SEROPREVALENCE OF HEPATITIS B AND C VIRUSES AMONG PATIENTS WITH CHRONIC LIVER DISEASE AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET KENYA

By

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SM/PGM/07/12

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Masters of Medicine in Internal Medicine,

Moi University

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Declaration

Student Declaration

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Dedication

I dedicate this thesis to my family for the support they gave me while pursuing my postgraduate studies.

Seroprevalence of Hepatitis B and C viruses among patients with Chronic Liver Disease at Moi Teaching and Referral Hospital, Eldoret Kenya

ABSTRACT

Background: Hepatitis B (HBV) and C (HCV) viruses are frequent causes of chronic liver disease (CLD) worldwide. It is estimated that one third of the world's population is infected with Hepatitis B virus (HBV) and more than 350 million have chronic infection resulting in 1.2 million deaths yearly. About 170 million persons have chronic Hepatitis C viral infection and 3-4 million new infections occur each year. Both viruses cause chronic hepatitis, liver cirrhosis and hepatocellular carcinoma which have high morbidity and mortality.
The prevalence of HBV is high in low-resource countries and Kenya is in the high endemic zone with a prevalence of more than 8%. There is need to establish the contributions of these infections to CLD for setting public health of priorities and guiding prevention programmes.

Objective: To determine the seroprevalence of HBV and HCV among adult patients with chronic liver disease attending Moi Teaching and Referral Hospital (MTRH), Eldoret, Kenya.

Methods: A cross-sectional descriptive study was conducted among patients with chronic liver disease attending MTRH Medical wards, Liver and Oncology outpatient clinics. Their socio-demographic and risk characteristics were recorded. Blood was tested for markers of HBV and HCV using direct ERBA LISA Hepatitis B and ERBA LISA Hepatitis C ELISA kits respectively. HIV (Human Immunodeficiency Virus) status of the participants was obtained from their charts. Data was analyzed using STATA Version 13 SE to determine the prevalence of HBV and HCV among the study participants.

Results: Between December 2013 and January 2015, 95 patients were screened out of whom 84 participants were enrolled into the study. Their mean age was 45 ± 14 years with a range of 18 to 81years. Majority of the participants, 64 (76%) were males. Fifty eight percent (58%) of the participants had history of harmful alcohol use. The prevalence of HBV was 44 %. None of the participants was infected with HCV. All the participants had not been immunized against HBV. Possible horizontal modes of HBV transmission were reported in low frequencies hence majority of the HBV-infected participants could have acquired it vertically. Seventeen percent (17%) of the participants had HIV infection and 8% were co-infected with HIV and HBV.

Conclusion: Almost half of patients with CLD at MTRH have HBV infection. In this cohort of CLD patients, none had HCV infection.

Recommendation: Preventive measures should be scaled up to reduce HBV infection and its complications such as CLD.

Table of Contents

Declaration....i
Dedication.... ii
Table of Contents iv
List of Tables.... vii
List of Figures.... viii
Acknowledgements....ix
List of Abbreviations x
Operational Definitions....x
CHAPTER ONE: INTRODUCTION....1
1.1 Background 1
1.2 Problem Statement....4
1.3 Justification 4
1.4 Research Question 5
1.5 Research Objectives....5
1.5.1 Broad objective....5
1.5.2 Specific objectives 5
CHAPTER TWO: LITERATURE REVIEW 6
2.1 Overview of prevalence....6
2.1.1 Introduction 6
2.1.2 Prevalence of HBV 6
2.1.3 Prevalence of HCV 6
2.2 Hepatitis B viral infection 7
2.2.1 Introduction 7
2.2.2 Modes of transmission....8
2.2.3 Diagnosis 9
2.2.4 Natural history....11
2.2.5 Risk stratification and disease progression....11
2.2.6 Hepatitis B viral infection and chronic liver disease 12
2.2.7 Treatment strategies 13
2.2.8 Prevention strategies 13

2.2.9 Counseling 14
2.3 Hepatitis C viral infection 14
2.3.1 Introduction. 14
2.3.2 Modes of transmission and risk factors. 15
2.3.3 Screening 15
2.3.4 Diagnosis 16
2.3.5 Natural history 17
2.3.6 Hepatitis viral infection and chronic liver disease 17
2.3.7 Treatment strategies 18
2.3.8 Prevention strategies 18
2.4 Summary of Literature Review 19
CHAPTER THREE: PARTICIPANTS, MATERIALS AND METHODS 20
3.1 Study site 20
3.2 Study population 20
3.3 Study design 20
3.4 Eligibility 20
3.4.1 Inclusion criteria..20
3.4.2 Exclusion criteria...20
3.5 Study period and Selection of the study participants..21
3.6 Study procedure 22
3.7 Data collection and analysis 23
3.7.1 Data collection...23
3.7.2 Data analysis...23
3.8 Ethical considerations 23
CHAPTER FOUR: RESULTS 25
4.1 Patient Enrolment..25
4.2 Socio-demographic characteristics. 26
4.3 Prevalence of Hepatitis B and C viruses 26
4.4 Risk characteristics of the study participants 26
4.5 HIV infection among the study participants. 27
CHAPTER FIVE: DISCUSSION 28

Study Limitations 32
CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS...........................33
6.1 Conclusions 33
6.2 Recommendations..33
REFERENCES 34
APPENDICES...40
Appendix I: Hepatitis B virus testing procedure...40
Appendix II: Hepatitis C virus testing procedure..46
Appendix III: Consent form (English version)..51
Appendix IV: Consent form (Kiswahili version)..53
Appendix V: Questionnaire 55
Appendix VI: Alcohol Use Disorders Identification Tool (AUDIT) questionnaire: ...59
Appendix VII: IREC approval to conduct the study 63
Appendix VIII: Permission from MTRH to conduct the study 64

List of Tables

Table 1: HBV serologic markers and their significance 10
Table 2: Socio-demographic characteristics of the study participants 26
Table 3: Risk Characteristics of the study participants 27
Table 4: HIV infection among the study participants................................... 27

List of Figures

Figure 1: World map showing prevalence of chronic infection with Hepatitis B virus 8

Figure 2: Natural history of chronic HBV infection 11

Figure 3: Schema showing the natural progression of chronic HBV infection 12

Figure 4: Algorithm showing the study procedure 22

Figure 5: Schema showing patient Screening and Enrolment 25

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List of Abbreviations

ALT : Alanine aminotransferase
anti- HBc : antibodies to hepatitis B core antigen
AUDIT : Alcohol Use Disorders Identification Tool
BMI : Body Mass Index
CDC : Centre for Disease Control
CLD : Chronic Liver Disease
DNA : Deoxyribonucleic Acid
EIA : Enzyme Immuno Assays
ELISA : Enzyme Linked ImmunoSorbent Assays
GBD : Global Burden of Disease
GSK : Gastroenterology Society of Kenya
HBeAg : Hepatitis B e-antigen
HBIG : Hepatitis B Immunoglobulin
HBsAg : Hepatitis B surface Antigen
HBV : Hepatitis B Virus
HCC : Hepatocellular carcinoma
HCV : Hepatitis C Virus
HIV : Human Immunodeficiency Virus
INR : International Normalized Ratio
IREC : Institutional Research and Ethics Committee, Moi University
KNH : Kenyatta National Hospital
LFTs : Liver Function Tests
MOPC : Medical Outpatient Clinic
MTRH : Moi Teaching and Referral Hospital
PCR : Polymerase Chain Reaction
RNA : Ribonucleic Acid
RPHA : Reverse Passive Hemagglutination Assays
rt- PCR :realtime Polymerase Chain Reaction
SPSS : Statistical Package for Social Sciences
USA : United States of America
WHO : World Health Organization

Operational Definitions

1. **Case definition**

Chronic Liver Disease was diagnosed if the patient had (Bukhtiari N, 2000).

1) Clinical features of CLD such as: ascites, splenomegaly, jaundice, palmar erythema, finger clubbing, oedema, axillary and pubic hair loss, spider nevi, flapping tremors, drowsiness, and confusion.
2) Impaired liver function tests including raised level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and low albumin.

   Abdominal ultrasound showing coarse hepatic texture, changes in liver size or increased portal vein diameter was used to determine the specific diagnosis.

   Moreover to diagnose HCC, patients with clinical and sonographic features of hepatocellular carcinoma had to have elevated α-fetoprotein.

2. Harmful alcohol use: A score of ≥8 in the Alcohol Use Disorder Identification Tool (AUDIT) screening questionnaire (Appendix VI).

CHAPTER ONE: INTRODUCTION

1.1 Background

Hepatitis B and C viruses are frequent causes of chronic liver disease (CLD) worldwide (Rantala & van de Laar, 2008). They create a significant burden on health care systems due to high cost of morbidity and mortality. According to World Health Organization (WHO), one third of the world's population has been infected with Hepatitis B Virus (HBV) and more than 350 million have chronic infection. Regarding Hepatitis C Virus (HCV), it has been estimated that 170 million persons have chronic infection and that 3-4 million new infections occur each year (Shepard, Simard, Finelli, Fiore, & Bell, 2006; Sy & Jamal, 2006). HBV causes 1.2 million deaths per year globally due to various complications including chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) (Hou, Liu, & Gu, 2005; Lavanchy, 2004; Michielsen, Francque, & van Dongen, 2005; Seeger & Mason, 2000).

The burden of HBV in Africa however is difficult to assess due to underreporting and inaccurate records (Te & Jensen, 2010). A study done by Kramvis et al; in 2007 estimated that 65 million of 350 million people infected with HBV reside in Africa and 250,000 HBV-related deaths occur in Africa (Kramvis & Kew, 2007).

Hepatitis B surface antigen (HBsAg) is a marker of HBV infection. Persistence of HBsAg six months after exposure indicates chronic carriage and is highest in patients with clinical consequences of HBV infections (Kramvis & Kew, 2007). The estimated global sero-prevalence of HBsAg ranges from 5-20%; with the total numbers of HBsAg carriers approaching 40-58 million worldwide (Custer et al., 2004; Silveira et al., 1999; Tanaka, 2000). Kenya is an area of high endemicity (≥8%). Western Africa is also an

area of high endemicity (>10%) and in Gambia 57% of HCC cases were attributed to HBV infection (Kirk et al., 2004).

There are 8 genotypes of HBV (A to H). These have a distinctive geographic distribution in Africa. Genotype A is predominantly in Southern, Eastern and Central Africa. Genotype D is dominant in Northern Africa, whereas isolates from Western Africa belong to genotype E. Genotype A and D are predominant in Kenya. HBV genotype B is associated with less active liver disease and a slower rate of progression to cirrhosis as compared to genotype C. Also patients infected with HBV genotype C are more likely to develop HCC (Schaefer, 2005).

The WHO estimated global prevalence of HCV infection was 3% or 170 million individuals in 2014(WHO, 2014). HCV is a major cause of chronic liver disease in the world(Te & Jensen, 2010). A review of HCV prevalence in sub-Saharan countries done by Madhava et al shows that sub-Saharan countries have the highest WHO estimated regional HCV prevalence (5.3%). Egypt is the most endemic with a prevalence of 17.7% followed by Central Africa at 6% and West Africa at 2.4%, while Southern and East Africa have the lowest estimate at 1.6% (Madhava, Burgess, & Drucker, 2002).

There are 11 HCV Genotypes (1 to 11). Genotypes 1 and 3 are widely distributed globally with genotypes 1a and 1b accounting for 60% of infections worldwide. Data regarding the role of viral genotype and quasi-species in predicting disease outcome are too contradictory to reach definitive conclusions. However, some studies have shown that genotypes 2 and 3 are more responsive to treatment than genotypes 1 and 4(T Muasya et al., 2008).

Chronic liver disease is a major cause of morbidity and mortality. Global mortality estimates done in 2002 showed that there were 929,000 deaths due to chronic HBV and HCV infections, including 446,000 cirrhosis deaths and 483,000 primary liver cancer deaths (Perz, Armstrong, Farrington, Hutin, & Bell, 2006).

An understanding of relative contributions of various etiologies to disease burden is important for setting public health priorities and guiding prevention programmes. The WHO, Global Burden of Disease (GBD) 2000 project aims to quantify the burden of premature morbidity and mortality from over 130 causes (liver cancer and cirrhosis included). However etiologies underlying these diseases are not well accounted for; in particular HBV and HCV (Perz et al., 2006).

Kenya has been mapped out as a high HBV endemic zone with an estimated overall prevalence of >8% (CDC, 2010). Locally in Kenya, a study by Biko et al (unpublished) in 2010; found a prevalence of 9.7% and 1.6% for HBV and HCV respectively among HIV-infected patients in MTRH, Eldoret (Biko, 2010). Among hemodialysis patients in KNH, a study by Otedo et al found a prevalence of 8% for HBV and 5% for HCV. A study by Okoth et al among pregnant women found a prevalence of 9.3% for HBV and 0% for HCV. Among blood donors in Western Kenya, a study by Karoney et al (unpublished), found a prevalence of 1.1% for HBV and 0.3% for HCV.

There are few local studies on the prevalence of HBV and HCV among patients with chronic liver disease. In a study at Kenyatta National Hospital (KNH) in 1995 by McLigeyo et al, a seroprevalence of 32% for HBV and 1% for HCV among patients with CLD was observed; none of the subjects was co-infected with HBV and HCV (Ilako et

al., 1995). An earlier study in Kenya reported that 77% of patients with CLD were positive for HBV antibodies when compared to 15% in a control group (Geoffrey Zambezi Mutuma, 2011).

This study therefore aims at establishing the prevalence of HBV and HCV among patients with chronic liver disease at Moi Teaching and Referral Hospital (MTRH), Eldoret Kenya.

1.2 Problem Statement

Chronic liver disease is a major cause of morbidity and mortality worldwide. High costs are incurred in treating patients with CLD.

Hepatitis B and C viruses are well-recognized causes of chronic liver disease worldwide(Lotz, Kiss, Novak, Sobel, & Schaff, 2001). Studies have shown that they contribute immensely to CLD; however there is a knowledge gap regarding the relative contributions of these infections to CLD in our set up. Moreover, there is low public awareness and education concerning these infections. Routine screening for these viral infections in the general population is not done, hence late diagnosis and treatment of cases.

1.3 Justification

There is need to establish the contributions of these infections to CLD to inform setting of public health priorities and guide prevention programmes. Prevention and early treatment of these infections reduce morbidity and mortality due to CLD(GSK, 2014). Moreover early diagnosis and treatment of cases decreases infectivity and transmission to others(GSK, 2014).

After introduction of universal vaccination against HBV in Kenya in 2002 and with increasing intravenous drug use, findings from earlier studies may have changed.

1.4 Research Question

What is the sero-prevalence of hepatitis B and C viruses among patients with chronic liver disease at Moi Teaching and Referral Hospital?

1.5 Research Objectives

1.5.1 Broad objective

To determine the sero-prevalence of hepatitis B and C viruses among adult patients with chronic liver disease at MTRH.

1.5.2 Specific objectives

i. To determine the sero-prevalence of hepatitis B virus in patients with chronic liver disease at MTRH.

ii. To determine the sero-prevalence of hepatitis C virus in patients with chronic liver disease at MTRH.

iii. To describe the socio-demographic and risk characteristics of the patients in the study.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of prevalence.

2.1.1 Introduction

End–stage liver disease accounts for 1 in forty deaths worldwide. Chronic infections with HBV and HCV are well recognized risk factors for cirrhosis and liver cancer (Perz et al., 2006) .

Globally 57% of cirrhosis was attributed to either HBV (30%) or HCV (27%) and 78% of HCC was attributed to HBV (53%) or HCV (25%) (Perz et al., 2006).

2.1.2 Prevalence of HBV

The prevalence of HBV varies with the study population as observed by a prevalence of 11% and 18% among medical students in Kenya and Uganda respectively (Lule, Okoth, Ogutu, & Mwai, 1989; Pido & Kagimu, 2005). Among HIV-infected icteric patients, a prevalence of 53% was observed (Otedo, 2004) and 26.2% among icteric patients in a general medical ward (Atina, Ogutu, Hardison, & Mumo, 2004). A prevalence of 4.3% was found among urban residents and 3 to 11.9% in a rural population (Bowry, 1983; F. A. Okoth et al., 1990; Wankya, Hansen, Ngindu, Feinstone, & Purcell, 1979; Yamanaka, Takayanagi, Nakao, Kobayashi, & Baba, 1991). A prevalence of 12.2% was observed among HIV-infected outpatients. Among hemodialysis patients in Kenya, seroprevalence of 8% and 5% for HBV and HCV respectively was observed (Otedo, Mc'Ligeyo, Okoth, & Kayima, 2003).

2.1.3 Prevalence of HCV

Egypt has the world's highest prevalence of HCV estimated at 17.7%.This is due to decades of schistosoma eradication using parenteral tartate. There is however indication

that owing to massive public health interventions this figure is on the decline (Ismail, Ziada, Sheashaa, & El-Din, 2009; Strickland, 2006).

Among a population of sexually active adults in Miami (U.S.A), a prevalence of 4.4% for HCV was observed; multiple sexual partners and HIV infection were identified as independent risk factors for HCV infection (Daikos, Lai, & Fischl, 1994). Among type 2 diabetics in a public hospital in Nigeria, 11% had HCV infection, suggesting a strong association between diabetes and HCV infection (Ndako et al., 2009). Kenyan studies indicate prevalence of between 0.2%-22.2%, in various patient groups, with genotype 1 and 4 being predominant (Ilako et al., 1995; Karuru, Lule, Joshi, & Anzala, 2005a, 2005b; T. Muasya et al., 2008; Stevens et al., 2008).

2.2 Hepatitis B viral infection

2.2.1 Introduction

Hepatitis B virus, the founding member of a small family of viruses called hepadna viruses is a small, enveloped, partially double stranded DNA virus. It has a remarkably compact genomic structure despite its small size. HBV DNA codes for four sets of viral products with a complex, multiparticle structure. HBV relies on a replicative strategy unique among DNA viruses but typical of retroviruses. Instead of DNA replication directly from a DNA template, hepadna viruses rely on reverse transcription effected by DNA polymerase.

HBV is associated with both acute and chronic hepatitis. HBV infection is the 9th leading cause of death worldwide (Otedo, 2004). Chronic HBV infection is defined as a person with positive HBsAg for a duration greater than 6 months (Lok & McMahon, 2009). The global prevalence of HBV infection is variable and regions are defined as having high,

intermediate or low prevalence based on prevalence of greater than 8%, 2 to 7% and less than 2% respectively (Carey, 2009; Lok & McMahon, 2009) (Figure 1).

HBV stands out as an important public health problem warranting high priority efforts, prevention and control. The burden of carriage falls predominantly in Asia where 75% of chronic HBV carriers live. Africa has the second largest number of chronic carriers.

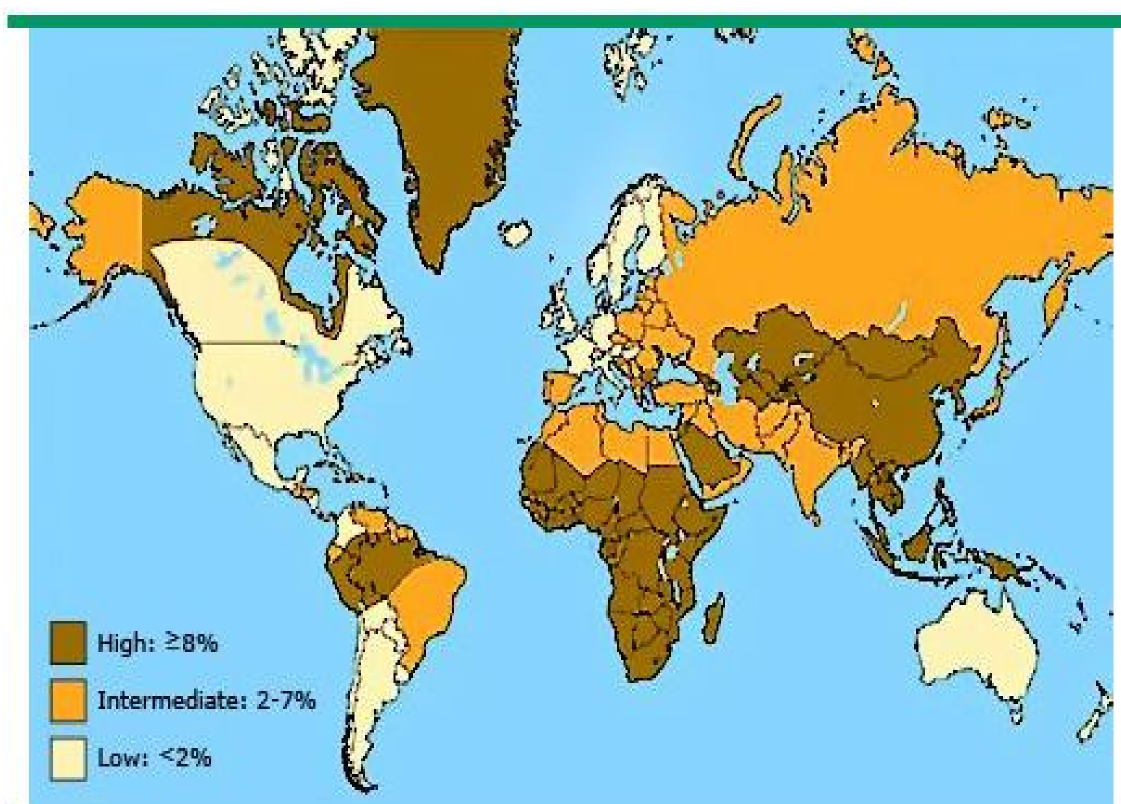

Figure 1: World map showing prevalence of chronic infection with Hepatitis B virus(CDC, 2010)

2.2.2 Modes of transmission

HBV is present in all body fluids and secretions including blood, saliva, semen, sweat, breast milk, tears and urine. The modes of transmission include sexual transmission

(Carey, 2009; Pido & Kagimu, 2005), needle stick injuries, perinatal transmission and percutaneous transmission (Greenfield et al., 1986; Lok & McMahon, 2009; Wankya et al., 1979). Hepatitis B virus can survive longer outside the human body and is transmitted 100 times more effectively than HIV (Lok & McMahon, 2009; Otedo, 2004). Most transmissions in developing countries are bimodal and are characterized by horizontal spread from person to person during childhood and sexually later on in adolescence and adulthood. In regions of high prevalence the rate of perinatal/vertical transmission can be particularly high reaching up to 90% (Lok & McMahon, 2009).

2.2.3 Diagnosis

Groups of people who should be tested for HBV include those from high prevalence areas, people with chronically elevated aminotransferases and high risk groups such as men who have sex with men, people with multiple sexual partners or history of sexually transmitted infections, inmates, dialysis patients, intravenous drug users, pregnant women, HIV and HCV infected patients, household contacts of HBV positive individuals and people with CLD (Lok & McMahon, 2009; F. Okoth et al., 2006).

Diagnostic approaches include laboratory tests whereby three classes of assays are used to diagnose HBV, notably:

1. **Serological assays** screening for HBV includes testing for HBV surface antigen (HBsAg) and antibodies to hepatitis B core antigen (anti-HBc) (Lok & McMahon, 2009). Occult HBV is defined by a negative HBsAg and positive HBV DNA and positive antibodies to HBc. HBsAg is detectable 6-10 weeks following exposure to the virus ; however modern Enzyme Immuno Assays

(EIA) have shortened the window period to approximately 9 days (F. Okoth et al., 2006) (Table 1).

Table 1: HBV serologic markers and their significance(CDC, 2010)

Marker	**Significance**
HBsAg	Antigen indicating infection
HBV DNA	Indicates active viral replication
Anti-HBc	Appears at the onset of symptoms of acute HBV infection. Indicates previous or ongoing infection in an undefined time frame.
HBeAg	Antigen correlating with HBV replication and infectivity
Anti-HBs	Indicates immunity

Chronic HBV infection is denoted by positive HBsAg, positive IgG anti-HBc, negative IgM anti-HBc and negative anti-HBs. On the other hand, acute HBV infection is denoted by positive HBsAg, positive IgG anti-HBc, positive IgM anti-HBc and negative anti-HBs.

2. **Molecular assays** include HBV DNA assays largely based on PCR techniques.
3. **Genotyping assays** identify specific genotypes of HBV and are used in epidemiological studies and in predicting the likelihood of treatment response. Genotyping of HBV has mapped out HBV infection regionally with the following distribution pattern observed:
    - Genotype A: Southern, Central and Eastern Africa
    - Genotype D: Northern Africa
    - Genotype E: Western Africa

HBV genotype B is associated with less active liver disease and a slower rate of progression to liver cirrhosis as compared to genotype C.

2.2.4 Natural history

The course of chronic HBV infection is considered to consist of 4 phases: immune tolerance, immune clearance (HBeAg-positive chronic hepatitis), inactive carrier, and reactivation (HBeAg-negative chronic hepatitis), although not all patients go through every phase (Figure 2).

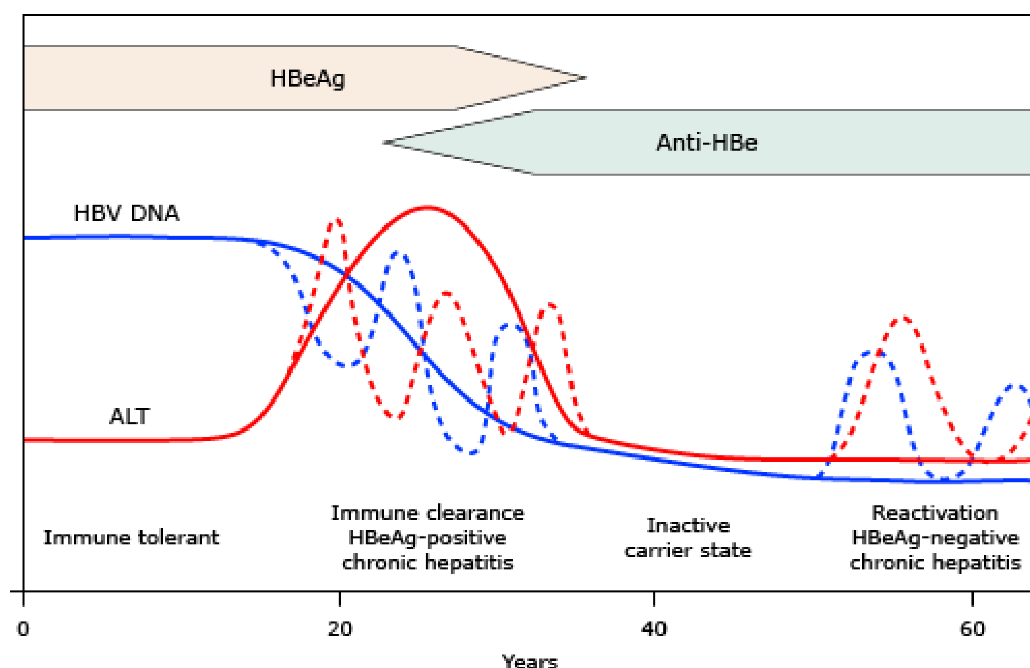

Figure 2: Natural history of chronic HBV infection (Lavanchy, 2004)

2.2.5 Risk stratification and disease progression

Risk of progression to chronic liver disease increases with decreasing age at infection such that the risk of HBV infection after acute exposure ranges from 90% in newborns of

HBeAg positive mothers to 25-30% in infants and children less than 5 years and 5 % in adults (Carey, 2009).

Complications are likely to be influenced by age, HBV genotype C, high levels of HBV DNA, habitual alcohol consumption, concurrent infection with HCV, HDV, or HIV, carcinogens like aflatoxins and cigarette smoking (Carey, 2009) (Figure 3).

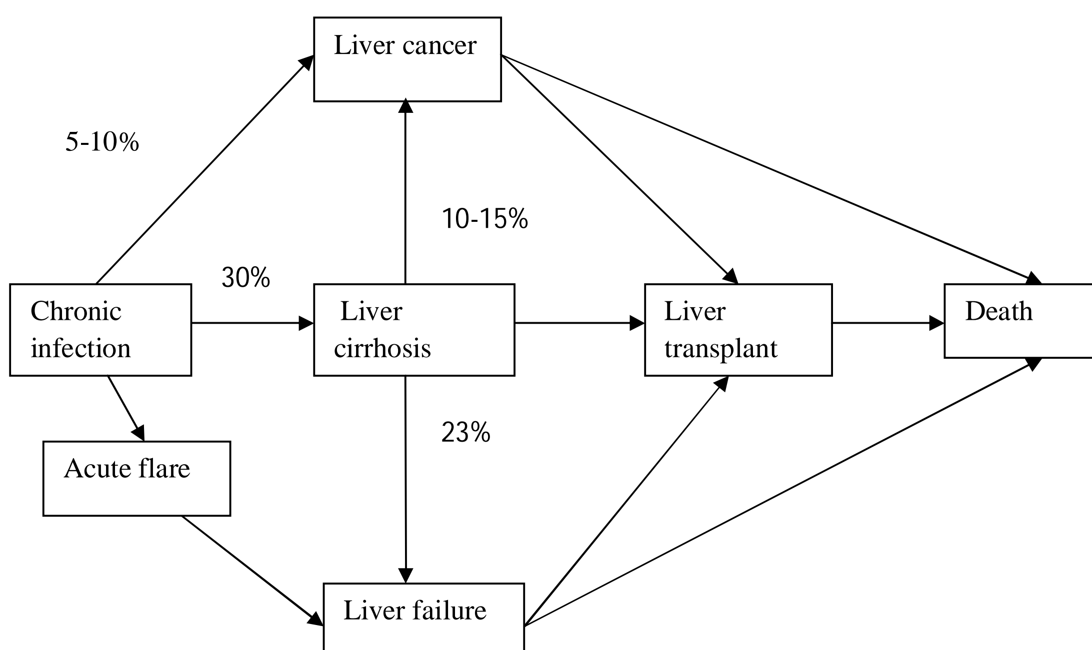

Figure 3: Schema showing the natural progression of chronic HBV infection (GSK, 2014)

2.2.6 Hepatitis B viral infection and chronic liver disease

Hepatitis B Virus is a widespread human pathogen that causes acute and persistent liver disease (Lotz et al., 2001). The spectrum of CLD associated with HBV include; chronic active hepatitis, liver cirrhosis and HCC. Epidemiological and experimental studies have demonstrated that chronic HBV infection is a major factor contributing to the development of primary liver cancer and liver cirrhosis (Geoffrey Zambezi Mutuma,

2011). A high HCC incidence has been demonstrated in regions with a high seroprevalence of HBV infection. In addition, patients with HCC show a 70-90% seroprevalence of HBV when compared to 10-20% HBV prevalence in the entire population in the same regions (Geoffrey Zambezi Mutuma, 2011).

Additionally a 10 to 100- fold risk of HCC has been observed in HBsAg carriers compared to non-carriers in different ethnic and social groups (Geoffrey Zambezi Mutuma, 2011). Studies have also demonstrated hepatocarcinogenesis associated with up-regulated expression of viral proteins (Geoffrey Zambezi Mutuma, 2011).

2.2.7 Treatment strategies

The treatment goals in patients with chronic HBV infection are to reduce the risk of progressive chronic liver disease, prevent transmission to others and to prevent long term complications such as cirrhosis and hepatocellular carcinoma. Complete eradication of HBV from host hepatocytes cannot be achieved because of the persistence of HBV covalently closed circular DNA (GSK, 2014).

Patients with chronic HBV infection are classified into two groups according to the HBeAg status to decide on therapy as below:

HBeAg positive; HBeAb negative- treat at HBV DNA > 20,000 IU/ml

HBeAg negative; HBeAb positive- treat at HBV DNA >2000 IU/ml

The recommended first line therapies for chronic HBV infection are pegylated interferon, entecavir and tenofovir but where there exists no contraindication, the drug of choice should be pegylated interferon (GSK, 2014).

2.2.8 Prevention strategies

1. Immunization of all at risk sero-negative individuals (Yamanaka et al., 1991)
2. Primary prevention in children by inclusion of HBV vaccine in the expanded programme of immunization.
3. Prevention of mother to child transmission by treating HBV-infected pregnant women and administration of hepatitis B immunoglobulin and hepatitis B vaccine to the newborn at birth(Suckling et al., 2006).
4. Treatment of HBV- infected individuals to reduce infectivity and transmission to sexual partners and close contacts (Lok & McMahon, 2009).
5. Condom use to reduce sexual spread.
6. Harm reduction strategies aimed at curtailing events that may prompt HBV infection for example discouraging intravenous drug use, scarification and tattooing.

2.2.9 Counseling

Patients should be counseled on transmission prevention, lifestyle modification and the importance of lifelong monitoring in patients with chronic hepatitis B viral infection.

Pregnant women should also inform their health care providers of their hepatitis B status so that hepatitis B immunoglobulin (HBIG) and hepatitis B vaccine can be administered to the newborn at birth (Lok & McMahon, 2009).

2.3 Hepatitis C viral infection

2.3.1 Introduction.

Hepatitis C virus is a single-stranded RNA virus in the lamiviridae family without any reverse-transcriptase activity (Lotz et al., 2001). There is evidence that HCV-RNA is integrated into the host genome.

The WHO estimated global prevalence of HCV infection was 3% or 170 million individuals in 2014(WHO, 2014). Sub-Saharan countries have the highest WHO estimated regional HCV prevalence (5.3%)(Madhava et al., 2002). HCV is a major cause of death due to liver disease and is among the leading indications for liver transplantation in the USA (Ghany, Strader, Thomas, & Seeff, 2009); 55-88% of individuals with acute hepatitis C infection will remain HCV- infected.

2.3.2 Modes of transmission and risk factors.

Hepatitis C viral infection is transmitted through blood and blood products and body fluids (Ghany et al., 2009; T. Muasya et al., 2008), and organ transplantation. Sexual transmission of HCV is low; however the rates of perinatal spread among HCV positive women who are also HIV positive is higher than in HIV negative women. This is attributed to the relatively higher HCV concentrations in cervico-vaginal fluids among HIV positive women (Minosse et al., 2006; Nowicki et al., 2005).

2.3.3 Screening

Persons at high risk who should be screened for HCV infection include intravenous injection drug users; persons with conditions with high predisposition for HCV infection like HIV infection, hemophiliacs and those who received factor transfusion prior to 1992; hemodialysis patients; persons with abnormally elevated aminotransferase (ALT) levels; children born to HCV positive mothers; health workers following needle stick injury or mucosal exposure to HCV positive blood and persons with tattoos and ritual scarification (Ghany et al., 2009).

2.3.4 Diagnosis

The optimal approach to detect HCV infection is to screen persons for a history of exposure to risk and then test these individuals for markers of HCV infection. Diagnostic approaches include laboratory tests whereby three classes of assays are used to diagnose hepatitis C, notably: (Forns & Costa, 2006)

1. **Serological assays** that detect specific antibodies to HCV. These are based on Enzyme Immunoassay techniques whose specificity is >99%. False positives are more likely when the testing is done in populations with low HCV prevalence and in severe immunosuppression. The window period between the acquisition of HCV and appearance of antibodies ranges from 3 to 8 weeks(Forns & Costa, 2006). The antibodies persist indefinitely but may decrease in patients who clear HCV either spontaneously or following antiviral therapy.
2. **Molecular assays** to detect viral nucleic acid. They can be either qualitative or quantitative assays with the former being more sensitive than the latter. The availability of real-time PCR assays (rt-PCR) and Transcription Mediated Amplification assays (TMA) have greatly improved sensitivities to greater than 10-50 IU/ml with specificity in the range of 98-99% (Ghany et al., 2009).
3. **Genotyping assays:** These identify specific genotypes of hepatitis C and are used in epidemiological studies and in predicting the likelihood of treatment response in addition to determining the optimal duration of treatment. Genotyping of HCV has mapped out HCV infection regionally, with the following distribution pattern observed: (Koziel & Peters, 2007; T. Muasya et al., 2008)
    - Genotype 1 and 3- predominant in Europe, Japan and USA.

- Genotype 4 and 5- predominant in Africa (Genotype 4 in Egypt and Congo, 5 in South Africa).
- Genotype 6- predominant in Asia.

Genotype 2 and 3 have a better treatment response, while genotypes 1 and 4 have a poor treatment response and require longer treatment durations of at least 1 year.

2.3.5 Natural history

Infection with HCV can result in both acute and chronic hepatitis. Acute infection is often asymptomatic and rarely causes hepatic failure. The risk of chronic infection after an acute episode of HCV infection is high; 60-80% of patients have persistently elevated liver enzymes.

The factors associated with fibrosis progression include fibrosis stage, age at infection (worse with advanced age than younger age), duration of infection, age at biopsy, and consumption of alcohol greater than 50g per day, HIV co-infection, male gender, high BMI and diabetes mellitus (GSK, 2014). Factors not associated with fibrosis progression include the level of the last serum viral load, mode of infection and liver viral load. Factors which have unclear association with fibrosis progression include inflammation, hemochromatosis, cigarette smoking, moderate alcohol consumption, genotype 3 infection and schistosomiasis (GSK, 2014).

2.3.6 Hepatitis C viral infection and chronic liver disease

Natural history studies have clearly demonstrated links between HCV infection and CLD (Lotz et al., 2001). Chronic HCV infection leads to a slowly progressive liver disease that over a period of up to 30 years may result in cirrhosis and perhaps HCC. Patients with more active and severe liver disease seem to be at higher risk of developing cancer(Lotz

et al., 2001). The mechanism of HCV infection causing HCC is still not exactly clear. Expression of viral proteins stimulates the host immune response and triggers liver inflammation. Cell death in the form of apoptosis is well known and recognized in association with HCV infection.(Lotz et al., 2001) Cycles of necroinflammatory lesions and hepatocellular regeneration might be prerequisites for genetic alterations resulting in malignant changes leading to HCC(Lotz et al., 2001).

2.3.7 Treatment strategies

All patients who are positive for HCV and have raised liver function tests should be offered treatment. The rationale for treatment in patients with chronic HCV is to reduce risk of progressive chronic liver disease, prevent transmission to others and prevent other long-term complications such as cirrhosis and HCC (GSK, 2014).

The first-line therapies for HCV are pegylated interferon-alpha 2A or pegylated interferon-alpha 2B. Many new drugs are in development; recently approved drugs include sofosbuvir and daclatasavir.

2.3.8 Prevention strategies

Currently, there is no vaccine to prevent hepatitis C viral infection. Preventive measures include(GSK, 2014):

1. Avoiding sharing of personal items like razors and tooth brushes.
2. Avoiding sharing of needles, syringes, water or equipment for intravenous drug use.
3. Following good health practice when tattooing or doing body piercings.
4. Screening of blood and blood products before transfusion.
5. Condom use to reduce sexual spread.

2.4 Summary of Literature Review

From the above literature review it is evident that hepatitis B and C viruses are well-recognized causes of chronic liver disease worldwide. However, there is paucity of published data locally regarding the relative contributions of these infections to chronic liver disease which would inform on care. Moreover, these viral infections are highly preventable. There is therefore need to establish the contributions of these infections to CLD locally to inform the setting and reviewing of prevention programmes.

CHAPTER THREE: PARTICIPANTS, MATERIALS AND METHODS

3.1 Study site

Participants were drawn from the adult medical wards, Medical Outpatient Clinic (MOPC), Liver and Oncology clinics of MTRH. MTRH is the second largest public hospital in Kenya. It is located in Eldoret town, Uasin-Gishu County in the western region of Kenya. It is a referral centre for most of the County hospitals in Western Kenya. Its catchment population is about 14 million people, approximately 40% of the total national population. MTRH is also the main teaching hospital for Moi University School of Medicine. The medical and oncology outpatient clinics are weekly clinics that offer care to adult patients with medical illnesses. These clinics receive most of the patients with medical conditions seen elsewhere, within the hospital, as well as referrals from other health institutions.

3.2 Study population

The study population was adult patients with suspected chronic liver disease.

3.3 Study design

This was a cross-sectional descriptive study

3.4 Eligibility

3.4.1 Inclusion criteria

1. Patients aged 18 years and above.
2. Patients diagnosed with chronic liver disease.

3.4.2 Exclusion criteria

1. Failure by patient to give consent to participate in the study.

2. Patients diagnosed with secondary liver cancer.

3.5 Study period and Selection of the study participants

A census was conducted where all patients diagnosed with chronic liver disease who presented between December 2013 and January 2015 were consecutively screened and enrolled if they met the eligibility criteria.

3.6 Study procedure

The figure below shows the study procedure.

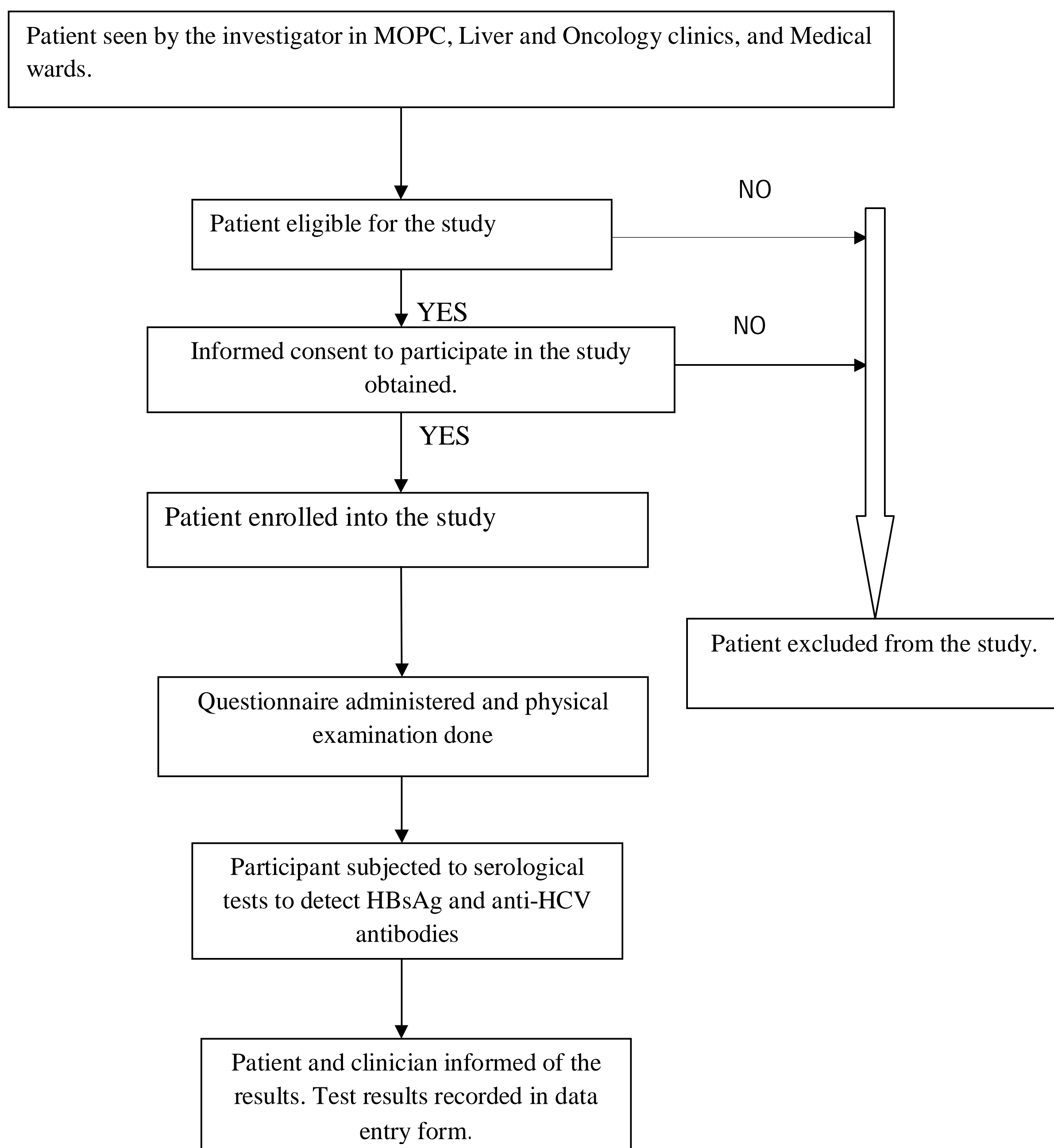

Figure 4: Algorithm showing the study procedure

3.7 Data collection and analysis

3.7.1 Data collection

Data was collected on a data entry form and later transferred to a computer database. Each entry form had a unique identifier, which is the participant number. Data collected included socio-demographic and risk characteristics of each participant and HBV and HCV serostatus. HIV serostatus of the study participants was obtained from their charts. Data was dually entered into Epidata software and validated.

3.7.2 Data analysis

Data analysis was done using STATA version 13 SE. Descriptive statistics (means and the corresponding standard deviation for normally distributed continuous variables and frequencies and the corresponding percentages and proportions and the corresponding 95% confidence limits for categorical variables) were used to summarize the data. Results were presented using tables.

3.8 Ethical considerations

Approval from IREC and permission from MTRH were obtained before the study commenced. Informed consent was sought from all participants before the procedure. For the illiterate patients, the researcher explained the contents of the consent form and if the patient consented a thumb print was used as an alternative to a signature. Participants were informed about the discomfort which may be experienced while drawing blood. They were also told that the procedures and tests were not associated with any significant risks. Participants were informed about the results and counseled accordingly depending on the outcome.

Tests and dissemination of results were done at no cost to the participants. Participants

were not given any inducements to participate in the study.

All patients participating in the study did so by free will and those who declined to participate were not discriminated against. All information from the study was handled confidentially. Only patients' codes were used in data entry form and no reference to their names was made. The interview was conducted in an environment that ensured privacy and confidentiality.

CHAPTER FOUR: RESULTS

4.1 Patient Enrolment

A total of 95 patients with suspected chronic liver disease were screened in the adult medical wards and medical outpatient clinics in MTRH between December 2013 and January 2015 of whom 84 participants were enrolled into the study (Figure 5).

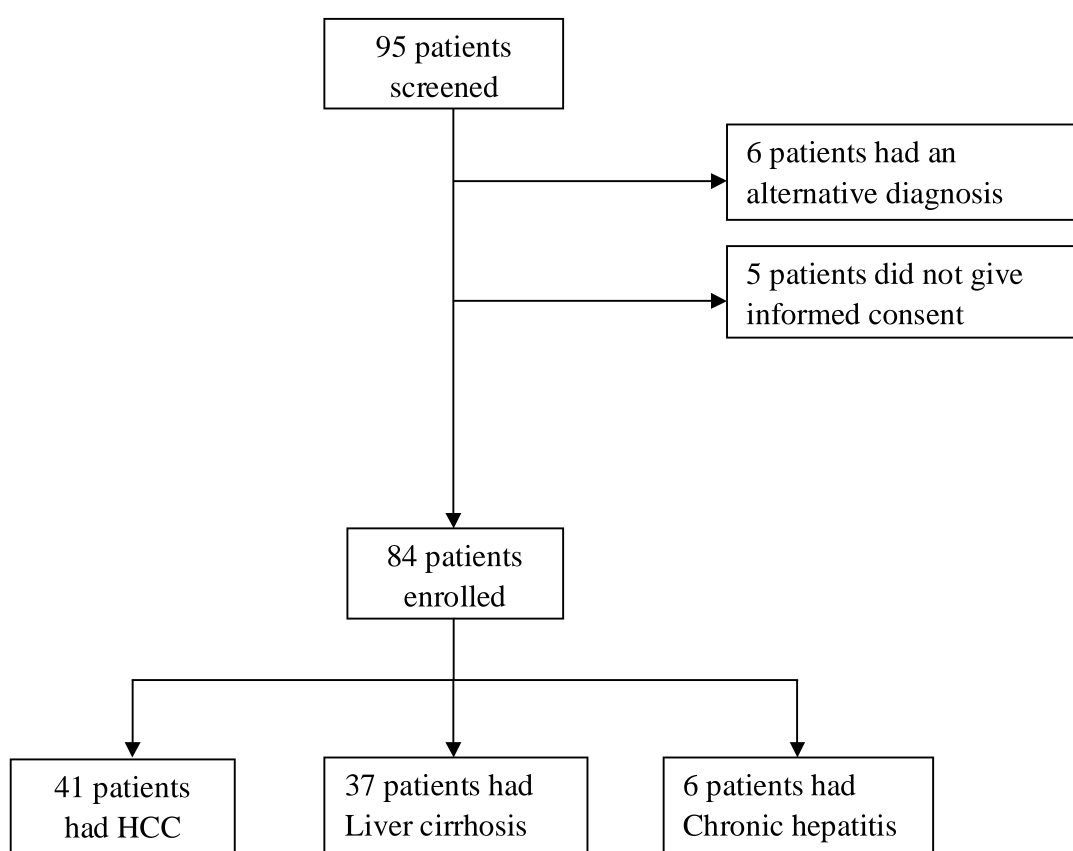

Figure 5: Schema showing patient Screening and Enrolment

4.2 Socio-demographic characteristics.

The mean age was 45 ± 14 years and the age ranged from 18 to 81 years. Majority of the participants 64 (76%), were male (Table 2).

Table 2: Socio-demographic characteristics of the study participants

Characteristic		**Mean (SD) or n (%)**
Age (yrs)		45 (14)
Residence	Rural	78 (93)
	Urban	6 (7)
Gender	Male	64 (76)
	Female	20 (24)
Highest level of education	None	5 (6)
	Primary	41 (49)
	Secondary	26 (31)
	Tertiary	12 (14)
Marital Status	Married	67 (79)
	Single	14 (17)
	Widowed	3 (4)
Harmful alcohol use		49 (58)

4.3 Prevalence of Hepatitis B and C viruses

The prevalence of hepatitis B virus was 44%. None of the participants was infected with hepatitis C virus.

4.4 Risk characteristics of the study participants

None of the participants had been vaccinated against Hepatitis B virus and more than half had undergone traditional circumcision. Among the study participants, none had history of intravenous drug use (Table 3).

Table 3: Risk Characteristics of the study participants

Characteristic	Mean (SD) or n (%)
Traditional circumcision	52 (62)
Intravenous drug use	0 (0)
History of blood transfusion	3 (4)
Scarification	12 (14)
Multiple sexual partners	15 (18)
Not immunized against HBV	84 (100)
History of imprisonment	3 (4)
Tattooing	0 (0)

4.5 HIV infection among the study participants.

The prevalence of HIV infection among the study participants was 17 %. HIV and HBV co-infection was found in 8% of the participants (Table 4).

Table 4: HIV infection among the study participants

HIV status	Study population n=84 (100%)	HBV-infected n=37 (100%)	HBV negative n=47 (100%)
Positive	14 (17)	3 (8)	11 (23)
Negative	70 (83)	34 (92)	36 (77)

CHAPTER FIVE: DISCUSSION

This study revealed that a younger population is affected by CLD locally compared to study findings from other regions. The mean age of the study participants in this study was 45 yrs. A study by Bukhtiari et al in Pakistan revealed that majority of CLD patients were in their 6th and 7th decade of life (Bukhtiari N, 2000). Possible explanation for this could be early childhood infection with the hepatitis viruses. The finding of a large proportion of participants having history of harmful alcohol use may mean a younger population misuse alcohol locally and develop CLD. The mean age was however comparable with earlier local studies (Ilako et al., 1995). In this study, majority (64%) of the participants were males. This can be attributed to the fact that males abuse alcohol more than females hence develop CLD as a consequence (Bloomfield, 2013).

The seroprevalence of HBV and HCV in this study was 44% and 0% respectively. Similar studies have yielded variable outcomes with a number of European, American and regional studies reporting inverse trends characterized by high HCV prevalence and low HBV prevalence (Perz et al., 2006). Some studies have however reported similar trends as were observed in this study (Geoffrey Zambezi Mutuma, 2011; Ilako et al., 1995).

In an earlier Kenyan study, McLigeyo et al at KNH in 1995 found a prevalence of 32% and 1% for HBV and HCV respectively. The prevalence of HBV and HCV infections in this study are comparable to earlier study findings. Despite the more than two decades gap with the KNH study, the prevalence of HBV is still high.

In a Pakistan study, Bukhtiari et al found a prevalence of 25% and 65% for HBV and HCV infections respectively and a HBV and HCV co-infection rate of 35%. These variations are due to geo-cultural and population related variations in the prevalence of hepatitis B and C (Forbi et al., 2007; Harania, Karuru, Nelson, & Stebbing, 2008; Ismail et al., 2009; T Muasya et al., 2008; Stevens et al., 2008).

None of the study participants had HCV infection or dual HBV and HCV co- infection. HCV infection is rare in this setting and dual HBV and HCV co- infection is even a rarer phenomenon; however, local and regional studies have documented this (Diop-Ndiaye et al., 2008; Forbi et al., 2007; Harania et al., 2008). Harania et al at Aga Khan University Hospital in Nairobi found a prevalence of 1.1% for HBV and HCV co-infection among HIV- infected patients (Harania et al., 2008). It is therefore not surprising that we did not establish HCV and dual infection in this study.

Population prevalence in other patient sub-groups other than patients with chronic liver disease indicated similar patterns of variation with lower prevalence (4.4- 16.9 %) for HBV (Lule et al., 1989; Ogutu, Amayo, Okoth, & Lule, 1990; Otedo et al., 2003; Stevens et al., 2008; Sutcliffe, Taha, Kumwenda, Taylor, & Liomba, 2002) and HCV prevalence of (0.9 – 22.2%) (Daikos et al., 1994; Ismail et al., 2009; T Muasya et al., 2008; Ndako et al., 2009; Stevens et al., 2008).

Biko et al found a prevalence of 9.7% and 1.6% for HBV and HCV respectively among HIV-infected patients at MTRH (Biko, 2010). Karoney et al found a prevalence and of 1.1% and 0.3% for HBV and HCV respectively among blood donors in Western Kenya (Karoney, 2014). It is not unusual that among different study populations, patients with

chronic liver disease have relatively higher prevalence of HBV as the virus is a well-recognized cause of CLD.

The high prevalence of Hepatitis B virus in this study may be attributed to the fact that none of the subjects had been immunized against Hepatitis B virus. HBV vaccination is protective for Hepatitis B viral infection and it was noted by Harania et al that no patient with prior Hepatitis B vaccination developed HBV infection (Harania et al., 2008). The lack of hepatitis B vaccination among the study participants may also be a reflection of low hepatitis B vaccine utilization in the general population. Possible reasons for lack of immunization against HBV include lack of awareness, high cost and unavailability of an effective vaccine in Kenya before the year 2000.

A significant proportion (62%) of the participants had been circumcised traditionally. This is a potential mode of HBV transmission due to the fact that traditional circumcisers may use the same instruments on several initiates and sterility may not be observed (Yamanaka et al., 1991). Other risk factors for hepatitis B viral infection considered in this study include low level of education, having multiple sexual partners, history of imprisonment, scarification and blood transfusion.

The finding of no HCV infection among the study participants can be attributed to the fact that potential modes of its transmission were reported in low frequencies with 14% of the participants having therapeutic scars and no reported cases of intravenous drug use or tattooing. Moreover, this can be attributed to low prevalence of HCV in the general population. The fact that blood in Kenya is screened before transfusion also lends

credence towards the unlikelihood of blood transfusion being a significant portal of Hepatitis C virus transmission (Ilako et al., 1995; Karoney, 2014; Karuru et al., 2005b).

A significant proportion (58%) of the participants had history of harmful alcohol use. This is not unusual because alcohol is a well recognized cause of chronic liver disease. Moreover alcohol abuse and concurrent HBV infection are synergistic in causing CLD and accelerating disease progression.

Majority of the participants (93%) had advanced chronic liver disease. This can be attributed to the health seeking behavior of patients of presenting late for care. Moreover, routine screening for HBV and HCV infections is not done hence late diagnosis and treatment of cases.

There was a high prevalence of HIV infection among the study population compared to the general population (NASCOP, 2014). This can be attributed to the similar modes of transmission of HIV and HBV. The HIV and HBV co-infection rate among the study participants is comparable to findings of earlier local studies (Harania et al., 2008). Biko et al in a study at MTRH found a HBV and HIV co-infection rate of 9.7% which is comparable to the findings of this study (Biko, 2010). This can be attributed to the fact that the study participants were drawn from a similar study site. Screening for HIV infection in HBV-infected patients is important because HIV co- infection hastens disease progression to CLD, lowers clearance of HBeAg, increases serum HBV DNA viral load, and is associated with increased liver injury and higher morbidity and mortality. Moreover, HIV co-infection influences the choice of HBV antiviral therapy (GSK, 2014).

Study Limitations

Abdominal ultrasounds were reported by different radiologists. Therefore the specific diagnoses may not have been accurate because of inter-observer variation in reporting.

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CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

There was a high prevalence of HBV infection among the study participants compared to findings in earlier local studies. All of the study participants had not been vaccinated against HBV.

None of the study participants had HCV infection.

6.2 Recommendation

Vaccination uptake should be scaled up to reduce HBV infection and its complications such as CLD.

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APPENDICES

Appendix I: Hepatitis B virus testing procedure.

Hepatitis B detection using ELISA kits to detect HBsAg in human serum and plasma(David et al., 1981; Fields, Davis, Bradley, & Maynard, 1983)

Manufacturer of Kit: ERBA Diagnostics Mannheim GmBh

Kit Name: ERBA LISA Hepatitis B

Introduction

The test kits detect HBsAg which is a set of lipoproteins of molecular weights ranging from 22kD to 96kD that constitute the envelope of the virus.

HBsAg is the first detectable marker in HBV infected serum and is detectable during the whole jaundice phase and becomes undetectable after the appearance of anti- HBsAg in serum.

ERBA LISA Hepatitis B use polyclonal antibodies to HBsAg as coating materials.

Principle

The test kit is a solid phase immunoassay for the qualitative detection of HBsAg in human serum and plasma.

The addition of positive control or HBsAg containing serum or plasma will form a stable complex with the bound antibody present in the well and with anti-HBsAg-HRPO.

A washing step will remove the unbound conjugate molecule.

Addition of color reagent will develop blue in positive control wells and in wells containing HBsAg in test specimen.

Upon addition of a stopping solution the blue changes to Yellow, and the intensity of the yellow is directly proportional to the presence of unbound HBsAg in the respective wells.

Contents of the kit

- Anti- HBsAg coated plate s
- Conjugate
- HBsAg positive control
- HBsAg negative control
- Colour reagent
- Sample diluent E
- Stopping solution
- Washing solution
- Washing solution D(20X) concentrate
- Black cover
- Adhesive strips

Additional materials required:

1. 0 to 20 and 50 to 200 microliter micropipettes and disposable tips
2. Automatic micropipette washing instrument
3. Precision ELISA reader
4. Disposable gloves
5. Timer
6. Measuring cylinder 500ml\

Storage

- Store at 2-8^{0}c
- Immediately after use return the reagents at 2-8^{0}c

Precautions

- Use disposable gloves throughout the procedure
- The test is for in vitro diagnostic use only
- Treat all serum as potentially infectious material
- Prior to disposal collect all waste and keep in 5% sodium hypochlorite solution for 30 minutes
- Don't use expired kits
- Don't interchange reagents between different lots

- Use clear serum- remove particulate matter by centrifuging
- Use separate tips for controls and individual test specimens
- Don't expose colour reagents to sunlight
- Use distilled or deionised water for dilution of the washing buffer
- After using the required strips the rest of the strips along with the activated silica gel should be kept in a sealed condition in the polythene zip lock bag

Collection and storage

- All test specimens should be handled as potentially biohazardous
- Early separation of serum from the clot prevent haemolysis of serum
- Use aseptic techniques to collect serum
- Store undiluted serum at 2-8^{0}c
- Frozen specimen should be thawed properly

Microplate washing procedure

- Dilute the washing solution in the ratio 1:20 with distilled or deionised water
- The washing solution may be crystallized at cool storage conditions. If so then use after thawing at 37^{0}c in a water bath
- At least 6 cycles with at least 0.35ml wash buffer per well per wash and a soak time of 30 seconds are recommended

- The plate should be inverted and tapped on an absorbent pad to remove the remaining washing solution

Test procedure

- Bring all test reagents and test specimens to room temperature before use.
- Add 50 microlitres of sample diluent to each well and in each run maintain 1 blank (100 microlitre sample diluent +50 microliter of conjugate), 3 negative controls and 1 positive control.
- Add 50 microlitres of control and test specimens to the respective wells.
- Add 50 microlitres of conjugate to each well.
- Cover the plate with a black cover and incubate for 60 minutes at 20-37^{0}c
- Wash the plate as per microplate washing procedure.
- Add 50 microlitre of colour reagent.Cover the plate with a black cover and incubate for 15 minutes in the dark at 20-30°C.
- Add 100 microlitres of stopping buffer to each well
- Read the absorbency at 450nm. Deduct the blank absorbency from the control and test wells

Calculation for cut off value determination

Blank value: Absorbency of the blank value should be <0.2

Positive control: Absorbency of individual positive control should be>1.0

Negative control : Absorbency of individual negative control should be <0.1

NCx= Average of the negative controls

Cut off value formula = 0.1 +NCx

Interpretation of results

Non reactive: If the absorbency of the test serum is less than the cut off value.

Reactive: If the absorbency of the test serum is equal to or greater than the cut off value, then it is considered as initial reactive.

The sample should be retested as duplicate. If the absorbency of the retest is less than the cut off value then the sample is considered to be non reactive.

If both the duplicate retest results are reactive then the specimen is repeatedly reactive.

Appendix II: Hepatitis C virus testing procedure

Hepatitis C detection using ELISA kits to detect anti-HC in human serum and plasma(Alter & Prince, 1988; Choo et al., 1989; Dawson et al., 1991; Dienstag, 1983; Kuo et al., 1989; Uyttendaele, Claeys, Mertens, Verhaert, & Vermylen, 1994)

Manufacturer of Kit: ERBA Diagnostics Mannheim GmBh

Kit Name: ERBA LISA Hepatitis C

ERBA LISA Hepatitis C: Uses synthetic peptides and recombinant proteins of Hepatitis C as coating materials.

Principle

The test kit is a solid phase immunoassay, utilizing a mixture of synthetic peptides and recombinant proteins of HCV i.e CORE, NS3, NS4 and NS5 for the detection of HCV antibodies present in human serum and plasma. When human serum is added to the well the bound antigen present in the well will form a stable complex with the anti-HCV present in the test or positive control specimen. After washing, anti human IgG–HRPO is added to the wells and only the bound antigen-antibody complex present in the well will react with the conjugate molecule. A second washing step will remove unbound conjugate molecule. Addition of color reagent will develop color only in positive control wells and wells containing anti-HCV in test specimen. The intensity of development of color is directly proportional to the presence of bound anti-HCV in the respective wells

Contents of the kit

- HCV antigen coated plate
- Conjugate

- Anti HCV positive control
- HCV negative control
- Color reagent
- Sample diluent
- Stopping solution
- Washing solution
- Washing solution D(20X) concentrate
- Black cover
- Adhesive strips

Additional materials required

1. 0 to 20 and 50 to 200 microlitre micropipettes and disposable tips
2. Automatic micropipette washing instrument
3. Precision ELISA reader
4. Disposable gloves
5. Timer
6. Measuring cylinder 500ml

Storage

- Store at 2-8^{0}c
- Immediately after use return the reagents at 2-8^{0}c

Precautions

- Use disposable gloves throughout the procedure
- The test is for in vitro diagnostic use only
- Treat all serum as potentially infectious material

- Prior to disposal collect all waste and keep in 5% sodium hypochlorite solution for 30 minutes
- Don't use expired kits
- Don't interchange reagents between different lots
- Use clear serum-remove particulate matter by centrifuging
- Use separate tips for controls and individual test specimens
- Don't expose color reagents to sunlight
- Use distilled or deionised water for dilution of the washing buffer
- After using the required strips the rest of the strips along with the activated silica gel should be kept in sealed condition into the polythene zip lock bag

Specimen colletion and storage

- All test specimens should be handled as potentially bioharzardous
- Early separation of serum from the clot prevents haemolysis of serum
- Use aseptic techniques to collect serum
- Store undiluted serum at 2-8^{0}c
- Frozen specimen should be thawed properly

Microplate washing procedure

- Dilute the washing solution in the ratio 1:20 with distilled or deionised water
- The washing solution may be crystallized at cool storage conditions. If so then use after thawing at 37^{0}c in a water bath
- At least 6 cycles with at least 0.35ml wash buffer per well per wash and a soak time of 30 seconds are recommended

- The plate should be inverted and tapped on an absorbent pad to remove the remaining washing solution

Test procedure

- Bring all test reagents and test specimens to room temperature before use
- Except for the blank add 100 microlitre of sample diluent F to each well and in each run maintain 1 blank (100 microlitre sample dilutent+ 50 microlitre of conjugate), 3 negative controls and 1 positive control.
- Add 10 microlitres of control and test specimens to the respective wells.
- Mix with a pipette and cover the plate with a black cover then incubate for 45 minutes at 20-30°C.
- Wash the plate as per micro plate washing procedure.
- Add 50 microlitres of the conjugate to each well except the blank. Cover the plate with a black cover and incubate for 15 minutes at 20-30^{0}C.
- Wash the plate as per the microplate washing procedure.
- Add 50 microlitres of colour regent. Cover the plate with a black cover and incubate for 15 minutes in the dark at 20-30°C
- Add 100 microlitres of stopping buffer to each well.
- Read the absorbency at 450nm. Deduct the blank absorbency from the control and test wells.

Calculation for cut off value determination

Blank value:Absorbency of the blank value should be <0.2.

Positive control: absorbency of individual positive control should be >0.1.

Negative control: Absorbency of individual negative control should be < 0.1

NCx= Average of the negative controls.

Cut off value formula= 0.1 + NCx.

Interpretation of results

Non reactive: if the absorbency of the test serum is less than the cut off value.

Reactive: if the absorbency of the test serum is equal to or greater than the cut off value, then it is considered as initially reactive.

The sample should be retested as duplicate. If the absorbency of the retest is less than the cut off value then the sample is considered to be non- reactive.

If both the duplicate retest results are reactive then the specimen is repeatedly reactive.

Appendix III: Consent form (English version)

Sero-prevalence of Hepatitis B and C viruses among patients with Chronic Liver Disease at Moi Teaching and Referral Hospital, Eldoret, Kenya

I am **Dr. Robert Kibet Yatich,** a post graduate student in Internal Medicine at Moi University. I am inviting you to participate in a study on the seroprevalence of Hepatitis B and Hepatitis C. Chronic viral hepatitis is a major cause of liver disease and its prevalence in patients with chronic liver disease at MTRH is presently unknown. It is therefore my intention, through your participation, to determine this as part of my postgraduate thesis.

The study will include the administration of a questionnaire, physical examination, and drawing of blood. Through this, I will be able to confirm or rule out the presence of Hepatitis B or C virus in your blood and you will subsequently be informed of the results.

It is however worth noting that other than slight pain during the blood drawing no harm will be inflicted on you, confidentiality will be observed and there will be no attraction (financial or otherwise) for participating in this study. You will benefit from the study by knowing your Hepatitis B and C status; and in the event that you are positive then your family members will be advised on immunization for Hepatitis B and on the safety measures to observe.

Are there any questions you may wish to ask at this point in time?

Do you therefore accept to participate in this study?

(If the respondent accepts participation then they will append their signature or thumb print below. If he/she declines then thank them for their patience, cooperation and audience).

Signature/ Thumb print of client/ relative/guardian:

…………………………………………………………………………………………

Signature of investigator:…………………………………………………………..

Date…………………………………………………………………………………..

Appendix IV: Consent form (Kiswahili version)

Utafiti wa kubaini kiwango cha virusi vya Hepatitis B na C kati ya wagonjwa wanougua ugonjwa wa maini na ambao wanatibiwa katika hospitali ya mafunzo na rufaa ya Moi, mjini Eldoret, Kenya.

Mimi ni Daktari Robert Kibet Yatich, na mimi ni mwanafunzi wa somo la uuguzi katika chuo kikuu cha Moi. Ninakualika kuhudhuria kikao cha utafiti uliobainishwa hapo awali. Hepatitis ni moja ya magonjwa makubwa yanayodhuru maini na kwa sasa kiasi cha ugonjwa huu kati ya wagonjwa wetu wanaohudumiwa katika hospitali ya MTRH hakichabainishwa; kwa hivyo ni lengo langu kufafanua hili swali hapa kwetu na nitahitaji msaada wako.

Utafiti huu utafuatilia maadili hayo- utaulizwa maswali kadha kulingana na orodha tulionayo, utapimwa mwili kisha utatolewa kiasi kidogo cha damu ambayo itapimwa kwenye maabara. Baadaye utaelezewa matokeo ya kipimo ya damu.

Ni vyema kuelewa kwamba utahisi uchungu kidogo wakati damu itakapotolewa, hata hivyo hakuna madhara yatakayotokea kuhusiana na tukio hilo; vilevile hadhi yako na siri zako zitalindwa kabisa. Hakuna manufaa ya kifedha utayopata kwa kutendakazi katika utafiti huu lakini utaweza kujua hali yako ya virusi vya Hepatitis B na C na ikiwa tutapata virusi hivi kwenye dama yako; jamii yako itaarifiwa na tahadhari pia itasisitizwa.

Kwa wakati huu uko na maswali yoyote ambayo ungetaka nijibu?

Je, unakubali kuorodheshwa katika huu utafiti?

(Ikiwa mgonjwa anakubali, tafadhali aidhinishe kwa sahihi yake ama alama ya kidole cha gumba kwenye nafasi iliyoko chini na ikiwa amekataa basi endelea na huduma yake ya afya vile ilivyo tarajiwa).

Sahihi ama alama ya kidole ya mgonjwa ama mlinzi aliye naye

…………………………………………………………………………………………………

Sahihi ya daktari anayehoji mgonjwa.

…………………………………………………………………………………………………

Tarehe:………………………………………………………………………………………

Appendix V: Questionnaire

Sero-prevalence of Hepatitis B and C viruses among patients with Chronic Liver Disease at Moi Teaching and Referral Hospital, Eldoret ,Kenya.

Administration time ~ 15-20 minutes

Outpatient/Inpatient identification no ___________ Date:_____/____/_______

dd mm yy

1. Demographic information

1.1 Sex: ☐ Male ☐ Female

1.2Age: _________ years ***(must be >18yrs)***

1.3 patient's current residence________

1.4 Occupation____________

1.5 level of education: ☐ primary school ☐ secondary school

☐ Tertiary education others____________

1.6 Marital status ☐ Polygamous ☐ married (one wife)

☐ Single ☐ Widowed

2.Social history

2.1 Have you ever used intravenous drugs of abuse? ☐ Yes ☐No ☐ still using

2.1.1 If still using intravenous drugs; then specify

2.2 Have you ever been imprisoned? ☐ Yes ☐ No

2.3 Are you circumcised? ☐Yes ☐ No

2.3.1 If yes to 2.3 above then how was it conducted? ☐ Traditionally ☐ In hospital

2.4 Do you have any history of unsafe or unwarranted injections? ☐ Yes ☐No

2.5 Do you have any tattoos on your body? ☐ Yes ☐ No

2.6 Have you engaged in any sexual activity over the past 6 months? ☐ Yes ☐No

2.6.1 If yes to 2.6 above then how many other sexual partners have you had in the past 6 months other than your usual sexual partner?

2.7 Have you ever used condoms? ☐ Yes ☐ No

2.8 Do you consume alcohol: ☐ Yes No ☐ (If YES, please go to AUDIT questionnaire)

2.8.1 If yes to the above what brands of alcohol do you consume ☐ Bottled wine and spirits ☐ local brew (chang`aa/ busaa)
Bottled beer ☐

3. Treatment history

3.1Have you been immunized against hepatitis B virus? ☐ Yes ☐ No

3.1.1 If yes to 3.1 above then how long ago were you immunized?

3.2 Have you ever been transfused blood?

☐ Yes ☐ No

4. Physical examination *(Relevant findings)*

4.1 Is the patient jaundiced? ☐ Yes ☐ No

4.2 Does the patient have ascites? ☐ Yes ☐ No

4.3 Does the patient have hepatomegaly? ☐ Yes ☐ No

4.5 Does the patient have flapping tremors, palma erythema or finger clubbing?

☐ Yes ☐ No

4.6 Has the patient lost axillary or pubic hair? ☐ Yes ☐ No

4.7 Does the patient have spider naevi or caput medusa?

4.8 Are there any scars in the patient's body? ☐ Yes ☐ No

4.81 If yes in 4.8 above, then are they therapeutic scars ☐ yes ☐ No

5. HIV status: ☐ +ve ☐ -ve

6. Samples

6.1 Sample ID No:____________ *(Use IP/OP No)*

6.2 Results: HBs Ag: ☐+ve ☐ -ve

Anti-HCV: ☐ +ve ☐ -ve

7. Verification details

Questionnaire administered by __________________________*(Initials)*

Appendix VI: Alcohol Use Disorders Identification Tool (AUDIT) questionnaire: Screen for alcohol misuse(Saunders, Aasland, Babor, de la Fuente, & Grant, 1993)

Please circle the answer that is correct for you

1. How often do you have a drink containing alcohol?

• Never

• Monthly or less

• 2−4 times a month

• 2 − 3 times a week

• 4 or more times a week

2. How many standard drinks containing alcohol do you have on a typical day when drinking?

• 1 or 2

•3 or 4

•5 or 6

•7 to 9

•10 or more

3. How often do you have six or more drinks on one occasion?

•Never

•Less than monthly

•Monthly

•Weekly

•Daily or almost daily

4. During the past year, how often have you found that you were not able to stop

drinking once you had started?

•Never

•Less than monthly

•Monthly

•Weekly

•Daily or almost daily

5. During the past year, how often have you failed to do what was normally expected of you because of drinking?

•Never

•Less than monthly

•Monthly

•Weekly

•Daily or almost daily

6. During the past year, how often have you needed a drink in the morning to get yourself going after a heavy drinking session?

•Never

•Less than monthly

•Monthly

•Weekly

•Daily or almost daily

7. During the past year, how often have you had a feeling of guilt or remorse after drinking?

•Never

•Less than monthly

•Monthly

•Weekly

•Daily or almost daily

8. During the past year, have you been unable to remember what happened the night before because you had been drinking?

•Never

•Less than monthly

•Monthly

•Weekly

•Daily or almost daily

9. Have you or someone else been injured as a result of your drinking?

•No

•Yes, but not in the past year

•Yes, during the past year

10. Has a relative or friend, doctor or other health worker been concerned about your drinking or suggested you cut down?

•No

•Yes, but not in the past year

•Yes, during the past year

Scoring the AUDIT

Scores for each question range from 0 to 4, with the first response for each question (eg never) scoring 0, the second (eg less than monthly) scoring 1, the third (eg

monthly) scoring 2, the fourth (eg weekly) scoring 3, and the last response (eg. daily or almost daily) scoring 4. For questions 9 and 10, which only have three responses, the scoring is 0, 2 and 4 (from left to right).

A score of 8 or more is associated with harmful or hazardous drinking, a score of 13 or more in women, and 15 or more in men, is likely to indicate alcohol dependence

Appendix VII: IREC approval to conduct the study

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2013/146
Approval Number: 0001064

19th September, 2013

Dr. Robert Kibet Yatich,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Dr. Yatich,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee have reviewed your research proposal titled:-

"Sero-Prevalence of Hepatitis B and C Viruses among Patients with Chronic Liver Disease at Moi Teaching and Referral Hospital".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1064*** on 18th September, 2013. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 17th September, 2014. 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,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc	Director - MTRH	Dean - SOM	Dean - SON
	Principal - CHS	Dean - SPH	Dean - SOD

Appendix VIII: Permission from MTRH to conduct the study

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
Ref: ELD/MTRH/R.6/VOL.II/2008

P. O. Box 3
ELDORET

19th September, 2013

Dr. Robert Kibet Yatich,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

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

"Sero-Prevalence of Hepatitis B and C Viruses among Patients with Chronic Liver Disease at Moi Teaching and Referral Hospital".

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

DR. J. KIBOSIA
DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM