PREVALENCE AND MOLECULAR CHARACTERIZATION OF HEPATITIS C INFECTIONS AMONG INJECTING DRUG USERS IN NAIROBI, KENYA

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(Infectious Diseases and Vaccinology)

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

2022

Prevalence and Molecular Characterization of Hepatitis C Infections among Injecting Drug Users in Nairobi, Kenya

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A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Infectious Diseases and Vaccinology of the Jomo Kenyatta University of Agriculture and Technology

2022

DECLARATION

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

Signature: ..... Date: .....

Alex Kattam Maiyo

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

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JKUAT, Kenya

DEDICATION

I would like to dedicate this work to my family. Most sincerely to my wife Nelly Maiyo for her care, support, and understanding in all possible ways, including having to shoulder most family responsibilities while I was away, allowing me to concentrate on my MSC research work. To my loving children: Joan Jepkorir, Duncan Kibet, Danson Kipkirui and Joy Cherotich for bearing with my chronic absence from home including when they needed me the most. To my father Simeon Kattam for encouragement and prayers and finally to my brother Dr. Geoffrey Maiyo for his support and encouragement throughout my academic life.

ACKNOWLEDGEMENT

I owe my deepest gratitude to the following persons and institutions for the role they played in the culmination of this thesis. I would like to thank Prof. Elijah Songok of KEMRI and Dr. Eddy Odari of JKUAT, for their unwavering supervision and support in taking me through the stages that led to the completion of this thesis. Your support, encouragement, and guidance enabled me to develop an understanding of the field of this research subject. I found your friendship and support invaluable on both academic and personal levels, for which I am extremely grateful. To Prof. E. Songok, I will always be very grateful to you for your financial support and for providing additional support on research resources that enabled the full implementation of this dissertation research. To Dr. E. Odari I feel so much indebted to you for the great institutional support. Thank you for your brotherly tireless guidance in my academics and research.

My sincere gratitude also goes to the Joyceline Kinyu; members of the KEMRI Hepatitis lab specifically; James Gikunda, Vincent Ruto, Robert Owira, and the KEMRI production department for their support throughout the project.

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ACRONYMS AND APPRECIATIONS

AASLD	American association for study of liver diseases
BLAST	Basic Local Alignment Search Tool
cDNA	Complimentary deoxyribonucleic acid
CVR	Centre for virus research
DAAS	Direct acting antiviral agents
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
EMA	European medical agency
ERC	Ethical review committee
FDA	Food and drug administration
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IDU	Injection drug users
IFN	Interferon
IAS	International antiviral society
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
LPC	Low positive control

MEGA	Molecular Evolutionary Genetics Analysis
MUSCLE	Multiple Sequence Comparison by Log- Expectation
NACADA	National authority for the campaign against alcohol and drug abuse
PWID	People who inject drugs
RNA	Ribonucleic acid
TAE	Tris base acetic acid and EDTA
USA	United states of America
UL	Microliter
UNODC	United Nations office on drugs and crime
WHO	World health organization

ABSTRACT

People who inject drugs (PWIDs) are increasingly becoming a public health concern in various parts of the world. It is estimated that between 11 and 21.1 million people inject drugs worldwide. This group of people is therefore considered a high-risk group mainly for Hepatitis C virus transmission since they act as a bridge of infection to the general population. Epidemiologically, the seven recognized genotypes (1-7) of HCV exhibits high genetic diversity, characterized by regional variations in genotype, posing a challenge to vaccine development and HCV treatment which in many countries is still genotype-specific. This cross-sectional study aimed to determine the prevalence of Hepatitis C and the circulating genotypes among PWIDs in Nairobi County. The snowball sampling technique was used to recruit 212 participants from two drop-in centers in Nairobi who consented to participate in the study. Up to 5 mL of blood was collected. The blood samples were screened using Bio-Elisa HCV 4.0 kit to determine the serostatus of the samples. Sero-positive samples were subjected to molecular analysis by amplifying the nucleic acids using PCR, where detecting the desired region was done by gel electrophoresis and finally sequencing analysis to determine the genetic diversity of the circulating strains. Socio-demographic characteristics, including individual parameters such as age, gender, and behavior on needle use, were determined using a questionnaire that was administered during recruitment. Summaries on socio-demographics were presented using descriptive statistics and multi multivariate analysis was performed to determine the association between the socio-demographic characteristics and the presence of anti-HCV in the selected population ($P< 0.05$ was considered significant). The Neighbor-Joining method on the MEGA6 programmer was used to generate a bootstrap consensus phylogenetic tree out of 1000 bootstrap replicates. For results, a total of 212 PWID were successfully recruited for the study, out of whom 29(13.7%) tested seropositive, with males accounting for 72.4% (n=21) and females were 27.6% (n=8). Age (OR=15; P=0.09), duration of injecting drugs (OR= 11.38; P=0.001), and the frequency of injecting drugs (OR=0.28; P=0.042) were found to be significantly associated with HCV infection. Of the 29 seropositive samples, 27(93.1%) were PCR-positive and were used for genotypic identification. The HCV circulating strains detected were genotype 1 at 51.8% (n=14), genotype 4 at 14.8% (n= 4), genotype 6 at 14.8% (n=4) genotype 5 at 7.4% (n=2), genotype 3 at 3.7% (n=1) and undefined genotypes at 7.4% (n=2). The study concludes that there is a potential emergence of circulation of foreign strains in Nairobi demonstrated by the identification of genotypes 5 and 6 which are not known to circulate in the region and the presence of the 2 unidentified genotypes. This calls for a change of treatment strategy from genotype-specific therapy to pangenotype therapy. The study recommends further genotype characterization with a larger number of samples to establish the magnitude of circulation of the genotypes considered not found in the region. These will influence policy on treatment strategy for HCV infection which is considered genotype-specific.

CHAPTER ONE

INTRODUCTION

1.1 Background information

An estimate of 270 million people used various types of drugs worldwide as reported in 2018, while over 35 million people suffer from drug use disorders, according to the United Nations office on drugs and crime (UNODC). Among the most used substances include; cannabis, cocaine, nicotine, alcohol, and the opioids (heroin, morphine, codeine, fentanyl, methadone, tramadol among others) though some of these substances are legal and others under prescription the have addictive potential to regular uses (Merz, 2018)

It is estimated that 35 million people suffer from drug abuse each year worldwide. In the year 2017 over 750,000 deaths were related to drug use. More than 70% of these deaths were associated with opioids abuse, with more than 30% of those deaths caused being a result of overdose (WHO 2019)(Organization).

Africa is believed to be a major route of drug trafficking and consumption of illegal drugs worldwide. The united nation estimates there are over 28 million drug users in Africa. Cannabis is the most widely used illicit substance in African. Some of the other most abused drugs include; Alcohol, nicotine, marijuana, nyope whoonga, heroin, methcathinone, Ritalin, mandrax, amphetamines, cocaine, methamphetamine, alprazolam, hydrocodone, benzodiazepines, oxycodone, methadone, clonazepam, propoxyphene, lorazepam, carisoprodol, diazepam, and trazodone. West and Central Africa are the most prevalent with rates between 5.2% and 13.5% (Peacock et al., 2018). Heroin consumption is mainly along the East African coast (in Kenya, Mauritius, Seychelles, and the United Republic of Tanzania). These region remains a significant destination and transit area point for heroin in Africa, heroin use has continued to grow notably in Comoros, Madagascar, Tanzania, Mauritius, Mozambique, South Africa, Lesotho, Kenya, and Senegal (Union, 2019).

In Kenya alcohol and drug abuse is recognized as a major threat to life and national development. According to the national authority for the campaign against alcohol and drug abuse (NACADA), the most commonly abused drugs and substances in Kenya are alcohol, tobacco, cannabis (bhang), glue, miraa (Khat,) and psychotropic substances (Kamenderi et al., 2021).

Injecting drug use (IDU) is increasingly becoming a public health concern in various parts of the world. It is estimated that between 11 and 21.1 million people might be injecting drug users worldwide (Mathers et al., 2008). China, the United States, the Russian Federation, and Brazil are estimated to have the largest populations of IDUs (Ruta & Cernescu, 2015). In 2008, injecting drug use was reported in 148 countries (Mathers et al., 2008) Of them the prevalence of injecting drug use ranged from 0.02% in India and Cambodia to 5.21% in Azerbaijan (Degenhardt et al., 2013; Gowing et al., 2015). Injecting drug users are high-risk population for infectious diseases such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and Hepatitis C virus (HCV) as well as blood-borne disease. An estimate of 6.1 million of people who inject drugs (PWIDs) are infected with HCV worldwide (Day et al., 2019; Nelson et al., 2011). In sub Saharan Africa injecting drug use is on the rise, though its' statistical burden has not been well established, Kenya, Nigeria, South Africa and Tanzania are among the countries who have reported HCV cases in injecting drug uses (Asher, Hahn, Couture, Maher, & Page, 2013).

Injecting drugs use in Kenya is rapidly increasing, with estimates suggesting there are over 18,000 people injecting heroin, with a human immunodeficiency virus (HIV) with men accounting for 18 % while women account for 44 % (Kenya, 2014)

The coastal region in Kenya has been the central point of most drug trafficking and use, with over 40 years' experience of drug trafficking. Studies on injecting drug use in these have shown an increase in blood bone viruses including Hepatitis B, Human immunodeficiency virus (HIV) and Hepatitis C. Prevalence of HCV among people who inject drugs (PWID) has been estimated to be 16.4% while the co-infection is 17.9% (Mwatelah et al., 2015).

1.1.1 Effects of the infection

Infecting drug use (IDU) is the most commonly reported HCV transmission route in Europe. In US Almost half of the 590000 people aged 18-29 years who reported to be IDUs have been found to be infected by HCV (Armstrong et al., 2006; Page, Morris, Hahn, Maher, & Prins, 2013). The sero-prevalence of HCV in people practicing injecting drug users for more than 3 decades is as high as 98.7% (Tseng et al., 2017). This infection account for 33.3% of acute and 83.7of % of chronic hepatitis C cases in European countries despite of there well establishment medical setup (Esteban, Sauleda, & Quer, 2018; Muhlberger et al., 2018).

1.2 Statement of the Problem

It is estimated that more than 180 million persons are chronically infected with hepatitis C virus worldwide. And an additional 3-4 million persons are estimated to be newly infected each year(Harnois, 2012) in areas where it is endemic it occurs as co-infections with human immunodeficiency virus (HIV) and hepatitis B virus (HBV). The co-infection modifies the progression of each infection. In Sub-Sahara Africa there is limited information on HCV infection. In Kenya, most of the studies done have been carried out on the co-infection of HCV among HIV patients (Harania, Karuru, Nelson, & Stebbing, 2008; Muriuki, Gicheru, Wachira, Nyamache, & Khamadi, 2013; Mwatelah et al., 2015) Information on infection among PWID is scarce and centered mainly from the coastal region of Kenya, yet the practice of drug use is common in various parts of the country including Nairobi. The sharing of needle is a common practice among PWIDs as they share the drugs, yet this is one of the route of hepatitis C virus infection (Tassiopoulos, Bernstein, & Bernstein, 2013).

World health organization (WHO) recommends use of pangenotypic direct-acting antiviral drugs (DAAs) which are very expensive and beyond rich for developing and middle income countries. These has made them to continue depending on genotype specific interferon combined therapy. Genotype therapy require constant genotyping assessment before prescription of drugs and these is expensive to individual facilities

who may have to handle a very small number of patients as a result of prevalence of HCV in Kenya being below 1%. Injecting drug use is legally and socially unacceptable leading to secrecy of the act and enhancing silent transmission to the general population.

1.3 Justification

Kenya being a middle income country is still using genotype specific regiments, for the treatment and management of HCV patients. These require the need to continuously document circulating genotypes to guide on effective management of the disease. People who inject drugs is a population that has been documented as a highly infected with HCV, jet they keep interacting with the general population unnoticeably. They therefore acts as a bridge of infection of HCV to the general population and thus documenting the burden of infection within this population for appropriate intervention, has an indirect effect in protecting the general population against the disease.

Nairobi, being a cosmopolitan city stands as a hub where various HCV genotypes could silently enter and circulate in the country. Hence the need for this study.

1.4 Research Question(s)

1. What is the sero-prevalence of hepatitis C virus among people who inject drugs from selected facilities in Nairobi?
2. What are the factors associated with hepatitis C virus infection among PWID from selected facilities in Nairobi?
3. What risk factors are associated with genotypes of hepatitis C virus circulating among people who inject drugs from selected facilities in Nairobi?

1.5 Objectives

1.5.1 Main objective

To determine the prevalence, genotypes and factors associated with hepatitis C infection among people who inject drugs from selected facilities in Nairobi.

1.5.2 Specific objectives

1. To determine sero-prevalence of Hepatitis C among people who inject drugs in the selected facilities in Nairobi.

2. To determine factors associated with Hepatitis C virus infection among people who inject drugs in the selected group facilities in Nairobi.

3. To determine the risk factors associated with Hepatitis C genotypes among people who inject drugs in the selected facilities in Nairobi.

CHAPTER TWO

LITERATURE REVIEW

2.1 Hepatitis C Virus

Hepatitis C Virus is an RNA virus belongs to the family *flaviviridae*, in the genus *Hepacivirus*. Humans and chimpanzees are the only known reservoir (Bokharaei Salim et al., 2010). HCV is the leading cause of chronic liver disease affecting 130–170 million people worldwide, between 3–4 million people are newly infected annually with more than 350,000 people estimated to die from hepatitis C-related liver diseases annually (Webster *et al.,* 2009).

HCV is transmitted mainly through parenteral exposure to blood of chronic carriers and evidence has shown certain groups to be at high risk including persons transfused with blood and blood products prior to 1992 (Engle *et al*., 2014). People who inject drug (PWIDs), persons involved in acupuncture and/or tattooing with unsterile tools, and risk sexual behavior have also been shown to transmit HCV. HCV/HIV co-infection is also an increasing problem in countries with concentrated HIV epidemics and PWIDs, as progression to chronic hepatitis is accelerated in these patients. Groups at risk for HCV infection are also those at risk for tuberculosis as the disease is endemic in countries that do not screen blood products routinely for blood-borne viruses (Wu *et al*., 2015).

2.2 Hepatitis C virus Structure

Hepatitis C virus is a small (55–65 nm in size) enveloped virus with a positive-sense, single-stranded RNA genome. Hepatitis C virus structure is made of envelope glycoprotein, core, envelope, and viral RNA. The envelope glycoprotein consists of two proteins E1 and E2, the two proteins play an important role in shielding the virus from immune system latching they are also thought to mediate cell entry by recognition of cellular membrane receptor proteins (figure 2.1). The core protein is a highly conserved basic protein that makes up the viral nucleocapsid (Moradpour *et al*., 2013).

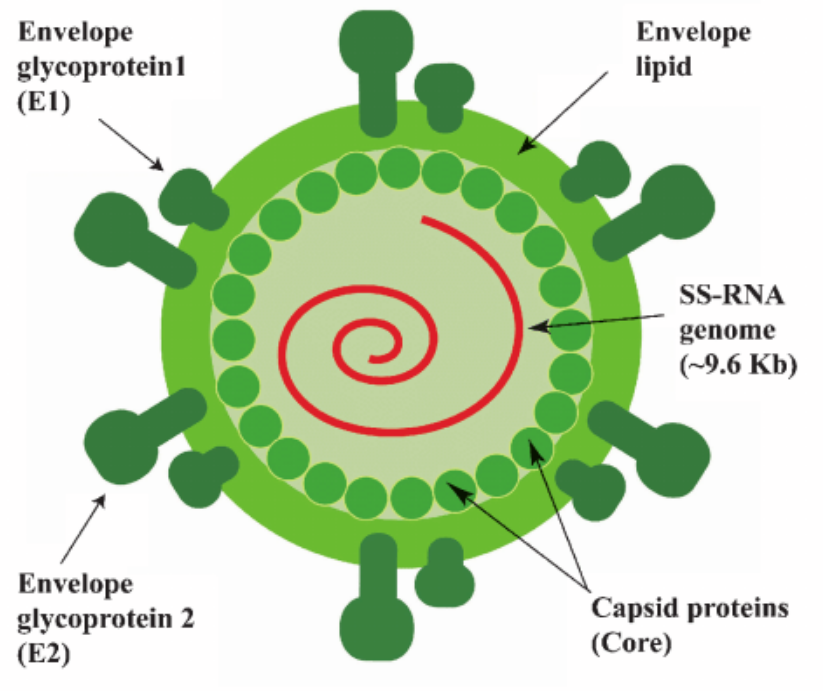


Figure: 2.1 shows a virus nucleic acid (ssRNA) enclosed in a capsid protein and a viral envelope (Bamford *et al*., 2021)

2.3 Hepatitis C Virus genome organization

Hepatitis C genome is a 9kb, the viral genome is translated into a single polyprotein of about 3010 amino acids, made up of 5'Untranslated Region (5'UTR), structural protein, nonstructural protein and the 3'Untranslated Region (3'UTR). The 5'UTR is the most conserved region of the genome contains 341 nt, it consists of four highly structured domains and contains the internal ribosome entry site (IRES) which has the capacity to form a stable pre-initiation complex by directly binding the 40s ribosomal subunit. The structural proteins, which form the viral particle, include the core protein and the envelope glycoproteins E1 and E2 which lie at the amino-terminal end of the polyprotein and are released co-translationally by host cell signal peptidase (Bamford *et al.*, 2021).

The 3'UTR consists of stem-loop structures and an internal poly(U)-poly(U/C) tract it is approximately 225nt organized in three regions from 5' to 3'. The 3′ X region and the 52 upstream nucleotide of the poly (U/C) tract are essential for RNA replication, the remaining sequence of the 3′ UTR are believed to enhance viral replication(Mendes,

2016). The nonstructural protein is made of p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B. NS2 protease is essential for full-length genome replication as it cleaves at the NS2/NS3 junction to release a free NS3 N-terminus for functional assembly (Isken, o. *et al*., 2015) (Figure 2.2). NS2 is a short-lived protein that loses its protease activity after self-cleavage from NS3 and is degraded by the proteasome in a phosphorylation-dependent manner by means of protein kinase casein kinase. NS3 is a multi-functional viral protein containing a serine protease domain in its N-terminal third and a helicase NTPase domain in its C-terminal two-thirds, NS3 helicase-NTPase has several functions, RNA binding, including RNA-stimulated NTPase activity and unwinding of RNA regions of extensive secondary structure by coupling unwinding and NTP hydrolysis. NS4B harbors at least four trans-membrane domains and an N-terminal amphipathic helix that are responsible for membrane association. It is an integral membrane protein of 261 aa with an ER or ER-derived membrane localization. NS5A play a role in interferon resistance by binding to and inhibiting PKR, an antiviral effecter of interferon-α. NS5A bears transcriptional activation functions and are believed to be involved in the regulation of cell growth and cellular signaling pathways (Maqbool *et al*., 2012). The NS5B is the catalytic core of the HCV replication machinery. NS5B is an important target for drug development, viral RNA-dependent RNA polymerase. NS5B can also unwind stable secondary and tertiary RNA structures(Shunmugam *et al*., 2019) (Figure 2.2).

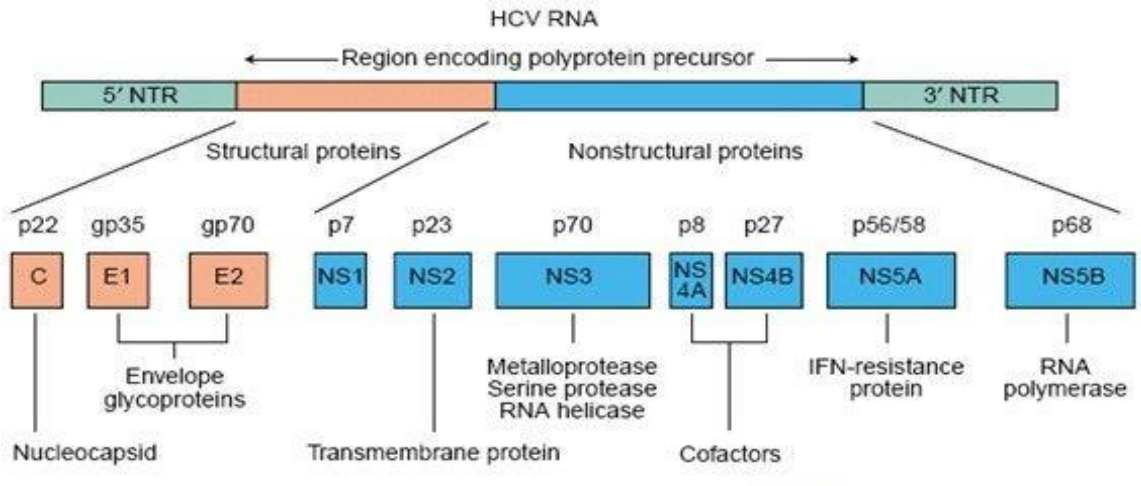


Figure 2.2 Genome organization of hepatitis C virus showing the different regions of the viral genome (Kato., 2000)

2.4 Genotypes and sub types

There are 7 HCV genotypes, with many subtypes, and about 100 different strains based on the sequence of the HCV genome (Donald B Smith *et al*., 2014).

HCV genotypes and subtypes exhibit a distinct geographical distribution (Gower *et al*., 2014) Studies have shown Genotypes 1a and 3a in majority of IDUs in European countries, England and Asian countries (May *et al*., 2015). Genotypes 1-3 are widely distributed globally, with genotypes 1a and 1b accounting for 60% of infections worldwide. Genotype 4 is characteristic for the Middle East, Egypt and Central Africa. Genotype 5 is mainly found in South Africa (Messina, J. P., *et al*., 2015). Genotype 1a and 2 have been identified among the blood donors in Kenya (Mwangi et al., 2016) (Figure 2.3).

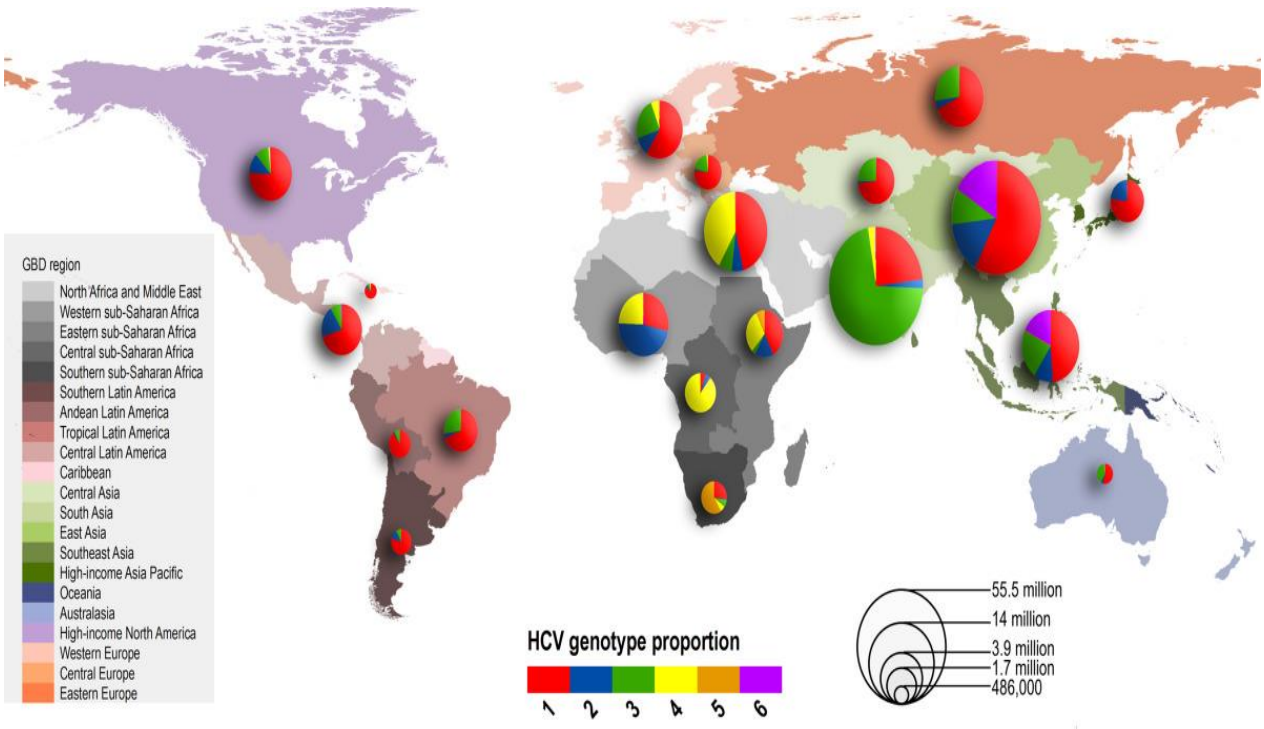


Figure 2.3 showing various hepatitis C genotypes and their most prevalent locality globally (Messina et al., 2015)

2.5 Hepatitis C Virus Life Cycle

HCV entry into the host cell involves a complex series of interactions including attachment, entry and fusion. The initial viral attachment to host cell receptor may involve HVR1 in HCV E2 with facilitation by heparan sulfate proteoglycans expressed on hepatocyte surface. The virus is internalized into the target cells after attachment via a pH-dependent and clathrin-mediated endocytosis (Zeisel, Felmlee, & Baumert, 2013). Class B type I (SRB1) and CD8 receptors, tight junction proteins, claudin-1 and occludin. Additional recently identified entry factors include the receptor tyrosine kinases epidermal growth factor receptor, ephrin receptor A2 and Niemann-Pick C1-like 1 cholesterol absorption receptors play a significant role in entry of the virus into the host cell(Crema, Ponzetto, Clementi, & Carloni, 2015).

HCV particle membrane fusion depends on acidic PH in endosomal compartment to release its RNA genome into the cytoplasm. cyclophilin A can modulate RNA-binding capacity of NS5B polymerase and interact with NS5A, HCV assembly and release process is closely linked to lipid metabolism (Kim & Chang, 2013).

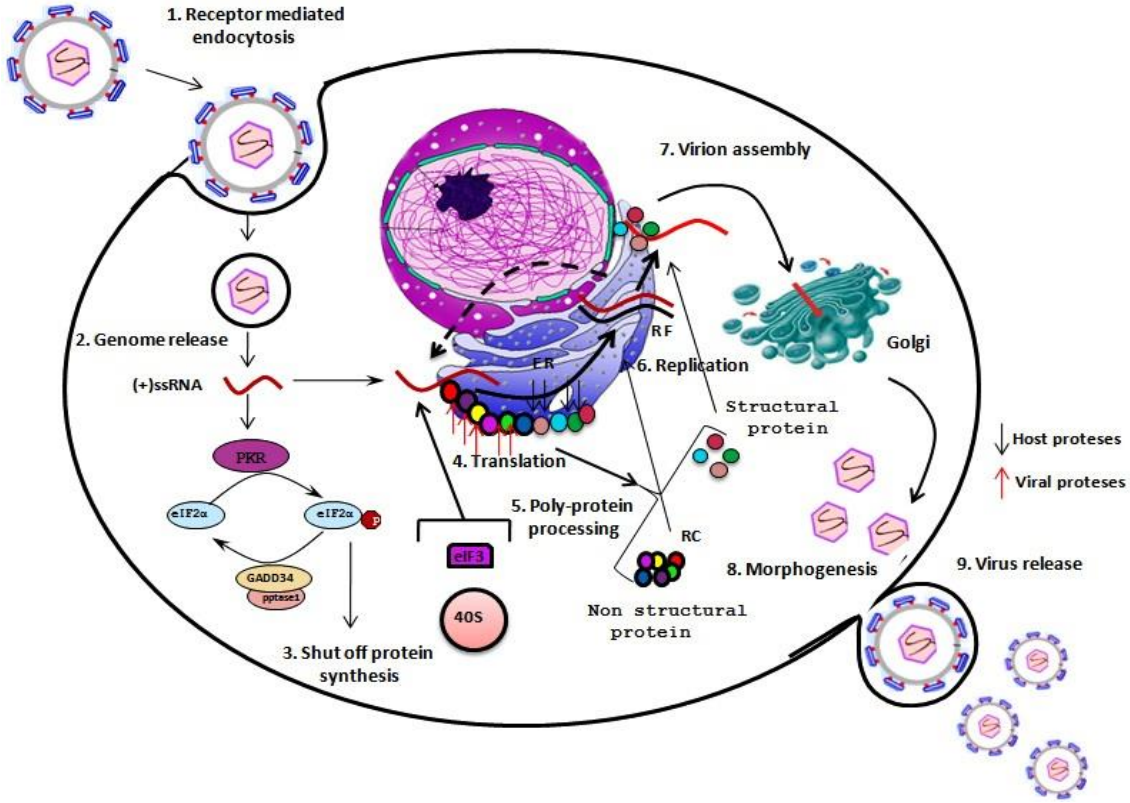


Figure 2.4 Overview of molecular mechanism of HCV infection. The virus mainly enters the cell through receptor mediated endocytosis and leave by budding. (Hayes *et al*., 2018)

2.6 Hepatitis C virus Epidemiology, transmission and disease profile

2.6.1 Epidemiology of HCV

2.6.1.1 Global prevalence of HCV

Hepatitis C virus (HCV) infection is on the rise as a global health burden. Recent studies postulate an annual increase in HCV sero-prevalence over the last decade to over 185 million infections worldwide of whom over 110 million are from the Asian countries, over 28 million from the Australia, over 26 million from sub-Sahara Africa, 12.7 form the north America and the middle east, over 12 million from America and over 0.5 million people from the European countries(Petruzziello *et al*., 2016). Persistent HCV infection can lead to progressive liver disease with the development of liver cirrhosis

and hepatocellular carcinoma, possibly accounting for up to 0.5 million deaths every year (Lozano *et al.*, 2012).

2.6.1.2 Distribution of HCV infection in Africa

A previous linear mixed model developed to estimate the burden of HCV and forecast appropriate interventions by examining at-risk cohorts and weighting with known populations showed an incremental prevalence estimates in southern Africa (0·72%), eastern Africa (3·00%), western Africa (4·14%), and central Africa (7·82%) (Burstow *et al.*, 2017).

Blood donors has the lowest documented sero-prevalence at 1·78%, followed by pregnant women (2·51%), people living with HIV (3·57%), and the general population (5·41%). western Africa has the highest sero-prevalence at 15·69%, in high-risk populations, whereas central and southern Africa has the highest sero-prevalence in the general population (Semugoma *et al*., 2017).

The prevalence of Hepatitis C virus in the African Region vary from country to country, Egypt which is the leading most affected worldwide remains the highly infected also in Africa with a prevent of 17.5%. Other countries that are highly infected include: Cameroon (13.8%), Burundi (11.3%), Gabon (9.2%), Morocco (7.7), Uganda (6.6%), DRC (5.5%) and Guinea (5.5%). CAR, Chad, Congo, Equatorial Guinea, Rwanda Sudan, Benin, Burkina Faso, Cote d'Ivoire, Gambia, Ghana Guinea, Mauritania, Niger, Nigeria, Senegal, Togo, Eritrea, Ethiopia, Kenya, Madagascar, Malawi, Mozambique, Somalia, South Africa, Swaziland, Tanzania, Zambia, Zimbabwe, Sudan, Libya, Tunisia, Algeria, Mauritania have reported a prevalent of less than 5%. No date is available or has not been reported for the rest of the countries according to(Karoney & Siika, 2013).

2.6.1.3 Hepatitis C Virus disease burden in Kenya

In Kenya, results from selected low risk population groups have shown that HCV prevalence is between 0.7 % and 0.9 % (Bartonjo, G. *et al*., 2019). Injecting drug users

in Kenya have been found to be highly infected with the virus. A study by Mwatelah found a prevalence of 16.4 % in IDUs (Mwatelah et al., 2015)

2.7 Transmission of Hepatitis C Virus

Hepatitis C is mainly spread when blood from a person infected with hepatitis C virus enters the body of someone who is not infected. This may be by sharing needles or other equipment that may bring blood conduct. (Kupek, 2004; Schillie, Wester, Osborne, Wesolowski, & Ryerson, 2020) . Hepatitis C was commonly spread through blood transfusions and organ transplants in the past. Although screening for HCV infection in blood donors has contributed immensely to the reduction of transfusion transmitted HCV infection, however HCV infection after blood transfusion is still a matter of concern (Garraud, Amorim Filho, Laperche, Tayou-Tagny, & Pozzetto, 2016). Horizontal transmission of HCV may occur through direct contact with infected blood and blood products, dialysis, sharing of needles among injecting drug users, needle stick accident among healthcare professionals while dealing with HCV positive sample (Indolfi, Nesi, & Resti, 2013). Sexual intercourse is believed to be a mode of HCV transmission however it is inefficient for transmission. This mode of transmission has been highly reported in people with unprotected multiple sex partners, people who engage in rough unprotected sex such as anal sex, having unprotected sex with partner having history of a sexually transmitted disease, being HIV positive, and also having unprotected sex during menstruation or whenever blood is present (Ghisla *et al*., 2017).
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 HCV 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% likely to transmit the virus to their babies(Elrazek et al., 2017).

2.8 Natural history of Hepatitis C Virus infection

The incubation period of HCV following successful infection typically is 8 to 12 weeks. At this stage, the clinical presentation will range from subclinical and asymptomatic carriage to acute self-limiting hepatitis or fulminant hepatitis to chronic hepatitis (Figure 2.5), which could lead to liver cirrhosis and HCC (Hajarizadeh *et al*., 2013).

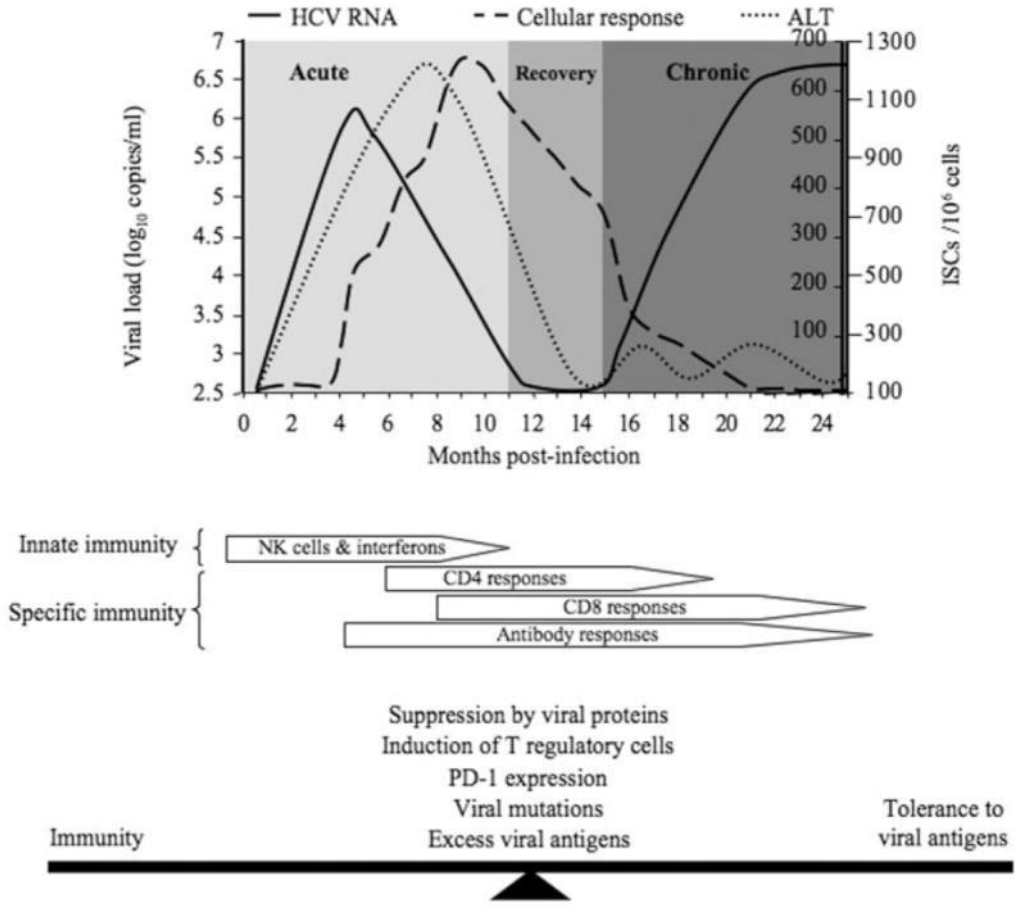


Figure 2.5 Figure showing the transition of Hepatitis C infection from acute face to chronic face with the various monitory markers (Blackard *et al.*, 2008)

2.8.1 Acute hepatitis C

Upon infection, persons infected with Hepatitis C enter into an acute hepatitis phase where they present with clinical signs and symptoms of hepatitis for a period of 6

months or fewer after the presumed time of HCV exposure (Corey *et al*., 2021). The clinical representation of early HCV infection was realized by early studies of post transfusion patients who developed non hepatitis A, non-hepatitis B viral infection (Westbrook & Dusheiko, 2014). After acute infection, HCV RNA may become detectable in the serum/plasma in as little as 2 weeks. Several weeks later, a high percentage of patients experience an increase in serum aminotransferase levels in a company with the development of acute hepatocellular injury. In the majority of cases, patients develop mild constitutional symptoms including; nausea, abdominal pain, fatigue, vomiting and anorexia. During this acute infection, serum aminotransferases often peak below 1000 IU/mL and may return to normal levels. A minority develops sufficient elevations in bilirubin to lead to presence of jaundice or the development of dark urine. For a prolonged un-cleared untreated infection may cause majority of infected patients entering chronic disease state development (Zibbell *et al*., 2018)

2.8.2 Chronic hepatitis

Chronic hepatitis C is the viral infection of the liver which can damage the liver over time and lead to scarring which may range from mild inflammation to severe liver damage and cirrhosis (Sebastiani *et al.*, 2014). It is marked by the persistence of HCV RNA in the blood for more than 6 months after onset of acute infection. HCV is self-limiting in only 25% of patients in whom HCV RNA in the serum becomes undetectable and ALT levels return to normal. Approximately 85% of infected patients do not clear the virus by 6 months, and chronic hepatitis develops (Vispo et al., 2014).

The progression of chronic infection from acute infection is dependent on host immune response, the age at time of infection, gender, ethnicity, and the development of jaundice during the acute infection (Lingala *et al.*, 2015). 30% in persons below the age of 20 years, and 76% for those older than 20 years infected with Hepatitis C proceed to the chronic stage of infection. Other studies have shown 55%-60% of children transfused with Hepatitis C infected blood remain HCV RNA positive to adulthood. For instance, age depending at the time of infection has been shown that majority of perinatal or childhood infections are found to be asymptomatic and may proceed to chronicity.

However, in adults the infection is usually acute and self-resolving (El-Guindi, 2016; Lauer, 2013) as shown in figure 2.6 below.

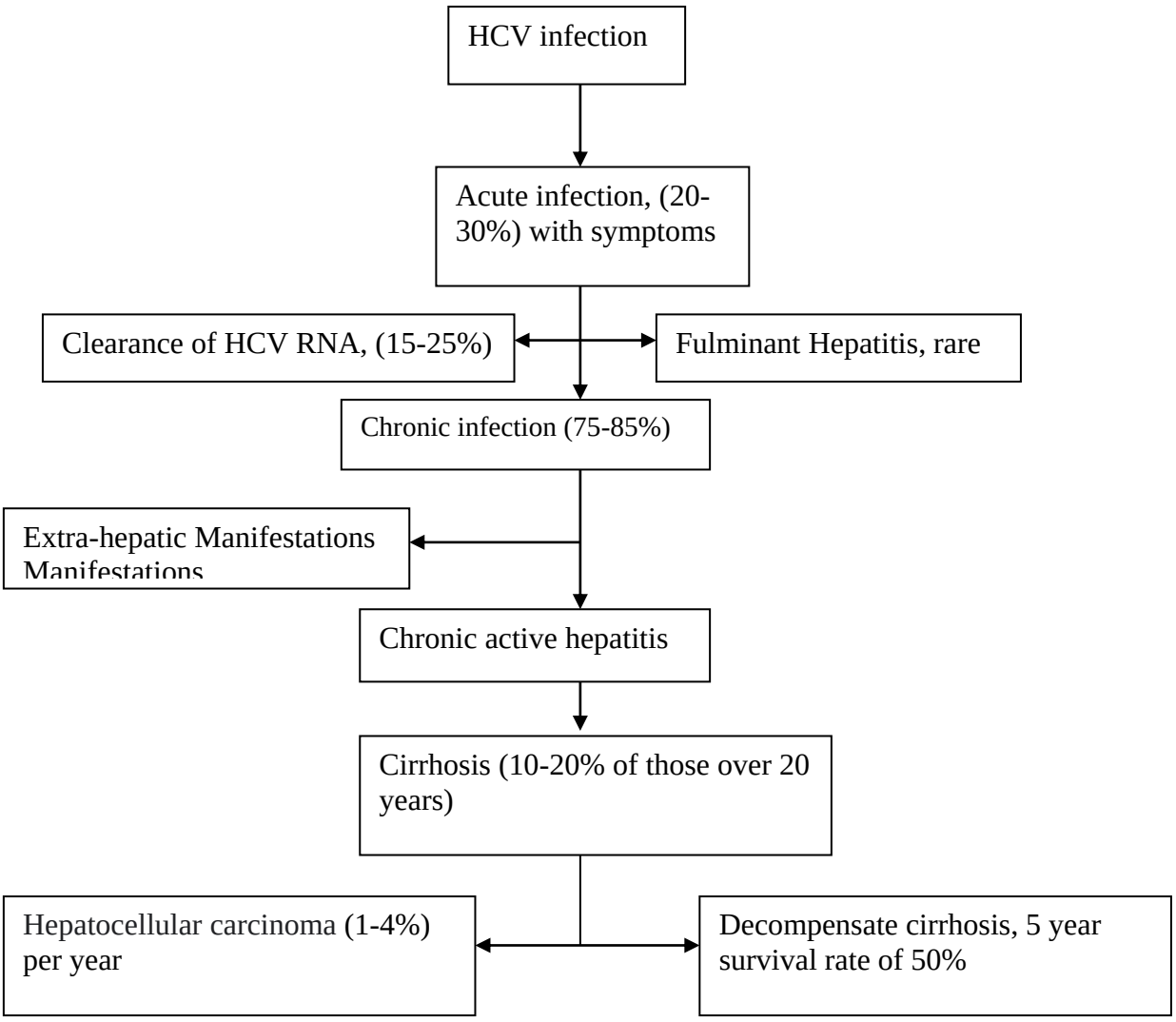


Figure 2.6 Natural progress of Hepatitis C virus infection with the percentage of those who progress to the second stage from infection to hepatocellular carcinoma (Sarin, S. K *et al* .,2012).

A Study on HCV infected people who inject drugs with viral clearance history, indicate the rate of chronic HCV infection being lower in patients who develop jaundice or symptoms during the acute onset of HCV infection as compared to those who are anicteric(Sarin, S. K *et al* .,2012). A study of women infected with contaminated Rh immune globulin in Germany exhibited a rate of chronicity in 43% of those with history

of jaundice, as compared to 60% among those who remained anicteric ($p<0.001$). Hypothetically jaundice may be triggering a more robust immune Th1 lymphocyte and cytokine response to the HCV leading to clearance of the infection(Ramya, Daniel, Ramalakshmi, & Usha, 2018).

2.8.5 Clinical relevance of HCV genotypes

Since the discovery of Hepatitis C virus, multiple genotypes and subtypes have been discovered on the basis of phylogenetic analysis of nucleotide sequences. Characterization of these genetic groups has enabled identification and distribution of the virus in the world. This is likely to facilitate and contribute to the development of an effective vaccine against infection with HCV besides management and treatment of the infected (Sorbo et al., 2018).

2.9 Prevention and treatment Of Hepatitis C Virus Infection

2.9.1 Vaccination as a preventive measures

Vaccination is a vital tool in preventing and managing of viral infection. However no vaccine against Hepatitis C virus has been found since the discovery of the virus, nevertheless a lot of effort is being put in place to develop HCV vaccine with two main aims: one is to protect people that are at high risk that have not yet been exposed to the virus; and secondly, is to immunize people that have been cured of the virus, this is to protect them against chronic HCV infection following a subsequent exposure (Cashman, Marsden, & Dustin, 2014).

The World Health Organization (WHO) has set 2030 to reduce the rate of new HCV infection by 90%, and is bumping its resources toward any effort working against the virus. Understanding of the morphology and immunogenicity has given opportunity to come up with noble ideas on vaccine development. The first vaccine is based on recombinant HCV gpE1/gpE2. This vaccine was one of the earliest tested in chimpanzees. Recombinant genotype 1a gpE1/gpE2 vaccination demonstrated effective immunogenicity and protective immunity against homologous or heterologous HCV re-challenge and even sterilizing immunity in some animals (Logan et al., 2017). Another

vaccine is aimed at priming HCV-specific CD4 and CD8 T cells, using an adenovirus-based vector approach and focusing on the virus NS (3,4A, 4B, 5A and 5B) proteins. These two vaccines have gotten approval for human trial (Shoukry, 2018).

2.9.2 Treatment of hepatitis C

Treatment is recommended for patients who are at risk of developing cirrhosis, generally defined by a measurable hepatitis C viral load and liver biopsy showing portal or ridging fibrosis along with moderate inflammation and necrosis (Ghany, Strader, Thomas, & Seeff, 2009). Some of the recommended regiments range from interferon monotherapy to interferon plus ribavirin given with the aim of achieving a sustained virological rate. The duration of the standard interferon plus ribavirin is based on the viral genotype (Manns, M., *et al*. 2011).

Different regiments have been designed to treat Hepatitis C viral infection. These regiments combination have been shown by different studies to be genotypic specific. Some of the recommended regiments by the American Association for the Study of Liver Diseases (AASLD), the Infectious Disease Society of America (ISDA), and the International Antiviral Society-USA (IAS-USA) are: for HCV genotype 1a and 1b infection is a combination of ombitasvir/paritaprevir/ritonavir and dasabuvir (Deeks, 2015).

For naive patients with HCV genotype 1a infection, a combination of ledipasvir (90 mg)/sofosbuvir (400 mg) for 12 weeks or combination of paritaprevir (150 mg)/ritonavir (100 mg)/ombitasvir (25 mg) plus twice-daily dasabuvir (250 mg) and weight-based ribavirin (RBV) (1000 mg [<75 k sofosbuvir (400 mg) plus simeprevir (150 mg), with or without weight-based for 12 weeks for patients with no cirrhosis or 24 weeks for patients with cirrhosis without Q80K polymorphism or daclatasvir (60 mg) and sofosbuvir (400 mg), for 12 weeks for patients with no cirrhosis or 24 weeks with or without ribavirin for patients with cirrhosis (Ahmed et al., 2017).

Naive patients with genotype 1b infections are recommended a combination of ledipasvir (90 mg)/sofosbuvir (400 mg) for 12 weeks or a combination of paritaprevir (150 mg)/ritonavir (100 mg)/ombitasvir (25 mg) plus twice-daily dasabuvir (250 mg) for 12 weeks. The addition of weight-based RBV (1000 mg [<75kg] to 1200 mg [≥75 kg]) is recommended in patients with cirrhosis or combination of sofosbuvir (400 mg) plus simeprevir (150 mg) for 12 weeks for patients with no cirrhosis or 24 weeks with or without weight-based RBV (cirrhosis) or a compination of daclatasvir (60 mg) plus sofosbuvir (400 mg) for 12 weeks for patients with no cirrhosis or 24 weeks, with or without weight-based ribavirin, for patients with cirrhosis (Ahmed et al., 2017).

Recommended regimen for patients with HCV genotype 2 infection is daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 12 weeks,for those with cirrhosis for 16 weeks or daily daclatasvir (60 mg) plus sofosbuvir (400 mg) for 12 weeks, in patients who cannot tolerate ribavirin (He, Lopez-Olivo, Hur, & Chhatwal, 2017).

In 2015 FDA approved daclatasvir (Daklinza), an NS5A inhibitor, with sofosbvir for use by patients with chronic HCV genotype 3 infections(M. A. Smith, Regal, & Mohammad, 2016). The dose is 60 mg once daily plus sofosbuvir 400 mg once daily (McCormack, 2015).

In 2015 FDA approved treatment of naive patients with HCV genotype 4 infection, is a combination of ledipasvir (90 mg)/sofosbuvir (400 mg) for 12 weeks, or a combination of paritaprevir (150 mg)/ritonavir (100 mg)/ombitasvir (25 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 12 weeks. Sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) combined is recommended for 24 weeks. FDA also approved simeprevir for the treatment of HCV genotype 4 monoinfected and HCV/HIV coinfected patients as a component of a combination antiviral treatment regimen that includes ribavirin and peginterferon(Hézode et al., 2015).

The recommended regimen for patients infected with HCV Genotypes 5 or 6 infection is a daily fixed-dose combination of ledipasvir (90 mg)/sofosbuvir (400 mg) for 12 weeks, or a daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG-IFN for 12 weeks (Asselah et al., 2019)

The World Health Organization (WHO) updated its guidelines for the treatment of persons with HCV infection to recommend DAA-based regimens in place of IFN-based regimens in 2016. These regimens that do not require ribavirin have continued to improve and several pangenotypic regimens, which successfully resolve HCV infection in over 85% of treated of the six major genotypes, were approved by regulatory bodies including the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). The use of this regiments has been associated with very low rates of virological relapse. Currently, the pangenotypic DAAs glecaprevir-pibrentasvir (8 week course), sofosbuvir-daclatasvir (12 week course), and sofosbuvir-velpatasvir (12 week course) are approved in most markets for the treatment of HCV-infected persons without cirrhosis. (WHO., 2018)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was a subset of a larger KEMRI study investigating prevalence and molecular epidemiology of viral hepatitis in Kenya. The study samples were collected from two dropping support centers targeting people who inject drugs (PWID) residing in Nairobi county. One of the center situated at Ngara approximately 1.5 kilometers to the east of Nairobi central business district, which serve clients from; Karioko, Majengo, Kamukunji, Mathare, Ngara East, Ngara West, West lands and its environs. The other dropping center is in Kawangware approximately 11 kilometers to the west of the city center serving clients from; Kawangware, Settilight, Kabiria, Lavington, Kangemi, Kibera, upper hill and its environs. The two centers are well organized by non-governmental organization supporting individuals who are addicted to injecting heroin drug in order to get high and perhaps get read of their troubles. The organization provides a daily support to the individuals by providing them with methadone, a narcotic pain reliever and counseling of the addicts which gives them an equal high feeling hence reducing the act of injecting heroin. A part from the selected region, there were clients who came from other parts of the county who were recruited into the study. Their area of residence was clustered as (Outside the selected block) (Figure 3.1

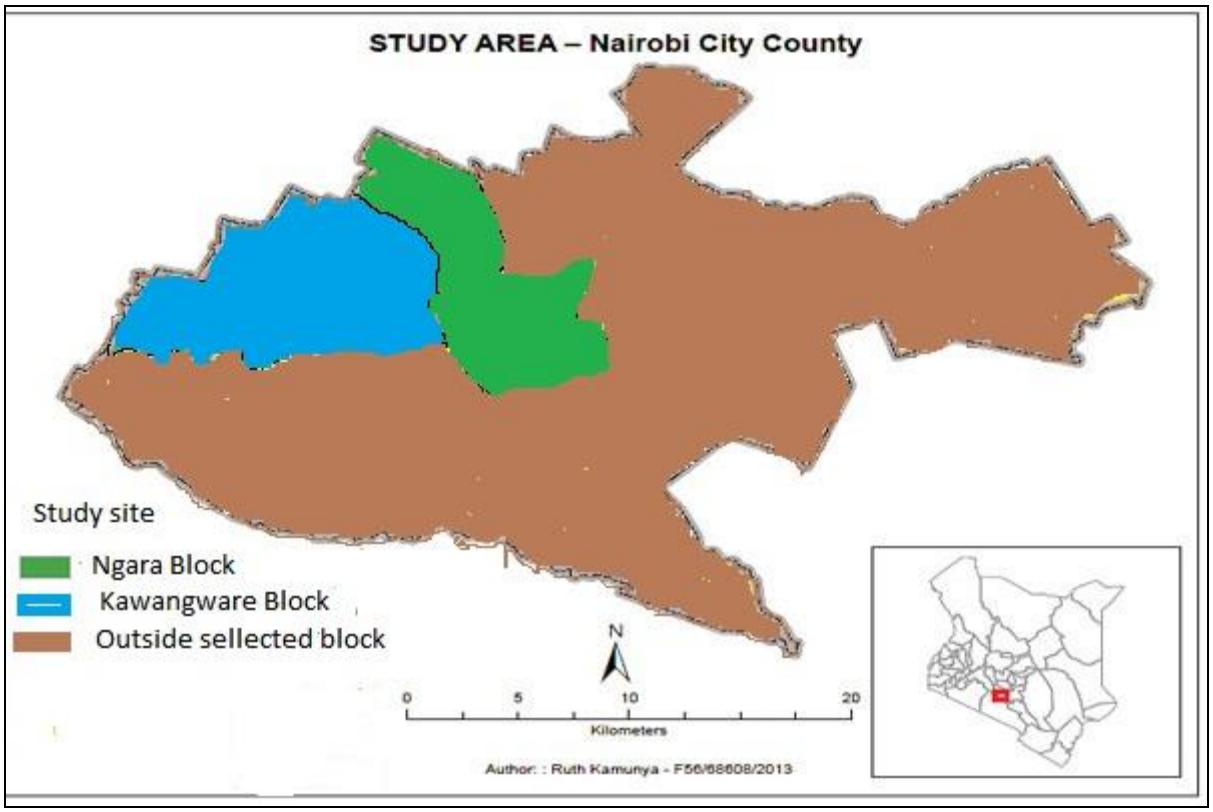


Figure 3.1: showing the map of Nairobi County indicating the coverage of the sample collection sites indicated in greens, blue and brown.

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

3.3.1 Inclusion criteria

1. The client must have recited in Nairobi County for more than one month
2. They must be over 18 years old
3. They must consent to the study

3.3.2 Exclusion criteria

1. Any client who does not recite in Nairobi County
2. Any client of less than 18 years old
3. Any client whose response seem to be under the influent of drugs

3.4 Sample size calculation

Sample size was determined using Fisher, 1998 standard statistical formula: $n=\frac{Z^2pq}{e^2}$

where;

n = the sample size,

Z = the abscissa of the normal curve (1.96)

e = the desired level of precision $\pm$ 5%

p = the estimated proportion of attribute present in the population, [16.4% (Mwatelah et al., 2015)].

q = 1-p.

$$n= \frac{Z^2pq}{e^2} \sim \frac{(1.96)^2(0.164)(0.836)}{(0.05)^2} = 210$$

Approximately a total of **210** samples

3.5 Sampling technique

Due to the complexity of getting the study clients, snowball sampling technique was used. The recruited study subjects were aged between 18 and 67 years old.

3.6 Participants distribution

Participants were recruited from two dropping centers (Kawangware and Ngara) with an assumption that all the participants resides within the selected dropping center. However participants who came from outside the region but within Nairobi County were considered into the study. Sample distribution was based on proportion of population based size. These lead to formation of 3 residential blocks ("block A" for Ngara residents, "block B" for Kawangware residents and "block C" for those who recited outside the sample dropping center

3.7 Data collection

Demographic factors data of the participants was collected using a structured questioner, the information obtained from the participants were; Age, gender, marital status, area of residence, length of using injectable drugs, the frequency of inject drugs, the frequency of sharing needle when injecting drugs.

3.8 Sample collection

At the dropping center, 3ml of blood sample were collected into 5ml vacutainer tube from each of PWIDs who consented to participate in the study. The blood samples were transported under cold chain condition, to maintain its integrity to center for virus researcher in KEMRI.

3.9 Sample processing

The collected whole blood samples were separated by centrifuging at 1500 RPM at room temperature for 10 minutes; two layers of cells and plasma were obtained. The top layer of plasma was transferred into two aliquot of 200ul into one vial (A) and the remaining into the other vial (B). The 200ul vial was used for serology work while the other vial was stored at -80^{o}c for further use in molecular analysis.

3.9.1 HCV Serology test

The Hepatitis C serology test was done using bio-Elisa HCV 4.0 kit as summarized;

This is an immuno-enzymatic method in which wells of a microplate are coated with recombinant antigens representing epitopes of HCV: Core, NS3, NS4 and NS5.

3.9.1.1 Serology Procedure

In summary, the procedure involved addition of 200 µl of sample diluent and 10 µl of each sample into the designated wells., 8 wells were reserved for blank and controls. 200 µl of negative control were transferred to 2 wells, 200 µl of low positive control were transferred to 3 wells and 200 µl of positive control to 2 wells. 1 well was Left empty for the substrate blank. The microplate was then covered with an adhesive seal, mixed gently and incubated for 1 hour at 37°C. The adhesive seal was removed to allow aspiration of the contents of the wells before filling them with approximately 350 µl of the diluted washing solution. The process of aspiration and washing was repeated 5 times. Each column of wells was allowed to soaks for at least 15 seconds before the next aspiration cycle. After the last washing the microplate was blotted on absorbent tissue to remove any excess liquid from the wells.

After which 100 µl of diluted conjugate was transferred into each well, except the one reserved for the substrate blank while avoiding bubbles upon addition. The microplate was covered with an adhesive seal and incubated for 30 minutes at 37°C.

After incubation the adhesive seal was removed and the wells washed. 100 µl of substrate-TMB solution was added to each well, including the blank and Incubated for 30 minutes at room temperature (18-25°C). The reaction was stopped by adding 100 µl of stopping solution in the same sequence and time intervals as for the substrate-TMB (Table 3.1).

The reader was blanked at 450 nm with a blank plate and the test plate was read and the absorbance of each well was read within 30 minutes in bichromatic mode using a 620 - 630 nm reference filter (BIOKIT, 2012).

Table 3.1 showing concentration of reagents used

Strips required	1	2	4	6	8	10	12	24
Substrate buffer ml	1.0	2.0	4.0	6.0	8.0	10.0	12.0	24.0
Chromogen (TMB) µl	20	40	80	120	160	200	240	480

3.9.1.2 Interpretation of results

The mean absorbance of the low positive control (LPC) was calculated. The obtained value, multiplied by 0.9. This was used as a cut-off value.

The absorbance of the sample was divided by the cut-off value.

Positive: ratio absorbance/cut-off ≥ 1.0

Negative: ratio absorbance/cut-off < 0.9

Equivocal: ratio absorbance/cut-off $\geq 0.9 < 1.0$ (BIOKIT, 2012)

3.9.2 Molecular analysis of the samples

3.9.2.1 Extraction of Viral RNA

Extraction was done using the Qiagen RNA extraction kit in summary, 560 µl of prepared Buffer AVL containing carrier RNA were pipet into a 1.5 ml micro centrifuge

tube. 140 µl plasma was added to the Buffer AVL–carrier RNA in the micro centrifuge tube, and Mix by pulse-vortexing for 15 seconds. The mixture was incubated at room temperature (15–25°C) for 10 min. briefly the tubes were centrifuged to remove drops from the inside of the lid. 560 µl of absolute ethanol was added to the sample, and mixed by pulse vortexing for 15 seconds. After mixing, the tube was briefly centrifuged to remove drops from inside the lid. 630 µl of the solution was carefully applied to the QIAamp Mini column in a 2 ml collection tube without wetting the rim. The tubes were then centrifuged at 8000 rpm for 1 min. The QIAamp Mini columns were removed and placed into a clean 2 ml collection tube, and the tube containing the filtrate was discarded. The QIAamp Mini columns were opened and carefully 500 µl of Buffer AW1 added and centrifuged at 8000 rpm for 1 min. 500 µl of Buffer AW2 was added to the QIAamp Mini column, and centrifuged at full speed (14,000 rpm) for 3 min.

The QIAamp Mini columns were then placed in a new 2 ml collection tube and Centrifuged at full speed for 1 min. The QIAamp Mini columns were placed in a clean 1.5 ml micro centrifuge tube, 60 µl of elution buffer AVE equilibrated to room temperature added and incubated at room temperature for 1 min. this was followed by Centrifugation at 8000 rpm for 1 min to obtain RNA. The final viral RNA was stored at -30^{o}c (Qiagen, 2014).

3.9.2.2 RNA Transcription

For the normal amplification of the extracted RNA, a complimentary DNA (cDNA) was synthesis using the extracted RNA as the template using a two-step RT-PCR using the reagent master mix and condition indicated in table 3.2.

Table 3.2: cDNA synthesis mix and condition

	Stage1 Mix	
Reagent	concentration	Volume (ul)
dNTP's	10Mm	1
Random primer	50 ng/l	2
RNA		10
	Stage 2 Mix	
First strand	5x	4
DTT		2
RNase inhibitor		0.25
RT Superscript		1
	RT-PCR	
Temperature(^{O}C)	Time (Mins)	
65	5	
4	5	Stage 1
25	5	
50	40	Stage 2
70	15	

3.9.2.3 Nested Polymerase chain reaction

Amplification of the HCV 5'UTR gene by nested PCR was done using an ABI Thermocycler 9700 system (Applied Biosystems, USA) where first round PCR as shown in table 3.3 below.

Table 3.3 Showing the first set of primer sequences used in PCR

Virus (Region) tested for	Primers used	Expected Fragment Size (bp)
HCV (5'UTR)	1. KY80 5'-GCAGAAAGCGTCTAGCCATGGCGT-3'	430 bp
	2. KY78 5'-CTCGCAAGCACCCTATCAGGCAGT-3'	(Mwangi et al., 2016)

A master mix was prepared depending on the number of samples as shown in Table 3.3

Table 3.4: PCR master mix preparation

Reagent	Stock concentration	Concentration	Volume per tube (µL)
PCR Buffer with $Mgcl_2$	10X	1	2.5
dNTPS	10mM	1	0.5
$Mgcl_2$	25 mM	0.5	0.5
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	-		15.4
RNA extract	-		5
Total	-		25

The thermocycling conditions for first round PCR consisted of 95 °C for 15 min; followed by 35 cycles at 95 °C for 30 seconds, 55 °C for 1 min, 72 °C for 2 min and final extension at 72 °C for 7 minutes. The nested PCR was done under the same conditions except that annealing was done at 58°C with 25µl master mix and 5µl of the first-round product using the primer as shown in table 3.5 below.

Table 3.5 The second set of primer sequences used in PCR

Virus (Region) tested for	Primers used	Expected Fragment Size (bp)
HCV (5'UTR)	1. hep21b 5'-GAGTGTYGTRCAGCCTCCAGG-3'	243 bp
	2. hep22 5'-GCRACCCAACRCTACTCTCG -3'	(Mwangi et al., 2016)

3.9.2.4 Detection of amplified HCV PCR products

Detection of the amplified PCR products was performed using 2.0% agarose gel in 1X TAE buffer (0.04M Tris acetate, 0.001 M EDTA). The gel was prepared by dissolving 2g of agarose into 100 mL of 1X TAE buffer by heating. After complete dissolving of

the agarose it was cooled to about 50^{O}C. The solution was powered onto a tray with gel comp and allowed to solidify, the gel comp was then removed forming wells. 2µl of the PCR product mixed with 2µl of 5x loading dye containing bromophenol blue, positive control and the molecular weight ladder was loaded into the wells on the gel and run in 1X TAE buffer on an electrophoretic tank for 30 minutes at 100 voltages. Once it was ^{3}/4 way the gel, it was removed and stained with ethidium bromide (1µl/100ml) for 15minutes. The expected bands was read against 100bp DNA ladder (Promega, Madison, USA) using ultraviolet (UV) trans-illumination and corresponded to positive controls (Cavallero, S. *et al*., 2013).

3.9.2.5 Purification of PCR product

The nested PCR products confirmed by agarose gel electrophoresis to be positive for the desired gene fragments were purified using the QIAGEN MinElute Purification Kit (Qiagen GmBH), according to the manufacturer's instructions. Briefly, 50ul of buffer PB was added to 10ul of PCR reaction and mixed. This was followed by the addition of 10ul of 3M Sodium acetate (PH 5.0) and mixed. In addition, washing was done by adding 750 µl buffer PE to the QIAquick column and then centrifuged for 30 seconds. Finally cDNA was eluted by adding 50ul of RNAse free water PH (7.0) and the column centrifuged at 13000 RPM for1 minute (Nagalakshmi, U., *et al*. (2010).

3.9.2.6 Sequencing of the amplicons

The purified products were used for sequencing. Briefly, a dideoxy terminator sequencing reaction were performed on the second round nested PCR products, making use of the second round primers and incorporating fluorescent chain termination dideoxynucleotides (ddNTPs). This was followed by exposure of the sequencing reaction products to a high resolution polyacrilamide electrophoresis reaction to separate short gene fragments (oligonuleotides). Using laser detection, the different wavelengths of the fluorescence emitted by each of the ddNTPs (ddATP, ddTTP, ddCTP, ddGTP) used were observed and then analyzed by a Spectrumedix computer software programme to generate sequenced data (Cui *et al*., 2013).

3.9.2.7 Alignment of sequenced product

The raw data generated from the sequencing were analyzed using software from Applied Biosystems, USA.

The forward and reverse sequence product of each sample were assembled into a single contig sequence using the GENETYX software (Tamura, Stecher *et al*. 2013). The assembled nucleotide sequences were aligned using MUSCLE(multiple sequence comparison by log-expectation) program 3.8 (Edgar, 2010). The quality of the sequences was improved by trimming and editing where necessary using BioEdit software (Hall, 1999). The Neighbour-Joining method on the MEGA6 program was used to generate a bootstrap consensus phylogenetic tree out of a 1000 bootstrap replicates, comparing the study sequences with the HCV references sequences which already had established genotype lineages (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013).

3.10 Data management and analysis

The demographic data were entered into an Excel spreadsheet and backed up in flash disk. 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.

3.11 Ethical consideration

Approval to conduct the study was granted by Kenya Medical Research institute KEMRI Scientific Steering committee (SSC) and Ethical Review committee (ERC) (SSC No. 2443- Appendix No. 3). Approval was also sought from the head of the KANCO to use their facility and their support group. Informed consent from the participants was also obtained. (Appendix No. 1). All the samples taken were given new unique identification number.

CHAPTER FOUR

RESULTS

4.1 Prevalence of HCV among the PWIDs in Nairobi

A total of 212 PWID participants were recruited for the study, the prevalence of HCV antibody detection among the participants was 9.9% (21/169) among men and 8% (3.8%) among women. An overall prevalence of 13.7% (29/212) was established from the population. Block A hard a prevalence of 6.6%, men having a prevalence of 4.7% and the female having a prevalence of 1.9%. Block B hard a prevalence of 5.7%, men having a prevalence of 4.2% and the female having a prevalence of 1.4%. Block C hard a prevalence of 1.4%, men having a prevalence of 0.9% and the female having a prevalence of 0.5%. (figure. 4.1).

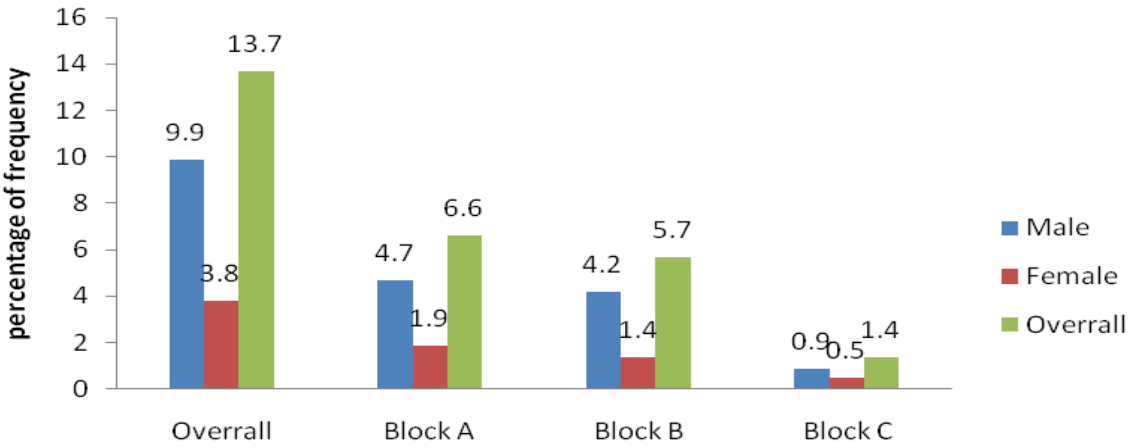


Figure 4.1 Prevalence of hepatitis C among PWIDs in Nairobi, Kenya (n=212)

Figure 4.1 Showing overall prevalence of hepatitis C and the prevalence per gender group among people who inject drugs in Nairobi, Kenya (n=212)

4.2 Demographic factors associated with HCV infection among PWIDs in Nairobi

Men accounting for 79.7% (169/212) and women 20.3% (43/212). The average age among the PWIDs was 33.60 years; male had an average of 34.7 years with the youngest being 20 years and the oldest being 67 years, while the female had an average of 29.40 years with the youngest being 18 years and the oldest being 62 years.(Table 4.1).

Majority of the cases were between 21 years and 39 years old 156 (73.6%), extreme age groups of below 20 years and above 60 years had the least representation of 7 (3.3%) and 6 (2.8%) respectively. (Table 4.1).

Table 4.1 showing the frequency distribution of various variables

Variable	Variable group	Frequency (n= 212)	Percentages (%)
Age	< 20 Years	7	3.3
	21 to 39 Years	156	73.6
	40 to 59 Years	43	20.3
	> 60 Years	6	2.8
Gender	Male	169	79.7
	Female	43	20.3
Marital status	Married	52	24.5
	Single	72	34
	Separated	88	41.5
Area of residence	Block (A)	98	46.2
	Block (B)	95	44.8
	Block (C)	19	9
For how long have you been using injectable drugs?	< 1Year	35	16.5
	Btw 1 and 5 Years	128	60.4
	Over 5 Years	49	23.1
How often do you inject drugs?	Daily	146	68.9
	Once a week	53	25.0
	Only when with my friends	13	6.1
	Every time I use drugs	5	2.4
How often do you share needle when injecting drugs?	When I cannot buy/ not given my own	111	52.4
	I have never shared	30	14.2
	I cannot remember	66	31.1

Within the marital status grouping, 52 (24.5%) accounted for the married, 72 (34%) for the singles and 88 (41.5%) for the Separated. Though the samples were collected from two centers, they were classified into three area of residence. Block (A) and Block (B) were the major area of residence with 98 (46.2%) and 95 (44.8%) respectively, while 19 (9%) of the participants resided outside the anticipated area forming Block (C), as shown in table 4.1.

Of those infected, average age among the males was 33.52 years with the youngest being 26 years and the oldest being 63 years. While the female had an average of 30.87 years, with the youngest participant infected being 20 years and the oldest participant infected was 41years. The rate of infection was high among those aged 21 to 59, who in total accounted for 89.7% of all the infections with the clusters of 21 to 39 and 40 to 59 years each recording an infection rate of 44.8% (13/29) of 21 to 39 years and 13 (44.8%) and the age group between 40 years and 59 years old 13(44.8%), extreme age groups of above 60 years had the least representation of 3 (10.3%) while those below the age of 20 years presented no member infected with hepatitis C as shown in table 4.2.

Within the marital status group, 11(37.9%) accounted for the married, 8(27.6%) for the singles and 10(34.5%) for the Separated. In relation to residential, majority of the infected participant recited in block A 14(48.3%) followed by those reciting in block B 12(41.4%), while 3(10.3%) of the participants that turned positive residing outside the anticipated area Block A and B forming a new block C, as shown in table 4.2.

The association between HCV sero-positivity and; age, gender, marital status and area of residence variables showed varied results (Table 4.2). Briefly, a significant association with age (OR 6.9; p= 0.001) was determined, where those above 60 years had a higher risk of infection compared to those below 20 years. A significant association with marital status (OR 0.4; p=0.008) was determined, where those who reported to be married were at high risk of infection compared to those who reported to be single or separated. There was no significance association between Area of residence and gender with HCV sero-positivity. (Table 4.2)

Table 4.2 showing the association of variables with HCV infection

Variable	Variable group	No./% Total Pos	% Pos within group	% Pos within HCV	P-Value	Odds ratio	95% CI for Odds
Age	below 20 Years	0 (0.0)	0.0%	0.0%	0.001	6.890	3.049
	21 Years to 39 Years	13 (6.1)	8.3%	44.8%			15.569
	40 Years to 59 Years	13 (6.1)	30.2%	44.8%			
	Above 60 Years	3 (1.4)	50.0%	10.3%			
Gender	Male	21 (9.9)	12.4%	72.4%	0.105	2.346	0.837
	Female	8 (3.8)	18.6%	27.6%			6.572
Marital status	Married	11 (5.2)	21.2%	37.9%	0.008	0.448	0.247
	Single	8 (3.8)	11.1%	27.6%			0.812
	Separated	10 (4.7)	11.4%	34.5%			
Area of residence	Block A	14 (6.6)	14.3%	48.3%	0.781	0.906	0.450
	Block B	12 (5.7)	12.6%	41.4%			1.823
	Block C	3 (1.4)	15.8%	10.3%			
For how long have you been using inject-able drugs?	< 1Year	2 (0.9)	5.7%	6.9%	0.001	7.454	3.275
	Btw 1 and 5 Years	7 (3.3)	5.5%	24.1%			16.964
	over 5 Years	20 (9.4)	40.8%	69.0%			
How often do you inject drugs?	Daily	26(12.3)	17.8%	89.7%	0.010	0.201	0.059
	Once a week	3 (1.4)	5.7%	10.3%			0.679
	Only when with my friends	0 (0.0)	0.0%	0.0%			
How often do you share needle when injecting drugs?	Every time I use drugs	0 (0.0)	0.0%	0.0%	0.818	0.946	0.572
	When I cannot buy/ not given my own	16 (7.5)	14.4%	55.2%			1.518
	I have never shared	1 (0.5)	3.3%	3.4%			
	I cannot remember	12 (5.7)	18.2%	41.4%			

Analysis of the association between HCV sero-positivity and the use of injecting needle also showed varied results; a significant association with the duration of injecting drug (OR 7.5; p=0.001) was determined, where those who had injected drugs for more than

five years were at the highest risk of infection with those who had injected for less than a year being at a lower risk of infection. Frequency of injecting drugs also showed a statistical significance with HCV infection (OR 0.2 p=0.010).Those who injected drugs on a daily basis being at most risk of infection while those who injected only when with friends where at least risk of infection.

Analysis of knowledge found no significance association between level of education (P<0.322; OR= 1.327; CI= 0.759-2.314), having had tested earlier (P<0.756; OR= 0.808; CI= 0.210-3.106) , people who may have influence to indulging in drug use (P<0.392; OR= 1.345; CI= 0.682-2.650), knowledge of the dangers related to sharing needles (P<0.815; OR= 1.078; CI= 0.572-2.032) and HCV infection, as summarized in table 4.2 above.

4.1.2 Factors associated with Hepatitis C genotypes among the PWIDs in Nairobi

All the 29 samples that were positive on ELISA were confirmed using PCR before proceeding for sequencing. Of the 29 ELISA antibody positive samples, 27 amplified to the expected band size of 243bp by PCR gel electrophoresis as shown by figure 4.2 and were successfully sequenced to determine the genotypes among the group.

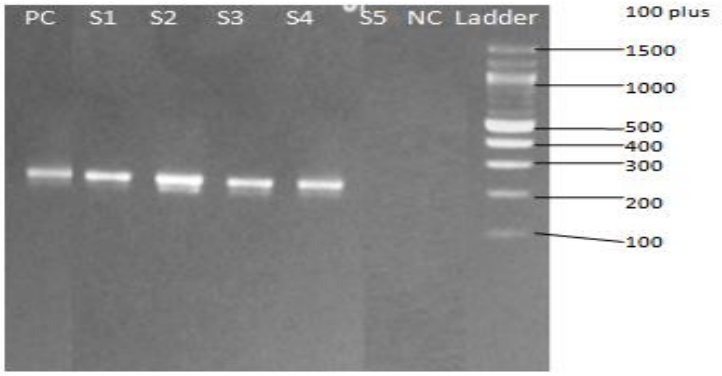


Figure 4.2 showing a representation of samples amplified in 2.0% gel positive sample are labeled S1,S2,S3,S4,S5, negative sample and an Invitrogen 100 bp plus ladder (cat. No. 239045).

The results of the successfully sequenced samples were aligned with selected reference from Hepatitis C data base center and presented in graphic form as shown in appendix (iv) and appendix (v).

The aligned sequences were used to construct a phylogenetic tree to determine their genotypes as shown in figure 4.3 below.

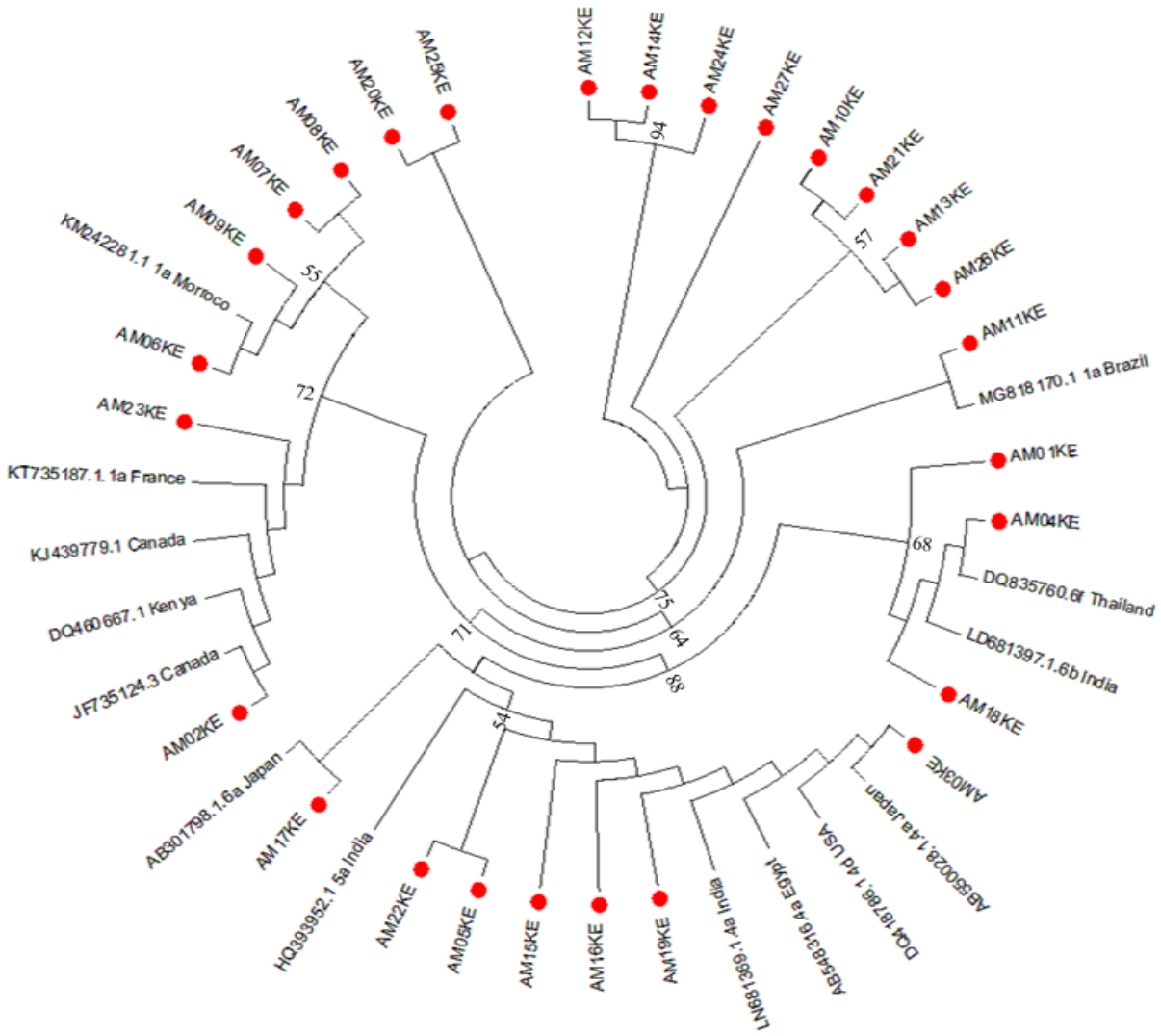


Figure 4.3 Phylogenetic tree of 27 samples marked in red aligned against representation of the different confirmed hepatitis C genotypes and subtypes tree constructed using maximum likelihood using MEGA6.

The most predominant genotype was genotype 1 subtypes a accounting for 51.85% (14/27); followed by genotype 4 subtype a accounting for 14.81% (4/27); genotype 6 with subtype a and f accounting for 14.81% (4/27); genotype 3 3.7(1/27). Two samples (7.4%) were classified as "undefined" genotypes since they did not feature within the known genotypes (Figure 4.3 above and Table 4.3 below).

Table 4.3 Showing association of various variables with HCV subtypes

Variable	Group	Genotype and Subtype						
		1a	3	4a	5a	6a	6f	Undefined
Age	21-39y	5(18.5%)	0.0%	3(11.1%)	1(3.7%)	2(7.4)%	2(7.4%)	1(3.7)%
	40-59y	8(29.6%)	1(3.7%)	1(3.7%)	1(3.7%)	0.0%	0.0%	0.0%
	Over 60	1(3.7%	0.0%	0.0%	0.0%	0.0%	0.0%	1(3.7)%
Gender	Male	11(40.7%)	1(3.7%)	2(7.4%)	1(3.7%)	2(7.4%)	2(7.4%)	2(7.4%)
	Female	3(11.1%)	0.0%	2(2.4%)	1(3.7%)	0.0%	0.0%	0.0%
Area of residence	Block A	9(33.3%)	1(3.7%)	1(3.7%)	1(3.7%)	0.0%	2(7.4%)	0.0%
	Block B	5(18.5%)	0.0%	3(11.1%)	1(3.7%)	0.0%	0.0%	1(3.7%)
	Block C	0.0%	0.0%	0.0%	0.0%	2(7.4%)	0.0%	1(3.7%)
Length of IDU	1 Year	1(3.7%)	0.0%	0.0%	0.0%	0(0.0%)	0.0%	0.0%
	Btw 1-5 y	4(14.8%)	0.0%	2(7.4%)	0.0%	0.0%	0.0%	0.0%
	Over 5 y	9(33.3%)	1(3.7%)	2(7.4%)	2(7.4%)	2(7.4%)	2(7.4%)	2(7.4)%
Frequency Of IDU	Daily	14(51.9%)	1(3.7%)	3(11.1%)	1(3.7%)	2(7.4%)	1(3.7%)	2(7.4%)
	Once/WK	0.0%	0.0%	1(3.7)%	1(3.7%)	0.0%	1(3.7%)	0.0%
Frequency Of sharing Needles	Can't buy or not given	7(25.9%)	1(3.7%)	3(11.1%)	1(3.7)%	2(7.4)%	2(7.4%)	1(3.7%)
	Have never	0.0%	0.0%	1(3.7%)	0.0%	0.0%	0.0%	0.0%
	Can't remember	7(25.9%)	0.0%	0.0%	1(3.7%)	0.0%	0.0%	1(3.7%)

Analysis of association between HCV risk factors and the detected HCV genotype showed that, those found with genotype 6 sharing most of HCV risk factors in common. Though the two clients were from different age group, both were male, from the same residential block area, both having been injecting drugs for over five years, injecting

drugs on a daily basis and they shared needle whenever they cannot buy or given their own needles. (Table 4.3)

The influence of age to the distribution of genotypes showed a varied distribution of the genotypes; genotype 1a was more common among the age group of 40-59 years; genotype 4a was more common among the age group of between 21 to 39 years. Genotype 6 a, 6f and the undefined were found among the age group of 21-39 years. In gender, genotype 1a,3,6a,6f and the undefined were mainly found among the male however genotype 4a, and 5a were relatively equally distributed among the sexes. In relation to their area of residence, genotypes 1a, 3 and 6f were mainly found among those living in block A. genotype 4a, were mainly found in block B while genotype 6a was found among those residing in block C. The undefined genotype were equally found in block B and block C The length of injecting drugs played a role, with each of the genotypes being found among those who injected drugs for over 5 years. (Table 4.3)

CHAPTER FIVE

DISCUSSION

This study reports a sero-prevalence of 13.7% which is similar to 13% that was reported previously in a study from the same region (Akiyama, Cleland, Lizcano, Cherutich, & Kurth, 2019). It is also worth noting that the sero-prevalence realized may only be a proportion of those infected since HCV has a 90 day sero-conversion period, with very sensitive antigen detection ELISA kits only able to reduce the window period to 32 days post infection and antibody based kits retaining a 90 day period (Odari, Budambula, & Nitschko, 2014), It is thus possible that PCR positive – Antibody negative samples were missed during the study. Further in the study, HCV infection rate appeared to be higher in male as opposed to female PWIDs. Indeed other studies have also demonstrated that males are more prone to HCV infection among the PWIDs compared to females (Aryan Esmaeili et al., 2017; Mainga et al., 2020). It is however notable that injecting drug use in Africa is legally and socially unacceptable, therefore this study having used a snowballing technique, there stands a possibility that more infected female PWIDs who did not come for the study and were therefore missed.

This study having been distributed into blocks for ease of sample collection, it is established a closely similar prevalence from the two collecting centers. This may be attributed to the fact that there residential area are more associated with the poor masses which drives the rate of disease transmission. Indeed other studies have revealed a high rate of transmission of HCV among the poor populations(Edmunds, Miller, & Tsourtos, 2019; Moore, Gauri, & Koru-Sengul, 2019). Poverty as a driver for transmission has several effects and raises a question on whether the current needle exchange program as a harm reduction strategy is effective. It appears that even with needle exchange, majority of poor PWIDs still exchange needles, perhaps selling their daily shares to the rich IDUs in exchange of money for more drugs a fact that need more investigation for reliable scientific evidence.

The rate of transmission among female (18.6%) was higher compared to male (12.4%). This finding could be attributed to the fact that majority (75%) of the positive female, reported to be sharing needles every time they inject drugs compared to (47%) of the HCV positive male who reported to be sharing needles every time they inject drugs. Similar finding were also realized in a study done in a systematic review and meta-analysis of 28 studies. (A. Esmaeili et al., 2017). However in their analysis they could not pinpoint the higher prevalence in female to any geographical or behavioral factors. Contrary to our finding, a study done in china on general population by (Cheng et al., 2017) found a higher prevalence in male than the female. This was postulated to be as a result of drug misuse being more common in men than women in the study.

Though participants between the ages of 21-39 years were found to indulge heavily in injecting drug use, they presented low percentage of HCV infection within the age group, this could be attributed to the fact that majority (83.3%) of those within the age group reported to have been using injecting drugs for less than 5 years, in addition 43.3% of the members in the age group reported to have neither never shared needle nor can remember the last time they share needles during drug use. Members above the age of 40 years showed an increase in prevalence of HCV. This may be due to the fact that majority of them (75.5%) reported to be injecting drugs on a daily basis and (53%) within the group shared needles every time they cannot buy or are not given their own needles. in concurrent with our finding, (Karoney & Siika, 2013; Thomas et al., 2000), found the same high prevalence among people above 40 years however no clear explanation was given in their findings. Marital status was found to be associated with HCV infection in our study, interestingly those who were married had the highest prevalence, perhaps this is a result of majority of the married who were HCV positive were found to have been injecting drugs for more than 5 years (63.3%), they inject drugs on a daily basis (81.9%) and also shared needles when cannot buy or given (36.4%), although majority could not remember when they last shared needles (54.5%), but at least have shared needles. These have been shown to be contributing factors to HCV transmission. (Aitken et al., 2017; Wenger, Rottnek, Parker, & Crippin, 2014).

The duration of drug use, the frequency of injecting drugs and the frequency of sharing drugs were found to be significantly associated with HCV infection. Majority (69%) of those who were positive had used drugs for more than five years, compared to those who have used drugs for less than five years (31%). 89.7% of the positive reported to be injecting drugs on a daily basis, and 55.2% reported to be sharing drugs when they cannot buy or given their own needles. This finding concurred with early findings in a study from New York City(Eckhardt et al., 2017), that found those who had injected for more than five years with a high frequency of injection to be at higher rich of HCV infection.

We report a significant proportion of PWID population in Nairobi, Kenya infected with a diverse groups of circulating HCV genotypes, among them, genotypes not previously known to circulate in Kenya. The existence of foreign genotypes in this population points towards a shift in the epidemiological and geographical distribution of HCV globally.

Finding of HCV distribution in Africa by (Karoney & Siika, 2013) has shown that east Africa predominantly having genotypes 1 to 3 findings which differed from our finding which mainly saw a high rate of genotype 1 and 4 circulation in the selected group. We note however that this is not the very first study reporting the circulation of genotype 1 and 4 in Kenya, since Muasya and colleagues (Tim Muasya, 2008) previously reported the circulation of genotypes 1 and 4 within a cohort of PWIDs. To the best of our knowledge however, this is the first study reporting the circulation of genotype 6a and 6f, which is predominantly known to circulate in south East Asia. The continuous finding of genotype 4 and now genotype 5 and 6 (genotypes considered foreign in the region) points towards importation of genotypes due to global migration and interactions raising concern on whether genotype specific treatment of HCV infections is still a noble idea. In concurrence to the study by Muasya et.al who found genotype 1a to be the most dominant in Kenya, in this study we also showed genotype 1a to be the most prevalent. However genotype 4 was also found to be higher than the other genotypes. (Tim Muasya, 2008).

Despite the inclusion of representatives from each of the genotype and subtypes, two of the sequences could not closely align with any of them.

CHAPTER SIX

CONCLUSION & RECOMMENDATION

6.1Conclusion

This study revealed a high risk of HCV infection among PIWDs than the general national prevalence, and associations of HCV with age, marital status, duration of injecting drugs and the frequency of injecting drugs were also found to be eminent in transmission of the disease.

This study showed a shift in HCV genotypes in the region. Although genotype 1 and 4 continues to be the most prevalent genotype, this study witnessed incidence of genotype 5 and 6 infection in the selected region. This temporal intrusion of other genotypes has fundamental implications with regard to translational efforts aimed at limiting and eradicating HCV in Kenya. Presents of samples that could not be assigned to a specific genotype or subtype demands further analysis in order to develop a better understanding of HCV genotype circulating among the selected population.

6.2 Recommendation

Preventive measures and interventions should be put in place to counter these factors among the selected group and other groups that are at high risk of infection.

Though we report intrusion of unexpected genotypes, this study did its genotypic analysis based on 5’UTR region which has however been considered not sufficient for conclusive declaration of genotypes. We therefore recommend analysis of the full genome or target other regions in order to make an informed decision from the population.

REFERENCES

Ahmed, H., Elgebaly, A., Abushouk, A. I., Hammad, A. M., Attia, A., & Negida, A. (2017). Safety and efficacy of sofosbuvir plus ledipasvir with and without ribavirin for chronic HCV genotype-1 infection: a systematicreview and meta-analysis. *Antiviral therapy, 22*(5), 369-379.

Aitken, C., Agius, P., Higgs, P., Stoove, M., Bowden, D., & Dietze, P. (2017). The effects of needle-sharing and opioid substitution therapy on incidence of hepatitis C virus infection and reinfection in people who inject drugs. *Epidemiology & Infection*, *145*(4), 796-801.

Akiyama, M. J., Cleland, C. M., Lizcano, J. A., Cherutich, P., & Kurth, A. E. (2019). Prevalence, estimated incidence, risk behaviours, and genotypic distribution of hepatitis C virus among people who inject drugs accessing harm-reduction services in Kenya: a retrospective cohort study. *The Lancet Infectious Diseases*, *19*(11), 1255-1263.

Armstrong, G. L., Wasley, A., Simard, E. P., McQuillan, G. M., Kuhnert, W. L., & Alter, M. J. (2006). The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med, 144*(10), 705-714.

Asher, A. K., Hahn, J. A., Couture, M. C., Maher, K., & Page, K. (2013). people who inject drugs, HIV risk, and HIV testing uptake in sub-Saharan Africa. *J Assoc Nurses AIDS Care, 24*(6), e35-44. doi:10.1016/j.jana.2012.09.003

Asselah, T., Lee, S. S., Yao, B. B., Nguyen, T., Wong, F., Mahomed, A.,Gane, E. (2019). Efficacy and safety of glecaprevir/pibrentasvir in patients with chronic hepatitis C virus genotype 5 or 6 infection (ENDURANCE-5, 6):

an open-label, multicentre, phase 3b trial. *The Lancet Gastroenterology & Hepatology, 4*(1), 45-51.

Bamford, D. and M. Zuckerman (2021). Encyclopedia of Virology, Academic Press.

Beaulieu, P. L., & Tsantrizos, Y. S. (2004). Inhibitors of the HCV NS5B polymerase: new hope for the treatment of hepatitis C infections. *Current opinion in investigational drugs (London, England: 2000), 5*(8), 838-850.

Bartonjo, G., & Oundo, J. (2019). Prevalence and associated risk factors of transfusion transmissible infections among blood donors at Regional

Blood Transfusion Center Nakuru and Tenwek Mission Hospital, Kenya. *The Pan African Medical Journal, 34*.

BIOKIT, S. A.-C. M. s. n.-L. d. A.-B. (2012). Bio ELISA hep c. *4.0*, 3-5.

Blackard, J. T., Shata, M. T., Shire, N. J., & Sherman, K. E. (2008). Acute hepatitis C virus infection: a chronic problem. *Hepatology (Baltimore, Md.), 47*(1), 321.

Bokharaei Salim, F., Keyvani, H., Amiri, A., Jahanbakhsh Sefidi, F., Shakeri, R., & Zamani, F. (2010). Distribution of different hepatitis C virus genotypes in patients with hepatitis C virus infection. *World J Gastroenterol, 16*(16), 2005-2009.

Cashman, S. B., Marsden, B. D., & Dustin, L. B. (2014). The humoral immune response to HCV: understanding is key to vaccine development.

Frontiers in immunology, 5, 550.

Cavallero, S., Snabel, V., Pacella, F., Perrone, V., & D'Amelio, S. (2013). Phylogeographical studies of Ascaris spp. based on ribosomal and mitochondrial DNA sequences. *PLoS neglected tropical diseases, 7*(4), e2170.

Chen, S. L., & Morgan, T. R. (2006). The natural history of hepatitis C virus (HCV) infection. *International journal of medical sciences, 3*(2), 47.

Cheng, W., Yang, Y., Zhou, Y., Xiao, P., Shi, Y., Gao, J., . . . Jiang, Q. (2017). Prevalence of hepatitis C virus infection and its correlates in a rural area of southwestern China: a community-based cross-sectional study. *BMJ Open, 7*(8), e015717. doi:10.1136/bmjopen-2016-015717.

Crema, A., Ponzetto, A., Clementi, M., & Carloni, G. (2015). Steps and routes of HCV infection: the great promise of new anti-viral targets. *Current drug targets, 16*(7), 757-770.

Day, E., Hellard, M., Treloar, C., Bruneau, J., Martin, N. K., Øvrehus, A., . . . Hickman, M. (2019). Hepatitis C elimination among people who inject drugs: challenges and recommendations for action within a health systems framework. *Liver International, 39*(1), 20-30.

Deeks, E. D. (2015). Ombitasvir/paritaprevir/ritonavir plus dasabuvir: a review in chronic HCV genotype 1 infection. *Drugs, 75*(9), 1027-1038.

Degenhardt, L., Whiteford, H. A., Ferrari, A. J., Baxter, A. J., Charlson, F. J., Hall, W. D., . . . Vos, T. (2013). Global burden of disease attributable to

illicit drug use and dependence: findings from the Global Burden of Disease Study 2010. *Lancet, 382*(9904), 1564-1574. doi:10.1016/s0140-6736 (13)61530-5.

Eckhardt, B., Winkelstein, E. R., Shu, M. A., Carden, M. R., McKnight, C., Des Jarlais, D. C., . . . Edlin, B. R. (2017). Risk factors for hepatitis C seropositivity among young people who inject drugs in New York City: Implications for prevention. *PLoS One, 12*(5), e0177341.doi:10.1371 /journal.pone.0177341.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics, 26*(19), 2460-2461. doi:10.1093/bioinformatics /btq461.

Edmunds, B. L., Miller, E. R., & Tsourtos, G. (2019). The distribution and socioeconomic burden of Hepatitis C virus in South Australia: a cross-sectional study 2010–2016. *BMC public health, 19*(1), 527.

El-Guindi, M. A. (2016). Hepatitis C viral infection in children: updated review. *Pediatric gastroenterology, hepatology & nutrition, 19*(2), 83-95.

Elrazek, A., Amer, M., El-Hawary, B., Salah, A., Bhagavathula, A. S., Alboraie, M., & Saab, S. (2017). Prediction of HCV vertical transmission: what factors should be optimized using data mining computational analysis. *Liver International, 37*(4), 529-533.

Esmaeili, A., Mirzazadeh, A., Carter, G. M., Esmaeili, A., Hajarizadeh, B., Sacks, H. S., & Page, K. A. (2017). Higher incidence of HCV in females

compared to males who inject drugs: A systematic review and meta analysis. *Journal of viral hepatitis, 24*(2), 117-127.

Esmaeili, A., Mirzazadeh, A., Carter, G. M., Hajarizadeh, B., Sacks, H. S., & Page, K. A. (2017). Higher incidence of HCV in females compared to males who inject drugs: A systematic review and meta-analysis. *J Viral Hepat, 24*(2), 117-127. doi:10.1111/jvh.12628.

Esteban, J. I., Sauleda, S., & Quer, J. (2008). The changing epidemiology of hepatitis C virus infection in Europe. *J Hepatol, 48*(1), 148-162. doi:10.1016/j.jhep.2007.07.033.

Garraud, O., Amorim Filho, L., Laperche, S., Tayou-Tagny, C., & Pozzetto, B. (2016). The infectious risks in blood transfusion as of today–a no black and white situation. *La Presse Medicale, 45*(7-8), e303-e311.

Ghany, M. G., Strader, D. B., Thomas, D. L., & Seeff, L. B. (2009). Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology, 49*(4), 1335-1374. doi:10.1002/hep.22759.

Ghisla, V., Scherrer, A. U., Nicca, D., Braun, D. L., & Fehr, J. S. (2017). Incidence of hepatitis C in HIV positive and negative men who have sex with men 2000–2016: a systematic review and meta-analysis. *Infection, 45*(3), 309-321.

Gower, E., Estes, C., Blach, S., Razavi-Shearer, K., & Razavi, H. (2014). Global epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol, 61*(1 Suppl), S45-57. doi:10.1016/j.jhep.2014.07.027.

Gowing, L. R., Ali, R. L., Allsop, S., Marsden, J., Turf, E. E., West, R., & Witton, J. (2015). Global statistics on addictive behaviours: 2014 status report. *Addiction, 110*(6), 904-919. doi:10.1111/add.12899.

Hajarizadeh, B., Grebely, J., & Dore, G. J. (2013). Epidemiology and natural history of HCV infection. *Nature reviews Gastroenterology & hepatology, 10*(9), 553.

Hall, T. A. (1999). BioEdit a user-friendly biological sequence alignment editor and analysis program. *oxford university press, 41*, 95-98.

Harania, R. S., Karuru, J., Nelson, M., & Stebbing, J. (2008). HIV, hepatitis B and hepatitis C coinfection in Kenya. *AIDS, 22*(10), 1221-1222. doi:10. 1097/QAD.0b013e32830162a8.

Harnois, D. M. (2012). Hepatitis C virus infection and the rising incidence of hepatocellular carcinoma. *Mayo Clin Proc, 87*(1), 7-8.doi:10.1016 /j.mayocp.2011.11.004.

He, T., Lopez-Olivo, M., Hur, C., & Chhatwal, J. (2017). Systematic review: cost-effectiveness of direct-acting antivirals for treatment of hepatitis C genotypes 2-6. *Alimentary pharmacology & therapeutics, 46*(8), 711-721.

Hézode, C., Asselah, T., Reddy, K. R., Hassanein, T., Berenguer, M., Fleischer-Stepniewska, K., . . . Pilot-Matias, T. (2015). Ombitasvir plus paritaprevir plus ritonavir with or without ribavirin in treatment-naive and treatment-experienced patients with genotype 4 chronic hepatitis C virus infection (PEARL-I): a randomised, open-label trial. *The Lancet, 385*(9986), 2502-2509.

Indolfi, G., Nesi, A., & Resti, M. (2013). Intrafamilial transmission of hepatitis C virus. *Journal of medical virology, 85*(4), 608-614.

Isken, O., et al. (2015). "A conserved NS3 surface patch orchestrates NS2 Protease stimulation, NS5A hyperphosphorylation and HCV genome replication." PLoS pathogens **11**(3): e1004736.

Kamenderi, M., Muteti, J., Okioma, V., Kimani, S., Kanana, F., & Kahiu, C. (2021). Status of drugs and substance abuse among the general population in Kenya. *EDITION 1: JULY 2019, 2*, 54.

Karoney, M. J., & Siika, A. M. (2013). Hepatitis C virus (HCV) infection in Africa: a review. *Pan African Medical Journal, 14*(1).

Kato, N. (2000). Genome of human hepatitis C virus (HCV): gene organization, sequence diversity, and variation. *Microbial & comparative genomics, 5*(3), 129-151.

Kenya, N. A. C. C. o. (2014). Kenya AIDS response progress report, 2014: Progress towards zero, Author Nairobi, Kenya.

Kim, C. W., & Chang, K.-M. (2013). Hepatitis C virus: virology and life cycle. *Clinical and molecular hepatology, 19*(1), 17.

Kupek, E. (2004). Transfusion risk for hepatitis B, hepatitis C and HIV in the state of Santa Catarina, Brazil, 1991-2001. *Brazilian Journal of Infectious Diseases, 8*(3), 236-240.

Lauer, G. M. (2013). Immune responses to hepatitis C virus (HCV) infection and

the prospects for an effective HCV vaccine or immunotherapies. *The Journal of infectious diseases, 207*(suppl_1), S7-S12.

Logan, M., Law, J., Wong, J. A. J.-X., Hockman, D., Landi, A., Chen, C., . Johnson, J. (2017). Native folding of a recombinant gpE1/gpE2 heterodimer vaccine antigen from a precursor protein fused with Fc IgG. *Journal of virology, 91*(1).

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., . . . Memish, Z. A. (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet, 380*(9859), 2095-2128. doi:10.1016/s0140-6736(12)61728-0

Mainga, R. O. a., Eddy, O. O., Peter, B. K., Allan, O. K., James, M. G., Vincent, R. K., . . . Raphael, L. W. (2020). Characterization of Hepatitis C Virus Circulating among Injecting Drug Users (IDU) in Kilifi County, Kenya.

Manns, M., Zeuzem, S., Sood, A., Lurie, Y., Cornberg, M., Klinker, H., . . . Merican, I. (2011). Reduced dose and duration of peginterferon alfa-2b and weight-based ribavirin in patients with genotype 2 and 3 chronic hepatitis C. *Journal of hepatology, 55*(3), 554-563.

Maqbool, M. A., Penin, F., & Lelievre, J.-d. (2012). *Impact of Hepatitis C Virus NS5A Genetic Variability on Liver Pathogenesis and Viral Replication.* Citeseer.

Mathers, B. M., Degenhardt, L., Phillips, B., Wiessing, L., Hickman, M., Strathdee, S. A., . . . Mattick, R. P. (2008). Global epidemiology of

injecting drug use and HIV among people who inject drugs: a systematic review. *Lancet, 372*(9651), 1733-1745. doi:10.1016/s0140-6736(08) 61311-2

May, S., Ngui, S. L., Collins, S., Lattimore, S., Ramsay, M., Tedder, R. S., & Ijaz, S. (2015). Molecular epidemiology of newly acquired hepatitis C infections in England 2008-2011: genotype, phylogeny and mutation analysis. *J Clin Virol, 64*, 6-11. doi:10.1016/j.jcv.2014.12.014.

McCormack, P. L. (2015). Daclatasvir: a review of its use in adult patients with chronic hepatitis C virus infection. *Drugs, 75*(5), 515-524.

Mendes, P. M. B. (2016). *Hepatitis C Virus (HCV) infection: molecular study of immune response mediators.*

Merz, F. (2018). United Nations Office on Drugs and Crime: World Drug Report 2017. 2017. *SIRIUS-Zeitschrift für Strategische Analysen, 2*(1), 85-86.

Messina, J. P., Humphreys, I., Flaxman, A., Brown, A., Cooke, G. S., Pybus, O. G., & Barnes, E. (2015). Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology, 61*(1), 77-87.

Moore, K. J., Gauri, A., & Koru-Sengul, T. (2019). Prevalence and sociodemographic disparities of hepatitis C in baby boomers and the US adult population. *Journal of Infection and Public Health, 12*(1), 32-36.

Moradpour, D. and F. Penin (2013). "Hepatitis C virus proteins: from structure to Function." Hepatitis C virus: from molecular virology to antiviral therapy: 113-142.

Muhlberger, N., Schwarzer, R., Lettmeier, B., Sroczynski, G., Zeuzem, S., & Siebert, U. (2009). HCV-related burden of disease in Europe: a systematic assessment of incidence, prevalence, morbidity, and mortality. *BMC Public Health, 9*, 34. doi:10.1186/1471-2458-9-34.

Muriuki, B. M., Gicheru, M. M., Wachira, D., Nyamache, A. K., & Khamadi, S. A. (2013). Prevalence of hepatitis B and C viral co-infections among HIV-1 infected individuals in Nairobi, Kenya. *BMC Res Notes, 6*, 363. doi:10.1186/1756-0500-6-363.

Mwangi, J., Nganga, Z., Mpoke, S., Lihana, R., Kinyua, J., Lagat, N. Ichimura , H. (2016). Hepatitis C virus genotypes in Kenya. *Arch Virol, 161*(1), 95-101. doi:10.1007/s00705-015-2623-8.

Mwatelah, R. S., Lwembe, R. M., Osman, S., Ogutu, B. R., Aman, R., Kitawi, R. C., . . . Ochieng, W. (2015). Co-Infection Burden of Hepatitis C Virus and Human Immunodeficiency Virus among Injecting Heroin Users at the Kenyan Coast. *PLoS One, 10*(7), e0132287. doi:10.1371/journal.pone.0132287.

Nelson, P. K., Mathers, B. M., Cowie, B., Hagan, H., Des Jarlais, D., Horyniak, D., & Degenhardt, L. (2011). Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. *Lancet, 378*(9791), 571-583. doi:10.1016/s0140-6736(11)61097.

Odari, E., Budambula, N., & Nitschko, H. (2014). Evaluation of an antigen-antibody “combination” enzyme linked immunosorbent assay for diagnosis of hepatitis C virus infections. *Ethiopian Journal of Health*

Sciences, 24(4), 343-352.

Organization, W. H. International Classification of Diseases for Mortality and Morbidity Statistics. Eleventh Revision. ICD-11. Reference guide. [Internet]. 2018[Consultado 27 Abr 2019].

Ozaras, R., & Tahan, V. (2009). Acute hepatitis C: prevention and treatment. *Expert review of anti-infective therapy, 7*(3), 351-361.

Page, K., Morris, M. D., Hahn, J. A., Maher, L., & Prins, M. (2013). Injection drug use and hepatitis C virus infection in young adult injectors: using evidence to inform comprehensive prevention. *Clin Infect Dis, 57 Suppl 2*, S32-38. doi:10.1093/cid/cit300.

Peacock, A., Leung, J., Larney, S., Colledge, S., Hickman, M., Rehm, J., . . . Griffiths, P. (2018). Global statistics on alcohol, tobacco and illicit drug use: 2017 status report. *Addiction, 113*(10), 1905-1926.

Petruzziello, A., Marigliano, S., Loquercio, G., Cozzolino, A., & Cacciapuoti, C. (2016). Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. *World journal of gastroenterology, 22*(34), 7824.

Poynard, T., Bedossa, P., & Opolon, P. (1997). Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet, 349*(9055), 825-832.

Qiagen. (2014). QIAGEN_protocol RNA., *4*, 22-24.

Ramya, E., Daniel, J. C., Ramalakshmi, S., & Usha, R. (2018). Prevalence and Risk Factors of Hepatitis C Virus in Irula Tribal Community, Tamilnadu, India. *Journal of PurE and aPPliEd Microbiology*, *12*(3), 1543-1552.

Ruta, S., & Cernescu, C. (2015). Injecting drug use: A vector for the introduction of new hepatitis C virus genotypes. *World J Gastroenterol*, *21*(38), 10811 -10823. doi:10.3748/wjg.v21.i38.10811.

Sarin, S. K., & Kumar, M. (2012). Natural history of HCV infection. *Hepatology international*, *6*(4), 684-695.

Schillie, S., Wester, C., Osborne, M., Wesolowski, L., & Ryerson, A. B. (2020). CDC recommendations for hepatitis C screening among adults—United States, 2020. *MMWR Recommendations and Reports*, *69*(2), 1.

Shoukry, N. H. (2018). Hepatitis C vaccines, antibodies, and T cells. *Frontiers in Immunology*, *9*, 1480.

Shunmugam, L. (2019). *In silico investigation of hepatitis c virus: a novel perspective into targeted viral inhibition of NS3 helicase, NS 3/4a protease and NS5b RNA dependent RNA polymerase.*

Smith, D. B., Bukh, J., Kuiken, C., Muerhoff, A. S., Rice, C. M., Stapleton, J. T., & Simmonds, P. (2014). Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. *Hepatology*, *59*(1), 318-327.

Smith, D. B., Pathirana, S., Davidson, F., Lawlor, E., Power, J., Yap, P. L., & Simmonds, P. (1997). The origin of hepatitis C virus genotypes. *J Gen*

Virol, 78 (Pt 2), 321-328. doi:10.1099/0022-1317-78-2-321.

Smith, M. A., Regal, R. E., & Mohammad, R. A. (2016). Daclatasvir: A NS5A replication complex inhibitor for hepatitis C infection. *Annals of Pharmacotherapy, 50*(1), 39-46.

Sorbo, M. C., Cento, V., Di Maio, V. C., Howe, A. Y., Garcia, F., Perno, C. F., & Ceccherini-Silberstein, F. (2018). Hepatitis C virus drug resistance associated substitutions and their clinical relevance: Update 2018. *Drug Resistance Updates, 37*, 17-39.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013).MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol, 30*(12), 2725-2729. doi:10.1093/molbev/mst197.

Tassiopoulos, K., Bernstein, J., & Bernstein, E. (2013). Age and sharing of needle injection equipment in a cohort of Massachusetts injection drug users: an observational study. *Addict Sci Clin Pract, 8*, 20. doi:10.1186/1940-0640-8-20.

Thomas, D. L., Astemborski, J., Rai, R. M., Anania, F. A., Schaeffer, M., Galai, N., . . . Johnson, L. (2000). The natural history of hepatitis C virus infection: host, viral, and environmental factors. *Jama, 284*(4), 450-456.

Tim Muasya, W. L., K Yano, Fredrick A Okoth. (2008). Prevalence of hepatitis c virus and its genotypes among a cohort of drug users in Kenya. *East African Medical Journal, 85*, 318-325.

Tseng, F. C., O'Brien, T. R., Zhang, M., Kral, A. H., Ortiz-Conde, B. A.,

Lorvick, J., . . . Edlin, B. R. (2007). Seroprevalence of hepatitis C virus and hepatitis B virus among San Francisco injection drug users, 1998 to 2000. *Hepatology, 46*(3), 666-671. doi:10.1002/hep.21765.

Union, A. (2019). African Union Plan of Action on Drug Control and Crime Prevention 2019-2023: Available upon request.

Vispo, E., Barreiro, P., Plaza, Z., Fernández-Montero, J. V., Labarga, P., de Mendoza, C., . . . Soriano, V. (2014). Spontaneous hepatitis C virus clearance in HIV patients with chronic hepatitis C bearing IL28B-CC alleles using antiretroviral therapy. *Aids, 28*(10), 1473-1478.

Webster, D. P., Klenerman, P., Collier, J., & Jeffery, K. J. (2009). Development of novel treatments for hepatitis C. *The Lancet infectious diseases, 9*(2), 108-117.

Wenger, P. J., Rottnek, F., Parker, T., & Crippin, J. S. (2014). Assessment of hepatitis C risk factors and infection prevalence in a jail population. *American journal of public health, 104*(9), 1722-1727.

Westbrook, R. H., & Dusheiko, G. (2014). Natural history of hepatitis C. *Journal of hepatology, 61*(1), S58-S68.

WHO. (2010). World Health Organization. Secretariat. Viral hepatitis. Sixty-Third World Health Assembly A63/15. Provisional agenda item 11.12. *A63 /15*, 1.

Williams, R. (2006). Global challenges in liver disease. *Hepatology, 44*(3), 521-526. doi:10.1002/hep.21347.

Zeisel, M. B., Felmlee, D. J., & Baumert, T. F. (2013). Hepatitis C virus entry *Hepatitis C Virus: From Molecular Virology to Antiviral Therapy* (pp. 87-112): Springer.

Zibbell, J. E., Asher, A. K., Patel, R. C., Kupronis, B., Iqbal, K., Ward, J. W., & Holtzman, D. (2018). Increases in acute hepatitis C virus infection related to a growing opioid epidemic and associated injection drug use, United States, 2004 to 2014. *American journal of public health, 108*(2), 175-181.

APPENDICES

APPENDIX I (A): INFORMED CONSENT FORM

INTRODUCTION:

Good morning /afternoon?

My name is Alex Maiyo, I am a master student of Jomo Kenyatta University of Agriculture and Technology (JKUAT). I am conducting a study on **Prevalence and molecular characterization of hepatitis c infection among people who inject drug in Nairobi** 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 HCV and HCV common genotypes among **infection among people who inject drug in Nairob**i. The results of this study were 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 was also be used in writing my thesis as part of 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 was used to find out if you are infected with hepatitis B or HCV viruses, genotype or the type of Hepatitis C virus in your body.

WHAT ARE THE RISKS OF THE STUDY?

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

WHAT ABOUT CONFIDENTIALITY?

All the information obtained was strictly confidential and data password protected only accessed by the Principal investigator, subjects/participants in the study was kept anonymous, being identified only by specific numbers assigned by the principal investigator and results obtained was 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 was transported to KEMRI for serological and the preparation of the samples for sequencing and genotype determination.

WHAT ARE THE COSTS?

There was no cost for the participants in the study.

ARE THERE BENEFITS IN TAKING PART IN THE STUDY?

The results of the study were communicated back to the group and also to the KEMRI for appropriate action. The overall results were 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 was 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:

Alex Maiyo (BSc. Microbiology)

Telephone: 0720 252 028

Email:kattammaiyo@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 CHECK ONE BOX 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 **Alex Maiyo**, 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 C miongoni mwa wanao tumia miadarati ya kujidunga jijini Nairobi. 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 wanao tumia dawa ya kujidunga. Na pia kuchunguza ili kujua ni aina gani ya virusi *(genotypes)* vya Hepatitis C 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.

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:

Alex Maiyo (BSc. Microbiology)

Simu: 0720 252 028

Pepe: kattammaiyo@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 C INFECTION AMONG PEOPLE WHO INJECT DRUG IN NAIROBI

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 ☐

002 For how long have you been using inject able drugs? _____years/months

003 How often do you use inject able drugs**? Daily** ☐ **once a WK** ☐ **only when with** ☐ **friends** ☐ **when under stress others**_____

☐

004 How often do you share needle when taking drugs**? Every time I take when**

I can't buy my own ☐ **I have never shared** ☐ **I cannot remember**

005 What do you think may have contributed to your engaging in the use of drugs?

_____

006 What dangers do you know that is related to injecting drug use? _____

007 Do you which to stop the use of drugs? YES☐ NO☐ .If yes how can you be helped to stop._____

APPENDIX II (B): KARATASI YA MASWALI (QUESTIONNAIRE)

KIWANGO CHA MAAMBUKIZI YA VIRUSI YA HEPATITIS C MIONGONI MWA WANAO TUMIA MIADARATI YA KUJIDUNGA JIJINI NAIROBI

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 ☐

002 Je umekuwa ukitumia madawa ya kujidunga kwa muda/miaka gani? _____

003 Je wewe utumia madawa ya kujitunga wakati gani? kilasiku ☐ mara moja kwa wiki Ninapokuwa na marafiki ☐ Ninapo tatisika ☐ Sababu ngingine ☐

_____

004 Je wewe utumia shindano moja wakati gani na wenzako unapo tumia dawa?

Kilawakati wakati siwez ☐ hunua yangu ☐ awai shiriki Si w ☐ kukumbuka

005 Je unathani ni nini iliyo sababisha utumie madawa ya kujitungia?

_____

006 Je ni hatari kani unayo jua inayo usika na utumiaji wa madawa ya kujidunga?

_____

007 Je ungependa kuwacha utumiaji wa madawa ya kujidunga? NDIO ☐ LA ☐. Ikiwa ndio ungependa kusaidiwa vipi?.

_____

APPENDIX III: ETHICAL APPROVAL

KENYA MEDICAL RESEARCH INSTITUTE

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

KEMRI/RES/7/3/1 **June 15, 2017**

TO: **BENARD KIBET LANGAT**
PRINCIPAL INVESTIGATOR

THROUGH **THE DIRECTOR, CVR,**
NAIROBI

21/6/17

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

Dear Sir,

RE: SSC PROTOCOL NO. 2209 *(RESUBMISSION REQUEST FOR AMENDMENT 4)*: EPIDEMIOLOGY OF HEPATITIS VIRUSES, MOLECULAR CHARACTERIZATION OF PREVAILING HEPATITIS A AND B VIRUSES AND EVALUATION OF HEPATITIS B VACCINE EFFICACY IN WESTERN KENYA

Reference is made to your letter dated April 19, 2017. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the following revised study documents on May 04, 2017;

The Committee noted the following amendments:

1. Inclusion of new study sites of Nairobi and Coastal regions.
2. Deletion of the word "western" from the study title.
3. Addition of two new KEMRI staff; James Gikunda and Alex Maiyoas co-investigators.

This is to inform you that the committee determines that the issues raised during the 261st Committee C meeting of the KEMRI Scientific and Ethics Review Committee held on **March 30, 2017** have been adequately addressed.

You are therefore **authorized** to implement the above amendments accordingly.

Please note that you are responsible for submitting any further changes to the approved version of the study protocol to SERU for review and the changes should not be initiated until written approval from the SERU is received

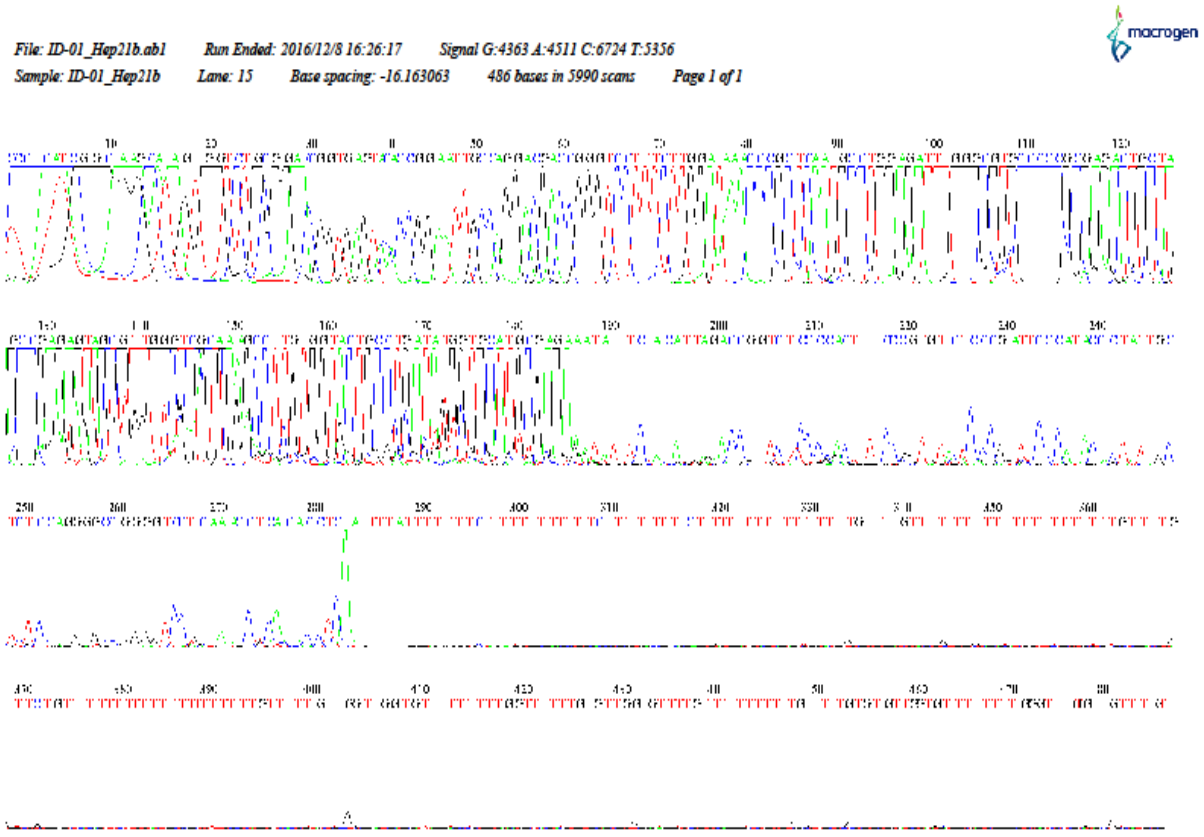
Yours faithfully,

DR. MERCY KARIMI NJERU,
ACTING HEAD,
KEMRI /SCIENTIFIC AND ETHICS REVIEW UNIT

CENTRE FOR VIRUS RESEARCH
20 JUN 2017
P.O. Box 54628 - NAIROBI

In Search of Better Health

APPENDIX IV: RAW SEQUENCE DATA.



APPENDIX V: SHOWING ALIGNED SEQUENCES WITH REFERENCE