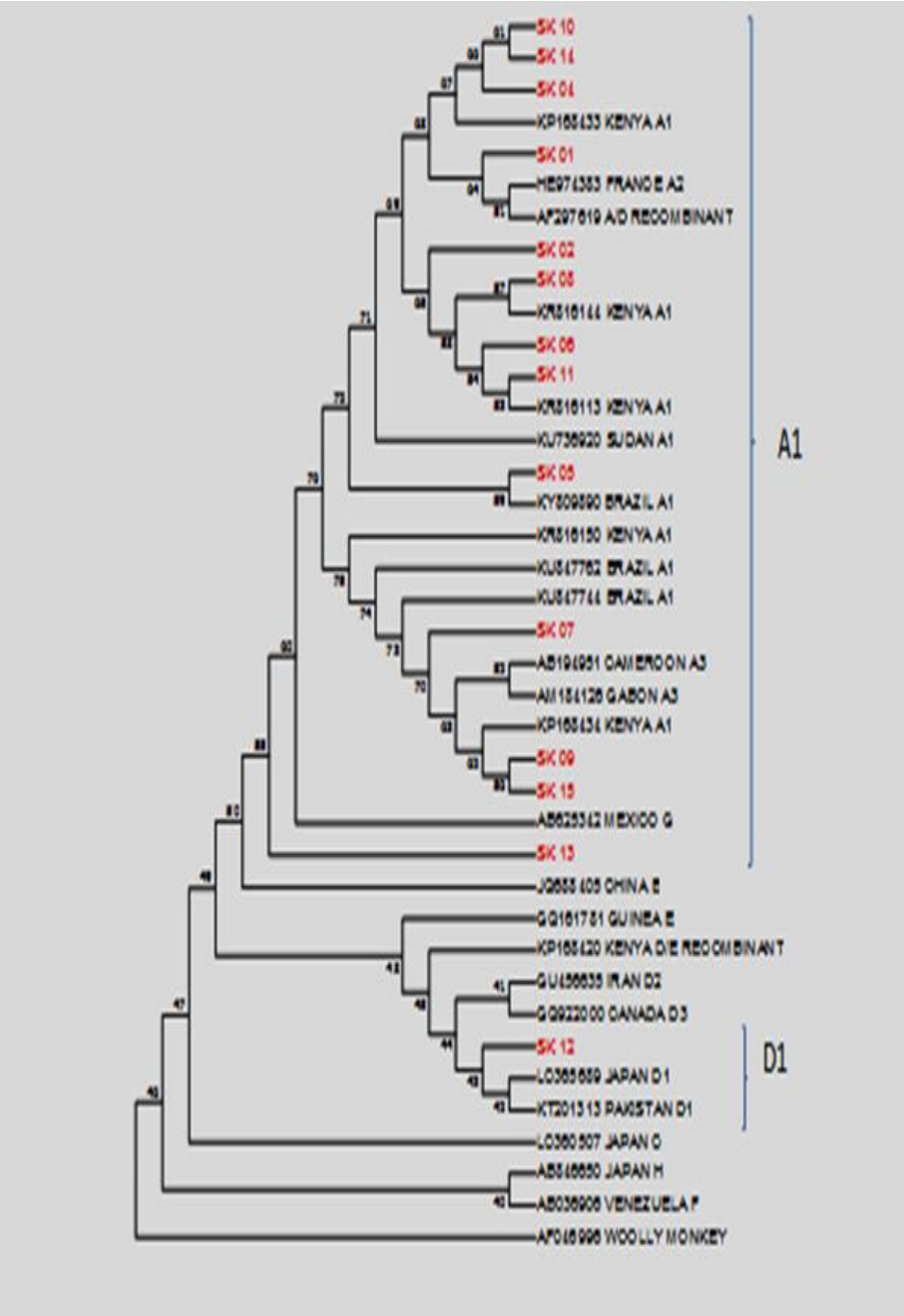

Figure 4.3: Phylogenetic tree of HBV samples constructed by Neighbour joining method. The evolutionary history was inferred using the Neighbor-Joining method. The analysis involved 39 nucleotide sequences. Evolutionary analyses were conducted in MEGA7.

4.3. Mutation in HBV S-region

In all the 14 isolates either one or more mutation was detected within the MHR (Figure 4.3). The most occurring amino acid substitution within the MHR was S114T which appears in 13/14 isolates (Table 4.3). One isolate SK 03 did not successfully align with the reference sequence. Mutation S114T is a point mutation found in the MHR and Mutations at MHR have been shown to be related with diagnostic problems, emergence of vaccine-escape mutants, and hepatitis B immunoglobulin (HBIG) therapy failure. No known mutations associated with occult HBV infection or vaccine escape were observed.

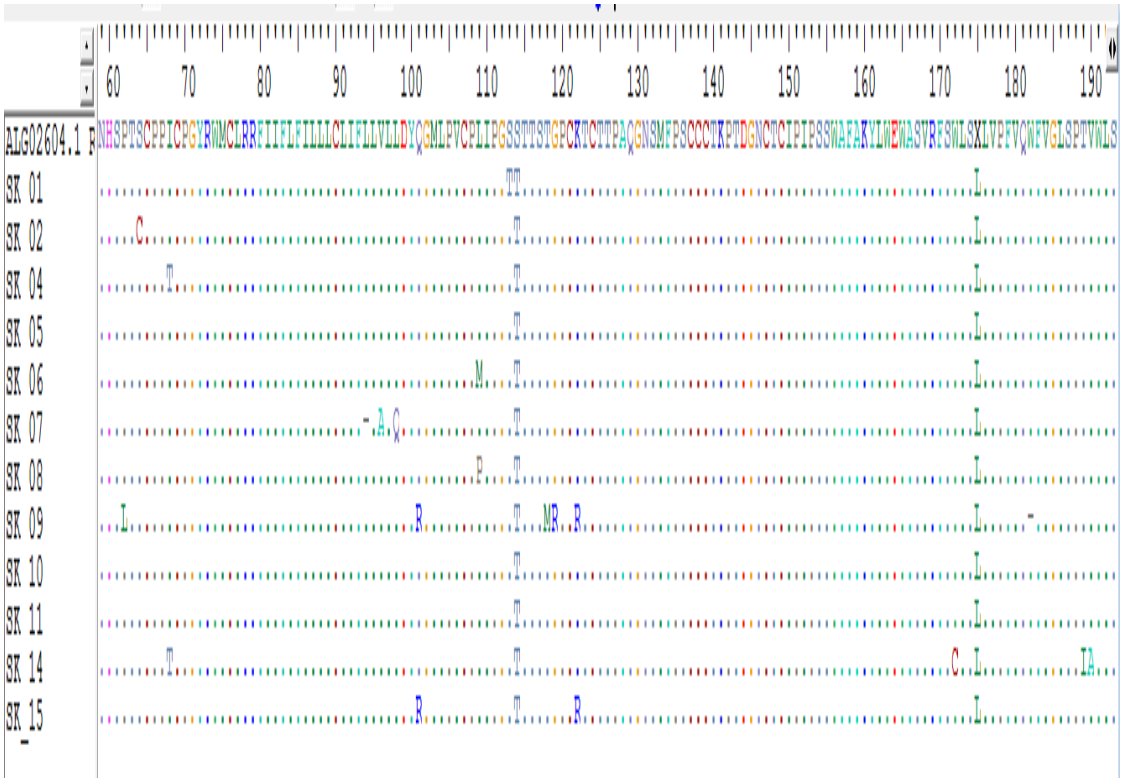

Figure 4.4: Multiple sequence alignment of the HBV S protein among the 12 genotypes. The amino acids substitutions are shown.

Table 4.3: Distribution of amino acid mutations in partial HBV S-gene among isolates from outpatients at MTRH.

HBV S-gene Region	Mutation	Sample/Sequence Identification
MHR (a.a positions 99-169)	Q101R	SK.09,15
	L109M	SK.06
	L109P	SK.08
	S113T	SK.01
	S114T	SK.01,02,04,05,06,07,08,09,10,11,12,14,15
	T118M	SK.09
	G119R	SK.09
	K122R	SK.09,15

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECCOMENDATIONS

5.1. Discussion

This study establishes HBV sero-prevalence of 10% occurring mainly among the married males aged between 31 – 40 years. Among HBV, the study further establishes genotype A1 as the circulating HBV within this population of outpatient attendees, mainly from the rural parts of the North rift region of Kenya. The prevalence established in this study is considered very high, especially due to the fact that it is determined among outpatients who were naive about their HBV status. It suffices to note that some previous studies (Ochwoto, *et al*., 2016) have also reported a high prevalence of HBV (50.6%) among populations in North Rift Kenya. Such high prevalence of HBV from a naïve rural population has an implication that, transmission of HBV could continuously be going on in the community unnoticed, since a lot of emphasis have been put on major infections such as HIV, Malaria and Tuberculosis (NASEM 2017). Further the realization of the high number among the married in their sexual reproductive age has an implication of a potential silent spread of HBV in families and if not checked, the risk of vertical transmission could also be increased, since HBV as it is currently, is not screened in routine antenatal care (ANC) services. Indeed the potential role of vertical transmission in HBV infection has been elucidated (Mor *et al.*, 2010; Tiele *et al.*, 2017), with up to 9.8% of children born to HBsAg-positive and/or HBV-DNA positive women turning HBV-DNA-positive by 48 weeks of age (Chasela *et al*., 2014).

In this study, all the sociodemographic variables were not significantly associated with the risk of HBV infection. This study demonstrates the presence of genotype A subtype A1 (92.9%) as the dominant genotype with a few cases (7.1%) presenting with genotype D subtype D1. The predominance of genotype A is consistent with recent HBV genotype distribution studies in other parts of Kenya like Nairobi,

Kericho and Mombasa (Mwangi, *et al*., 2008; Ochwoto, *et al*., 2016; Kibaya, *et al*., 2015; Nyairo, *et al* 2016). Genotype D distribution of this study is also comparable to certain extent with aforementioned studies. The HBV genotypes show a distinct geographical distribution in Africa, with genotype A predominating in the South-East, genotype D in the North and genotype E in the West (Kramvis *et al*., 2005). HBV genotype A has been reported to be predominant in Kenya (Mwangi *et al*., 2008; Kwange *et al*., 2013), Uganda (Calisti *et al*., 2015) and Tanzania (Hasegawa, *et al*., 2006), while genotype D is prevalent in Sudan (Yousif *et al*., 2013; Yousif *et al*., 2014) and Egypt (Saudy *et al*., 2003).

Knowing about the predominant HBV molecular variants present at specific area is significant for the assessment of diagnostic capabilities and vaccine efficacy (Stanaway *et al*., 2016). Mutations at MHR have been shown to be related with diagnostic problems, emergence of vaccine-escape mutants, and hepatitis B immunoglobulin (HBIG) therapy failure (Coleman, 2006; Wu *et al*., 2010; Ma *et al*., 2012). The mutation analysis of this study showed that all 14 outpatients had amino-acid substitutions within the MHR. Another study conducted on HBV/HIV mono or co-infected Ethiopians reported occurrence of immune-escape mutants at different levels (Belyhun *et al*., 2017). Amino-acid substitutions at MHR are associated with immunological pressure resulting from both natural and HBV vaccination (Coleman, 2006; Ma *et al*., 2012). The finding of amino acid substitutions in the MHR in this study which represents immune escape variants should be taken into account as there is a possibility for these variants to spread more and consequently influence vaccine efficacy and treatment strategy in the country. There was no G145R mutation found in the samples sequenced and this may be a rare mutation in Kenya. G145R has been found in various studies shown to contribute to occult hepatitis B infection (OBI) (Nyairo *et al*., 2016).

Different studies have shown that genotype A1 is associated with high rates of HCC in sub-Saharan Africa, this association of genotype A with advance disease outcome is probably a course to worry since this is the main genotype in Kenya. The participants of this study were outpatients who were seeking treatment for other different ailments at the hospital and were not aware of their HBV status. Presence of

genotype A among some of them could therefore indicate a long standing transmission circulating in the population without notice. This could consequently lead to development of advanced disease over time. Early detection is critical to limit the HCC disease pathology. Since testing for hepatitis B and genotype determination is not routinely done in Kenya, the population could be exposed to a potential chronicity of infections. Therefore, testing and genotyping would be important in HBV programs especially in the implementation of diagnostic measures in the population. This genotype has also been shown to be the most prevalent among patients with acute hepatitis B (Lyra *et al*., 2005).

The present study suggests that the outpatients at MTRH may develop chronic HBV infections. HBV genotyping that was previously regarded as a research tool has been suggested as an important element to guide the choice of therapy by several investigators and national professional associations (Cooksley *et al*., 2010). Genotype information of chronic HBV infections enables practicing physicians to identify those patients at risk of disease progression and to determine the appropriate and optimal anti-viral therapy (Tanwar, & Dusheiko. 2012).

Thus this study will become an input in narrowing the existing gap of HBV molecular studies in Kenya.

5.2. Conclusions

(i) The prevalence of HBsAg was 10% among the outpatients attendees at MTRH.
(ii) The socio demographic variables were not significantly associated with the risk of HBV infection.
(iii) The most predominant genotype found among the outpatient attendees at MTRH is HBV/A1.

5.3. Recommendations

(i) Campaigns for VCT in HBV should be enhanced among the public just as we see in HIV.

(ii) Knowledge on HBV transmission could also be provided to the public by trained specialists.

(iii) The HBV Diversity in the country should be considered when designing interventions for HBV.

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APPENDICES

Appendix I (A): Informed Consent Form

INTRODUCTION:

Good morning /afternoon?

My name is Sella Koech, I am a master student of Jomo Kenyatta University of Agriculture and Technology (JKUAT). I am conducting a study on P**revalence and Genotypic Characterization of HBV among outpatient attendees at Moi Teaching and Referral Hospital, Eldoret** as part of my thesis. I would like to seek your permission, please read the consent form below. I would be very grateful if you will assist me by agreeing being my volunteer in my study.

THE PURPOSE OF THE STUDY

The purpose of this study is to determine the prevalence of HBV and the common genotypes among **outpatient attendees at MTRH inEldoret**. The results of this study will be communicated back to the group organization for necessary action by the physician and to KEMRI who will also take action depending on the outcome. The results will also be used in writing my thesis as part of a requirement by the university.

The study will therefore be of benefit to you and aid in formulation of policies in the health sector of Kenya.

PROCEDURE

The purpose of this form is to obtain your consent to participate. If you choose to participate I will provide a questionnaire for you to fill to the best of your knowledge. For all the questions in the questionnaire there is no right or wrong answer. To ensure confidentiality, your name will not appear anywhere on the

questionnaire. In addition to the questionnaire, I will also take blood sample from your blood vein using the venipuncture procedure. The blood will be used to find out if you are infected with hepatitis B virus and the type of genotype in your body.

WHAT ARE THE RISKS OF THE STUDY?

During this process slight pain will be experienced when drawing blood, apart from this there is no other known risk in participating in this study. All the procedure will be conducted in a sterile environment.

WHAT ABOUT CONFIDENTIALITY?

All the information obtained is strictly confidential and data is password protected and will be only accessed by the Principal investigator. Subjects/participants in the study will be kept anonymous and being identified only by specific numbers assigned by the principal investigator. The results obtained will be made available to the health care provider only with consent from the subjects.

HOW LONG WILL THE STUDY TAKE?

The study is projected to take one year but I will only take your blood sample once. Once you begin the exercise and you wish to stop at any time, you are free to do so; you will not be penalized in any way.

WHERE WILL THE SAMPLES BE ANALYZED?

The blood sample will be transported to KEMRI for serological tests and for preparation of the samples for sequencing and genotype determination.

WHAT ARE THE COSTS?

There will be no cost for the participants in the study.

ARE THERE BENEFITS IN TAKING PART IN THE STUDY?

The results of the study will be communicated back to the group and also to the KEMRI for appropriate action. The overall results will be used to advice the general public and the government health sector.

CONTACT INFORMATION

For any inquiries in the event of any research related questions, comments or complaints, the following persons are available for contact:

The secretary

KEMRI Ethics and Research committee

P.O. BOX 54840-00200 Nairobi

Tel. (254) (020) 2722541 or 0722 205901 or 0733 400003

Email: director@kemri.org

Principle Investigator:

Sella Koech (BSc. Microbiology)

Telephone: 0728 890 260

Email:sellakoech@gmail.com.

Co – Investigators:

1. Prof. Elijah M. Songok

CVR-KEMRI

Telephone: 0711 870 333

Email: Esongok@kemri.org.

2. Dr. Eddy Odari

Department of Medical Microbiology

Jomo Kenyatta University of Agriculture and Technology

Telephone: 0725 259 296

Email:kodarie04@yahoo.com

Subject permission:

I have read and understood the consent form. I have been informed that completion of this form is voluntary and I therefore make my decision.

(PLEASE TICK ONE ONLY)

Consent for blood collection **YES_____ NO_____**

Consent for Transportation of blood Samples to KEMRI **YES_____NO_____**

Signature of the Participant _____ Date _____

Signature of the person obtaining consent _____ Date _____

(Must be the investigator or individual who has been designated to obtain consent)

Signature of the principle investigator _____ Date _____

(*Affirming subject's eligibility to participate in the study and that informed consent has been obtained).*

Appendix I (B): Karatasi Ya Kufahamisha Idhini

Habari ya asubuhi/mchana?

Jina langu ni Sella Koech, mwanafunzi wa chuo kikuu cha Jomo Kenyatta ukulima na teknolojia ambapo ninasomea shahada ya pili. Kwa wakati huu ninafanya utafiti wa shahada la pili kwa kuchunguzakiwango cha maambukizi ya virusi ya hepatitis B miongoni mwa wagonjwa wanao tembelea kliniki la nje katika hospitali la Moi Teaching and Referral Hospital jijini Eldoret. Kwa hivyo nakuomba idhini usome karatasi hili la kukufahamisha. Nitashukuru sana iwapo utanisaidia kwa kujitolea na kuwa mshiriki.

MADHUMUNI YA UCHUNGUZI HUU

Lengo la uchunguzi huu ni kuhakikisha viwango na idadi ya virusi hivi miongoni mwa wagonjwa wanao tembelea kliniki la nje katika hospitali la Moi Teaching and Referral Hospital jijini Eldoret. Na pia kuchunguza ili kujua ni aina gani ya virusi *(genotypes)* vya Hepatitis B vinavyopatikana miongoni mwa hao wagonjwa. Matokeo ya uchunguzi huu yatawasilishwa moja kwa moja kwa Zahanati ili hatua zifaazo zichukuliwe na madaktari wahusika. Vile vile matokeo hayo yatatumika kuandika tasinifu (thesis) ambayo inahitajika na chuo kikuu. Matokeo hayo pia yatasaidia sekta ya Afya katika kutengeneza mikakati mwafaka haswa virusi hivi vinapoambatana.

TARATIBU ZA UTAFITI

Lengo la hili karatasi ni kukuomba idhini ya kushiriki. Iwapoutakubalikushiriki, basi utajaza fomu ya maswali na kishautatolewa damu na wauguzi. Damu iyo itatumika kwa uchunguzi. Damu iyo itasafirishwa hadi taasisi ya uchunguzi wa matibabu (KEMRI) ambapo maandalizi ya kuchunguza idadi na aina ya virusi *(genotypes)* vya Hepatitis C itafanjika. 65

FAIDA

Faida ya binafsi mshiriki atakayo pata ni kuwa, majibu ya utafiti huu yatatumwa kwa hospitali ya kikundi yenu ili wachukue hatua zifaazo. Faida nyingine ni kuwa, matokeo hayo yatasaidia sekta ya Afya katika kutengeneza mikakati mwafaka haswa virusi hivi vinapoambatana, kuelimisha na kusaidia umma.

MADHARA

Kando na uchungu mdogo mshiriki atahisi wakati wa kutolewa damu, hakuna madhara mengine. Hata hivyo utaratibu wa kutoa damu utafanyika katika mahali safi na hatua zote za kapunguza uchungu zitatumika.

SIRI YA HAKI YAKO

Majibu ya utafiti huu yatawekwa kwa siri kuu. Mchunguzi mkuu ndiye pekee atakua na idhini. Hakuna jina litakalochapishwa popote wakati hata baada ya uchunguzi kukamilika. Washiriki watajulikana kwa nambari za siri zitakazopeanwa na mchunguzi mkuu.

GHARAMA

Hakuna ada au gharama yoyote mshiriki atatozwa katika uchunguzi huu.

HAKI ZAKO KAMA MSHIRIKI?

Kushiriki katika uchunguzi huu ni kwa hiari na mshiriki anaweza kujiondoa wakati wowote na mshiriki atahujumu haki zake kwa kutia kidole kwenye stakabali hii.

UTAFITI HUU UTAFANYWA KWA MUDA GANI?

Utafiti huu utafanywa kwa muda wa mwaka moja. Lakini mshiriki anaruhusiwa kutolewa damu mara moja tu.

HABARI ZAIDI AU MASWALI

Iwapo utakuwa na swali lolote kuhusu mradi huu linastahili kuelekezwa kwa wafuatao:

Mchunguzi mkuu:

Sella Koech (BSc. Microbiology)

Simu: 0728 890 260

Pepe: sellakoech@gmail.com

WasaidiziwaUchunguzi:

1. Prof. ElijahM.Songok

CVR-KEMRI

simu: 0711-870333

Pepe: Esongok@kemri.org

2. Dkt.Eddy Odari

Jomo Kenyatta University of Agriculture and Technology

Simu: 0725- 259296

Pepe:kodarie04@yahoo.com

Kwa Karani,

KEMRI Ethics and Research committee

S.L.P. 54840-00200. Nairobi

Simu. 2722541 au 0722 205901 au 0733 400003

IDHINI YA MUHUSIKA

Nimeisoma fomuhii na masharti ya utafiti huu na kwahivyo natoa idhini ya sampuli yangu kutumiwa kwa hiari yangu. ***(CHAGUA MOJA***)

Idhini ya kuchukua damu Ndio……. La…….

Idhini ya kusafirisha damu Ndio……La……

sahihi ya Mshiriki_____tarehe_____

Sahihi ya mtu anayechukua idhini_____tarehe_____

Jina la mtu anayechukua idhini_____

(*Lazimaawemtafiti/mchunguzi ama mtualiyepewajukumu la kupewaidhini*)

Sahihi ya mchunguzi mkuu _____ tarehe_____

Appendix II (A): Questionnaire

PREVALENCE AND MOLECULAR CHARACTERIZATION OF HEPATITIS B VIRUS INFECTION AMONG OUTPATIENT ATTENDEES AT MOI TEACHING AND REFERRAL HOSPITAL IN ELDORET.

Date _____ Patient's Identification Number _____

(a) Individual Background and Demographic Information:

01 Year of birth _____

02 Sex: Male Female

03 Area of residence _____

04 Marital status: Married_____ Single_____

05 Level of Education: Primary_____Secondary_____ College_____

06 Occupation _____

(b) **Medical History**

001 Have you ever been tested for Hepatitis? YES_____ NO_____

Appendix II (B): Karatasi Ya Maswali (Questionnaire)

KIWANGO CHA MAAMBUKIZI YA VIRUSI YA HEPATITIS B MIONGONI MWA WANAOTEMBELEA KLINIKI LA NJE KATIKA HOSPITALI KUU LA MOI TEACHING AND REFERRAL HOSPITAL JIJINI ELDORET.

Tarehe _____ Nambari ya mgonjwa _____

(a) Ujumbe wa kibinafsi na habari ya makaazi

01 Mwaka wa kuzaliwa _____

02 Jinsia Mme Mke

03 Wilaya au eneo unaloishi _____

04 Hali ya unyumba Nimeoleka/nimeolewa Sijaolewa/sijaoa

05 kiwango ya elimu shule ya msingi shule ya upili chuo kikuu

06 Kazi unayo fanya _____

(b) Historia ya matibabu

001 Je Umewahi pimwa virusi vya Hepatitis? Ndio_____ La_____

Appendix III: Publication in Peer Reviewed Journal Arising from this Work

Japanese Journal of Gastroenterology and Hepatology

Research Article

Viral Hepatitis B Genotypes among Outpatient Clinic Attendees in North Rift, Kenya

Koech S[1,2], Odari E[2], Lihana R[3], , Maiyo A[3], Cheriro W[4], Kiptoo M[5], Azumi I[6] and Songok EM[1,3]*

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[4]Moi Teaching and Referral Hospital (MTRH), Department of Medical Laboratory services, Eldoret, Kenya

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Published: 31 Mar 2020

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1. Abstract

1.1. Objective: Most hospital outpatients unaware of their Hepatitis B Virus (HBV) status could be seeking treatment for other different ailments in Kenya. To evaluate the HBV prevalence and the genetic diversity, the genetic analysis of the partial HBV S gene was conducted.

1.2. Methods: Two hundred blood samples were collected from consenting outpatients who were unaware of their HBV status at Moi Teaching and Referral Hospital (MTRH), Kenya, between September 2015 and October 2016. The serum was tested for the HBV surface antigen (HBsAg) using the ELISA test. DNA was extracted from HBsAg positive samples, amplified and sequenced for HBV S gene. The sequences were then compared with reference sequences retrieved from the GenBank.

1.3. Results: Ten percent of subjects (20/200) were positive for HBsAg. Thirteen of the 14 isolates (92.9%) belonged to HBV sub genotype A1 (HBV/A1). In all the 14 isolates either one or more mutation was detected within Major Hydrophilic Region (MHR). The most occurring mutation was S114T which appeared in 13 of 14 isolates. No known mutations associated with occult HBV infection or vaccine escape were observed.

1.4. Conclusion: Ten percent of outpatients at MTRH could be a source of unaware HBV transmission in the community. HBV/A1 remains the most predominant genotype. The findings that the HBsAg mutations in MHR were observed in all isolates revealed the importance of monitoring the MHR mutations in this country. The development of an optimized HBV screening, vaccine program and a monitoring system of MHR mutation are urgently needed in North Rift Kenya.

2. Keywords: HBV genotypes; MHR mutations; Outpatient attendees; Kenya

3. Introduction

Global estimates in the year 2015 suggest that quite 2 billion people are infected with Hepatitis B Virus (HBV) and 250 million of those people are chronically infected, of which 65million live in Africa [1, 2, 3].

The Prevalence of HBV among the rural population in Kenya stood at 8.8% in the year 2011 [4] while that of pregnant women attending antenatal clinic at Moi Teaching and Referral Hospital (MTRH) in Eldoret, North Rift Kenya stood as 9.3% in the year 2006 [5].

Appendix IV: Ethical Approval Letter

KENYA MEDICAL RESEARCH INSTITUTE

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

KEMRI/RES/7/3/1 **October 29, 2015**

TO: **SELLA KOECH,**
PRINCIPAL INVESTIGATOR

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

Forwarded
Raphaelkibana
5TH Nov. 2015

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

Dear Madam,

RE: **KEMRI/SERU/CVR/004/3125 (*RESUBMISSION2 OF INITIAL SUBMISSION*): PREVALENCE OF VIRAL HEPATITIS B AND HBV/HIV CO-INFECTION AMONG OUTPATIENT ATTENDEES AT MOI TEACHING AND REFFERAL HOSPITAL IN ELDORET, KENYA-(*VERSION 1.2 DATED 13TH OCTOBER, 2015*)**

Reference is made to your letter dated 13th October, 2015. KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised protocol on 26th October, 2015.

This is to inform you that the Committee notes that the issues raised during the 242nd meeting of the KEMRI/Ethics Review Committee (ERC) held on 18th August, 2015 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **29th October, 2015** for a period of one year. Please note that authorization to conduct this study will automatically expire on **October 28, 2016.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **September 16, 2016**.

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

You may embark on the study.

Yours faithfully,

FOR:

PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

CENTRE FOR VIRUS RESEARCH
05 NOV 2015
P.O. Box 54628 - NAIROBI

Appendix V: Seminar Presentation Minutes

MINUTES FOR SCHOOL OF BIOMEDICAL SCIENCES SEMINAR PRESENTATION HELD AT SCC100 ON 4TH MARCH 2019.

Members present

1. Prof. Daniel Kariuki
2. Prof. Ongus
3. Prof. Fred Omunyokoli
4. Dr. Kinyua
5. Dr. Caroline Ngugi
6. Dr. Samson Chebon
7. Dr. Kyama
8. Dr. Celestine Makobe
9. Dr. Eddy Odari
10. Dr. Steven Ger

AGENDA:

1. Postgraduate Seminar.
2. AOB

Prof. Kariuki welcomed members to the seminar and advised the students to follow the Board of Postgraduate Studies (BPS) standards on the structures of thesis and manuscript publication in impact factor journals.

	Deliberation on MSc project presentation from SELLA KOECH TM303-2125/2014 **PREVALENCE OF VIRAL HEPATITIS B AND HBV/HIV COINFECTION AMONG OUTPATIENT ATTENDEES AT MOI TEACHING AND REFERRAL HOSPITAL IN ELDORET.** **The following recommendations were made:** The student was asked to state the sample size of the study and how the samples were collected. The sequencing section was missing in the presentation and the student was asked to do further research.	Student Supervisor

AOB

The dean thanked the members for their attendance and the staff for being patient. This was the first postgraduate seminar in the School of Biomedical Sciences, helpful to students because they had an opportunity to share their research experience. The students were informed that they should always request their supervisors to attend. The students were also informed of the new rule from BPS whereby they have to get a Plagiarism certificate and must publish work before defence.

Appendix VI: Seminar Presentations Minutes

MINUTES FOR SCHOOL OF BIOMEDICAL SCIENCES SEMINAR PRESENTATION HELD AT SCC100 ON 4TH MARCH 2019.

Members present

1. Prof. Daniel Kariuki
2. Prof. Ongus
3. Prof. Fred Omunyokoli
4. Dr. Kinyua
5. Dr. Caroline Ngugi
6. Dr. Samson Chebon
7. Dr. Kyama
8. Dr. Celestine Makobe
9. Dr. Eddy Odari
10. Dr. Steven Ger

AGENDA:

1. Postgraduate Seminar.
2. AOB

Prof. Kariuki welcomed members to the seminar and advised the students to follow the Board of Postgraduate Studies (BPS) standards on the structures of thesis and manuscript publication in impact factor journals.

	Deliberation on MSc project presentation from SELLA KOECH TM303-2125/2014 **PREVALENCE OF VIRAL HEPATITIS B AND HBV/HIV COINFECTION AMONG OUTPATIENT ATTENDEES AT MOI TEACHING AND REFERRAL HOSPITAL IN ELDORET.** **The following recommendations were made:**	Student Supervisor

	The student was asked to state the sample size of the study and how the samples were collected. The sequencing section was missing in the presentation and the student was asked to do further research.	

AOB

The dean thanked the members for their attendance and the staff for being patient. This was the first postgraduate seminar in the School of Biomedical Sciences, helpful to students because they had an opportunity to share their research experience. The students were informed that they should always request their supervisors to attend. The student were also informed of the new rule from BPS whereby they have to get a Plagialism certificate and must publish work before defense.

Appendix VII: Showing Aligned Sequences with Reference