INCIDENCE OF HEPATITIS C VIRUS INFECTION IN A SEMI-URBAN COMMUNITY OF SOUTHWESTERN NIGERIA

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ABSTRACT

Hepatitis C Virus (HCV) infection is an important cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma. Its epidemiology has been well described in developed countries. In Nigeria, previous studies on the virus were hospital-based or point prevalence from which the burden of HCV cannot be accurately determined. A population-based prospective study was therefore designed to assess the burden of HCV infection in a semi-urban community in southwestern Nigeria.

A cohort of 490 purposively recruited consenting participants in Saki, a border town in Nigeria were enrolled and followed up for nine years (2003-2012). Blood samples were collected and tested for the presence of HCV antibodies using the ELISA technique from each participant at baseline, one year, 2 years and 9th year. The participants included 299 male and 191 female members of two occupational groups, auto-mechanics (n=236) and fashion designers (n=254) with age range of 15 to 65 years (median age=26years). A structured questionnaire was administered to capture information on awareness of HCV infection as well as predisposing factors including sharing of sharp objects, transfusion of blood and blood product, polygamy and multiple sexual partnership. The cohort was continuously provided education on prevention of sexually transmitted diseases and blood borne pathogens during the follow-up period. Data were analysed using descriptive statistics and ANOVA at p=0.05. Incidence of infection was reported as number of HCV cases/1000 person years.

The rate of HCV infection at baseline was 8.4%. A total of 27 new cases of infection were identified in the cohort giving an overall incidence of 27.8 per 1000 person years. Incidence of HCV increased from first to the second (9.0 Vs 24.7 per 1000 person years) year but declined thereafter (11.3 per 1000 person years). Incidence of the infection increased with age and peaked among persons 45-54

years (34.5 and 38.5 per 1000 person years). The incidence was higher among male than female (21.2 Vs 14.5 per 1000 person years). Incidence in both male and female groups increased from first to second point but declined sharply thereafter. Incidence of HCV infection was higher among auto-mechanics (31.4 per 1000 person years), a male occupational group than fashion designers (23.9 per 1000 person years), a female dominated occupational group. Similarly, HCV incidence was higher in male (49.9 per 1000 person years) than female (14.6 per 1000 person years) members of the fashion designer group (Risk Ratio = 2.7, CI=1.32-5.87). The only significant risk factor identified was sharing of sharp objects (RR=2.4, CI=1.0-5.56, $\chi 2_{0.05:1}$=4.329, p=0.04).

There was a substantial burden of HCV infection in the studied community. Sharing of sharp objects is a significant predisposing factor for HCV infection among the study populations. The high burden of the infection indicates the need for urgent implementation of measures to control HCV infection in Nigeria.

Keywords: Hepatitis C virus, Incidence, Community-based, Occupational groups, Nigeria

Word count: 459

CERTIFICATION

We certify that Dr. Adeleye Solomon Bakarey carried out this work in the Department of Virology, College of Medicine University of Ibadan under our supervision.

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DEDICATION

This work is dedicated to God almighty for providing me life, good health and strength to get to the end of the programme. May Thy Name be glorified.

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I am also grateful to my family for the patience, perseverance, understanding, moral and financial support till this moment. I pray that all our hopes and aspirations will never be in vain. At the end of it all, we shall have cause to rejoice in the Lord and glorify His Name.

Above all, I give glory and adoration to Almighty God, for seeing me through the end of the programme and hoping that success will be my ultimate, Amen. Without thee where would I have been today? May Thy Holy Name be glorified?

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ABBREVIATIONS

ALP	**Alanine aminotransferase**
AST	**Aspertate amino transferase**
CD	**Clusters of Differentiation**
CDC	**Centres for Disease Control and prevention, Atlanta**
cDNA	**complimentary Deoxyribonucleic acid**
CI	**Confidence limit**
COM	**College of Medicine**
DNA	**Deoxyribonucleic acid**
EIA	**Enzyme Immunoassay**
ELISA	**Enzyme-Linked Immunosorbent assay**
HBsAg	**Hepatitis B surface Antigen**
HBV	**Hepatitis B Virus**
HCC	**Hepatocellular carcinoma**
HCV	**Hepatitis C Virus**
HIV	**Human Immunodeficiency Virus**
IgG	**Immunoglobulin G**
IRB	**Institutional review board**
Mab	**Monoclonal antibody**
NS5a	**Nonstructural protein 5a**
NS5b	**Nonstructural protein 5b**
ORF	**Open reading frame**
PBS	**Phosphate buffered saline**

PBS-GT	**PBS, gelatin & Tween 20**
PBS-T	**PBS & Tween 20**
PCR	**Polymerase chain reaction**
RNA	**Ribonucleic acid**
RR	**Risk Ratio**
SK	**Saki**
UCH	**University College Hospital**
UI	**University of Ibadan**
WHO	**World Health Organization**

CHAPTER ONE

INTRODUCTION

Hepatitis is an inflammation of the liver parenchyma caused by a variety of pathogens including viruses. Viral hepatitis is a general term that is reserved for infections of the liver whose etiological agents are viruses. Eight distinct groups of viral hepatitis namely A-H have been identified (Okamoto *et al*., 1988; Norder *et al*., 1994; Grosheid and Van Damme, 1996; Tobler and Hirsch, 1997; Kao, 2002; Thio, 2003). Hepatitis C virus (HCV) infection is known to cause liver cancer, liver cirrhosis and hepatocellular carcinoma (HCC) in chronic conditions and is mainly transmitted through blood and blood products (WHO, 2002; Petrus *et al.,* 2005; UNAIDS, 2006; Filippini *et al.,* 2007; Magiorkinis *et al.,* 2009; Opaleye *et al.,* 2010; Sanaullah *et al.,* 2011; Forbi *et al.,* 2012; Oh *et al.*, 2012; Kowdley *et al.,* 2014).

Studies (Melita *et al*, 2000; Vandelli *et al.,* 2004; Vincent *et al.,* 2007; Magiorkinis *et al.,* 2009; Opaleye *et al.,* 2010; Forbi *et al.*, 2012; Stamouli *et al.*, 2012; Kowdley *et al.*, 2014) have also shown that HCV is an important cause of morbidity and mortality worldwide resulting in chronic liver disease associated with liver cirrhosis and HCC. However, little information regarding its incidence is available in developing countries where incidence of HCV is estimated to be 5.9 to 9.7 times higher than in the developed countries like the United States of America and Australia (Alter *et al.,* 1992; Crofts *et al.,* 1993; Jawetz *et al.,* 1998; Sarwar *et al.,* 2008; Magiorkinis *et al.,* 2009; Williams and Bells, 2011; Yousra *et al.*, 2013; Abdelwahab *et al.*, 2013; WHO, 2014). Also, testing for HCV infection is

rarely requested in general medical practice in most developing countries unlike HIV and HBV. It is therefore pertinent to know that HCV is spread parenterally, heterosexually, homosexually, neonatally, through contact in infected household and among injection drug users (Vandelli *et al.,* 2004; Petrus *et al.,* 2005; Sima *et al.,* 2007; Sanaullah *et al.,* 2011; Cifuentes *et al.*, 2012; Newshome, 2013; WHO, 2014).

Hepatitis C virus was discovered in the late 1970's after the introduction of sensitive assays for screening blood for hepatitis B virus. It was anticipated that post transfusion hepatitis would be virtually eliminated but this was not to be. There remained a substantial residue of cases, which were called non-A, non-B hepatitis (NANBH). The causative agent remained frustratingly elusive for over a decade until 1989 when a team of molecular biologists in the United States of America succeeded in the ambitious assignment. The ingenious protocol they devised serves as prototype to discover non-A, non-B, hepatitis viruses such as hepatitis C virus (Hollinger *et al.,* 2000; Petrus *et al.,* 2005; Davis *et al.,* 2010; Rein *et al.,* 2011; Oh *et al.,* 2012).

Acute hepatitis C is clinically similar in hepatitis B. The major differences are that the incubation period of hepatitis C ranges up to several months with an average of 6-8 weeks and about 75% of infections are subclinicals (Mellita *et al.,* 2000; Hollinger and Liang, 2004) while clinical infections of Hepatitis C are generally less severe than hepatitis B, having a shorter preicteric period, milder symptoms, absent or less marked jaundice, and somewhat lower serum alanine aminotransferase (ALT) levels, which often fluctuate widely (Ola *et al.,* 2002; Hoffnagle and Liang, 2004). There is also a clear correlation

between chronic HCV infection and the development of hepatocellular carcinoma (HCC). The most remarkable and alarming aspects of HCV infection are its high rate of persistence and its ability to induce chronic liver disease (Ayodele and Salako, 2003; Vandelli *et al.,* 2004; Sanaullah *et al.,* 2011; Casey and Lee, 2013; Yousra *et al.,* 2013).

Epidemiological studies have established that HCV is established as the major parenteral type and it is the major cause of post transfusion Non-A Non-B hepatitis (Alter *et al.,* 1989; Kuo *et al.,* 1989; Ayodele and Salako, 2003; Magiorkinis *et al.,* 2009). Transmission of HCV predominantly takes place as a result of blood transfusion and exposure to blood derivatives. The disease was first recognized in recipients of blood and blood products such as factor VIII and immunoglobulins. Also organs transplants and needle stick injuries have been implicated in transmission of the virus (Crofts *et al.,* 1993; Dieterich *et al.,* 2002; Hadi, 2004; Thorburna *et al.,* 2001). A small risk through sexual contact and mother to child transfer also occurs. Drug misusers and patients in dialysis and surgical units have also been reported to be at higher risk in HCV transmission {Mclean *et al.,* 1997; WHO, 2002; Abdelwahab *et al.,* 2013).

HCV may also be transmitted by means of acupuncture, tattooing and sharing razors. Although needle stick injuries in the health care setting result in a 3% risk of HCV infection (Volker *et al.,* 2006; Theodore *et al.,* 2006; Filippini *et al.,* 2007), the prevalence of hepatitis C among health workers is similar to that of general population (Olubuyide *et al.,* 1997; Dieterich *et al.,* 2002). Nosocomial patient-to-patient transmission may occur by means of a contaminated endoscopy/colonoscopy; dialysis or surgical instruments

(Olubuyide *et al.*, 1997; Alter *et al.*, 1999; Mostafa *et al.*, 2010; Oh *et al.*, 2012; Casey and Lee, 2013).

WHO has estimated that about 3% of the world population has been infected with HCV, with subgroups in Africa having prevalence rate as high as 10% (Magiorkinis *et al.*, 2009). It has also been estimated that 130-180 million people are infected worldwide and that 4 to 5 million people in the United States are at risk of developing liver cirrhosis, liver cancer or both (Jawetz *et al.*, 1998; CDC, 1998; Alter *et al.*, 1999; Petrus *et al.*, 2005; Volker *et al.*, 2006; Theodore *et al.*, 2006; Magiorkinis *et al.*, 2009; Opaleye *et al.*, 2010). Studies have also projected a substantial burden from HCV disease and related complications, including liver failure and hepatocellular carcinoma (HCC), over the next 10 to 20 years as an indications for endemic nature of this desease among the adult population in developing countries such as in Africa and Asia (Volker *et al.*, 2006; Theodore *et al.*, 2006; Filippini *et al.*, 2007). In a study conducted among the paediatric cohort in an inner city of human immunodeficient virus (HIV)-infected persons, as well as the demographic characteristic of the cohort, the prevalence and transmission mode of hepatitis B and C were evaluated. Hepatitis B or C was found in 13 (5.8%) of 228 children suggesting that chronic hepatitis is prevalent in the paediatric HIV-infected population (Sima *et al.*, 2007).

Furthermore, more than 3 million people in the United States are chronically infected with the hepatitis C virus (HCV) (Holmberg *et al.*, 2013; Institute of Medicine, 2010). Although the number of new infections has been declining for decades, HCV-related

morbidity and mortality are projected to continue rising for another 20 years (Davis *et al.*, 2011). One half to three quarters of persons currently infected with HCV have not received a diagnosis and are untreated; many will have progression to decompensated cirrhosis, hepatocellular carcinoma, and other liver complications (Davis *et al.*, 2010; Rein *et al.*, 2011. It is therefore desirable that early diagnosis and treatment are essential to improve long-term health outcomes in this population.

Figures from epidemiological studies in different regions of the world show wide variance in HCV prevalence patterns, though it is clearly evident that the incidence of HCV is higher among less developed nations (Yousra *et al.*, 2013; WHO, 2014). The prevalence of hepatitis C is lowest in Northern European countries, including Great Britain, Germany and France. According to one survey, the prevalence of HCV antibodies in blood donors averages less than 1% for the region when compared to 3% reported worldwide (Margiofinins *et al.*, 1999). However, other studies (Alter *et al.*, 1992; Williams and Bells, 2011) have suggested that rates of infection may be much higher, comparable to rates in the United States which were approximately 2.5%. Higher rates have been reported in Southeast Asian countries, including India (1.5%), Malaysia (2.3%), and the Philipines (2.3%) (WHO, 2014). Moreover, alarming rates were reported for many African nations, reaching as high as 14.5% in Egypt (Yousra *et al.*, 2013).

HCV is regarded as a leading hepatotropic pathogen which predominant causes of severe pathological consequences such as acute hepatitis, chronic liver diseases and cirrhosis (Ali *et al.*, 2010; Umar *et al.*, 2010). According to Hussain *et al.* (2010), approximately 10

million people in Pakistan are suffering from HCV, covering 6% of the overall population and it falls in the intermediate endemic zone (Hussain *et al.,* 2010). Such a high prevalence of HCV has earlier been attributed to the absence of good preventive measures, inadequate funding for health care resources, tremendous increase in work load, inadequate provision of barrier devices for the Health Care Workers. Moreover, high frequency of HCV among the general population put them at a high risk of acquiring HCV infection (Raja *et al.,* 2008; Waheed *et al.,* 2008).

Soni *et al.*, in 1995 reported that hepatitis C virus infection is found in 0.5 - 8% of blood donors worldwide. The presence of antibodies to various hepatitis C viral antigens indicates infection with the virus and in most cases signifies a chronic infection (McLean *et al.*, 1997; WHO, 2002). However, HCV antibodies develop slowly up to 1-3 months after the onset of clinical illness in some patients and may not be detected for up to one year afterwards. This situation has been reported in 60% of patients with sporadic Non-A Non-B hepatitis (McLean *et al.*, 1997; Hadi, 2004). Similarly, the course of chronic hepatitis in HCV infection can also be prolonged and insidious and infected persons may not develop symptoms for many years after infection (McLean *et al.*, 1997; Onyekwere *et al.*, 2002; Cookley *et al.*, 2003; Opaleye *et al.*, 2010; McHutchison *et al.*, 2013).

Rates of HCV infection in Africa, Southern East Asian, the Eastern Mediterranean or the Western pacific, South America and Asia are high in comparison with industrialized countries (WHO, 1999; Dieterich *et al.*, 2002). Since the addition of anti HCV assay to the screening algorithm of blood donors in developed countries such as USA and Canada, the

same cannot be said of developing countries like Nigeria where the screening is not compulsory and hence not done routinely. More than 70% of haemophiliacs treated with blood products during the 1970 and 1980s became infected with HCV (Alter *et al.,* 1992). Hoffnagle and Liang (2000) in a study reported that more than 80% haemophiliacs over 18 years of age are infected with HCV in India in 1998.

Ignorance of the risk factors of HCV infection is one of the numerous factors responsible for the high prevalence among the inmates, injecting drug users and those with multiple sex partners and unprotected sex (Burtler *et al.,* 1997). It has also been reported that there are many chronic carriers of HCV worldwide, who are at risk of developing liver cirrhosis, liver cancer or both (Jawetz *et al.,* 1998; Ayodele and Salako, 2003; Poordad *et al.,* 2013). An estimated 1.8% of the population in the United States of America is positive for HCV antibodies; this rate corresponds to an estimated 3.9 million persons with HCV nationwide. Infection due to HCV accounts for 20% of all cases of acute hepatitis, an estimated 30,000 new acute infections, and 8,000-10,000 deaths each year in the United States (Drosten *et al.,* 2004; Petrus *et al.,* 2005; Rein *et al.,* 2011; Holmberg *et al.,* 2013; Institute of Health, 2014).

The prevalence of co-infection varies according to the risk of exposure (Dieterich *et al.,* 1999; Chung *et al.,* 2001). Groups at highest risk of co-infection include individuals who receive multiple transfusions with blood or blood products, injection drug users (IDUs) and persons who have multiple sexual partners (Hoofnagle *et al.,* 2000). Among haemophiliacs in the United States, infection with HCV was found to be 85% of many

individuals with HBV infection (Dieterich *et al.,* 1999; Vincent *et al.,* 2007). This association suggests that both infections were acquired through the same route. Hadi (2004) performed a prospective follow-up amongst an IDU population in Pakistan and showed that the levels of baseline HBV and HCV infections were 34% and 42% respectively among the 500 individuals recruited to the study. The group also reported high levels of risky behaviors such as sharing of injecting equipment among these individuals.

Hepatitis C is a global disease. While not every nation in the world has had adequate means to survey its population for incidence of the virus, enough statistics have been compiled to demonstrate the enormous threat posed by hepatitis C (Williams and Bells, 2011). It has been established that Hepatitis C, in combination with hepatitis B, now accounts for 75% of all cases of liver disease around the world (Vincent *et al.,* 2011). The rise in the incidence of HCV globally has been reported in previous studies (Abdelwahab *et al.,* 2013; Yousra *et al.,* 2013). The incidence of HCV-related HCC is continuing to rise in United States and worldwide, in part because of the increasing numbers of persons who have been chronically infected for decades, the presence of comorbid factors, and the longer survival of persons with advanced liver disease due to improved management of complications. Risk factors for HCC in persons with chronic HCV infection are largely the same as those for the development of End Stage Liver Disease (ESLD) (Sanaullah *et al.,* 2011; Casey and Lee, 2013).

Alcohol use plays an important role in increasing the risk of progressive liver disease, with strong evidence for the detrimental effects of 60 g/day in men (equivalent to six beers, four glasses of wine, or three mixed drinks) and 40 g/day in women, but there is suggestive evidence that lower amounts can also increase the risk of liver damage associated with HCV. Other factors, including iron overload, nonalcoholic fatty liver disease, schistosomal coinfection, potentially hepatotoxic medications, and environmental contaminants, may also have important effects (Oh *et al.*, 2012; Poordad *et al.*, 2013).

In the United States, deaths associated with chronic HCV are currently more likely to be due to ESLD than to HCC. Data from death certificates in 1999 found that approximately 4,000 deaths were attributed to HCV infection, but this is likely to be an underestimate. The only treatment option for persons who have developed ESLD (decompensated cirrhosis) is transplantation (Sanaullah *et al.*, 2011). Currently, HCV is the primary reason for liver transplantation in the United States. Little is known about the clinical course and risks of HCV-related complications in persons who have been infected longer than two decades. HCV accounts for an estimated one-third of HCC cases in the United States and HCC rarely occurs in the absence of cirrhosis or advanced fibrosis (Willams and Bells, 2011).

Earlier reports indicated that the burden of viral hepatitis in Africa and indeed Nigeria most especially on HCV is difficult to quantify precisely because of inaccurate statistical data and under-reporting (Bojuwoye, 1997; Ayodele and Salako, 2003). Furthermore in Nigeria, available statistics from most of the studies conducted so far on HCV have also been so difficult to quantify precisely due to inaccurate statistical data as well as under-reporting too (Ayodele and Salako, 2003; Ola *et al.*, 2002; Magiorkinis *et al.*, 2009; Forbi *et al.*, 2012; Oh *et al.*, 2012). In addition, most of the available information on the rate of HCV infection had been hospital-based studies. Similarly, there is scanty information on the incidence associated with the spread of HCV infection at the rural, semi-urban and urban communities As a result of this, all the previous works in Nigeria were either hospital-based or point prevalence studies from which incidence of HCV could not be ascertained.

AIM AND OBJECTIVES OF THIS STUDY

The aim of the study was to determine the burden of HCV infection among Semi-urban community dwellers in Nigeria.

The specific objectives of the study are to:

1. determine the prevalence of HCV infection at community level in a semi-urban community (Saki) of Southwestern Nigeria.

2. determine the incidence of HCV infection in a semi-urban community of Southwestern Nigeria.
3. identify risk factors associated with HCV infection in the study community.

CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND OF HCV

Hepatitis C virus (HCV) was discovered by accident when patients treated against the infection caused by agents of hepatitis A and B as well as other viruses associated with hepatitis such as Cytomegalovirus (CMV) and Epstein Barr Virus (EBV) had been excluded, yet a number of patients still had acute hepatitis (Tabor *et al,,* 1978). With the introduction of more sensitive assay techniques for screening blood for hepatitis A and B viruses by the 1970s, it was anticipated that post transfusion hepatitis would be virtually eliminated but this was not to be. There remained a substantial residue of cases, which were called hepatitis Non-A Non-B (White and Fenner, 1994). It was also known that hepatitis could occur in individuals who had previously recovered from hepatitis B (Hoofnagle *et al.*, 1977; Mosley *et al.*, 1977).

However studies in Chimpanzees further confirmed these findings and showed conversely that hepatitis B could be transmitted to animals that had recovered from hepatitis Non-A Non-B (Hoofnagle *et al.*, 1977; Tabor e*t al,,* 1978; Khuroo, 1980; White and Fenner, 1994). After many years of confusion concerning the putative causative agent, the genome of the virus responsible for the majority of this type of hepatitis was finally isolated and characterized. Its RNA was cloned in California from the serum of a chimpanzee that had been known to be highly infectious in serial transmission studies in experimental animals

(Escobar, 1992; White and Fenner, 1994). The reasonable assumption was made that plasma from a Chimpanzee that had developed chronic hepatitis following inoculation of factor VIII (antihaemophilic globulin) contaminated with an agent that had caused Non-A Non-B hepatitis in haemophiliac, would constitute a good source of the putative virus, and that virus could be concentrated from the plasma by ultracentrifugation. This causative agent remained frustratingly elusive for over a decade until 1989 when a team of molecular biologists in the United States of America succeeded ambitious assignment. The ingenious protocol they devised serves as prototype to discover non-A, non-B, hepatitis viruses such as hepatitis C virus. This hepatitis C virus was cloned in 1989 and testing became available in early 1990. Many of the patients previously diagnosed as either non-A non-B or post transfusion hepatitis were retested, (or stored serum was tested) and found to be hepatitis C (White and Fenner, 1994). Hepatitis C is recognized as one of the most common types of hepatitis with up to 1.5% - 3% of the population being positive and 0.5% - 8% prevalence reportedly found in blood donors worldwide (Soni *et al.*, 1995).

2.2 STRUCURE OF HCV

The morphology of Hepatitis C virus shows that it has a diameter of 55-65nm and consists of a core containing the viral RNA genome enclosed within an envelope which also has glycoproteins with short spikes as revealed through an electron microscope (McLean *et al.*, 1977) fig 1. The viral particles are spherical and have morphological features similar to those of flavivirus (Kaito *et al.*, 1994; White and Fenner, 1994).

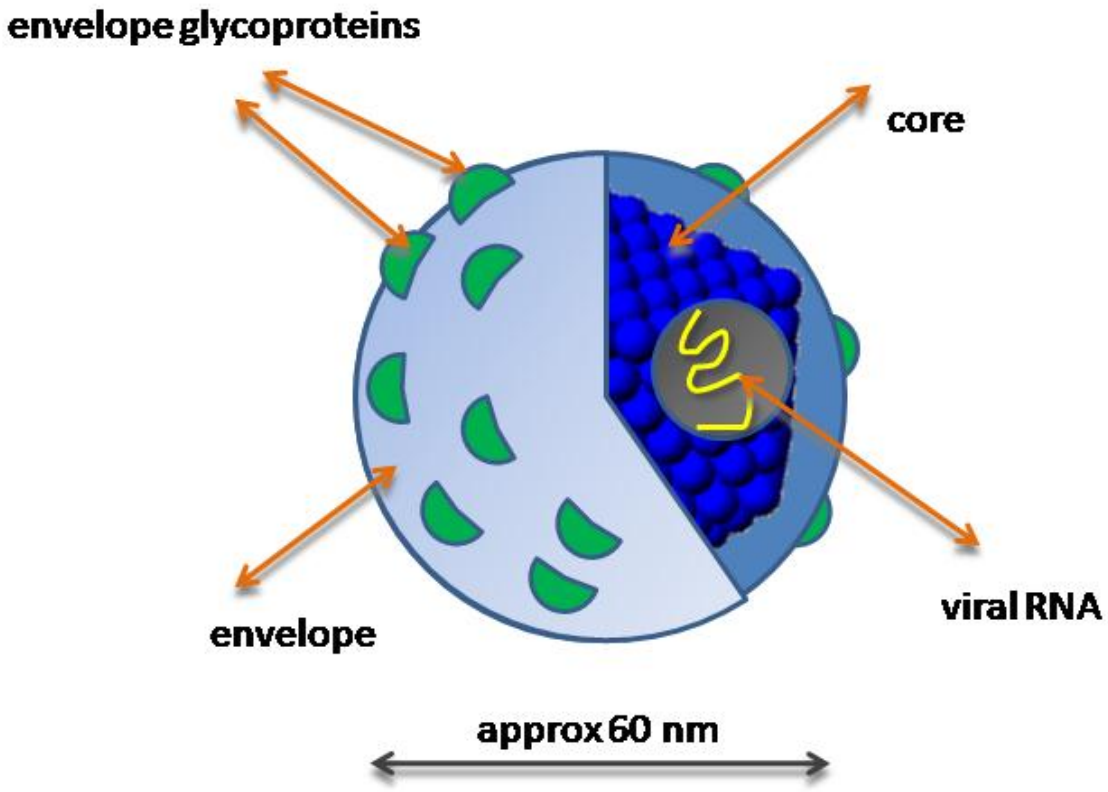

FIGURE 1: Simplified diagram of the structure of Hepatitis C virus

Source: Adopted from GrahamColm 2008

The genome is a single 9.5 kilobase molecule of single stranded RNA of positive polarity with a single long open reading frame (OFR) encoding a glycoprotein of about 3000 amino acids flanked by untranslated 5' and 3' sequences each containing short direct repeats and taking the form of a hair pin. The RNA is approximately 9379 nucleotides long (Dusheiko, 1995). The 3' is not polyadenylated and the structural proteins occupy the 5' quarter of the open reading frame and the non-structural proteins as the remainder (White and Fenner). The polyprotein is cleaved posttranslational by viral and cellular proteases into the putative structural core, the envelope and six non- structural proteins (McLean *et al.,* 1977). The structural proteins include the capsid protein that is highly conserved, with amino acids similarity of approximately 90% among different isolates of HCV, and E1 (192-383) and E2 (384 -729/746) proteins.

The putative virion envelope has sequence variation with amino acid similarity of 49-70% among different HCV isolates, including the hypervariable region in the N-terminal region of E2 (Cuthbert, 1994). The non-structural proteins are NS1, NS2 (viral protein and helicases) NS4A, NS4B (co-factors), NS5A (Interferon resisting protein) and the NS5B (the RNA polymerase) (Cuthbert, 1994; Dubuisson *et al.,* 1994) Fig 2. The NS3 carries the serine protease activity in amino terminal half and the helicase activity in its carboxyl-terminal half while the NS2 and NS4 may be comparable with the membrane binding proteins, postulated to be required by other flaviviruses during the membrane associated replication (White and Fenner, 1994). The proteins E1 and E2 (gp33-35 and gp70) are putative virion glypoproteins that may be targets for neutralizing antibodies (Kuo *et al.,*

1989). The NS3 protease produces cis and trans cleavages during the processing and assembly of hepatitis C.

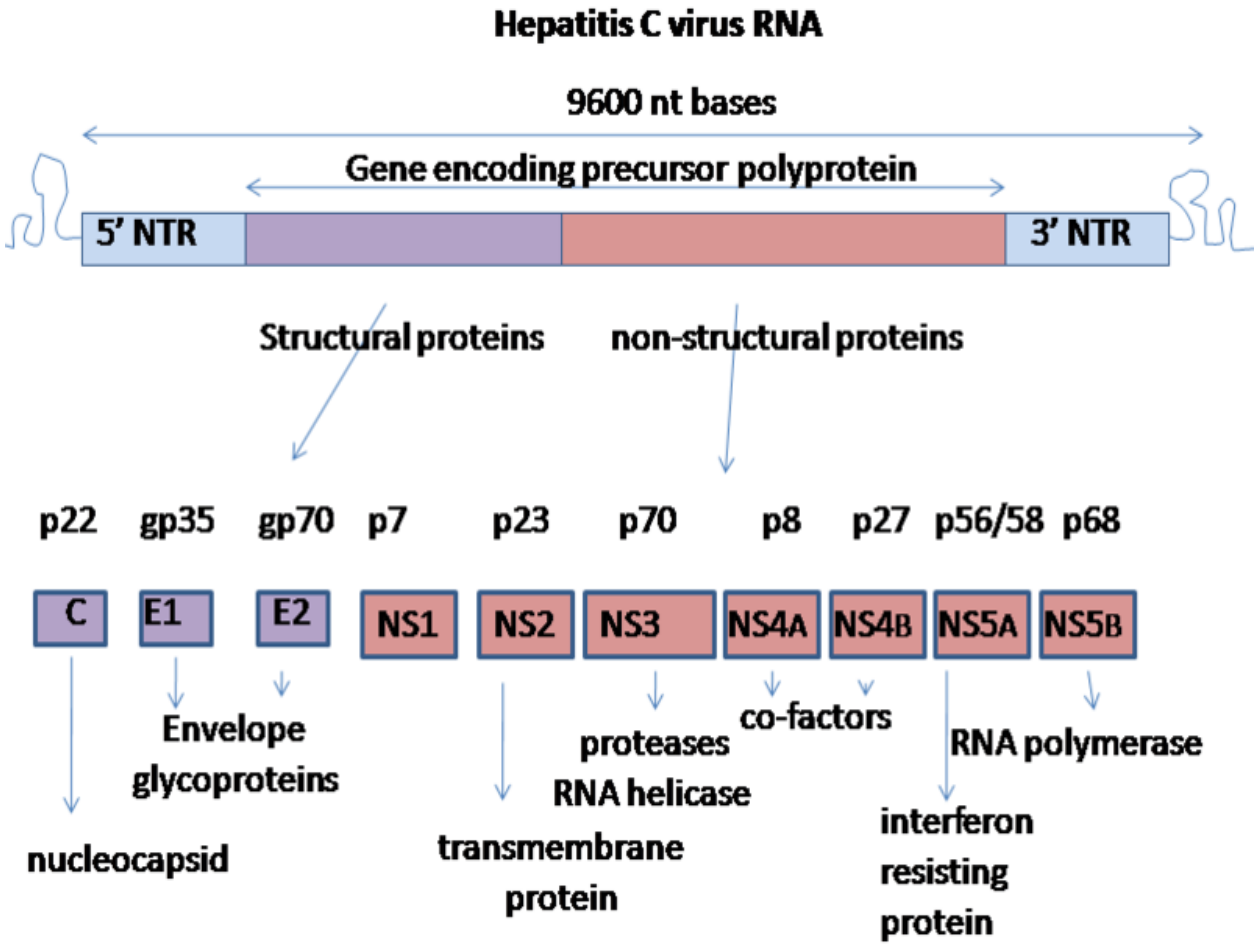

FIGURE 2: **The genome organization of Hepatitis C virus**

Source: Adopted from GrahamColm, 2007

2.3 GENOME AND PROTEINS OF HCV

HCV is a spherical, enveloped, single stranded RNA virus belonging to family Flaviviridae. Its genome unlike that of HBV has shown considerable sequence variation between individual HCV isolates and more than ten different HCV genotypes which have been discovered worldwide (Okamoto *et al.*, 1992; Mori *et al.*, 1992; Simmonds *et al.*, 1993; Tokita *et al.*, 1994; Bukh *et al.*, 1994). Identification of these HCV genotypes is based mainly on the nucleotide sequence diversity and phylogenetic analysis of specific portions of the HCV genome i.e. the 5' non-coding (5' NC) region of the core region, the envelope glycoprotein 1 (E1) region or the non-structural protein 5 (NS5) regions (Stuyver *et al.*, 1993; Van Doorm *et al.*, 1994).

Several methods such as sequence analysis (Van Doorm *et al.*, 1994), restriction fragment length polymorphism (McOmish *et al.*, 1994), subtype specific PCR (Okamoto *et al.*, 1992), hybridization of PCR products with specific probes, and reverse hybridization (Stuyver *et al.*, 1993; Van Doorm *et al.*, 1994), have been developed for the identification of HCV genotypes. A unified nomenclature system derived by Simmonds *et al.* (1994) in which types are denoted by numbers such as 1, 2, 3 etc and subtype by letters is currently applied in naming the genotypes. Seven major HCV genotypes (1-7) and numerous subtypes have been identified based on molecular relatedness (WHO, 2002; Vandelli *et al.*, 2004; Petrus *et al.*, 2005). Molecular differences between genotypes are relatively large, and they have a difference of at least 30% at the nucleotide level. Genotypes I, 2, and 3 have a worldwide distribution, while genotypes 4, 5, and 6 are localized to specific geographic locations. Genotype 1 is the most common genotype in the United States. HCV genotype 1, particularly 1b, does not respond to therapy as well as genotypes 2 and

3. Genotype 1 also may be associated with more severe liver disease and a higher risk of hepatocellular carcinoma. Genotype 4 is the most prevalent genotype in Egypt, genotype 5 is found in South Africa, and genotype 6 is found in Southeast Asia (Suglyama *et al.*, 1995).

The genotypes differ from each other by approximately 30% over the entire genome, and the genotype may correspond to the serotypes of other RNA viruses. In a study carried out among 43 pregnant women out of which 40 (93%) of them were viraemic, the result of serotyping by ELISA showed complete agreement with those determined by PCR genotyping (Lin *et al.*, 1996). The genotypes may be further divided into a series of more closely related subtypes, differing from each other by around 20% (Chan *et al.*, 1992; Stuyver *et al.*, 1993). In persons who are infected, HCV may produce approximately a trillion new viral particles each day in a steady state of viral replication. The RNA-dependent RNA polymerase, an enzyme critical in HCV replication, lacks proof reading capabilities and thus generates a large number of mutant viruses known as quasispecies. Viral quasispecies represent minor molecular variations with only 1-2% nucleotide heterogeneity. These quasispecies pose a major challenge with respect to immune-mediated control of HCV and may explain the variable clinical course and the difficulties in vaccine development.

HCV encodes a single polyprotein of 3011 amino acids that is processed into 10 structural and regulatory proteins. Structural components include the core and two envelope proteins, E1 and E2. Two regions of E2 protein have an extremely high rate of mutation;

these are designated hypervariable regions 1 and 2. Envelope protein E2 contains the binding site for CD-81, a receptor expressed on hepatocytes and B-lymphocytes.

2.4 ANTIGENIC CHARACTERISTICS OF HCV

HCV antigen for immunoassay probing is not sufficiently sensitive to pick up the low titre of virus in the serum and in 1990, a cDNA clone representing part of HCV genome was expressed in yeast. This produced a recombinant C100-3 protein antigen (fusion antigen corresponding to a large portion of the non-structural protein NS4). Another antigen was cloned based on recombinant yeast numeric protein comprising the three most conserved HCV proteins, Capsid (C), NS3 and NS4. This cloned protein lacks the hyper variable E1 and E2 proteins (White and Fenner, 1994). Peptides representing linear epitopes encoded by the HCV structural proteins have been synthesized and used to detect antibodies in acute and chronic infections in proteins, and also studied (Okamoto *et al.,* 1992; Ishida *et al.,* 1993; Salberg *et al.,* 1993). Antibodies to these peptides usually appear within ten weeks of onset of infection (Okamoto *et al.,* 1992; Ching *et al.,* 1992; Hosein *et al.,* 1992). Overlapping peptides were synthesized for amino acids 1-727 encompassing the putative structural region of HCV H strain (Nasoff *et al,* 1991; Ishida *et al.,* 1993; Inchauspe *et al.,* 1991). The GOR of the GOR protein from the cDNA clone GOR 47-1 has a sequence homologous with amino acids 5-23 of HCV. The GOR epitope is considered to be an immune epitope (Mishiro *et al.,* 1990). In many cases, production of antibodies to the core and NS3 proteins precedes production of NS3 antibodies (Van Doorm *et al.,* 1996).

2.5 REPLICATION CYCLE OF HCV

HCV is a spherical, enveloped, single-stranded RNA virus belonging to family Flaviviridae. Little is known about the replication cycle of HCV; however, its replication probably takes place exclussively in the cytoplasm (White and Fenner, 1994). In persons who are infected, HCV may produce approximately a trillion new viral particles each day in a steady state of viral replication. The RNA-dependent RNA polymerase, an enzyme critical in HCV replication, lacks proofreading capabilities and thus generates a large number of mutant viruses known as quasispecies. Viral quasispecies represent minor molecular variations with 1-2% nucleotide heterogeneity. These quasispecies pose a major challenge with respect to immune-mediated control of HCV and may explain the variable clinical course and the difficulties in vaccine development (HCV Fact sheet, 2002).

HCV encodes a single polyprotein of 3011 amino acids that is processed into 10 structural and regulatory proteins. Structural components include the core and 2 envelope proteins E1 and E2. Two regions of the E2 protein have an extremely high rate of mutation; these are designated hypervariable regions 1 and 2. Envelope protein E2 cotains the binding site for CD-81, a receptor expressed on hepatocytes and B lymphocytes. In hepatocytes of infected chimpanzees, dense reticular cytoplasmic inclusions and convoluted membranes are conspicous by electron microscopy and immunoflouresence reveals viral proteins, mainly NS3 and NS4, confined to the cytoplasm (White and Fenner, 1994). Full-length plus and minus RNA strands as well as full-length double-stranded RNA (replicative form) have been found by in-situ hybridization in the cytoplasm of infected liver, and apparently also in T-lymphocytes (White and Fenner, 1994; Rein *et al.*, 2011).

Consistent with the transcription stategy of other flaviviruses, no subgenomic RNAs have been detected by northern blot. HCV also encodes a virus specific helicase, protease and polymerase, all of which are critical in viral replication. These enzymes are attractive targets for antiviral therapy. Similarly, the untranslated regions at both ends of the viral RNA, 5'-UTR and 3'-UTR, are highly conserved. These sites are involved in critical stages of viral replication and may be logical targets for therapy.

2.6 EPIDEMIOLOGY AND INCIDENCE OF HCV INFECTION

Hepatitis C virus (HCV) is a common cause of post-transfusion hepatitis. It has infected about 170 million people worldwide with an estimated 32 million in Africa. The risk of infection is high among intravenous drug users, haemophiliac, blood transfusion and organ recipients. Infection has also been associated with social and cultural practices using percutaneous procedures such as ear piercing, body piercing, circumscision etc if equipment used is not sterilized (Simmonds *et al.*, 1993; Mostafa *et al.*, 2010; Opaleye *et al.*, 2010). The incidence of HCV infection is difficult to estimate as <25% of acute cases of hepatitis C are clinically apparent. About 20% have normal liver function tests despite infection. In the United States the peak incidence of Hepatitis C was ~240,000 per year (in 1989). It is currently ~ 20-30,000 new cases per year. In Canada the reported rates were 25 per million population in 2004, 16 per million in 2006 and 22 per million in 2008 (Annonymous, 2009).

Hepatitis C virus (HCV) infections are excessive throughout the world. Estimation by WHO in 1997 shows that about 3% of the world population has been infected, with population subgroups in Africa having prevalence rates as high as 10%. Other high prevalence areas are found in South America and Asia. It is estimated that there are more than 170 million chronic carriers worldwide, who are at risk of developing liver cirrhosis, liver cancer or both (Jawetz *et al.*, 1998; Oh *et al.*, 2012). HCV infection is found in 0.5 to 8% of blood donors worldwide (Dusheiko, 1995). In most western countries, the prevalence of antibodies to HCV is around 1% in blood donors, but the prevalence has been declining as a result of improving and more rigorous screening and selection of blood donors in blood banks. Presently, the most clearly identifiable cohort is the intravenous drug users, the majority of whom are infected (White and Fenner, 1994; Davis *et al.*, 2010; WHO, 2014).

The distribution of HCV genotypes varies in different parts of the world. Blood donors and patients with chronic hepatitis from European countries and U.S.A are commonly infected with genotypes 1a, 1b, 2a and 3a, although the distribution of each may vary. There is a tendency for type 1b to be prevalent in Southern and Eastern Europe (Dusheiko, 1995), while in the United States and Northern Europe the predominant HCV genotype is type 1a (Davidson *et al.*, 1995). In many European countries genotype distributions vary with age of patients, possibly reflecting the introduction of different genotypes through

practices such as intravenous drug use (Chan *et al.,* 1992; McOmish *et al.,* 1994; Simmonds *et al.,* 1994).

Within Japan, Taiwan and probably parts of China genotypes 1a, 2a and 2b are the most frequently found. In Thailand Singapore, and possibly in Bangladesh and Eastern India, it is type 3; in the Middle East, predominantly Egypt, and central Africa; it is the type 4 (Bukh *et al.,* 1993). The genotype 4 comprises of an array of subtypes. The type 5 has been found in South Africa (Chan *et al.,* 1992; Bukh *et al.,* 1993; Simmonds *et al.,* 1993; Xu *et al.,* 1994), whereas genotype 6a has been found so far only in Hong kong, Macau and Vietnam (Bukh *et al.,* 1993; Simmonds *et al.,* 1993). Other newer genotypes have also been reported from Vietnam (Kato *et al.,* 1990; Houghton *et al.,* 1991; Simmonds *et al.,* 1993).

The increase in incidence HCV within communities and from one population to the other has been reported from previous studies globally (Hadi, 2004; Petrus*et al.,* 2005). According to the report of Sanaullah *et al.* (2011), the incidence of HCV-related HCC is continuing to rise in United States and worldwide, in part because of the increasing numbers of persons who have been chronically infected for decades, the presence of comorbid factors, and the longer survival of persons with advanced liver disease due to improved management of complications. Risk factors for HCC in persons with chronic HCV infection are largely the same as those for the development of End Stage Liver Disease (ESLD) (Davis *et al.*, 2010).

In Nigeria, three different novel subtypes of genotype 1 (EUNIG13, EUNIG14, and EUNIG45) have been discovered in blood donors (McOmish *et al.,* 1994). Also in Nigeria according to Forbi *et al.* (2012), a total of 60 HCV isolates were characterized by Molecular method. Genotypes 1 was 85% (51) and genotype 2 was 15% (9) (n=60) when they investigated an 'Epidemic history of Hepatitis C Virus Infection in two remote communities in Nigeria' by Molecular characterization.The pattern of HCV variation in central Africa and South-East Asian regions appear to be distinct from that in Europe and other western countries. In the latter, a restricted number of HCV genotypes occur, of which type 1a; 1b and 3a are the most frequent. The occurrence of additional genotypes in these countries is often attributable to recent travel or immigration; for example, a Canadian blood donor infected with type 6 was found to be a recent immigrant from Vietnam (Murphy *et al.,* 1994). In South-east Asia e.g. (Thailand, Nepal and India) and Central Africa, HCV infection within a population is predominantly of a single major genotype, but a wide range of subtypes are found. For example, six subtypes of type 3 were found in ten hepatitis C patients in Nepal (Tokita *et al.,* 1994b) and six subtypes from eleven individuals in Bangladesh, India and Pakistan. An analogous situation is taking place in Central Africa, where nine subtypes of type 4 have been found from relatively small number of HCV infected individuals. The discovery of three subtypes from three donors in Nigeria is preliminary evidence for the same phenomenon elsewhere in Africa (Mellor *et al.,* 1995).

The high level of diversity of particular major genotypes within a restricted geographic region may be interpreted as long term endemicity of the infection within a community,

while epidemic spread of HCV, such as its entry into new risk groups might be interpreted by the presence of only one or two subtypes of a genotype in a wide area of a community. As an example, this widespread occurrence of type 1b genotype worldwide is an evidence for relatively recent and rapid transmission of this particular variant throughout Europe, North America, parts of Africa and Far East. The degree of diversity of type 1b sequence is around 9% over the complete genome, which dates the start of its dissemination to relatively recent times, perhaps over the last 40 to 50 years (Mellor *et al*., 1995). How the 1b genotype was transmitted remain unclear, although examples are known of type 1b transmission to large sections of particular populations through the use of contaminated blood products (Hohne *et al.,* 1994; Power *et al.,* 1994).

The existence of discrete subtypes in countries showing the presence of only one or two subtypes of a few genotypes may reflect the epidemiology of HCV transmission, where the founder effect from a single introduction of HCV may be apparent for several decades. Consequently, classification of HCV into subtypes provides convenient epidemiological labels for variants within countries where it has been relatively and recently spread, but may turn out to be of different relevance in areas where HCV infection shows a more epidemic pattern of variability. The existence of numerous subtypes within a single geographical area suggests the long-term presence of HCV in the population.

Phylogenetic calculation of mean pair wise distances between subtypes reveal differences in the degree of diversity of sequences within a major genotypes, as well as the number of

component subtypes. For instance, type 3 sequences are in general, much more divergent from each other than are subtypes of type 1 or 2 (Chan *et al.,* 1994). The relatedness of variant (subtypes) of a genotype with each and with another subtype that is present within a continuous relatively restricted geographical area will strongly indicate if the variants were originally from a single subtype that has been differentiated by the process of diversification (Mellor *et al.,* 1995).

2.7 GENOTYPES OF HCV

Based on genetic differences between HCV isolates, the hepatitis C virus species is classified into seven genotypes (1-7) with several subtypes within each genotype (represented by lower-cased letters) (Simmonds *et al.,* 1993; Sakano *et al.,* 1999). Subtypes are further broken down into quasispecies based on their genetic diversity. Genotypes differ by 30-35% of the nucleotide sites over the complete genome (Ohno *et al.,* 2007). The difference in genomic composition of subtypes of a genotype is usually ~20-25%. Currently there are over 80 subtypes recognized. Subtypes 1a and 1b are found worldwide and cause 60% of all cases.

While there were initially multiple proposed typing schemes a consensus was reached in 1994 at the 2nd International Conference of HCV and Related Viruses (Simmonds *et al.,* 1993). Over 85% of the world's nearly 170 million hepatitis C virus case lives in African, Southeast Asian and Middle Eastern countries where genotypes 4-6 are common. Genotype 4 is highly prevalent in Egypt with more than 19% of the population infected and chronic HCV representing one of the top five leading causes of death. This is due in

part to ineffective response to interferon alpha treatment against this genotype (Timm *et al.*, 2007).

Genotype distribution

The preponderance and distribution of the various HCV genotypes varies considerably between countries and regions. Additionally it tends to differ by route of transmission: genotypes 1a and 3a are closely associated with intravenous drug use and genotype 1b is seen more often in patients who acquire HCV through blood transfusion (Berg *et al.*, 1997). Recombinant strains have also been reported.

Genotype 1

Thirteen (13) subtypes have been identified: these have been termed 1a to 1m. In North America, genotype 1a predominates followed by 1b, 2a, 2b, and 3a (Burguete-García *et al.*, 2011). In Europe, genotype 1b is predominant followed by 2a, 2b, 2c, and 3a. This pattern is also found in South Korea (48% cases) Shin, 2006: Oh *et al.*, 2012), Mongolia (Takahashi *et al.*, 2004) and Morocco (Brahim *et al.*, 2011).

In Uzbekistan, type 1 is predominant followed in frequency by type 3 (Kurbanov *et al.*, 2003). This pattern is also found in Belarus, Moldova and Russia (Viazov *et al.*, 1997) China (Zhou *et al.*, 2009) Indonesia (Utama *et al.*, 2008) Iran (Samimi-Rad *et al.*, 2004) and Taiwan (Liu *et al.*, 2008). In Germany 70% of cases are due to type 1 (20% 1a and 80% 1b) and 25% to type 3 (Berg *et al.*, 1997). This pattern is also found in Brazil (Campiotto *et al.*, 2005) and Western Siberia (Shustov *et al.*, 2005).

Genotypes 1 and 2 are both common in West Africa and have considerable genomic variability (Jeannel *et al.,* 1998). In the United States genotype 1 is responsible for ~75% of cases. In Ireland the genotype associated with an outbreak due to the use of contaminated anti-D immunoglobulin was 1b (Power *et al.*, 1995). In Romania ~90% of cases are due to type 1b (Sultana *et al.,* 2011). This appears to have been the use of blood transfusions in pregnancy and surgery. In Greece genotype 1 accounts for ~40% of cases (Karatapanis *et al.*, 2012). In Poland, 55% of cases are due to type 1 (Chlabicz *et al.*, 2008).

In Italy type 1b is the most common genotype (Maio *et al.*, 2000). The prevalence of this genotype is thought to be due to the use of improperly sterilised reusable syringes in the past. In the Netherlands this genotype accounts for ~50% of cases (de Vries *et al.,* 2006). In Croatia this genotype accounts for ~60% of cases (Vince *et al.,* 2006). In Tunisia 90% of cases are due to type 1b (Mejri *et al.*, 2005) while 95% of isolates in Turkey are type 1(Bozdayi *et al.*, 2004).

In Japan, subtype 1b is responsible for up to 73% of cases (Takada *et al.,* 1993). In Syria 19% of cases were genotype 1a and 27% were genotype 1b (Abdulkarim *et al.*, 1998). In Jordan 40% of cases were type 1a, 33% were type 1b and 26.7% were type 4(Bdour, 2002). In Lebanon this genotype is the second most common (after genotype 4) in non drug users: genotype 1a accounts for 12.5%-43.3% of cases and 1b for 8.0-34.4% (Sharara *et al.,* 2007). Genotype 1a and 1b have been reported from Bahrain but their prevalence is not known (Qadi *et al.,* 2004).

Genotype 2

Eighteen subtypes are known (2a-2r). This genotype accounts for 8% of cases in Europe (Mangia and Mottola, 2012). In Greece genotype 2 accounts for ~7% cases (Karatapanis*et al.,* 2012). In Belgium type 2 infection (6% of cases) has been associated with invasive medical examinations (Putzeys *et al.,* 2011). In the Netherlands this type accounts for ~10% of cases (de Vries *et al.,* 2006).

Subtypes 2a and 2b are relatively common in North America, Europe and Japan. Subtype 2c is common in northern Italy. Subtype 2k/1b was originally isolated from St Petersburg, Russia but has also been identified in Cyprus (Demetriou *et al.,* 2011). It is associated with intravenous drug use. 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, Burkina Faso and Ghana';(3) The Gambia, Guinea, Guinea-Bissau and Senegal;(4) Madagascar (Markov *et al.,* 2009.)

In Guinea-Bissau type 2 accounts for the vast bulk of the isolates (Plamondon *et al.,* 2007). In Ghana 85% of the isolates are type 2 (Candotti *et al.,* 2003). In South Korea type 2 accounts for almost 50% of cases (Oh *et al.,* 2012).

Genotype 3

Eleven subtypes are known (3a-3j). This genotype is the predominant type in India (Das *et al.,* 2002) and Pakistan (Idrees and Riazuddin, 2008) followed by type 1. In Pakistan type 3 accounts for 55-85% cases and type 1 10% (Ali *et al.,* 2010; 2011). It has been suggested that the pattern in Pakistan is due to the reuse of medical equipment without

adequate sterilization (Idrees and Riazuddin, 2008). Type 3 accounts for 60% of isolates in India (Narahari *et al.,* 2009). This pattern is also found in north east Brazil where type 3 is the most common isolate (50% cases) followed by type 1 (40% cases) (Zarife *et al.,* 2006).

Subtype 3a is particularly prevalent in intravenous drug abusers in Europe and the United States (Pawlotsky *et al.,* 1995). In Greece and Poland genotype 3 accounts for ~30% cases (Karatapanis *et al.,* 2012; Chlabicz *et al.,* 2008). The proportion of type 3 infections in Greece may be higher than this with other studies reporting rates of 40% (Stamouli *et al.,* 2012. In the Netherlands this type accounts for ~30% cases (de Vries *et al.,* 2006). In Croatia this genotype accounts for ~35% of cases (Vince *et al.,* 2006).

In Iran genotype 3a accounts for 58% of cases (Samimi-Rad *et al.,* 2012). Genotype 3 is the predominate isolate among drug users in Lebanon where it is responsible for 57.1% of cases (Mahfoud *et al.,* 2010). Among non drug users genotype 4 is the most common (34.2-53.3% of cases) followed by type 1 (genotype 1a: 12.5-43.3%; genotype 1b: 8.0-34.4%)(Sharara *et al.,* 2007).

Genotype 4

Worldwide genotype 4 accounts for ~20% of all chronic infections (Smith *et al.,* 1997). Eighteen subtypes are known (4a - 4r).

Middle East

Curiously genotype 4 is the most common genotype in the Arab countries of the Middle East while rare in the non Arab countries (Sharara *et al.*, 2007; Altuglu *et al.*, 2008). In Egypt (where the prevalence is ~13% of the population) (Kamal, 2009), the distribution of this genotype (specifically subtype 4a) is thought to be due to the mass treatment programmes for schistosomiasis in that country from the 1930s until the 1980s when oral treatment became available (Tanaka *et al.*, 2004; Antaki *et al.*, 2010). In Egypt genotype 4 is responsible for >90% of cases.

It is the most common genotype in Saudi Arabia (62% of all cases) (Shobokshi, 2003). In the United Arab Emirates this genotype accounted for 46% of isolates from females (Alfaresi, 2011). Curiously in the same study genotype 3a was the most common type in the males. This genotype has been identified in Yemen but its overall prevalence has not yet been reported (Ohno *et al.*, 1996). In Syria this genotype accounts for 30% of cases (Abdulkarim *et al.*, 1998). In Jordan this genotype accounts for 26.7% of cases (Bdour, 2002). In Lebanon this genotype is the most common in non drug users (34.2-53.3% of cases) (Sharara *et al.*, 2007). In Kuwait this genotype may account for 64% of cases (Hasan *et al.*, 1999). It is the most common genotype in Qatar (John *et al.*, 2010). It has been reported from Bahrain but its prevalence is not known (Qadi *et al.*, 2004).

Africa

Within Africa both types 1 and 4 are common in Cameroon (Ndjomou *et al.*, 2003) and Kenya (Muasya *et al.*, 2008). Type 4 is also found in the Central African Republic (Fretz *et al.*, 1995), Gabon (Xu *et al.*, 1994), Nigeria (Oni and Harrison, 1996), the Republic of the Congo (Cantaloube *et al.*, 2010), South Africa (Gededzha *et al.*, 2012), Sudan

(Mudawi *et al.*, 2007), Tanzania (Rapicetta *et al.*, 1998) and Uganda (Biggar *et al.*, 2006). The majority of isolates from the Central African Republic (82%) are of this genotype (Njouom *et al.*, 2009). This is similar to that found in south Cameroon where 75% of isolates are type 4 (Njouom *et al.*, 2003). 35% of cases in Libya are of genotype 4 (Elasifer *et al.*, 2010).

Europe

Outside of Africa and the Middle East this genotype has been isolated in several European countries including Austria (Haushofer *et al.*, 2001), Belarus (Olinger *et al.*, 2008), Belgium (Mathei *et al.*, 2005), Croatia (Vince *et al.*, 2006), Cyprus (Demetriou *et al.*, 2009), Denmark (Eriksen *et al.*, 2010), France (Nicot *et al.*, 2008), Greece (Katsoulidou *et al.*, 2006), Italy (Ansaldi *et al.*, 2005; Sereno *et al.*, 2009), Portugal (Calado *et al.*, 2011), the Netherlands (de Bruijne *et al.*, 2009), Romania (Sultana *et al.*, 2011), Sicily (Pizzillo *et al.*, 2009), Spain (Franco *et al.*, 2007) and Turkey (Bozdayi *et al.*, 2004).

Within Europe the prevalence of type 4 varies between countries from 7% in northern Europe to 24% in southern Europe (van Asten *et al.*, 2004). In Greece genotype 4 accounts for ~15% cases (Karatapanis *et al.*, 2012). A similar proportion has been reported in Poland (Chlabicz *et al.*, 2011). In the Netherlands this type accounts for ~10% of cases.(de Vries *et al.*, 2006). In Croatia this genotype accounts for ~3% of cases (Vince *et al.*, 2006). In Spain this genotype has been reported to be responsible for ~20% of cases (Cifuentes *et al.*, 2012).

Americas

In the Americas it has been isolated in Brazil (Campiotto *et al.*, 2005), Canada (Murphy *et al.*, 2007), Martinique (Martial *et al.*, 2004), the United States (Zein *et al.*, 1996) and Venezuela (Sulbara *et al.*, 2010).

Asia

It has also been isolated in Asia: India (Narahari *et al.*, 2009), Iran (Zali *et al.*, 2000) and South Korea (Oh *et al.*, 2012).

Genotype 5

No subtypes have yet been identified for this genotype. It is found mainly in South Africa (Chamberlain *et al.*, 1997) (40% of cases) but it is also found in Austria (Haushofer *et al.*, 2001), Belgium (Verbeeck *et al.*, 2006), Canada (Murphy *et al.*, 2007), Cyprus (Demetriou *et al.*, 2009), Ethiopia (Abreha *et al.*, 2011), France (Henquell *et al.*, 2004), Germany (Mauss *et al.*, 2012), Martinique (Martial *et al.*, 2004), Namibia (Vardas *et al.*, 1999), Pakistan (Attaullah *et al.*, 2011), Saudi Arabia (Osoba, 2002), Spain (Jover *et al.*, 2001) and Syria (Antakali *et al.*, 2009). An unusually large number of cases of type 5a have been reported from Rhodes (Karatapanis *et al.*, 2012).

Genotype 6

Twenty-one (21) subtypes of genotype 6 have been recognised. It is most common genotype in Asia and represents perhaps 1/3 of all cases (Anonymous, 1997). It is found in Cambodia (Akkarathamrongsin *et al.*, 2011), China (20% cases) (Yan *et al.*, 2012), Hong Kong, India (Narahari *et al.*, 2009), Indonesia (Tokita *et al.*, 1996), Laos (Huschen *et al.*, 2011), Myanmar (48% of cases) (Lwin *et al.*, 2007), Pakistan (Attaullah *et al.*, 2011), Thailand (Apichartpiyakul *et al.*, 1996) and Vietnam (52% of cases) (Noppornpanth *et al.*, 2006). It is also found in Americans and Australians (Kaba *et al.*, 1998) of Asian origin. It has occasionally been isolated in Canada (Murphy *et al.*, 2007) and Martinique (Martial *et al.*, 2004). It is a diverse genotype and now contains genotypes that were originally classified as genotypes 7, 8, 9 and 11 (Tokita *et al.*, 1996). It has been reported in Germany (Mauss *et al.*, 2012).

Genotype 7

This is the most recently identified genotype and only a single subtype has yet been described (WHO, 2014).

2.8 CLINICAL DISEASES OF HCV

The clinical syndrome of HCV reveals that it may cause both acute and chronic hepatitis but majority of cases are asymptomatic. The incubation period of Hepatitis C Virus on the other hand, though ranging up to several months, averages between 6-8 weeks. The infection is principally in adults, and occurs throughout the year (Jawetz *et al.*, 1998). The onset of HCV is insidious (Jawetz *et al.*, 1998) and the infection is usually clinically mild

with only minimal to moderate elevation of liver enzymes. Fever is not common and duration of slight alanine amino transferase rise of fluctuation is over six months to a year or two. Immunoglobulin (IgM) levels remain normal or slightly elevated (Jawetz *et al.*, 1998). Hospitalization is unusual, and jaundice occurs in less than 2.5% of patients. The case fatality from fulminant HCV which is rare and presents as acute disease characterized by hepatic failure and symptoms of hepatic encephalopathy (Escobar, 1992) is 1% or less. Chronic infection occurs in 80% of cases and typically runs a variable course with fluctuation in the extent of liver damage (McLean *et al.*, 1997).

Most patients with HCV infection are asymptomatic, but histological evaluation often reveals evidence of chronic active hepatitis, especially in those whose disease is acquired following blood transfusion (Jawetz *et al.*, 1998), while most of these are asymptomatic carriers, or mild cases of chronic persistent or chronic active hepatitis which spontaneously revolve (White and Fenner, 1994). Chronic liver damage can progress to cirrhosis and hepatocellular carcinoma (White and Fenner, 1994). Progression is slow taking 10 years for cirrhosis and 15 years for carcinoma to develop (McLean *et al.*, 1997), and this progression can be more rapid in the immuno-compromised, while some patients may have persistent mild hepatitis for 30 tears. Viraemia is detectable by PCR within days of infection and lasts for weeks or months before resolution in most cases, but it may persist for years in chronic carriers, often fluctuate erratically (White and Fenner, 1994). About 75% of infections are sub-clinical, clinical infections are generally less severe than hepatitis B. having a shorter Icteric period, milder symptoms, absent or less marked

jaundiced and somewhat lower serum alanine aminotransferase (ALT) levels, which often fluctuate widely (White and Fenner, 1994).

2.8.1 High Risk Groups of HCV In The United States

Many of the high risk groups for hepatitis C are easily identified, due to practices resulting in frequent exposures to blood or risk factors for transmission. Blood transfusions currently seem to account for only approximately 5-10% of all cases of hepatitis C. Prior to 1990, there were no tests for hepatitis C against the blood supply, and the rate of post-transfusion hepatitis was between 8% and 10%. Anyone who received a blood transfusion prior to that time is at risk for having been infected. Incidence among hemophiliacs, who receive frequent transfusions of blood and blood products, is particularly high, ranging between 25-40%. Women who have had Cesearean sections prior to 1990 represent another significant risk group, as these operations were frequently accompanied by blood transfusion (Kiyosawa *et al.,* 1991; Niederau *et al.*, 1998).

Blood tests have greatly reduced the rate of post-transfusion hepatitis C. CDC estimates the risk factor for transfusion-contracted HCV during the 1990-1993 period at 5%, and risk of infection was brought down to less than 1% after 1993 (Panlilio *et al.*, 1995; Shapiro *et al.,* 1996). Today, the risk of post-transfusion infection is negligible, at approximately 1 per 100,000 units of blood. Blood banks now also notify donors if they detect the virus (Opaleye *et al.,* 2010; WHO, 2014).

Intravenous (IV) drug users represent the largest single risk group. Hepatitis C infection among intravenous drug users occurs at an alarming rate. As with HIV, the sharing of contaminated needles and syringes increases the chance of infection dramatically: incidence of HCV antibody rates among I.V. drug users has surpassed 50 percent in many studies and almost reached 100 percent in others. Within only six months to a year after beginning intravenous drug use, 50-80 percent of drug users test positive for the hepatitis C antibody. I.V. drug users account for about 30-40% of all identified cases, and about 50 percent of all new cases of the disease (Thomas *et al.*, 1992; Cooper *et al.*, 1993).

Sexual contact has been clearly identified as a means of transmitting hepatitis C. Several studies of risk factors in sexual activity found rates of infection between 1 and 18% for homosexually active individuals, 1 to 10% among heterosexually active individuals, and 1 to 12% among female prostitutes, with the primary risk factors for infection being greater numbers of partners, unprotected sex, simultaneous infection with other STD's, and traumatic sexual activity. Seroprevalence for long term partners of hepatitis C patients was found to be around 5% and household contact with another household member that has hepatitis C (Zuckerman *et al.*, 1994; Lanphear *et al.*, 1994; Thomas *et al.*, 1996; Mostafa *et al.*, 2010).

Risk factors for hepatitis C have also been strongly implicated, and these, in combination with heterosexual exposure, are believed to be responsible for approximately 13% of all infections. The incidence of household-member transmission cases has more than doubled since 1990. Maternal-infant transmission has also been documented as a mode of spread, occuring in no more than six percent of children of hepatitis C positive mothers (Frischer *et al.,* 1993; Goldberg *et al.,* 1998).

Around 2% of all cases of hepatitis C are thought to be contracted through the occupational risk (needle-stick injuries, blood spills, etc) involved with the health care profession. Prisoners have enormous incidence of infection - rates reported in some California prisons exceed 80%, with certain institutions reporting nearly 100% of their prisoners infected (Camilleri *et al.,* 1991; McOmish *et al.,* 1993; *et al.,* 1993).

Some skin piercing practices, notably tattooing, body piercing, and acupuncture, have contributed significantly to the spread of HCV, particularly in less industrialized nations. Tattooing in particular poses a serious risk. Even in the presence of good sterilization, studies have suggested that the ink used in tattooing can become contaminated and transmit the virus (Dow *et al.,* 1993; Garcia *et al.,* 1996; Liu *et al.,* 1997).

Several studies have shown that adequate blood can be present in other body secretions to transmit infection. Cocaine users have an abnormally high risk of infection due to the fact that they frequently share snorting straws, which may have small amounts of blood-

carrying mucous on them. Such indirect sources of blood may explain many cases of inter-household transmission (Neal *et al.,* 1997; Davis *et al.,* 2010 Mostafa *et al.,* 2010; Opaleye *et al.,* 2010; WHO, 2014).

Less obvious, specialized risk factors have been identified resulting from indirect exposures to blood - including manicures, shared toothbrushes and razors, and straight razors in barber shops. Particular racial, ethnic, and income groups are at higher risk of infection. An ethnic analysis in one earlier, somewhat underestimated study (1994) determined that Caucasian Americans statistically accounted for the most number of infected persons, while the highest incidence rates incidence of hepatitis C in some prisons exceeds 80% (Davis *et al.,* 2010). Health care professionals that come into regular contact with blood are at increased risk for contracting hepatitis C were among African and Hispanic Americans (Mitsui *et al.,* 1992). The highest prevalence of the disease was found in middle-aged people (30 to 49 years old) who accounted for 3% -4% of the cases. Prevalence among black men in this age group approached 9% to 10%. Gender, however, did not emerge as a significant risk factor in the population as a whole. In the United States, blacks have the highest incidence rates, followed by Native Americans, Hispanics, and whites (Grellier *et al.,* 1997; Bosch *et al.,* 1998).

Similarly, low income groups seem to have the highest risk of infection. In one study at the inner city VA Hospital in Washington, D.C., one in five people admitted tested positive for HCV. Similar results (18%) were obtained at the John Hopkins University Hospital, located in Baltimore's inner city (Davis *et al.,* 2010; WHO, 2010). The higher

incidence among certain racial, ethnic, and income groups is probably the result of higher rates of other cofactors, but may also be the result of unidentified modes of transmission. Many groups showing high incidence of infection do not have obvious correlations with known modes of transmission, pointing towards the existence of unknown routes of transmission. For example, there is a serious question as to why many alcoholics are infected with HCV. In many surveys, about a third of people who are alcoholics are also infected with HCV. Whether alcoholics are in fact more prone to infection has not been firmly established (Hayashi *et al.*, 1995; Esteban *et al.*, 1996).

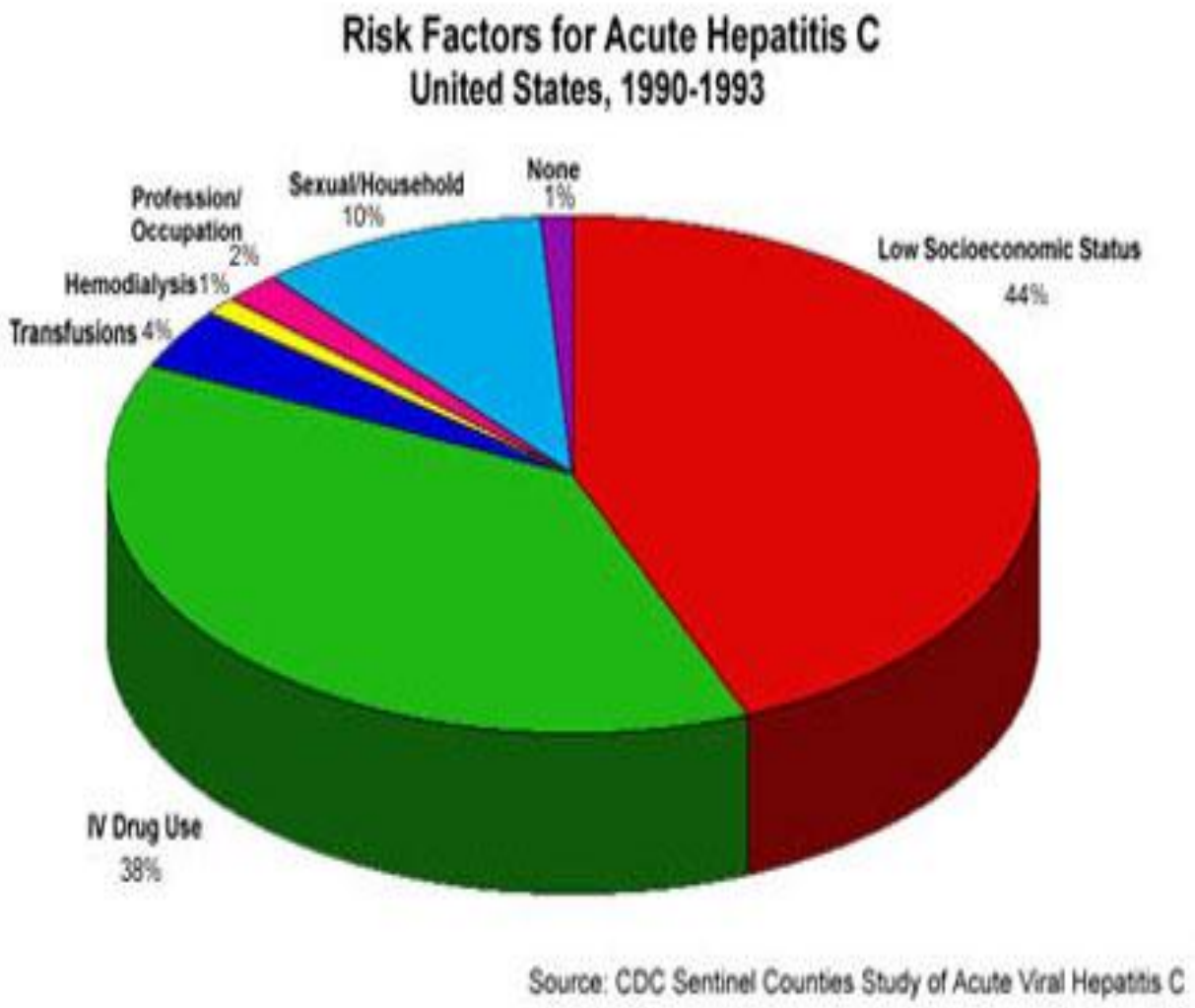
Risk Factors for Acute Hepatitis C
United States, 1990-1993
None 1%
Sexual/Household 10%
Profession/ Occupation 2%
Hemodialysis 1%
Transfusions 4%
Low Socioeconomic Status 44%
IV Drug Use 38%
Source: CDC Sentinel Counties Study of Acute Viral Hepatitis C

Figure 3: Risk Factors for Acute HCV

2.9 ACUTE HCV INFECTION

The course of acute viral hepatitis infection is conventionally divided into three phases: preicteric, sub-clinical (asymptomatic or prodromal), Icteric (flu-like) and convalescent (fulminant) symptomatic (McMahon *et al.,* 1985, White and Fenner, 1994). The incubation period is 6-26 weeks and is followed by preicteric phase characterized by malaise, lethargy, anorexia, nausea and vomiting, pain in the right upper abdominal quadrant, with evidence of serum sickness. The Icteric phase commences before the end of prodromal phase, heralded by dark urine (bilinubinuria), pale stools and jaundice 1% of icteric results in fulminant hepatitis and death. About one third of acute adult infections are completely asymptomatic; one third present as flu- like illness without jaundice that is rarely diagnosed as hepatitis C, and one third present as full blown hepatitis with typical signs and symptoms (McMahon *et al.,* 1985) including signs and symptoms of elevated liver transferases such as alanine amino transferases (ALT). The third phase is the convalescent phase with malaise and fatigue lasting for weeks (White and Fenner, 1994). The incubation period of hepatitis C virus infection ranges from 15 to 150 days. The common symptoms include fatigue and jaundice. Mortality rate is less than 1% but serum transaminase levels can be as high as 10 to 20 times the normal values (McMahon *et al.,* 1985).

In acute Hepatitis C infection the preicteric (prodromal) phase commences with malaise, lethargy, anorexia, nausea, vomiting and pain in the right upper abdominal quadrant following the incubation period (White and Fenner, 1994). A minority of patients develop at this time a type of serum sickness characterized by mild fever, urticarial rash, and polyarthritis, resembling a benign fleeting form of acute rheumatoid arthritis including glomerulonephritis. Circulating immune complexes have been suggested as the cause of the syndromes. Between 2 days in 2 weeks after the prodromal phase begins, the icteric phase commences, heralded by dark urine (biliribinuria) closely followed by pale stools and jaundice. The convalescent phase may be long and drawn out with malaise and fatigue lasting for weeks. Only 5% of HCV infected patients become jaundiced (White and Fenner, 1994).

Fulminant hepatitis occurs in a very small proportion of cases and could be due to super infection of HCV, HAV or HEV infections. It runs an exceptionally severe course, which is often fatal within days especially in the first four weeks. It is related to enhanced immune response with more rapid clearance of the virus (Breachol *et al.,* 1984). Furthermore, fulminating hepatitis is present in acute disease characterized by hepatitis hepatic failure and symptoms of hepatic encephalopathy (Escobar, 1992). The case fatality from fulminant hepatitis C is 1% or less (McLean *et al.,* 1997). Some high risk groups have been identified in the United States. Many of the high risk groups for hepatitis C are easily identified, due to practices resulting in frequent exposures to blood or risk factors

for transmission. Sexual contact has been clearly identified as a means of transmitting hepatitis C.

Several studies of risk factors in sexual activity found rates of infection between 1 and 18% for homosexually active individuals, 1 to 10% among heterosexually active individuals, and 1 to 12% among female prostitutes, with the primary risk factors for infection being greater numbers of partners, unprotected sex, simultaneous infection with other STD's, and traumatic sexual activity (McLean *et al.,* 1997; Davis *et al.,* 2010; Mostafa *et al.,* 2010; Newshome, 2013; WHO, 2014). Seroprevalence for long term partners of hepatitis C patients was found to be around 5%. Household contact with another household member that has hepatitis Risk factors for hepatitis C has also been strongly implicated, and this, in combination with heterosexual exposure, is believed to be responsible for approximately 13% of all infections. The incidence of household-member transmission cases has more than doubled since 1990 (Mostafa *et al.,* 2010). Maternal-infant transmission has also been documented as a mode of spread, occurring in no more than six percent of children of hepatitis C positive mothers. Around 2% of all cases of hepatitis C are thought to be contracted through the occupational risk (needle-stick injuries, blood spills, etc) involved with the health care profession as shown in Fig 3 (Newshome, 2013; WHO, 2014).

In acute HCV infection after initial exposure, HCV RNA can be detected in blood in 1 to 3 weeks and is present at the onset of symptoms. Antibodies to HCV are detected by enzyme immunoassay (EIA) in only 50 to 70 percent of patients at the onset of symptoms, increasing to approximately 90 percent of these patients after 3 months. Within an average of 2 to 8 weeks, liver cell injury is manifested by elevation of serum alanine aminotransferase (ALT) (White and Fenner, 1994; MClean *et al.*, 1997). Acute infection can be severe but is rarely fulminant. Symptoms are uncommon but can include malaise, weakness, anorexia, and jaundice. Symptoms usually subside after several weeks as ALT levels decline (Mosmier *et al.*, 1993).

Most people with acute HCV infection are asymptomatic or have mild symptoms (fatigue, nausea, jaundice) but are unable to clear the virus, leading to chronic infection in approximately 80% of cases (Petrus *et al.*, 2001). Chronic HCV infection progresses at a variable rate to cirrhosis in 15 to 20% of patients, who then have a 1 to 4% annual risk of developing hepatocellular carcinoma (Hadi, 2004).

2.10 CHRONIC HCV INFECTION

In chronic cases of HCV infection, 40% show persistent features, which leads much more commonly to chronic liver disease than does HBV (White and Fenner, 1994). Chronic liver damage can proceed to cirrhosis or hepatocellular carcinoma (White and Fenner, 1994). There is also a clear correlation between chronic HCV infection and the development of hepatocellular carcinoma (HCC); with more than 90% of HBV-negative

cases in some countries being HCV antibody positive. Because the HCV genome is RNA, and reverse transcriptase is not involved in its replication, it is unlikely that integration of cDNA occurs in HCV as with HBV associated HCC (White and Fenner, 1994). About half the patients with acute hepatitis C develop chronic hepatitis, 10 to 20% of chronic hepatitis cases develop cirrhosis and 1% to 5% of cases develop liver cancer within 20 to 30 years (McLean *et al.,* 1997; Newshome, 2013).

The chronic infection in 80% of cases runs a variable course with fluctuation in the extent of liver damage. With chronic infection, a patient is at risk of developing sequalae of cirrhosis and late liver failure including ascites, variceal bleeding or HCC (Dushieko, 1994). In chronic cases, 40% show persistent features, 40% chronic active hepatitis and 20% cirrhosis (McLean *et al.,* 1997), a proportion of about 10% die of hepatic failure or complications of portal hypertension. The course of the chronic hepatitis C can also be prolonged and insidious, and patient may not develop for many years after onset of chronic infection (Escobar, 1992). Long term consequences of infection are highly variable from patient to patient, and the likelihood of progression to chronic hepatitis appears to be unrelated to the clinical severity of acute illness (Brillanti *et al.,* 1993; Alter *et al.,* 1995). Chronic infection with HCV be it symptomatic or not leads to chronic liver disease (70% of cases), cirrhosis (20-30%) or HCC after decades (Van der Poel *et al.,* 1994; Newshome, 2013).

Chronic HCV infection is diagnosed by the detection of HCV RNA at least intermittently in the blood by either qualitative or quantitative tests for a period of at least 6 months. In general, prospective studies have shown that the majority of HCV-infected persons develop chronic infection. Factors associated with spontaneous clearance of HCV infection appear to include younger age, female gender, and certain major histocompatibility complex genes. African-American men appear to be least likely to spontaneously clear the virus (Davis *et al.*, 2010).

The most important sequelae of chronic HCV infection are progressive liver fibrosis leading to cirrhosis, end stage liver disease (ESLD), and HCC. Estimates of the proportion of chronically infected persons who develop cirrhosis 20 years after initial infection have been substantially higher from retrospective studies (17 to 55 percent) than from prospective studies (7 -16 percent). The actual risk of progressive disease at 20 years is now considered to be closer to the estimates from prospective studies (Newshome, 2013).

There is little evidence that the risk of progression of liver disease is affected significantly by virologic factors, including viral load, viral genotype, and quasispecies diversity. However, many host factors are observed to increase this risk, including older age at time of infection; male gender; and an immunosuppressed state, such as HIV infection. Hepatitis B appears to increase the risk of progressive liver disease (McLean *et al.*, 1997; Davis *et al.*, 2010).

Alcohol use plays an important role in increasing the risk of progressive liver disease, with strong evidence for the detrimental effects of 60 g/day in men (equivalent to six beers, four glasses of wine, or three mixed drinks) and 40 g/day in women, but there is suggestive evidence that lower amounts can also increase the risk of liver damage associated with HCV. Other factors, including iron overload, nonalcoholic fatty liver disease, schistosomal coinfection, potentially hepatotoxic medications, and environmental contaminants, may also have important effects (WHO, 2014).

In the United States, deaths associated with chronic HCV are currently more likely to be due to ESLD than to HCC. Data from death certificates in 1999 found that approximately 4,000 deaths were attributed to HCV infection, but this is likely to be an underestimate. The only treatment option for persons who have developed ESLD (decompensated cirrhosis) is transplantation (Sanaullah *et al.*, 2011).

For now, HCV is the primary reason for liver transplantation in the United States. Little is known about the clinical course and risks of HCV-related complications in persons who have been infected longer than two decades. HCV accounts for an estimated one-third of HCC cases in the United States. HCC rarely occurs in the absence of cirrhosis or advanced fibrosis (Davis *et al.*, 2010; WHO, 2014).

2.11 TRANSMISSION OF HCV

Several routes of transmission have been identified. These include intravenous drug use, blood and blood product transfusions, sexual activity, hemodialysis, perinatal and organ transplantion. Certain genotypes have been associated with certain transmission routes: subtypes 1a and 3a are associated with intravenous drug use; subtype 1b mainly spread via blood transfusion and various other nosocomial modes of transmission. It should be noted that this association is not absolute (Mauss *et al.*, 2012).

Intravenous drug use

The prevalence of Hepatitis C among intravenous drug users is ~80% (Touzet *et al.*, 2000). The rate differs somewhat between countries with Australia reporting a 75% rate (Maher *et al.*, 2004) and 90% in Pakistan (Muhammad and Jan, 2005). In the United States ~60% of all hepatitis C is due to intravenous drug use (Davis *et al.*, 2010).

A prospective study in the United Kingdom estimated the rate of acquisition of the virus to be 0.4% per year of drug use (Judd *et al.*, 2005; Newshome, 2013). A study in Ireland estimated the rate to be 0.66% per year (Smyth *et al.*, 2003).

Blood transfusions

Before the introduction of routine hepatitis C screening of blood the incidence of post transfusion hepatitis in the US was about 5% per patient (1 per 200 units) (Donahue *et*

al., 1992). This risk has now dropped to ~1/30 million units. In haemophiliacs the prevalence of hepatitis C is >90% (Newshomw, 2013).

Sexual activity

Hepatitis C appears to be transmitted sexually but the rate of transmission appears to be low (Magder *et al.*, 2004; Vandelli *et al.*, 2004). The rate among men who have sex with men is higher (4-8%) than the general population.

Hemodialysis

Prevalence rates in hemodialysis patients have been reported to be as high as 50% (Hayashi *et al.*, 1999). With the introduction of tests and treatments for Hepatitis C this prevalence has been reduced considerably. The annual risk still remains high at 1.38-1.9%/year (Halfon *et al.*, 1994). Transmission within dialysis units has been reported (Kalinina *et al.*, 2001).

Perinatal

The virus appears to be transmissible perinatally. Caesarian section does not appear to reduce the risk of transmission.

Transplants

Renal transplants in Italy have been associated with 33% prevalence (Angelico *et al.*, 1997). A confounding factor here is that all the recipients had been on dialysis for some time before being transplanted.

2.12 MODES OF TRANSMISSION OF HCV INFECTION

Hepatitis C is a major cause of parenterally transmitted Non-A Non-B hepatitis (Kuperan *et al.*, 1996). The disease was recognized by the late 1970s in blood recipients and blood products such as factor VIII and immunoglobulin (McLean *et al.*, 1977). Non-A Non-B hepatitis causes over 90% of cases of post transfusion hepatitis and 20-35% of sporadic viral hepatitis (Dienstag, 1883a, 1883b; Kuo *et al.*, 1989). It is recognized that HCV is a major aetiological agent of post transfusion hepatitis Non-A Non-B. The HCV infection frequently progresses to chronic liver disease (Choo *et al.*, 1990; Saito *et al.*, 1990). Transplanted organs and needle stick injuries have also been implicated in transmission. Intravenous drug use is also a risk factor and the most identifiable infected cohorts are intravenous drug users (White and Fenner, 1994; Mauss *et al.*, 2012). There is a small risk of transmission through sexual contact and mother to baby transmission is also low. However risk in both, may be increased in the immuno compromised (McLean *et al.*, 1977). HCV has a higher efficiency of transmission via blood contact than HBV. It may also be transmitted through in apparent blood contact during injecting processes, such as sharing equipment other than needles and syringes. The lower prevalence of transmitted Hepatitis C virus than hepatitis B virus in sexual partners suggests a lower efficiency for sexual transmission than for other viruses such as HBV and HIV (MacDonalel *et al.*, 1996).

HCV can be transmitted through blood and blood products recipients, transmission in injecting drug miss-users, tattooing and skin piercing, transmission in health care setting, sexual transmission, household contact, mother to child transmission, breastfeeding and transmission through mode of delivery. Early studies of HCV prevalence among transfusion recipients and in people with medical conditions associated with blood transfusion such as haemophiliacs, thalassemia, chronic renal failure and cardiac surgery showed that the high rate of Non-A Non-B hepatitis in this group was largely related to the acquisition of HCV infection (Alter *et al.,* 1992; Leslie *et al.,* 1992; Ismay *et al.,* 1995).

Cross-sectional survey and cohort studies among groups of injecting drug miss-users from Europe (Van Der Poel *et al.,* 1991; Tor *et al.,* 1990) North America (Wormsers *et al.,* 1991; Kelen *et al.,* 1992), Asia (Lee *et al.,* 1991; Chan *et al.,* 1992) and Australia (Crofts *et al.,* 1993; Bell *et al.,* 1990) have found extremely high prevalence of HCV antibody from 50-90%. Hence the most clearly identifiable infected cohorts are intravenous drug miss-users (White and Fenner, 1994). The presence of tattooing has been independently associated with an increased risk of HCV infection (Kaldor *et al.,* 1992). In a study specifically examining tattoos as a risk for HCV (Thompson *et al.,* 1996), there was a high risk with multiple tattoos compared with a single site, and tattooing carried out by non professionals compared with professional tattooists.

Transmission of HCV from patient to health care worker has been generally documented following percutaneous exposure to blood (Gerberding, 1994). In addition to being

detected in blood, HCV-RNA has been detected in ascetic fluid, semen, and the urine of HCV-positive patients with chronic liver damage (Liou *et al.,* 1992). Sexual transmission is uncommon but sequence analyses of HCV in sexual partners indicate that sexual transmission can occur. There is some evidence that HCV may be shed in genital secretions and saliva as well as in blood. A case control study among blood donors showed a significantly higher risk of HCV in people with more than one lifetime sexual partner, compared with those with one or less, after people with history of injecting drug use; blood transfusion and tattoo were excluded (Kaldor *et al.,* 1992). Transmission of HCV to household contacts of people with HCV infection is low and it is difficult to rule out blood borne transmission as the route of infection (MacDonalel *et al.,* 1996). A prevalence of 2.2% was detected among 181 household contacts of patients with chronic liver disease in Korea. Comparison with 102 household contacts of people with non-HCV liver disease showed an increased risk with a history of hepatitis, blood transfusion or acupuncture (Kim *et al.,* 1994).

The vertical (Mother to Child) transmission of HCV has been documented in several studies (MacDonalel *et al.,* 1996). It has been proved by examining sequence homology in the most variable region of the HCV genome, the envelope glypoprotein (E2 region) (Dusheiko *et al.,* 1996) as determined by detection of HCV-RNA in the child, range widely from 0-7% in Europe and Asia (Marcellin *et al.,* 1993; Lin *et al.,* 1994; Manzini *et al.,* 1995). The HCV-RNA is usually used as marker of HCV infection in infants and not HCV antibody because the passively acquired maternal HCV antibody cannot be distinguished from antibody produced by the infant. The passively acquired maternal

antibody progressively disappeared from birth until 15 months, in all children but one in an Italian study of 45 infants and gave a transmission rate as determined by detection of HCV-RNA of 2.2% (Manzini *et al.,* 1995).

In breastfeeding there is little about the role in modifying transmission of HCV from mother to child because of small numbers of infants in individual studies and the low rate of HCV transmission in these studies (McDonalel *et al.,* 1996). A German study showed that none of the 76 breast milk samples from 73 chronically HCV infected women showed HCV-RNA by RT-PCR analysis, while 37 (59.7%) of 62 mothers had HCV viraemia, but only 1 of the 76 breast fed infants was detected to have HCV infection one month after birth, making it seem unlikely that the virus was transmitted by breastfeeding (Polywka *et al.,* 1996).

In the case of mode of delivery there is limited information about this effect on HCV transmission as only small numbers of children in some studies. In a study of 66 children, 2 of them delivered by caesarian section delivery had no evidence of maternal acquired HCV, compared with 6% HCV transmission in children who were born by vaginal delivery (Ohto *et al.,* 1994). Both current and post maternal intravenous drug abuse are risk factors for paediatric infection; vertical transmission seems to be the most common route of infection (Bortolotti *et al.,* 1998).

2.13 PATHOGENESIS OF HCV INFECTION

The major target cells in the pathogenesis of HCV infection are the hepatocytes but there is also evidence that viral replication takes place in leucocytes and perhaps other cells. Viraemia is detectable by PCR within days of infection and lasts weeks or months before resolution in most cases, but it may persist for years in chronic carriers, often fluctuating erratically. Whether these swings reflect reactivation of virus, with accompanying relapses in clinical hepatitis, and if so, what triggers them has to be resolved (White and Fenner, 1994). Antibody and T-cell responses can be detected but are usually unable to clear the virus and do not protect against re-infection even with the same strain of virus. Variation of the envelope glycoproteins may aid persistence, as may the binding of virus to lipid. Immuno-suppressed patients show evidence of liver disease indicating a direct cytotoxic effect of the virus. The hepatitis C infection has been linked to several other conditions such as immune complex deposition (vasculitis and glomerulonephritis), Sjorgen's disease, and immunologically mediated renal disease, most commonly cryoglobulinaemic and non-cryoglobulinaemic MPGN, MGN and proliferative GN (McLean *et al.*, 1997).

The pathogenesis of Hepatitis C virus infection shows that the virus transmission is by parenteral route and the infection can also be transmitted sexually and from mother to child at delivery. Viraemia is detectable between 1 to 3 weeks after infection and this may last between 4 to 6 months in acute infection and up to 10 years in chronic infection. Production of chronic active hepatitis may progress to liver cirrhosis and liver failure in chronically infected patients (Hoofnagle and Alter, 1984).

The most cases of acute hepatitis C anicteric and asymptomatic with fewer than 25% are being clinically apparent. Fulminating hepatitis C are rare. The most remarkable and alarming aspects of HCV infection are its high rate of persistence and its ability to induce chronic liver disease (Ayodele and Salako, 2003). The infection persists in about 80% of cases leading to chronic hepatitis (Van der Poel *et al.*, 1994). Approximately 20-30% of those chronically infected subjects will develop cirrhosis and a portion of these will develop hepatocellular carcinoma (Heathcote *et al.*, 2000; Ayodele and Salako, 2003). The pathogenesis and clinical course of HCV infection have been associated with the following main features:

(a) The characteristic pattern of enzyme elevations with marked spontaneous fluctuation in serum alanine amino transferase levels. This enzyme pattern is rare in hepatitis B, except during period of reactivation (Hoofnagle and Alter, 1984).

(b) The histological pattern characterized by fatty metamorphosis. This feature is said to be more typical of cytopathic, rather than immunologically mediated, injury (Dienstag *et al.*, 1983).

(c) The high incidence of chronicity with 50-70% of the patients developing liver disease following the acute form of this type of hepatitis (Dienstag, 1983).

The course of HCV infection in an individual patient seen at one point in time is often difficult to predict. Interpretation of liver biopsy samples from symptom less patients or

blood donors needs to take into account how progressive HCV disease can be symptom less, and that serological tests do not reliably predict the histology. The specimen should show anything from minimal change to cirrhosis. Patients who are not viraemic or whose recombinant immunoblot assay (RIBA) results are indeterminate are less likely to have significant liver disease on biopsy. The progression of the disease and response to therapy will need closer examination in symptom free "carriers" (Di Biceglie *et al.,* 1991).

The pathology of HCV infection indicates that the inflammation in mild hepatitis corresponding with persistent infection of HCV is characterized by a portal inflammatory response with a variable amount of lobular inflammation with parenchymal apoptoses. The portal inflammatory infiltrate can form lymphoid aggregates, and periportal necrosis is usually mild. Necrosis during the bouts of inflammation establishes fibro-inflammatory connective tissue connections across the lobule. Acinar zone 3 is increased with much lobular disarray. There may be much more "ballooning" degeneration as well as canalicular cholestasis. The histological appearance can come to resemble closely the traditional picture of "acute" hepatitis, such that the distinction between acute and chronic hepatitis is difficult on morphological differences alone. Despite the difficulties, the typical features of HCV infection, including apoptosis of follicles, and fibrosis make the disease easily recognizable. The histologic alterations found in human liver biopsy specimens for the most part are similar to those seen in cases of HBV infections, except that the lesions in Non-A Non-B are the result of virus mediated cytotoxicity and is characteristic of hepatitis B by light microscopy with the former being less severe (Heathcote *et al.,* 2000).

The morphological features of chronic hepatitis C were reported by Govindarajan (1990) from a respective study of serologically documented chronic HCV infection on the basis of liver biopsies which consistently revealed proliferation of atypical bile products including serpinginous growth patterns, hydropic changes of the epithelium and rarely, hyperplastic changes. In addition, in all cases there was prominent periportal or periseptal sinusoidal fibrosis, and only occasionally perivenular fibrosis. In approximately 60% of the cases, there were micro-vesicular fatty changes with a random distribution. The author suggested that morphological features such as fat, sinusoidal collagen, glycogen nuclei, and a typical duct were unusual for other types of chronic hepatitis (Escobar, 1992). Dense reticular cytoplasmic inclusions and convoluted membranes are conspicuous by electron microscopy in hepatocytes from infected Chimpanzees (only 1% of cells are productively infected) (Escobar, 1992).

Immunoflorescence reveals viral proteins, mainly NS3 and NS4, confined to the cytoplasm (White and Fenner, 1994). Virological evidence suggests that parenchymal inflammation and necrosis are related to the presence of replicating virus in liver cells (Haruna *et al.*, 1993) together with corresponding MHC class 1 restricted cytotoxic CD6+ T-cell response, rather than being a result of a cytopathic effect. The parenchymal changes are probably important in disease progression. The effect of a fall in HCV-RNA levels in reducing hepatic inflammation would not refute the hypothesis of immune-mediated change. The portal changes (including the lymphoid follicles) are unlikely to be due to

immunological changes, possibly of the autoimmune type, since HCV shares sequence histologies with the endogenous liver proteins such as cytochrome p45011D6 (Lunel *et al.,* 1992). "Antiviral" and "autoimmune" mechanisms are not mutually exclusive, and there may be elements of each in the same patient at the same time (Dhillon, 1995).

The liver is the major site of HCV replication, and it contains a high abundance of HCV RNA (typically 10^8-10^{11} copies per gram of tissue) (Dhillon,1995). However, the proportion of hepatocytes infected with HCV in patients with chronic hepatitis C is very uncertain. HCV RNA was detected by in situ hybridization in most hepatocytes as early as 2 days following parenteral challenge of chimpanzees with a high-titer inoculum, prior to its appearance in the serum (Haruna *et al.,* 1993). Immunohistochemical studies also suggest that HCV infection is not limited to hepatocytes because viral antigens and viral RNA have also been detected in a small percentage of mononuclear cells and biliary epithelial or sinusoidal lining cells (Heathcote *et al.,* 2000).

2.14 HOST IMMUNE RESPONSES TO ACUTE AND CHRONIC HCV INFECTION

In HCV infection, antibody and T-cell responses can be detected but are usually unable to clear the virus and do not protect against re-infection even with the same strain of the virus (McLean *et al.,* 1997). Antibodies to peptides representing linear epitopes encoded by the HCV structural proteins usually appear within 10 weeks of onset (Okamoto *et al.,* 1992; Hosein *et al.,* 1992). Antibodies to the virus develop late up to 6 months after onset

of jaundice. There is increasing evidence that the course of the disease with hepatocellular damage during chronic hepatitis C are mediated by the immune response of persons infected by HCV (Botarelli *et al.,* 1993; Mosnier *et al.,* 1993). It has been suggested that HCV induces auto immune phenomena because of the high occurrence of antibodies which are commonly associated with chronic hepatitis (Lenzi *et al.,* 1990 and 1991; Magin *et al.,* 1991). In particular, it has been reported that patients with chronic hepatitis C frequently present a humoral response to a host derived epitope designated as GOR (Mishiro *et al.,* 1990; Michel *et al.,* 1992). The sequence of the GOR epitope has a partial homology with the HCV-encoded core protein sequence (Mishiro *et al.,* 1991).

Chronic carriers of HCV make antibodies to a variety of virus specific proteins and this include the structural nucleocapsid protein and various non structural proteins. However, individuals with persistent HCV infection may also be seropositive against the surface glycoproteins of the virion. Antibodies to the antigens from the conserved capsid and NS3 and NS4 regions are also produced (Harrison, 1995). Seroconversion may not be detectable until several months after exposure to the virus (Escobar, 1992). Although the antibodies continue to be made, and most of the virus is bound in virus –antibody complexes, the infection is not eliminated (White and Fenner, 1994). The host produces antibodies to the antigens corresponding to the three most conserved HCV proteins C, NS3 and NS4; and also of the hypervariable E_1 and E_2 proteins. Because of the slow and variable development of antibodies post infection, there is a window phase of 3 months before test registers positive. In many cases, production of antibodies to the core and NS3 proteins precedes production of NS4 antibodies (Van Doorm *et al.,* 1996). Retrospective

studies of Chimpanzees used for transmission of non-A, non-B hepatitis show that repeated acute infections may occur in one individual (Farci *et al.,* 1992). Furthermore, the amino end terminus of the surface glycoprotein E_2 (gp 70), which may be a major antigenic domain of the virion surface, is highly variable (Harrison, 1995).

According to immune response to HCV infection, homologous viral populations have been observed at the start of infection, with sequence homogeneity developing some years back. It is uncertain whether the observed genetic drift reflects inherent viral evolution, or whether immune selection "drives" the sequence variability of the amino terminus of E_2/NS1. Careful mapping experiments have shown epitope shifts. Apparently the result of the presence of antihypervariable region antibodies which support the hypothesis that the humoral response to HCV is directed against envelope epitopes, and that the antibody neutralization allows the selection of variants. Whether cytotoxic or humoral response predominates in hepatitis C is not well defined, although a T-lymphocyte response is likely (Dusheiko, 1995).

Cytotoxic T-lymphocytes (CTLs) are thought to be a major host defense against viral infection and have also been implicated in the immunopathogenesis of viral infection. HCV-specific CTLs are present in both the peripheral blood (Kita *et al.*, 1993), and among lymphocytes infiltrating the liver (Koziel, *et al.*, 1992) in patients with chronic hepatitis C. Epitopes in HCV antigens recognized by CTLs cultured from peripheral blood lymphocytes (PBLs) or lymphocytes infiltrating the liver of patients with HCV infection

have been identified in association with human leucocytes-antigens B44 (Kita *et al.*, 1993; 1995), A29, B35 (Koziel *et al.*, 1993), A11, B7, B50, B51 (Koziel *et al.*, 1993), A3, A23, B53, E8 (Koziel *et al.*, 1995) and A2 (Shiral *et al.*, 1994). It was found that the nucleoprotein NS3-specific CTLs are CD8+ T-cells and that NP12-specific and NP17-specific CTLs are CD4+ T-cells (Kaneko *et al.*, 1996).

CTLs have been shown to be involved in the pathogenesis of viral infection and the immune clearance of infected cells, although CD4+ CTLs may be less effective for viral clearance than CD8+ CTLs (Muller *et al.*, 1992). Thus NP12-stimulated CD4+ CTLs and NP17-stimulated CD4+ CTLs may play a role in the host defense against HCV infection, although whether or not these CTLs can recognize endogenously synthesized HCV nucleoprotein remains a mirage. Alternatively, CD4+ HCV nucleoprotein peptide–specific CTLs may be involved in the immunopathogenesis of viral infection rather than viral clearance as postulated for herpes simplex virus infection (Yasukawa *et al.*, 1989).

It has been observed that Chimpanzees that recovered from HCV infection can be re-infected with the homologous or heterologous strain of the virus. Humans also frequently experience multiple episodes of acute hepatitis C, but it is unclear whether these are exogenous re-infections with the same or another strain or reactivation of the original infection (White and Fenner, 1994). Also though virus-specific T-cells populations continue to be present in the liver, they seem unable to eradicate HCV infection in the most persistently infected carriers (Erickson *et al.*, 1993).

2.15 DIAGNOSIS OF HCV INFECTION

The variety of laboratory tests used for the diagnosis of HCV infectivity includes serology, serotyping, detection of HCV-RNA, biochemistry (liver biopsy histologic), and haematology. Laboratory diagnosis involving serology requires specimen such as serum or plasma and this is based on the detection of HCV antibody. The lab tests include ELISA and Immunoblot assay (for confirmation of ELISA test results). The nucleic acid detection on the other hand requires specimen such as serum or plasma. The lab diagnosis is based on the detection of HCV nucleic acid sequences in serum or plasma. The laboratory tests include reverse transcriptase PCR (RT-PCR) and DNA probes. The first serological diagnostic test for hepatitis C virus (HCV) was licensed for the screening of blood donors in the United States in 1990 (White and Fenner, 1994). A cDNA done representing parts of the HCV genome was expressed in yeast to produce a recombinant antigen (fusion protein) corresponding to a large portion of the non structural protein NS4 of the C100-3 region and also to the 5-1-1 region, in one of the kits (Escobar, 1992; Sulkowski *et al.*, 2012), and this antigen was employed in an enzyme Immunoassaay to detect antibody to that particular protein in the serum or plasma of the blood or organ donors.

The first generation EIA (ELISA) kit was replaced by second-generation version on a recombinant yeast chimeric protein comprising the three most conserved HCV proteins, C, NS3 and NS4. This antigen, used in enzyme immunosorbent assay (ELISA) detects up to 95% of post-transfusion Non-A Non-B hepatitis and replaces the first generation ELISA/ EIA kits in the blood banks and elsewhere (White and Fenner, 1994). They provided improved sensitivity and specificity compared with the first generation kits and shorten

the time for detection of seroconversion (Aach *et al.,* 1991; Chaudhary *et al.*, 1993). The third generation HCV antibody assays were introduced in 1993 in Europe. In 1995, third and fourth generation assays had become available (MacDonalel *et al.,* 1996).

Recombinant immunoblot assay can also be used in detecting HCV infection and the genotypes of the virus but Vrielink *et al.,* (1997) in their studies found that ELISA-2 and 3 were significantly sensitive than second and third generation recombinant immunoassays. The development of HCV antibodies post-infection is slow and variable, and there is a window phase of 3months before the tests register positive; assay methods detect chronic infections and are less sensitive in the early stages of infection. An alternative approach is to determine viral RNA by amplification of DNA after reverse transcription polymerase chain reaction (RT-PCR) (McLean *et al.,* 1997). The HCV genotyping can also be carried out by analyzing amplicons from the conserved 5'-non-translated region generated by nested PCR (Datz *et al.,* 1999). Sera can be tested from HCV-RNA by reverse transcriptase" nested" PCR and the envelope characterized by rescription fragment length polymorphism (Natov et al, 1998). Since HCV-RNA is usually detected very soon after infection, the genotyping analysis is the only method available during the window phase (Van Doorm *et al.,* 1996)

On the basis of branched type-specific oligopeptide, a serotyping assay has been developed (Simmonds *et al.,* 1993). At first, this allows the detection of only type 1 to 3, but it has been extended to types 4, 5 and 6 (Bukh *et al.,* 1993; Simmonds *et al.*, 1993; McHutchison *et al.,* 2013). A new analysis is based on serological discrimination between

the major types 1 to 6 by measuring the type-specific NS4 antibodies. Serotyping analysis of HCV isolates provides an indirect typing method based on the production of the type specific antibodies by the infected host. Therefore the type ability depends on the immunocompetence of the infected host. Whereas genotyping detects only present viral genomes, the indirect nature of serotyping may allow it to also detect antibodies related to post infection. However, the presence of cross reacting antibodies cannot be excluded (Van Doorm *et al.,* 1996). Serotyping results correlate with genotyping method and may be useful in characterization of HCV isolates, especially in laboratories that lack the specific expertise to perform genotyping methods. The serotype test is rapid and relatively easy to perform. However, the sensitivity of the test may be limited by the immunocompitence of the infected host.

Examination of liver biopsy helps to exclude other diseases, and to determine the degree and progress of liver diseases as well as response to therapy. The histologic appearance comes to resemble closely the traditional picture of acute hepatitis, such that the picture between acute and chronic hepatitis is difficult on morphological differences alone (Dhillon, 1995). Assessment of overall prognosis, and activity of the diseases, is difficult from the study of single biopsy specimens because of the unpredictability of the inflammatory episode from clinical data, and the possibility that any one sample is not representative of the overall illness in a given patient.

The episode of the acute may in fact be exactly that and might reflect a further acute, immunological response against a genomically altered HCV (Dhillon, 1995). Despite

these difficulties, the typical features of HCV infection, including apoptosis, fat, follicles, and fibrosis makes the diseases readily recognizable. Liver biopsy findings also reveal lymphocytic infiltration, portal or bridging fibrosis (Houghton *et al* 1991) and moderate degree of inflammation and necrosis. Regenerating nodules are noted in patients with cirrhosis. Findings of hepatocellular carcinoma may be present in some patients (Heathcote *et al.*, 2000). In symptom free patients, the biopsy could show any thing from minimal change to cirrhosis as is seen in symptom free blood donors. In chronic cases 40% show persistent features, 40% chronic active hepatitis especially in those whose diseases are acquired after transfusion, and 20% cirrhosis (McLean *et al.*, 1997; Jawetz *et al*, 1998; Annonymous, 1999).

Using biochemistry as a tool for HCV diagnosis, tests from abnormal liver function, such as alanine aminotransferase (ALT) and bilirubin can supplement the clinical and pathological data. Alanine aminitransefase level in acute hepatitis range from 500 and 200 unit and they are usually higher than those of the aspartate aminotransferase (AST). A gradual rise with prolongation (35 to 200 days) appears to characterize hepatitis B and C infections (Jawetz *et al.*, 1998). Fluctuating levels are more characteristic of hepatitis C while a sharp elevation within a short duration of 3 to 19 days is more suggestive of hepatitis A Infection (Escobar, 1992). The prevalence of HCV-RNA in plasma of asymptomatic blood donors with elevated ALT, and its correlation with the presence of anti-HCV, hepatitis B surface antigen and antibodies to hepatitis B core antigen has been reported (Olubuyide *et al*., 1997). In diagnosis of HCV through the detection of HCV-RNA, immunoflourescence and in situ nucleic acid hybridization are also of diagnostic

value when used on biopsy (White and Fenner, 1994). However the insensitivity of conventional techniques including electron microscopy has been attributed to the low copy of virus at any one site. The only reliable technique is PCR amplification, which is more sensitive in frozen materials than in routine fixed and processed materials (Dhillon, 1995)

In haematology, leukopaenia is typical in the preicteric phase and may be followed by a sensitive lymphocytosis. Large apical lymphocytes similar to those found in infectious mononucleosis may occasionally be present in smears prepared for differential white blood cell count, but these generally do not exceed 10% of the total lymphocyte population (Escobar, 1992). In terms of diagnosis of its infection through cell culture, HCV has been successfully cultured in lymphocytes (Purcell, 1997). Evidence of invitro replication has been shown in three human T cell lines namely MOLT-4 cell, HPB-MA cell (Shimzu *et al.*, 1993) and H9 cells (Nissen *et al*, 1994). In two other studies, replication of HCV was observed in fetal hepatocytes (Manzini *et al.*, 1995) and in human bone marrow derived B cell line (Bertolini et *al.*, 1993). Cribier *et al.*, (1995) have also demonstrated the infection of cultured peripheral blood mononuclear cells (PBMC) using sera from HCV-infected patients, but the replication level detected by measuring HCV-RNA by RT-PCR was very low. However it was also shown that when MOLT-4 cells were infected by a Murine retrovirus, the replication of HCV was more efficient (Shimizu *et al.*, 1993; McHutchison *et al.*, 2013), suggesting that co-factors play an important role in HCV replication in PBMC.

Prevalence

The WHO has estimated the prevalence of this virus to be ~170 million (Annonymous, 1999). In the United States it has been estimated that 2.7 million people are chronically infected (Alter *et al.,* 1999). In the US it accounts for 8000 to 13,000 deaths each year and the majority of liver transplants performed in the United States are for chronic HCV. In South America the rate is ~6% (Perez *et al.,* 1999). Currently, approximately 10 million people in Pakistan are infected with HCV, covering 6% of the overall population and it falls in the intermediate endemic zone (Hussain *et al.,* 2010). There is a high prevalence of HCV in Japan, Mediterranean countries of Europe, the Middle East and Africa (Van der Poel *et al.,* 1994; Sharara *et al.,* 1996).

In Europe ~1% of the population has antibodies to this virus (Touzet *et al.,* 2000).It is estimated that there are 2-5 million HCV-positive persons in Europe. In Italy the prevalence tends to be higher with rates of up to ~20% in the south of the country (Maio *et al.,* 2000). In the United Kingdom at least 200,000 adults are chronic carriers (Dept of Health, UK 2002). In Pakistan the antibody positive rate is ~5%. The prevalence of HCV in the general population in Africa is about 10%. Egypt has the highest prevalence of hepatitis C virus (HCV) in the world, estimated nationally at 14.7% (Abdelwahab *et al.*, 2013). In Nigeria, the rate of HCV across various centres ranges from 0.5% to 12% (Oni and Harrison, 1996, Olubuyide *et al.*, 1997a, Ola *et al.*, 2002; Opaleye *et al.*, 2010).

Incidence of HCV

The incidence of this infection is difficult to estimate as <25% of acute cases of hepatitis C are clinically apparent. 20% have normal liver function tests despite infection. In the United States the peak incidence of Hepatitis C was ~240,000 per year (in 1989). It is currently ~ 20-30,000 new cases per year. In Canada the reported rates were 25 per million population (2004), 16 per million (2006) and 22 per million (2008) (Annonymous, 2009; WHO, 2014).

The incidence of HCV on a global scale is not well known, because acute infection is generally asymptomatic (Yousra *et al.*, 2013). As many as 2 to 4 million persons may be chronically infected in the United States, 5 to 10 million in Europe, and about 12 million in India and most do not know they are infected. About 150 000 new cases occur annually in the US and in Western Europe, and about 350 000 in Japan. Of these, about 25% are symptomatic, but 60 to 80% may progress to chronic liver disease, and 20% of these develop cirrhosis. About 5%-7% of patients may ultimately die of the consequences of the infection (Newshome, 2013; WHO, 2014).

Approximately 35,000 new cases in the United States each year, 4,000,000 people in the United States chronically infected and 9,000 deaths a year in United States from HCV-related liver disease. African Americans and Latinos have the highest rates of chronic hepatitis in the United States, especially in urban environments where limited access to health care, crowded housing and intravenous drug use are prevalent (Davis *et al.*, 2010; Newshome, 2013).

2.16 HBV AND HCV CO-INFECTION

Co-infection of HCV and HBV infections has been reported and the interaction of the viruses may determine the pattern of the clinical presentation of the patients. In a recent study, the presence of high rate of combined HBV and HCV infections in patients compared to the apparently healthy (control) individuals and the higher rate of single HCV infection in the latter group (controls) might be a reflection of the natural history of HCV infection which is often mild and asymptomatic in the acute form and indolent in the course to chronicity (Ola *et al.*, 2002; Opaleye *et al.*, 2010). This study has further re-affirmed that both HBV and HCV are efficiently transmitted by the parenteral route with HBV being more readily transmitted than HCV. Olubuyide *et al.* (1997) in their own findings suggested a high prevalence of hepatitis B virus (HBV) with a high potential of transmissibility, as well as a high prevalence of hepatitis C virus (HCV) infection. In another study carried out by Olubuyide *et al.*, (1997), 10.9% of patients with hepatocellular carcinoma (HCC) were positive for both HCV and HBsAg. This shows the extent at which HBV co-infected with HCV has been on the increase at the community level in this part of the world.

Co-infection with HCV in HBV infected individuals is common, presumably due to the shared route of transmission of these viruses. The prevalence of coinfection varies according to the risk of exposure (Dieterich *et al.*, 1999; Gao *et al.*, 2002). Groups at highest risk of coinfection include individuals who receive multiple transfusions with blood or blood products, such as person with haemophilia, as well as injection drug users

(IDUs) and persons who have multiple sexual partners (Hoofnagle and Liang, 2000). Among haemophiliacs in the United States, infection with HCV was found to be 85% of individuals with HBV (Dieterich *et al.,* 1999). This association suggests that both infections were acquired through the same route.

In a long term follow-up study the clinical and virological presentation of HBV/HCV coinfection in anti-HIV positive patients was evaluated. Plasma HBV-DNA, HCV-RNA, and HIV-RNA were determined by PCR in 5 HBsAg/anti-HCV/anti-HIV positive patients, in 4 HBsAg/anti-HIV positive patients and in 82 anti-HCV/anti-HIV positive patients first observed at a Unit of Infectious Diseases I Naplies (Italy) in a follow up between 6-16 years (1990 to 2000). All five Hepatitis B and C coinfected patients showed reciprocal inhibition of viral replication on admission and during the follow up. At the end of the follow up a clearance of HBsAg from serum was observed in four patients and a clearance of anti-HCV in one of them. In anti-HIV positive patients HBV/HCV coinfection is characterized by reciprocal inhibition of viral replication, more evident in HBV expression in plasma and at times by progression to occult HBV infection (Filippini *et al.*, 2007; Opaleye *et al.*, 2010).

2.17 PREVENTION OF HCV INFECTION

Unlike HBV infection, neither viral vaccines nor protective immunoglobulin is currently available to prevent or control HCV infection (Jawetz *et al.,* 1998). However, various precautionary measures are being explained to stem the increase of HCV infection especially in the developed countries, while efforts are being intensified to develop

effective vaccines against HCV infection. In the area of universal precaution, simple environmental procedures can limit the risk of infection to health care workers, laboratory personnel and others. All blood, body fluids and materials contaminated with them are treated as if they are infectious to HBV, HCV, HIV and other blood borne pathogens. Wearing of gloves should be used when handling potentially infectious materials and protective garment should be worn and removed before leaving the work area. Face masks and eye protectors are also worn whenever splashes or droplets from infectious materials pose a risk. Only disposable needles should be used and discarded directly into special containers without reheating, and work surfaces are decontaminated using appropriate dilution of bleach solution. Mouth pipetting, eating, drinking and smoking are prohibited in all work areas of the laboratory and metallic objects and instrument are disinfected by autoclaving or exposure to ethylene oxide gas where available (Jawetz *et al.*, 1998).

Blood and blood products screening and treatment should also be encouraged before use post-transfusion HCV has become relatively rare in the developed countries since donors with risk factors for blood borne viral infections and those found to have HCV, HIV or hepatitis antibody have been excluded from donating blood (Donahue *et al.*, 1992). The heat treatment of blood products has also eliminated the transmission risks from these sources (McLean *et al.*, 1997). Further efforts to reduce the current risks of HBV, HCV and other viral infections are continuing. These include restricting transfusions to those which are necessary or appropriate, utilizing alternatives to transfusion, employing novel assays to detect viral nucleic acids and implementing various microbial inactivation techniques on blood and blood components and plasma derivatives (Holland, 1998).

Nevertheless, the preventive measures against hepatitis C include screening and testing of blood and organ donors, virus inactivation of plasma-derived products, infection control in health settings and counseling for persons with high-risk drug and sexual practices on risk reduction i.e to reduce risk of acquiring it. However, for immunization, there is no vaccine against hepatitis C for now (Judd *et al.*, 2005; Opaleye *et al.*, 2010; WHO, 2014).

2.18 GLOBAL PREVENTION AND CONTROL OF HCV INFECTION

Many countries have yet to address primary prevention of HCV infection, particularly in the healthcare setting. However, even when control of HCV transmission is realized in these countries, HCV-associated morbidity and mortality from cirrhosis and HCC will continue to increase for years, even decades, in the absence of effective care and treatment programs. Linking prevention and control to testing, care, and treatment of HCV infection requires a comprehensive, cohesive approach tailored to meet the needs of individual countries. Public health officials must be familiar with the epidemiology of HCV infection within their borders and know whether widespread transmission is ongoing and in what settings it is occurring. Addressing transmission should be the first priority for any country.

Unlike global efforts to prevent and control HIV infection and other infectious diseases, those for HCV infection are limited and largely unaccompanied by community advocacy and awareness. This lack of grassroots support complicates efforts to ensure that

populations most affected by hepatitis C, which typically are hard to reach and disenfranchised, receive needed services. Also challenging is the absence of viral hepatitis control programs at the Ministry of Health level in many lower-to-middle income countries. This absence leads to fragmented and indirect efforts to prevent and control not only hepatitis C but all forms of viral hepatitis. As an example, efforts to address HCV transmission in healthcare settings may be performed by infection control programs, whereas efforts to test IDUs may be coordinated by a different Ministry of Health entity, such as the HIV program. This lack of coordination was first recognized in 2009, when the World Hepatitis Alliance surveyed its 193 member countries to determine the state of global viral hepatitis prevention and control (Davis *et al.*, 2010; Newshome, 2013).

This WHO-funded survey revealed that although most countries have hepatitis C policies and goals in place, most existing programs are disconnected; 60% of these countries requested assistance from the WHO in establishing a more coordinated approach to prevention and control of HCV infection. In response, in 2010 the WHO passed World Health Assembly Resolution 63.18, calling for increased viral hepatitis education and improved testing and provision of care and treatment to the 500 million persons infected with hepatitis B virus and HCV worldwide (Sulkowski *et al.*, 2003). The WHO has since formed a gblobal Hepatitis Program to assist member countries in achieving control of these diseases (WHO, 2014).

2.19 TREATMENT OF HCV INFECTION

The treatment of HCV infection is possible through drug therapy and liver transplant. Interferon alpha (IFα) is widely used to treat chronic hepatitis, but high doses (3-6

megaunits, thrice weekly) and long term treatment (6-12 months) are required (McLean *et al.*, 1997; Hu *et al.*, 2001; Casey and Lee, 2013; Kowdley *et al.*, 2013b). It is the only licensed therapy for chronic hepatitis therapy but an efficiency of such treatment is highly variable (Stanczakc, 1999). The interferon injected subcutaneously at a dose of 2 to 3 million units thrice weekly for 6 months, reduces ALT levels to normal in about half of chronic hepatitis C patients but all expect 20 to 25% of these relapse after withdrawal of the drug. Recombinant interferon in combination with Ribavirin is effective for the treatment of chronic hepatitis C in 30% to 50% of patients. However, treatment with interferon alone is effective in 10% to 20% of patients suffering from chronic hepatitis C infection (Zeuzem *et al.*, 2012).

Patients with less severe disease or those treated before cirrhosis set in, appear to have a better chance of long- term response. However, higher doses have no advantage. Ribavirin may have a comparable effect (White and Fenner, 1994; Cummings *et al.*, 2001; Poordad *et al.*, 2013). Ribavirin meekly suppresses HCV (Reichard *et al.*, 1991; Kowdley *et al.*, 2013a), but doses above 1.2g per day are associated with mild haemolytic anaemia. However, combination therapy of Tribarin with interferon seems to induce more sustained results especially in patients without cirrhosis, than interferon alone (Dusheiko, 1995; McHutchison *et al.*, 2013; Kowdley *et al.*, 2013b). In the case of liver transplant, this may be necessary in those with advanced disease, cirrhosis alone or accompanying hepatocellular carcinoma. Nevertheless, infection of the graft through endogenous recurrence usually occurs (McLean *et al.*, 1997; Zeuzem *et al.*, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY SITE

A cohort of 490 purposively recruited consenting participants at Saki, a border town in Oyo State of Nigeria were enrolled and followed up for nine years (2003-2012). This stable community has been selected to represent semi-urban communities in Southwestern Nigeria. The city is also dominated by traders, artisans, transporters and civil servants predominantly by Yorubas. Some government establishments and industries are sited in Saki in Oke Ogun area of the state which is about 180 km NW from Ibadan (the capital of Oyo State and the largest city in West Africa). The total population of Saki is about 300,000 inhabitants (estimated from 2006 census). Saki is a Yoruba ethnic community with residents coexisted with other tribes (Igbo and Hausa) from Eastern and Northern Nigeria and other foreign nationals mainly from neighboring Republic of Benin. The citizens in this community live in modern and mud houses with cement plastering and efficient transportation network.

3.2 STUDY POPULATION

Apparently healthy individuals who consented to participate were enrolled in the study. These were of different ethnic groups, gender and age range of 15 to 65years with median age of 26 years.The demographic features by ages and gender of the participants have been categorized into six groups as shown in table 2. The different occupational groups however with their code and location are shown in table 1.

TABLE 1: OCCUPATION, LOCATION/CODE AND CHARACTERISTICS OF THE STUDY POPULATIONS

OCCUPATION	LOCATION/CODE	SEXUAL CHARACTERISTICS
AUTOMECHANICS	IGBORO OLD (SK/AMA/IO)	MALE DOMINATED
	IGBORO NEW (SK/AMA/IN)	MALE DOMINATED
	AJEGUNLE OLD (SK/AMA/AO)	MALE DOMINATED
	AJEGUNLE NEW (SK/AMA/AN)	MALE DOMINATED
FASHION DESIGNERS	AJEGUNLE (SK/FDA)	FEMALE DOMINATED
	IGBORO (SK/FDI)	FEMALE DOMINATED

The participants included 299 male and 191 female members of two occupational groups, auto-mechanics (n=236) and fashion designers (n=254) located within two large stable communities namely Ajegunle (Old and New) and Igboro (Old and New).

TABLE 2: DEMOGRAPHIC FEATURES OF THE STUDY POPULATION

AGE (YEARS)	MALE	FEMALE	TOTAL
15-24	74	117	191
25-34	118	53	171
35-44	61	17	78
45-54	29	2	31
55-64	15	1	16
≥ 65	2	1	3
TOTAL	299	191	490

3.3 SOCIO-DEMOGRAPHIC DATA

A structured questionnaire was administered to capture information on awareness of HCV infection as well as predisposing factors including sharing of sharp objects, transfusion of blood and blood product, polygamy and multiple sexual partnership. The cohort was continuously provided education on prevention of sexually transmitted diseases and blood borne pathogens during the follow-up period. The data generated from each questionnaire were analysed for Risk Ratio (RR), Confidence Interval (CI), Chi-Square (X^2) and P-value.

3.4 STUDY PERIOD

The subjects were enrolled and followed up for nine years (2003-2012) at baseline and three other points. Samples were collected at baseline and then at intervals of one year each from 2003 to 2005 (First and Second points in 2004 and 2005) respectively. The Third point was carried out in 2012 after an interval of 7 years. These three points are significant in the determination of the incidence of HCV in the study population within the period.

3.5 SAMPLING METHOD

The entry points were chairmen/chairladies, group head and community leaders. We adopted work place to work place (workshop) and meeting hall enumeration for enrollment of the participants in this community into the study. The purpose of the study was explained to the participants. Active surveillance for HCV infection, incidence and risk factors was intensified. Registration and Follow up cards were provided for each

participant. There was daily visitation of participants at their workplace. There were initial counseling, voluntary recruitments of participants and signing of consent forms followed by the administration of structured questionnaires by trained counselors before sample collection. Ethical approval was obtained from UI/UCH and Oyo State IRB committee prior to the commencement of the study.

A cohort of 490 participants at Saki who consented to participate in the study was enrolled and that was followed up for 9 years. Blood samples were collected from each of the participants at baseline, one year, two years and then at the ninth year. The participants included 299 male and 191 female members of two occupational groups Automechanics (n=236) and Fashion Designers (n=254) with age range of 15 to 65 years (Median age=26 years). A total of 490, 475, 438 and 188 subjects participated from baseline to the fourth point respectively. The cohort was provided education on prevention of sexually transmitted diseases and blood borne pathogens during the follow-up period.

Five mililitres (5ml) of blood sample was collected from each person by venepuncture into a sterile EDTA specimen labeled bottle with the subject's laboratory identity number and date of collection. The labeled bottle with specimen was temporarily kept in rack placed in Jablow box containing cold iced pack and taken to Baptist Medical Centre laboratory where the Plasma was extracted from whole blood by centrifugation at 2000rpm for 20minutes. The extracted plasma was separated into three aliquots immediately to prevent haemolysis. For each specimen, three aliquots of plasma and one

for red cells were also stored temporarily in liquid Nitrogen tank (-196^{o}C) and -20^{O}C freezer before transported to Virology Department, University College Hospital (UCH), Ibadan where they were transferred to -86^{o}C and -20^{o}C ultra-low and deep freezers respectively until analyzed. An aliquot of the plasma specimen was used for the detection of Anti-HCV, while the other aliquots and the red cell were stored as reference samples.

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3.6 LABORATORY ANALYSIS

All the samples collected at baseline and thereafter at three other points were tested for HCV infection using commercially available test kits.

3.6.1 DIAGNOSIS OF HCV INFECTION

HCV infection was determined by detection of anti-HCV using anti-HCV HUMAN ELISA test kit DIA. PRO Diagnostic Bioprobes Sri via columella n^{o}31, 20128 Milano-Italy.

3.6.2 PRINCIPLES OF DIA-PRO ANTI-HCV EIA

The use of different HCV recombinant antigens and synthetic peptides from the virus has shown to be effective in identifying a greater number of acute and chronic non-A non B hepatitis than single antigen assays. The primary use of DIA-PRO EIA Test kit is to detect patients infected with HCV early in the course of infection, a tool used for the mandatory screening of blood units to prevent post transfusion hepatitis in blood donors. It is also currently used to follow-up risk individuals and patients under treatment with interferon. The human DIA-PRO Bioprobes ANTI-HCV detection assay is a 3rd generation Enzyme Linked Immunosorbent Assay (ELISA) that detects circulating anti-

HCV IgG and IgM antibodies, which are considered to be indicatives of infection with Hepatitis C virus.

The test which is based on the indirect sandwich principle, utilizes HCV recombinant antigens (NS3, NS5) and synthetic peptides (core, NS4) in the coating of the polysterene microtitre wells. On addition of the specimen, anti-HCV IgG and IgM antibodies, if present bind to the HCV specific antigens derived from "core" and the "non structural" regions encoding for conservative and immunodominant antigenic determinants (core, NS3, NS4, and NS5), which are coated on the surface of the microtitre wells. After the incubation, unbound specimen components are removed by washing and then antihuman IgG or IgM HRP conjugate is added which binds to the specific human anti HCV IgG or IgM antibody labeled with peroxidase (HRP) at the surface, and forms a sandwich complex. Unbound components are removed by washing and substrate comprising H_20_2 and TMB chromogen is added. Reaction of the bound enzyme on the substrate leads to development of a blue colour. On addition of stop solution (sulphuric acid), the colour changes from blue to yellow and the absorbance is read at 450nm. The intensity of the colour is correlated to the level of anti-HCV antibodies in the specimen

3.6.3 REAGENTS SOURCE FOR HCV

Reagents and contents per kit as supplied by manufacturers are as follows:

(a) 12 Microtitre strips

8 well strips, coated with synthetic and recombinant HCV antigens.

(b) 0.5ml Negative control

Non reactive for anti-HCV human serum or plasma potentially infectious (preserved with sodium axide).

(c) 0.5ml Positive control

Anti-HCV reactive human serum or plasma (inactivated), potentially infectious (preserved with sodium axide).

(d) 50ml Sample diluent

Contains proteic buffered solution and Tween-20

(e) 60ml Wash buffer Concentrate (20x)

Constitute in sterilized distilled water contains phosphate buffered saline Tween-20 in presence of kathon GC as preservative.

(f) 2ml ANTI-human-IgG-HRP conjugate

Contains Horse-raddish peroxidase conjugated polyclonal antibody to human IgG and IgM antibodies (bovine serum albulmin).

(g) Enzyme Conjugate diluent (20x)

Tris-buffered solution of bovine serum albumin (0.3mg/ml), Gentamycin sulphate and 0.1% Kathon GC as preservative.

(h) 10ml Substrate reagent A

Hydrogen peroxide (H_2O_2)

Buffered solution (citric acid buffer)

(i) 10ml Substrate reagent B

3.3, 5.5, Tetramethylbenzidine (TMB)

DM50

(j) 10ml Stop Solution

0.3M sulphuric acid

(k) 1 Strip holder

3.6.4 REAGENT PREPARATION

All reagents were brought to room temperature including the sealed microtitre plates before use and the various reagents reconstituted as follows:

a. Working wash solution (20x)

This was separated by diluting the concentrated wash solution 1 in 20 with clean sterile distilled water e.g. 50ml concentrated wash solution + 950ml distilled water.

b. Working conjugate solution.

The conjugate was diluted 1 in 20 with conjugate diluent e.g. 0.5ml + 9.5ml of the diluent for 96 well microtitre plate.

c. Working substrate solution

Equal volumes of substrate with chromogen i.e 5ml of each were mixed in a clean dry trough 5 minutes prior to use away from intense light.

3.6.5 Anti-HCV TEST PROCEDURE

Before the Anti-HCV test was carried out, the reagents, controls, and plasma were placed on the working bench to stabilize at room temperature. The controls specimen distribution and identification plan were properly arranged and recorded on the log sheet. The microplate frame and well strips were removed from their protective foil bag. Two hundred microlitres (200ul) of sample diluent was dispensed into each of the wells except well A1 (blank), E12 and F12(negative control), G12 and H12 (positive control). Two

hundred microlitres of Negative controls were added into each of wells E12 and F12 while 200ul of Positive controls were added into each of wells G12 and H12. Ten microlitres of sample was then dispensed in each properly identified well except wells labeled A12, E12, F12, G12, and H12 starting from B1. The microtitre plate was gently tapped for 15 seconds to ensure proper mix of the sample and diluent. The plate was sealed with adhesive film and incubated at 37^{o} C for 45 minutes.

After the incubation, microtitre plate was removed from the incubator, and the adhesive film removed. The contents of the wells were all aspirated into a liquid waste trough containing 5% sodium hypochlorite. About 300ul wash solution was added to each well and allowed to soak for 10 seconds and then aspirated into the liquid waste container. The process of washing was repeated five times. The remaining liquid in the wells was removed by tapping the plate upside down on a paper soaker or tissue paper. One hundred microlitres of working conjugate solution was then dispensed into each well except A1. The plate was sealed with a new adhesive film and incubated for the second time at 37^{0}C for another 45 minutes. The microtitre plate was then removed from the incubator and washed six times as described above. One hundred microlitres (100ul) of working substrate was dispensed into all the wells except well A1 and the plate was incubated in the dark at room temperature for 15minutes. One hundred microlitres (100ul) of stop solution was added into each well, then the microtitre plate was tapped gently to mix the contents and the absorbance read at 450nm wavelength in an ELISA microtitre plate reader. The readings for the blank, controls and tests were recorded.

3.6.6 CALCULATION OF CUT OFF VALUE AND INTERPRETATION OF HCV RESULTS

Validation of the test: The colour in A_1 should be colourless and have an OD (450nm) value of less than 0.100.

The cut off mean absorbance at 450nm (A450) for each batch of test was calculated as follows:

a. Mean Negative Control (MNC) [A450 (NC1) + A450 (NC2) ÷ 2

b. Cut off value CoV) =

Cut off value (Co) = MNC + 0.250

The cut off index (S/Co)

S/Co = A sample/Co

The interpretation of the result which is based on S/Co ratio (value) which is calculated by the Sample OD value (S) divided by Cut off value (Co) is shown in table 3.

TABLE 3: INTERPRETATION OF HCV RESULTS

Sample OD value/Cut Off value (S/Co)	Interpretation
<1.0	Negative
1.0 – 1.2	Equivocal
>1.2	Positive

3.7 STATISTICAL ANALYSIS

Data were analysed using descriptive statistics and ANOVA at p=0.05. Incidence of infection was reported as number of HCV cases/1000 person years. Frequencies of variables were calculated for p-value with one-degree of freedom at 5% value using Chi Square ($\chi 2$) test for comparison.

3.7.1 Formular for calculating Incidence Rate:

Incidence rate = Total number of new cases of a disease per unit of time/

Total population at risk per 1000 person years

(Time in years).

3.7.2 Formular for calculating Risk Ratio or Relative Risk:

Risk Ratio is:

(Cumulative incidence in the exposed/(Cumulative incidence in the unexposed)

Calculating Risk Ratio or Relative Risk:

Relative Risk (Risk Ratio) can be calculated from a 2x2 table using the following formular: (A/(A+B)/(C/(C+D))

TABLE 4: CALCULATION OF RISK RATIO OR RELATIVE RISK

	Disease Yes	Disease No	Total
Exposed Yes	A	B	A+B
Exposed No	C	D	C+D
Total	A+C	B+D	A+B+C+D

CHAPTER FOUR

RESULTS

4.1: OVERALL PREVALENCE OF HCV INFECTION BY GENDER AND YEAR OF COLLECTION IN OYO STATE

During the four stages of this study (2003 to 2012), a cohort of 490 (Male=299; Female=191) consented apparently healthy individuals aged 15 years and above was followed up from two large occupational groups for Incidence of Hepatitis C Virus through the baseline and subsequently at three other points in Saki, a semi-urban community in Southwestern Nigeria. Of the 490 sera collected at baseline, a total of 41 (8.4%) were positive for HCV antibodies at baseline. Subsequently, out of 475, 438 and 188 sera collected at first, second and third point, a total of 40(8.4%), 33(7.5%) and 23(12.3%) were also positive for anti-HCV respectively (Table 5). By gender at baseline and at first point, HCV antibody rate was respectively higher in the female (12.0% and 11.3%) than male (6.0% and 6.6%) while at second and third points, the male (8.1% and 15.4%) Anti-HCV prevalence was higher than their female (6.5% and 5.2%) counterparts respectively (Tables 5 and 6).

A total of 236 automechanics and 254 fashion designers were recruited to voluntarily participate in the study at baseline giving rise to a cohort of 490 participants. At first point, 9 automechanics and 6 fashion designers dropped out of the group with attrition rates of 1.8% and 1.2% respectively at an interval of one year. Also, 26 Automechanics and 24 Fashion Designers declined from participating in the study at second point with attrition rates of 5.3% and 4.9% respectively after an interval of two years. However, at

third point after an interval of seven years, 132 Automechanics and 170 Fashion Designers dropped out of the study with attrition rates of 26.9% and 34.7% respectively. Total attrition rates of 3.1%, 10.2% and 61.6% were obtained from baseline to third point across the study population. The rates of Attrition were higher among Automechanics than Fashion Designers at first and second points. However at third point, the rate of Atrition among Fashion Designers (34.7%) was higher than Automechanics (26.9%) (Table 7).

4.2: INCIDENCE OF HCV INFECTION BY GENDER IN 2004, 2005 AND 2012 IN SAKI OYO STATE

The incidence of HCV at first point was 9.2 per 1000 person years. However, it increased to 24.7 per 1000 person years after a year at second point but declined thereafter to 11.3 per 1000 person years at third point after seven years interval of sample collection (Table 6). Gender distribution of HCV incidence showed that the incidence at first, second and third points was higher in male (11.1, 32.3 and 14.3 per 1000 person years) than female (6.1, 12.7 and 5.2 per 1000 person years) participants respectively. Also, the number of seroconversions (new cases of disease) was respectively higher in male (3, 8 and 11) than female (1, 2 and 2) throughout the three points of contact (Table 7). Overall incidence for the study was 27.8 per 1000 person years. These differences were however not significant (p=0.904).

TABLE 5: PREVALENCE OF HCV INFECTION BY GENDER AND YEAR OF COLLECTION IN SAKI

GENDER	BASELINE 2003		FIRST POINT 2004		SECOND POINT 2005		THIRD POINT 2012	
	NT	N(%)P	NT	N(%)P	NT	N(%)P	NT	N(%)P
MALE	299	18(6.0)	289	19(6.6)	270	22(8.1)	130	20(15.4)
FEMALE	191	23(12.0)	186	21(11.3)	168	11(6.5)	58	3(5.2)
TOTAL	490	41(8.4)	475	40(8.4)	438	33(7.5)	188	23(12.3)

TABLE 6: INCIDENCE OF HCV INFECTION BY GENDER IN 2004, 2005 AND 2012 IN SAKI OYO STATE

GENDER	FIRST POINT 2004			SECOND POINT 2005			THIRD POINT 2012		
	PERSONS AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years
MALE	270	3	11.1	248	8	32.3	110	11	14.3
FEMALE	175	1	5.7	157	2	12.7	55	2	5.2
TOTAL	445	4	9.0	405	10	24.7	165	13	11.3

Overall HCV Incidence for the study: 27.8 per 1000 person years

TABLE 7: ATRITION RATES ACROSS THE THREE POINTS OF THE STUDY

OCCUPATIONAL GROUP	BASELINE 2003	ATRITION/ RATE (%)	FIRST POINT 2004	ATRITION/ RATE (%)	SECOND POINT 2005	ATRITION/ RATE (%)	THIRD POINT 2012
AUTOMECHANIC	236	9(1.8)	227	26(5.3)	208	132(26.9)	104
FASHION DESIGNER	254	6(1.2)	248	24(4.9)	230	170(34.7)	84
TOTAL	490	15(3.1)	475	50(10.2)	438	302(61.6)	188

4.3 OVERALL AGE DISTRIBUTION OF HCV INFECTION IN SAKI

Table 8 shows the age distribution pattern of HCV infection from baseline to third point of follow up. At baseline, highest prevalence of HCV infection was observed in age group 15-24 years (11.0%) closely followed by age group 25-34 years (10.5%) while lowest HCV rate was observed in age group 35-44 years (1.3%). Also at first point, highest prevalence of HCV infection was observed in age range 15-34 years (10.3%) while lowest rate of Anti-HCV was detected in age group 35-44 years (2.7%). Furthermore, at second point, highest prevalence of HCV infection was observed in age group 45-54 years (10.3%) while lowest prevalence of HCV infection was observed in age group 35-44 years (2.9%). However at third point, highest prevalence of HCV infection was observed in age group 45-54 years (28.6%) while lowest prevalence of HCV infection was observed in age group 15-24 years (9.4%). None of the individuals in the age group 55 years and above from baseline to second point had detectable Anti-HCV while at third point, HCV infection was not detected in age group 65 years and above in Saki.

4.4 OVERALL AGE DISTRIBUTION OF HCV INCIDENCE IN SAKI

Table 9 shows the overall age distribution pattern of incidence of HCV across the three points of follow up study. A total of 27 new cases of HCV infection were identified during the period. At first point, highest incidence (34.5 per 1000 person years) of HCV infection was observed in age range 45-54 years while lowest incidence (11.9 per 1000 person years) of HCV was detected in age group 15-24 years.

TABLE 8: OVERALL AGE DISTRIBUTION OF HCV INFECTION IN SAKI

AGE GROUP (YRS)	BASELINE 2003		FIRST POINT 2004		SECOND POINT 2005		THIRD POINT 2012	
	NT	N(%)P	NT	N(%)P	NT	N(%)P	NT	N(%)P
15-24	191	21(11.0)	185	17(9.2)	169	14(8.3)	65	5(7.7)
25-34	171	18(10.5)	165	17(10.3)	152	14(9.2)	71	7(9.9)
35-44	78	1(1.3)	75	4(5.3)	69	2(2.9)	29	5(17.2)
45-54	31	1(3.2)	31	2(6.5)	29	3(10.3)	15	5(33.3)
55-64	16	0(0.0)	16	0(0.0)	16	0(0.0)	7	1(14.3)
≥ 65	3	0(0.0)	3	0(0.0)	3	0(0.0)	1	0(0.0)
TOTAL	490	41(8.4)	475	40(8.4)	438	33(7.5)	188	23(12.2)

TABLE 9: OVERALL AGE DISTRIBUTION OF HCV INCIDENCE IN SAKI

AGE GROUP (YRS)	FIRST POINT 2004			SECOND POINT 2005			THIRD POINT 2012		
	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years
15-24	168	2	11.9	155	3	19.4	60	3	7.1
25-34	148	0	0	138	5	36.2	64	3	6.7
35-44	71	1	14.1	67	1	14.9	24	3	17.9
45-54	29	1	34.5	26	1	38.5	10	2	28.6
55-64	16	0	0	16	0	0	6	2	47.6
≥65	3	0	0	3	0	0	1	0	0
TOTAL	435	4	9	405	10	25	165	13	11.3

Overall HCV Incidence for the study: 27.8 per 1000 person years

Also, at second point, highest incidence (38.5 per 1000 person years) of HCV infection was observed in age range 45-54 years while lowest incidence (14.9 per 100 person years) of HCV was detected in age group 35-44 years. However at third point, highest incidence (47.6 per 1000 person years) of HCV infection was observed in age range 55-64 years while lowest incidence (6.7 per 1000 person years) of HCV was observed in age group 25-34 years. Incidence of HCV was not observed in age range 55 years and above at first and second points while at third point, HCV incidence was not observed in age group ≥65 years in Saki.

4.5: OVERALL AGE DISTRIBUTION OF HCV INFECTION AMONG MALE PARTICIPANTS IN SAKI

A total of 299 sera from male participants were tested for HCV antibodies of which 18(6.0%) were positive for HCV infection at baseline. Highest anti-HCV rate (9.3%) was observed in age group 25-34 years while lowest prevalence (1.0%) of HCV infection was detected in age group 35-44 years. At first point, out of a total of 289 sera from male participants tested for HCV antibodies 21(7.3%) were positive. Highest anti-HCV rate (8.8%) was observed in age group 25-34 years while lowest prevalence (6.8%) of HCV infection was detected in age group 35-44 years. However at second and third points, highest anti-HCV rates (11.1% and 30.8%) were observed in age groups 45-54 years while lowest prevalence (3.8% and 11.3%) of HCV infection were detected in age groups 35-44 and 25-34 years respectively. In addition, out of 270 and 130 male participants tested for HCV antibodies at second and third points respectively, 22(8.1%) and 20(15.4%) were positive for Anti-HCV in Saki. HCV rates in male participants declined from baseline to third point (Table 10).

4.6: OVERALL AGE DISTRIBUTION OF HCV INFECTION AMONG FEMALE PARTICIPANTS IN SAKI

A total of 191 sera from female participants were tested for HCV antibodies at baseline of which 23(12.0%) were positive. Highest anti-HCV rate of 13.7% was observed in age group 15-24 years while lowest prevalence (13.2%) of HCV infection was detected in age group 25-34 years. At first point, out of a total of 186 sera from female participants tested for HCV antibodies, 21(11.3%) were positive. Highest prevalence (8.8%) of Anti-HCV

TABLE 10: AGE DISTRIBUTION OF HCV INFECTION AMONG MALE PARTICIPANTS IN SAKI

AGE GROUP (YRS)	BASELINE 2003		FIRST POINT 2004		SECOND POINT 2005		THIRD POINT 2012	
	NT	N(%)P	NT	N(%)P	NT	N(%)P	NT	N(%)P
15-24	74	5(6.8)	71	3(4.2)	69	7(10.1)	30	4(13.3)
25-34	118	11(9.3)	113	10(8.8)	105	10(9.5)	53	6(11.3)
35-44	61	1(1.6)	59	4(6.8)	52	2(3.8)	26	5(19.2)
45-54	29	1(3.4)	29	2(6.9)	27	3(11.1)	13	4(30.8)
55-64	15	0(0.0)	15	0(0.0)	15	0(0.0)	7	1(14.3)
≥ 65	2	0(0.0)	2	0(0.0)	2	0(0.0)	1	0(0.0)
TOTAL	299	18(6.0)	289	19(6.6)	270	22(8.1)	130	20(15.4)

was observed in age group 25-34 years while lowest prevalence (12.3%) of HCV infection was detected in age group 15-24 years. Similarly at second and third points, highest anti-HCV rates (8.5% and 50.0%) were detected in age groups 25-34 and 45-54 years while lowest rates (7.0% and 2.9%) of HCV infection were detected in age group 15-24 years respectively. Furthermore, out of 168 and 58 female participants tested for HCV antibodies at second and third points respectively, 11(6.5%) and 3(5.2%) were positive for Anti-HCV in Saki (Table 9). HCV rates in female participants declined from baseline to third point. Overall, HCV rates in female (12.0% and 11.3%) were higher than in male (6.0% and 7.3%) counterparts at first and second points while that of the male rates (8.1% and 15.4%) were higher than female (6.5% and 5.2%) participants at second and third points respectively. These differences by gender of HCV antibodies were however not significant (p= 0.395) (Tables 10 and 11).

TABLE 11: AGE DISTRIBUTION OF HCV INFECTION AMONG FEMALE PARTICIPANTS IN SAKI

AGE GROUP (YRS)	BASELINE 2003		FIRST POINT 2004		SECOND POINT 2005		THIRD POINT 2012	
	NT	N(%)P	NT	N(%)P	NT	N(%)P	NT	N(%)P
15-24	117	16(13.7)	114	14(12.3)	100	7(7.0)	35	1(2.9)
25-34	53	7(13.2)	52	7(13.5)	47	4(8.5)	18	1(5.6)
35-44	17	0(0.0)	16	0(0.0)	17	0(0.0)	3	0(0.0)
45-54	2	0(0.0)	2	0(0.0)	2	0(0.0)	2	1(50.0)
55-64	1	0(0.0)	1	0(0.0)	1	0(0.0)	0	0(0.0)
≥ 65	1	0(0.0)	1	0(0.0)	1	0(0.0)	0	0(0.0)
TOTAL	191	23(12.0)	186	21(11.3)	168	11(6.5)	58	3(5.2)

4.7: AGE DISTRIBUTION OF HCV INCIDENCE AMONG MALE PARTICIPANTS IN SAKI

A total of 270, 248 and 110 male participants were at risk at first, second and third points respectively among the consented participants during the nine years follow up study for incidence of HCV in Saki. The numbers of new cases of HCV infection were 3, 8 and 11 at first, second and third points with HCV incidence of 11.1, 32.3 and 14.3 per 1000 person years respectively (Table 10). At first point, highest incidence (37 per 1000 person years) of HCV in male participants was observed in age group 45-54 years while lowest incidence (14.7 per 1000 person years) of HCV was observed in age group 15-24 years. Highest incidence (42.1 per 1000 person years) of HCV in male participants was observed in age groups 25-34 and 45-54 years while lowest incidence (20 per 1000 person years) of HCV was observed in age group 35-44 years at second point. On the other hand among male population, highest incidence (47.6 per 1000 person years) of HCV was observed in age group 55-64 years while lowest incidence (6.1 per 1000 person years) of HCV was observed in age group 25-34 years at third point. However, there was no incidence of HCV at first point among the male participants at risk of HCV infection (Table 12).

4.8: AGE DISTRIBUTION OF INCIDENCE OF HCV AMONG FEMALE PARTICIPANTS IN SAKI

Furthermore, a total of 165, 157 and 55 female participants were at risk HCV infection at first, second and third points respectively among the consented participants during the nine years follow up study for HCV incidence in Saki. However, unlike their male

counterparts, one person was seroconverted for HCV infection at first point (Tables 11 and 12) while numbers of new cases of HCV infection were 2 each at second and third points. A total of HCV incidence of 6.1, 12.7 and 5.2 per 1000 person years respectively were observed at first, second and third points (Table 13). Incidence (10 per 1000 person years) of HCV in female was observed in age group 15-24 years at first point. At second point, highest incidence (23.3 per 1000 person years) of HCV was observed in age group 25-34 years while lowest incidence (10.8 per 1000 person years) of HCV was observed in age group 15-24 years. On the other hand among female population at risk of HCV infection at third point of this study, highest incidence (142.9 per 1000 person years) of HCV was observed in age group 45-54 years while lowest incidence (8.4 per 1000 person years) of HCV was observed in age group 25-34 years (Table 13). Overall gender distribution of incidence of HCV in Saki showed that, HCV incidence was higher in male (11.1, 32.3 and 14.3 per 1000 person years) than female (6.1, 12.7 and 5.2 per 1000 person years) participants from first to third point respectively (Tables 12 and 13). Overall incidence of HCV in both sexes increased from first to second point but declined considerably at third point after an interval of seven years.

TABLE 12: AGE DISTRIBUTION OF HCV INCIDENCE AMONG MALE PARTICIPANTS IN SAKI

AGE GROUP (YRS)	FIRST POINT 2004			SECOND POINT 2005			THIRD POINT 2012		
	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years
15-24	68	1	14.7	62	2	32.3	26	3	16.5
25-34	103	0	0	95	4	42.1	47	2	6.1
35-44	55	1	18.2	50	1	20	21	3	20.4
45-54	27	1	37	24	1	41.7	9	1	15.9
55-64	15	0	0	15	0	0	6	2	47.6
≥65	2	0	0	2	0	0	1	0	0
TOTAL	270	3	11.1	248	8	32.3	110	11	14.3

Overall HCV Incidence for Male in Saki: 21.2 per 1000 person years

TABLE 13: AGE DISTRIBUTION OF HCV INCIDENCE AMONG FEMALE PARTICIPANTS IN SAKI

AGE GROUP (YRS)	FIRST POINT 2004			SECOND POINT 2005			THIRD POINT 2012		
	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years
15-24	100	1	10	93	1	10.8	34	0	0
25-34	45	0	0	43	1	23.3	17	1	8.4
35-44	16	0	0	17	0	0	3	0	0
45-54	2	0	0	2	0	0	1	1	142.9
55-64	1	0	0	1	0	0	0	0	0
≥65	1	0	0	1	0	0	0	0	0
TOTAL	165	1	6.1	157	2	12.7	55	2	5.2

Overall HCV Incidence for Female in Saki: 14.5 per 1000 person years

4.9 COMPARISON OF HCV PREVALENCE BY GENDER AT POINTS OF CONTACT IN SAKI

Comparism of HCV infection by gender in Saki shows that the rates of infection increased from baseline to third point among the male participants while in female counterparts, it decreased from the baseline across the three other points of sample collection. At baseline and at first point, HCV infection was higher in female (12.0% and 11.3%) than male (6.8% and 6.0%) participants. However at second and third points of this follow up study, prevalence of HCV infection was higher in male (8.1% and 15.7%) than their female (6.5% and 4.9%) counterparts (Fig. 4).

4.10: COMPARISON OF HCV INCIDENCE BY GENDER AT POINTS OF CONTACT IN SAKI

In Figure 5, incidence of HCV by gender was higher in male (11.1 per 1000 person years) than their female (6.1 per 1000 person years) counterparts at first point. Also at second and third points, incidence of HCV among the male (32.3 and 14.3 per 1000 person years) were higher than in female (12.7 and 5.2 per 1000 person years) respectively. Altogether in this study, incidence of HCV increased along the points in both sexes at first and second points and declined at third point but that of the male was higher than their female counterparts at each stage along the three points of the follow up (Fig. 5).

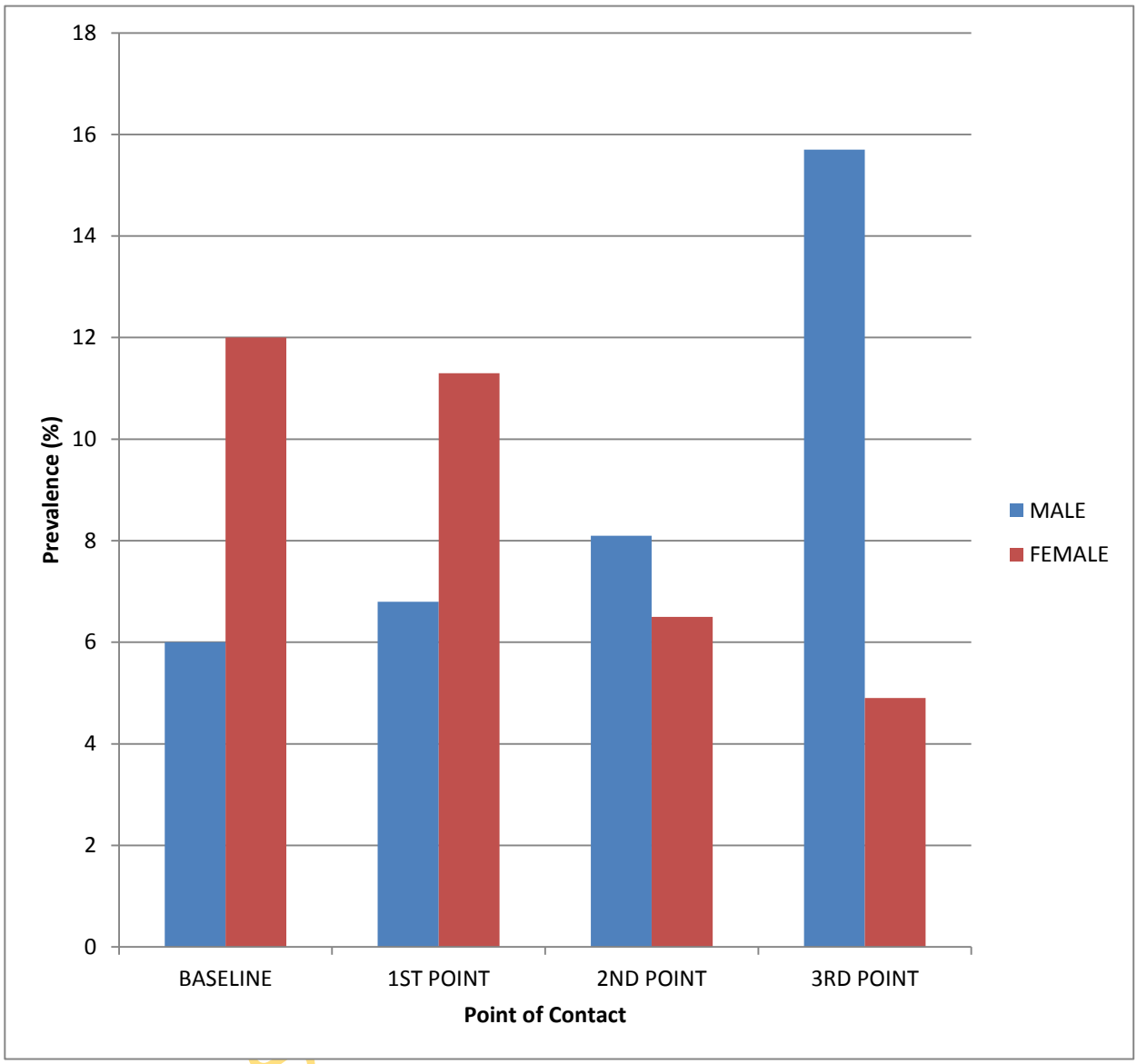

FIGURE 4: COMPARISON OF HCV INFECTION BY GENDER IN SAKI

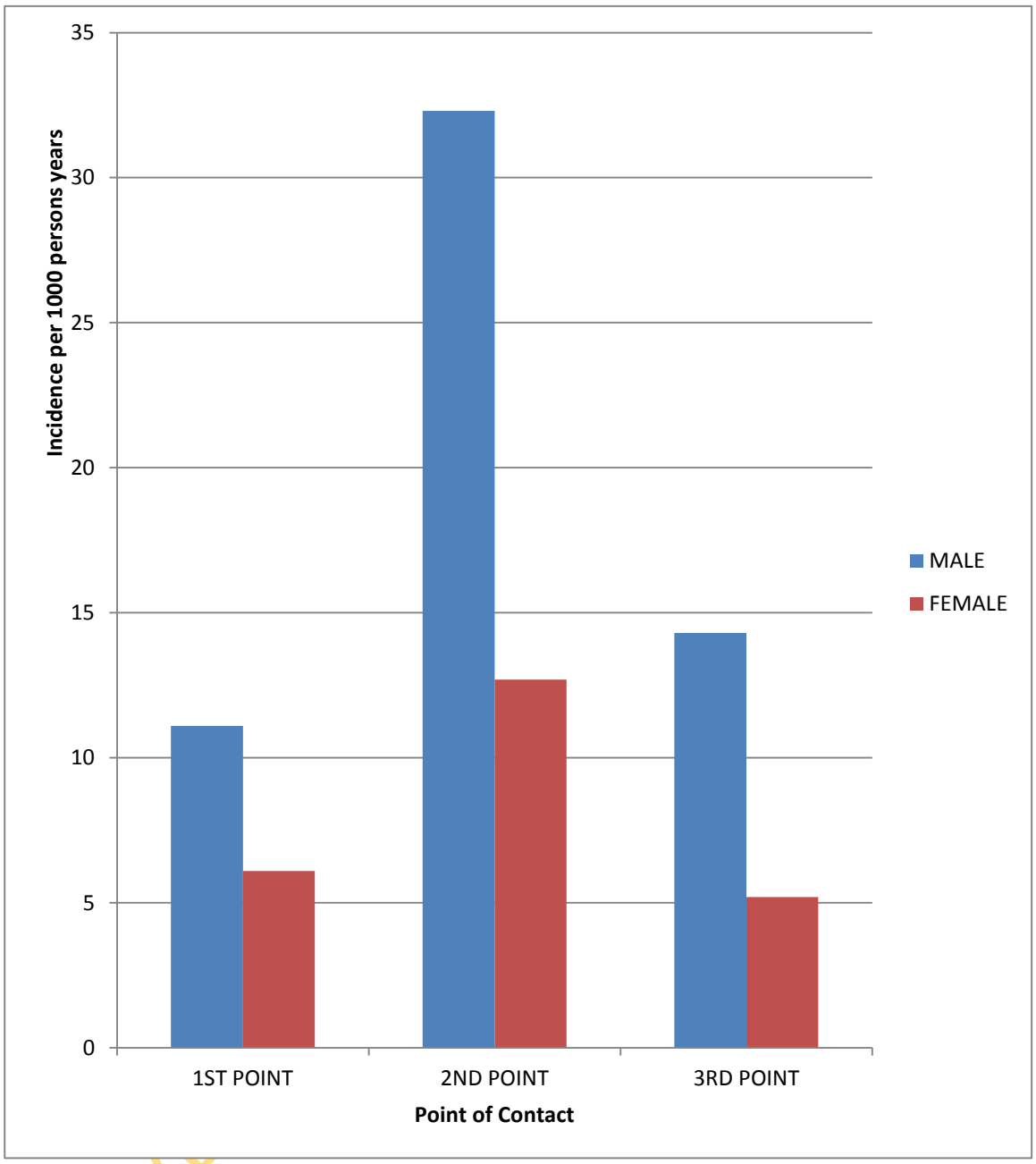

FIGURE 5: COMPARISON OF INCIDENCE OF HCV BY GENDER IN SAKI

4.11 DISTRIBUTION OF HCV INFECTION BY AGE AND GENDER AT POINTS OF CONTACT IN SAKI

Figure 6 shows the overall age and gender distribution of HCV infection. Highest prevalence of 9.3% for HCV antibodies was observed in age group 25-34 years in male while lowest prevalence of 3.4% was observed in age group 45-54 years. Also, highest prevalence of 13.7% for HCV antibodies was observed in age group 15-24 years in female while lowest prevalence of 13.2% was observed in age group 25-34 years. At first point, highest rate of 8.8% for HCV antibodies was observed in age group 25-34 years in male while lowest prevalence of 3.4% was observed in age group 35-44 years. Among the female participants, highest prevalence of 13.5% for HCV antibodies was observed in age group 25-34 years in female while lowest prevalence of 12.3% was observed in age group 15-24 years. Furthermore, at second point highest rate of 11.1% for HCV antibodies was observed in age group 45-54 years in male while lowest prevalence of 3.8% was observed in age group 35-44 years. Among the female counterparts, highest rate of 8.5% for HCV antibodies was observed in age group 25-34 years while lowest prevalence of 7.0% was observed in age group 15-24 years. Also at third point, highest rate of 25.0% for HCV antibodies was observed in age group 45-54 years in male participants while lowest prevalence of 11.5% was observed in age group 35-44 years. Among the female counterparts, highest prevalence of 50.0% for HCV antibodies was observed in age group 45-54 years in female while lowest prevalence of 2.9% was observed in age group 15-24 years.

4.12 DISTRIBUTION OF INCIDENCE OF HCV BY AGE AND GENDER AT POINTS OF CONTACT IN SAKI

Figure 7 shows age and gender distribution of incidence of HCV in Saki. In male at first point, highest incidence (37 per 1000 person years) of HCV was observed in age group 45-54 years while lowest incidence (14.7 per 1000 person years) of HCV was observed in age group 15-24 years. However in female at first point, incidence (10 per 1000 person years) of HCV was only observed in age group 15-24 years. At second point, highest incidence (42.1 per 1000 person years) of HCV in male was observed in age groups 25-34 years while lowest incidence (20 per 1000 person years) of HCV was observed in age group 35-44 years. Also in female at second point, highest incidence (23.3 per 1000 person years) of HCV was observed in age group 25-34 years while lowest incidence (10.8 per 1000 person years) of HCV was detected in age group 15-24 years. On the other hand at third point, highest incidence (47.6 per 1000 person years) of HCV in male was observed in age group 55-64 years while lowest incidence (6.1 per 1000 person years) of HCV was detected in age group 25-34 years. Also in female population during the third point of this study, highest incidence (142.9 per 1000 persons years) of HCV was observed in age group 45-54 years while lowest incidence (8.4 per 1000 person years) of HCV was observed in age group 25-34 years (Fig 5; Table 13). By gender distribution, the incidence (11.1 per 1000 person years) of HCV observed in male was higher than (6.1 per 1000 person years) for female counterparts at first point. Also at second and third points respectively the incidence (32.3 and 14.3 per 1000 persons years) of HCV in male were higher than that of their female (12.7 and 5.2 per 1000 person years) counterparts (Tables 12 and 13).

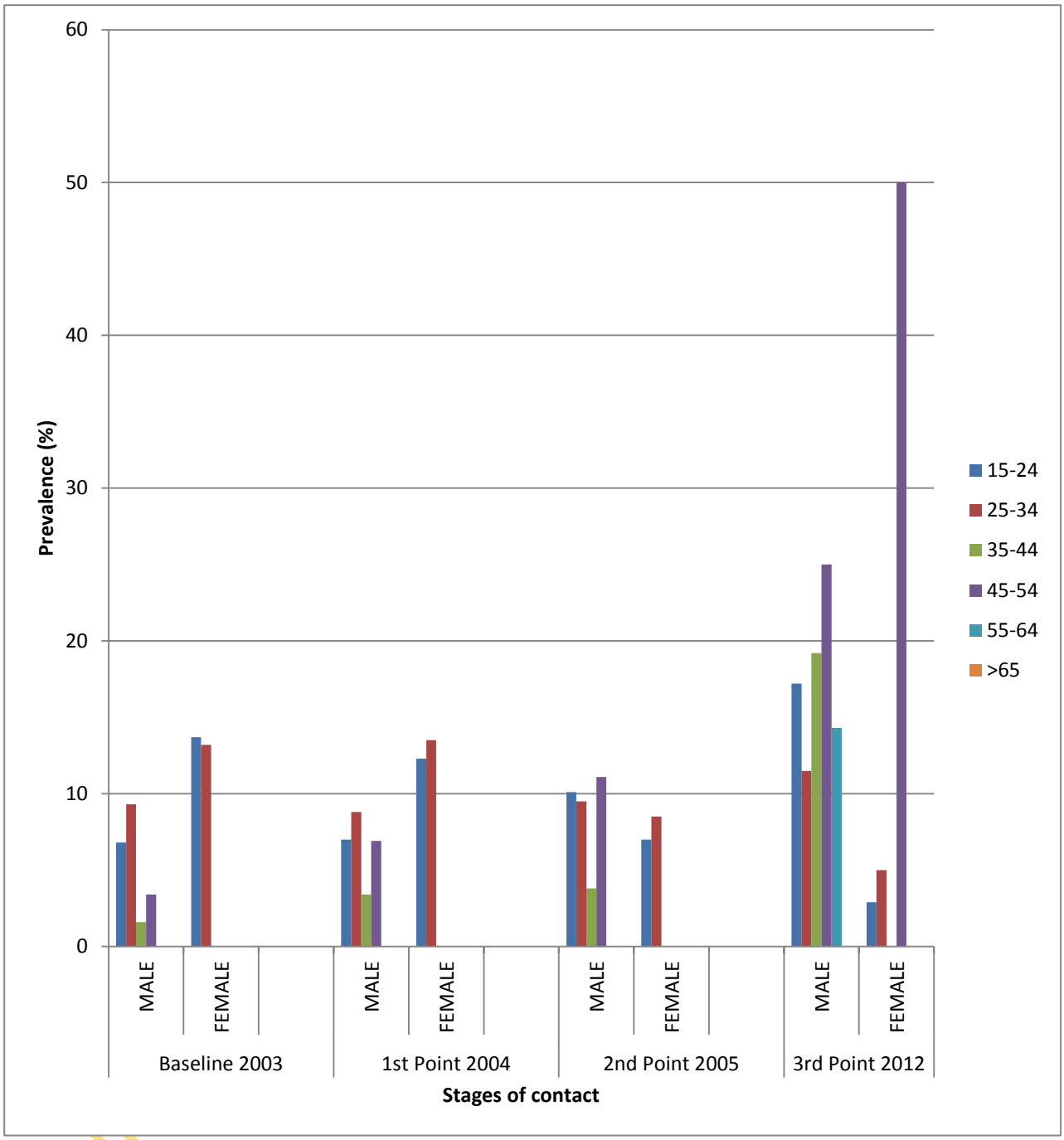

FIG 6: OVERALL AGE AND GENDER DISTRIBUTION OF HCV INFECTION IN SAKI

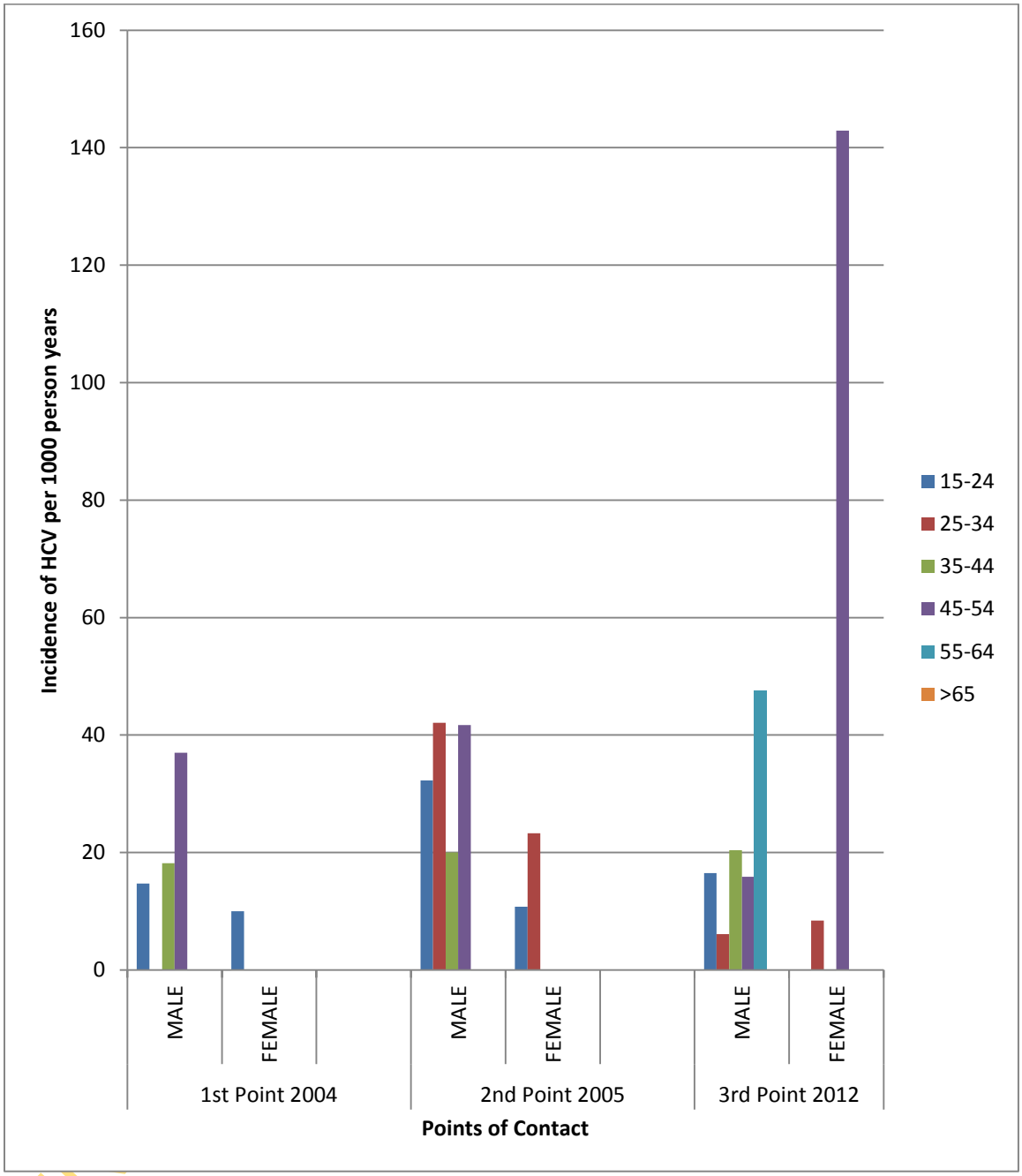

FIGURE 7: INCIDENCE OF HCV BY AGE AND GENDER AT POINT OF CONTACT IN SAKI

4.13: AGE DISTRIBUTION OF HCV INFECTION AMONG AUTOMECHANICS IN SAKI

Table 14 shows the age distribution pattern of HCV infection among Automechanics from baseline to third point of the follow up study in Saki. A total of 236, 227, 208 and 104 consented individuals were tested for Anti-HCV of which 1(4.2%), 13(5.7%), 12(5.8%) and 14(13.5%) were respectively positive from baseline to third point. Age distribution shows that highest prevalence (7.4%) of HCV infection was observed in age group 25-34 years while lowest rate (1.9%) was detected in age group 35-44 years at baseline. Also at first point, highest prevalence (9.5%) of HCV infection was observed in age group 45-54 years while lowest rate (3.9%) was detected in age group 35-44 years. Furthermore, highest Antibodies to HCV prevalence (15.8%) of HCV infection was observed in age group 45-54 years while lowest rate (4.4%) was detected in age group 35-44 years at second point Similarly, highest prevalence (30.0%) of HCV infection was observed in age group 45-54 years while lowest rate (5.1%) was detected in age group 25-34 years at third point. However, antibodies to HCV were not detected in age range 55 years and above among Automechanics from baseline to second point while at third point, HCV antibodies were not detected in age group ≥65 years and above (Table 14).

4.14: AGE DISTRIBUTION OF HCV INFECTION AMONG FASHION DESIGNERS IN SAKI

Age distribution pattern of HCV infection among Fashion Designers from baseline to third point of the follow up study in Saki shows that total of 254, 248, 230 and 84

consented apparently healthy individuals were tested for Antibodies to HCV of which 30(11.8%), 27(10.9%), 21(9.1%) and 9 (10.7%) were respectively positive from baseline to third point (Table 14). At baseline, highest prevalence (14.5%) of HCV infection was observed in age group 25-34 years while lowest rate (14.2%) was detected in age group 15-24 years. Similarly, highest prevalence (14.9%) of HCV infection was observed in age group 25-34 years while lowest rate (12.2%) was detected in age group 25-34 years at first point.

Furthermore, highest Anibodies to HCV prevalence (14.8%) of HCV infection was observed in age group 25-34 years while lowest rate (9.4%) was detected in age group 15-34 years at second point. Finally, highest prevalence (20.0%) of HCV infection was observed in age group 45-54 years while lowest rate (7.7%) was detected in age group 15-24 years at third point. However, antibodies to HCV were not detectable in age range 35 years and above among Fashion Designers from baseline to second point while at third point, HCV antibodies were not detected in age range 55 years and above (Table 15).

TABLE 14: AGE DISTRIBUTION OF HCV INFECTION AMONG AUTOMECHANICS IN SAKI

AGE GROUP (YRS)	BASELINE 2003		FIRST POINT 2004		SECOND POINT 2005		THIRD POINT 2012	
	NT	N(%)P	NT	N(%)P	NT	N(%)P	NT	N(%)P
15-24	57	2(3.5)	54	3(5.6)	52	3(5.8)	26	3(11.5)
25-34	95	7(7.4)	92	6(6.5)	83	4(4.8)	39	2(5.1)
35-44	54	1(1.9)	51	2(3.9)	45	2(4.4)	24	5(20.8)
45-54	21	1(4.8)	21	2(9.5)	19	3(15.8)	10	3(30.0)
55-64	8	0(0.0)	8	0(0.0)	8	0(0.0)	4	1(25.0)
≥ 65	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)
TOTAL	236	1(4.2)	227	13(5.7)	208	12(5.8)	104	14(13.5)

TABLE 15: OVERALL AGE DISTRIBUTION OF HCV INFECTION AMONG FASHION DESIGNERS IN SAKI

AGE GROUP (YRS)	BASELINE 2003		FIRST POINT 2004		SECOND POINT 2005		THIRD POINT 2012	
	NT	N(%)P	NT	N(%)P	NT	N(%)P	NT	N(%)P
15-24	134	19(14.2)	131	16(12.2)	117	11(9.4)	39	3(7.7)
25-34	76	11(14.5)	74	11(14.9)	70	10(14.8)	32	5(15.6)
35-44	24	0(0.0)	23	0(0.0)	23	0(0.0)	5	0(0.0)
45-54	10	0(0.0)	10	0(0.0)	10	0(0.0)	5	1(20.0)
55-64	8	0(0.0)	8	0(0.0)	8	0(0.0)	3	0(0.0)
≥ 65	2	0(0.0)	2	0(0.0)	2	0(0.0)	0	0(0.0)
TOTAL	254	30(11.8)	248	27(10.9)	230	21(9.1)	84	9(10.7)

4.15: AGE AND GENDER DISTRIBUTION OF HCV INFECTION AMONG FASHION DESIGNERS IN SAKI

Age and gender distribution of HCV infection among Fashion Designers from baseline to third point in this study in Saki shows that a total of 63 males and 191 females were recruited for the study at baseline in which 7(11.1%) and 23 (9.7%) were positive. At first point however, out of a total of 62 males and 186 females tested for HCV infection, 6(9.7%) and 21(11.3%) were positive for anti-HCV. Also at second point, out of a total of 62 males and 168 females tested for HCV infection, 9(14.5) and 11(6.5%) were positive. Furthermore, at third point, 5(19.2%) and 3(5.2%) were positive for HCV infection out of 26 males and 58 females respectively tested for anti-HCV in Saki (Table 15).

At baseline, highest rates (18.8% and 13.6%) of HCV infection were observed in age group 15-24 years while lowest rates (16.7% and 13.5%) were detected in age group 25-34 years in males and females respectively. However at first point, highest rates (16.7% and 13.7%) of HCV infection were observed in age group 25-34 years while lowest rates (12.5% and 12.3%) were detected in age group 15-24 years in males and females respectively. Also at second point, highest rates (26.1% and 8.5%) of HCV infection were observed in age group 15-24 years while lowest rates (18.8% and 7.9%) were detected in age group 25-34 years in males and females respectively. Furthermore at third point, highest rates (25.0% and 11.1%) of HCV infection were observed in age group 15-24 and 25-34 years while lowest rates (21.4% and 5.7%) were detected in age groups 15-24 and 25-34 years in males and females respectively. However, antibodies to HCV was

not detectable in age range 35 years and above among Fashion Designers from baseline to second point while at third point, HCV antibodies were not detected in age range 55 years and above (Fig. 8).

4.16: AGE AND GENDER DISTRIBUTION OF HCV INCIDENCE AMONG FASHION DESIGNERS IN SAKI

Figure 9 shows Age and gender distribution of HCV incidence among Fashion Designers from first point to third point in this study in Saki of which 56 males and 165 females were at risk of HCV infection at first point. The only new case among female population was in age group 15-24 years with HCV incidence of 9.9 per 1000 person years while there was no new case of HCV in males. An overall female incidence at first point was 6.1 per 1000 person years.At second point, there were 4 and 2 new cases of HCV out of a total of 53 male and 156 female population at risk resulting in HCV incidence of 75.5 and 12.8 per 1000 person years for males and females respectively. Highest HCV incidence (176 and 23.3per 1000 person years) and lowest incidence (76.9 and 10.8 per 1000 person years) were observed in age groups 25-34 and 15-24 years respectively at second point. However at third point, there were 2 and 2 new cases of HCV out of a total of 21 male and 54 female participants at risk resulting in incidence of 13.6 and 5.3 per 1000 person years for males and females respectively. Highest HCV incidence (47.6 and 71.4 per 1000 person years) and lowest incidence (13.0 and 8.9 per 1000 person years) were observed in age groups 15-24 and 45-54 and that of 25-34 and 15-24 years respectively at third point.There were no new cases in age range ≥35 years among male and female participants in all the three points except in age group 45-54 years at third point (Fig.9; Table 16).

TABLE 16: INCIDENCE OF HCV BY GENDER AMONG FASHION DESIGNERS IN SAKI

AGE GRP (YRS)	FIRST POINT 2004						SECOND POINT 2005						THIRD POINT 2012					
	MALE			FEMALE			MALE			FEMALE			MALE			FEMALE		
	POP .AT RISK	NEW CASES	INC PER 1000	POPL. AT RISK	NEW CASES	INC PER 1000	POP .AT RISK	NEW CASES	INC PER 1000	POP AT RISK	NEW CASES	INC PER 1000	POPL AT RISK	NEW CASES	INC PER 1000	POP AT RISK	NEW CASES	INC PER 1000
15-24	14	0	0	101	1	9.9	13	1	76.9	93	1	10.8	3	1	47.6	33	0	0
25-34	20	0	0	44	0	0	17	3	176	43	1	23.3	11	1	13	16	1	8.9
35-44	7	0	0	16	0	0	7	0	0	16	0	0	2	0	0	3	0	0
45-54	8	0	0	2	0	0	8	0	0	2	0	0	3	0	0	2	1	71.4
55-64	7	0	0	1	0	0	7	0	0	1	0	0	3	0	0	0	0	0
≥65	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0
TOTAL	56	0	0	165	1	6.1	53	4	75.5	156	2	12.8	21	2	13.6	54	2	5.3

POP AT RISK= POPULATION AT RISK
NEW CASES= NO OF SEROCONVERSIONS
INC PER 1000= INCIDENCE PER 1000 PERSON YEARS
RR* = RISK RATIO OR RELATIVE RISK

Overall HCV Incidence for male fashion designers: 49.9 per 1000 person years

Overall HCV Incidence for female fashion designers: 14.6 per 1000 person years

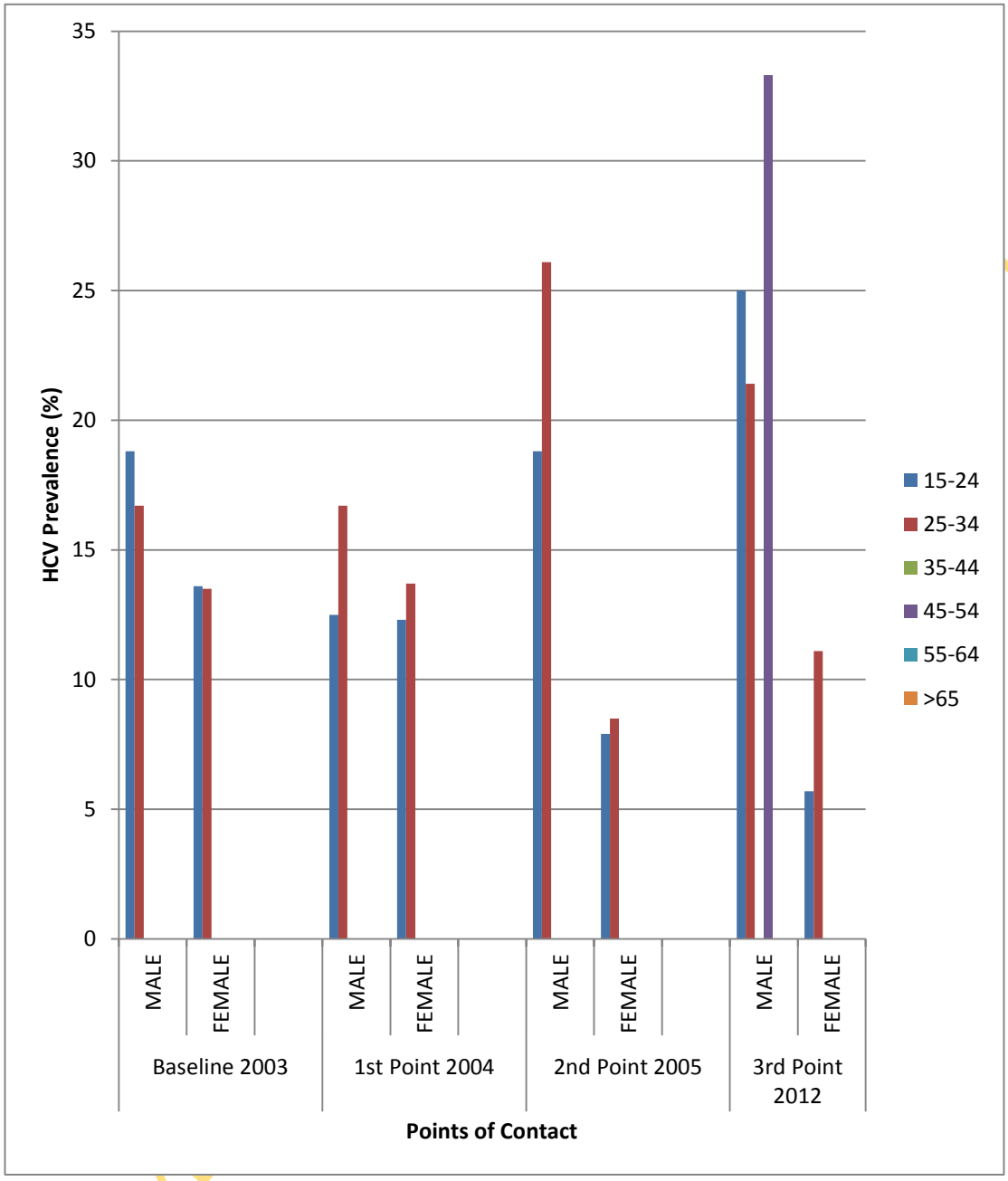

FIGURE 8: GENDER DISTRIBUTION OF HCV INFECTION AMONG FASHION DESIGNERS IN SAKI

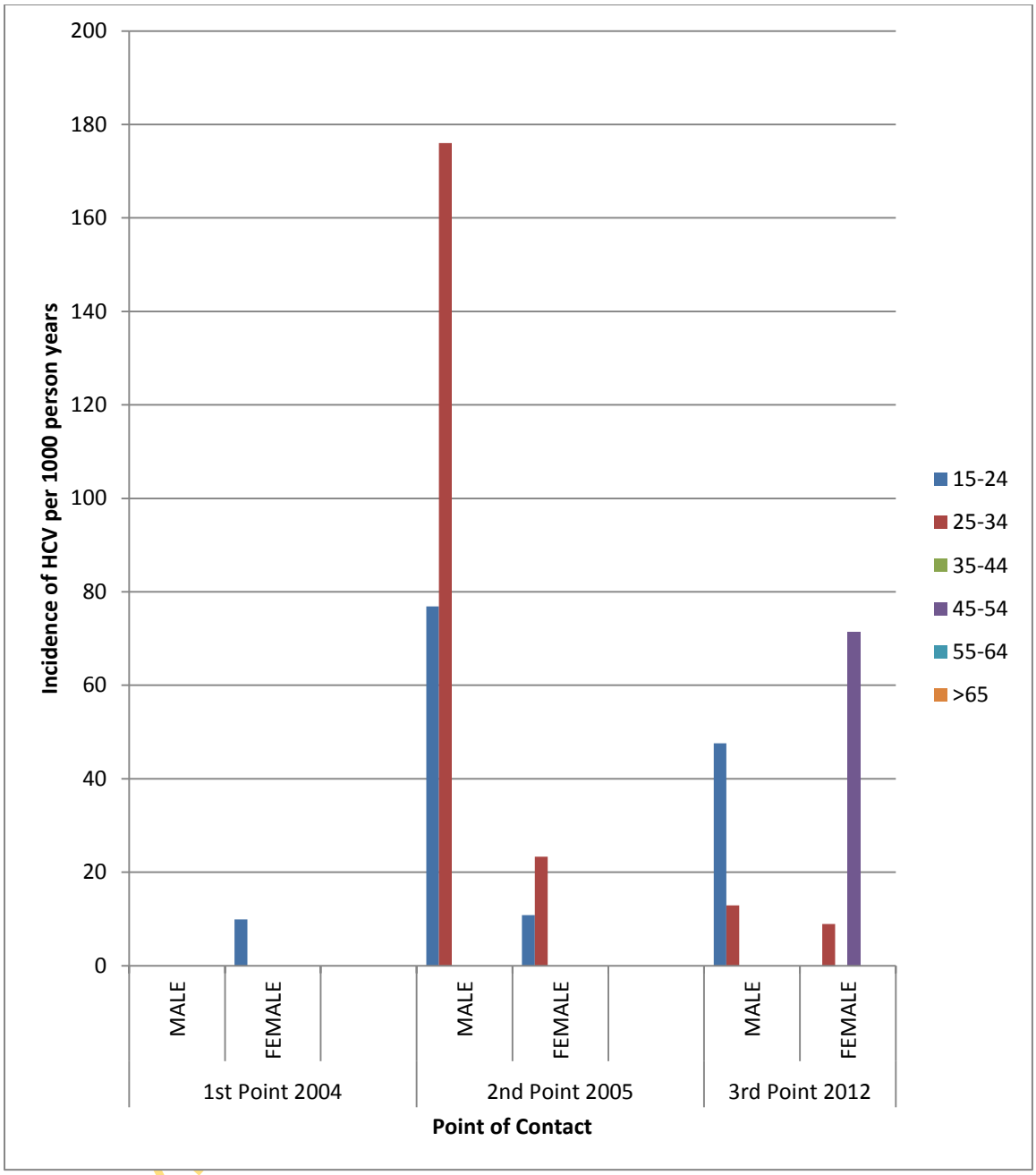

FIGURE 9: GENDER DISTRIBUTION OF HCV INCIDENCE AMONG FASHION DESIGNERS IN SAKI

4.17: AGE DISTRIBUTION OF HCV INCIDENCE AMONG AUTOMECHANICS IN SAKI

Table 17 shows the age distribution pattern of incidence of HCV among automechanics in Saki from first to third points during the nine years of the follow up study. A total of 214, 196, and 90 consented population at risk of HCV followed up out of which 3, 4 and 9 subjects seroconverted giving overall HCV incidence (14, 20.4 and 14.3 per 1000 person years) at first, second and third points respectively among automechanics. At first point, age distribution among automechanics shows that highest incidence (52.6 per 1000 person years) of HCV was observed in age group 45-54 years while lowest HCV incidence (19.6 per 1000 persons years) was observed in age group 15-24 and 35-44 years. However at second point, highest incidence (62.5 per 1000 person years) was observed in age group 45-54 years while lowest incidence (12.7 per 1000 person years) of HCV was observed in age group 25-34 years. Furthermore, at third point highest incidence (95.2 per 1000 person years) was observed in age group 55-64 years while lowest incidence (3.9 per 1000 person years) of HCV was observed in age group 25-34 years. HCV incidence was not observed in age groups 25-34 years at first point, 55 years and above at second point while age group ≥65 years and above at third point among automechanics.

4.18: AGE DISTRIBUTION OF HCV INCIDENCE AMONG FASHION DESIGNERS IN SAKI

Age distribution pattern of incidence of HCV among Fashion Designers in Saki from first to third points during the nine years of the follow up study shows that a total of 219, 209, and 75 consented apparently healthy population at risk of HCV were followed up of which

1, 6 and 4 subjects seroconverted giving overall HCV incidence (4.5, 28.7 and 7.6 per 1000 person years) at first, second and third points among Fashion Designers respectively (Table 17). At first point, age distribution pattern among Fashion Designers shows that incidence (8.7 per 1000 person years) of HCV was observed only in age group 15-24 years. However at second point, highest incidence (66.7 per 1000 person years) was observed in age group 25-34 years while lowest incidence (18.9 per 1000 person years) of HCV was observed in age group 15-24 years. At third point however, highest incidence (35.7 per 1000 person years) was observed in age group 55-64 years while lowest incidence (4.0 per 1000 person years) of HCV was observed in age group 15-24 years. Furthermore, incidence of HCV was not observed in age range 25 years and above at first point, 35-44 years and 55 years and above at second point. Also at third point, age group 55 years and above was also affected without HCV incidence (Table 18).

TABLE 17: INCIDENCE OF HCV BY AGE AMONG AUTOMECHANICS IN SAKI

AGE GROUP (YRS)	FIRST POINT 2004			SECOND POINT 2005			THIRD POINT 2012		
	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years
15-24	51	1	19.6	49	1	20.4	23	2	12.4
25-34	86	0	0	79	1	12.7	37	1	3.9
35-44	49	1	20.4	43	1	23.3	19	3	22.6
45-54	19	1	52.6	16	1	62.5	7	1	20.4
55-64	8	0	0	8	0	0	3	2	95.2
≥65	1	0	0	1	0	0	1	0	0
TOTAL	214	3	14	196	4	20.4	90	9	14.3

Overall HCV incidence for automechanics: 31.4 per 1000 person years

TABLE 18: INCIDENCE OF HCV BY AGE AMONG FASHION DESIGNERS IN SAKI

AGE GROUP (YRS)	FIRST POINT 2004			SECOND POINT 2005			THIRD POINT 2012		
	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years	POPL. AT RISK	NO OF SEROCONVERSION	INCIDENCE per 1000 person years
15-24	115	1	8.7	106	2	18.9	36	1	4
25-34	63	0	0	60	4	66.7	27	2	10.6
35-44	23	0	0	23	0	0	5	0	0
45-54	10	0	0	10	0	0	4	1	35.7
55-64	8	0	0	8	0	0	3	0	0
≥65	2	0	0	2	0	0	0	0	0
TOTAL	221	1	4.5	209	6	28.7	75	4	7.6

Overall HCV Incidence for fashion designers: 23.9 per 1000 person years

4.19: A COMPARISON OF HCV INFECTION BY AGE GROUPS AMONG OCCUPATIONAL GROUPS IN SAKI

Figure 10 compares the incidence of HCV among the occupational groups. Overall HCV rates (11.8, 10.9 and 9.1%) obtained for fashion designers were higher than (4.6, 5.7 and 5.8%) observed for automechanics from baseline to second points respectively. However at third point, HCV prevalence (13.5%) was higher in automechanics than 10.7% observed for fashion designers. Also, highest Anti-HCV rates (14.5, 14.9 and 14.3%) in age group 25-34 years and lowest rates (14.2, 12.2 and 9.4%) in age group 15-24 years were observed among fashion designers compared with highest Anti-HCV rates (7.4, 9.5 and 15.8%) in age group 25-35, 45-54 years and lowest rates (1.9, 3.9 and 4.4%) in age groups 35-44 years observed among automechanics from baseline to second point respectively. However at third point highest Anti HCV rate (20.0%) in age group 45-54 years and lowest rate (7.9%) were observed among fashion designers compared with highest 33.3% prevalence of HCV in age group 45-54 years and lowest (5.0%) in age group 25-34 observed among automechanics.

4.20: A COMPARISON OF HCV INCIDENCE BY AGE GROUP AMONG OCCUPATIONAL GROUPS IN SAKI

Comparing the incidence of HCV among the occupational groups, overall incidence (14 per 1000 person years) observed among automechanics was higher than (4.5 per 1000 person years) observed for fashion designers at first point. Also at second point, HCV incidence (20.4 and 28.7 per 1000 person years) observed among automechanics were higher than incidence (14.3 and 7.6 per 1000 person years) observed among fashion designers at

second and third points respectively. By age distribution at first point, highest HCV incidence (52.6 per 1000 person years) and lowest incidence (19.6 per 1000 person years) were observed in age groups 45-54, and 15-24 years among automechanics unlike 8.7 per 1000 persons observed only in age group 15-24 years among fashion designers at first point.

Similarly at second point, highest HCV incidence (62.5 per 1000 person years) and lowest incidence (12.7 per 1000 person years) were observed in age groups 45-54, and 25-34 years among automechanics compared with highest and lowest incidence 66.7 and 18.9 per 1000 person years observed in age groups 25-34 and 15-24 years respectively among fashion designers. Finally at third point however, highest HCV incidence (95.2 per 1000 person years) and lowest incidence (3.9 per 1000 person years) were observed in age groups 55-64, and 25-34 years among automechanics when compared with highest and lowest HCV incidence 35.7 and 4 per 1000 person years observed in age groups 45-54 and 15-24 years respectively among fashion designers. HCV incidence spread through all age groups among Automechanics except age group ≥65 years while in fashion designers, incidence of HCV was not observed in age groups 35-44 years and ≥55 years among fashion designers (Tables 17 and 18; Fig. 11).

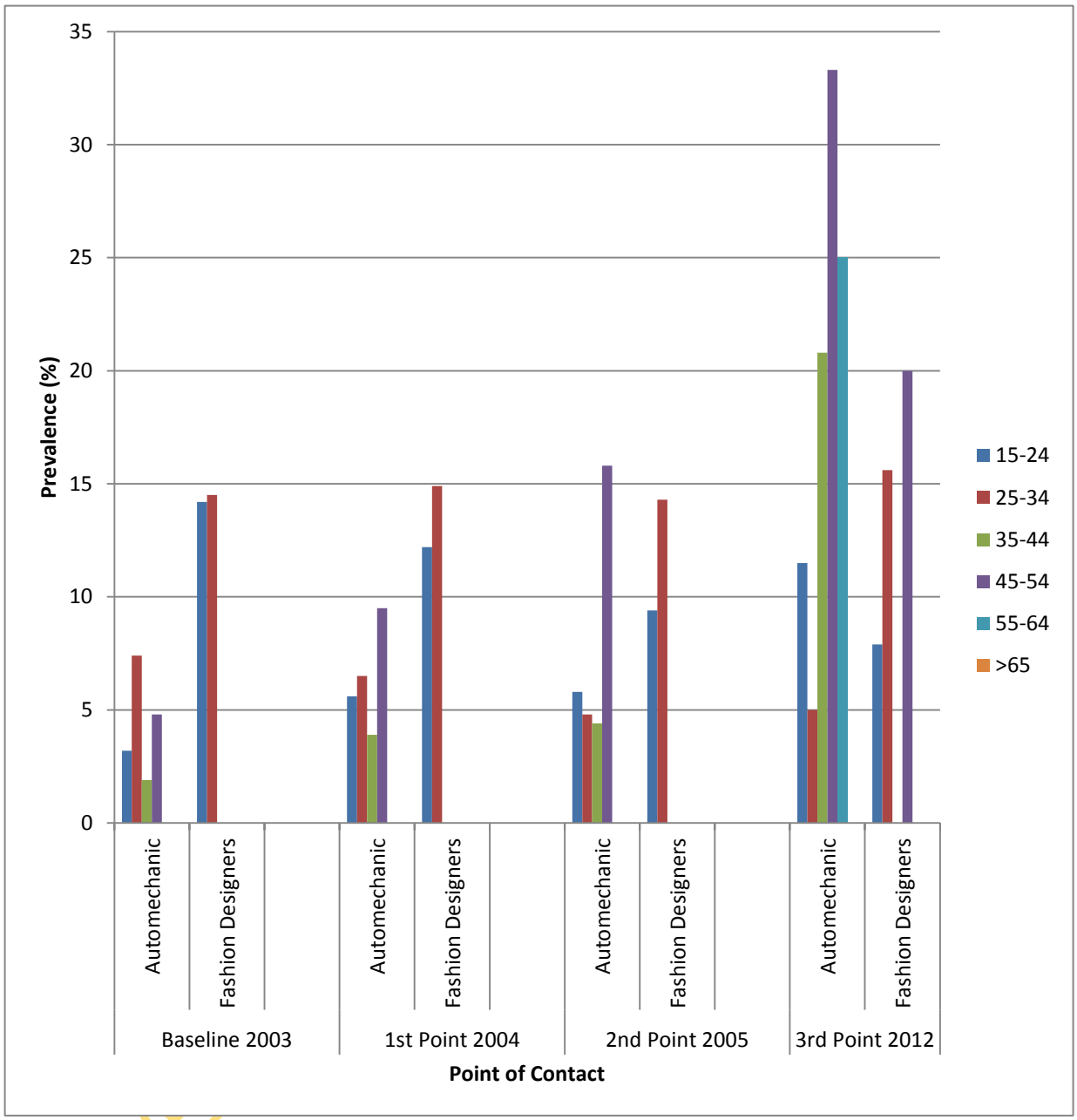

FIGURE 10: COMPARISON OF HCV INFECTION BY AGE AMONG OCCUPATIONAL GROUPS IN SAKI

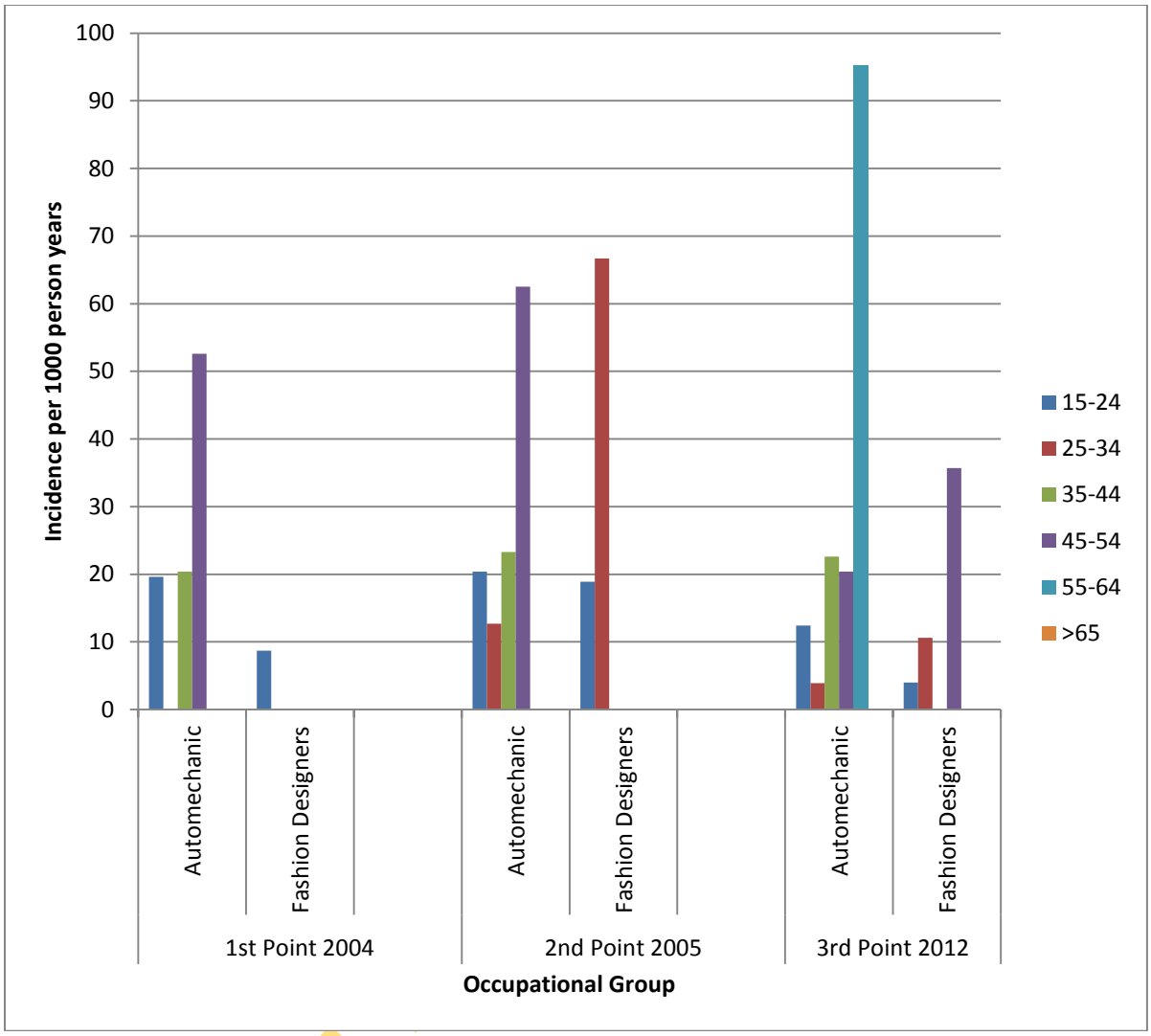

FIGURE 11: INCIDENCE OF HCV BY AGE AMONG THE DIFFERENT OCCUPATIONAL GROUPS IN SAKI

4.21: RISK FACTORS ASSOCIATED WITH HCV INFECTION IN SAKI

We sought to identify potential risk factors associated with HCV infection in the study community during the 9 years of the study. Members of the tow occupational groups namely automechanics (a male dominated) and fashion designers (a female dominated) in this community at different locations were compared with one another in relation to risk factors for HCV infection. From baseline to the third point, a total of 41, 40, 33 and 23 individuals were positive for HCV infection at different points out of 490, 475, 435 and 188 participants respectively recruited for the study.

The predisposing risk factors associated with HCV infection included multiple sexual partnership (RR =1.4, CI=0.92-3.96, $X^2_{0..5:1}$ =3.922, p=0.428), blood and blood product transfusion (RR=1.2, CI=0.64-2.36, $X^2_{0..5:1}$ =0.380, p=0.537) polygamy (RR=1.4, CI=0.67-2.47, $X^2_{0..5:1}$ =0.580, p=0.611), sharing of sharp objects (RR=2.4, CI=1.0-5.56, $X^2_{0..5:1}$ =4.329, p=0.0351) and knowledge of HCV at baseline (0%). However, the only significant risk factor identified in this study community was sharing of sharp objects such as razor blade (Table 19).

TABLE 19: POTENTIAL RISK FACTORS ASSOCIATED WITH HCV INFECTION IN SAKI

Potential Risk Factor	Risk Ratio (RR)	Confidence Interval (CI)	Chi-Square ($\chi 2$)	P-value
Blood and blood product transfusion	1.23	0.64-2.36	0.380	0.537 Not significant
Multiple sexual partnership	1.4	0.92-3.96	3.92	0.428 Not significant
Polygamy	1.4	0.67-2.47	0.580	0.611 Not significant
Sharing of sharp objects	2.4	1.0-5.56	4.329	0.0351 Significant
Knowledge of HCV at baseline	0	0	0	0

CHAPTER FIVE

DISCUSSION

The results of this study show an overall HCV incidence of 27.8 per 1000 person years among occupational groups for the nine years of the community-based study in Saki. As far as it can be ascertained, this is the first incidence data on HCV in Nigeria. Previous studies on HCV infection rate in Nigeria were either hospital-based or point prevalence studies from which incidence of HCV could not be established. However, most of the HCV incidence reports in the literature are from developed countries and very little is known about the situation in most African countries. The incidence of 27.8 per person years found in the study community indicates a very high burden of HCV infection among asymptomatic members of the population in Nigeria. This finding is higher than HCV incidence reported in a community-based study in similar population in the west of Scotland (2.8 per 1000 person years), Khyber pakhtunkhwa in Pakistan (4.13 per 1000 person years) and in Egypt which ranged from 0.8 to 6.8 per 1000 person years (Thorburna *et al.*, 2011; Yousra *et al.*, 2013; Abdelwahab *et al.*, 2013).

The prevalence of 8.4% of HCV infection at baseline and 12.3% at third point found among the study population (occupational groups) is higher than previous findings in other population-based studies in Nigeria (Oni and Harrison, 1996; Olubuyide *et al.*, 1997b; Forbi *et al.*, 2012). In addition, several studies from various centres in Nigeria and worldwide have shown that the prevalence of Hepatitis C virus infection among occupational groups in different regions ranges from 0.5% to 39% and are in concordance with the results of this study (Soni *et al.*, 1995; Neal *et al.*, 1997; Vandelli *et al.*, 2004;

Pérez *et al.,* 2005; Puro *et al.,* 2010; Williams *et al.,* 2011). The rate (8.4%) of anti-HCV detected in this study at baseline is lower than 12.6% reported in a community-based study in southern Italy (WHO, 2014) and 14.7% in Egypt (Yousra *et al.,* 2013) but higher than 4.1% rate of HCV among similar population in Pakistan (Sanaullah *et al.,* 2011) and in Hong kong with a prevalence of 5.8% (Corwin *et al.,* 1996). This therefore agrees with the recent report (WHO, 2014) that HCV is endemic in developing countries and also connotes the endemicity of this infection in this region of the country.

Also, the prevalence of HCV infection are lower than 14.7% reported in Egypt (Yousra *et al.,* 2013) and rates obtained in cross-sectional survey and cohort studies among high risky groups from Europe (Van Der Poel *et al.,* 1991; Tor *et al.,* 1990) North America (Wormsers *et al.,* 1991; Kelen *et al.,* 1992), Asia (Lee *et al.,* 1991; Chan *et al.,* 1992) and Australia (Crofts *et al.,* 1993) in which they found extremely high prevalence of HCV antibody ranging from 50-90%. The study population in Saki community comprises of apparently healthy individuals with same findings when compared with hospital-based studies earlier reported (Ola *et al.,* 2002; Pérez *et al.,* 2005) and therefore indicates high level at which the virus is circulating in this region and confirms the asymptomatic nature of HCV infection. It has been reported that high risk population groups in the community are the most clearly identifiable infected cohorts of HCV infection worldwide and this category of people also cohabit and interact with the general population thereby aiding the circulation of the virus within the study community (Van der Poel *et al.,* 1994; Sharara *et al.,* 1996; Thorburna *et al.,* 2001; Sanaullah *et al.,* 2011; Oh *et al.,* 2012; Casey and Lee, 2013).

Furthermore, the high rates of HCV infection detected in this study indicate a high level of activity of this virus in Nigeria, and compares with less than 1% seroprevalence of HCV antibodies in Europe, America and Australia (Alter *et al.,* 1989; Crofts *et al.,* 1993; WHO, 1997; Jawetz *et al.,* 1998; Motta-Castro *et al.,* 2003; Williams *et al.,* 2011). The rate of HCV obtained in this study agrees with earlier studies in Nigeria (Olubuyide *et al.,* 1997; Ola *et al.,* 2002; Opaleye *et al.,* 2010) and in Africa (Derdas *et al.,* 1999; Mostafa *et al.,* 2010; Stamouli *et al.,* 2012; Yousra *et al.,* 2013) which indicate that the rates of HCV vary between 0.4 -14.7%. However, the rates of HCV obtained in this study from baseline to the third point were higher than 3% observed among blood donors and comparable with what have been reported for hospital-based studies in Nigeria (Olubuyide *et al.,* 1997b; Opaleye *et al.,* 2010; Oh *et al.,* 2012; Casey and Lee, 2013. This therefore connotes HCV as a leading hepatotropic virus with attendant consequences such as acute hepatitis, chronic liver diseases and hepatocellular carcinoma (Ali *et al.,* 2010; Umar *et al.,* 2010; Newshome, 2013).

The incidence of HCV increased from first to the second year but declined significantly at 9th year from the second to third point. This decline in the incidence of HCV in the community during these periods could be attributable to a greater awareness and interventions instituted in the community. The cohort was provided education on prevention of sexually transmitted diseases and blood borne pathogens at baseline and during the follow-up period. It is therefore likely that, the transmission of HCV at the community level could be interrupted with basic intervention measures and active

surveillance. This is similar to the observation in US study where the overall incidence of HCV declined from 7 to 0.7 cases per 100,000 people as a result of active surveillance and massive education of the public on the routes and risks of transmission of HCV (Williams *et al.*, 2011). Therefore, the higher incidence of HCV in the study community indicates that there could be some lifestyle and predisposing factors that potentiate the spread of HCV in such an environment.

The high incidence of HCV in this study may not be unconnected with some risk factors responsible for the transmission of the virus within the study community which includes sharing of sharps such as razor, injection needle, blood transfusion, multiple sexual partnership or polygamy, household sharing of toiletries, and occupational risks among others. Household contact between one HCV infected person and another who has not been infected may also have strong implication in HCV transmission. It has also been reported that the incidence of household-member transmission cases has more than doubled since 1990 (Smyth *et al.*, 2003). According to earlier report, heterosexual exposure in combination with some of the aforementioned risk factors is believed to be responsible for approximately 13% of all infections in Dublin (Smyth *et al.*, 2003). Studies (Williams *et al.*, 2011, WHO, 2014) have reported in the US that two percent of all cases of hepatitis C have been contracted through the occupational risks (needle-stick injuries, blood spills) involved with the health care profession; less obvious, specialized risk factors have been identified resulting from indirect exposures to blood - including manicures, shared toothbrushes and razors, and straight razors in barber shops which are commonly practiced in Saki community. Above all however, ignorance on the mode and

routes of transmission of HCV plays a major role in maintaining the circulation of the virus within a community (Cooper *et al.*, 1992; Forbi *et al.*, 2012).

Gender distribution of HCV incidence in this study indicates that the rates at the different sampling points were higher among male participants which translates to an overall male: female ratio of 2:1, 2.5:1 and 2.8:1. This indicates a slight but insignificant increase in the proportion of infected males with HCV ($p>0.05$) than female in the study community. Also in this study, there was higher incidence of HCV among male than female (21.2 Vs 14.5 per 1000 person years). This is in concordance with the results of previous hospital based studies in Nigeria that showed a slightly higher rate of HCV infection in males (Olubuyide *et al.*, 1997; Ola *et al.*, 2002; Opaleye *et al.*, 2010) indicating that HCV infection is higher in the male than female. Another study also reported a higher HCV rates in males than females among patients attending General Outpatients Clinic at the University College Hospital (UCH), Ibadan (Ayodele and Salako, 2003). The male prepondance found in most studies corresponds to the generally higher incidence of acute HCV infection in male than female (Williams *et al.*, 2011; Yousra *et al.*, 2013). This could be attributed to the involvement of males in high risk practices and some lifestyles which predispose them to HCV infection.

In the Tunisia study, the sex ratio among HCV cases was nearly identical at southern region of the country in which the community-based studies were carried out when compared with the northern region; however, there was a slight but insignificant preponderance of males than females among the study population and contrasting

patterns of HCV infections in the two regions (Mejri *et al.*, 2005). Also in this study, it was observed that HCV incidence increased in both sexes from first to second points but declined sharply at third point over the years. This decline in HCV incidence by gender overtime may be attributable to a greater awareness and massive intervention strategies instituted in the community during the first and second points of the study which was sustained till the end. On the other hand, the increase in incidence of HCV in male than female participants overtime may not be unconnected with various socioeconomic activities and risky lifestyle engaged by the males including multiple sexual partnership, polygamy and alcoholism which sustain the spread of the infection in the community (Van-Asten *et al.*, 2004; Cifuentes *et al.*, 2012). Moreover, high sexual promiscuity, practice of polygamy among this category of participants coupled with higher carrier rate among male than their female counterparts may be contributory to the spread of HCV infection in this regards.

Many factors have been adduced for the transmission and maintenance of the HCV in the study community. Furthermore, cultural practices such as female and male circumcision and polygamy also have serious impact in HCV transmission. Nevertheless, Polygamy and Multiple sexual partnership as predisposing factor were not statistically significant ($P>0.05$). The chronic nature of HCV infection poses more danger coupled with ignorance on the routes of its transmission. All the volunteered participants tested in this study were apparently healthy with no signs of illness, not suspected of any hepatitis C infection and could be qualified as prospective blood donors which may have public health implication. This portends greater risks to the general population in the community

with low level of education as HCV transmission predominantly occurs parenterally as a result of blood transfusion, exposure to blood derivatives and through sex (Chickwem *et al.*, 1997; WHO Fact Sheet-VHPB, 1997; Alter *et al.*, 1989; Kuo *et al.*, 1989; Mahoney, 1999; Ola *et al.*, 2002, Petrus *et al.*, 2005).

Information on the age distribution of the study population with HCV infection in developing countries is very scarce. The results of this study indicate that participants aged 45-54 years were mostly affected in the cohort at first and second points, although there were also substantial infections in the age group 25-34 years at second point. The age at peak of incidence of HCV was slightly delayed at third point with participants in age group 55-64 years mostly affected even though there was substantial number of cases in the 45-54 years age group. This observation is in agreement with the finding in the US study (WHO, 2000) which showed that 65% of persons with HCV infection are aged 30-49 years. Although the peak age of occurrence of the infection was in participants with 45-54 years age group, the incidence of HCV was also considerable among the other age groups. Similarly, another finding on the age of peak incidence of HCV carried out among population group who are at high risk of HCV infection according to Abdelwahab *et al.* (2013) also reported that high incidence of HCV was peaked at 40-50 years and this is in agreement with the present study. The variation in the peak of occurrence of the infection is in agreement with earlier finding in the Egypt study (Yousra *et al.*, 2013) where the peak age varied depending on the duration at which the infection remains asymptomatic and advancement in age of the infected population. It is therefore pertinent to note that many of the participants may have been infected with the virus earlier than

expected but due to asymptomatic nature of HCV infection, the virus continues to circulate in the community. This scenario is therefore indicative of an ongoing HCV infection circulating in the study community overtime but with time the infection will extend to all age groups irrespective of gender and occupation. Also, the pattern of distribution of HCV infection by age varied among the occupational groups enrolled into the study. However, this spread HCV from one age group to the other was observed to be higher among automechanics than fashion designers at third point after a long period of seven years with risk ratio of 2.4

The incidence of HCV on a global scale is not well known, because acute infection is generally asymptomatic. According to WHO (2002), age specific incidence data in sexually active and economically productive age groups are essential for national planning and intervention so as to educate the young ones on the burden of HCV before approaching active sexual age and maturity including public health issues associated with transfusion of unscreened blood contaminated with HCV infection in the community. At baseline, the knowledge about HCV in the study community was very low but increased thereafter with the intervention, it is therefore obvious from the available information that many of the participants were ignorantly infected through transfusion of unscreened blood and blood products (RR=1.4, CI=0.64-2.36, $\chi^2_{0..5:1}$ =0.38, p=0.54). It has been reported that 3% of blood donors worldwide are HCV infected (WHO, 2002) but in developing countries of Africa, the rate is high. In Egypt study (Yousra *et al.,* 2013), it was reported that HCV infection among blood donors is between 5-25%, but among other general population groups, it ranges between 0-40%; this is due to the asymptomatic

nature of the infection among prospective blood donors in communities where screening of blood for HCV is inadequate. A similar situation may be observed in a semi-urban community like Saki which may not guarantee safety in blood banking due to level of ignorance on HCV before the intervention.

Additionally, high rate of HCV observed in a semi-urban community such as that of Saki could be attributable to greater involvement of urban dwellers and cross border interaction including activities that promote the transmission of the virus within the community despite the zero percent awareness. However, during the first contact with the study participants, creation of awareness on the routes of HCV transmission, other blood borne pathogens and sexually transmitted infections was instituted and this yielded results later on. It is therefore likely that the transmission of HCV at the community level could be interrupted with basic conservative measures and active surveillance as instituted in the study community. This is similar to the observations in the Japan study where the overall burden of HCV was lower in the study community from 35.8% during the first point to 17.5% (Petrus *et al.*, 2005). The high burden of HCV in the urban setting indicates that there could be some cultural practices, environmental or lifestyle factors that potentiate the spread of HCV in such places. Also, the high burden of HCV found in this study is worrisome because HCV infections are frequently complicated by HBV coinfection and superinfections often leading to complicated liver disease conditions (Ayodele and Salako, 2003; Vandelli *et al.*, 2004; Judd *et al.*, 2005).

It is a common practice by many of the study participants to patronize unqualified health personnel due to poverty. Also, the influx of traders from other regions of Nigeria and alliens from the Republic of Benin to Saki on business mission who cohabit with the indigenes who engage in risky sexual practice may also contribute to the spread of HCV among sexually active and economically productive population in the study community. Therefore, considering the economic and educational status with age of the infected population vis-à-vis route, ease of exposure and transmission of HCV to susceptible contacts, this senario connotes public health significance. The high HCV rate among the sexually active age groups may be due to horizontal transmission and their involvement in risky sexual behaviours such as multiple sexual partnerships coupled with unprotected sex (Vandelli *et al.*, 2004; Petrus *et al.*, 2005; Oh *et al.*, 2012). Due to asymptomatic nature of HCV infection without prominent clinical presentation, the advancement in age of the infected population may tilt towards the development of liver associated disease conditions leading to serious health implications (Oh *et al.*, 2012). This might also be a reflection of the natural history of HCV infection which is often mild and asymptomatic in the acute form and indolent in the course to chronicity (Montallo *et al.*, 2002; Ola *et al.*, 2002).

The finding of higher incidence of HCV among automechanics (31.4 per 1000 person years), a male occupational group than fashion designers (23.9 per 1000 person years), a female dominated occupational group in the present study can also be explained based on the fact that the members of the group are known to engage themselves in risky lifestyle (RR=1.4, CI=0.67-2.47, $\chi^2_{0..5:1}$ =0.580, p>0). It has also been reported that the male

preponderance found in most studies corresponds to the generally higher incidence of acute infections of any etiology in the men when compared with their female counterparts (Tor and Carunell, 1990; Vandelli *et al.*, 2004; Petrus *et al.*, 2005; Filippini *et al.*, 2007; Stamouli *et al.*, 2012). In this study, the male participants have higher risk ratio of 2.7 in contracting HCV infection than their female counterparts among the study occupational groups which may not be unconnected with their involvement in risky lifestyle. This finding is also in agreement with earlier studies (Vandelli *et al.*, 2004; Petrus *et al.*, 2005; Filippini *et al.*, 2007).

The HCV infected participants in Saki also put the general population in the community at risk of HCV because they interact and cohabit together as observed in the present study and this connotes public health importance. Another population-based studies carried out in developed countries also reported in their findings that economically productive age groups are mostly infected with HCV with ultimate liver conditions (Vandelli *et al.*, 2004; Filippini *et al.*, 2007; Umar *et al.*, 2010). The finding from this study is also in concordance with the hospital-based study carried out by Olubuyide *et al.*, (1997), in which 10.9% of patients with hepatocellular carcinoma (HCC) were positive for HCV who were ignorantly infected for a long period of time before they came down with the disease. This shows the extent at which HCV infection has been on the increase at the community level in this part of the world and the earlier the better for intervention of this magnitude. This finding further agrees with the study by Fashola *et al.* (2003) who carried out viral Hepatitis among high risk population group in Nigeria which reported

high burden of HCV among those that had been ignorantly infected for age in their community.

It has been proposed that high risk occupational groups who perform exposure prone procedures, where injury to the worker may result in exposure of the client's open tissues to the blood of the worker, are theoretically at increased risk of infection with blood borne viruses (Oh *et al.*, 2012). If occupational transmission of HCV was common, staff performing exposure prone procedures might be expected to have a higher incidence of hepatitis C compared with staff in less exposed occupations such as automechanics and fashion designers, yet similar incidence was observed in the study population with the high risk occupational groups. The fact that the occupational groups enrolled for this study could have similar incidence with those considered to be at high risk, the implication of this is that they are also high risk population when considering their risky social behaviors and this is an indication that this virus is spreading fast in this study community. Also, this is in support of the findings from previous study that rates were higher in developing countries than in developed countries (Volker *et al.*, 2006; Raja and Janjua, 2008). This may be attributed to the socio-economic problems and high risky attitudes which include the use of unscreened blood and blood products, lack of basic amenities, multiple usages of syringes and needles in some areas, use of unsterilised needles for tattooing, acupuncture, circumcision or ear piercing.

The present study also evaluated some predisposing factors for HCV transmission. Potential HCV risk factors were addressed in a multivariate analysis of the results of this study. Whether a participant had been transfused with blood and blood products at one time or the other was not a significant factor for HCV infection (RR=1.2, CI=0.64-2.36, $X^2_{0..5:1}$ =0.380, p=0.537). Multiple sexual partnership was also not a significant risk factor for acquiring HCV infection (RR =1.4, CI=0.92-3.96, $X^2_{0..5:1}$ =3.922, p=0.428). In Saki community, the fact that most of the participants in this study engage in multiple sexual partnership (either single or married) and majority of those already married are involved in polygamous family set up but the correlation and effect of multiple sexual partnership and polygamy as risk factors for HCV infection could not be ascertained in this study (p>0.05). However, incidence studies in Egypt and Parkistan ((Mostafa *et al.*, 2010; Hussain *et al.*, 2010; Yousra *et al.*, 2013) have contrary reports which implicated these risk factors that aid the transmission of HCV infection. Rates of multiple sexual partnership and polygamy are also reported to be very high in those countries thereby associating HCV burden with these predisposing factors. In industrialized or advanced countries such as US, awareness and knowledge on the mode of transmission of HCV including risk factors such as multiple sexual partnership, blood and blood products transfusion are on the increase despite the use of sophisticated equipments for detecting HCV infection even at molecular level. Hence, a very low HCV rate and incidence were observed in this part of the world (WHO, 2014) and this fit was achieved through active surveillance and sensitization of the public on HCV. Although it is worth mentioning that most of the participants in this study are sexually active and mostly involved in polygamous set up in which sexual transmission may be implicated ((RR=1.4, CI=0.67-

2.47, $\chi^2_{0..5:1}$ =0.580, p=0.611); however, this predisposing risk factor was not significant (p> 0.05) but the risk at which HCV infection could be contracted through sex was 1.4 times higher.

Furthermore, sharing of sharp object (RR=2.4, CI=1.0-5.56, $X^2_{0..5:1}$ =4.329, p=0.0351) was identified as a significant predisposing factor that played a major role in the transmission of HCV in the study community. Majority of our participants share sharp objects such as razor or blade between friends, colleagues and partners particularly among members of the occupational groups enrolled into the study. However, none of the participants was engaged in organ transplant despite their low level of education which was not significant. Nevertheless, many other studies have found an association between the level of education and HCV infection (Mostafa *et al.*, 2010; Sanaullah *et al.*, 2011; Forbi *et al.*, 2012). This may explain the consistent evidence of education vis-à-vis HCV infection. Although, habit of alcoholism was not captured in this study as a risk factor for HCV, some findings have posed a serious question as to why many alcoholics are infected with HCV. In many surveys, about a third of people who are alcoholics are also infected with HCV. Whether alcoholics are in fact more prone to infection has not been firmly established (Puro *et al.*, 2010; Umar *et al.*, 2010; Sanaullah *et al.*, 2011). Unavailability of proper education and sensitization on the mode of transmission are serious constraints that promote the spread of HCV infection in the study community.

The findings from this study have also reaffirmed the fact that HCV infection is very common among population groups of this caliber (automechanics ans fashion designers), many of which with low educational background and strong belief for cultural myths;

these two factors also aided the spread of HCV within the study community before the intervention. It has been reported recently that Egypt has the highest prevalence of hepatitis C virus (HCV) in the world, estimated nationally at 14.7% which was higher than 8.4% detected in this study at baseline. In contrast, Egypt (Yousra *et al.*, 2013) was reported to have highest incidence of HCV worldwide with 6.8 per 1000 person years which is far lower than 27.8 per 1000 person years reported in the present study in Saki community. This alarming rate may have put Nigeria ahead of Egypt which portends great danger for the study community and Nigeria as a whole considering the geographical location of Saki. This therefore does not imply that the risk of contracting HCV infection in the study community was higher before the intervention.

Moreover, inadequate enlightenments and sensitization from health care personnel and non involvement of Federal Ministry of Health in HCV campaign as it is done in the case of HBV and Polio for control and eradication are not helping matter at all in the study community knowing that there is no vaccine against HCV for now. This may have accounted for the high HCV rates in the community within which this study was carried out. This therefore calls for urgent action and the need to control the spread of this infection among the community dwellers. It is also desirable as a matter of urgency for the stakeholders to note that National healthcare awareness and infection control programmes should be strengthened to prevent further transmission of HCV.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

The study shows that HCV infection remains endemic and on the increase in our rural and urban communities. A total of 27 new cases of infection were identified in the cohort during the period giving an overall incidence of 27.8 per 1000 person years. The incidence of HCV increased from first to the second point but declined thereafter. The incidence of the infection increased with age and peaked among persons 45-54 years in the first and second year and among the 55-64 years age group at third point, with some substancial infections in the 45-54 years age group at third point. HCV incidence was higher among male than female. Similarly, HCV incidence was higher in male than female members of the fashion designer group. Also, overall incidence of HCV infection was higher among automechanics, an all male occupational group than fashion designers, a female dominated occupational group. Sharing of sharp objects has been identified as a significant predisposing factor for HCV infection among the study populations.

The results of this study have provided for the first time the incidence of HCV infection among occupational groups at the community level in Nigeria. This study has therefore highlighted the substantial burden of disease attributable to Hepatitis C virus infection as a public health problem especially in a socio-economic derived setup. Therefore the following recommendations are partinent:

a. The public should be properly educated on the route of transmission of HCV and the predisposing risk factors associated with the infection. This should be done through the media especially radio jingles on the need to intesify screening of

blood and blood products for HCV before transfusion and it must be free or subsidized and compulsory at all levels.

b. Continuous and active surveillance for HCV should be made routine with a 'watch group' in a place; research strategy at discovering HCV vaccine should be intensified to provide for prophylaxis against HCV infection at subsidized rate by the three tiers of government if it cannot be absolutely free.

c. Screening centres should be provided in all the local governments and wards throughout the country; screening of families of infected HCV subjects (and their contacts) should be an essential part of case management for early detection, management and control.

d. Blood banks should be provided with modern and sophisticated equipment for diagnosis of early detection of HCV to prevent pre and post transfusion hepatitis. Screening and confirmatory tests for HCV in recognized virology laboratories with the issuance of tenable certificates should be advocated for as the only tool to solemnize couples together in holy matrimony as is the case for HIV.

e. Medical scientists should be provided with funds by governments, international organizations and well meaning Nigerians to carry out community based studies of this kind on HCV throughout the federation and not only routine hospital-based studies commonly reported in Nigeria.

f. Basic conservative prevention strategy should be directed towards the avoidance of risk factors (sharing of sharp objects, transfusion of HCV infected Blood/ blood products etc) that promote the spread of HCV.

g. National healthcare awareness and infection control programmes should be strengthened to prevent further transmission of HCV infection in our communities in Nigeria.

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APPENDIX

Incidence of Hepatitis C Virus Infection-A community based Project

QUESTIONAIRE/Baseline survey

This is a questionnaire to weigh the perceptions of some occupational groups on the above topic. Please fill out objectively. Thank you.

Introduction of the Interviewer: "My name is… I'm working for… We're interviewing people here in [Saki, a southwestern community] in order to find out about…[describe purpose of study].

Confidentiality and consent:
"I'm going to ask you some very personal questions that some people find difficult to answer. Your answers are completely confidential. Your name will not be written on this form, and will never be used in connection with any of the information you tell me. You do not have to answer any questions that you do not want to answer, and you may end this interview at any time you want to. However, your honest answers to these questions will help us better understand what people think, say and do about certain kinds of behaviors. We would greatly appreciate your help in responding to this survey. The survey will take about XX minutes to ask the questions. Would you be willing to participate?"

_____
(Signature of interviewer certifying that informed consent has been given verbally by respondent)

Date -----/-----/---- Study Number ----------

Gender: 1=Male, 2=Female

Site in Saki : 1=Igboro New, 2=Igboro Old, 3=Ajegunle Old, 4=Ajegunle New

Name of subject: Surname ---------- Other Names ----------

Age (Yrs) -------- Date of birth ----/------/-------

Occupation: 1=Automechanic, 2= Fashion Designer

Address ----------

Nationality: 1= Nigerian 2= Non-Nigerian

Marrital Status: 1=Single, 2=Married, Divorced

Number of sexual partner: 1=None, 2=1, 3= More than one

Education: 1=None, 2= Koranic, 3= Primary, 4= Secondary, 5= Tertiary

Type of Religion: Christianity=1 Islam= 2 Traditional= 3

Ethnic group: 1=Yoruba 2= Igbo 3= Hausa

Have you shared sharp objects e.g razor blade, hypodermic needle before?: 1=Yes, 2=No

Family set-up: 1= Monogamy, 2= Polygamy, 3= Others (specify)

Have you been transfused with blood & blood products before? 1=Yes, 2=No

Have you heard of Hepatitis C Virus (HCV) before? 1=Yes, 2=No

Do you know anyone who is infected? 1= YES 2= No

Has anyone in your family died or suffered from HCV infection before?

1= Yes 2= No 3=Don't know 4= No response

Have you had of organ transplant before? 1=Yes, 2=No

Have had of Sexually Transmitted Infections/Deseases before? 1=Yes, 2=No

Some people have tried injecting drugs using a syringe. Have you injected drugs in the last 12 months?
1=Yes 2= No

DRUGS INJECTED FOR MEDICAL PURPOSES OR TREATMENT OF AN ILLNESS DO NOT COUNT:
1=YES 2= No 3= Don't know 4= No response

Thank you very much for your time