DETECTION OF HEPATITIS C AND HEPATITIS B VIRUS INFECTION AMONG PRISON INMATES AND PSYCHIATRIC PATIENTS IN KADUNA METROPOLIS, NIGERIA

BY

REBECCA WADA BITIYONG

DEPARTMENT OF MICROBIOLOGY

AHMADU BELLO UNIVERSITY, ZARIA

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BY

Rebecca Wada BITIYONG, B. Sc 2010, PGDE 2012 (ABU)

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DECLARATION

I declare that the dissertation entitled "Detection of Hepatitis C and B Viruses among Prison Inmates and Psychiatric Patients in Kaduna Metropolis" was carried out by me in the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work was previously presented for another degree or diploma at this or any other Institution.

Bitiyong, Rebecca Wada _____ _____

Name of Student **Signature** **Date**

CERTIFICATION

This thesis entitled “Detection of Hepatitis C and B Virus Infection among Prison Inmates and Psychiatric Patients in Kaduna Metropolis” by Bitiyong, Rebecca Wada meets the regulation governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Dr M. Aminu-Mukhtar, Chairperson, Supervisory Committee, Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria.	_____ Signature	_____ Date
Prof. E. D. Jatau, Member, Supervisory Committee, Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria.	_____ Signature	_____ Date
Prof. I. O. Abdullahi, Head of Department, Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria.	_____ Signature	_____ Date
Prof. Kabiru Bala, Dean, School of Postgraduate Studies, Ahmadu Bello University, Zaria.	_____ Signature	_____ Date

DEDICATION

This work is dedicated to my beloved, unique and outstanding parents, His Royal Highness Mr. and Mrs. Tobias Nkom Wada (Agwam Takad I), for their love and selfless contributions to my life.

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LIST OF ABBREVIATIONS

HCV	-	Hepatitis C Virus
RNA	-	Ribonucleic Acid
E1	-	Envelope protein 1
E2	-	Envelope protein 2
NS	-	Non structural Protein
ORF	-	Open Reading Frame
NTR	-	Non-translated Region
IRES	-	Internal Ribosomal Entry Site
STD	-	Sexually Transmitted Disease
IDU	-	Injection Drug Usage
DNA	-	Deoxyribonucleic Acid
HBsAg	-	Hepatitis B Surface Antigen
HBcAg	-	Hepatitis B Core Antigen
HBV	-	Hepatitis B Virus
PCR	-	Polymerase Chain Reaction
RT-PCR	-	Reverse Transcription Polymerase Chain Reaction
UTR	-	Untranslated Region
TCR	-	T-cell Receptor
TNK	-	Natural Killer T-Cell
HIV	-	Human Immunodeficiency Virus
SNP	-	Single Nucleotide Polymorphism
IL28B	-	Interleukin 28 B

KASL	-	Korean Association for Study of Liver
HCC	-	Hepatocellular Carcinoma
IFN	-	Interferon
EIA	-	Enzyme Immunoassay
RIBA	-	Recombinant Immunoblot Assay
TMA	-	Transcription Mediated Amplification
ALT	-	Alanine aminotransferase
LiPA	-	Line Probe Assay
RFLP	-	Restriction Fragment Length Polymorphism
CLIA	-	Chemiluminescent immunoassay
ECLIA	-	Electrochemiluminescent immunoassay
ELISA	-	Enzyme Linked Immunosorbent Assay
ELASA	-	Enyyme Linked Aptosorbent Assay
dsDNA	-	Double Stranded Deoxyribonucleic Acid
cccDNA	-	Covalently Closed Circular Deoxyribonucleic Acid
anti-HBsAg	-	Antibodies to Hepatitis B Surface Antigen
HBcAb	-	Hepatitis B Core Antibody
rpm	-	Revolutions Per Minute
anti-HCV	-	Antibodies to Hepatitis C Virus
IgM	-	Immunoglobulin M
IgG	-	Immunoglobulin G
HRP	-	Horse Raddish Peroxidase
TMB	-	Tetramethylbenzidine

OD	-	Optical Density
cDNA	-	Complimentary Deoxyribonucleic Acid
EDTA	-	Ethylene Diamine Tetraacetic Acid
µl	-	Microlitre

ABSTRACT

There is high risk of contracting hepatitis B and C among individuals with psychotic disorders due to lifestyle factors and prisoners globally continue to demonstrate a higher prevalence of Hepatitis B and C than the general population. This study was aimed at determining the seroprevalence of HCV and HBV and to detect hepatitis C virus (HCV) among prison inmates and psychiatric patients in Kaduna Metropolis. A total of 276 (153 prison inmates and 123 psychiatric patients) serum samples were tested for anti-HCV and Hepatitis B surface antigen (HBsAg) using third generation Enzyme Linked Immunosorbent Assay (ELISA) and RDT method respectively. Hepatitis C virus genome was detected in ten (10) serum samples using reverse transcription polymerase chain reaction (RT-PCR). An overall anti-HCV IgM prevalence of 10.14% (28/276), anti- HCV IgG prevalence of 8.69% (24/276) and HBsAg prevalence of 6.15% (17/276) was established. A 0.36% (1/276) HCV/HBV co-infection rate was obtained. Among the inmates, an anti-HCV IgM and IgG prevalence of 10.45% (16/153) and 8.5% (13/153) respectively was obtained with a 9.2% (14/153) HBsAg prevalence. An HBsAg, anti-HCV IgM and anti-HCV IgG prevalence of 2.4% (3/123), 9.75% (12/123) and 8.9% (11/123) respectively was obtained among the psychiatric patients. The highest HCV antibody prevalence was obtained among the female subjects (14.1% for IgM and 8.4% for IgG). No female tested positive for HBsAg. Subjects aged ≥48 years had the highest HCV prevalence (28.9%: 13/45 for IgM and 31.1%: 14/45 for IgG) while those within age group 28-32 years had the highest HBsAg prevalence (11.7%: 7/60). Age was observed to be associated with HCV infection (p=0.00). Viremia was evaluated by amplifying conserved untranslated region of HCV genome and bands of 244bp were observed. There was no statistically significant association between the viral infections and demographics. Presence of tattoo/scarification and alcohol intake were statistically associated with HCV infection while clothes sharing was associated with HBsAg among the inmates. Hepatitis C virus infection was statistically associated with blood transfusion, alcohol intake, presence of tattoo/scarification, sexual experience and shaving equipment sharing among the psychiatric patients while HBsAg was associated with only clothes sharing. This study established the circulation of HBV and HCV among inmates and psychiatric patients in Kaduna State. These individuals should therefore be screened for these viruses for appropriate clinical management and effective prevention.

CHAPTER ONE

1.0 INTRODUCTION

Hepatitis C virus (HCV) is a small enveloped virus measuring 55-65nm in size. It is a positive sense single stranded RNA virus of the family *Flaviviridae* and genus *Hepacivirus* (Kapoor *et al*., 2011). It is the causative agent of human hepatitis C infection, although it has been found to infect chimpanzees, dogs, horses, and rodents (Rogo, 2011; Burbelo *et al*., 2012; Kapoor *et al.*, 2013; Quan *et al.*, 2013). Hepatitis C virus particle is made up of an RNA core of genetic material, surrounded by an icosahedral protective protein. This is further encased in a lipid envelope derived from the host. The viral glycoproteins, E1 and E2 are embedded in the lipid envelope (De-Beeck *et al*., 2003; Igwe *et al*., 2010). Hepatitis C virus encodes a single polyprotein of 3010-3011 amino acids which is processed into structural and non-structural proteins (NS). This is made possible with the aid of cell signalases and viral proteases.

Hepatitis C genome consists of a single open reading frame (ORF) that is made up of 9600 nucleotide base long (Kato, 2000). The ORF possess highly conserved nontranslated regions (NTR) in its 5′ and 3′ ends. In the 5′ end, there is an internal ribosome entry site (IRES) which allows the RNA to bind to the ribosomes close to the codon to start codon of the ORF. Based on genetic differences between HCV isolates, the virus is classified into seven genotypes (1-7) (Nakano, 2011). These subtypes are further broken into quasi species based on genetic diversity (Christian, 1996) and high error rate on the part of the virus RNA dependent RNA polymerase. The entry of the virus into the host is as a result of complex interaction between virions and cell surface molecules (Zeisel *et al.*, 2009;

Kohaar *et al*., 2010). The structure and replication of HCV is poorly known due to lack of efficient cell culture system, difficulty to grow or develop in cell culture as well as striking heterogenicity in density (Kawo *et al*., 2012). The virus replicates mainly in the hepatocytes of the liver, where it is estimated that daily each infected cell produces approximately fifty (50) virions with a calculated total of one trillion virions generated (Bartenschlager and Lohmann, 2000).

Hepatitis C virus was formally known as non A non B hepatitis (Alter *et al*., 1990; Brooks *et al.,* 2013). In the 1980s investigators from the Centres for Disease Control and Prevention (CDC) identified the virus. Hepatitis C infection ranges in severity from a mild illness lasting a few weeks to a serious lifelong illness and hepatocellular carcinoma (Alter, 2007; WHO, 2013). This virus is most commonly transmitted through exposure to infected blood. This can occur through contaminated blood transfusion, blood products and organ transplant (WHO, 2013), although the most common route of HCV infection is through injection drug use (IDU) (Afolabi *et al*., 2012; Suresha *et al*., 1996). The virus may be sexually transmitted, although this is rare and usually only occurs when sexually transmitted diseases (STD) that causes open sores and bleeding is present (Liu and Wei, 2007). Other risk factors involve parenteral exposures with contaminated equipment, tattooing, body piercing, accidental needle stick injury among health workers and perinatal transmission in infants born to HCV positive women (Prati, 2006; Igwe *et al*., 2010).

The incubation period for hepatitis C is 2 weeks to 6 months and approximately 80% of people do not exhibit any symptoms following initial infection (Gao, 2011; Kawo *et al*.,

2012; WHO, 2013). The viral infection does not really manifest symptoms in majority of people, the infection becomes chronic in most cases and is typically characterized by a prolonged period of no symptoms.

Hepatitis B infection is a liver disease that results from infection with a virus called Hepatitis B virus (CDC, 2013). It is a DNA virus of the family *Hepadnaviridae*, genus *Orthohepadnavirus* and species Hepatitis B virus (Pungpapong *et al*., 2007; Willey *et al*., 2013). The viral particle consists of an outer lipid envelope and an icosahedral nucleocapsid core which is composed of protein. The outer envelope contains proteins that are involved in viral binding and entry into susceptible cells. The outer surface or envelope contains hepatitis B surface antigen (HBsAg) and surrounds the inner nucleocapsid core that contains hepatitis B core antigen (HBcAg) (Willey *et al*., 2013).

The viral genome consists of a partially double stranded circular DNA, with up to 3020-3320 nucleotides and viral isolates share 80-90% nucleotide homology (Brooks *et al*., 2013; Willey *et al*., 2013). WHO (2013) posited that people at high risk include prison inmates, IDUs, people who frequently require blood and blood products, mentally ill persons, people with multiple sexual partners and health-care workers. The virus is found in body fluids and blood and it is transmitted from person to person through sex, tattooing, body piercing with unsterilized equipment, sharing of equipment like razor, toothbrush and IDU. Transmission from mother to child during childbirth where equipment are not adequately sterilized can also occur (Ugbebor *et al*., 2011).

The disease is either acute or chronic (Shepard *et al*., 2006). The acute disease is a short term illness which occurs within six months after the individual is exposed to the virus and

does not always lead to chronic infection (CDC, 2013). The likelihood of developing a chronic infection depends on the age at which a person becomes infected (Shepard *et al*., 2006). Young children less than six years old are most likely to develop a chronic infection when infected with the virus and infants infected during the first year of life develop chronic infection by 80%-95% (Brooks *et al*., 2013). Five percent of adults develop chronic infection and 15-25% of adults who become chronically infected during childhood die of liver cirrhosis or cancer related to HBV (CDC, 2013).

1.2 Statement of the Research Problem

It is estimated that about 200 million people in the world (about 3% of the world population) are infected with HCV (Ugbebor *et al*., 2011; WHO, 2013). Of the 2 billion people who have been infected with the HBV globally, more than 350 million have chronic infection and over 20 million people are infected annually with this virus (Ugbebor *et al*., 2011; Akinsegun *et al*., 2012). It occurs frequently in Nigeria and about 12% of the Nigerian population is infected with HBV (Akinsegun *et al*., 2012).

Hepatitis C virus induces end stage chronic liver disease and it is the leading cause of liver failure (Sheyin *et al*., 2012) even hepatocellular carcinoma and this is a major growing public health problem (Afolabi *et al*., 2012). The major problem with HCV infection is that 85% of individuals initially infected with the virus become chronically infected, usually for decades (Gao, 2011; CDC, 2012; WHO, 2013) and the other 15% simply have acute infection which resolves in few weeks or months (CDC, 2012). The infection is often asymptomatic but once established, chronic infection can progress to scarring of the liver

(fibrosis) and advanced scarring (cirrhosis) which is generally apparent after many years (Ray and Ryan, 2004) or life threatening oesophageal varices and gastric varices which is responsible for thousands of death each year (Kawo *et al*., 2012). Those who develop cirrhosis or liver cancer may require a liver transplant and the virus universally recurs after transplantation (Afolabi *et al*., 2012).

The Department of Health and Human Services, CDC (2011) posited that hepatitis C is the most common type of hepatitis found in jails and prisons. People with mental illness are likely to be over represented in high-risk categories for infection with pathogens with similar routes of transmission, such as HBV and HCV (Stanley *et al*., 2001). Occurrence of this virus among inmates in the United States is 10 to 20 times that of the occurrence observed in the general population; this has been attributed to high risk behaviour in prisons such as IDU and tattooing with non sterile equipment (Vescio *et al*., 2008; Imperial, 2010). It is estimated that nearly half of prison inmates share unsterilized tattooing equipment (Jafari *et al*., 2010).

Hepatitis B virus and HCV co-infection is very common because of the shared modes of transmission and subjects with high risk of parenteral infections tend to have this (Chi-Jen and Shou-Dong, 2008). A clinical and laboratory study showed that HBV and HCV co-infection causes severe liver disease than mono infection with either HBV or HCV and it is associated with a higher prevalence of liver cirrhosis and hepatocellular carcinoma as compared with mono infection (Chi-Jen and Shou-Dong, 2008).

Hepatitis C virus is spread by blood -to- blood contact (Gao, 2011; Brooks *et al*., 2013). The viral infection is common in prison populations, mostly among those with history of IDU, tattooing and sharing of shaving equipment (Teutsch *et al*., 2010). Nearly 50% of conviction of French prisoners is as a result of drug trafficking (Semaille *et al*., 2013). Intravenous drug use is the major route of HCV transmission worldwide after the introduction of approved blood screening measures before transfusion in 1992 (Maheshwari *et al.*, 2010; Gravitz, 2011; Afolabi *et al*., 2012).

In Nigeria, studies have been carried out on the prevalence of HCV among pregnant women and blood donors. Sheyin *et al*. (2012) reported a prevalence rate of 4.5% among pregnant women in Kaduna State, Bala (2012) reported a prevalence of 2.5% among pregnant women in Maiduguri. Afolabi *et al*. (2012) reported 2.7% among blood donors in Ibadan, and a prevalence of 6.0% among blood donors in Jos was reported by Egah *et al*. (2004). Pennap *et al*. (2010) had reported 13.3% prevalence among people of a local community in Keffi while Adoga *et al*. (2009) carried out a study among inmates in Nasarawa State and had a prevalence of 12.3%. The prevalence of HCV among mentally ill individuals in Nigeria has been found to be 4.2% (Omoaregbe *et al*., 2013).

Many Studies on HCV and HBV have been conducted in Nigeria but among pregnant women and blood donors. However, there is dearth of published work on the prevalence of HCV among prison inmates and psychiatric patients in Kaduna State.

1.3 Justification

Since there is dearth of published information on the prevalence of HCV and HBV in Kaduna State among inmates and psychiatric patients, this study will provide data on the prevalence of HCV and HBV among these populations. The data generated by this study will serve as baseline information for the prevention and control of these viruses and for further studies among prison inmates and psychiatric patients.

1.4 Aim

This study aimed at determining the prevalence of HCV and HBV infections among prison inmates and psychiatric patients in Kaduna Metropolis, Nigeria.

1.5 Specific Objectives

The specific objectives of this research were to:

1. Screen for HCV antibodies and HBsAg among prison inmates and psychiatric patients in Kaduna Metropolis.
2. Detect HCV RNA using reverse transcription polymerase chain reaction (RT-PCR).
3. Determine the risk factors that predispose individuals to HCV and HBV infections.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Hepatitis C Virus

The discovery of Hepatitis C virus in 1989 was a major breakthrough. Before that point, it was clear that a major cause of acute hepatitis after a blood transfusion was neither related to Hepatitis A nor to Hepatitis B, hence the early names for this disease, non-A, non-B hepatitis (Rogo *et al*., 2010). This was only possible following the development of serological diagnostic tests for hepatitis A and B in 1965 and 1973 respectively (Eze *et al*., 2010). Hepatitis C virus, a relatively new virus discovered about 25 years ago has become a major cause of chronic liver disease worldwide and the main reason for liver transplantation in the western world (Godwin *et. al*., 2013). Its evolution is believed to be mediated by several processes such as genetic drift caused by lack of proof-reading activity in the viral RNA-dependent RNA-polymerase during replication, immune pressure and recombination (Suwanna *et al*., 2010). Hepatitis C virus formerly designated Non-A-Non-B hepatitis is a linear, single stranded RNA virus belonging to the *Flaviviridae* with at least six genotypes identified (Eze *et al*., 2010). The agent was named Hepatitis C virus by Choo and his co-workers in 1989 (Ejiofor *et al*., 2010). Before 1992, when widespread screening of blood supply began in the United States, Hepatitis C was also commonly spread through blood transfusion and organ transplants (CDC, 2013).

2.2 Hepatitis C Virus

The virus is a small (55-65nm in size), enveloped, positive sense, single stranded RNA virus of approximately 9.6kb (Ji-Hoon *et al*., 2013) from the family *Flaviviridae*, genus

Hepacivirus, and *Hepatitis* C virus type species (Simmonds *et al*., 2005; Eze *et al*., 2010). Its complete genome organization resembles that of the Pestiviruses and Flaviviruses and encodes untranslated conserved regions (5′UTR and 3′UTR) (Suwanna *et al*., 2010). The viral proteins are translated as a polyprotein of about 3010 amino acids that is processed co-and post-translationally by cellular and viral proteases into structural and non structural proteins (Ji-Hoon *et al*., 2013).

The open reading frame of the virus encodes the structural proteins, core (C), envelope (E1, E2), and the nonstructural proteins (p7, NS2, NS3, NS4a, NS4b, NS5a and NS5b) (Rogo *et al.,* 2010; Hoshida *et al*., 2014). The viral genome is extremely heterogeneous (Casanova *et al.,* 2014) and is enclosed in a protein coat which is wrapped in a lipid envelope derived from the host cell (Ejiofor *et al*., 2010). Based on genetic differences between HCV isolates, the genotype has been recently updated to seven main genotypes that differ by more than 30% in their nucleotide sequence (Casanova *et al*., 2014). The subtypes are further broken down into quasispecies based on their genetic diversity (Kato, 2000; Rogo, 2010) and high error rate on the part of the virus RNA dependent RNA polymerase.

Hepatitis C virus genotyping is an important tool for the diagnosis and follow-up of infected patients (Campiotto *et al*., 2005). The structure and replication of HCV are incompletely understood due to low viral titers found in sera and livers of individuals infected and lack of an efficient cell culture system or small animal model permission for HCV infection (Kawo *et al*., 2012). The virus principally replicates in the hepatocytes even

though other sites (haematopoietic cells such as B lymphocytes and stem cells) of replication have been reported (Greenwood *et al*., 2012) Hepatitis C infection is a contagious liver disease that results from infection with the hepatitis C virus.

2.2.1 Modes of Transmission

Hepatitis C virus is spread parenterally (Ejiofor *et al*., 2010) or when blood from an infected person enters the body of someone who is not infected (CDC, 2010; CDC, 2011; CDC, 2013). This infection was spread through blood transfusion and organ transplant before the advent of blood screening in 1992, but today the virus is spread through sharing of needles to inject drugs (Nelson *et al*., 2011; Afolabi *et al*., 2012), tattooing, body piercing or scaring with unsterilized equipment (Vescio *et al*., 2008; Imperial, 2010; CDC, 2010). Centers for Disease Control and Prevention (2010) has however posited that there is little evidence that HCV can spread by getting tattooed in licensed commercial facilities. The most efficient mode of the viral transmission is through large or repeated direct percutaneous exposure to blood such as transfusion or transplantation from infectious donors or injecting drugs use (Rogo *et al.*, 2010). Unfortunately, the screening of blood donors for HCV is not yet routinely performed by some blood banks in developing countries and most new cases in developed countries are related to intravenous drug use (Daw, 2014).

Transmission through sex may be rare but usually occurs when an STD that causes sores or ulcers is present (CDC, 2011; Afolabi *et al*., 2012). The Centres for Disease Conrol and Prevention in 2010 documented that sexual transmission can also occur in people with multiple sex partners and among gaymen. Mother to child transmission can occur during

childbirth when aseptic techniques are not observed (Ugbebor *et al*., 2011). This infection can also be acquired in health care facilities and residential care facilities due to poor infection control mechanism (CDC, 2013). Other routes of contracting this infection include intranasal cocaine use, accidental needle-stick injury and sharing of household items such as nail and hair clippers, razor blades and tooth brushes (Ejiofor *et al*., 2010).

Current major risk factors for infection include IDU (more than 80% of infections) and other procedures requiring skin penetration, such as non sterile injections, tattooing, and other body art (Awofeso, 2010; Brooks *et al*., 2013). In industrialized countries, transmission of HCV is largely attributed to injection drug use (Bretana *et al*., 2015). Prolonged survival of HCV on blood contaminated skin, cloth, and other object emphasizes the importance of fomites in nosocomial spread (Rogo *et al*., 2010). Contact Sport and Slam Dancing that may lead to accidental blood-to-blood exposure have been reported by Udeze *et al*. (2011) to be potential sources of HCV as well as common practices like scarification and circumcision which are common practices among African countries including Nigeria.

2.2.2 Pathogenesis

The mechanisms for the onset and progression of hepatic lesions during chronic HCV infection are not fully understood. Several arguments suggest that HCV itself is not cytopathic, including the lack of any relationship between HCV viral load and the severity of liver disease (Poynard *et al.,* 2003). Brooks *et al*. (2013) also reiterated the non-cytopathogenic nature of the virus and it is believed that the cellular damage seen is

immune mediated even though less is known about the host response because most acute infections are mild and chronic infections are insidious.

Liver damage in chronic hepatitis C is mainly represented by portal lymphoid infiltration, focal and bridging necrosis, and degenerative lobular lesions. The lesions seem to be due to the local immune response, which might be amplified by the accumulation of specific T cells that are recruited by adhesion molecule and chemokine expression. The lymphoid infiltrate is majorly composed of CD4 T cells located within the periportal space (Fiore *et al.*, 1997).

The severity of liver lesions is related to local expression of Th1 cytokines. Cytotoxic T lymphoid responses also appear to play an important role by killing both infected and bystander cells. However, various studies using different approaches have failed to establish a clear relationship between the abundance of CTLs in the liver and the severity of liver lesions. Other cells, such as CD4, natural killer T (TNK) cells and T-cell receptor (TCR) cells might therefore be involved (Tian *et al*., 2013).

2.2.3 Complications of HCV infection

Fibrosis is the main complication of chronic HCV infection that occurs as a result of the deposition of extracellular matrix components within the liver parenchyma (Bedossa and Paradis, 2003). Fibrogenesis is characterized by significant increases in collagen and other extracellular matrix constituents, such as laminin, fibronectin and proteoglycans (Rockey, 2000). This process leads to the involvement of neighboring mesenchymal structures and to the formation of fibrous septa. The progression fibrosis results directly from chronic

inflammation of the liver, which is associated with chronic destruction of liver cells and local production of cytokines and growth factors. During HCV infection, hepatic stellate cells are activated as a result of liver inflammation and cell injury. This complex activation process leads to the generation of myofibroblasts that are capable of producing large amounts of extracellular matrix components and smooth muscle a-actin. The role of viral factors in the progression of the fibrosis process is unclear (Pawlotsky, 2004). The main complications of HCV include the following:

Cirrhosis: This is a frequent complication of HCV infection. Cirrhosis is characterized by the replacement of normal liver tissues by scarred tissues leading to loss of liver function (Gores and Talwalkar, 2014). Approximately 20% to 50% of individuals who are HCV-infected will develop cirrhosis within approximately 20 years of becoming infected (Brooks *et al*., 2013). The progression to cirrhosis is known to be more rapid in HCV/HIV coinfected patients who have not received protease inhibitors, who have consumed excess alcohol, or who have a low $CD4^+$ cell count (Hann and Civan, 2014).

In cirrhosis, fibrotic scars are formed within the parenchyma of the liver. The liver becomes increasingly stiffer and noncompliant due to the fibrotic scarring. Cirrhosis increases resistance to blood flow and higher pressure in the portal venous system leading to portal hypertension that further results in splenomegaly, hemorrhoids and esophageal varices (Friedman, 2014). Hepatic decompensation ensues, with ascites as the salient marker. Other signs and symptoms of cirrhosis include anorexia, weight loss, nausea, spider veins, weakness, fatigue, jaundice, hemorrhage, decreased libido, itching, dependent edema, and mental confusion (Phillips and Brewer, 2003).

Hepatic Failure: Liver failure occurs in approximately 10% of individuals who are HCV infected. Some individuals learn of their HCV infection many years after they were originally infected when the signs of cirrhosis or hepatic failure appear. When hepatic function becomes severely compromised, it loses its normal functions. The liver is unable to filter pathogens and clear toxins, drugs, and metabolic waste products from the blood. In hepatic failure, the liver is unable to metabolize fats, proteins, and carbohydrates to provide nourishment. Symptoms of hepatic failure may include jaundice, ascites, variceal bleeding, or hepatic encephalopathy (Phillips and Brewer 2003).

Hepatocellular Carcinoma is the ultimate complication of chronic HCV infection. It occurs at an annual incidence of 1–8% in patients with HCV-related cirrhosis (Hann and Civan, 2014). Cirrhosis appears to be the main risk factor for hepatocellular carcinoma, but exogenous factors could also play a role, such as chronic alcohol consumption, viral co-infection, diabetes and obesity. The specific role of the virus in triggering or facilitating oncogenic events is controversial. Several viral proteins have been shown *in vitro* to possess functions that could favor hepatocarcinogenesis (Hann and Civan, 2014).

In particular, the core protein, NS3, NS4B and NS5A can transform various cell lines, either alone or in cooperation with oncogenes (Pawlotsky, 2004). Cardinal signs and symptoms of hepatic carcinoma are abdominal pain, ascites, anorexia, weight loss, portal hypertension, and erythrocytosis. Hepatocellular carcinoma metastasizes through circulation to the heart, lungs, brain, kidney, and spleen (Phillips and Brewer, 2003).

2.2.4 Clinical Presentation

According to CDC (2010), hepatitis is the inflammation of the liver and is often caused by viruses although heavy alcohol use, toxins, some medications and medical conditions can also cause hepatitis. Exposure to certain chemicals, autoimmune diseases and bacterial infections can be a foundation of this infection (Agarry and Lekwot, 2010; Ugbebor *et al*., 2011). This contagious liver disease ranges from very mild illness showing few or no symptoms to a serious condition requiring hospitalization (Alter, 2007; CDC, 2010; WHO, 2013). The incubation period for hepatitis C ranges from 2 weeks to 6 months (Kawo *et al*., 2012; WHO, 2013). About 20% of HCV-infected individuals recover from acute infection, and the cell-mediated immune response has been recognized as the major factor allowing this recovery. In contrast, a defective immune response is associated with persistent infection in 80% of infected individuals, leading to the development of chronic hepatitis infection and the occurrence of cirrhosis in 0.5 to 30% of cases and development of hepatocellular carcinoma at a rate of 1 to 3% per year (Breiman *et al*., 2005).

Acute infection is mostly undetectable and some acutely infected patients will experience spontaneous resolution (spontaneous clearance) while the majority will develop chronic HCV infection (Kamal *et al*., 2014). Acute infection occurs within the first 6 months after exposure and it is a short term illness with 15-25% of the infected persons resolving the infection without treatment (CDC, 2013). Genome-wide association studies have recently identified single-nucleotide polymorphisms (SNPs) in proximity to the interleukin-28B (IL-28B) gene that can predict or is strongly related to spontaneous clearance of acute

infection (Greenwood *et al*., 2012; Kamal *et al*., 2014). The IL28B is located on chromosome 19 and expresses interferon-lamda-3 (KASL, 2014).

When symptoms occur during this stage, they include fever, fatigue, abdominal pain, nausea, vomiting, dark urine and jaundice (yellowed eyes and skin) and liver enzymes level may be normal (Collins and Swan, 2009). Asymptomatic female under age 40 years are likely to have spontaneous clearance. Factors related to spontaneous recovery are hepatitis accompanying jaundice, female, low viral load, and genotype (KASL, 2014). Approximately 75-85% of people who become infected with HCV develop chronic or lifelong infection (Gao, 2011; WHO, 2013; Casanova *et al*., 2014) and 1-5% develop liver cancer (CDC, 2010). According to Ray and Ryan (2004) and Sheyin *et al.* (2012), chronic disease occurs over time and can lead to liver problems including liver damage (fibrosis), cirrhosis, hepatocellular carcinoma (cancer) or liver failure alongside depression, confusion (brain fog), steatosis (development of fat in the liver), and anorexia (Collins and Swan, 2009). Most people do not show symptoms when having chronic hepatitis C, symptoms and signs of the chronic stage can take up to 30 years to develop (CDC, 2010).

George and Bruce (2001) opined that factors that accelerate clinical progression include alcohol intake (affects the course of disease), co-infection with HIV-1 or HBV, male sex, and an older age at infection. Excessive alcohol intake by chronic hepatitis C patients is strongly related to occurrence of cirrhosis, and increases risk of HCC. Co-infection of HIV or HBV in chronic HCV infection causes faster progression of liver diseases and increases the risk of HCC compared to HCV single infection (KASL, 2014). Extrahepatic

manifestation may be seen in minority of infected individual such as vasculitis, glomerulonephritis caused by immune complex and association between HCV and mix cryoglobulinaemia has been suggested (Greenwood *et al*., 2012)

2.2.5 Epidemiology

As of 2010, the epidemiology of HCV infection in Nigeria is limited (Ejiofor *et al*., 2010). The virus has no ethnic, socio-economic, gender, age or geographic boundaries (Rogo *et al*., 2010). It appears to have a narrow host range, human and chimpanzees are the only known species susceptible to infection, with both species developing similar disease (Pennap *et al*., 2010; Kawo *et al*., 2012). About 3% (200 million) of the world's population are infected with chronic HCV (Bala *et al*., 2012; Godwin *et al*., 2013), this represents a viral pandemic (George *et al.,* 2001). The highest prevalence of HCV found in Egypt (20%-30%) is attributed to unsterilized needles that were used for the mass treatment of schistosomiasis (Shalaby *et al*., 2010; Godwin *et al*., 2013). There are seven major genotypes of HCV and many hundreds of subtypes distributed globally, with genotype 1a being the most prominent virus in North America and genotype 1b infecting most people worldwide (Yu and Chuang 2009; Law *et al*., 2013). Genotype -1 is prevalent in West African countries and patients with HCV genotype 1 and 4 have more rapid development of fibrosis (Nwezi, 2006). Genotype 4 was found to be prevalent in Egypt (Yu and Chuang 2009).

Yu and Chuang (2009) also posited that genotype 1b is the dominant type in Asia-pacific particularly Japan, South Korean, China and Taiwan. According to Collins and Swan

(2009), genotypes 1a and 1b are predominant in Europe, North America and Japan; while in Southeast Asia it is genotype 3; genotype 4 leads in Egypt, Middle East and Central Africa; South Africa has genotype 5 as predominating. Pybus *et al*. (2009) reported that genotype 6 is prevalent in East Asia and no region has yet been found to contain high levels of HCV genotype 5 Greenwood *et al.* (2012) posited that genotype 5a and 6a are predominant in South Africa and East Asia.

It has also been observed that certain variants respond poorly to available prophylactic treatment and genotype 1, which is prevalent in West African countries, has been implicated. Patients with genotypes 1 and 4 were found to be less likely to respond to interferon (IFN) therapy than genotypes 2 and 3 (Sheyin *et al*., 2012; Brooks *et al*., 2013). Genotype 2 responds best to interferon treatment, type 3 shows the highest rate of spontaneous clearance and type 4 has the highest frequency of chronic infection (Brooks *et al*., 2013).

The prevalence of anti-HCV among IDUs varies among countries, although rates are constantly very high. The proportion ranges between 50% in Cyprus to >80% in Germany (Francesco, 2014). Globally, the prevalence of HCV infection among incarcerated populations has been reported to range from 9.6% to 41% (Alvarez *et al*., 2014) and accounts for more than 90% of hepatitis after blood transfusion (Willey *et al*., 2013). The prevalence rate of HCV infection in Nigeria is considerably high ranging from 5.8% to 12.3% (Ojide *et al*, 2015). Alvarez *et al*., (2014) reported HCV prevalence in the U.S. general population to be 1% to 2%.

2.2.6 Diagnosis

The infected populations are mostly unaware of their status and thus are more likely to come down with complications. This is because patients do not develop symptoms until they have progressed to advanced cirrhosis therefore, early diagnosis of the disease is essential to curb the progression of the disease (Nwezi, 2006). In the diagnosis of HCV, the first screening test (serological assay) is aimed at assaying for antibodies to hepatitis C virus (Ejiofor *et al*., 2012). The various tests to detect the antibodies include Enzyme Imunoassays (EIA) which contains antigens from core and non structural genes, Recombinant Immunoblot Assay (RIBA) (Ejiofor, *et al*., 2010) that detects HCV-specific antibodies on nitrocellulose strips (KASL, 2014). In cases of positive antibody screening, different blood tests (Follow- up tests) are needed to determine whether the infection has been cleared or has become a chronic infection (CDC, 2013). Specific antibodies can be detected 7 to 8 weeks after infection and HCV RNA becomes detectable about one or two weeks after infection, then usually peaks before disappearing in cases that spontaneously resolve and (Pawlotsky, 2004)

Ribonucleic acid tests (molecular assays that detect, quantify, and characterize HCV RNA genotypes and subtypes) such as Transcription Mediated Amplification (TMA) and Reverse Transcription–Polymerase Chain Reaction (RT-PCR) are used to assay for RNA in those with reactive antibody test (Ejiofor *et al*., 2010). A negative RNA test implies that an infection has occurred but spontaneous clearance has taken place while a positive RNA test means the individual is currently having the virus in the blood (CDC, 2013).

Other diagnosis to be carried out as specified by CDC (2010) include Liver Function Test (liver panel) to tell how well the liver is working, Serum alanine aminotransferase level (ALT) which is elevated when there is liver injury and reveals efficacy of therapy, an Ultra Sound to create a visual image of the liver, Liver Biopsy (Removal of a tiny bit of the liver for direct examination), a Genotype Test to determine the type of HCV and a viral load test to ascertain how much virions one has in the body. Widely used laboratory procedures for genotyping HCV that target various HCV genomic regions include the line probe assay (LiPA) and UTR sequencing. Restriction fragment length polymorphism (RFLP) analysis of the 5´UTR of the HCV genome is an old assay for genotyping that involves digesting a PCR-amplified hepatitis C virus dribonucleic acid (HCV-DNA) into fragments with restriction enzymes that recognize cleavage sites specific for each genotype (Casanova *et al*., 2014).

Recently, use of enhanced chemiluminescent immunoassay (CLIA) or electro chemiluminescence immunoassay (ECLIA) is increasing since antigen-antibody reaction is detected more sensitively by these assays compared to third generation EIA (KASL, 2014). Meanwhile, there is point-of-care test using saliva or finger stick blood producing rapid results within 20 minutes. Ji Hoon *et al*. (2013) documented a new assay system suitable for quantifying infectious HCV particles using aptamers against HCV E2. In this new assay, the Enzyme Linked Apto-Sorbent Assay (ELASA) which is similar to ELISA assay, two different aptamers that recognize HCV E2 proteins are used instead of antibodies.

2.2.7 Treatment

Decisions about the commencement of antiviral treatment are based on the genotype of virus, the condition of the liver and other health conditions (CDC, 2010). Viral genotype determination before commencement of treatment is advised as a routine assay and patients infected with genotypes 1 and 4 having viral loads higher than 800,000IU/ml must be treated for one year while those infected with other genotypes may be treated for 6 months (Campiotto *et al*., 2005). The treatment is aimed at achieving a sustained virologic response (undetectable HCV-RNA 6 months after termination of therapy), improvement of histology and prevention of hepatocellular carcinoma (Ejiofor *et al.*, 2010).

The European Consensus Development and National Institute of Health suggested that patients with elevated aminotransferase level, detectable RNA and chronic inflammation with some degree of fibrosis should be offered treatment (NHI, 2002). According to George and Bruce (2001), the first successful treatment of HCV was documented in 1989 with interferon alfa but relapse occurred. Present antiviral agents that are used are pygelated interferon in combination with ribavirin which gives a better treatment response than monotherapy (George and Bruce, 2001; Godwin *et al*., 2013). Pegylated interferon (peginterferon alfa), a product of the combination of polyethylene glycol and interferon-alfa extends the half-life and duration of therapeutic activity of interferon alfa (George and Bruce, 2001; Collins and Swan, 2009). Those who develop cirrhosis or liver cancer may require a liver transplant, though the virus universally recurs after transplantation (Afolabi *et al*., 2012).

Treatment consists of 3 million U of interferon alfa administered subcutaneously three times a week and 1200 mg of ribavirin orally per day for patients who weigh at least 75 kg and 1000 mg of ribavirin orally per day for those weighing less than 75 kg. Usually, ribavirin is taken in divided doses, given in the morning and evening, and interferon is given before bedtime (George *et al.,* 2001). Side effects such as flu-like symptoms, depression, weight loss, rash, anaemia and insomnia can disrupt one's life and day-to-day activities (CDC, 2010).

Ribavirin causes side effects like ribavirin induced anaemia, nasal congestion, pruritis and gout while seizures, loss of libido, cardiotoxicity, hearing impairment, alopecia, myalgia leucopenia thrombocytopenia and neutropenia among others are attributed to Interferon-alpha (George and Bruce, 2001). In treating HCV/HBV co-infection, it is essential to determine the dominant virus by serological and virological testing before initiation of therapy, treatment guides in existence for patients with chronic hepatitis C and chronic hepatitis B mono-infection can be applied to co-infected patients (Chi-Jen and Shou-Dong, 2008)

2.2.8 Prevention and Control

Understanding the modes of transmission of HCV and the ways it spreads among communities and healthcare settings, plays important steps in formulating the strategies of prevention of such infectious virus. Hepatitis C transmission is preventable (Daw, 2014). There is no vaccine for the prevention of HCV (Muhktar *et al*., 2006). Hepatitis C virus can be prevented by avoidance of sharing of tattooing, piercing or cutting equipment that

has been used by someone else (CDC, 2011). Education and greater availability of disposable needles will go a long way in the prevention of this infection (Greenwood *et al*., 2013). Implementation of international and national guidelines regarding the prevention of HCV particularly at special hospital settings as blood banks, hemodialysis units and high risk groups at the community will be of paramount importance in the prevention and control of this infection (Daw, 2014)

In the control of HCV infection, the most logical thing to do is to prevent contracting the virus as there is no vaccine against it due to its high genome mutability. Health education campaign on the different routes of transmission and other preventive measures can help in controlling the spread especially in developing countries like Nigeria (Ejiofor *et al*., 2010). Careful handling of blood and body fluids should be encouraged, communal sharing of shaving equipment, blades and sharp instrument, barbing and manicure apparatus, body piercing/tattooing should be discouraged.

2.3 The Hepatitis B Virus

Hepatitis B virus (HBV) is classified as an *Orthohepadnavirus* belonging to the family *Hepadnaviridae* with a very high transmissibility (Isa *et al*., 2015). The infecting virus is a 42nm spherical particle which contains DNA and DNA polymerase called the Dane particle. The virus is composed of 27nm nucleocapsid core surrounded by an outer lipoprotein coat containing the Hepatitis B surface antigen (HBsAg) (Ado *et al*., 2010). The viral genome is 3.2 kb in length, consisting of four partially overlapping, open reading frames that encode viral proteins. Viral replication takes place predominantly in hepatocytes. The infecting virus encases its double-shelled Dane particles within

membrane envelopes coated with hepatitis B surface antigen. The inner nucleocapsid core antigen encloses a single molecule of double-stranded HBV DNA and an active DNA polymerase.

Serum from individuals infected with hepatitis B contains three distinct antigenic particles: the Dane particle, a spherical 22 nm particle, and tubular or filamentous particles that vary in length (Willey *et al*., 2013). The virus particle consists of an outer lipid envelope and an icosahedral nucleocapsid core which is composed of protein. The outer envelope contains proteins that are involved in viral binding and entry into susceptible cells (Howard, 1986). The outer surface or envelope contains hepatitis B surface antigen (HBsAg) and surrounds the inner nucleocapsid core that contains hepatitis B core antigen (HBcAg) (Geo *et al*., 2007).

2.3.1. Replication

Hepatitis B virus is quite different from other DNA viruses in terms of replication, this is because they have circular dsDNA genomes that consist of one complete but nicked strand and a complementary strand that has a large gap that is, it is incomplete. After infecting the host cell, the virus's gapped DNA is released into the nucleus. There, host repair enzymes fill the gap and seal the nick, yielding a covalently closed, circular DNA (cccDNA). Transcription of viral genes occurs in the nucleus using host RNA polymerase and yields several mRNAs, including a large 3.4 kilobase RNA known as the pregenome (plus-strand RNAs). The RNAs move to the cytoplasm, and the mRNAs are translated to produce viral proteins, including core proteins and a polymerase having three activities (DNA

polymerase, reverse transcriptase, RNase H). Then the RNA pregenome associates with the polymerase and core protein to form an immature core particle. Reverse transcriptase subsequently reverse transcribes the RNA using a protein primer to form a minus-strand DNA from the pregenome RNA. After almost all the pregenome RNA has been degraded by RNase H, the remaining RNA fragment serves as a primer for synthesis of the gapped dsDNA genome, using the minus-strand DNA as template. Finally, the nucleocapsid is completed and the progeny virions are released. (Willey *et al*., 2013)

2.3.2 Transmission

Hepatitis B virus is spread mainly by percutaneous or mucosal exposure to infected blood and other body fluids with numerous forms of human transmission (Aba and Aminu, 2016). Hepatitis B virus is normally detected in saliva, sweat, semen, breast milk, urine, feces, peripheral mononuclear cells, tissue of pancreas, kidney, tears, vaginal secretions and contaminated equipment including shared intravenous needles (Willey *et al*., 2013; Aba and Aminu, 2016). Hepatitis B virus can also be transmitted among family members within households from carriers, possibly by contact of non intact skin or mucous membrane with body secretions containing HBV, oral route sexual or intimate exposure (Aba and Aminu, 2016).

The virus can also pass through the placenta to the fetus of an infected mother and in highly endemic areas and is most commonly spread from mother to child at birth, or from person to person in early childhood (WHO, 2013). Perinatal or early childhood transmission may also account for more than one third of chronic infections in areas of low

endemicity, although in those settings, sexual transmission and the use of contaminated needles, especially among injecting drug users, are the major routes of infection. The HBV can survive outside the body for at least seven days and during this time, the virus can still cause infection if it enters the body of a person who is not protected by the vaccine. The HBV is not spread by contaminated food or water, and cannot be spread casually in the workplace (WHO, 2013).

All HBsAg-positive persons are infectious (CDC, 2015), but those who are also positive for hepatitis B envelope antigen (HBeAg) circulate HBV at high titers (greater than 1million IU/mL) in their blood (Greenwood *et al*., 2012). The HBV is relatively stable in the environment at room temperature and remains viable for at least 7 days on environmental surfaces in the absence of any visible blood therefore, surfaces also play an important role in the transmission of hepatitis B infection (Shepard *et al*., 2006).

2.3.3 Clinical Manifestation

Hepatitis B infection is a liver disease that results from infection with Hepatitis B virus (CDC, 2013). The virus infects liver hepatic cells and causes liver tissue degeneration and the release of liver-associated enzymes (transaminases) into the bloodstream (Willey *et al*., 2009). The disease is either acute or chronic (Sunbul, 2014; CDC, 2015). The acute disease is a short term illness and occurs within 6 months after the individual is exposed to the virus and does not always lead to chronic infection (CDC, 2013). Persistence of HBsAg, HBeAg and high levels of HBV DNA in serum for more than 6 months after the primary infection is defined as chronic HBV infection. Shepard *et al*.

(2006) reported that the likelihood of developing a chronic infection depends on the age at which a person becomes infected. Young children are most likely to develop a chronic infection when infected with the virus and about 80-95% of infants infected during the first year of life develop chronic infection (Brooks *et al*., 2013). Five percent of adults develop chronic infection and 15-25% of adults who become chronically infected during childhood die of liver cirrhosis or cancer related to HBV. More than 90% of healthy adults who are infected with the HBV will recover and be completely rid of the virus within six months (WHO, 2013).

Most cases are asymptomatic. Most people do not experience any symptoms during the acute infection phase. However, some people have acute illness with symptoms that last several weeks, including fever, loss of appetite, abdominal discomfort, nausea, extreme fatigue, vomiting, dark urine, changes in stool color, and hepatomegaly or splenomegaly and other symptoms gradually appear following an incubation period of 1 to 3 months (Shepard *et al.*, 2006; WHO, 2013). This is followed by jaundice, the accumulation of bilirubin (a breakdown product of hemoglobin) in the skin and other tissues with a resulting yellow appearance. Extra hepatic manifestations of disease such as skin rashes, arthralgias, and arthritis can also occur (CDC, 2001). In some people, hepatitis B infection causes a chronic liver infection that later develop into primary liver cancer, known as hepatocellular carcinoma (Willey *et al.*, 2013).

2.3.4 Hepatitis B Virus Genotypes

The virus is divided into ten genotypes (A–J) according to overall nucleotide sequence variation of the genome and four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope proteins (Sunbul, 2014). The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination (Kramvis *et al*., 2005).

Different genotypes may respond to treatment in different ways (Palumbo, 2007; Mahtab *et al*., 2008). The genotypes differ by at least 8% of the sequence and have distinct geographical distributions (Sunbul, 2014). Type F which diverges from the other genomes by 14% is the most divergent type known. Type A is prevalent in Europe, Africa and South-east Asia, including the Philippines. Type B and C are predominant in Asia; type D is common in the Mediterranean area, the Middle East and India; type E is localized in sub-Saharan Africa; type F (or H) is restricted to Central and South America. Type G has been found in France and Germany. Genotypes A, D and F are predominant in Brazil and all genotypes occur in the United States with frequencies dependent on ethnicity (Sunbul, 2014).

Within genotypes, 24 subtypes have been described which differ by 4–8% of the genome (Sunbul, 2014). Type A has two subtypes: Aa (A1) in Africa/Asia and the Philippines and Ae (A2) in Europe/United States. Type B has two distinct geographical distributions: Bj/B1 ('j'—Japan) and Ba/B2 ('a'—Asia). Type Ba has been further subdivided into four clades. Type C has two geographically subtypes: Cs (C1) in South-east Asia and Ce (C2)

in East Asia. The C subtypes have been divided into five clades (C1–C5). A 6th clade (C6) has been described in the Philippines but only in one isolate (Cavinta *et al*., 2009). Type C1 is associated with Thailand, Vietnam, and Myanmar; type C2 with Japan, Korea and China; type C3 with New Caledonia and Polynesia; C4 with Australia; and C5 with the Philippines (Lucida *et al*., 2008).

Type D has been divided into 7 subtypes (D1–D7). Type F has been subdivided into 4 subtypes (F1–F4). Subtype F1 has been further divided into 1a and 1b. In Venezuela, subtypes F1, F2, and F3 are found in East and West Amerindians. Among South Amerindians only F3 was found (Sunbul, 2014).

2.3.5 Epidemiology of Hepatitis B Virus

Of the 2 billion people who have been infected with HBV globally, more than 350 million have chronic (lifelong) infections and over 20 million people are infected annually with this virus (Ugbebor *et al*., 2011). It is a major disease of serious global public health proportion and preventable with safe and effective vaccines that have been available since 1982 (Ugbebor *et al*., 2011). The proportion of patients who develop chronic HBV infection varies with age at infection ranging from more than 90% of infants born to highly infectious HBeAg-positive mothers to less than 1% in adults, with an intermediate frequency of approximately 30% in children infected after the neonatal period but before the age of 5 years (Brooks *et al*., 2013: Willey *et al*., 2013).

Humans are the sole reservoir of HBV. Routes of infection include vertical transmission (such as through childbirth), early life horizontal transmission (bites, lesions, and sanitary habits), and adult horizontal transmission (sexual contact, intravenous drug use) (Custer *et*

al., 2004). In low prevalence areas such as the continental United States and Western Europe, injection drug abuse and unprotected sex are the primary modes of transmission, although other factors may also be important (Redd *et al*., 2007).

Aba and Aminu (2016) posited that Nigeria has been classified as one of the groups of countries with highly endemic HBV infection and has a carriage rate in the range of 9-39%. In moderate prevalence areas, which include Eastern Europe, Russia, and Japan, where 2-7% of the population is chronically infected, the disease is predominantly spread among children. The prevalence of chronic HBV infection in areas of high endemicity is at least 8% with 10-15% prevalence in Africa/Far East (Komas *et al*., 2013). The worldwide prevalence of HBV infection has been described and HBsAg prevalence of < 2% s regarded as low prevalence, 2-7% as intermediate prevalence and ≥ 8% as high prevalence (Hwang and Cheung 2011).

2.3.6 Diagnosis of Hepatitis B Virus

A number of blood tests are available to diagnose, monitor people with hepatitis B (Ado *et al*., 2010) and distinguish acute and chronic infections. Laboratory diagnosis of hepatitis B infection centers on the detection of the hepatitis B surface antigen (HBsAg) which is the first detectable viral antigen to appear during infection. However, early in an infection, this antigen may not be present and it may be undetectable later in the infection as it is being cleared by the host.

Acute HBV infection is characterized by the presence of HBsAg and immunoglobulin M (IgM) antibody to the core antigen, HBcAg. If the host is able to clear the infection,

eventually the HBsAg will become undetectable and will be followed by IgG antibodies to the HBsAg and core antigen (anti-HBs and anti HBc IgG) (Zukerman, 1996). A person negative for HBsAg but positive for anti-HBs has either cleared the infection or has been vaccinated previously. Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers (Lok and McMahon, 2007). Chronic infection is reflected by elevated serum alanine aminotransferase (ALT) levels and inflammation of the liver in carriers.

During the initial phase of infection, patients are also seropositive for HBeAg. Chronic infection is characterized by the persistence (>6 months) of HBsAg (with or without concurrent HBeAg). Persistence of HBsAg is the principal marker of risk for developing chronic liver disease and hepatocellullar carcinoma (HCC) later in life. The presence of HBeAg indicates that the blood and body fluids of the infected individual are highly contagious (WHO, 2013). reaction assays are used to detect and quantitate the amount of HBV DNA, called the viral load, in clinical specimens. These tests are used to assess a person's infection status and to monitor treatment (Zoulim, 2006). Individuals with high viral loads, characteristically have ground glass hepatocytes on biopsy.

2.3.7 Treatment

There is no specific treatment for acute hepatitis B. Care is aimed at maintaining comfort and adequate nutritional balance, including replacement of fluids that are lost from vomiting and diarrhoea (CDC, 2013). Acute hepatitis B infection does not usually

require treatment and most adults clear the infection spontaneously (Hollinger and Lau 2006). Treatment in chronic cases may be necessary to lessen the risk of developing cirrhosis and liver cancer and improve long term survival (WHO, 2013). Chronically infected individuals with persistently elevated serum alanine aminotransferase (a marker of liver damage) and high HBV DNA levels are candidates for therapy. Treatment may last from six months to a year, depending on medication and genotype (Alberti and Caporaso, 2011).

The World Health Organization recommended a combination of tenofovir and entecavir as first line agents (WHO, 2015). Other drugs used include antiviral drugs lamivudine (Epivir), adefovir (Hepsera), telbivudine (Tyzeka) and two immune system modulators interferon alpha-2a and PEGylated interferon alpha-2a (Pegasys). Response to treatment differs between the genotypes. Interferon treatment may produce an e antigen seroconversion rate of 37% in genotype A but only a 6% seroconversion in type D. Genotype B has similar seroconversion rates to type A while type C seroconverts only in 15% of cases. Sustained e antigen loss after treatment is 45% in types A and B but only 25–30% in types C and D (Cao, 2009).

Monotherapy with alpha interferon or lamivudine is approved by the U.S. Food and Drug Administration (FDA) to treat patients with chronic hepatitis B. Although the dosage of lamivudine should be modified based on creatinine clearance in patients with renal impairment, no additional dose modification is necessary after routine hemodialysis (CDC, 2001).

2.3.8 Prevention of Hepatitis B Infection

The best way to prevent Hepatitis B is by getting vaccinated (Greenwood *et al*., 2012). It can also be prevented by avoiding contact with infected blood and body fluids, including semen and vaginal secretions of infected individuals (Ado *et al*., 2010). WHO (2013) recommends that all infants receive the hepatitis B vaccine as soon as possible after birth preferably within 24 hours especially infants of HBV infected mothers (Aspinall *et al*., 2011). The birth dose should be followed by 2 or 3 doses to complete the primary series. A protective response to the vaccine is defined as an anti-HBs antibody concentration of at least 10 IU/ml in the recipient's serum. The vaccine is more effective in children and 95% of those vaccinated have protective levels of antibody. This drops to around 90% at 40 years of age and to around 75% in those over 60 years.

Testing to verify effective immunization is recommended and further doses of vaccine are given to those who are not sufficiently immunized (Aspinall *et al*., 2011). Those at high risk of infection should be tested before vaccination, they include people who frequently require blood or blood products, dialysis patients, recipients of solid organ transplantations, people interned in prisons, injecting drug users, people with multiple sexual partners, as well as health-care workers and travelers who have not completed their hepatitis B vaccination series should be offered the vaccine before leaving for endemic areas (WHO, 2013).

2.4 HCV and HBV Co-infection

Cancer and cirrhosis of the liver due to hepatitis has been announced as the ninth cause for mortality in the world and HBV and HCV are among the most important and dangerous types of viral hepatitis (Ataei *et al*., 2015). Because they share similar modes of transmission, co-infection is not uncommon especially in areas of high prevalence and among people at high risk for parenteral infection (Pennap *et al*., 2010) such as injection drug use, sexual contact, or from mother to child during pregnancy or birth (Ojide *et al*., 2015). Co-infection of HBV and HCV seems to result in more severe disease than either infection alone. In some co-infected patients, each virus might exert its own pathogenetic role, causing a cumulative effect in terms of liver injury that may explain the high grade of disease severity frequently observed in case of co-infection (Chi-Jen and Shou-Dong, 2008).

Hepatitis B virus and HCV infections are important global public health problems (Chi-Jen and Shou-Dong, 2008). The worldwide prevalence of co-infection with HBV and HCV is unknown due to lack of large-scale population-based studies. Moreover, occult or clinically silent HBV infection (undetectable HBsAg with detectable HBV DNA in serum) has been described in patients with chronic hepatitis C infection (Brooks *et al*., 2013) by highly sensitive molecular techniques (Chi-Jen and Shou-Dong, 2008). Co-infection of HBV/HCV is frequently found in several high-risk populations, such as injection drug users, patients on hemodialysis, patients undergoing organ transplantation.

Chi-Jen and Shou-Dong (2008) affirmed that HBV and HCV interact with each other and affect immune responses. Infection with HCV can suppress HBV replication, as demonstrated by acute HCV superinfection in chimpanzees with chronic HBV infection that resulted in a significant reduction of serum HBsAg titer (Chi-Jen and Shou-Dong, 2008). This suppression and inhibitory effect is mediated by HCV core protein (Chen *et al*., 2003).

2.5 Prison Inmates and Psychiatric Patients

The worldwide prevalence of infections caused by HBV and HCV among psychiatric patients is of concern due to their endemic nature in residential institutions for persons with mental illness (Kakisi *et al*., 2009). Sero-prevalence of HCV among mentally ill patients is approximately 11 times that of the general adult population (Daw, 2014). The characteristics of psychiatric patients as posited by Marc *et al*. (2011) include intravenous drug use, being single or divorced, practicing risky sexual behavior, and having reduced awareness about the potential modes of transmission of infectious diseases. Others include reduced awareness on relevant protective measures, exposure to violent social behavior as well as substandard living or hospitalization conditions among others. Individuals with psychotic disorders are particularly at risk of contracting hepatitis B and C due to lifestyle factors (Omoaregba *et al*., 2013).

Compared to the general population, prisoners globally continue to demonstrate a significantly higher prevalence of HBV, HCV and HIV infections (Francesco, 2014; Kakar *et al*., 2014; Rana *et al*, 2015) yet routine screening for HCV infection among prisoners is not generally available (Alvarez *et al*., 2014). The disease-amplification

function of prisons is seen in disproportionately high disease incidence and prevalence, which are documented in most inmate health surveys, and in reports of high rates of infectious disease transmission in prisons (Awofeso, 2010). The prevalence rate may be connected to risk factors before incarceration such as low socioeconomic status, poor access to health care, high-risk sexual behaviors and injection drug use. Factors such as crowded living conditions, sexual harassment, non-professional tattooing, body piercing and poor ventilation systems increase the infection risk after entrance to prison (Nokhodian *et al*., 2012).

Intravenous drug use is the most important factor for HCV infection and its spread whilst in prison (Francesco, 2014). Imprisonment is considered a major risk factor for HCV infection, with the risk of infection directly proportional to the length of incarceration. Prisons with poorly implemented policies in relation to illicit drug use make inmates more vulnerable to contracting HCV infection (Awofeso, 2014). Marco *et al*. (2014) stated that incarceration does not equate the absence of drug consumption. Prisoners may acquire new infections during incarceration due to poor medical diagnosis and treatment, insufficient infection control, an absence of harm reduction efforts like condom use or needle exchange, and sudden transfer to other facilities or discharge into the community without provisions for ongoing therapy (Unakal *et al*., 2013).

2.6 Review of Empirical Findings

Published data on HCV and HBV infections in correctional facilities and psychiatric patients in Nigeria is scarce. However, Adoga *et al.* (2009) carried out a study on the

prevalence of HCV and HBV among inmates in Nassarawa State and found HBsAg prevalence of 23%, a 12.3% prevalence of HCV and 0.7% of HCV and HBV co-infection rate was recorded. Nimzing *et al*, (2015) found a HCV prevalence of 35% in a study conducted among inmates in Makurdi, Benue State, Nigeria. Monday and Francis (2014) carried out a study of HBV among inmates in Bali prison in Taraba State, a seroprevalence of 18% HBsAg was recorded

Champion *et al.* (2004) found HCV prevalence of 16% in saliva among Scottish inmates as a prevalence of 16% and an incidence of 14.6% were obtained. Bretana *et al.* (2015) found no significant association between HCV infection and demographics and lifetime risk behaviours among Australian inmates. In Barcelona, Spain, Marco *et al*. (2014) found the prevalence of HBsAg and HCV among inmates to be 2.6% and 4.9% respectively. Guimaraes *et al.* (2001) conducted studies on Brazilian prisons and found HBsAg prevalence of 68.1% and 41% for HCV. Rana *et al.* (2015) carried out a study in Tihar Jail, New Delhi on the prevalence of HBsAg and found no inmate positive for HCV and HIV while 2.72% prevalence of HBsAg was found. Unakal *et al.* (2013) found HBsAg seroprevalence of 10.4% among inmates in Ethiopia. Macalino *et al.* (2004) found HBV prevalence of 20.2% and 23.1% of HCV in inmates in Rhodes Island.

In New York, prevalence of 1.3% and 10.1% were obtained for HBsAg and HCV respectively among inmates in a correctional facility (Alvarez *et al.,* 2014). Ataei *et al.* (2015) carried out a study on HBV/HCV co-infection in Iran among inmates and obtained an HBsAg prevalence of 3.3% while HCV was 19.4% with 2.1% co-infection rate. In

Scotland, a 19% HCV prevalence was found among drug users while 3% was found among other prisoners and no inmate accepted to be an IDU (Taylor *et al.,* 2013). Kakar *et al.* (2014) found no HCV/HBV co-infection among jailed inmates in Quetta, Balochistan but obtained a 6.0% and 4.0% prevalence for HBV and HCV respectively.

Semaille *et al.* (2013) worked with French inmates and found a higher HCV prevalence among females with an overall prevalence of 4.8%. Teutsch *et al.* (2010) reported an overall prevalence of 31.6% in Australian prison and observed tattooing to be significantly associated with HCV infection. Stanley *et al.* (2001) carried out a study on the prevalence of HBV and HCV among severe mentally ill patients in U.S.A and obtained 18.8% and 16.1% prevalence of HBcAb and HCV respectively. Sexual trading and IDU were significantly associated with these viral infections. Kakisi *et al.* (2009) carried out a retrospective study on psychiatric patients in Thassaloniki and obtained 2% and 9% prevalence of HBsAg and anti-HCV respectively. Marc *et al.* (2011) obtained a prevalence of 19.6% HCV and 23.4% HBV among patients with severe mental illness. Omoaregbe *et al.* (2013) found a 4.2% HCV and 3.1% HBV prevalence among psychiatric patients even though interaction analysis did reveal statistical association with transfusion.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in Kaduna State, Nigeria. Kaduna State (Figure 3.1) is located in the north west of Nigeria with the capital city as Kaduna which had its name derived from a Hausa word, 'Kada', meaning Crocodile. The State has a land mass of 44,567 square kilometers and on it lies between latitude 10^{o} 20′ N and longitude 7^{o} 45′ E. The estimated population is 6,113,503 as of 2006 census. The state was created on 27th May, 1967 and has 23 Local Government Areas (LGAs) divided into three geo-political zones and senatorial districts namely: Northern zone, Central Zone and Southern Zone (Franklin, 2014).

The State has one Neuro-Psychiatric Hospital and 14 prisons namely; Kaduna convict prison, Open Prison Camp Barnawa, Zaria prison, Kafanchan prison, Birnin Gwari prison, Prison Farm Centre Kujama, Gwantu Satelite Prison, Kwoi Satelite Prison, Manchok Satelite Prison, Kachia Satelite Prison, Saminaka Satelite Prison, Soba Satelite Prison, Ikara Satelite Prison, and Makarfi Satelite Prison. The Kaduna Convict Prison and the Federal Neuro-Psychiatric Hospital were used for this study. One prison out of the many was used for this study due to some restrictions by the prisons management in Kaduna State as a result of the high insurgency in the country as at the time of sample collection.

3.2 Study Design

The Study was a cross-sectional survey where the subjects were selected using random sampling method.

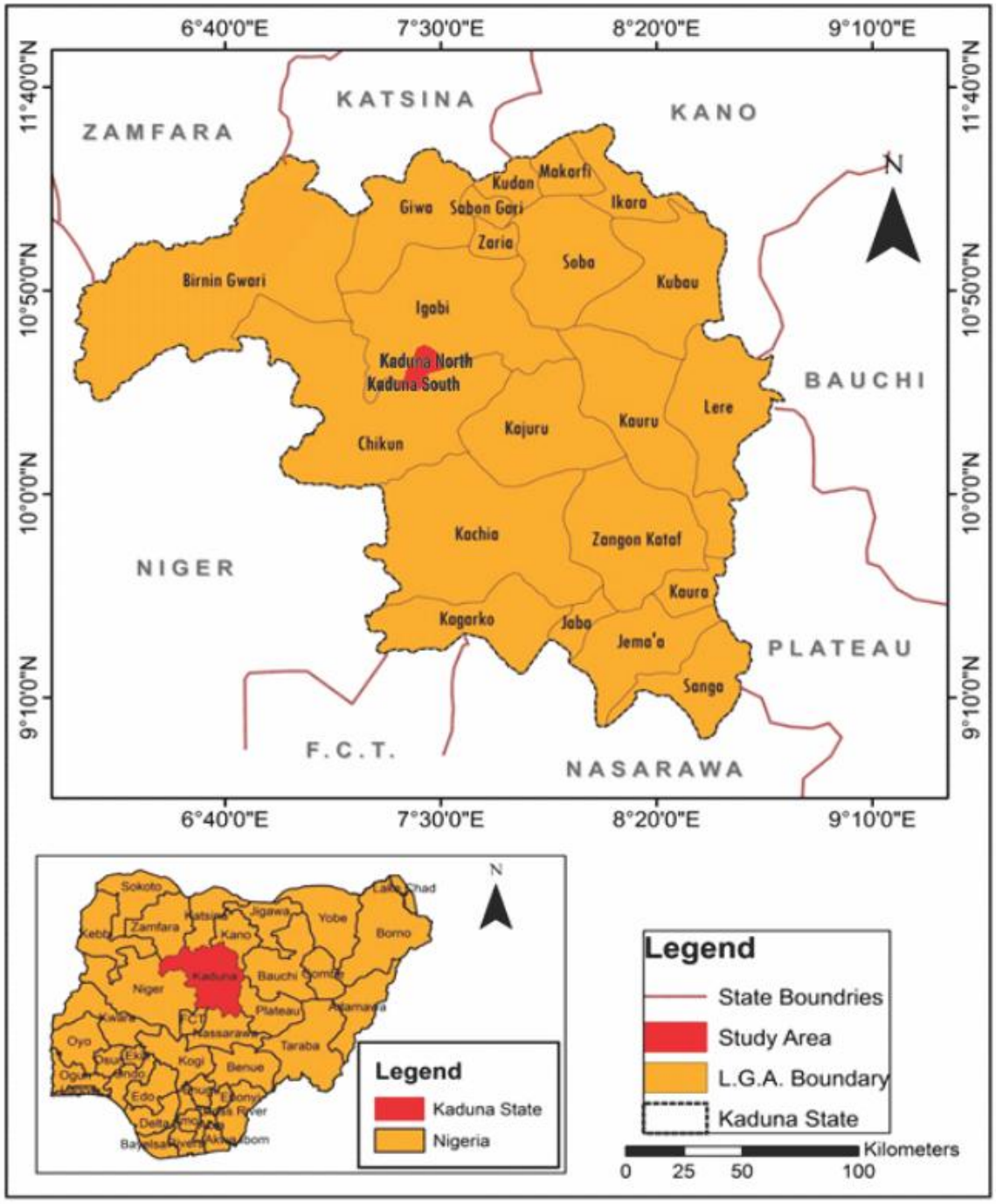


Figure 3.1: Map of Kaduna State showing the study area

Source: Adapted from the administrative map of Kaduna State.

3.3 Study Population

The study population comprised of male and female inmates of Kaduna Convict Prison and out patients attending the Federal Neuro-Psychiatric Hospital, Kaduna State.

3.4 Ethical Approval and Consent

Ethical approval (Appendices Ia, Ib, Ic) was obtained from the Ethical Committees of Kaduna State Ministry of Health, Nigerian Prisons Service and Federal Neuro-Psychiatric hospital, Kaduna. Informed Consent Form (Appendix II) was administered to participants who enrolled in the study for their consent.

3.5 Inclusion and Exclusion Criteria

The inclusion criterion was consenting male and female of 18 years and above, inmates and, psychiatric patients of the selected prison and hospital respectively. The exclusion criterion included non inmates, non-psychiatric patients and non-consenting individuals

3.6 Sample Size

The sample size for this study was determined using a prevalence of 10.1% for the prisoners (Alvarez *et al*., 2014) and 4.2% (Omoaregba *et al*., 2013) for the psychiatric patients at 95% confidence interval using the formula by Naing *et al.* (2006) below:

$$n = \frac{Z^2 pq}{d^2}$$

Where:
n= Sample size
Z= Standard normal distribution at 95% Confidence limit=1.96
P= prevalence rate
q= 1-p
d= allowable error= 0.05

For prisoners:

$$n = \frac{(1.96)^2 X\ 0.101\ X(1 - 0.101)}{(0.05)^2}$$

$$n = \frac{3.8416\ X\ 0.101\ X\ 0.899}{0.0025}$$

$$n = \frac{0.3488134}{0.0025}$$

=139.525

For Psychiatric patients:

$$n = \frac{(1.96)^2 X\ 0.042\ X(1 - 0.042)}{(0.05)^2}$$

$$n = \frac{3.8416\ X\ 0.042\ X\ 0.958}{0.0025}$$

$$n = \frac{0.154577067}{0.0025}$$

=61.8

Sum of sample sizes= 140+61.8

n= 201.8

The sample size calculated for inmate was 140 while that for the psychiatric patients was 62. To curtail dropout rate, 153 samples were collected from inmates and 123 collected from psychiatric patients giving a total of 276 samples.

3.7 Sample Collection

Samples were collected from the months of January- March, 2015. About 5ml of blood sample was aseptically collected into plain containers from each consenting inmate and psychiatric patients by a laboratory technician. The samples were allowed to clot

transported immediately in a cold box to the Department of Microbiology, Ahmadu Bello University, Zaria for processing.

3.8 Sample Processing

The clotted blood samples were spun at 1000rpm for 5min using a Centrifuge (80-3 Techmel and Techmel, USA). Each serum was separated and divided into two aliquots, one aliquot for ELISA and HBsAg assays and the other for RT-PCR. The sera were stored in the Department of Microbiology, Ahmadu Bello University, Zaria at -20^{o}C until required for analysis.

3.9 Data Collection

Data related to socio-demographic factors and probable risk factors associated with HCV and HBV infections were collected using a self designed questionnaire (Appendix III).

3.10 Detection of anti-HCV and HBsAg in Serum

The anti-HCV IgM and anti-HCV IgG were detected using qualitative ELISA while HBsAg was assayed for using the Rapid Diagnostic Test method. The ELISA and rapid test kits used were manufactured by Diagnostic Automation, Inc., USA.

3.10.1 ELISA Assay for Detection of Anti-HCV IgM and IgG

Hepatitis C virus ELISA test involves a two-step incubation procedure in which an indirect ELISA assay for IgM and IgG antibodies to HCV is established. Before incubation, recombinant, highly immunoreactive antigens corresponding to the core and non-structural regions of HCV are pre-coated to the polystyrene micro wells. During the first incubation stage, HCV-IgM specific antibodies (if present) will bind to the solid phase pre-coated HCV antigens. The next step is to wash the wells to remove unbound materials. What is

added next is the rabbit anti-human IgM antibodies (anti-IgM) conjugated to horseradish peroxidase (HRP-Conjugate).

The second incubation stage involves these labeled antibodies, which will be bound to any antigen-IgM complexes previously formed. The next step involves washing the wells to remove any unbound HRP-Conjugate. This is followed by the addition of chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide. A blue-coloured product develops in the presence of the antigen-antibody-anti-IgM-HRP immunocomplex and when the colourless chromogens are hydrolyzed by the bound HRP conjugate. After stopping the reaction with sulphuric acid, the blue colour turns yellow. The colour intensity is proportional to the amount of antibody captured and that in the sample. The wells remain colourless if the HCV-IgM result is negative.

3.10.2 ELISA Procedure for detection of HCV IgM and IgG Antibodies

Reagents were allowed to equilibrate at room temperature for 30min. Well A1 was blank; A2, A3, and A4 of the microtitre plates were negative controls. Wells A5 and A6 were positive controls, the remaining wells were used for samples. One hundred μl of specimen diluent was added into each well except the blank well for anti- HCV IgG. For anti-HCV IgM, sample diluent was not added to the blank, positive and negative control wells. Ten microliter of positive and negative controls were added into their respective wells in the IgG coated plates while 100μl of positive and negative control were added to respective wells in the IgM coated plates. Separate disposable pipette tips were used for each specimen, negative control and positive control to avoid cross-contamination. Ten μl of the samples were added to the labeled wells. This was mixed by tapping the plate gently. The plates were covered with the plate cover and incubated for 30min at 37°C. At the end of

the incubation, the plate cover was removed and discarded. Diluted buffer (Phosphate Buffered Saline containing Tween -20 as a detergent) was added to the wells, allowed to stay for 30-60 seconds and washed 5 times with diluted wash buffer. After the final washing cycle, plates were blotted to remove any remaining liquids. One hundred microlitre of horse radish peroxidase-conjugate was added to each well except the blank. The plates were covered with new plate cover and incubated for 30 minutes at 37°C. The wells were washed 5 times with diluted wash buffer. Fifty microliter of Chromogen A and 50µl Chromogen B solution were added into each well including the blank and mixed by tapping the plate gently. The plates were further incubated at 37°C for 15min in the dark. The enzymatic reaction between the Chromogen A and B solutions produced a blue colour in the positive control, HCV IgM and HCV IgG positive sample wells. Fifty microlitres stop solution was added into each well and mixed by tapping the plates gently. Intensive yellow colour developed in positive control, HCV IgG and HCV IgM positive wells. The optical Density (OD) was read at 450nm and 630nm wavelengths within 5min of stopping the reaction using GF-M3000 microtitre plate reader and results were read according to the manufacturer's manual.

3.10.3 Interpretation of ELISA Results

Each microtitre plate was considered separately when the results were calculated. Results were calculated by relating the Cut-off (C.O.) point calculated to samples' O.D. Samples giving an absorbance greater than, or equal to the Cut-off value are considered initially reactive which indicates that antibodies to hepatitis C virus have been detected.

3.10.4 Principle of Rapid Test for the Detection of Hepatitis B Surface Antigen (HBsAg)

The HBsAg rapid test employs a chromatographic lateral flow device. Colloidal gold conjugated monoclonal antibodies reactive to HBsAg are dry-immobilized onto a nitrocellulose membrane strip. When the sample is added, it migrates by capillary diffusion through the strip, rehydrating the gold conjugate. If present, HBsAg will bind with the gold conjugated antibodies forming particles. These particles will continue to migrate along the strip until the Test Zone (T) where they are captured by anti-HBs antibodies immobilized there and a visible red line appears. If there is no HBsAg in the sample, no red line will appear in the Test Zone (T). The gold conjugate will continue to migrate alone until it is captured in the Control Zone (C) from immobilized goat, anti-mouse IgG antibody and aggregating in a red line, which indicates the validity of the test.

3.10.5 Hepatitis B Surface Antigen screening using the Rapid Test

The test cassettes were allowed to equilibrate to room temperature before opening the pouch. The strips were dipped into the serum samples below the maximum line. The strips were placed on flat surface and results were read within 15min. For positive results, one red line next to the Test Zone (T) indicates that HBsAg has been detected. Non appearance of red line within 10 minutes next to the Test Zone (T) indicates that no HBsAg have been detected.

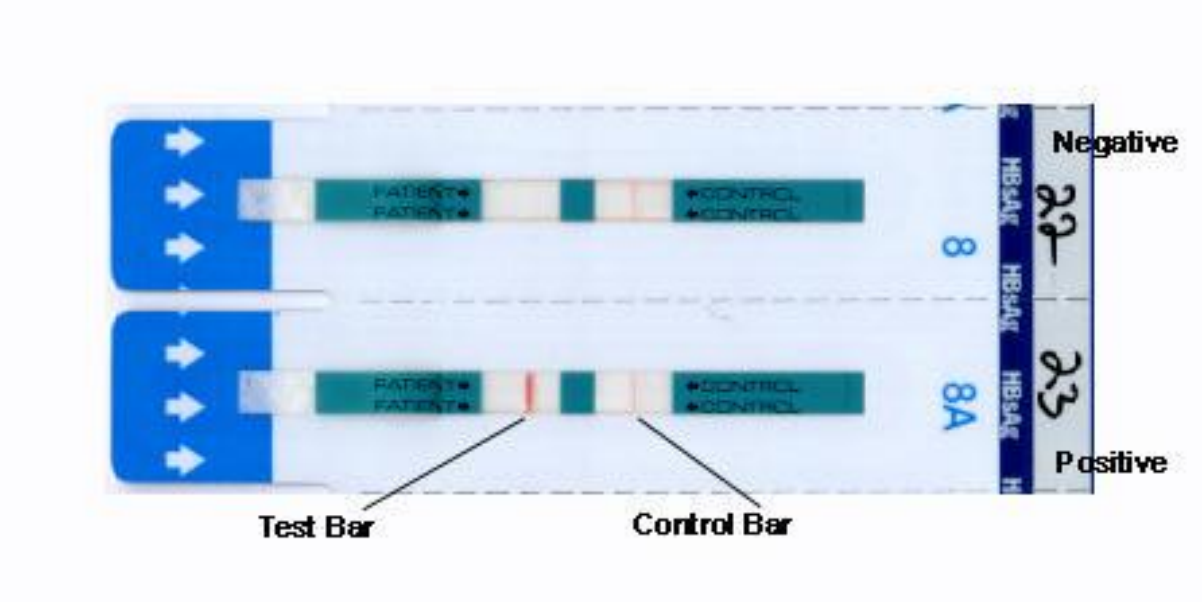


Figure 3.2 **Photo of HBsAg RDT showing a positive and negative result.**

Source: WHO/BCT/BTS/01.4

3.11 Detection of HCV RNA

The Invitrogen whole blood extraction kit (Invitrogen Life Technologies California, USA) was used for the RNA extraction after which reverse transcription was carried out. Polymerase chain reaction products were run on agarose gel to check for amplification of the untranslated conserved region.

3.11.1 HCV RNA Extraction

Out of the 28 anti-HCV IgM positive samples, 10 representative samples (five from Psychiatric and five from prisons) were used for the RT-PCR due of financial constraints. Two hundred µl of each serum sample was dispensed into 1.5ml RNase free microcentrifuge tube and 0.2ml of lysis buffer prepared with 2-marceptoethanol was added to each tube. The tubes and content were vortexed and centrifuged at 12,000rmp at room temperature. Each supernatant was transferred to a clean 1.5ml micro-centrifuge tube, 200µl of 100% ethanol was added to all tubes and vortexed to disperse precipitate. Samples were transferred into spin cartridge and centrifuged at 12,000 xg for 1min. The flow through was discarded and 700µl of wash buffer I was added to spin cartridge and centrifuged at 12,000rpm for 1min at room temperature. The flow through with collection tubes was discarded. Spin cartridge was transferred to a new collection tube, 500µl of wash buffer II with ethanol was added and centrifuged for 1min at 12,000rpm. Five hundred µl of wash buffer II was added and centrifuged again. The flow through was discarded and spin column was spun for 1min at room temperature to dry the membrane with attached RNA. Spin cartridge was inserted into a recovery tube and 150µl of RNase free water was added to the centre of the spin cartridge for elution. The eluted RNA was incubated at

room temperature for 1minute. The spin cartridge still inside the recovery tube was centrifuged for 2min at ≥ 12,000rpm at room temperature and placed on ice immediately for reverse transcription.

3.11.2 Reverse Transcription

Primers HepC – 939 (forward) and HepC – GEN 209 (reverse) were used for the first round reverse transcription. Fifteen µl of deionised water was added to all tubes and 5µl of extracted RNA was added to the respective tubes. Five µl of positive control (hepatitis C positive sample) was added to the positive control tube and 1µl each of forward and reverse primers (HepC – Gen – 939 and Gen - 209) were added. One drop of mineral oil was added to each tube to prevent evaporation. Tubes were placed in an already set thermocycler (PTC – 100^{TM} MJ – Research, INC, Peltier – Effect cycling). Reverse transcription was done at 42^{o}C for 1hr to obtain a complementary DNA with Moloney Murine Leukemia Virus as enzyme reverse transcriptase enzyme.

3.11.3 Polymerase Chain Reaction

Seventeen µl of premix was added to each tube and 1µl of each primer was added to the tubes including the negative control (Premix) tube. Seven µl of cDNA was added to the respective tubes making a final volume of 25µl. The premix used contained 1X Tri- actate-EDTA (TAE) buffer, MgCl, dNTPs (GTP, CTP, ATP and TTP) and Tag polymerase. Tubes were quickly spun and placed in the thermocycler for amplification. The cycling steps are presented on Table 3.1. The primers used for the RT-PCR is shown in Table 3.2. Primers with sequences gotten from conserved untranslated region of the HCV genome were used, HEPC- GEN 939 (sense) and HEPC- GEN 209 (antisense) were used for reverse transcription while KY-80 (forward) and KY-78 (reverse) were used for PCR.

Table 3.1: Cycling Plan for HCV Genome Amplification

Step	Temperature (^{o}C)	Time	Cycles
Pre-denaturation	94	5min	
Denaturation	94	30 sec	
Annealing	60	30 sec	35 cycles
Extension	72	30 sec	
Final extension	72	5 min	
Hold	4	Infinity	

Table 3.2: Primer sequence used for RT-PCR

Primer	Sequence	Basepair	References
HEPC-GEN939	5′-CTGTGAGGAACTACTGTCTT-3′	240	Yang *et al*., 2014
HEPC-GEN 209	5′ATACTCGAGGTGCACGGTGCACGGTCTACGAGACT-3′		
KY-80	5′-GCAGAAAGCGTCTAGCCATGGCGT-3′	244	Young *et al*, 1993
KY-78	5′-CTCGCAAGCACCCTATCAGGCAGT-3′		

3.11.4 Agarose Gel Electrophoresis

A microwave oven was used to solubilize 1.5% agarose (QD LE Agarose, Green BioResearch, USA) by heating for 2min. The resultant gel was allowed to cool and 10µl of ethidium bromide (intercalating agent) was added. The molten agarose was transferred into the casting tank, well-forming combs were inserted and allowed to solidify for 50min. The gel was submerged into the electrophoresis tank containing 1XTris Acetate EDTA buffer and the combs were removed. Fifteen µl PCR products were mixed with loading dye (bromophenol blue) and loaded into the respective wells. The negative control, positive control and 100 base pair marker were also added to labeled wells after which 120V electromotive force was applied for 40min. Gels were examined under UV transilluminator (Gel Doc 2000, BIORAD, USA) and photographs were taken to determine the 244bp of the untranslated conserved region.

3.12 Data Analysis

Data obtained from questionnaire and the results of the laboratory analysis were analyzed using Statistical Package for Social Sciences Version 16.0. (SPSS Incorporation, 2007 Chicago, USA). Chi-Square test was employed to test for association between the variables and seroprevalences obtained at 95% confidence interval and a p value ≤ 0.05 was considered significant.

CHAPTER FOUR

4.0 RESULTS

Out of 276 subjects tested for both HCV and HBV infections, 28 were positive for anti-HCV IgM giving a seroprevalence of 10.14% while 24 were positive for anti-HCV IgG giving an IgG seroprevalence of 8.7%. Seventeen subjects tested positive for HBsAg giving a seroprevalence of 6.15%. One person out of the 276 subjects had a co-infection giving a seroprevalence of 0.36%. (Figure: 1).

Among the inmates, 6 subjects were reactive for both anti-HCV IgM and IgG seromarkers, 16 and 13 inmates were positive anti-HCV IgM and IgG respectively. With regard to psychiatric patients, 12 and 11 patients were reactive to anti-HCV IgM and IgG respectively while 6 patients were reactive to both anti-HCV IgM and IgG. The above result is presented on Table 4.1.

Seroprevalence of HBV and HCV infections was analysed according to study population and the result as shown on Table 4.2. There was no statistically significant association ($P = 0.848$, $\chi^2 = 0.37$, $df = 1$ for IgM and $P = 0.896$, $\chi^2 = 0.017$, $df = 1$ for IgG) between HCV infection and study population. A significant association was observed ($\chi^2 = 5.313$, $df = 1$, $P = 0.021$) between the prevalence of HBsAg and study population. For IgM antibodies, prison inmates had a higher prevalence (10.45%: 16/153) than patients attending Psychiatric Hospital (9.75%: 12/123). The HCV-IgG prevalence of 8.5% (13/153) was obtained among the prison inmates while the psychiatric patients had 8.9% (11/123). With regard to HBsAg, the inmates had a higher prevalence of 9.2% (14/153) than the psychiatric patients who had a prevalence of 2.4% (3/123).

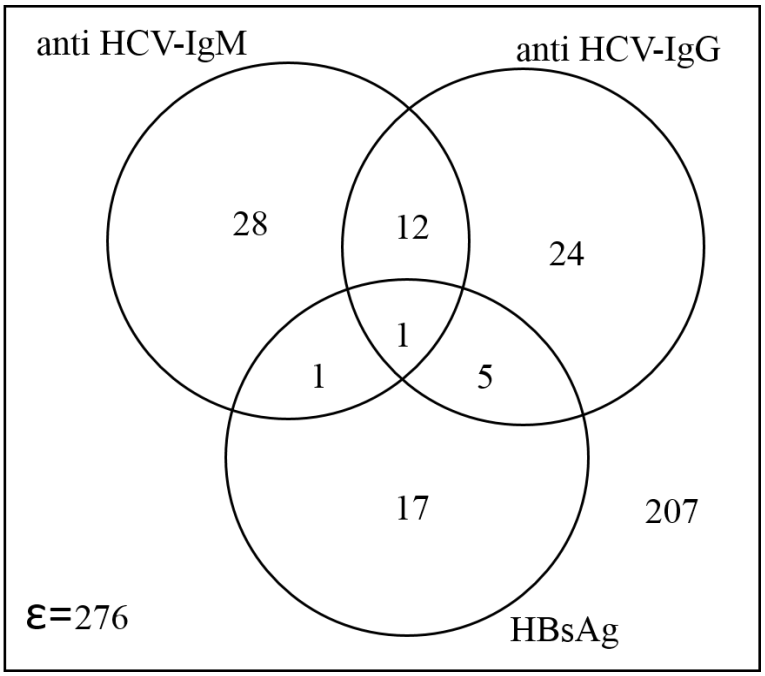


Figure 4.1: Venn Diagram Depicting the Seroprevalence of anti- HCV IgM, anti-HCV IgG and HBsAg among Inmates and Psychiatric Patients in Kaduna Metropolis.

Table 4.1: Distribution of HCV Seromarkers among Inmates and Psychiatric Patients

Seromarkers	Inmates	Psychiatric patients
Anti-HCV IgM	16	12
Anti-HCV IgG	13	11
IgM/IgG	6	6

Table 4.2: Seroprevalence of HCV IgG, IgM, and HBsAg among Inmates and Psychiatric Patients in Kaduna Metropolis

Population	Total	HCV IgM		HCV IgG		HBsAg	
		NP (%)	P value/ OR	NP (%)	P value/ OR	NP (%)	P value/ OR
Inmates	153	16(10.5)	0.848/0.926	13(8.5)	0.896/1.058	14(9.2)	0.021/0.248
Psy. Patients	123	12(9.8)		11(8.9)		3(2.4)	
Total	276	28(10.1)		24(8.7)		17(6.2)	

Key: OR = Odds Ratio

NP = Number Positive

Table 4.3 depicts the prevalence of anti-HCV IgM, IgG and HBsAg according to gender. Even though there was no significant association (χ^2 = 2.127, df = 1, P = 0.145) between the observed seroprevalence of HCV IgM among subjects by gender, female subjects had a higher prevalence (14.1%: 12/85) compared to male subjects (8.4%: 16/191). However, HCV IgG was similarly distributed by gender. In this study, HBsAg was significantly associated with gender (χ^2 = 8.062, OR = 0.911, P = 0.002). None of the 85 female subjects enrolled in this study was infected with HBsAg while 17 of the 191 male subjects were infected.

The age distribution of anti-HCV IgM, anti-HCV IgG and HBsAg among prison inmates and psychiatric patients in Kaduna metropolis was also analysed and the results is shown on Table 4.4. The IgM and IgG antibodies was significantly associated (P = 0.00, df = 6, χ^2 = 26.37 for IgM and P = 0.00, df = 6, χ^2 = 36.516 for IgG) with age while HBsAg was not (P = 0.339, df = 6, χ^2 = 6.805). For anti-HCV IgM, subjects that are 48 years and above had the highest prevalence of 28.9% (13/45) while no subject within age group 23-27 years had the infection. For anti-HCV IgG, subjects within age group 48years and above had the highest prevalence of 31.1% (14/45) while no subject within age groups 38-42 and 43-47 years had anti HCV IgG antibodies while for HBsAg, subjects within age group 28-32 years had the highest prevalence of 11.7% (7/60) and subjects found within age group 48 years and above had the lowest prevalence of 2.2% (1/45).

Table 4.3: Seroprevalence of HCV IgG, IgM, and HBsAg among Inmates and Psychiatric Patients in Relation to Gender in Kaduna Metropolis

		HCV IgM		HCV IgG		HBsAg	
Gender	**Total**	**No. positive (%)**	**P value**	**No. positive (%)**	**P value**	**No. positive (%)**	**P value**
Male	191	16(8.4)	0.145	16(8.4)	0.778	17(8.9)	0.002
Female	85	12(14.1)		8(9.4)		0(0.0)	
Total	276	28(10.1)		24(8.7)		17(6.2)	

Table 4.4: Age Distribution of anti-HCV IgG, IgM, and HBsAg among Inmates and Psychiatric Patients in Kaduna Metropolis

		HCV IgM		HCV IgG		HBsAg	
Age (years)	**Total**	**No. positive (%)**	**P value**	**No. positive (%)**	**P value**	**No. positive (%)**	**P value**
18-22	40	1(2.5)	0.00	1(2.5)	0.00	4(10.0)	0.339
23-27	45	0(0.0)		2(4.4)		2(4.4)	
28-32	60	5(8.3)		4(6.7)		7(11.7)	
33-37	36	4(11.1)		3(8.3)		1(2.8)	
38-42	31	2(6.5)		0(0.0)		1(3.2)	
43-47	19	3(15.8)		0(0.0)		1(5.3)	
≥ 48	45	13(28.9)		14(31.1)		1(2.2)	
Total	276	28(10.1)		24(8.7)		17(6.2)	

Plate 4.1 shows the agarose gel electrophoresis of RT-PCR products of HCV positive samples from inmates and patients attending Psychiatric Hospital. Lane 1 is the ladder (100bps), lanes P2-P92 are amplicons of samples from prison inmates and lanes 12-63 are amplified products of samples from psychiatric patients that were positive for ELISA anti-HCV IgM.

The association between some demographic factors and the viral infections among inmates is shown on Table 4.5. A statistically significant association between anti-HCV-IgM and marital status was observed (χ^2 = 6.574, df = 1, P = 0.010) and the married inmates were almost six times likely to be infected with HCV than those that were single (OR = 5.959; 95% CI = 1.304-27.227). The married inmates had a higher HCV IgM prevalence of 15.9% (14/88) while a lower prevalence was recorded among inmates that are single (3.1%: 2/65). However HCV IgG and HBsAg were not statistically associated with marital status (χ^2 = 2.190, df = 1, P = 0.139, OR = 2.650; 95% = 0.699-10.044 for IgG and χ^2 = 2.998, df = 1, P = 0.083 for HBsAg). Considering HCV IgG, a prevalence of 11.4% (10/88) was recorded among the married subjects while the single subjects had a prevalence of 4.6% (3/65). The HBsAg prevalence (13.8%: 9/65) was higher among single subjects than the married subjects (5.7%; 5/88).

There was no statistically significant association (χ^2 = 1.837, P = 0.607, df = 3 for IgM, χ^2 = 2.859, P = 0.414, df = 3 for IgG and χ^2 = 7.450, P = 0.059, df = 3 for HBsAg) between the viral infections and occupation. For anti-HCV IgM, public servants had the highest prevalence (15.6%: 5/32) while the lowest (4.5%: 1/22) was recorded among the civil

servants. For IgG, the highest prevalence (15.6%: 5/32) was obtained among public servants while the lowest prevalence (5.9%: 2/34) was recorded among the businessmen. The HBsAg prevalence of 20.6% (7/34) was recorded among the businessmen while the lowest prevalence of 3.1% (1/32) was recorded among the public servants.

A non statistically significant association was observed between anti-HCV IgM and HBsAg, and educational status (χ^2 = 2.852, P = 0.415, df = 3 for IgM and χ^2 = 1.493, P = 0.684, df = 3 for HBsAg). Inmates with tertiary education had the highest HCV-IgM prevalence of 16.3% (7/43) while the lowest prevalence (4.8%: 1/21) was recorded among those with non-formal education. For IgG, inmates with tertiary level of education had the highest prevalence of 18.6% (8/43) while none of the 21 subjects with non-formal education was positive. The highest HBsAg prevalence of 14.3% (3/21) was obtained among inmates with non-formal education while the lowest (6.8%: 5/74) was recorded among those with secondary education.

The result of the analysis of seroprevalence of HCV-IgG, IgM and HBsAg in relation to possible risk factors among prison inmates is shown on Table 4.6. There was a statistically significant association between tattooing and the occurrence of HCV IgM (P = 0.033, df = 1, χ^2 = 4.522). Inmates with tattoo/scarification were three times more likely to be infected with HCV than those without it (OR = 3.182; 95% CI = 1.048-9.662). A non-statistically significant association was observed with HCV-IgG and HBsAg (χ^2 = 0.164, P = 0.686, df = 1 for IgG and χ^2 = 0.408, P = 0.523, df = 1 for HBsAg). Inmates with tattoo/scarification had a higher HCV IgM prevalence (16.4%: 11/67) than those without (5.8%: 5/86).

Inmates without tattoo/scars had an HCV IgG prevalence of 9.3% (8/86) while those with it had 7.5% (5/67). For HBsAg, a prevalence of 10.5% (9/86) was obtained among inmates without tattoo/scars as against the 7.5% (5/67) among those with it.

Blood transfusion was also a risk factor that was analysed in this study. A Non statistically significant relationship was observed between history of transfusion and the infections (p=0.623, χ^2 =0.241, df=1 for IgM, χ^2 =0.664, p=0.415, df=1 OR=1.939; 95%CI=0.38-9.787 for IgG and χ^2 =1.552, p=0.213, df=1for HBsAg). Inmates with history of transfusion had a higher IgM prevalence of 14.3% (2/14) as against 10.1% (14/139) obtained from those with no history. A higher HCV IgG prevalence (14.3%: (2/14) was recorded among inmates with history of transfusion which is lower than that obtained among those with no history of transfusion (7.9%: 11/139). Inmates who had never been transfused had HBsAg prevalence of 10.1% (14/139) while none of the 14 subjects with a history of transfusion was positive for HBsAg.

There was no statistically significant association between IDU and the viral infections in this study (χ^2 = 0.840, P = 0.359, df = 1 for IgM, χ^2 = 0.179, P = 0.672, df = 1 for IgG and χ^2 = 0.317, P = 0.573, df = 1 for HBsAg). A higher IgM prevalence (16.7%; 3/18) was obtained among inmates with history of IDU as against a prevalence of 9.6% (13/135) found among non-IDU. For HCV IgG, a higher prevalence of 11.1% (2/18) was recorded among IDUs compared to the 8.1% (11/135) obtained among non-IDUs. The HBsAg prevalence (5.6%: (1/18) found among the IDUs was higher than 9.6% (13/135) of non-IDUs.

Hepatitis C virus IgM and HBsAg were not statistically associated with alcohol intake in this study (χ^2 = 2.413, P = 0.120, df = 1 for IgM and χ^2 = 1.623, P = 0.203, df = 1 for HBsAg). However, a higher IgM prevalence of 17.6 (6/34) was obtained among inmates that take alcohol and they were two times more likely to be infected with HCV than those that do not take alcohol (OR = 2.336; 95% CI = 0.782-6.975). A statistically significant association was observed between HCV IgG and alcohol intake (χ^2 = 4.708, df = 1, P = 0.030) and inmates that take alcohol were three times more likely to be infected than those who do not take alcohol (OR = 3.429; 95% CI = 1.068-11.007). For HBsAg, a higher prevalence (14.7%: 5/34) was recorded among inmates that take alcohol compared with that obtained among those that do not take alcohol (7.6%: 9/119). Inmates that take alcohol were two times likely to be infected with HBsAg than those that do not take alcohol (OR = 2.107; 95% CI = 0.656-6.771).

There was no statistically significant association between sexual experience and occurrence of these infections (χ^2 = 3.658, P = 0.056, df = 1 for IgM, χ^2 = 0.871, P = 0.351, df = 1 for IgG and χ^2 = 1.464, P = 0.226, df = 1 for HBsAg). An HCV IgM prevalence of 12.6%: 16/127) was found among inmates with sexual experience while none of the 26 subjects with no sexual experience was positive. Inmates with sexual experience were almost three times likely to be infected with HCV than those without experience (OR = 2.609; 95% CI = 0.324-20.993). For IgG, the prevalence among sexually active inmates was higher (9.4%: 12/127) than the obtained among non-sexually active inmates 3.8% (1/26). Inmates without sexual experience had a higher HBsAg prevalence of 15.4% (4/26) compared to the sexually active inmates (7.9%: 10/127).

A non-significant statistical relationship was observed between equipment sharing and HBV and HCV infections ($\chi^2 = 0.168$, P = 0.682, df = 1 for IgM, $\chi^2 = 0.275$, df = 1 P = 0.600 for IgG and $\chi^2 = 3.146$, P = 0.076, for HBsAg). Inmates who shared shaving equipment had a lower IgM prevalence of 8.9% (4/45) as against the 11.1% (12/108) recorded among those who did not share. Inmates who did not share shaving equipment had a higher IgG prevalence of 9.3% (10/108) while a lower prevalence (6.7%: 3/45) was obtained among those who shared equipment. A higher HBsAg prevalence (15.6%: 7/45) was obtained among inmates who shared shaving equipment and they were almost three times likely to be infected with HBV than those who do not share (OR = 2.658; 95% CI = 0.874-8.082).

There was a statistically significant association between sharing of clothes and HBsAg infection whereas, a non-significant association was observed with HCV infection in this study ($\chi^2 = 4.542$, P = 0.033, df = 1 for HBsAg and $\chi^2 = 0.012$, P = 0.912, df = 1 for IgM). A higher HBsAg prevalence (17.5%: 7/40) was obtained among inmates that shared clothes than that (6.2%: 7/113) recorded among those who do not and subjects that shared clothes were three times likely to be infected with HBV than those that do not (OR = 3.212; 95% CI = 1.050-9.826). The HCV-IgM antibodies were similarly distributed among inmates who shared clothes and those who do not.

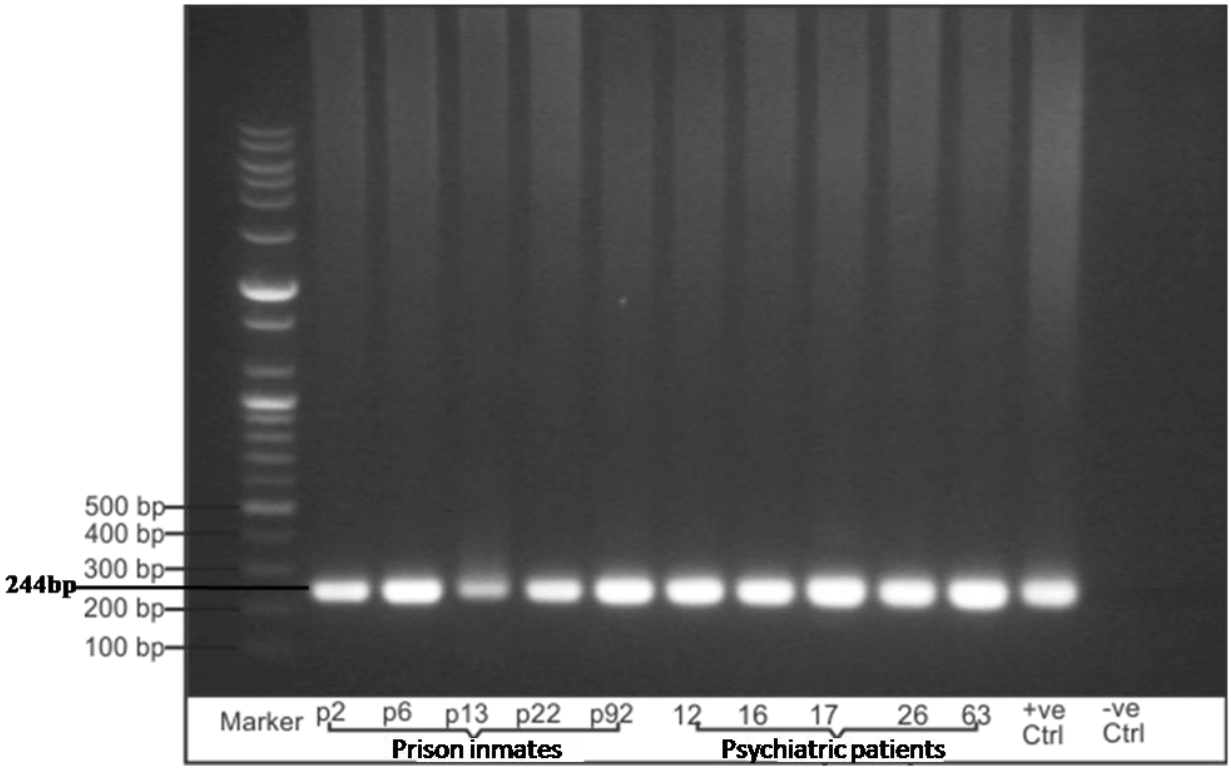


Plate 4.1: Agarose gel showing HCV amplified RT-PCR products of prison inmates and psychiatric patients by electrophoresis

Table 4.5: Seroprevalence of HCV IgG, IgM, and HBsAg in Relation to some Demographic Factors Status among inmates in Kaduna Metropolis

Factor	Total	HCV IgM		HCV IgG		HBsAg	
		No. Positive (%)	P value	No. positive (%)	P value	No. Positive (%)	P value
Marital status							
Married	88	14(15.9)	0.010	10(11.4)	0.139	5(5.7)	0.082
Single	65	2(3.1)		3(4.6)		9(13.8)	
Occupation							
Civil servants	22	1(4.5)	0.607	2(9.1)	0.414	2(9.1)	0.059
Public servants	32	5(15.6)		5(15.6)		1(3.1)	
Business	34	3(8.8)		2(5.9)		7(20.6)	
Unemployed	65	7(10.8)		4(6.2)		4(6.2)	
Educational status							
Primary	15	2(13.3)	0.415	1(6.7)	0.036	2(13.3)	0.684
Secondary	74	6(8.1)		4(5.4)		5(6.8)	
Tertiary	43	7(16.3)		8(18.6)		4(9.3)	
Non-formal	21	1(4.8)		0(0.0)		3(14.3)	

Table 4.6: Seroprevalence of HCV IgG, IgM, and HBsAg and Possible Risk Factors among Prison Inmates in Kaduna Metropolis

		HCV IgM		HCV IgG		HBsAg	
Risk Factor	**Total**	**No. positive (%)**	**P value/ OR**	**No. positive (%)**	**P value/ OR**	**No. positive (%)**	**P value/ OR**
Tattoo /scarification							
Yes	67	11(16.4)	0.033/ 3.182	5(7.5)	0.686/ 0.786	5(7.5)	0.523/ 0.690
No	86	5(5.8)		8(9.3)		9(10.5)	
Transfusion							
Yes	14	2(14.3)	0.623/ 1.488	2(14.3)	0.415/ 1.939	0(0.0)	0.213/ 1.112
No	139	14(10.1)		11(7.9)		14(10.1)	
IDU							
Yes	18	3(16.7)	0.359/ 1.877	2(11.1)	0.167/1. 409	1(5.6)	0.358/ 0.552
No	135	13(9.6)		11(8.1)		13(9.6)	
Alcohol intake							
Yes	34	6(17.6)	0.120/ 2.336	6(17.6)	0.030/3. 429	5(14.7)	1.466/ 2.107
No	119	10(8.4)		7(5.9)		9(7.6)	
Sexual experience							
Yes	127	16(12.6)	0.056/ 0.874	12(9.4)	0.351/ 2.609	10(7.9)	0.226/ 0.470
No	26	0(0.0)		1(3.8)		4(15.4)	
Equipment sharing							
Yes	45	4(8.9)	0.682/ 0.780	3(6.7)	0.600/ 0.700	7(15.6)	0.076/ 2.658
No	108	12(11.1)		10(9.3)		7(6.5)	
Clothes sharing							
Yes	40	4(10.0)	0.912/ 0.935	0(0.0)	0.025/ 1.130	7(17.5)	0.033/ 3.212
No	113	12(10.6)		13(11.5)		7(6.2)	

Key: OR-Odds ratio at 95% confidence interval

Table 4.7 shows the distribution of HCV IgM, IgG and HBsAg in relation to demographics among patients attending Psychiatric Hospital in Kaduna Metropolis. The highest HCV-IgM prevalence (13.9%: 10/72) was recorded among married patients, while none of the divorced patients was infected. For HCV IgG, the highest prevalence of 15.3% (11/72) was found among married patients too and no HCV IgG was detected among single and separated patients. The highest HBsAg prevalence (9.1%: 1/11) was found among patients who could not respond to the question on marital status while the least (1.4%: 1/72) was recorded among the married patients. Statistically, there was no significant association of IgM, and HBsAg with marital status ($\chi^2 = 3.673$, P = 0.299, df = 3 for IgM, $\chi^2 = 2.435$, P = 0.487, df = 3 for HBsAg) but the reverse was the case for IgG ($\chi^2 = 8.557$, P = 0.036, df = 3).

With respect to educational status, a non-statistically significant association was observed between the viral infections and educational status ($\chi^2 = 2.409$, P = 0.661, df = 4, for IgM, $\chi^2 = 0.966$, P = 0.915 df = 4 for IgG, $\chi^2 = 2.528$, P = 0.640, df = 4 for HBsAg). For IgM, the highest prevalence of 13.3% (2/15) was obtained among patients with non-formal education while no IgM antibody was detected among patients with primary and secondary education. Patients with secondary education had the highest IgG prevalence of 13.3% (2/15) compared to the least obtained among patients with tertiary education (6.5%: 2/31). The lowest HBsAg prevalence (3.3%: 2/60) was obtained among patients who could not respond to the question on educational status while the highest was recorded among subjects with secondary education (6.7%: 1/15).

There was no statistically significant association between occupation and the prevalence of the infections (χ^2 = 1.632, P = 0.803, df = 4 for IgM, χ^2 = 2.616, P = 0.624, df = 4 for IgG and χ^2 = 0.846, P = 0.932, df = 4 for HbsAg). The highest IgM prevalence (11.8%: 2/17) was found among civil servant while no positive result was found among patients that were public servants and businessmen. Patients that were civil servants had the highest HCV IgG prevalence of 17.6% (3/17) and the least (6.9%: 2/29) was recorded among the unemployed. For HBsAg, the highest prevalence (3.4%: 1/29) was obtained among the unemployed patients compared to the least (3.4%: 1/29) recorded among those who could not respond to the question on occupation.

The association between the seroprevalence of anti-HCV IgM, IgG and HBsAg and some risk factors among psychiatric patients was analysed and the result is shown in Table 4.8. Considering tattoo/scarification, there was a statistically significant association between HCV infection and tattoo/scar while a non-significant association was observed between HBV infection and tattoo/scarification in this study (χ^2 = 7.234, P = 0.027, df = 2 for IgM, χ^2 = 10.284, P = 0.006, df = 3 for IgG and χ^2 = 1.097, P = 0.578, df = 4 for HBsAg). For IgM, the highest prevalence of 21.9% (7/32) was found among patients with tattoo/scarification while the lowest (5.2%: 3/58) was recorded among those without tattoo/scarification. Highest prevalence of IgG (21.9%: 7/32) was also obtained among patients that had tattoo/scarification on their body while the lowest (1.7%: 1/58) was found among those without tattoo/scarification. The prevalence of HBsAg was similarly distributed among patients without tattoo/scarification and patients who could not respond to the question on tattoo while no HBsAg was detected among those with tattoo.

Transfusion as a risk factor was significantly associated with HCV infection but not with HBsAg (χ^2 = 16.05, P = 0.00, df = 2 for IgM χ^2 = 17.826, P = 0.00, df = 2 for IgG and χ^2 = 3.771, P = 0.152, df = 2 for HBsAg). A high IgM prevalence of 50% (4/8) was found among patients that had a history of transfusion as against the 4.9% (2/41) found among those who did not respond to the question on transfusion. Patients with history of blood transfusion had an IgG prevalence of 50% (4/8) compared with the 4.9% (2/41) recorded among those that did not respond to the question on transfusion. The highest HBsAg prevalence (12.5%: 1/8) was obtained among patients with history of transfusion while the lowest (1.4%: 1/74) was recorded among those with no history.

None of the psychiatric patient agreed to be an IDU. No statistically significant association was observed between IDU and the viral infections (χ^2 = 3.170, P = 0.075, df = 2 for IgM, χ^2 = 0.092, P = 0.762, df = 2 for IgG and χ^2 = 0.068, P = 0.794, df = 2 for HBsAg). The highest IgM prevalence of 13.7%: 10/73) was found among the patients who are non-IDU while the lowest (4.0%: 2/50) was recorded among those that did not respond to the question on IDU. Higher IgG and HBsAg prevalence (9.6%: 7/73 for IgG and 2.7%: 2/73 for HBsAg) were obtained among non-IDU compared to the lower values (8.0%: 4/50 for IgG and 2.0%: 1/50 for HBsAg) found among patients that did not respond to the question on IDU.

A statistically significant association was observed between HCV infection and alcohol intake (χ^2 = 21.961, P = 0.00, df = 2 for IgM and χ^2 = 8.848, P = 0.012, df = 2 for IgG) while a non-significant association was found with HBsAg (χ^2 = 0.445, P = 0.800, df = 2).

Patients that take alcohol had the highest IgM prevalence of 46.2% (6/13) as against 4.4% (2/45) found among those that did not respond to the question on alcohol consumption. For IgG, a prevalence of 30.8% (4/13) was also obtained among patients that take alcohol compared to the 4.4% (2/45) found for those that did not respond to the question. The reverse was the case for HBsAg where patients do not take alcohol had the highest prevalence of 3.1% (2/65) while those that did not respond to the question had a prevalence of 2.2% (1/45).

No significant association was observed between sexual experience and HBsAg but a significant relationship was observed with HCV infection in our study (χ^2 = 5.150, P = 0.076, df = 2 for HBsAg, χ^2 = 9.914, df = 2, P = 0.009 for IgM and χ^2 = 8.557, P = 0.014, df = 2 for IgG). A prevalence of 9.1% (2/22) for HBsAg was obtained among the non-sexually active patients while no HBsAg was detected among patient that did not respond to the question on sexual experience. Also, IgM prevalence of 16.7% (12/72) and IgG prevalence of 15.3% (11/72) was recorded among the sexually active patients while no IgM and IgG antibodies were detected among the non active patients and those that did not respond to the question on sexual experience.

Sharing of equipment was associated with HCV infection (χ^2 = 9.843, P = 0.007, df = 2 for IgM and χ^2 = 30.098, P = 0.00, df = 2 for IgG). A prevalence of 25% (6/24) was found among patients who shared equipment for IgM and the lowest prevalence of 9.0% 6/67) was found among those who do not share. For IgG, the highest prevalence (37.5%: 9/24) was obtained among patients that shared equipment and the least (3.0%: 2/67) recorded

among those that do not share. There was no significant association between HBsAg and sharing of equipment in this study ($\chi^2 = 5.242$, P = 0.073, df = 2). However, a high HBsAg prevalence of 8.3% (2/24) was found among patients who shared equipment compared to the low prevalence (3.1%: 1/32) found among those who did not respond to the question on equipment sharing.

A statistically significant association was observed between sharing of clothes and HBsAg but not with HCV infection ($\chi^2 = 6.446$, P = 0.04, df = 2 for for IgG and $\chi^2 = 7.010$, P = 0.030, df = 2 for HBsAg and $\chi^2 = 3.778$, P = 0.15, df = 2 for IgM). For IgM, the highest prevalence of 13.4% (11/82) was found among patients who did not share clothes while IgM was not detected among patients that did not respond to the question on clothes sharing. A 40.0% (2/5) prevalence of IgG was recorded among subjects that shared clothes compared to the 5.6% (2/36) found among patients who did not respond to the question. The highest HBsAg prevalence of 20.0% (1/5) was found among those who shared clothes while the least 1.2% (1/82) was obtained among the patients that did not share clothes.

Table 4.7: Seroprevalence of HCV IgG, IgM, and HBsAg according to Demographics among Patients attending Psychiatric Hospital in Kaduna Metropolis

	Total	HCV IgM		HCV IgG		HBsAg	
		No. Positive (%)	P value	No. Positive (%)	P value	No. Positive (%)	P value
Marital status							
No Response	11	0(0.0)	0.299	0(0.0)	0.036	1(9.1)	0.487
Married	72	10(13.9)		11(15.3)		1(1.4)	
Single	38	2(5.3)		0(0.0)		1(2.6)	
Separated	2	0(0.0)		0(0.0)		0(0.0)	
Educational status							
No Response	60	6(10.0)	0.661	6(10.0)	0.915	1(9.1)	0.640
Primary	2	0(0.0)		0(0.0)		1(1.4)	
Secondary	15	0(0.0)		2(13.3)		1(2.6)	
Tertiary	31	4(12.9)		2(6.5)		1(2.6)	
Non formal	15	2(13.3)		1(6.7)		0(0.0)	
Occupation							
No Response	68	8(11.8)	0.803	6(8.8)	0.624	2(9.1)	0.932
Civil servants	17	2(11.8)		3(17.6)		0(0.0)	
Public servants	4	0(0.0)		0(0.0)		0(0.0)	
Business	5	0(0.0)		0(0.0)		0(0.0)	
Unemployed	29	2(6.9)		2(6.9)		1(3.4)	

Table 4.8: Relationship between the Seroprevalence of HCV IgG, IgM, and HBsAg and some Risk Factors among Patients attending Psychiatric Hospital in Kaduna Metropolis

Risk Factor	Total	HCV IgM (%)No. positive	P value	HCV IgG (%)No. positive	P value	HBsAg (%)No. positive	P value
Tattoo/scarification							
No response	33	2(6.1)	0.027	3(9.1)	0.006	1(3.0)	0.578
Yes	32	7(21.9)		7(21.9)		0(0.0)	
No	58	3(5.2)		1(1.7)		2(3.4)	
Transfusion							
No response	41	2(4.9)	0.000	2(4.9)	0.000	1(2.4)	0.152
Yes	8	4(50.0)		4(50.0)		1(12.5)	
No	74	6(8.1)		5(6.8)		1(1.4)	
IDU							
No response	50	2(4.0)	0.075	4(8.0)	0.762	1(2.0)	0.794
Yes	0	0(0.0)		0(0.0)		0(0.0)	
No	73	10(13.7)		7(9.6)		2(2.7)	
Alcohol intake							
No response	45	2(4.4)	0.000	2(4.4)	0.012	1(2.2)	0.800
Yes	13	6(46.2)		4(30.8)		0(0.0)	
No	65	4(6.2)		5(7.7)		2(3.1)	
Sexual experience							
No response	29	0(0.0)	0.009	0(0.0)	0.014	0(0.0)	0.076
Yes	72	12(16.7)		11(15.3)		1(1.4)	
No	22	0(0.0)		0(0.0)		2(9.1)	
Equipment sharing							
No response	32	0(0.0)	0.007	0(0.0)	0.000	1(3.1)	0.073
Yes	24	6(25.0)		9(37.5)		2(8.3)	
No	67	6(9.0)		2(3.0)		0(0.0)	
Sharing clothes							
No response	36	1(2.8)	0.151	2(17.6)	0.04	1(2.8)	0.030
Yes	5	0(0.0)		2(0.0)		1(20.0)	
No	82	11(13.4)		7(0.0)		1(1.2)	

CHAPTER FIVE

5.0 DISCUSSION

With the paucity of published information on the epidemiology of HCV and HBV infections among inmates and psychiatric patients in Nigeria, this study has shown that the infections are prevalent among these populations. Anti-HCV IgM was detected with a prevalence of 10.14%, anti-HCV IgG 8.7% and HBsAg 6.15%. The presence of these viruses among these subjects indicates that they can be infectious to others. These findings cannot be over emphasized because subjects are at risk of developing hepatocellular carcinoma if preventive measures are not taken. The anti-HCV IgM and IgG seroprevalence in this research were higher than the 4.8% found among inmates in France (Semaille *et al.*, 2013) and 4.2% among mentally ill persons in Nigeria (Omoaregba *et al.*, 2013). In contrast, the HCV seroprevalence in this study is lower than the 12.3% reported among inmates in Nasarawa State, Nigeria (Adoga, *et al.*, 2009) and the 19.4% recorded in Iran (Ataei *et al.*, 2015). The anti HCV prevalence rates of this study corroborates the 5.8-12.3% range for HCV prevalence rate in Nigeria as reported by Ojide *et al.* (2015). The high prevalence rate of HCV infection in this study could be as a result of high risk behaviour or life style common among inmates and psychiatric patients such as intravenous drug use, being single or divorced, practicing risky sexual behavior, and having reduced awareness about the potential modes of transmission of infectious diseases (Marc *et al*., 2011).

The overall 6.2% HBsAg prevalence obtained in this study was higher than the 2.72% reported by Rana *et al.* (2015) among inmates in New Delhi but similar to 6.0% found by

Kakar *et al.* (2014). However it was at variance with the 23.4% found among patients with severe mental disorders (Marc *et al.*, 2011) and 10.4% among inmates in Ethiopia (Unakal *et al*, 2013).

An HBV/HCV co-infection rate of 0.36% was obtained in this study. The low co-infection rate could be due to the presence of occult HBV infection which this study did not determine or as a result of suppression of HBV DNA multiplication by HCV (Chi-Jen and Chou-Dong 2008) leading to reduction in serum HBsAg titre. The low co-infection rate could also be due to global infant childhood vaccination, post exposure prophylaxis and antiviral therapy to HBV. This finding is similar to the 0.57% HCV/HBV co-infection rate reported by Ugbebor *et al*. (2011) and the 0.4% reported by Kakisi *et al*. (2009). The rate however disagrees with the 8.8% prevalence found by Pennap *et al*. (2010) among people of a local community Keffi, Nassarawa State.

Subjects reactive to both anti-HCV IgM and IgG seromarkers are having chronic infection. This implies that they are infectious and are candidates for HCC if preventive measures are not taken. Those that were reactive to only anti-HCV IgG must have spontaneously cleared the infection, treated or recovered from the infection while those positive for anti-HCV IgM only are having current infection and are of public health importance.

This study found among the prison inmates an ant-HCV IgM prevalence of 10.5% and IgG prevalence of 8.5%. These prevalence rates confirm the circulation of HCV among the inmates in Kaduna Metropolis. These anti-HCV IgM and IgG prevalence rate among the

inmates in this study were lower than the 35% recorded among inmates in Benue State, Nigeria (Nimzing *et al*., 2015), the 31.6% prevalence rate among inmates in Australia (Teutsch *et al.*, 2010) and the 19.2% rate reported in Ghana by Adjei *et al.* (2006). The HCV-IgM prevalence rate among inmates indicates the presence of current infection in this correctional facility. This finding is similar to the 10.1% obtained by Alvarez *et al.* (2014) among inmates in New York. With respect to HBsAg among the inmates a prevalence of 9.2% was obtained. This shows that HBV is endemic among the inmates in Kaduna Metropolis. This prevalence rate is higher than the 2.72% reported among inmates in New Delhi (Rana *et al*., 2015) and the 2.6% reported among Barcelona prisons (Marco *et al*., 2014) but lower than the 17.4% HBsAg prevalence reported among inmates in Ghana (Adjei *et al*., 2006) and 18% among inmates in Taraba State, Nigeria (Monday and Francis, 2014).

Among the psychiatric patients in this study, an HCV IgM and IgG prevalence of 9.8% and 8.9% respectively were obtained. The anti-HCV IgM and IgG prevalence rates among psychiatric patients in this study agrees with the 9% found by Kakisi *et al*. (2009) among patients in psychiatric hospitals in Thassaloniki, Greece but higher than 4.2% among mentally ill persons in Nigeria (Omoaregbe *et al*., 2013) and lower than 12.6% among mentally ill patients in Ilorin, Nigeria (Durotoye *et al*., 2014) and 19.6% found by Marc *et al*. (2011). A very low HBsAg prevalence of 2.4% was recorded in this study among the psychiatric patients in Kaduna Metropolis. This rate agrees with the HBsAg prevalence of 2% reported among mentally ill persons in Thessaloniki (Kakisi *et al*., 2009) but disagrees with 10.0% among mentally ill patients in Ilorin, Nigeria (Durotoye *et al*., 2014) and

23.4% prevalence reported among mentally ill persons by Marc *et al.* (2011). This low HBsAg prevalence among the psychiatric patients could be that HBsAg testing has been carried out during the patients' follow-up clinic and the patients must have been probably vaccinated or treated as the case may be.

There was no significant association between gender and HCV infection in this study even though women recorded a higher anti-HCV IgM and IgG seroprevalence rates of 14.1% and 9.4% respectively against 8.4% and 8.4% respectively for males. This result could be due to chance and may probably be the reason for the non significant association. This finding agrees with that of Semaille *et al.* (2013) but contradicts that reported by Rogo *et al.* (2010) and Kakisi (2009) where male subjects had the higher seroprevalence of HCV infection. On the other hand, no female tested positive for HBsAg in this research, whereas male subjects had a seroprevalence of 8.9%. This might be that male subjects were more exposed to the risk factor that predisposed them to this infection or the females clear it easily.

In this study, anti-HCV and IgG infection was significantly associated with age (P = 0.00) whereas HBsAg infection was not (P = 0.339). The highest HCV infection prevalence found among subjects aged 48 years and above could be due to the fact that people of older age are more susceptible to infectious disease or due to the progression of HCV infection with age. This finding contrasts that of Kawo *et al.* (2012) who found the highest prevalence of HCV infection among subjects within age groups 24-33 and 34-43 years. The highest HBsAg prevalence among inmates within 28-32 years of age may be due to

youthfulness which is associated with adventure and quest for recreation which manifest in substance abuse and greatest sexual activities thus supporting the role of sexual transmission of the virus (Dawaki and Kawo 2006). This finding contrasts the result of Ugbebor *et al.* (2011) where the highest prevalence of HBsAg was found among subjects within age group 32-36 years.

Amplified HCV PCR products were observed on the gel photo in form of bands at 244bp. This confirms viremia among these subjects among anti-HCV positive subjects. The presence of RNA confirms viral replication which indicates that subjects are infectious and risky behaviours could be highly practiced by these groups. These subjects could be a source of infection to the general population if measures to curb the spread are not taken. Only five samples were processed for the molecular assay due to financial constraints.

5.1 Prison Inmates

In relation to marital status among the prison inmates, the married inmates had the highest prevalence of HCV infection. This is indicative of heterosexual intercourse among the married inmates or due to sexual activities with multiple sex partners who probably had STD with open sores and ulcers and were infected with HCV. This finding agrees with Kakar *et al,* (2014) who found that married subjects had more HCV infection than the singles. In contrast, the highest prevalence of HBsAg was found among the single subjects than the married in this study could be may be due to the fact that the unmarried individuals are more likely to engage in behaviours that put them at risk of all these infections than the married.

Although there was no association between occupation and HBV and HCV infections, the highest HCV prevalence rate was observed among the inmates who were public servants while highest prevalence of HBsAg was obtained among inmates who were into business.

Inmates with tertiary education had the highest prevalence of HCV infection. This may be attributable to the fact that the legal age of incarceration is 18 years and most of the inmates must have completed their primary and perhaps their secondary education and were in tertiary institutions. Inmates with non-formal education had the highest HBsAg prevalence rate in this study although this did not reveal any statistical significant association. The non-significant association indicates that education was not a risk factor for the acquisition of HCV and HBV infections among the subjects in this study.

The statistically significant association observed between HCV infection and tattoo/scarification may suggest that the infection was probably acquired through the use of unsterilized or shared tattoo equipment which is a common practice among inmates. This finding has further confirmed that tattooing might be one of the ways inmates acquire HCV infection (Imperial *et al*., 2010; CDC, 2010).

With regards to blood transfusion among inmates in this study, the highest HCV infection prevalence was among inmates that had a history of blood transfusion even though a significant association was not established. This could be that some inmates responded incorrectly (eg may have been transfused at an early age and so do not know) or the viruses

were in their window period (amount of time from first infection with HCV and the appearance of detectable evidence of infection e.g presence of HCV antibodies) of infection at the time of transfusion. During this period, a false negative result is obtained and patients are transfused with infected blood. This finding agrees with findings of Godwin *et al.* (2013) who did not record any statistical association with blood transfusion. The highest HBsAg prevalence was found among inmates who had not been transfused. However, the association was not significant. This implies that these inmates must have contracted HBV infection through means other than blood transfusion.

A non-significant association was found between the viral infections and IDU even though there was a higher anti-HCV IgM seroprevalence among inmates who were IDUs than non-IDUs. This finding could be attributable to the fact that IDU has been described as a norm within prisons (Treloar *et al.,* 2015) and injection tools are not conveniently available in prisons therefore, prisoners have to share needles which helps in spreading viruses among inmates (Ataei *et al.,* 2015).

A higher seroprevalence of the infections was recorded among inmates that take alcohol than inmates who do not take alcohol. Drinking alcohol was shown to be associated with HCV IgG in this study. This finding agrees with the assertion that alcohol is among factors that lead to disease progression and development of hepatocellular carcinoma (George and Bruce, 2001; KASL, 2014). This finding agrees with the findings of Kawo *et al.* (2012) that alcohol consumption is associated with HCV infection.

In relation to sexual experience, a higher prevalence of HCV infection was found among the sexually active inmates thereby making sexual intercourse an important factor in HCV infection in this study. This finding may suggest that inmates engage in sexual activities with partners that have sores as a result of STD in their genitals since HCV is blood borne. However, the above association was not significant.

Higher prevalence of HBsAg was found among inmates that shared clothes as against those who did not. Infection with HBV has been shown to be acquired through body fluids (Aba and Aminu, 2016) and these inmates might have this infection through sweat or other body fluids since they wear each other's clothes. The significant association observed implicates clothes sharing as a risk factor for HBV infection. Clothes sharing was not associated with HCV infection among inmates in this study. This confirms the assertion that HCV is blood borne (Ejiofor *et al*., 2010, CDC, 2013).

5.2 Psychiatric Patients.

For most of the sociodemographic and risk factors studied, a significant percentage of the patients did not respond to the study questions. Consequently, the statistics may not be significant. Therefore, it may be difficult to proffer explanations for the lack of association.

The married patients attending Neuro-Psychiatric Hospital, Kaduna Metropolis had higher prevalence of HCV infection than the singles although the association was not significant for IgM but significant for IgG. This could be that close person-person contact with infected spouse as the virus can be spread through body fluids and close contact (Aba and

Aminu 2016). The highest prevalence of HBsAg was obtained among subjects that did not respond to the question on marital status no HBsAg was detected among subjects that were separated. However, this was not statistically significant with marital status.

Level of education was not significantly associated with HCV and HBV infections among psychiatric patients in this study. This implies that level of education is not a factor in the transmission of these infections. The highest anti-HCV IgM prevalence was found among patients with non-formal education while none was reactive to it among subjects with primary and secondary education. The highest HCV IgG and HBsAg prevalence were found among patients with secondary education. This could be the patients stopped their education after the secondary school level as a result of their health challenge or might be due to chance.

With regards to occupation, the highest IgM prevalence was found among civil servants and patients that did not respond to the question on occupation while HCV-IgG was more prevalent among the civil servants. The unemployed had the highest HBsAg prevalence although there was no significant association between the viral infections and occupation. This finding could be due to chance since they are out-patients who come from different parts of the country and different works of life.

A significant association was found between HCV infection and tattooing among psychiatric patients in Kaduna metropolis (P=0.027 for IgG and P=0.006 for IgG). Hepatitis C virus infection prevalence was higher among patients with tattoo/scarification

than those without it. This finding implies that tattooing is a factor in the transmission of HCV infection in this study. It could also be that the patients must have been infected through use and sharing of unsterilized sharp objects for their body art. This finding agrees with the finding of Pennap *et al*. (2010) who found significant association between HCV and tattooing/scarification. A higher HBsAg prevalence recorded was among patients with no tattoo/scarification than among patients with it. This is indicative of infection through other risk factors.

With respect to blood transfusion, anti-HCV IgG, IgM and HBsAg were found to be more prevalent among patients with history of blood transfusion. This could be that the blood samples were not properly screened before transfusion or the virus infections were in their window period at the time of transfusion of the screened blood. This association was significant for HCV but not significant for HBsAg infection. This finding disagrees with that of Omoaregba *et al.* (2013) who did not record any association between transfusion and HCV infection in Nigeria.

No psychiatric patient agreed he was an injection drug user. However some of them were found to be reactive to anti-HCV IgM, IgG and HBsAg. Insincere responses could be the reason for the non-significant relationship between the viral infections and IDU even though IDU is a common behaviour among psychiatric patients (Stanley *et al.,* 2001) and has been found to be the leading mode of transmission after the implementation of blood and blood products screening (Bretana *et al.*, 2015). This finding concurs with the finding of Adoga *et al,* (2009) but disagrees with that of Stanley *et al*, (2001) who found a

significant association between IDU and HCV and HBV infections among psychiatric patients in U.S.A.

In this study, the patients who take alcohol had the highest prevalence of HCV infection. Alcohol could lead to progression of HCV and HBV infection and viral replication due to overproduction of free radicals that destroy liver cells (Collins and Swan 2009). However, a significant association was found between alcohol intake and HCV infection among psychiatric patients. Patients that do not take alcohol had the highest prevalence HBsAg though the association was not significant.

Hepatitis C virus infection was recorded only among sexually active patients and not those that had no sexual experience. This result could be due to patient's involvement in high risky sexual activities that may have led to infection with this virus. Stanley *et al.* (2001) posited that people with mental illness have increased rates of sexually transmitted diseases and are likely to engage in high-risk behaviours like multiple sexual partners and are known to be victims of sexual assaults (Durotoye *et al*., 2014). This finding is in consonance with that of Stanley *et al.* (2001) who found a significant association between sexual exposure and HBV and HCV infections. The highest HBsAg prevalence among patients who have no sexual experience does not implicate sexual experience as a risk factor for HBV infection among psychiatric patents in this study. This finding contradicts that of Stanley *et al,* (2001) among mentally ill persons in USA.

Hepatitis C virus and HBsAg prevalence were found to be higher among patients that shared shaving equipment. This has implicated equipment sharing as a culprit in the acquisition of these infections. This finding disagrees with that of Ataei *et al*. (2015) where sharing equipment sharing was not significantly associated with the infection.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

Hepatitis C virus is a positive sense single stranded RNA virus of the family *Flaviviridae* and Genus *Hepacivirus* (Kapoor *et al*., 2011). It is the causative agent of human hepatitis C infection and most commonly transmitted through exposure to infected blood. Hepatitis B is a DNA virus of the family *Hepadnaviridae*, Genus *Orthohepadnavirus* and species Hepatitis B virus (Pungpapong *et al*., 2007). Hepatitis C and hepatitis B virus co-infection is very common because of the shared modes of transmission and subjects with high risk of parenteral infections tend to have this (Chi and Shou, 2008). It is estimated that about 200 million people in the world are infected with HCV and over 350 million with HBV leading to more than one million deaths annually (Akinsegun *et al*., 2012). The Department of Health and Human Services, CDC (2011) posited that hepatitis C is the most common type of hepatitis found in jails and prisons. People with mental illness are likely to be over represented in high-risk categories for infection with pathogens with similar routes of transmission such as HBV and HCV (Stanley *et al*., 2001).

This study has provided data on the prevalence of HCV and HBV among prison inmates and psychiatric patients in Kaduna Metropolis. The data generated in this research will serve as baseline information for the prevention and control of these viruses and for future studies. This study aimed at determining the prevalence of HCV and HBV and to evaluate HCV viremia among prison inmates and psychiatric patients in Kaduna Metropolis. The specific objectives of this research were to screen for HCV antibodies and HBsAg, to

detect HCV RNA using RT-PCR, and to determine the possible risk factors that predispose individuals to HCV and HBV infections.

A total of 276 subjects were tested for both HCV and HBV infections. Antibodies to HCV were detected using ELISA and the Rapid test method was used to detect HBsAg. Hepatitis C virus genome of ELISA positive samples was amplified using RT-PCR. Data on demographic and risk factors were obtained via a self-designed questionnaire. Twenty-eight subjects were positive for anti-HCV IgM giving a seroprevalence of 10.14% while 24 subjects were positive for anti-HCV IgG giving an IgG seroprevalence of 8.7%. Seventeen persons tested positive for HBsAg giving a seroprevalence of 6.15%. One subject out of the 276 subjects had a co-infection of both viruses giving a seroprevalence of 0.36%.

There was no statistically significant association between the occurrence of HCV IgM and HCV IgG and study population. For IgM antibodies, prison inmates had a prevalence of 10.45% (16/153) while psychiatric patients 9.75% (12/123). A HCV-IgG prevalence of 8.5% (13/153) was obtained among prison inmates while the psychiatric patients had 8.9% (11/123). With regard to HBV infection the inmates had a higher prevalence (9.2%: 14/153) than the psychiatric patients (2.4%: 3/123).

Even though there was no significant association between the observed seroprevalence of HCV IgG among subjects by gender, the females were two times more likely to be infected with HCV than males. However, HCV IgG was similarly distributed (8.4% for males and

9.4% for females) by gender. None of the 85 female subjects who enrolled in this study was infected with HBV while 17 of the 191 male subjects were infected with HBV.

For anti-HCV IgM, subjects within age group 48 years and above had the highest prevalence of 28.9% (13/45) while no subject within the age group 23-27 years had the infection. For anti-HCV IgG, subjects within age group 48 years and above had the highest prevalence of 31.1% (14/45) while no subject within age groups 38-42 and 43-47 years had anti HCV IgG antibodies. For HBsAg, participants within age group 28-32 years had the highest prevalence of 11.7% (7/60) and subjects found within age group 48 years and above had the lowest prevalence of 2.2% (1/45).

Amplification of the HCV RT-PCR products was observed on the gel with band size of 244bp confirming the presence of HCV-RNA in the serum of patients with positive anti-HCV IgM test. There was no statistically significant association between the viral infections and all demographic factors studied except age where the highest HCV and HBsAg prevalence were obtained among subjects aged $\geq$ 48 years and 28-32 years respectively. Having tattoo/scarification and alcohol intake were statistically associated with HCV while clothes sharing was associated with HBsAg among the inmates. For the psychiatric patients, having tattoo/scarification, history of transfusion, alcohol intake, sexual experience and shaving equipment sharing were found to be statistically associated with HCV while clothes' sharing was associated with HBsAg.

6.2 CONCLUSION

This study has shown prevalence of 6.15% for HBsAg, 10.14% for anti-HCV IgM and 8.7% for anti-HCV IgG among prisons inmates and psychiatric patients in Kaduna metropolis between the months of February and March, 2015. A co-infection rate of 0.36% was obtained. This confirms the circulation of these viruses in these populations. A high prevalence of 9.2% for HBsAg, 10.5% for IgM and 8.9% for IgG prevalence was obtained among the prison inmates while HCV IgM prevalence of 9.8%, 8.9% IgG and HBsAg 2.4% was found among psychiatric patients in this study.

This study detected HCV-RNA in ten (10) serum samples. More women had HCV infection (14.1%) than men while no female subject was infected with HBV. Subjects within age group 28-32 years had the highest HBsAg prevalence (11.7%) while those ≥ 48 years recorded the highest HCV infection.

Marital status was significantly associated with HCV infection among other demographic factors. Tattoo/Scarification and alcohol intake were statistically associated with HCV while clothes sharing was associated with HBsAg among the inmates. For the psychiatric patients, tattoo/scarification, history of transfusion, alcohol intake, sexual experience and equipment sharing were found to be statistically associated with HCV while clothes sharing was associated with HBsAg.

6.3 RECOMMENDATIONS

In view of the above findings it is recommended that:

1 Inmates should be screened for these infections at the point of incarceration in order to ascertain their status and routine tests should be considered for the psychiatric patients during their follow-up clinics to detect new health problems.

2. General public health and prevention programmes should be intensified to create awareness of these viruses especially in correctional facilities and psychiatric hospitals since the prevalence of these viruses is higher in these populations than the general population.

3. Government should encourage and support HCV vaccine development research and make HBV vaccine available and affordable to all.

4. Effective implementation of surveillance to prevent the smuggling of drugs and drug equipment could minimize HCV and HBV infection risk in these facilities and the community

5. Further should be carried out to proffer reasons as to why the prevalence of HCV is higher among females and HBV among males.

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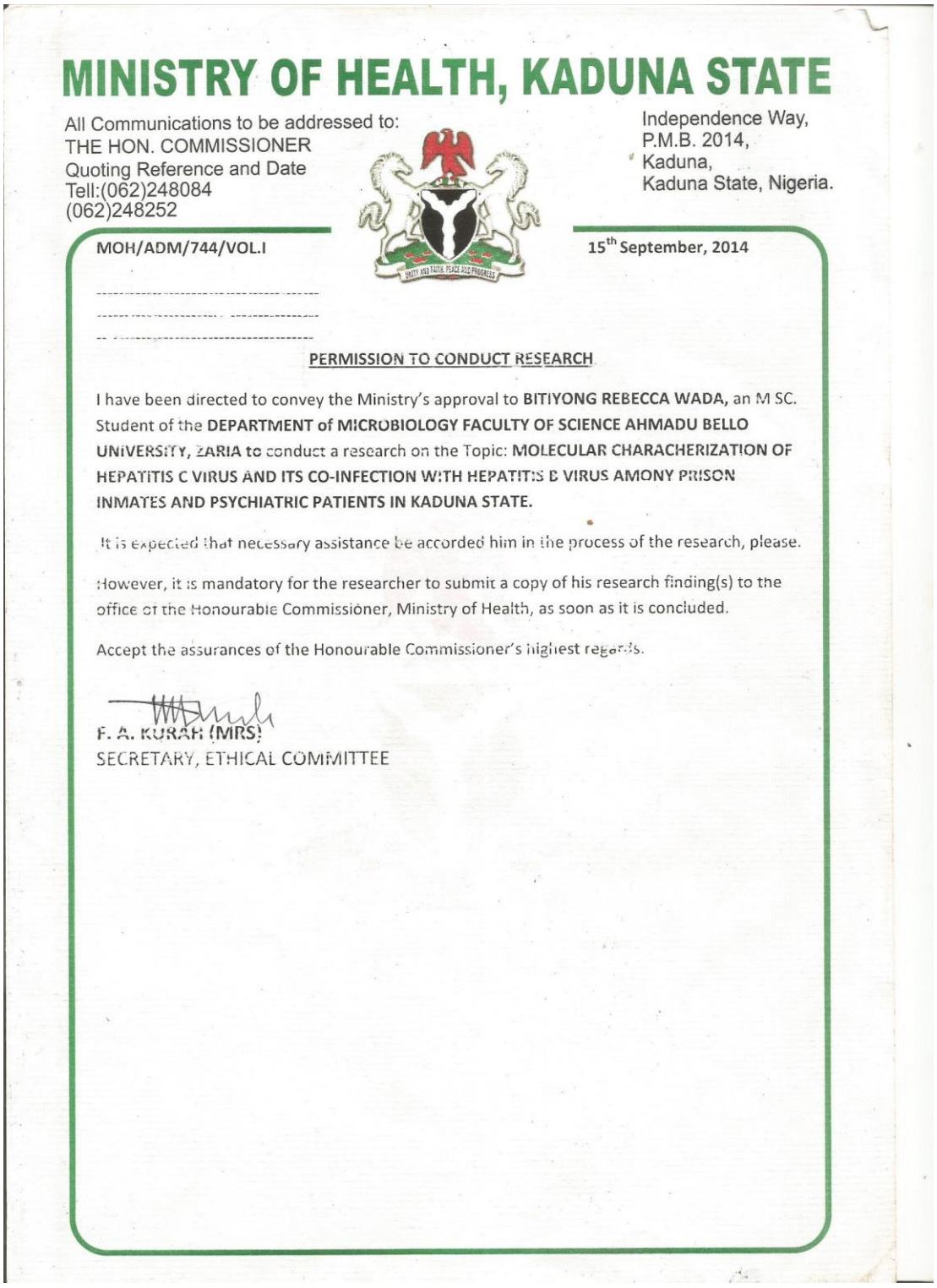
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APPENDIX Ia: Ethical Approval



MINISTRY OF HEALTH, KADUNA STATE

All Communications to be addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Tell:(062)248084
(062)248252

Independence Way,
P.M.B. 2014,
Kaduna,
Kaduna State, Nigeria.

MOH/ADM/744/VOL.I

15th September, 2014

PERMISSION TO CONDUCT RESEARCH

I have been directed to convey the Ministry's approval to **BITIYONG REBECCA WADA,** an M SC. Student of the **DEPARTMENT of MICROBIOLOGY FACULTY OF SCIENCE AHMADU BELLO UNIVERSITY, ZARIA to** conduct a research on the Topic: **MOLECULAR CHARACHERIZATION OF HEPATITIS C VIRUS AND ITS CO-INFECTION WITH HEPATITIS B VIRUS AMONY PRISON INMATES AND PSYCHIATRIC PATIENTS IN KADUNA STATE.**

It is expected that necessary assistance be accorded him in the process of the research, please.

However, it is mandatory for the researcher to submit a copy of his research finding(s) to the office of the Honourable Commissioner, Ministry of Health, as soon as it is concluded.

Accept the assurances of the Honourable Commissioner's highest regards.

F. A. KURAH (MRS)
SECRETARY, ETHICAL COMMITTEE

APPENDIX Ib: Ethical Aproval

FEDERAL NEURO-PSYCHIATRIC HOSPITAL, KADUNA

BARNAWA, KADUNA NIGERIA.

OFFICE:
Opposite Shagari Low Cost
P.M.B. 2187,
Barnawa Kaduna.
☎:062-230001-3, 237308-10
e-mail: fnphkaduna@yahoo.com

18th August, 2014

Medical Director
DR. T. L. SHEIKH
MBBS (ABU) M.Sc., FWACP
Cert. Gmh (Harvard)

Head of Clinical Services
DR. EBITI W.N.
MBBCH (CAL) FWACP

Deputy Director of Administration/
Secretary to Board
ALHAJI MAHMUD ALHASSAN
B.Sc. MPA, CHPM, AHAN, MNIM, MNIPR

HEALTH RESEARCH ETHICS COMMITTEE

Notice of Full Approval

Re: **Molecular Characterization of Hepatitis C Virus and its Co-Infection with Hepatitis B virus Among Prison Inmates and Psychiatric Patients in Kaduna State**
Name of Principal Investigator: **Bitiyong, Rebecca Wada**
Address of Principal Investigator: **Department of Microbiology**
Date of receipt of valid application: **1st August, 2014**
Date of meeting when final determination of research was made:

This is to inform you that the research described in the submitted protocol, the consent procedure, and other participant information materials have been reviewed and *given full approval by the Health Research Ethics Committee.*

This approval dates from **12th August, 2014 to 12th January, 2015.** If there is delay in starting the research, please inform the HREC so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the HREC. No changes are permitted in the research without prior approval by the HREC. The HREC reserves the right to conduct compliance visit your research site without previous notification.

Dr. Ebiti William N.
Chairman, HREC

Dr. Folorunsho T. Nuhu
Secretary, HREC

THE BOARD OF MANAGEMENT
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APPENDIX Ic: Ethical Approval

NIGERIAN PRISONS SERVICE

NATIONAL HEADQUARTERS
Bill Clinton Drive, Airport Road, Abuja
(P. M. B. 16, Garki - Abuja)

Phone:

Your Ref: MSC/Sc1/29339/12-13

Our Ref: NPS 569/T.45

Date: 18/12/2014

BITIYONG REBECCA WADA

U.F.S Prof S Ado,
Head of Department,
Dept of Microbiology,
ABU, Zaria.

APPROVAL OF ETHICAL CLEARANCE

I am directed to refer to your request in respect of the above stated subject matter.

Approval has been granted for you to carry out the study titled 'Detection of Hepatitis B and Hepatitis C viruses among Prison inmates and mentally ill persons in Kaduna state Prison.

Wishing you all the best in your study.

Dr Aham-Neze N.C
CP (Med 1/Clinical)
For: Controller General of Prisons

APPENDIX II: Consent Form

Detection of Hepatitis C and Hepatitis B Virus Infection among Prison Inmates and Psychiatric Patients in Kaduna Metropolis, Nigeria

This consent form gives information about the study that will be discussed with you. Once you understand the study, and if you agree to take part, you will be asked to sign your name or make your mark on this form. The researcher is from the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria.

I, ---------- permit Miss Bitiyong Rebecca of Dept. of Microbiology, ABU, Zaria to take my blood as sample for her research. This doesn't stop me from my daily activities and it will enable me know my status.

---------- ----------

Sign Date

Witness ----------

Sign

APPENDIX III: QUESTIONNAIRE

Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria

Detection of Hepatitis C and Hepatitis B Virus Infection among Prison Inmates and Psychiatric Patients in Kaduna Metropolis, Nigeria

This questionnaire requires information about the aforementioned study. We request you fill it to the best of fact. You should be rest assured that the information provided will be treated with utmost confidentiality. Please fill in the spaces and tick where necessary.

1. Age----------
2. Date---------- 3.Sex: Male [ ] Female [ ]
4. Marital Status: Married [ ] Single [ ] Separated [ ]
5. Highest level of Education: Primary [ ] Secondary [ ] Tertiary [ ] Non-formal [ ]
6. Occupation: Civil Service [ ] Public Service [ ] Business [ ] Unemployed [ ]
7. Tattooing/ scarification Yes [ ] No [ ]
8. History of transfusion Yes [ ] No [ ]
9. Are you an injection drug user? Yes [ ] No [ ]
10. Do you or have you been consuming alcohol? Yes [ ] No [ ]
11. Sexual experience Yes [ ] No [ ].
12. Do you share shaving equipment? Yes [ ] No [ ]
13. Do you share clothes? Yes [ ] No [ ]