PREVALENCE, SOCIAL DEMOGRAPHICS AND CHARACTERIZATION OF HEPATITIS C GENOTYPES CIRCULATING AMONG INJECTING DRUG USERS IN KILIFI COUNTY, KENYA

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Prevalence, Social Demographics and Characterization of Hepatitis C Genotypes Circulating Among Injecting Drug Users in Kilifi County, Kenya

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DECLARATION

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

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DEDICATION

This work is dedicated to my wife Rose Mogoi Arika and my children Tiffany Kemunto, Stephany Kerebi and Kalyha Kerubo. I also dedicate this work to my parents Alfred Mainga Ntabo and Dorica Nyanchama Obare who believe and know the value of education.

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LIST OF ABBREVIATIONS AND ACRONYMS

aa	Amino acid
CBO	Community Based Organization
CDC	Centers for Disease Control and Prevention
EASL	European Association for the Study of the Liver
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
ESLD	End-stage liver disease
FBOs	Fixed-base operator
HCC	hepatocellular cancer
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
IDU	Injecting Drug Users
IDPC	International Drug Policy Consortium
IRES	Internal ribosome entry site
KANCO	Kenya AIDS NGOs Consortium
LDL	Low density lipoproteins
βME	beta-mercaptoethanol
MEWA	Muslim Education Welfare Association
NS	Non-structural (NS) proteins
nt	Nucleotides
OR	Odds ratio
OECD	Organization for Economic Co-operation and Development
ORC	Omari rehabilitation center

ORF	Open reading frame
PCR	Polymerase Chain Reaction
Peg-IFN-a	Pegylated interferon a
RBV	Ribavirin
RNA	Ribonucleic acid
RdRp	RNA-dependent RNA polymerase (one of non-structural protein)
RT-PCR	Reverse transcription and PCR
SD	Standard deviation
SEM	Standard error mean
SPSS	Statistical Package for the Social Sciences
SVR	Sustained virological response
UNDOC	United Nations Office on Drugs and Crime
UTR	Untranslated regions
VLDL	Very-low density lipoproteins
WHO	World Health Organization

ABSTRACT

Hepatitis C virus is a major global health problem estimated to infect over 170 million people globally with the most common route of infection being injecting drug use (IDU). Treatment for HCV infection has traditionally been shown to be genotype specific; however the available drugs are still expensive and out of reach in many developing countries. To improve on monitoring, there is need to continuously document the genotypic burden and epidemiology in different populations. This study aimed to determine HCV prevalence and circulating genotypes and link the data to the socio-demographic characteristics of injecting drug users in Kilifi County along the Kenyan coastline. A cross-sectional survey was conducted among 127 injecting drug users in Watamu, Malindi and Kilifi County. Serology was done for detection of HCV antibodies followed by amplification of HCV RNA using RT-PCR and eventual sequencing of amplified nucleic acid at the ORF conserved region to establish the circulating genotypes. Socio-demographic data was collected using questionnaire administered at the sample collection facilities. In the study, demographic characteristics for Tout/drivers/bodaboda group of IDUs had the highest HCV infection 12(21.8%, OR=1) compared to Beach boy, Fisherman and other IDUs with different occupations. Percentage OR for HCV infection were 1.3 (95% CI: 0.4-4.3) for beach boys, 0.8 (95% CI: 0.2-3.1) for fishermen and 0.9 (95% CI: 0.3-2.6) for IDUs with other occupations. In education, lower primary had 13 (20.0%) infection compared to IDUs with upper primary 6 (16.7%) and secondary and tertiary education 9 (34.6%). Percentage ORs for HCV infection were 1.0 for IDUs with education level of lower primary, 0.8 (95% CI: 0.3-2.3) for upper primary, 2.1 (95% CI: 0.8-5.8) for IDUs with secondary and tertiary education. A total of 28 (23 males and 5 females) samples out of 127 samples were positive for HCV giving a sero-prevalence of 22.1%. 11 (39.3%) samples, all from the male participants were detected as antibody positive PCR positive, showing potential for acute infection and the remaining 17 (60.7%) antibody positive PCR negative. The most prevalent genotype was genotype 4a accounting for 87% with genotype 1a accounting for the remaining 13%. Kilifi town only recorded genotype 4a with Watamu and Malindi towns recoded multiple genotypes of 1a and 4a. Phylogenetic analysis established 100% concordance between the local strains and strains from, Portugal, France, Egypt, Cyprus, Southeast Asia, Middle East, Saudi Arabia, America, Japan, Indonesia, China and India. In the study, prevalence of HCV infection among IDUs in Kilifi County is not reducing despite the fact that a lot of investment has been put on harm reduction strategies such as the needle exchange programs; the study also reveals a potential importation of genotype 4a which has been previously considered foreign in the region. Finally, this study recommends continuous molecular surveillance of circulating HCV genotypes among Injecting drug users which act as a bridge of infection to the general population.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Hepatitis C is a global health problem as World Health Organization (WHO) reported that 3-4 million people are newly infected with hepatitis C virus (HCV) per year and 130-170 million people are chronically infected (Amal, 2015). Hepatitis C virus (HCV) causes 308000 deaths due to liver cancer and 758000 deaths due to cirrhosis every year (Seyed and Farshid, 2014). Totally, 170 million people have HCV which is not considered a malignant infectious disease while compared to its annual mortality rate (Longo et al., 2011). Hepatitis C virus is the cause of hepatitis C disease and some cancers such as hepatocellular carcinoma, abbreviated HCC and lymphomas in humans (Ferri, 2015; Rusyn, 2014). It is estimated that about 80% of HCV infected victims will develop chronic hepatitis. About 3-11% of those will progress to liver cirrhosis during 20-year time interval and the risk of liver failure and hepatocellular carcinoma increases as a life-threatening factor (Te and Jensen, 2010). Hepatitis C is estimated to be the leading cause of liver transplantation in the world (Carmen et al., 2013). The main route for HCV transmission is blood-borne including contaminated blood transfusion or blood products, needle or syringe sharing among members of intravenous drug parties or undergoing a needle stick by health workers. Other risk factors are high-risk sexual behaviors, tattooing, shaving in contaminated barber, reused and unsterilized dental and surgical instruments, and carelessly prepared laboratory equipment (Samimi-Rad *et al*., 2012 and Rahimi-Movaghar *et al*., 2010). Currently, the major contributors and driving risk factor for localized and universal spread of HCV infection are injection drug users (IDUs). A study done on the prevalence of HCV and its genotypes among a cohort of drug users in Kenya estimates the prevalence in the general population to be between 0.2% - 0.9% (Muasya, *et al*., 2008). A study done on co infection of HCV, HIV and HBV among IDUs in major cities that is Nairobi, Mombasa and Kisumu in Kenya refills that HCV is 6.5% (Micah *et al.*,2018) Factors such as the immune response, determined by host genetics, gender, mode of acquisition, the severity of the acute illness, presentation with jaundice, a poorly defined weak immune response, immunosuppression with for

example corticosteroid treatment, which can affect clearance of HCV and HIV co-infection, are all determinants of the acute response. This means that the time course of clearance is difficult to establish with certainty.

People who clear HCV infection generally have limited viral diversity, which also points to enhanced immune-mediated response to acute infection (Karla *at al*., 2018). The rate of HCV production is high, 10^{10} to 10^{12} virions in a person per day, and the lack of proofreading by the viral polymerase leads to enormous genetic diversity, which in turn creates a major challenge for the host immune response. This broad genetic diversity contributes to the high likelihood of developing chronic infection. Host factors also play an important role in whether or not an individual will go on to spontaneously clear HCV (Karla *at al*., 2018). Human and animal studies indicate that clearance of HCV is associated with strong and persistent HCV-specific cytotoxic T-lymphocyte and CD4 lymphocyte responses (Karla *at al*., 2018). The aim of this study was to invest get the prevalence of HCV among IDUs in Kilifi County, estimated HCV genotypes that are circulating in the region and correlate the genotypes with Social demographics of IDUs in the area.

1.2 Statement of the problem

It is estimated that 71 million people globally have chronic hepatitis C infection (Tun *et al*., 2013), who are at risk of developing liver cirrhosis and liver cancer (Meryem *et al.,* 2018). Hepatitis C course liver cancer, predominantly hepatocellular carcinoma (HCC). It is the second most deadly cancer worldwide (Yujin *et al*., 2014). The developing countries mostly in sub-Saharan Africa, chronic infection with hepatitis C virus (HCV) has been responsible for the increasing HCC (Yujin *et al*., 2014). Eastern Africa leads in opiate use in Africa, with Kenya among the top two heroin using countries according to the 2011 World Drug Report **(**World Drug Report 2016). Conservative statistics suggest that substance use continues to rise in Kenya particularly in the cosmopolitan coastal region including Kilifi County, mostly influenced by tourism boom, illicit drug trafficking and escalated by cultural attributes (Mwatelah *et al.,* 2015). This accounts for 399000 deaths every year. Among various genotypes of HCV, genotype 1 is the most prevalent which accounts for 46% of all HCV infections, followed by genotype 3, which is 22% prevalent. Genotype 2 and 4 each has 13% prevalence (Mahajan *et al.,* 2014). As death occurs decades after being

infected, people dying of liver conditions are often not linked to underlying viruses (Meryem *et al.,* 2018). Symptoms of acute infection are generally mild and majority of cases of acute infection are asymptomatic and remain undiagnosed. Spontaneous clearance of acute HCV infection occurs within 6 months of infection in 15–45% of infected individuals in the absence of treatment (Westbrook and Dusheiko, 2014). Infection with HCV does not result in long-term immunity. This is because virus-specific T cells are exhausted and functionally impaired because of sustained antigenic stimulation. As a result, virus-specific T cells have a poor proliferation capacity, weak cytolytic activity and suppressed cytokine production. This leads to re-infection after recovery has been largely reported (Lanini *et al*., 2016). Furthermore, in settings with high HCV prevalence, mixed HCV genotype infections, are major consequence of multiple exposures as a result of asymptomatic which is also becoming common (Layden *et al*., 2015). The inability to mount an effective protective immune response and the high variability of HCV genotypes (7 genotypes and 67 confirmed subtypes) (Lanini *et al.,* 2016), has hindered progress in vaccine development.

1.3 Justification of the study

As opposed to HIV and HBV infections, HCV is mainly transmitted via blood with needle sharing being the main reason (Yehia *et al.,* 2014). In Kenya, needle sharing through injecting drug use is gradually becoming a public health burden (Matthew *et al*., 2019). The fact that the data on the burden of HCV among the IDU population in Kenya is still scanty justifies the reason to conduct and document this burden among this population (Matthew *et al*., 2019). Further, Kenyan coast is a major tourist destination with sex tourism becoming a norm. The latter coupled with injection of drugs and sexual act among the beach populations ensures a potential for infection and transmission of foreign HCV genotypes in this region (Matthew *et al*., 2019). The fact that treatment of HCV is genotype specific and the realization that circulating genotypes in the region are not fully documented further justifies the need to document such circulating genotypes and correlate them to the socio-demographic characteristics of the infected patients (Matthew *et al*., 2019). Knowledge on this study will ensure improved surveillance mechanisms that would lead to effective mitigation measures. Genotyping of HCV in patients is necessary, for proposing therapeutic

protocols (Longo *et al*., 2011). An argument has been advanced that if the same trend continues on youth and drug abuse for the next 10 years, then the coast region will miss a whole generation (Korir, 2013). The high number of chronically infected individuals, the burden of disease and the absence of a vaccine indicates that treatment will form part of the control of the disease. However, the asymptomatic patients with persistent infection are unaware of the infection and screening programs to identify the infected will be required to prevent silent progression of the disease (Mohd *et al*., 2013 and D 2011). This study aimed to understand the circulating genotypes of HCV among IDUs undergoing voluntary rehabilitation care in Kilifi County, Kenya. Data from this study will form basis on policy formulation by the ministry of Health on antiviral treatment to eradicate the disease from the area.

1.4 Research Questions

1. What is the prevalence of HCV in injecting drug users (IDUs) in Kilifi County, Kenya?
2. What are the socio demographic characteristics of HCV among the IDUs in Kilifi County, Kenya?
3. What are the genotypes of HCV circulating among IDUs patients in Kilifi County, Kenya?

1.5 Objective

1.5.1 Broad Objective

To determine HCV prevalence, socio demographics and the circulating genotypes among IDUs in Kilifi County

1.5.2 Specific objectives

1. To determine prevalence of HCV in injecting drug users **(**IDUs**)** in Kilifi County, Kenya
2. To establish the social demographics of IDUs populations with HCV living in Kilifi County, Kenya
3. To identify different HCV genotypes circulating among IDUs in Kilifi County, Kenya

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology of Hepatitis C Virus

The hepatitis C virus (HCV) is an- enveloped, single-stranded, positive-sense RNA virus (Rosen, 2011). It is a member of the Hepacivirus genus in the family Flaviviridae (Peter *et al.*, 2017). The Flaviviridae family is divided into three genera: flavivirus, pestivirus, and hepacivirus. Flaviviruses include yellow fever virus, dengue fever virus, Japanese encephalitis virus, and Tick-borne encephalitis virus. Pestiviruses include bovine viral diarrhea virus, classical swine fever virus and Border disease virus. Since the identification of HCV in the late 1980s, six major genotypes have been recognized. Genotypes 1, 2, 3, 4, and 6 are further subdivided into a series of subtypes. The complete genomes of genotypes differ from each other by ≥30% at the nucleotide level, while those of subtypes within a given genotype differ typically by 15% to 25% (Smith *et al*., 2014). Based on genetic differences between HCV isolates, the hepatitis C virus species is classified into six genotypes (1–6) with several subtypes within each genotype (represented by lower-cased letters) (Nakano *et al*., 2011). Subtypes are further broken down into quasispecies based on their genetic diversity. Subtypes 1a and 1b are found worldwide and cause 60% of all cases. The genotypes differ from each other by 15% of their sequences (Smith *et al.*, 2014; Charlotte *et al*.,2019). Below (Table 1) gives a summary of CHV genotypes and their sub types with their locus as distinguishing futures, access number and their references.

Table 2.1 Confirmed Hepatitis C Virus genotypes/subtypes (Courtesy of Smith *et al.*, 2014)

Genotype[1]	**Locus/Isolate(s)**[2]	**Accession number(s)**	**Reference(s)**
Genotype 1			
1a	HPCPLYPRE, HPCCGAA	M62321, M67463	(Choo *et al.*, 1991; Inchauspe *et al.*, 1991)
1b	HPCJCG, HPCHUMR	D90208, M58335	(Kato *et al.*, 1990; Takamizawa *et al.*, 1991)
1c	HPCCGS, AY051292	D14853, AY051292	(Okamoto *et al.*, 1994)(Guntaka et al. unpublished 2001)
1d	QC103	KJ439768	(Lu *et al.*, 2014)
1e	148636, QC172	KC248194, KJ439769	(Li *et al.*, 2013), (Lu *et al.*, 2014)
1g	1804, QC78	AM910652, KJ439770	(Bracho *et al.*, 2008) (Lu *et al.*, 2014)
1h	EBW443, EBW9	KC248198, KC248199	(Li *et al.*, 2013)
1i	QC181	KJ439772	(Lu *et al.*, 2014)
1j	QC329	KJ439773	(Lu *et al.*, 2014)
1k	QC82	KJ439774	(Lu *et al.*, 2014)
1l	136142, EBW424	KC248193, KC248197	(Li *et al.*, 2013)
1m	QC196, QC87	KJ439778, KJ439782	(Lu *et al.*, 2014)
1n	QC113, QC74	KJ439775, KJ439781	(Lu *et al.*, 2014)
1o	QC316, DE/17-0414	KJ439779, MH885469	(Lu *et al.*, 2014; Wang *et al.*, 2019)
Genotype 2	**Locus/Isolate(s)**[2]	**Accession number(s)**	**Reference(s)**
2a	HPCPOLP, JFH-1	D00944, AB047639	(Kato *et al.*, 2001; Okamoto *et al.*, 1991)
2b	HPCJ8G, JPUT971017	D10988, AB030907	(Murakami *et al.*, 2001; Okamoto *et al.*, 1992)
2c	BEBE1	D50409	(Nakao *et al.*, 1996)
2d	QC259	JF735114	(Li *et al.*, 2012)
2e	QC64	JF735120	(Li *et al.*, 2012)
2f	ZS542, GZ98799	KC844042, KC844050	(Xu *et al.*, 2013)
2i	D54	DQ155561	(Noppornpanth *et al.*, 2006)
2j	C1799, QC232	HM777358 JF735113	(Sulbarán *et al.*, 2010) (Li *et al.*, 2012)
2k	VAT96	AB031663	(Samokhvalov *et al.*, 2000)
2l	MRS89, PTR7904	KC197235, KC197240	(Jordier *et al.*, 2013)
2m	QC178, BID-G1314	JF735111, JX227967	(Li *et al.*, 2012; Newman *et al.*, 2013)
2q	963, 852	FN666428, FN666429	(Martró *et al.*, 2011)
2r	QC283	JF735115	(Li *et al.*, 2012)
2t	MRS40	KC197238	(Jordier *et al.*, 2013)
2u	QC182	JF735112	(Li *et al.*, 2012)
Genotype 3	**Locus/Isolate(s)**[2]	**Accession number(s)**	**Reference(s)**
3a	HPCEGS, HPCK3A	D17763, D28917	(Sakamoto *et al.*, 1994; Yamada *et al.*, 1994)
3b	HPCFG	D49374	(Chayama *et al.*, 1994)

3d	NE274	KJ470619	(Li *et al.*, 2014a)
3e	NE145	KJ470618	(Li *et al.*, 2014a)
3g	BID-G1243, QC260	JX227954, JF735123	(Lu *et al.*, 2013; Newman *et al.*, 2013)
3h	QC29	JF735121	(Lu *et al.*, 2013)
3i	IND-HCV, BID-G1244	FJ407092, JX22795	(Arankalle & Gupte 2008 unpublished)(Newman *et al.*, 2013)
3k	HPCJK049E1, QC105	D63821, JF735122	(Lu *et al.*, 2013; Tokita *et al.*, 1996)
Genotype 4	**Locus/Isolate(s)[2]**	**Accession number(s)**	**Reference(s)**
4a	ED43	Y11604	(Chamberlain *et al.*, 1997a)
4b	QC264	FJ462435	(Li *et al.*, 2009a)
4c	QC381	FJ462436	(Li *et al.*, 2009a)
4d	03-18, QC382	DQ418786, FJ462437	(Timm *et al.*, 2007) (Li *et al.*, 2009a)
4f	IFBT88, PS6	EF589161, EU392175	(Hmaied *et al.*, 2007) (Kuntzen *et al.*, 2008)
4g	QC193	FJ462432	(Li *et al.*, 2009a)
4k	PS3, QC383	EU392173, FJ462438	(Kuntzen *et al.*, 2008) (Li *et al.*, 2009a)
4l	QC274	FJ839870	(Li *et al.*, 2009a)
4m	QC249	FJ462433	(Li *et al.*, 2009a)
4n	QC97	FJ462441	(Li *et al.*, 2009a)
4o	QC93	FJ462440	(Li *et al.*, 2009a)
4p	QC139	FJ462431	(Li *et al.*, 2009a)
4q	QC262	FJ462434	(Li *et al.*, 2009a)
4r	QC384	FJ462439	(Li *et al.*, 2009a)
4s	QC361	JF735136	(Lu *et al.*, 2015)
4t	QC155	FJ839869	(Li *et al.*, 2009a)
4v	CYHCV073, BID-G1248	HQ537009, JX227959	(Demetriou & Kostrikis, 2011) (Newman *et al.*, 2013)
Genotype 5	**Locus/Isolate(s)[2]**	**Accession number(s)**	**Reference(s)**
5a	EUH1480, SA13[4]	Y13184 ,AF064490	(Bukh *et al.*, 1998; Chamberlain *et al.*, 1997b)
Genotype 6	**Locus/Isolate(s)[2]**	**Accession number(s)**	**Reference(s)**
6a	EUHK2, 6a33	Y12083, AY859526	(Tokita *et al.*, 1998)
6b	Th580	D84262	(Tokita *et al.*, 1998)
6c	Th846	EF424629	(Lu *et al.*, 2007a)
6d	VN235	D84263	(Tokita *et al.*, 1998)
6e	GX004	DQ314805	(Li *et al.*, 2006)
6f	C-0044	DQ835760	(Lu *et al.*, 2007b)
6g	HPCJK046E2	D63822	(Tokita *et al.*, 1996)
6h	VN004	D84265	(Tokita *et al.*, 1998)
6i	Th602	DQ835770	(Lu *et al.*, 2007b)
6j	Th553	DQ835769	(Lu *et al.*, 2007b)
6k	VN405	D84264	(Tokita *et al.*, 1998)

6l	537796	EF424628	(Lu *et al.*, 2007a)
6m	B4/92	DQ835767	(Lu *et al.*, 2007b)
6n	KM42, D86/93	DQ278894, DQ835768	(Lu *et al.*, 2007b; Lu *et al.*, 2006)
6o	QC227	EF424627	(Lu *et al.*, 2007a)
6p	QC216	EF424626	(Lu *et al.*, 2007a)
6q	QC99	EF424625	(Lu *et al.*, 2007a)
6r	QC245	EU408328	(Li *et al.*, 2009b)
6s	QC66	EU408329	(Li *et al.*, 2009b)
6t	VT21, D49	EF632071, EU246939	(Lu *et al.*, 2008; Noppornpanth *et al.*, 2008)
6u	D83	EU246940	(Noppornpanth *et al.*, 2008)
6v	NK46, KMN-02	EU158186, EU798760	(Lu *et al.*, 2008) (Wang *et al.*, 2009)
6w	GZ52557, D140	DQ278892, EU643834	(Lee *et al.*, 2010; Lu *et al.*, 2006)
6xa[5]	DH012, DH028	EU408330, EU408332	(Xia *et al.*, 2008)
6xb	TV476, VN110	JX183552, KJ567645	(Li *et al.*, 2014b; Wang *et al.*, 2013)
6xc	TV520	KJ567651	(Li *et al.*, 2014b)
6xd	L23, L347	KM252789, KM252790	(Li *et al.*, 2015)
6xe	DH027, KM98	JX183557, KM252792	(Li *et al.*, 2015; Wang *et al.*, 2013)
6xf	VN214, TV469	KJ567647, KJ567646	(Li *et al.*, 2014b)
6xg	KS27, KS81	MH492360, MH492361	(Ye *et al.*, 2019)
6xh	1350-1	MG879000	(Wu *et al.*, 2018)

2.2 Hepatitis C Virus Structure

Hepatitis C virus particles are 50–80 nm in diameter (Catanese *et al*., 2013) and contain the single-stranded RNA genome, core and the envelope glycoproteins, E1 and E2 (Vieyres *et al*., 2014). The HCV genome interacts with the core protein to form the nucleocapsid that is surrounded by a lipid membrane, called the viral envelope, in which the envelope glycoproteins are anchored (Figure 2.1). Importantly, due to virion association with lipoproteins, apolipoproteins such as apoE, apoB, apoA1, apoC1, apoC2, and apoC3 can also be found in association with HCV particles (Meunier *et al*., 2008). Furthermore, a characterization of cell culture-produced particles indicates that their lipid composition resembles very-low density lipoproteins (VLDL) and low-density lipoproteins (LDL) with cholesteryl esters accounting for almost half of the total HCV lipids (Merz *et al*., 2011). Electron microscopy analyses of purified infectious virions confirm the pleomorphic nature of HCV particles and show virions with a rather smooth and even surface (Catanese *et al*., 2013). HCV virion could be a

hybrid particle composed of a virion moiety and a lipoprotein moiety (Bartenschlager *et al*., 2011). However, alternative models have also been suggested, with lipoproteins peripherally associated with canonical viral particles via interaction between apolipoproteins and HCV envelope lipids or proteins (Lindenbach, 2013). In both particle types, the interaction with lipoproteins could contribute to the shielding of HCV glycoproteins from the host immune response and explain the poor detection or availability of HCV glycoproteins at the virion surface (Catanese *et al*., 2013; Merz *et al*., 2011; Dao *et al*., 2012). Hepatitis C Virus envelope glycoproteins are the major viral determinants of HCV entry. They indeed play a role in receptor binding and mediate the fusion process between the viral envelope and an endosomal host cell membrane. Hepatitis C Virus glycoproteins E1 and E2 are type I transmembrane proteins, which form a non-covalent heterodimer within infected cells, whereas they assemble as large covalent complexes stabilized by disulfide bonds on the viral particle (Vieyres *et al*., 2010). At least, a fusion partner of an E1E2 fusion complex formed upon conformational rearrangements (Douam *et al*., 2014).

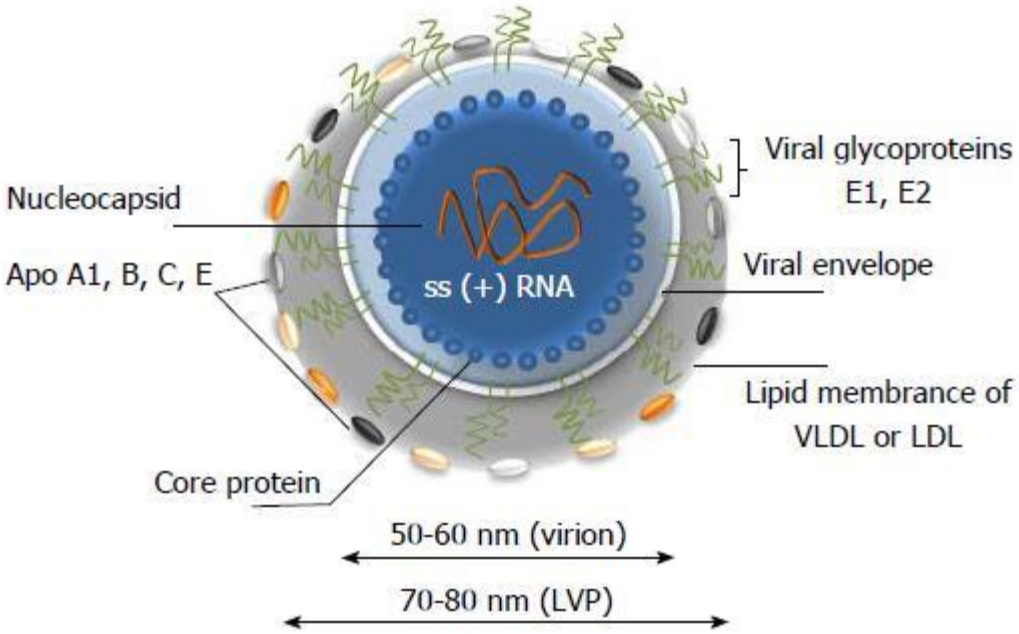

Figure 2.1 Viral structure of Hepatitis C Virus (Vladimir and Sylvie, 2018)

2.3 Genome organization of Hepatitis C Virus

Hepatitis C Virus is a positive-strand RNA virus encoding a single polyprotein precursor (Simmonds, 2013) that is generated by RNA translation at the rough endoplasmic reticulum (ER). This polyprotein is proteolytically processed into 10 mature proteins in a preferential, but not obligatory order (Figure 2.2) (Niepmann, 2013; Moradpour and Penin, 2013). Of these, p22 separates the structural proteins (that is, Core and the envelope glycoproteins E1 and E2) from the nonstructural proteins that save for NS2 are essential for viral RNA replication (NS3, NS4A, NS4B, NS5A and NS5B) (Lohmann, 2013). The core domain of the hepatitis C virus (HCV) IRES contains a four-way helical junction that is integrated within a predicted pseudoknot (Berry *et al*., 2011).

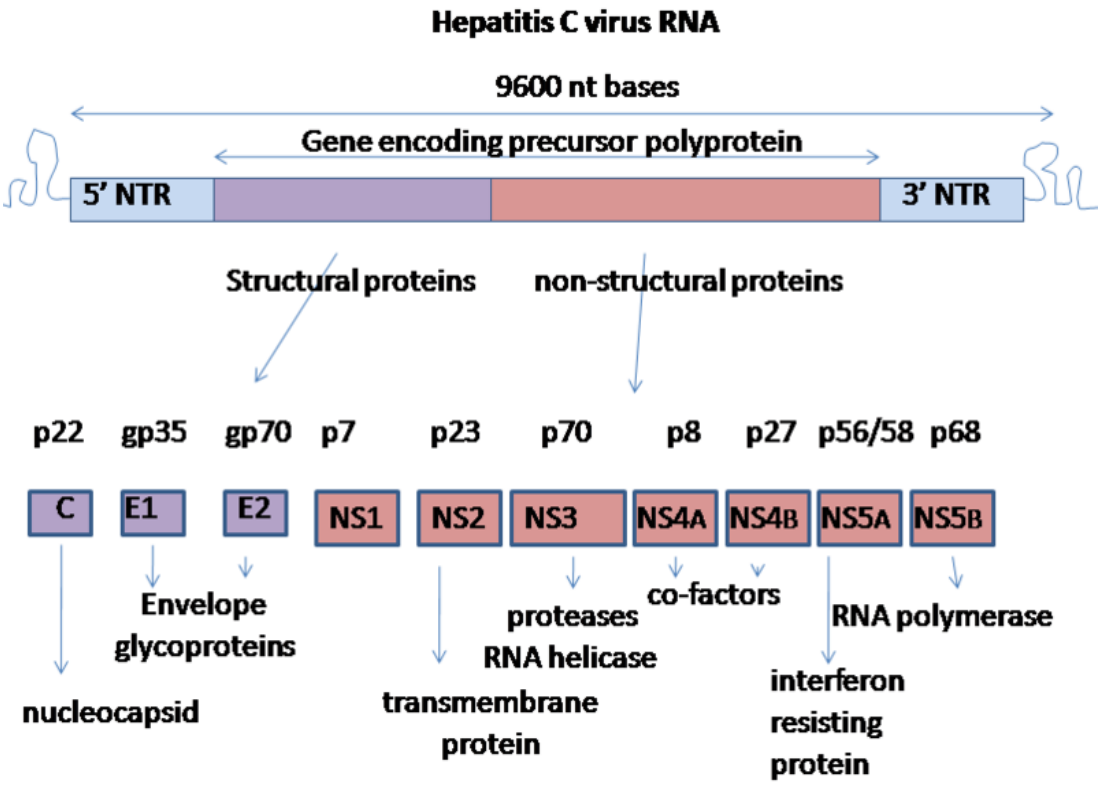

Figure 2.2 Genome organization of Hepatitis C virus (Usman, 2011)

2.4 Epidemiology of Hepatitis C Virus genotypes

Hepatitis C virus (HCV) is one of the major causes of death and morbidity globally (Cooke *et al*., 2013) and recent estimates showed an increase in its seroprevalence over the last decade to 2.8%, corresponding to > 185 million infections worldwide (Mohd *et al*., 2013). The "epidemic subtypes" - specifically 1a, 1b, 2a, and 3a - are widely distributed worldwide and account for a great proportion of the totality of HCV cases, especially in high income countries. They were probably spread in the 70's and 80's, before HCV sequencing, through transfusion, blood products and drug abusers (Smith *et al*., 2014). The genotype 2 strains from Africa can be divided into four clades that correlate with their country of origin: (1) Cameroon and Central African Republic (2) Benin, Ghana and Burkina Faso (3) Gambia, Guinea, Guinea-Bissau and Senegal (4) Madagascar (Markov *et al*., 2009). There is also strong evidence now for the dissemination of hepatitis C virus genotype 2 from West Africa to the Caribbean by the Trans-Atlantic slave trade (Markov *et al*., 2012). Genotype 3 is thought to have its origin in South East Asia (Li *et al*., 2014). Genotype 4 is responsible for most of the infections in IDUs of north-eastern of Poland (Chlabicz *et al*., 2008). In Kenya, a study which was done by Muasya *et al*.,2008 among IDUs refills that genotype 1a and 4were present. A study done by Joseph Mwangi *et al*.,2015 among blood doners refills genotype 1a and 2b. Further the study carried out by Micah *et al*.,2018 indict that genotype 1a and 4 are circulating in Kenya. The "endemic" strains, instead, are comparatively rare and have been restricted for long time in specific regions, as West Africa, Southern Asia, Central Africa and South Eastern Asia (Smith *et al*., 2014). A study done on the prevalence of HCV and its genotypes among a cohort of drug users in Kenya which was genotype 1a and 4 estimates the prevalence in the general population to be between 0.2%-0.9% (Muasya, *et al*., 2008). In Malindi Sub County, Kenya, the prevalence of HCV among IDUs is 16.4% (Mwatelah *et al.,* 2015) but there is no data on prevalence of HCV infections among IDUs in Kilifi County as a whole. Conservative statistics suggest that drug use continues to rise in Kenya particularly in the cosmopolitan coastal districts; this continue to raise HCV infection in the area and is mostly influenced by tourism boom, illicit drug trafficking and escalated by cultural attributes (Chesang, 2013).

2.5 Transmission of Hepatitis C virus

Hepatitis C virus is most often transmitted parenterally, through blood transfusion, tattooing and injecting drug use (IDUs) by sharing of sharp objects like needles, but it is also transmitted vertically and sexually (CDC 2017). Hepatitis C Virus is up to 4 times more infectious than Human Immunodeficiency Virus (HIV). It also requires less exposure than HIV to cause infection (Te and Jensen, 2010). The incubation period for hepatitis C is between 2 weeks to 6 months, following initial infection. Approximately 80% of people do not exhibit any symptoms. Those who are acutely symptomatic may exhibit fever, fatigue, decreased appetite, nausea, vomiting, abdominal pain, dark urine, grey-coloured faeces, joint pain and jaundice (yellowing of skin and the whites of the eyes) (Lozano *et* al., 2012). The acute illness is clinically mild and typically unrecognized and thus, it is only infrequently diagnosed, particularly in those who progress to chronic hepatitis. Accurate studies of the time course for clearance of acute hepatitis C are difficult to carry out because of the silent onset of the acute disease. Studies to determine the rate of persistence are few and may be biased by the mode of ascertainment. They frequently involve the prospective study of symptomatic individuals, who are more likely to clear the virus (J *et al*., 2014). Asymptomatic individuals are more difficult to identify. In the studies that are available, it is frequently stated that 15–40% of individuals resolves their acute disease and do not progress to chronic hepatitis, based largely on retrospective studies of post-transfusion hepatitis. This range points to a degree of uncertainty.

2.5.1 Injection Drug Use

Intravenous drug use (IDU) is a major risk factor for hepatitis C in many parts of the world (Kimberly *et al*., 2013). The 77 countries reviewed, 25 (including the United States) were found to have prevalences of hepatitis C in the intravenous drug user population of between 60% and 80% (Nelson *et al*., 2011; Xia *et al*., 2008) Twelve countries had rates greater than 80% (Nelson *et al*., 2011). It is believed that ten million intravenous drug users are infected with hepatitis C; China (1.6 million), the United States (1.5 million), and Russia (1.3 million) have the highest absolute totals (Nelson *et al*., 2011). Occurrence of hepatitis C among prison inmates in the United States is 10 to 20 times that of the occurrence observed in the general population. This

has been attributed to high-risk behavior in persons such as IDU and tattooing with nonsterile equipment (Imperial, 2010; Vescio *et al*., 2008). Shared intranasal drug use may also be a risk factor (Moyer, 2013).

Increasing prevalence of HCV infection overall and in new IDUs, appear to have preceded the HIV outbreaks by several years (Malliori *et al*., 2011). This suggests that prevalence of HCV infections in new IDUs may be a timely indicator of injecting risk among IDUs (Vickerman *et al*., 2010). HCV infection incidence among IDUs appears to remain generally high, probably due to much higher infectivity of HCV. Recent evidence suggests that sustained provision of combined prevention measures at high coverage levels can reduce the incidence of HCV infection (ECDC 2011; Hagan *et al*., 2011; Turner *et al*., 2011). In Kenya, NACADA is trying to fulfilled its plan through the following Strategic Focus Areas; to do public education and advocacy to general population and IDUs, continue counseling in rehabilitation centers and reintegration of IDUs, compliance, quality control and standards to the patients, research, policy and planning to diseases like Hepatitis C, to strengthening rehabilitation institutions, and finally to do leadership and integrity which will help law enforcement to interact with IDUs and not to use force for eradicate drugs in the region (Strategic, 2019).

2.5.2 Sexual Transmission

Sexual transmission of hepatitis C is uncommon (Kim, 2016). Studies examining the risk of HCV transmission between heterosexual partners, when one is infected and the other is not, have found very low risks (Kim, 2016). Sexual practices that involve higher levels of trauma to the anogenital mucosa, such as anal penetrative sex, or that occur when there is a concurrent sexually transmitted infection, including HIV or genital ulceration, present greater risks (Kim, 2016; Tohme and Holmberg, 2010). The risk of men who have sex with men and other people who might have unprotected anal sex is higher than to the general population. A history of a sexually transmitted disease, sex contact for more than five sexual partners per year, or a combination of these has been independently associated with positive HCV serology (Kimberly and Rachel, 2017). The frequency of HCV transmission between monogamous sexual partners is very low (Ghany *et al*., 2009).

2.5.3 Health care exposure

Blood transfusion, transfusion of blood products and organ transplants without HCV screening carry significant risks of infection (Wilkins et al., 2010). This low risk remains as there is a period of about 11–70 days between the potential blood donor's acquiring hepatitis C and the blood's testing positive depending on the method (Pondé, 2011). Those who have experienced a needle stick injury from someone who was HCV positive have about a 1.8% chance of subsequently contracting the disease themselves (Wilkins *et al*., 2010). Hospital equipment has also been documented as a method of transmission of hepatitis C, including re use of needles and syringes; multiple-use medication vials; infusion bags; and improperly sterilized surgical equipment, among others.

2.5.4 Vertical transmission of Hepatitis C Virus

Mother-to-infant transmission has become the primary route of HCV infection in children. Intrauterine and intrapartum transmission have been documented in several studies (Chun-Yan et al., 2014) (Le Campion et al., 2012). Mechanisms for transplacental HCV transmission such as viral transcytosis, maternal mononuclear cell trafficking, receptor-mediated entry into and possibly infection of trophoblasts, and direct or indirect injury of the placental barrier. The virions may reach the placenta and have contact with the fetus, HCV vertical transmission rates remain low, suggesting that the placenta has a key role in controlling HCV infection.

2.6 Hepatitis C Virus Therapy

2.6.1 Hepatitis C Virus genotype 1 infections

Patients with a history of treatment failure cirrhosis and after liver transplantation are treated with Sofosbuvir / Ledipasvir + Ribavirin for 12 week (Schwarz *et al*., 2016). In cases where Ribavirin (RBV) may not be used, the period of treatment is extended to 24 weeks (Flisiak *et al*., 2017). Asunaprevir + Daclatasvir therapy lasts for 24 weeks for patients with genotype 1b without cirrhosis that may lead to the selection of drug-resistant strains has not been confirmed in Poland as yet (Kumada *et al*., 2014; Manns *et al*., 2014). Grazoprevir + Elbasvir therapy in GT1-infected patients lasts for 12

weeks and used in combination with Ribavirin for patients with baseline viraemia > 800,000 IU/ml and the period of treatment is extended to 16 weeks (American Association for the Study of Liver Diseases/Infectious Disease Society of North America, 2016). Sofosbuvir/Velpatasvir therapy is used for 12 weeks regardless of the stage of fibrosis and failure of previous treatment. A 48-week Pegylated interferon a + Ribavirin therapy is recommended in children over 3 years of age, and Pegylated interferon α -2 α + Ribavirin may be used in children over 5 years of age (Schwarz *et al*., 2016).

2.6.2 Hepatitis C Virus genotype 2 infection

A 12-week Sofosbuvir/Velpatasvir regimen is the therapy of choice regardless of the stage of fibrosis both in treatment- naive patients and for retherapy. Ribavirin is added to therapy in cases of decompensated cirrhosis (Charlton *et al*., 2015; Manns *et al*., 2015). An alternative therapeutic regimen is a 12-week course of Sofosbuvir + Ribavirin treatment which is successful in the majority of treatment-naive patients. The treatment is extended to 24 weeks in patients after liver transplantation and with high HCV viraemia or previously treated with Pegylated interferon α + Ribavirin (Mitchell and Gurakar, 2015). If Sofosbuvir + Ribavirin is ineffective, a 12-week Sofosbuvir/Velpatasvir regimen or a 24-week Sofosbuvir + Daclatasvir + Ribavirin regimen is recommended (EASL, 2018). In children the recommended duration of treatment is 24 weeks: Pegylated interferon β + Ribavirin is used in children over 3 years of age, and Pegylated interferon α -2 α + Ribavirin in children over 5 years of age (Schwarz *et al*., 2016).

2.6.3 Hepatitis C Virus genotype 3 infection

The optimum therapeutic option is a 12-week Sofosbuvir/Velpatasvir regimen, combined with RBV in patients with cirrhosis. A 12-week Sofosbuvir + Pegylated interferon α + Ribavirin treatment ensures an equally high efficacy, particularly in cirrhosis- free patients. Patients with contraindications to interferon may be treated with Sofosbuvir + Ribavirin alone for 24 weeks (Cornberg *et al*., 2017). Patients failing therapy with Sofosbuvir + Ribavirin ± Pegylated interferon α are required to receive a 12-week therapy with Sofosbuvir / Velpatasvir ± Ribavirin. Alternatively, a

24-week Sofosbuvir + Daclatasvir + Ribavirin regimen may be considered. In children the recommended duration of treatment is 24 weeks: Pegylated interferon α -2 β + Ribavirin is used in children over 3 years of age, and Pegylated interferon α -2 α + Ribavirin – in children over 5 years of age (Schwarz *et al*., 2016).

2.6.4 Hepatitis C Virus genotype 4 infection

Therapy with Sofosbuvir/Ledipasvir lasts 12 weeks in treatment-naive cirrhosis-free patients. In patients with cirrhosis, with history of treatment failure or after liver transplantation Sofosbuvir/Ledipasvir is used for 12 weeks, and if there are contraindications to ribavirin, the duration of therapy should be extended to 24 weeks (Charlton *et al*.,2015). Regardless of the stage of liver fibrosis the Sofosbuvir/Velpatasvir drugs are used for 12 weeks and RBV added to therapy in patients with decompensated cirrhosis (Fazia *et al*.,2017). Therapy with Grazoprevir+Elbasvir lasts 12 weeks, however in patients previously unsuccessfully treated with interferon + Ribavirin it is extended to 16weeks, and ribavirin added to the regimen (Lawitz *et al*., 2014). In children the recommended duration of treatment is 48 weeks: Pegylated interferon α -2 β + Ribavirin is used in children over 3 years of age, and PegIFNa-2a+ Ribavirin in children over 5 years of age (Schwarz *et al*., 2016).

2.6.5 Infection with Hepatitis C Virus genotypes 5 and 6

Treatment-naive, cirrhosis free patients usually receive Sofosbuvir/Ledipasvir therapy for 12 weeks, however those with cirrhosis or post liver transplantation additionally receive ribavirin or their treatment should be extended to 24 weeks (Flisiak *et al*., 2016). The treatment with Sofosbuvir/Velpatasvir last for 12 weeks regardless of the stage of liver fibrosis, but Ribavirin should be considered as an addition to therapy in patients with decompensated liver function (Fazia *et al*., 2017). Currently there is no vaccine for hepatitis C, therefore prevention of HCV infection depends upon reducing the risk of exposure to the virus in health-care settings and in higher risk populations, for example, people who inject drugs, and through sexual contact (Lozano *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This study was conducted in three sites in 4 major towns within Kilifi County (Figure 3.1) including Omari rehabilitation center (ORC) in Malindi town, the Kenya AIDS NGOs Consortium (KANCO) in Watamu town and Muslim Education Welfare Association (MEWA) situated both in Kilifi and Mtwapa towns. (Note that Kilifi town and Mtwapa is one centre splited into two with one register called Muslim Welfare Association (MEWA). Its main offices are at Kilifi town. Sample collection sites mainly support the injecting drug users in the region. The leading economic activities in Kilifi County are tourist industries and fishing (Kenya-Census, 2009) this creates a market for drugs like heroin and many other drugs for both tourist and IDUs. The fact that the major harm reduction centers like KANCO and MEWA are situated at the area and HCV is known to spread through injecting of drugs, it triggered me to carry out research on HCV at the region.

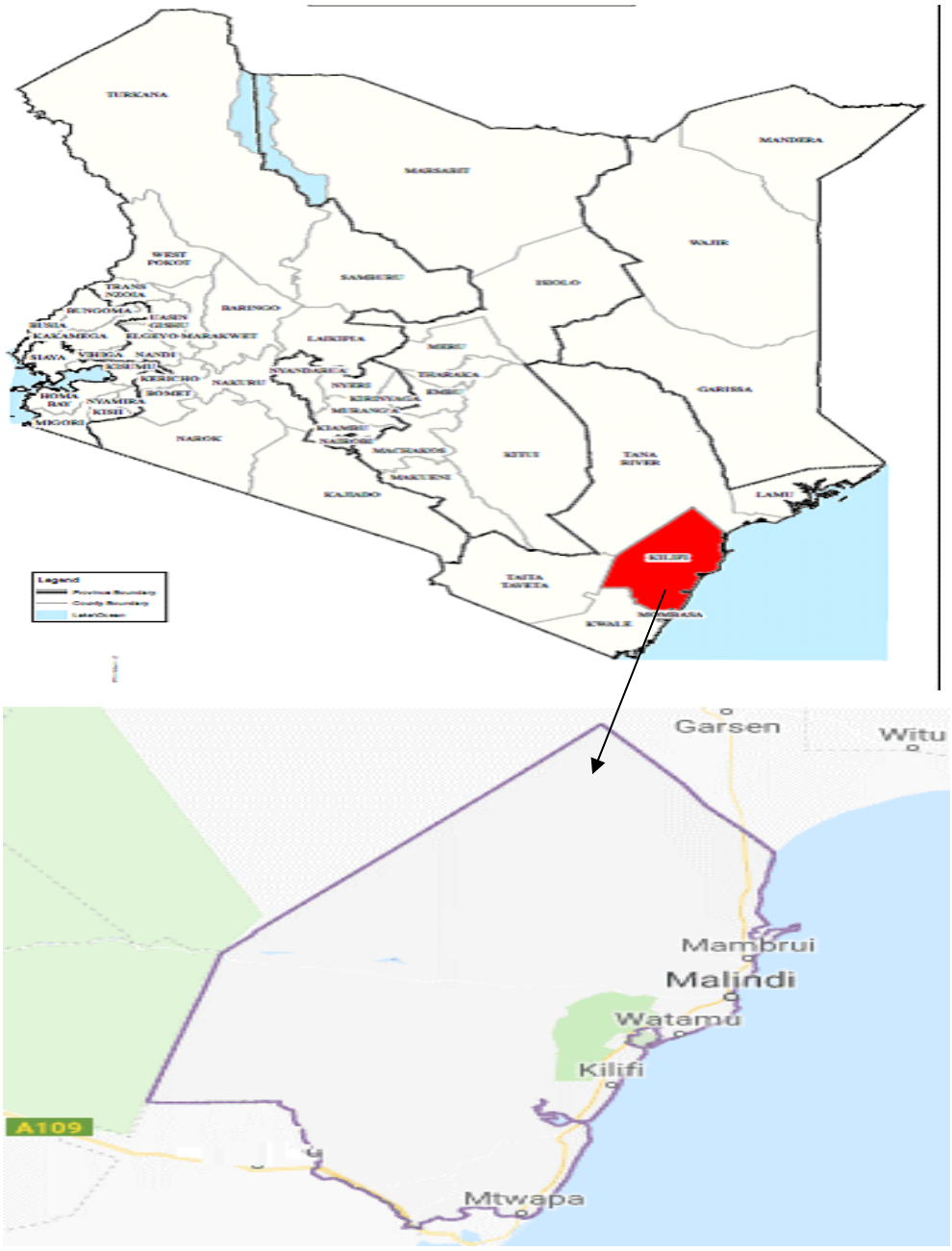

Figure 3.1 Map of Kilifi County in the Kenyan coast (Kenya-Census, 2009)

3.2 Study Design

This was a cross sectional survey, which involved collection of blood samples for laboratory analyses and administration of questionnaire for determination of the socio-demographic characteristics of the study population (Appendix 2).

3.3 Target population

Blood samples were collected from Injecting Drug Users (IDUs) registered within the study site facilities in Kilifi County.

3.4 Sample size

The sample size was calculated using prevalence of 0.9% among HCV IDUs in Kenya (Muasya, *et al*., 2008) using Fisher *et al*., 1998 formula.

$$N=Z^2PQ/d^2$$

$$N= \frac{(1.96^2 x\ 0.09) x (1.0-0.09)}{0.05^2} = 126$$

Where

N = Sample size required

Z = Confidence level at 95% (standard value of 1.96)

P= Prevalence figure for HCV in Kenya is 0.9%.

Q= [1-p]

3.5 Inclusion and exclusion criteria

The inclusion criteria included the persons who had been enrolled and were participating in the site facilities for at least 3 months during the prior to the study, they were above 18 years of age and were willing to consent. While exclusion criteria included the participants who were not IDUs, they were not willing to consent; they were below 18 years and not assisted by guardian and those who were not attending the selected rehabilitation centers.

3.6 Sampling criteria

Samples were distributed per site based on proportion of the population of IDUs in the specific towns as shown in Table 3.1

Table 3.1 Sample size distribution based on population of IDUs and collection in each sites at Kilifi County

Sample collection site	Number of IDUs (N)	% of the total (N)	Number of samples
Malindi	(2006)	2006/4587*100= (43.7%)	(55)
Watamu (KANCO)	(1409)	1409/4587*100= (30.7%)	(39)
Kilifi town (MEWA a) (MEWA b) drop-in Centres	(598)	598/4587*100= (13%)	(17)
Mtwapa (MEWA b) Drop-in Centres	(574)	574/4587*100= (12.5%)	(16)
TOTAL	**(N = 4587)**	**(100%)**	**(127)**

(33)

3.7 Sample collection

Samples were collected from 127 participants who were willing to consent and were available in each center according to the inclusion criteria. The clinical officers who were stationed in each centre by the NGOs to offer medical treatment for IDUs were involved in collecting of samples. . Five milliliters of venous blood was collected into EDTA vacutainer tubes. The blood was fractionated to separate serum. Proper labeling of samples was done with labels containing the following information: Container identification number, Sampling date and time, Sampling location, Field sampler's name and Name of the witness. The samples were stored in a freezer at -20^0C which was at the rehabilitation centers health facility before being transported to KEMRI.

3.8 Socio-demographic participants

Participants were selected according to inclusion criteria using a questionnaire. They were then grouped as per gender, age groups, marital status, religion occupation and education.

3.9 Sample transportation and storage

Frozen serum samples were transported in dry ice from rehabilitation Centers to KEMRI headquarters laboratories in Nairobi for molecular characterization. The Field Sampling Data Sheet was placed in a waterproof bag, sealed and taped under the lid of the cooler along with the samples to which it applies. Serum samples were frozen at -80^{0}C till use.

3.10 Quality assurance for Laboratory Operations

Separate space was provided at KEMRI Hepatitis laboratory for this research work. This work was to be separated from routine work which was clinical study in the laboratory. Space was provided for archives, limited to access by authorized personnel only, for the storage and retrieval of all raw data and specimens from completed studies (WHO, 2011). All reagents and solutions in the laboratory areas were tracked/managed to include date received/opened/prepared, labeled to indicate identity, titer or concentration, storage requirements, and expiration date. Deteriorated or outdated reagents and solutions were not used and were properly disposed of in accordance with standard operating procedures while observing all safety precautions. Purchased chemicals, and reagents were received and tracked according to the Materials Management SOP (Appendix 6). Solutions and other reagents prepared in the laboratory were also labeled to indicate date of preparation and the preparer's initials. Other information that may be pertinent was also indicated on the label. Reagents and solutions that contain hazardous components and required special protective measures while handling and storing them (e.g., need for protective equipment, use in hood, peroxide-forming compound) were having appropriate warning sticker affixed to each container. Each month, equipment were adequately inspected, cleaned, maintained, tested, calibrated and/or standardized (WHO, 2011).

3.11 Serological tests

Blood from the EDTA tube was placed in a Tomy centrifuge and spun at 3000 rpm for 10 minutes to separate plasma from the whole blood (Mwatela *et al.*, 2015). Fifty microliter (μL) of the serum from 127 samples was used for HCV detection using

dipstick rapid serology kit. Serum specimen was collected in a clean test tube and the sealed pouch was brought to the room temperature, then open to remove the dipstick. Once the dipsticks are opened, they must be used immediately. The dipsticks were submerged into each serum specimen living about 1cm high submerging only the absorbent area. After at least 10 seconds the dipstick were taken out and laid on a flat on clean, dry, non-absorbent surface. At the end of fifteen minutes the results were read was follows;

NEGATIVE: Only one pink-purple colored control band appeared on the dipstick

POSITIVE: Two distinct pink-purple colored bands appeared on the dipstick.

INVALID: the test was considered invalid if no control band appears, it was to be repeated. Depending on the concentration trial of HCV in the specimen, positive results was to start appearing as early as 2 minutes, negative results must be confirmed only at the end of fifteen minutes. In case of doubt of results at 15 minutes, the test was to be extended up to 30 minutes to get a clear background. The HCV kit consisted of recombinant HCV antigen-coated strips that reacted with anti-HCV antibodies in the tested serum (Eurostrip West Harrow Middx, U.K).

3.12 Hepatitis C RNA extraction

Viral RNA was extracted from 28 serum samples, which tested positive on rapid test kit by using Zymo Research Kit (Irvine17062 Murphy Ave.Irvine, CA 92614, U.S.A.) ccording to manufacturer's instructions. βME was added to Viral RNA Buffer[1] to a final dilution of 0.5% (v/v) and 24ml of 100% ethanol to 6ml Viral Wash Buffer in RNA free environment. 300 µl volumes Viral RNA Buffer were added to each 100 µl serum sample and mix. Then they were transfer the sample to the Zymo-spin IC Column in a Collection Tube and centrifuge at 13000rpm for 2 minutes. The flow-through was discarded. 500 µl Viral Wash Buffer was added to the column and centrifuged at 13000rpm for 2 minutes. Then carefully it was transferred to the column into the DNase/RNase-Free Tube and Centrifuge13000rpm for 2 minutes again to remove any remaining Viral Wash Buffer before adding DNase/RNase-Free Water. 15µl RNase-Free Water was added directly to the column matrix and centrifuge 13000rpm for 30 seconds. Eluted RNA was stored at -70^0C for use. All the reactions components were on ice and mixed individually prior to use as shown in the Table 3.3.

Table 3.3: Protocol for a Routine PCR

Components	**25µl RXN**	**FINAL CONCENTRATION (15 RXN)**
2x Reaction Buffer	(12.5µl)	(187.5 µl)
Forward Primer	(1.0µl)	(15 µl)
Reverse Primer	(1.0µl)	(15 µl)
RNase-Free Water	(5µl)	(75 µl)
Taq Polymerase Enzyme	(0.5 µl)	(7.5 µl)
RNA Template (Sample**)**	(5µl)	(5 µl)
Reverse Transcriptase Enzyme	(0.5 µl)	(7.5µl)
TOTAL	**(25.5µl)**	**(312.5µl)**

3.13 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Nested RT-PCR was carried out using Zymo Research PCR Kit (Irvine CA U.S.A.) and GeneAmp PCR system 9700 (Fabienne, 2017). In reverse transcription and PCR reactions, both 5′UTR universal primers: forward KY80 and revese KY78 (Table 3.4) were used. 35 cycles of PCR (94°C for 30 seconds, 54°C for 1 minute, and 68°C for 2 minutes) were performed HCV PCR products of 244 bp were visualized, and electrophoresed on 1.5% agarose gel (Figure 4.1). The PCR products were purified and sent to Netherland for sequencing.

Table 3.4: Oligonucleotide primers and PCR product size used to detect HCV for both forward and reverse primer and their positions

Primer name	Primer sequence	Primer position
Forward KY80	5'-GCA GAA AGC GTC TAG CCA TGG CGT-3'	nt68-nt91
Reverse KY78	5'-CTC GCA AGC ACC CTA TCA GGC AGT-3'	nt288-nt311

3.14 Ethical Consideration

Ethical clearance was sought from Kenyatta National Hospital/ University of Nairobi Ethical and Research review committee (KNH/UON-ERC), ethical number P366/07/2017(Appendix 4). Participants were adequately informed and their voluntary consent obtained in writing. Patient data was treated with strict confidence, including code labeling of questionnaires and specimen.

CHAPTER FOUR

RESULTS

4.1 Gel Electrophoresis

The gels containing samples were transferred to an ultra-violet (UV) transilluminator an equipment used in life science laboratories for visualization of target DNAs and proteins. It emits high levels of UV radiation through the viewing surface. Out of the 28 samples subjected to PCR only 11 samples tested positive.

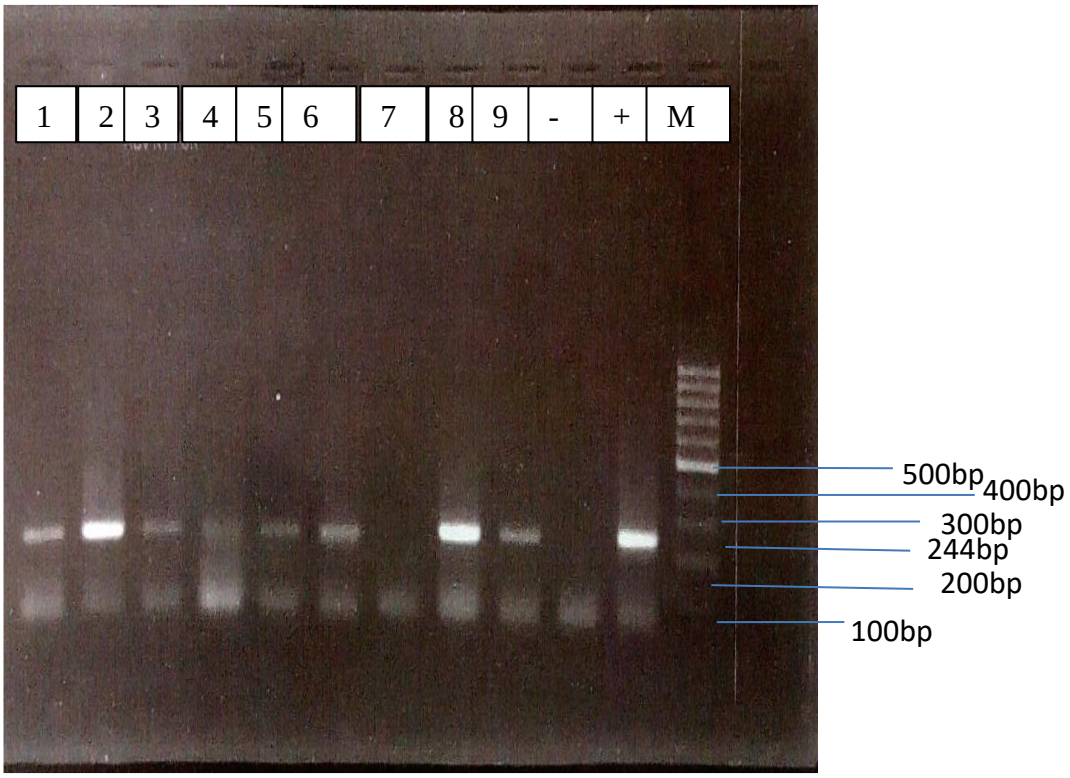

Figure 4.1: Electrophoresis pattern of some samples performed for qualitative analysis.

Lanes **1, 2, 3, 4, 5, 6, 7, 8 and 9 -CV**=Negative control, +**CV**=Positive control and **MW**= Molecular weight marker. Sample number 7 is negative.

4.2 Sequence 5′UTR analysis

The eleven samples were aligned with the representative number of sequences for each major genotype and subtype selected from LOS ALAMOS HCV database and Gene Bank using the Multiple Sequence Alignment Program. The original alignment of

HCV reference sequences were downloaded from the HCV database under the subheading “alignments” (Figure 4.2). Homology and evolutionary distance pair-wise comparisons for percent nucleotide were made (Medhat *et al.*, 2014). Phylogenetic analysis of a 243-bp segment (nucleotides −311 to −68) of the 5′UTR was performed on a subset of the sequences generated in this study. Included in this analysis were 11 prototype sequences obtained from GenBank. The prototype sequences used are presented here along with their designations, where they originated from and GenBank accession numbers as follows: 1a, HCV1 (EF407419**)** America; 1a, HCV1 **(**M67463**)**America and Japane; 1a, HCV **(**D14853**)** Indonesia; 1a, HCV (AY587016)China; 1a, HCV (AY051292**)** India; 4a, HCV (FJ025856**)**Portugal; 4a, HCV **(**FJ025855**)**Portugal; 4a, HCV **(**Y11604)Egypty, South East Asia and Middle East; 4a, HCV **(**HQ537008) Cyprus; 4a, HCV **(**HQ537009) Cyprus; 4a (EU392173**)** Egypt and SoudArabia. The analysis of these sequences was performed using the Molecular Evolutionary Genetics Analysis (MEGA) computer program, version 7.0.14 (Sudhir 2016). Both the unweighted pair group method with arithmetic means (UPGMA) described by Sneath and Sokal (Muniba,2019) and the neighbor-joining method of Saitou and Nei (Saiton, 2020**)** were used to construct trees depicting the relationships between sequences generated from the 5′UTR and the prototype sequences. In each case, the Jukes-Cantor algorithm was used to estimate the number of nucleotide substitutions between sequences. The branching of both trees was tested by bootstrap analysis (5000 replicates).

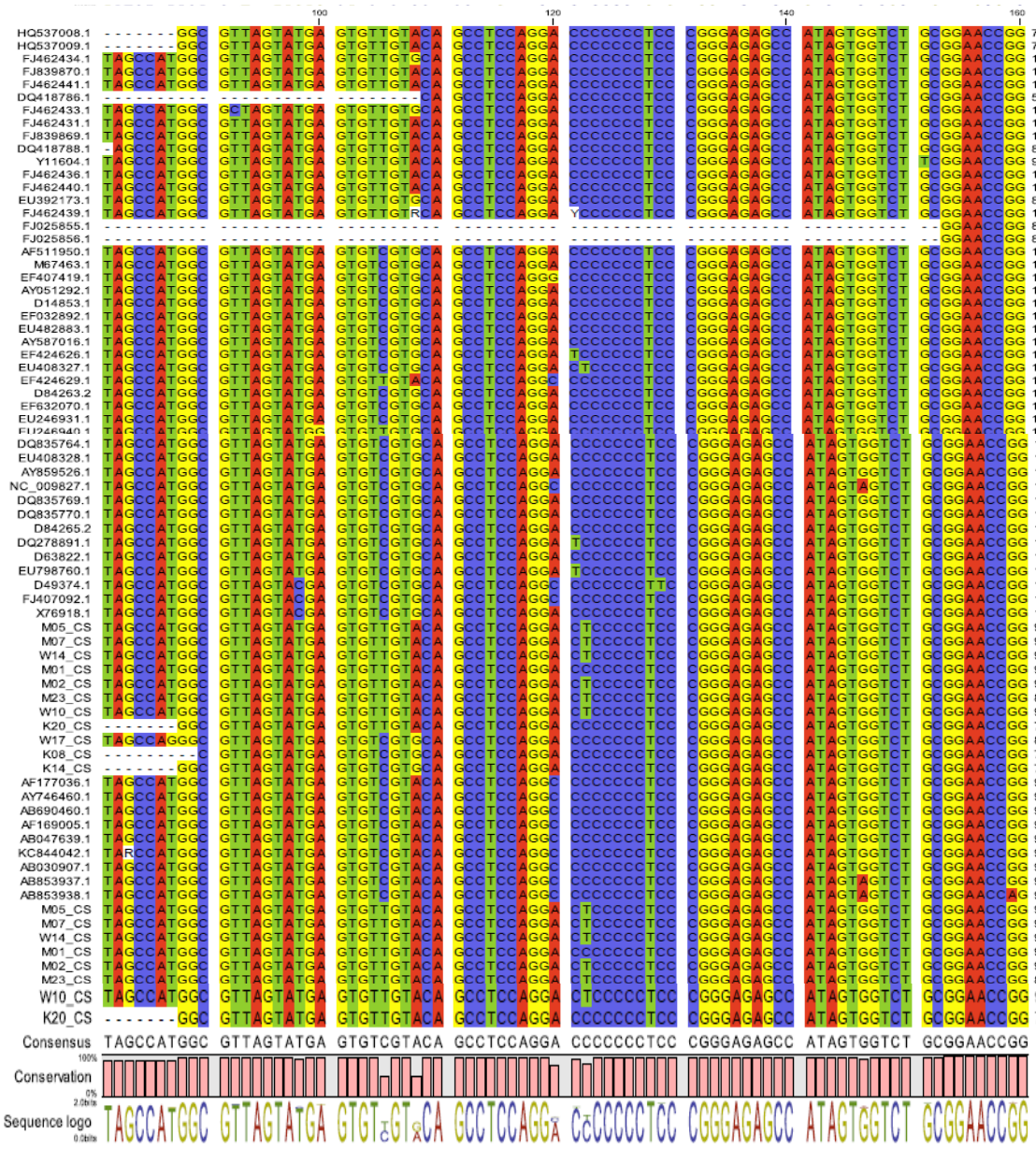

Figure 4.2: Multiple Sequence Alignment Program, ClustalW

It includes the 11 samples, which were done in the laboratory and there was no mutation among the samples.

4.3 Data management and Analysis

The participants' data was coded immediately they arrived in the lab. Demographic data that included age, sex, marital status, religion and occupation data were entered into a Microsoft excel database. The errors were checked and removed. Data was stored in password protected computer. Analysis was done using Statistical Package for the Social Sciences (SPSS) version 20 software (SPSS Inc., Chicago, IL, USA). Non-parametric Mann-Whitney U test was used to compare between genotypes with respect to quantitative variables. Chi square was used to analyze association between social demographics and circulating HCV genotypes. Odds ratios were reported as estimates of relative risk with 95% confidence interval. All statistical tests interpreted at 5% level of significance (p value less or equal to 0.05 was considered statistically significance (Sylvie *et al*., 2019). The analyzed data was presented in tables and figures. PCR positive amplicon were send to Macrogen Inc. Netherlands for sequencing and purification. The amplification primers (10uL) were used for sequencing. The sequenced data was assembled using GENTYX V.9 software. The alignment was done using MEGA7.0.14 version. The phylogenetic analysis of the sequenced HCV samples and downloaded sequences were done using neighbourhood joining tree. The genotype of each sequence was determined by the phylogenetic tree. Data was presented for various genotypes circulated within IDUs population in Kilifi County.

4.4 Phylogenetic tree construction

With reference sequences for different HCV genotypes (including 1, 2, 3, 4, 5 and 6), there was only genotype 1 and 4 (Figure 4.3 below) There was no evident on the tree because there were no associated isolates. The original alignment of HCV reference sequences were downloaded from the HCV database Gene Bank using the Multiple Sequence Alignment Program, ClustalW (Figure 4.3) under the subheading "alignments". Homology and evolutionary distance pair-wise comparisons for percent nucleotide were made. Maximum composite likelihood algorithms were utilized, and phylogenetic trees were constructed by the neighbor-joining method. The reliability of different phylogenetic groupings was evaluated by using the bootstrap re-sampling test from the MEGA program (5000 bootstrap replications).

Figure 4.3: Phylogenetic neighbor-joining trees of HCV 5′UTR partial sequences in Kilifi County

4.5 Socio-demographic characteristics of the study participants

A total of 127 participants were recruited 107 (84.2%) male and 20 (15.7% female). Distribution by key towns had Watamu town with 33.2%, Malindi town 35.5 %, Kilifi 32.7% (Table 4.1). The overall mean age for the three centers was 33.7years (range: 16years –55 years). On marital status, 53.5% were single with those divorced accounting for 18.9%. In terms of religion, occupation and education level, majority of participants were Muslims (60%), in passenger service (43.3%) and of lower primary school education (49.6%) respectively (Table 4.1).

Table 4.1: Demographic characteristics of participants in Kilifi County

		Kilifi county						Overall	
		Kilifi, n=49		Watamu, n=43		Marindi, n=35			
Variable		**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
Gender	Male	(45)	(91.8)	(38)	(88.4)	(24)	(68.6)	(107)	(84.3)
	Female	(4)	(8.2)	(5)	(11.6)	(11)	(31.4)	(20)	(15.7)
Age	Mean (SD)	(32.7)	(7.2)	(33.2)	(7.8)	(35.5)	(6.7)	(33.7)	(7.3)
Age groups	16-20 years	(1)	(2.0)	(1)	(2.3)	(1)	(2.9)	(3)	(2.4)
	21-25 years	(7)	(14.3)	(5)	(11.6)	(0)	(.0)	(12)	(9.4)
	26-30 years	(11)	(22.4)	(11)	25.6	(7)	(20.0)	(29)	(22.8)
	31-35 years	(17)	(34.7)	(13)	(30.2)	(9)	(25.7)	(39)	(30.7)
	36-40 years	(6)	(12.2)	(5)	(11.6)	(10)	(28.6)	(21)	(16.5)
	41-45 years	(5)	(10.2)	(4)	(9.3)	(5)	(14.3)	(14)	(11.0)
	46-50 years	(1)	(2.0)	(3)	(7.0)	(3)	(8.6)	(7)	(5.5)
	51-55 years	(1)	(2.0)	(1)	(2.3)	(0)	(.0)	(2)	(1.6)
Marital status	Single	(17)	(34.7)	(20)	(46.5)	(31)	(88.6)	(68)	(53.5)
	Married	(21)	(42.9)	(10)	(23.3)	(4)	(11.4)	(35)	(27.6)
	Divorced	(11)	(22.4)	(13)	(30.2)	(0)	(.0)	(24)	(18.9)
Religion	Pagan	(2)	(4.1)	(2)	(4.7)	(0)	(.0)	(4)	(3.1)
	Muslim	(36)	(73.5)	(21)	(48.8)	(20)	(57.1)	(77)	(60.6)
	Christian	(11)	(22.4)	(20)	(46.5)	(15)	(42.9)	(46)	(36.2)
Occupation	Tout/driver/rider	(32)	(66.7)	(6)	(14.0)	(17)	(48.6)	(55)	(43.3)
	Beach boy	(0)	(.0)	(15)	(34.9)	(4)	(11.4)	(19)	(15.0)
	Fisherman	(4)	(8.3)	(11)	(25.6)	(2)	(5.7)	(17)	(13.4)
	Other	(12)	(25.0)	(11)	(25.6)	(12)	(34.3)	(35)	(27.6)
Education	College	(0)	(.0)	(0)	(.0)	(1)	(2.9)	(1)	(0.8)
	Secondary	(9)	(18.4)	(9)	(20.9)	(7)	(20.0)	(25)	(19.7)
	Upper Primary	(18)	(36.7)	(15)	(34.9)	(3)	(8.6)	(36)	(28.3)
	Lower Primary	(20)	(40.8)	(19)	(44.2)	(24)	(68.6)	(63)	(49.6)
	No Education	(2)	(4.1)	(0)	(.0)	(0)	(.0)	(2)	(1.6)

4.6 Prevalence of HCV among study participants

The overall antibody sero-prevalence was 22.1% (n=28) with 7.9 % (n=10) from Kilifi, 3.2% (n=4) from Watamu and 11.0% (n=14) from Malindi. Prevalence by each town was therefore 15.2% (n=5) for Kilifi, 7.7% (n=3) for Watamu and 5.5% (n=3) for Malindi. In terms of gender, a majority of those sero-positive were male (82.1%, n = 23) with 17.9% (n=5) being female. Sero-positivity among males was 18.3% while in females 3.9%. In terms of correlates of sero-positivity, although not significant, those who reported being single were 3 times more likely to be infected with HCV compared to the married (OR = 3; p=0.06), with the infection more likely among those 16 to 20 years compared to all other age groups (Table 4.2) Notable was also the fact that although majority of the participants were mainly Muslims in terms of religion, those professing Christian faith had 5.3% higher chance of getting infected than their Muslim counter-parts (OR = 1.4; P= 0.31) (Table 4.2).

Table 4.2 Correlation between serological outcome and demographic characteristics of IDUs Participants in Kilifi County

Variable	N	(n/N) %	Positive n (%)	Negative n (%)	OR (95%CI)	P value
Age						
16-20 years	(3)	(66.7)	2 (66.7)	1 (33.3)	1.0	
21-25 years	(12)	(25.0)	3 (25.0)	9 (75.0)	0.2 (0-2.6)	(0.171)
26-30 years	(29)	(10.3)	3 (10.3)	26 (89.7)	0.1 (0-0.8)	(0.011)
31-35 years	(39)	(20.5)	8 (20.5)	31 (79.5)	0.1 (0-1.6)	(0.070)
36-40 years	(21)	(33.3)	7 (33.3)	14 (66.7)	0.3 (0-3.3)	(0.265)
41-45 years	(14)	(7.1)	1 (7.1)	13 (92.9)	0.04 (0-0.9)	(0.014)
46-50 years	(7)	(57.1)	4 (57.1)	3 (42.9)	0.7 (0-11.3)	(0.778)
51-55 years	(2)	(0)	0	2 (100.0)	-	(0.136)
Gender						
Male	(107)	(21.5)	23 (21.5)	84 (78.5)	1.0	
Female	(20)	(25.0)	5 (25.0)	15 (75.0)	1.2 (0.4-3.7)	(0.729)
Marital status						
Married	(35)	(11.4)	4 (11.4)	31 (88.6)	1.0	
Single	(68)	(27.9)	19 (27.9)	49 (72.1)	3.0 (0.9-9.7)	(0.065)
Divorced	(24)	(20.8)	5 (20.8)	19 (79.2)	2.0 (0.5-8.6)	(0.330)
Religion						
Muslim	(77)	(0)	16 (20.8)	61 (79.2)	1.0	(0.497)
Christian	(46)	(26.1)	12 (26.1)	34 (73.9)	1.4 (0.6-3.2)	(0.309)
Pagan	(4)	(20.8)	0	4 (100.0)	-	
Education						
Lower primary and below	(65)	(20.0)	13 (20.0)	52 (52.5)	1.0	
Upper primary	(36)	(16.7)	6 (16.7)	30 (30.3)	0.8 (0.3-2.3)	(0.682)
Secondary and above	(26)	(34.6)	9 (34.6)	17 (17.2)	2.1 (0.8-5.8)	(0.146)
Occupation						
Tout/driver/bodaboda	(55)	(21.8)	12 (21.8)	43 (78.2)	1.0	
Beach boy	(19)	(26.3)	5 (26.3)	14 (73.7)	1.3 (0.4-4.3)	(0.688)
Fisherman	(17)	(17.6)	3 (17.6)	14 (82.4)	0.8 (0.2-3.1)	(0.712)
Other	(35)	(20.0)	7 (20.0)	28 (80.0)	0.9 (0.3-2.6)	(0.837)

4.7 Polymerase chain reaction (PCR) and genotyping

The eleven (11) sero-positive samples which were only male participants accounting for 39.3% were determined as PCR-Antibody positive samples – potentially indicating acute infections. Only two genotypes were established with genotype 4a accounting for 72.7% (n=8) being the most prevalent genotype and genotype 1a accounting for the remaining 27.3% (n=3). All groups were more likely to be infected by genotype 4a compared to genotype 1a (Table 4.3).

Table 4.3 PCR and Genotypes prevalence with participant demographic characteristics in Kilifi County

Variable	HCV1a, n=3 n (%)	HCV4, n=8 n (%)	OR (95% CI)	P value
Age				
≤35 years	1 (16.7)	5 (83.3)	0.3 (0-4.9)	(0.387)
>35 years	2 **(**40.0)	3 (60.0)	1.0	
Gender				
Male	3 (27.3)	8 (72.7)	-	-
Female	0	0		
Marital status				
Single	2 (40.0)	3 (60.0)	1.0	
Married	0	3 (100.0)	-	(0.206)
Divorced	1 (33.3)	2 (66.7)	0.8 (0-15.0)	(0.850)
Religion				
Muslim	2 (22.2)	7 (77.8)	0.3 (0-6.9)	(0.424)
Christian	1 (50.0)	1 (50.0)	1.0	
Education				
Lower primary and below	1 (25.0)	3 (75.0)	0.2 (0-4.5)	(0.270)
upper primary	0	4 (100.0)	-	(0.053)
Secondary and above	2 (66.7)	1 (33.3)	1.0	
Occupation				
Tout/driver/bodaboda	2 (40.0)	3 (60.0)	1.0	
Beach boy	0	3 (100.0)	-	(0.205)
Fisherman	1 (50.0)	1 (50.0)	1.5 (0.1-40.6)	(0.809)
Other	0	1 (100.0)	-	(0.438)

CHAPTER FIVE

DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1 Discussion

This study reveals that majority of participants were male (84.2%) compared to female who were (15.7%). A similar observation was seen by Micah et al., 2018 in a study done in three major cities in Kenyan, that is Nairobi, Mombasa and Kisumu among IDUs. Single participants recoded high percentage (27.6%) of HCV transmission compared to married (18.9%) persons, this was also seen in a retrospective cohort study. study carried out by Matthew *et al.*,2019 in hepatitis C virus among IDUs who were accessing harm-reduction services in Kenya. The study established a sero-prevalence rate of 22.1% of HCV infection among injecting drug users in Kilifi County, with only 2 genotypes circulating. Further this is among the few studies that document the circulation of genotype 4a in the coastal towns of Kenya. The prevalence of 22.1% determined in this study falls within the +/- margin of error also previously determined in previous studies (Mwatela *et al.,* 2015) among IDUs. Since HCV is mainly transmitted via needle exchange, the prevalence here raises a number of public health questions including, are the harm reduction strategies such as needle exchange program effective in this population? What would be the true prevalence of HCV in the general population or is HCV only concentrated within the IDU population? What would be the best public health intervention measures? It suffices to note further that, although the prevalence was seen to be higher in male than female gender, the number of female participants was significantly lower with male being 5 times more in the study as compared with the study which was done by Mwatelah *at al*.,2015 in Kilifi Malindi sub County and even in the co infection study by Beatrice *et al.*,2013 in Nairobi whereby female were more than male. Another study carried by Micah *et al*.,2018 in major cities in Kenya also indicate that female were more than male. The latter would indicate a potential stigmatization associated with injecting drug use among women. If the latter is true, then this would have a major negative implication for public health intervention, where majority of drug users

remain in secrecy with the net results that HCV infections could silently spread within the IDU female population (United Nations Office of Drug and Crime, 2015). Many adverse health outcomes of HCV are associated with injecting drug users especially in many developing countries that continue to weigh heavily on this disease. In this study we have seen that the population screened using rapid test kit for male is five times high 107 (84.2%) compared to female 20 (15.7%). This is perhaps the stigma from female who don't want to be seen or known by the public on the same knot they know that it is illegal to use these drugs, as others complained about police harassment and others having suffered social injustice through law enforcement institutions (Emmy, 2015). Although the confirmation by PCR shows that there was only11 (8.7%) male IDUs confirmed to be HCV positive by PCR as compared to none for female IDUs (0%).

Long-term endemicity in some regions is reflected in the diversity and multiplicity of subtypes. Subtype patterns have been used to trace the origin of genotypes 1 and 2 to West Africa, of genotype 4 to Central Africa (James *et al.,* 2014). Genotype diversity is particularly high in China and many Southeast Asian countries and also in Western Europe and Australia, perhaps as a result of population immigration from Africa and/or Asia (International migration data, 2012). In countries like China, Western Europe and Australia, vaccine efficacy at a population level would be dependent on the generation of cross reactive immunity. An alternative approach could also be the development of vaccines hosting different immunogens. In contrast, in countries where diversity is constrained such as Egypt (low diversity here is likely to be as a result of the rapid amplification of subtype 4a during the twentieth century as a result of specific iatrogenic events (Jane *et al.,* 2015) vaccine immunogens designed to most closely align with population HCV sequence may be the optimal strategy. In this study, HCV genotype 4 subgenotype 1a is predominant among the population of drug users, this suggest that there is a need for continuous monitoring for better treatment outcomes that entirely depend on HCV genotyping results. Similar observations were reported from a study conducted in Pakistan to determine the distribution of HCV genotypes in different geographic locations and social demographics. This will help in improving and eradicating of HCV among IDUs in Kilifi County (Micah *et al.,*

2018). Majority of Kenyan IDUs use heroin, which is most widely available and accessible substance locally (Mwatelah *et al.,* 2015). A recent surveillance of 'most-at-risk population (MARPS) identified illicit substance use as a key behavior risks, with initiation to injecting drug use occurring as early as 11 years of age (NASCOP, 2012). Some risk groups associated with specific genotypes may be more readily sampled than others, and the impact of this may be strongest in those countries with the fewest number of genotyped cases. A mechanism for removing studies of certain risk groups would be required, and such a subjective decision could lead to additional sources of bias. In addition, we have not stratified studies according to the methodology used to genotype patients. Since the HCV RNA 5'UTR (untranslated region) is the most common target for diagnostic HCV RNA assays, it is also the viral region most commonly used to define viral genotype. While this region is relatively conserved, single nucleotide differences assessed using sequencing or probe-based assays are most commonly used in assays to define viral genotype (Jane *et al.,* 2015). Importantly, these assays have been deemed adequate for current clinical applications. Although clinical assays that assess viral subtype also rely on 5'UTR as a substrate, the accuracy of HCV subtype will be improved by additional sequencing of other viral regions such as core (Hara *et al.,* 2013). Whether social networks mitigate the HCV transmission or not is a fact of controversy. While some authors denied the role of these networks in HCV spread (Pilon *et al*., 2011), a recent study strongly confirmed the central role of injecting network traits, not only as a transmission factor but also as a predictor of HCV genotype in different communities (Sacks-Davis *et al*., 2012).

5.1 Conclusion

The prevalence of HCV in this study remains high in Kilifi County 22.1% as compared to previous studies. Genotype 4a which was considered to be foreign is mainly in Kilifi town with Watamu and Malindi recoding both HCV genotype 1a and 4a. On social demographics, the infection burden remains high among the younger population between 16 to 20 years and among those who are single.

5.2 Recommendations

Since the sero-prevalence remain high in Kilifi County, the Government should screen and treat IDUs in order to reduce continue spreading of the disease. People or tourists, born of history or a region where hepatitis C prevalence is known to be high like in, Central, East and South Asia, Australasia and Oceania, Eastern Europe, Subsaharan Africa, North Africa or Middle East are supposed to be screened before accessing entry to our Country or in Kilifi County.

A program of counseling and bringing of patients with history of sexual contact or sharing of personal care items with someone who is HCV-infected should be started in order to minimize the spread of HCV in the region. Since HCV remain infectious for up to 6 weeks on surfaces at room temperature, (Ronald, 2014). Precautions should be made and people advised especially in areas where these IDUs are to avoid further contamination.

In all cases, HCV RNA (or HCV core antigen) can be detected during the acute phase, although their levels may vary widely and there may be interludes (up to several weeks) of undetectable HCV RNA (or HCV core antigen). Thus, HCV RNA-negative (or HCV core antigen-negative) individuals should be retested for HCV RNA (or HCV core antigen) 12 and 24 weeks after a negative result to confirm definitive clearance as they are treated using genotypes (EASL 2018). Hepatitis C Virus in developed countries is very expensive using RNA detection, as such Rapid test kit can be used for detection of this virus.

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Irvine 17062 Murphy Ave.Irvine, CA 92614, U.S.A.17062. *Murphy Ave is a miscellaneous (industrial) located in Irvine,* CA 92614. Built in 1977, *this property features* 1.17 *acres lot, and* 19,011 *sq ft of living space.*

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APPENDICES

Appendix 1: Consent Form- English

Title of the study: PREVARENCE, SOCIAL DEMORGRAPHICS AND CHARACTERIZATION OF HEPATITIS C GENOTYPES CIRCULATING AMONG INJECTING DRUG USERS IN KILIFI COUNTY, KENYA

CONSENT FORM No**.**

Good morning/ evening?

My name is _____

We are currently carrying out a study on Viral Hepatitis C in Kilifi County Rehabilitation Centres, in order to find out how many people are infected (prevalence) and the strains or types of the infection (Genotypes). The study is designed to evaluate the contribution of Hepatitis C to chronic infection in the area.

How many people will take part in the study? This study will involve more than 126 participants attending Rehabilitation Centres in Kilifi County.

Procedure: The purpose of this form is to obtain your consent to participate. Participation in the study is voluntary. 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. The responses you give to these questions will be kept completely confidential and will only be accessed by the principal investigators. To ensure confidentiality, your name will not appear anywhere on the questionnaire. The questionnaires will be coded and only your facility or Clinic admission number (OP) will be used to relay the results to you. You can answer all questions in the questionnaire, but in case there is a question that you are not comfortable to answer you have the rights to do so.

In addition to the questionnaire, we will also take approximately 7mL of blood sample from your blood vein to be used for testing whether you are infected with Hepatitis C, later I will find the strain or the type of the Hepatitis C.

What are the risks of the study? There is no known risk in participating in this study except the slight pain that you will experience when drawing blood.

What are the costs? There will be no costs for the participants in the study. It is FREE.

What are the benefits in taking part in the study? The results of the study will be communicated back to you as a participant through your facility or Clinic. The same result will be communicated KEMRI for appropriate action. The overall results will be used to advice the public and the government health sector on the impact of Hepatitis C and Liver Cancer.

How long will the study take? The study is projected to take one year. 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 sample be analyzed? The blood sample will be transported to KEMRI for laboratory analysis.

It is important to emphasize that your information will be very useful in establishing the Hepatitis C in Kilifi County. Your acceptance is most appreciated. To answer all questions in this form, it might take 10-15 minutes. For any clarification feel free to contact Robert Mainga (**the principal investigator),** on mobile **+254 723934180,** or gmail: **rmainga2000@gmail.com** and Dr Eddy Odari (**Supervisor**), on mobile +**254 725259296** or email: **kodarie04@yahoo.com** or the following contact will be available:

The Secretary,

Kenyatta National Hospital/ University of Nairobi (KNH-UON ERC),

Ethical and Review Committee (ERC)

KENYATTA NATIONAL HOSPITAL
(KNH)
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272

OR

UNIVERSITY OF NAIROBI (UoN)
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

Subject Permission

I have read the consent form and I therefore make my decision

Consent to fill the questionnaire **YES** ☐ **NO** ☐

Consent for blood collection **YES** ☐ **NO** ☐

Consent to transport the samples to KEMRI **YES** ☐ **NO** ☐

Signature/fingerprints of participant: _____Date:_____

Signature of the principle investigator: _____ Date: _____

Nyongeza 1: Fomu ya ridhaa

Kichwa cha utafiti: TABIA YA VIRUSI VYA HOMA YA MAJANO KIKUNDI C KULINGANA NA CHEMBECHEMBE ZA KIBIOLOGIA KATI YA WATUMIAJI WA MIHADARATI KWA KUJICHOMA SIDANO KATIKA VITUO VYA UREKEBISHAJI NASAHA JIMBO LA KILIFI, KENYA

FOMU YA RIDHAA NAMBA.

Habari za asubuhi/njioni?

Mimi ninaitwa _____

Kwa kipindi hiki,tupo kwenye utafiti uaolenga homa ya majano inayosababishwa na kirusi kwenye kikundi C jimbo la Kilifi kwenye vituo vya kutoa marekebisho nasaha au ushauri.Hili zoezi litatuwezesha kujua kiwango cha maambukizi,haina ya virusi na chembechembe za uai ambazo kila kirusi kimebeba.Utafiti huu utachangia pakubwa jinsi maambukizi ya homa ya majano inayosababishwa na kirusi katika kikundi C katika eneo hii.

Ni watu wagapi ambao watashiriki katikam huu utafit? Utafiti huu utahusisha washikiki zaidi ya 126 ambao wanafanyiwa marekebisho katika vituo mbali mbali hapa jimbo la Kilifi.

Utaratibu: Mdhumuni ya hili fomu ni kupata ridhaa yako wewe unayeshiriki.Ushiriki katika utafiti huu ni wa hiari.Ukiamua kushiriki,nitakupatia dodoso ili uweze kujaza maswali yaliopo kulingana na uleewa wako kikamilifu.Majibu ambayo utatoa yatatuzwa kwa usiri mkubwa ambapo mtafiti mkuu ndiye anamamlaka ya hayo majibu. Ili kuhakukusha kuna usiri,majina yako hayatakuwepo kwenye dodoso hili.Dodoso litapata namba tu na namba ya kliniki itatumika au namba ya mshiriki itatumika ili upate majibu yako.Unaweza ukajibu maswali yote lakini kama kuna swali ambalo hauko sawa nalo,una uhuru wa kuliacha.

Pia tutachukua sampuli ya damu kiasi cha mililita saba katika mshipa wako ambayo itatumika katika upimaji kubaini maambukizi ya homa ya majano ka kirusi cha kikundi cha C

.Baadaye nitabaini haina ya kirusi.

Hatari kwenye utafiti huu ni zipi? Hamna hatari zozote kushiriki katika utafiti huu ila tu utapata maumivu madogo unapochukuliwa damu.

Gharama zake ni gani? Hamna garama zozote kwa mshiriki.

Kuna faida gani kushiriki katika utafiti huu? Matokeo ya utafiti huu yatarudishwa kwako kupitia kliniki yako.Hayo matokeo pia yatapelekwa kwenye shirika la kitaifa la utafiti la KEMRI kwa ajili ya hatua ifaayo.Majibu yote kwa ujumla yatatumika kutoa ushauri kwa serikali na washika dau wengine kuhusu maambuklizi ya homa ya majano na pia saratani ya ini.

Utafiti huu utachukua muda gani?Utafiti huu utachukua muda wa mwaka mmoja?Mara tu unapoanza ushiriki wa utafiti huu na unajihisi kana kwamba uache,una uhuru wa kujiondoa ushiriki na hutatozwa faini yoyote.

Sampuli za damu zitafanyiwa uchambuzi wapi? Sampuli zote zitapelekwa maabara ya KEMRI kwa uchambuzi. Ni vyema nisisitize ya kwamba taarifa zako ni za umuhimu ili kutadhimini maambukizi ya homa ya majano haina ya C,jimbo la Kilifi.Tuheshimu ukubali wako.

Utachukua muda wa dakika 10-15 kujaza hili fomu.

Kwa ufafanuzi zaidi,wasiliana na Robert Mainga (**Mtafiti mkuu),** simu **+254 723934180,** au barua pepe: **rmainga2000@gmail.com** na Dr Eddy Odari (Msimamizi) simu +**254 725259296** au barua pepo: **kodarie04@yahoo.com** au mawasiliano yafuatayo yatapatikana:

Kwa katibu,

Hospitali kuu ya Kenyatta/Juo Kikuu cha Nairobi (KNH-UoN)

Kamati ya Kimaadili na Ukaguzi (ERC)

KENYATTA HOSPITAL NATIONAL (KNH)
Sanduku la Posta 20723 Kanuni 00202
Simu: 726300-9Faksi: 725272

au

JUO KIKUU CHA NAIROBI (UoN) Kitengo cha Matibabu
Sanduku la Posta 19676 Kanuni 00202
Barua pepo: Juokiku (254-020)2726300 Kuongezea 44355

Nimesoma ya fomu ya ridhaa na hivyo nimetoa maamuzi yangu.

Ridhaa ya kujaza dodosa **Ndio** ☐ **La** ☐

Ridhaa ya kuchukua sampuli ya damu **Ndio** ☐ **La** ☐

Ridhaa ya usafirishaji wa sampuli hadi KEMRI **Ndio** ☐ **La** ☐

Sahii/Ndole gumba la mshiriki: _____Tarehe: _____

Sahihi ya mtafiti mkuu _____ Tarehe_____

Appendix II: Questionnaire

Code:

QUESTIONNAIRE FOR A STUDY TITLED "PREVARENCE, SOCIAL DEMORGRAPHICS AND CHARACTERIZATION OF HEPATITIS C GENOTYPES CIRCULATING AMONG INJECTING DRUG USERS IN KILIFI COUNTY, KENYA"

QUESTIONNAIRE

Date _____

Enumerator administering the questionnaire_____ **Sign**_____

Rehabilitation Centre…….....Questionnaire Number……

1. Clinic ☐ ANC

2. Date of specimen collection Day …….. Month……Year……

3. Residence1.District……2Division……

3.Location……4. Village……..

4. Year of Birth: (dd/mm/yyyy) _____

5. Marital Status ***(Tick one only)***

1. ☐ Single 2. ☐ Married (Polygamous) 3. ☐ Married (Monogamous)

4. ☐ Separated / Divorced 5. ☐ Widowed

6 Religion Islam ☐ Christian ☐ Hindu ☐ African tradition ☐

7. Occupation House wife ☐ Business ☐ Casual ☐ Professional ☐

8. Education: ***(Tick one only)*** ***(Years completed)***

1.☐ No Education 2.☐ Lower Primary Education Class1-

Class5-

3.☐ Upper Primary Education 4. ☐ Secondary Education

5. ☐ Tertiary Education (or other Higher Education Institution)

9. Accepted Testing for Hepatitis? 1. ☐ Yes 2 ☐ No

10. Risk Factors Information

1. Have you ever been transfused ☐ Yes ☐ No

If yes, how many time? 1. ☐ 2. ☐ 3. ☐

Have you had any history of surgical procedure? ☐ Yes ☐ No

Any history of dental procedure ☐ Yes ☐ No

If yes, how many times_____

Do you have any tattoo on your body? ☐ Yes ☐ No

Have you ever had history of unsafe injection? ☐ Yes ☐ No

Have you ever under gone Caesarean section procedure ☐ Yes ☐ No

Any history of ear piercing ☐ Yes ☐ No

If yes, where 1.In jeweller's shop ☐ 2.At home ☐

Have you ever have had liver problem or jaundice? ☐ Yes ☐ No

Is there any history of liver disease among the members of your family? ☐ Yes

☐ No

Any history of Abortion or Miscarriage? ☐ Yes ☐ No

Any history of circumcision? ☐ Yes ☐ No

If yes, was it done individually or in a group of other ladies?

11. Screening Results: 1. Positive ☐ 2. Negative ☐

Kiambatisho 2: Hojaji

Usajili:

DODOSO LA UTAFITI WENYE KICHWA "KUZUIA, PICHA ZA JAMII NA KUZUIA, PICHA ZA JAMII NA TABIA YA VIRUSI VYA HOMA YA MAJANO KIKUNDI C KULINGANA NA CHEMBECHEMBE ZA KIBIOLOGIA KATI YA WATUMIAJI WA MIHADARATI KWA KUJICHOMA SIDANO KATIKA VITUO VYA UREKEBISHAJI NASAHA JIMBO LA KILIFI,KENYA"
DODOSO

TAREHE _____

Mwelekezi anayesimamia dodoso_____Sahihi_____

Kituo cha marekebisho nasaha…….....Namba ya Dodoso……

1. Kliniki ☐ Kliniki ya wamama

2. Tarehe ya kuchukuliwa sampuli Tarehe……. Mwezi……Mwaka……

3. Makazi1.Wilaya……2Tarafa……3. Eneo……4.Kijiji…….

4. Mwaka wa kuzaliwa: (dd/mm/yyyy) _____

5. Hali yako ya ndoa *(weka alama ya vema)*

1. ☐ Sijaolewa 2. ☐ Nimeolewa(wake wengi) 3. ☐ Nimeolewa(mke mmoja)

4. ☐ Nimeachika 5. ☐ Widowed

6 Dini Uislamu ☐ Mkristo ☐ Uhindu ☐ Kitamanduni ☐

7. Kazi Mke wa nyumbani☐ Biashara ☐ Kibarua ☐ Mtaalamu ☐

8. Elimu: *(weka vema)* ***(Miaka uliomaliza)***

1. ☐ sina elimu yoyote 2.☐ Elimu ya msingi kiwango cha chini darasa1-

darasa5-8

3☐ Elimu ya msingi kiwango cha chini 4. ☐ Elimu ya shule ya upili

5. ☐ Elimu ya chuo (Elimu nyingine)

9. Umekubali kupima virusi vya homa ya majano? 1. ☐ Ndio 2 ☐ La

10. Taarifa ya mambo hatarishi

Umeshawahi kuongezewa damu ☐ Ndio ☐ La

Kama ndio, mara gapi? 1. ☐ 2. ☐ 3. ☐

Je,unahistoria ya kufanyiwa upasuaji wowote? ☐ Ndio ☐ La

Je,unahistoria ya kug'olewa meno au matibabu ya meno ☐ Ndio ☐ La

Kama ndio, mara gapi_____

Je,unaalama yoyote aina ya chale kwenye mwili wako? ☐ Ndio ☐ La

Je,unahistoria yoyote ya uchomaji sindano ambao sio salama? ☐ Ndio ☐ la

Je,umeshawahi kufanyiwa upasuaji wakati wa kijufungua? ☐ Ndio ☐ La

Je,unahistoria yoyote ya kujitoboa masikio? ☐ Ndio ☐ La

Kama ndio, wapi 1.Duka la sonara ☐ 2.Nyumbani ☐

Je,umeshawahi kuwa na tatizo ya ini au kuwa na majano? ☐ Ndio ☐ La

Je,kuna historia yoyote ya kuwa na matatizo ya ini kwenye familia yako? ☐ Ndio ☐ La

Je,unahistoria yoyote ya utoaji au kuharibika kwa mimba? ☐ Ndio ☐ La

Je,unahistoria ya kutahiriwa? ☐ Ndio ☐ La

Kama ndio, ulitahiriwa peke yako au kwenye kikundi na wanawake wegine?

---

11. Matokeo ya mahabara: 1. Anavirusi ☐ 2. Hana virusi ☐

Appendix III: ROLE OF ETHICAL RESEARCH COMMITTEE

(a) Human Subjects

In all investigations involving human subjects, the following guidelines should be observed:

"First, do no harm."

Direct benefit to study subjects or community should exist.

Informed consent by subjects and/or community leaders including possible benefits, risks and inconveniences (the protocol should be accompanied by consent-seeking information sheet and informed consent form).

Indicate the method of maintaining confidentiality of information obtained during the study.

In case of new drugs and/or procedures to be used on human subjects, any possible side effects, untoward reactions and results of previous use even in animals should be stated.

(b) Animal Subjects.

In all investigations involving animals, the following guidelines should be observed:

Methods to minimize pain and distress must be specified:

If applicable, a strong justification must be made for not using proper drugs to alleviate pain and distress;

If applicable, the method of euthanasia should be specified.

For any clarification feel free to contact Robert Mainga (**the principal investigator),** on mobile **+254 723934180,** or gmail: rmainga2000@gmail.com and Dr Eddy Odari (**Supervisor**), on mobile +**254 725259296** or email: kodarie04@yahoo.com or the following contact will be available:

The Secretary,

Kenyatta National Hospital/ University of Nairobi (KNH-UON ERC), Ethical and Review Committee (ERC)

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OR

UNIVERSITY OF NAIROBI (UoN)
COLLEGE OF HEALTH SCIENCES
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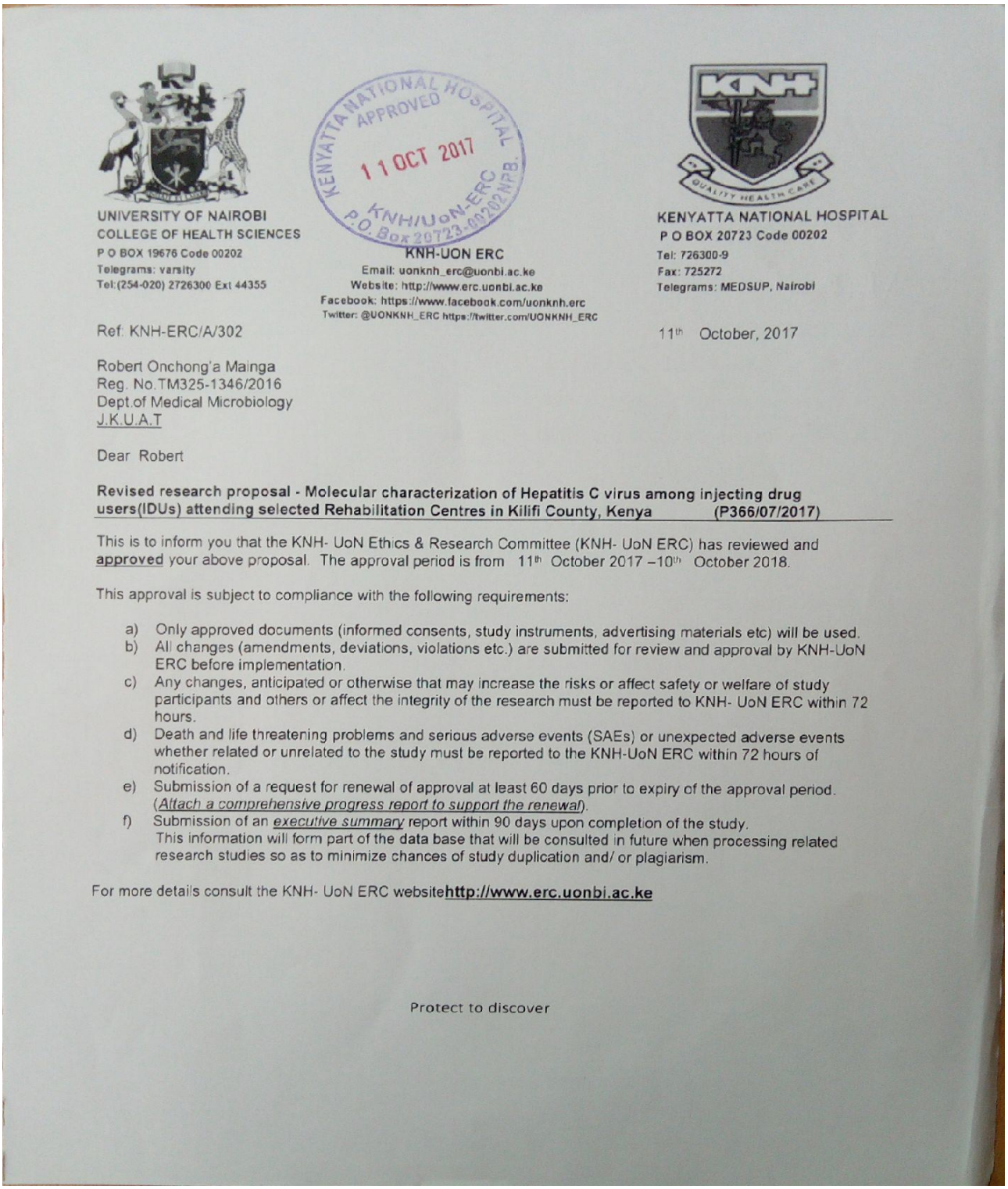
Appendix IV: Ethical Clearance



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Ref: KNH-ERC/A/302

11th October, 2017

Robert Onchong'a Mainga
Reg. No.TM325-1346/2016
Dept.of Medical Microbiology
J.K.U.A.T

Dear Robert

Revised research proposal - Molecular characterization of Hepatitis C virus among injecting drug users(IDUs) attending selected Rehabilitation Centres in Kilifi County, Kenya (P366/07/2017)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and approved your above proposal. The approval period is from 11th October 2017 –10th October 2018.

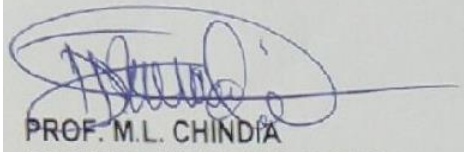
This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
d) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website**http://www.erc.uonbi.ac.ke**

Protect to discover

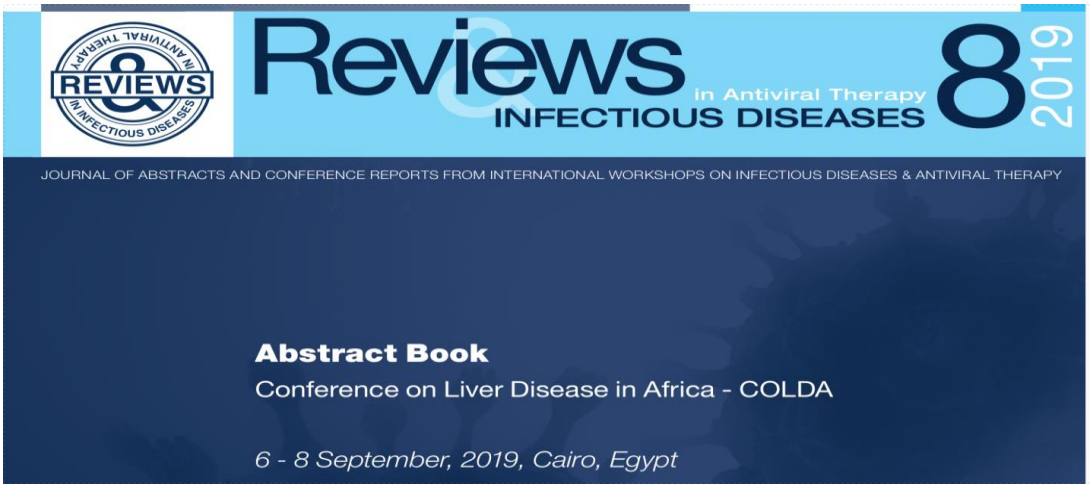
Yours sincerely,



PROF. M.L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Assistant Director, Health Information, KNH
The Chairperson, KNH-UoN ERC
Supervisors: Dr. Eddy Odari, (JKUAT), Dr. Raphael Lihana(KEMRI)

Appendix V: Abstract book Number 64, conference on liver Diseases in Africa (COLDA)



Characterization of Hepatitis C Virus Among Injecting Drug Users Attending Selected Rehabilitation Centers in Kilifi County, Kenya.

Mainga R[1], *Lihana R[1], Odari E[2], Borus P[1], Ole Kwalla A[1], Osero B[1],Charles M[1]1Kenya Medical Research Institute, Nairobi, Kenya, 2Jomo Kenyatta University of Agriculture and Technology (JKUAT) ,Nairobi, Kenya.*

Introduction: Hepatitis C virus is a major global health problem that is responsible for an estimated 170 million people infected around the world. It is known that some genotypes are more common in certain areas or in certain cohorts. Studies have shown that injecting drug use (IDUs) is a strong transmission route for HCV infection. Hepatitis C genotypes and subtypes have different prevalence considering the risk factors such as blood transfusion, hemodialysis, root of acquisition and others that are detected in intravenous drug users IDUs. The aim of this study was to determine the prevalence, social demographics and HCV genotypes circulating among IDUs in rehabilitation centres in Kilifi County Kenya.

Methodology: Questionnaire was used to collect social demographic characteristics. As this was a cross sectional study, a total of 127 HCV infected IDUs was selected randomly. Statistical analysis was done using non parametric Mann Whitney U test and associations analyzed using chi square. Up to 5ml of blood was collected and serum was screened for HCV antibodies using rapid test kit. RNA was extracted from positive samples, reverse transcribed, amplified and sequenced.

Results: Serology (28) samples tested positive were done and 11 samples which reflected only male tested positive for PCR. The prevalence of this study was 22.1% (n=28). The highest number of injecting drug users occurred in the age group of 31-35 compared to other age brackets. 35 (27.6%) of IDUs married, 68 (53.5%) single and 24 (18.9%) divorce. Single group and divorce group had odds ratio (OR) of 3.0 and 2.0 (OR>1), this indicated that exposure was associated with higher odds of outcome. IDUs of the Muslim faith were 16(OR=1.4. P=0.309),

Christian 12 (OR=1.0.P=0.497.) and pagans 4. (OR<1) Tout/drivers/bodaboda group of IDUs had highest HCV infection 12(46%, OR=1) compared to other IDUs. On education, lower primary had high level infection compared to upper primary and secondary and tertiary level. In the 11samples subjected to PCR, three male had HCV genotype1a in which 2 fall in age between 31-40 and one above 41 years. Eight were genotype 4 positive where 2 were less than 30 years, 5 between 31-40 years and one above 40 years. Two IDUs with HCV1a belonged to single group and one to divorced category. Eight IDUs with genotype 4, three belonged to single group, three married and two divorced. The three genotype 1a, two were Muslims and one Christian. Seven Muslim and one Christian were positive for genotype 4. Lower primary, had one genotype 1a and three genotype 4, Upper primary with only four genotype 4 and secondary and above there was two genotype1a and one genotype 4. In occupation Tout/Driver/Bodaboda, had three genotype4, Beach boys, one genotype 1a and one genotype 4 to Fisherman then the rest had only 1 genotype 4.

Conclusion: The study reveals the true picture of the burden of HCV infection among IDUs in Kilifi County and therefore the Government should formulate policies for intervention on testing and treatment.

Appendix VI: Publication Paper

Research Article

Characterization of Hepatitis C virus circulating among injecting drug users (IDU) in Kilifi county, Kenya

Abstract

Background: Hepatitis C virus is a major global health problem estimated to infect over 170 million people globally with the most common route ofinfection being injecting drug use (IDU). Treatment for HCV infection has traditionally been shown to be genotype specific; however the available drugs are still expensive and out of reach in many developing countries. To improve on monitoring, there is need to continuously document the genotypic burden and epidemiology in different populations arises.

Objectives: This study aimed to determine the circulating genotypes and link the data to the socio-demographics of injecting drug users in Kilifi County along the Kenyan coastline.

Methods: Using a random sampling method, this was a cross-sectional prospective study conducted among 127 injecting drug users, whereby ethical clearance was sought from Kenyatta National Hospital/ University of Nairobi Ethical and Research review committee (KNH/UON-ERC), and Reference number P366/07/2017 on 25th September 2017. Serology for HCV was done followed by nucleic acid amplification and eventual genotyping. Socio-demographic data was collected using questionnaire administered at the sites.

Results: A total of 28 (23 males and 5 females) samples out of 127 samples were positive for HCV giving a prevalence of 22.1%. The modal age group was 25– 49 years. Of the positive samples, 11 were amplified by PCR, all from the male IDUs. Prevalent genotypes included genotype 1a (13%) and genotype 4a (87%). Both genotype 1a and 4a were only found in males.

Conclusion: Although it is the first time to report HCV4a in Kilifi town and Mtwapa as compared to Watamu and Malindi which had both HCV1a and HCV4a. Tourists born in countries where HCV prevalence is high are supposed to be screened before accessing entry to Kilifi County. No clinical trial was conducted. The study reveals the burden of HCV infection among IDUs inKilifi County. The Government should formulate policies for intervention on testing and treatment of HCV in Kilifi County targeting IDUs in order to minimize spread to other populations.

Keywords: characterization, hepatitis cvirus, genotyping, prevalence

Volume 8 Issue 2 - 2020

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Abbreviations: Bp, base pair; CI, confidence interval; CSW, commercial sex workers; dNTPS, nucleoside triphosphate; EDTA, ethylenediaminetetraacetic acid; DNA, deoxyribonucleic acid; HCC, hepatocellular cancer; HCV, Hepatitis C virus; HCV1a, Hepatitis C virus Genotype one Subtype a; Hcv4a, HCVFour Subtype a; IDUs, injecting drug users; KANCO, Kenya AIDS NGOs Consortium; KEMRI, Kenya Medical Research Institute; MEWA, muslim education welfare association; PCR, polymerase chain reaction; RNA, ribonucleic acid; RT-PCR, ribonucleic acid; SPSS, statistical package for the social sciences; TE buffer, buffer used to solubilize DNA or RNA, while protecting it from degradation; µl, microliters; 5'UTR, five prime untranslated region; WHO, world health organization; ZR, zymo research

Introduction

Hepatitis C virus causes liver cancer such as hepatocellular carcinoma (HCC) and lymphomas in humans.[1,2]Hepatitis C is a global health problem, over130 million people are chronically infected yearly.[3] Studies show that every year, approximately 308,000 deaths occur due to liver cancer while up to 758,000 deaths are estimated to occur due to liver cirrhosis.[4]Approximately 80% of HCV infected persons will develop chronic hepatitis. Eleven percent will progress to liver cirrhosis for the period of 20-year time interval. Life-threatening will be as a result of liver failure which leads to hepatocellular carcinoma.[5,6] Blood products, needle or syringe sharing among members of intravenous drug parties or undergoing a needle stick by health workers leads to transmission of HCV. Other risk factors are high-risk sexual behaviors, tattooing, reused and unsterilized dental and surgical instruments, and unsterilized laboratory equipment.[7]About 80% of HCV infected persons are asymptomatic. Infected persons with acute HCV exhibit symptom ranging from fever, joint pain and jaundice. Asymptomatic individuals are more difficult to identify.[8]

Most IDUs with persistent infection are unaware of the infection, screening programs to identify patients will be required to prevent silent progression of the disease.[9–11]In most case HCV is often first diagnosed in late stage. Due to slow and silent onset, many patients are unaware of their infection and at least 40% cases remain undetected. Chronic hepatitis C infection is difficult to assess, because it is frequently subclinical.[5] Patients with chronic hepatitis C are at risk of cirrhosis and hepatocellular carcinoma and their contacts (especially injecting drug users, IDUs and commercial sex

workers CSWs) at risk of acquiring the infection through exposure to the virus.[12]Given this situation, it appears that there is no clear understanding of the contextual factors that continue to fuel the upsurge in HCV infections among the key populations such as IDUs and CSWs.[13]Molecular genotyping of HCV identified in patients is necessary, for proposing therapeutic options.[4] It is assumed that some genotypes are more common in certain areas or groups of people. The high number of chronically infected individuals, the burden of disease and the absence of a vaccine indicates that treatment will form part of the control of the disease.[14] The aim of this study was to determine the prevalence, social characteristics and HCV genotypes circulating among IDUs in rehabilitation centers in Kilifi County Kenya.

Material and methods

Study site and study design

This study was conducted in Kilifi County located within the Kenyan coastline. The design was cross sectional. Participants were recruited from harm reduction/rehabilitation centers within Malindi, Watamu, Kilifi town and Mtwapa {note that Kilifi town and Mtwapa is one centre divided into two called Muslim Welfare Association (MEWA)} located at Kilifi County.

Sample collection, transportation and storage

After informed consent, Socio-demographic data was captured using questionnaire which was administered at the facilities. The data captured included, name of centers, date of birth, residential area, marital status, religion, occupation and the level of education. Further five milliliters of venous blood was collected into EDTA vacutainer tubes. They were then transported in dry ice from rehabilitation Centers to KEMRI laboratories for analysis.[15]

Serological tests

Blood in the EDTA tube was centrifuged at 3000 rounds per minute (rpm) for 10 minutes to separate plasma from the whole blood. Plasma was transferred into duplicate aliquots. Five microliter (µL) of the serum was used for HCV detection using diaspot rapid serology kit.[16]based on the manufacturer's instructions as previously described.[17]

Hepatitis C RNA extraction

Viral RNA was extracted from 170 µl of serum using Zymo Research Kit (IrvineCA, U.S.A.) according to the manufacturer's instructions. The RNA pellets were resuspended in 60 µl of TE buffer. The RNA concentrations were measured in ng/µl using NanoDrop spectrophotometer (ThermoFisher Scientific, Wilmington, Delaware, USA).

Polymerase Chain Reaction (RT-PCR) and Sequencing reaction

Nested RT-PCR was carried out using Zymo Research PCR Kit (IrvineCA U.S.A.)and GeneAmp PCR system 9700.[18] In reverse transcription and PCR reactions, both 5'UTR universal primers: forward KY80 (5'-GCA GAA AGC GTC TAG CCA TGG CGT-3') and reverse KY78 (5'-CTC GCA AGC ACC CTA TCA GGC AGT-3') were used. 35 cycles of PCR (94°C for 30 seconds, 54°C for 1 minute, and 68°C for 2 minutes) were performed HCV PCR products of 244 bp were visualized, and electrophoresed on 1.5% agarose gel.[19,20] The positive amplicons were purified using the ZR DNA sequencing clean up kit.[20](Zymo, Irvine, CA, USA) according to the manufacturer's protocol to eliminate unincorporated primers and dNTPs.[21] The purified amplicons were sequenced with Big Dye kit, and the ABI PRIS M 3100 genetic analyzer (Applied Biosystems) according to manufacturer's instructions.

Sequence analysis

Samples were aligned with the representative sequences for each major genotype and subtype selected from the HCV database and Gene Bank using the Multiple Sequence Alignment Program, ClustalW. Homology and evolutionary distance pair-wise comparisons for percent nucleotide were made.[22]The phylogenetic analysis of HCV isolates was performed with MEGA7.0.14software.

Statistical methods

Statistical Package for the Social Sciences (SPSS) version 20 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Non-parametric Mann-Whitney U test was used to compare between genotypes with respect to quantitative variables. Chi square was used to analyze association between social demographics and circulating HCV genotypes. A p-value of less or equal to 0.05 was considered statistically significant (Valentine *et al.*, 2018).

Results

Demographic characteristics of study participants

A total of 127 IDUsregistered patients were recruited for the study {107 (84.2%) were male and 20 (15.7%) were female}. The samples in each site were as follows; Malindi, (n =35), Watamu (n =43), and Kilifi and Mtwapa (n =49) respectively. The mean age for the three centers was found to be 33.7 years.

Prevalence of HCV among study participants

Anti-HCV positive included 10 IDUs from Kilifi, 4 from Watamu and 14 from Malindi making a total of 28 out of 127 participants. Twenty three IDUs (82.1%) were male while 5 (17.9%) were women. Of the 28 IDUs, 11 (8.7%) male were confirmed to be HCV positive using PCR as compared to none for female IDUs.A total 28 participants screened, seroprevalence was established at 22.1%. With the highest prevalence found in males (18.1%; n=23) and low in females at (3.9%; n=5) (P=0.004). The seroprevalence in Malindi was 40%, Watamu 9.3% and Kilifi and Mtwapa 20.4% respectively(Table 1).

Serology outcome and demographic characteristics

The highest number of injecting drug users was in the age group of 31-35(39%),it also had a higher number of IDUs who tested positive 8 (28.5%) as compared to other age brackets.In the study, 55 IDUs sampled were married, 68 single and 24 divorced. (OR=1.0, 3.0, 2.0. P = 0.065, 0.330 and CI: 0.9-9.7, 0.5-8.6 respectively). Those of the Muslim faith had higher number of HCV infections, 16 compared to Christians, 12 and pagans zero. (OR= 1.0, 1.4 and P= 0.497, 0.309 respectively). In education, Lower primary had 13, Upper primary 6 and Secondary and Tertiary 9 (OR=1.0, 0.8, 2.1, P = 0.682, 0.146. CI: 0.3-2.3) for upper primary, CI: 0.8-5.8 for secondary and tertiary respectively).Touktdrivers/riders group of IDUs had highest HCV infection 12, (OR=1), Beach boys were5, Fishermen were 3 and

other IDUs with different occupations were 7 respectively. (OR=1.0, 1.3, 0.8, 0.9. P= 0.688, 0.712, 0.851 CI: 0.4-4.3, 0.2-3.1, 0.3-2.6 respectively)(Table 2).

Polymerase chain reaction (PCR) and genotyping

The 28 samples which were positive were subjected to PCR., 11 (39.3%) were confirmed positive (all males) and none of the 5 female IDUs was positive. The 11 samples were then subjected for sequencing and results gave 3 out of the 11 samples as HCV Genotype 1 subtype a (HCV1a) while 8 were HCV Genotype 4 subtype a (HCV4a)(Table 3).

Table 1 Prevalence of Hepatitis C Virus (HCV) which is 22.1% and its Genotypes among study participants in Kilifi County

	Rehabilitation centers			Overall	P Value
	KILIFI, n=49	WATAMU, n=43	MALINDI, n=35		
Variable	n (%)	n (%)	n (%)	n (%)	
Rapid test					
Negative	39 (79.6)	39 (90.7)	21 (60.0)	99 (78.0)	0.004
Positive	10 (20.4)	4 (9.3)	14 (40.0)	28 (22.1)	
PCR					
HCV1a	0	1 (22.2)	2 (66.7)	3 (27.3)	0.121
HCV4a	5 (100.0)	2 (66.7)	1 (22.2)	8 (72.7)	

Table 2 Correlation between Serological outcome and demographic characteristics of Participants in Kilifi County

Variable	N	(n/N) %	Positive n (%)	Negative n (%)	OR (95%CI)	P value
Age						
16-20 years	3	66.7	2 (66.7)	1 (22..2)	1.0	
21-25 years	12	25.0	3 (25.0)	9 (75.0)	0.2 (0-2.6)	0.171
26-30 years	29	10.3	3 (10.3)	26 (89.7)	0.1 (0-0.8)	0.011
31-35 years	39	20.5	8 (20.5)	31 (79.5)	0.1 (0-1.6)	0.070
36-40 years	21	33.3	7 (33.3)	14 (66.7)	0.2 (0-2.2)	0.165
41-45 years	14	7.1	1 (7.1)	12 (92.9)	0.04 (0-0.5)	0.014
46-50 years	7	57.1	4 (57.1)	3 (42.9)	0.7 (0-11.2)	0.778
51-55 years	2	0	0	2 (100.0)	-	0.136
Gender						
Male	107	21.5	23 (21.5)	84 (78.5)	1.0	
Female	20	25.0	5 (25.0)	15 (75.0)	1.2 (0.4-3.7)	0.729
Marital status						
Married	35	11.4	4 (11.4)	31 (88.6)	1.0	
Single	68	27.9	19 (27.9)	49 (72.1)	3.0 (0.9-9.7)	0.065
Divorced	24	20.8	5 (20.8)	19 (79.2)	2.0 (0.5-8.6)	0.330
Religion						
Muslim	77	0	16 (20.8)	61 (79.2)	1.0	
Christian	46	26.1	12 (26.1)	34 (73.9)	1.4 (0.6-3.2)	0.497
Pagan	4	20.8	0	4 (100.0)	-	0.209

Citation: Robert HO, Eddy OO, Peter BK, et al. Characterization of Hepatitis C virus circulating among injecting drug users (IDU) in Kilifi county, Kenya. J Hum Virol Retrovirology. 2018;6(2):22–30. DOI: 10.15406/jhvrv.2018.06.00117

Table Continued

Variable	N	(n/N) %	Positive n (%)	Negative n (%)	OR (95%CI)	P value
Education						
Lower primary and below	65	20.0	12 (20.0)	52 (51.5)	1.0	
Upper primary	36	16.7	6 (16.7)	30 (20.2)	0.8 (0.2-2.2)	0.682
Secondary and above	26	34.6	9 (34.6)	17 (17.2)	2.1 (0.8-5.8)	0.146
Occupation						
Tuktrivershriders	55	21.8	12 (21.8)	43 (78.2)	1.0	
Beach boy	19	26.3	5 (26.3)	14 (73.7)	1.3 (0.4-4.3)	0.688
Fisherman	17	17.6	3 (17.6)	14 (82.4)	0.8 (0.2-3.1)	0.712
Other	35	20.0	7 (20.0)	28 (80.0)	0.9 (0.3-2.6)	0.837

Key: N,number of participants, n, participants who are either Positive or negative (n/N) percentage of participants, OR, odds ratio, CI, confidence intervals

Table 3 Correlation of Polymerase Chain Reaction (PCR) and Genotypes with participant demographic characteristics in Kilifi County

Variable	HCV1a (%)	HCV4a (%)	P value
Age			
16-20 years	0	0	
21-25 years *	0	0	
26-30 years	0	2 (100.0)	
31-35 years	1 (25.0)	3 (75.0)	
36-40 years	1 (33.3)	2 (66.7)	0.426
41-45 years	0	1 (100.00	
46-50 years	1 (100.0)	0	
51-55 years	0	0	
Gender			
Male	3 (27.3)	8 (72.7)	
Female	0	0	-
Marital status			
Single	2 (40.0)	3 (60.0)	
Married	0	2 (100.0)	0.452
Divorced	1 (33.3)	2 (66.7)	
Religion			
Pagan	0	0	
Muslim	2 (22.2)	7 (77.8)	0.425
Christian	1 (50.0)	1 (50.0)	

Citation: Robert HO, Eddy OO, Peter BK, et al. Characterization of Hepatitis C virus circulating among injecting drug users (IDU) in Kilifi county, Kenya. J Hum Virol Retrovirology. 2018;9(2):33–39. DOI: 10.15406/jhvrv.2018.09.00317

Table Continued

Variable	HCV1a (%)	HCV4a (%)	P value
Education			
Lower primary and below	1 (25.0)	3 (75.0)	
Upper primary	0	4 (100.0)	0.145
Secondary and above	2 (66.7)	1 (33.3)	
Occupation			
Tuktriverhriden	2 (40.0)	3 (60.0)	
Beach boy	0	2 (100.0)	0.488
Fisherman	1 (50.0)	1 (50.0)	
Other	0	1 (100.0)	

Phylogenetic tree construction

With reference of Full-genome consensus sequences for different HCV genotypes (including genotypes 1, 2, 3, 4, 5 and 6), there was only genotype 1 and 4 (Figure 1).The rest there was no evident on the tree because there were no associated isolates. Maximum composite likelihood algorithms were utilized, and phylogenetic trees were constructed by the neighbor-joining method. The reliability of different phylogenetic groupings was evaluated by using the bootstrap re-sampling test from the MEGA program (1,000 bootstrap replications).[19,20,21]

Discussion

In this study we have managed to establish a higher prevalence of HCV in male injectors107 (84.2%) than females 20 (15.7%). We have also shown a changing trend of HCV genotype distribution with genotype 4a becoming more prevalent in this region as opposed to the traditional genotypes 1a, genotype 1a can be seen in Watamu and Malindi but not in Kilifi, it also shows that it is the first time to report HCV4a in Kilifi. Hepatitis C patients were found to be illiterate, it shows that lack of knowledge about the disease and its precautions become a strong cause for disease. In this study, there is an increase of HCV infection as compared to the study which was done by Sacks-Davis R[15]in the entire Coast which showed a prevalence of 16.7%.We have shown that there is increased diversity along the coastal strip centers like Mtwapa which is part of Kilifi town both are under Muslim welfare association (MEWA).

Genotyping of hepatitis C virus (HCV) is considered an important tool for epidemiological and clinical studies and valuable marker for disease progression and response to antiviral therapy.[20]However, development of antiviral drugs, vaccines, and genotyping assays has a major impact on HCV this is because HCV has high levels of genetic diversity.[6,21,22]In this study, we have confirmed that only two genotypes circulating in Kilifi County; this can form a basis for more targeted control for the 2 subtypes in the region.Hepatitis C Virus diversity is very essential in response to antiviral therapy.[14,20] More severe liver disease and more elevated liver enzymes have been highly associated with HCV genotype 1.[20] The endemicity of HCV genotypes in some areas is revered with multiplicity and diversity of subtypes and strains. A good example is origin of genotypes 1 and 2 to West Africa and genotype 4 to Central Africa.[23] This can be done by using a phylogenetic analysis to revealed two monophyletic clusters (bootstrap value,~87) containing HCV infected patients in Kilifi County from which a partial 5'UTR sequence was available.

The largest cluster contained 87% of HCV sequences were identified as genotype 4, with isolates M01, M02, M05, M07, M23, K20, W10, and W14. This isolates clusters with isolates from: Portugal, France, Egypt, Cyprus, Southeast Asia, Middle East and Saudi Arabia. On the other hand genotype1a Isolates were K08, K14 and W17 Clusters with isolates from: America, Japan, Indonesia, China and India. Genotype diversity is particularly high in China and many Southeast Asian countries and also in Western Europe and Australia, perhaps as a result of population immigration from Africa and/or Asia.[24]In countries like these, vaccine efficacy at a population level would be dependant on the generation of cross reactive immunity; an alternative approach could also be the development of vaccines hosting different immunogens. In this study, HCV genotype 4a and genotype1a is predominant among the population of drug users, this suggest that there is a need for continuous monitoring of these for better treatment outcomes that entirely depend on HCV genotyping results. With global travel, increasing of tourist to Kilifi Count and the geographic zone of this region dictates the distribution of these genotypes in this region.[25]

Due to lack of vaccine and effective therapy, the prevention of HCV infection has been a great challenge to developing country and owns one-fifths of the world's population.[14,20]World Health Organization Assembly approved on 2016 the Global Health Sector Strategy to eliminate hepatitis infection by 2030.[26]It introduced global targets for the care and management of HCV including 90% reduction in new cases of chronic hepatitis C, 65% reduction in hepatitis C deaths, and treatment of 80% of eligible people with chronic hepatitis C infections.[27]To achieve these goals, the country need to develop rational policies based on reliable epidemiological evidence.[28] In Kilifi County, Screening populations at risk and Counseling program to IDUs with history of sexual contact or sharing of items should be done to stop spread of HCV in the region. Tourists born in countries where HCV prevalence is high are supposed to be screened before accessing entry to Kilifi County.However, data are often outdated and conflicting, making evidence-based policy and resource allocation difficult.

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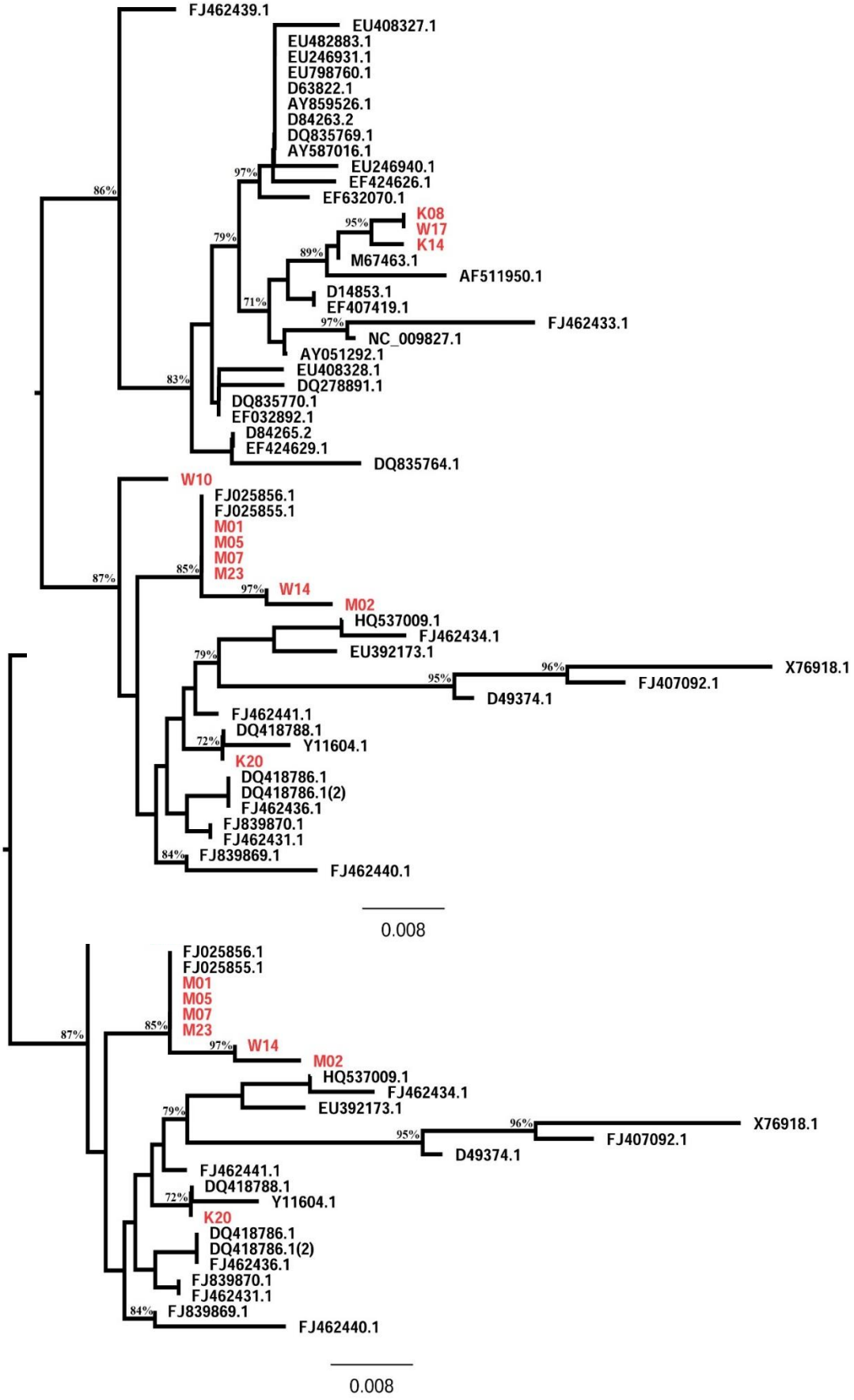
FJ462439.1
EU408327.1
EU482883.1
EU246931.1
EU798760.1
D63822.1
AY859526.1
D84263.2
DQ835769.1
AY587016.1
EU246940.1
EF424626.1
EF632070.1
K08
W17
K14
M67463.1
AF511950.1
D14853.1
EF407419.1
FJ462433.1
NC_009827.1
AY051292.1
EU408328.1
DQ278891.1
DQ835770.1
EF032892.1
D84265.2
EF424629.1
DQ835764.1
W10
FJ025856.1
FJ025855.1
M01
M05
M07
M23
W14
M02
HQ537009.1
FJ462434.1
EU392173.1
X76918.1
FJ407092.1
D49374.1
FJ462441.1
DQ418788.1
Y11604.1
K20
DQ418786.1
DQ418786.1(2)
FJ462436.1
FJ839870.1
FJ462431.1
FJ839869.1
FJ462440.1
86%
97%
79%
95%
89%
71%
97%
83%
87%
85%
97%
79%
96%
95%
72%
84%
0.008
FJ025856.1
FJ025855.1
M01
M05
M07
M23
W14
M02
HQ537009.1
FJ462434.1
EU392173.1
X76918.1
FJ407092.1
D49374.1
FJ462441.1
DQ418788.1
Y11604.1
K20
DQ418786.1
DQ418786.1(2)
FJ462436.1
FJ839870.1
FJ462431.1
FJ839869.1
FJ462440.1
87%
85%
97%
79%
96%
95%
72%
84%
0.008

Conclusion

This study reveals the burden of HCV infection among IDUs population inKilifi County. HCV infection seroprevalence rate seem to be increasing up to (22.1%) as compared to the previous studies 16.4% by Mwatelah[7]and 22% by Akiyama,[8]this might become a major health predicament in future. Although screening and treatment is expensive, the Government should put control measures like conducting constant surveillance, educating, screening and treatment of the affected population in order to control the disease from the area.

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Declaration

Ethical consideration

Ethical clearance was sought from Kenyatta National Hospital/ University of Nairobi Ethical and Research review committee (KNH/ UON-ERC), Reference number P366/07/2017 on 29th September 2017.

Consent

Consent to participate is attached together with the worksheet and Manuscript.

Availability of data and materials

It is available in IDUs worksheet that I have attached.

Conflicts of interest

There were no Conflicts ofinterest in this project.

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The project was funded by Children investment fund foundation (CIFF). File Number: E- 2008. The owner of the project frontier to buy for me reagents since I was a student, I dint have a source of funds for my project.

Author's Contributions

M. O. R. He is the author, his work was to collect samples, do laboratory work (both serological and molecular work) and writing of manuscript.

O. O. E. He supervised laboratory work, assisted in field work, review manuscript writing and data analysis.

B. K.P. His work was to review manuscript and administrative work.

O. K. A. His work was to review manuscript and laboratory work analysis.

M. G. J. His work was field work and laboratory analysis

O.O. B. His work was review manuscript writing, data analysis and field work.

R. K. V.His work was to assist in laboratory analysis.

M. S. C. He assisted in funding of the project, field work and administrative work.

L. W. R. He supervised laboratory work, review manuscript writing and data analysis.

All authors have read and approved this manuscript for publication.

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