# CHAPTER ONE

## 1.0 INTRODUCTION

HIV/AIDS is one of the clinical conditions of public health importance with high morbidity and mortality worldwide, especially in developing countries. It is a socio-economic burden, and a serious threat to development. There are more than 33.4 million people living with HIV/AIDS and more than 95% of them live in developing countries, about 70% in Sub-Saharan Africa (WHO, 2012; WHO, 2013; Global AIDs 2016). There are 5 different viruses that cause hepatitis virus namely; the hepatitis A virus (HAV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Hepatitis D virus (HDV) and Hepatitis E Virus (HEV) (Ochei and Kolhatkar, 2008). Among these 5 different viruses, it is only the Hepatitis B Virus (HBV), Hepatitis C Virus and Hepatitis D virus that share certain epidemiological characteristics such as risk factors and transmission routes with Human Immunodeficiency Virus (HIV).

Among the 3 viruses that share certain transmission route with HIV, the Hepatitis B and C virus infections are major public health problem worldwide. It was estimated that approximately 2 billion people (about 30% of the world's population) have serological evidence of past or present HBV infection. More than 350 million are chronic carriers of HBV. This HBV infection may lead to a variety of clinical pictures, ranging from asymptomatic carrier state to acute hepatitis, fulminant hepatitis, chronic hepatitis, liver cirrhosis (LC) and hepatocellular carcinoma (HCC). Progression of chronic hepatitis B (CHB) disease to severe liver diseases, such as LC and HCC, is determined by the genetic characteristics of the host, as well as by viral and environmental factors (Biswas*et al*, 2013;

WHO, 2017). Between 600,000 and 1.2 million death cases are reported each year as a result of the complications of acute or chronic hepatitis (WHO 2017).

Hepatitis B Virus (HBV) is an enveloped, non-cytopathic, hepatotropic virus which can cause acute and chronic hepatitis. Hepatitis B virus (HBV) is a serious public health problem worldwide, though there is a safe vaccine currently in use against hepatitis B virus (HBV); it remains a severe public health issue, especially in Asia, Africa and South America, and may result in death. Just like the HIV, the HBV and HCV share common route of transmission. As the same transmission routes are shared by all these viral infections, co-infection is not uncommon. The transmission route can be through blood and blood products, body piercing, intravenous drug abuse, unsafe injections and sexual activity (Jafari*et al*, 2010); Lam *et al*, 2010; Olokoba*et al*, 2008). This puts HIV positive individuals at risk of co-infection with hepatitis B or hepatitis C or both.

Infection with hepatitis C virus (HCV)is estimated to affect 2% of the world population and we have about 170 million HCV carriers worldwide (Rotman and Liang, 2009) and is a leading cause of liver-related morbidity and mortality, most of them are thought to be in the developing countries. Despite the effective decline of the mortality and morbidity rate from HIV/AIDS as the result of highly active antiretroviral therapy (HAART), liver diseases due to chronic HBV and HCV infections become a leading cause of death. Although the direct impact of HCV upon HIV disease progression remains controversial in many reports the complex interactions between HIV/HBV/HCV co-infection and HAART are increasingly apparent in HIV disease progression.In HIV/HBV co-infections, HIV infection causes increased rates of persistent HBV infection, increased cirrhosis and liver-related mortality and

increased risk of hepatocellular carcinoma. Similarly in HIV/HCV co-infections, there is a more rapid progression to cirrhosis, end-stage liver disease and hepatocellular carcinoma (Wondimeneh *et al,* 2013).

The impact of HBV and HCV could not be limited in causing liver hepatotoxicity but also results in failure in immunological recovery in HIV positive patients. For example, Christian *et al,* (2010) reported slow rate of immunologic recovery after initiation of HAART treatment and higher risk of hepatotoxicity among HIV/HBV and HIV/HCV co-infected patients in their study in Tanzania. Thus the management of HBV and HCV in HIV infection is complicated and bring high burden in particular where HIV is rampant. As a result, HIV, HBV and HCV become the major public health concerns globally. In some countries, screening of HIV-infected individuals for HBV and HCV is highly recommended before initiation of antiviral treatment.

The complications from HCV and HBV infections are serious. Approximately 10% to 40% will develop chronic hepatitis and experience gradual progression to liver cirrhosis and hepatocellular carcinoma (HCC) of which HBV is considered to be the main agent. Also, HCV plays an important role in the causation of chronic liver disease, and has become the leading cause of liver cirrhosis and primary liver cell carcinoma in North America, Southern Europe and Japan (Chen, 2011). Patients with HCV and HBV co-infections with HIV have an increased risk of progression of HCV and HBV-related liver disease (chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC)), compared with HCV mono-infected patients (Omland*et al*., 2009; Ananthakrishnan*et al.,* 2010). There have been debatable reports concerning whether HCV or HBV infection changes the natural history of HIV disease (Lo *et*

*al.*, 2008; Soriano *et al.*, 2008). Thus it is unclear whether the improvement of the long-term outcome of HIV-positive patients is influenced by co-infection with hepatotropic viruses. Some authors reported that, "HCV infection may contribute to faster progression of HIV infection", while it has been established that hepatitis B infection does not hasten HIV disease progression or severity unlike hepatitis C (Carter, 2011). With prolonged survival of HIV infected patients, co infection with either HBV or HCV correlates with reduced survival rate (Schmidt, 2008). For HIV and HBV co-infection (HIV/HBV), the seroprevalence ranges from 6.3% to as high as 39%.

Based on the endemicity of hepatitis B virus (HBV) infection, countries are classified into high (8% or more), intermediate (2-7%) or low (less than 2%) prevalence countries. In areas of low endemicity, such as North America, Australia and Europe, HBV and HIV infection are usually acquired in adulthood through sexual or percutaneous transmission and the prevalence of chronic co-infection is around 5-7%. In countries with intermediate and high HBV endemicity, the main routes of transmission of HBV are perinatal or in early childhood. In these countries, HBV co-infection rates are 10-20% (Lee, 2008). The rate of progression and complications from viral hepatitis has been reported to be accelerated in patients with HIV co-infection (Thio, 2009). HIV/HBV co-infected individuals are 6 times more likely to develop chronic hepatitis B than HIV negative individuals and the incidence of HCC and cirrhosis is nearly 6.5 to 11 times higher among HIV co-infected patients than those without HIV co-infection and this is more likely to occur in HIV infected men with lower CD4+ cells. Decreased rates of clearance of HBeAg and increased HBV replication are also seen, with higher HBV DNA viral load. In addition, HIV infected individuals are more likely to lose

previously developed protective anti-HBs antibody and develop acute hepatitis B infection; this risk is also associated with lower CD4+ counts (Biggar*et al.,* 2007).

Hepatitis C virus (HCV) and hepatitis B virus (HBV) infection are very common in HIV-infected participants (Soriano *et al*., 2008). However, results in studies of HCV co-infection are varied depending on the geographical area, type of exposure and different risk behavior populations. In China, prevalence of HCV co-infection among HIV-infected patients ranging from 12% to 85% has been reported. The differences in co-infection rates may result from various primary transmission routes (Shang *et al*., 2010; Liu *et al*., 2008). As a consequence, regardless of whether these microorganisms increase HIV disease progression or not, it is necessary to determine the prevalence of co-infections in HIV-infected participants.

Recent studies showed that in antenatal clinic, the prevalence of hepatitis B surface antigen (HBsAg) was 4 - 6% and that of HCV 0.5 - 2% (Khan *et al.*,2011; Khan *et al.*, 2014; Ramos *et al.*, 2011). In Ghana, the prevalence of antibodies to HIV and HCV was 19% for each infection among prison inmates. In Taiwan, the latest sero-epidemiologic survey that was conducted 25 years after implementation of the universal HBV vaccination program to evaluate the effectiveness of program in the general population has shown that the prevalence of HBsAg positivity was 0.9%, and that of anti-HBs and anti-HBc positivity was 55.9% and 10.0%, respectively, in the subjects born in the vaccination era (Ni *et al,* 2012).

Estimates of the prevalence of HBV co-infection among adults with HIV in South Africa range from 5% in urban cohorts to 20% in a cohort of gold miners from rural areas (Boyles and Cohen, 2011).Nigeria is a very large country of about 167 million people with multiple ethnic groups, religion and cultural behaviors(Wondimeneh *et al,*2013). The sero-prevalence

of HIV/HBV and HIV/HCV and HIV/HBV/HCV has been studied in many parts of the countryWondimeneh *et al,* (2013), reported that the rates of co-infection of HIV with either HCV or HBV vary from region to region, study population and risk factors for HIV acquisition. A systematic review in 18 sub Saharan African countries also showed that the prevalence of HBV and HCV in HIV-infected individuals ranged from 3.9-7.3% and 6.9%, respectively (Barth *et al,* 2013).Diwe *et al,* (2013) reported HCV co-infection prevalence of 0.7%, HBV co-infection of 2.2% and no triple infection in their study in the South-East in Nigeria. This perhaps may be related to the low incidence of intravenous drug abuse and needle sharing amongst our people compared to the developed countries. HBV co-infection has been shown to occur in 10-70% of HIV infected individuals. In Lagos, Nigeria, theprevalence of HIV/HBV and HIV/HCV among HIV patients was estimated to be 28.4% and 14.7% respectively as against the prevalence of 6.0% and 0.8% for HIV/HBV and HIV/HCV respectively for non-HIV volunteers. (Balogun *et al,* 2012). In Ekiti State,Nigeria,Adekunle*et al,* (2011) documented thatthe prevalence of HIV/HBV and HIV/HCV was 6.6% and 0.7% respectively. The frequency of positive serum HBsAg was 8.1% and 6% among males and females respectively. When the HBsAg sero-negative patients were compared to those who were HBsAg sero-positive, there was no significant difference in their mean serum Alanine Aminotransferase(ALT) (20.84± 18.26 vs. 13.39± 11.20), Aspartate Aminotransferase (AST) (19.43 ± 15.17 vs. 12.14± 5.49).Furthermore, in Owerri, (Nwako*et al,* 2013) reported the prevalence rate of HIV/HBV co-infection to be 10.6%. In Abuja, Tremeau-Bravard *et al,*(2012) reported a prevalence of 7.9% for HIV/HBV co-infection, 2.3% for HIV/HCV co-infection, and 0.7% for HBV/HCV/HIV triple infections. The overall prevalence of HIV/hepatitis co-infection in the studied population is 10.8%.

In demorgraphic study, Tremeau-Bravard *et al*, (2012) documented in his study, the co-infected population (HIV and one or two of the studied hepatitis) is distributed as follows: 9% of men and 7% of women have hepatitis B, 3.5% of men and 1.2% of women have hepatitis C, 0.5% of men and 0.8% of women have both hepatitis (HBsAg and Anti-HCV) and there are no significant differences in the mean age of the HIV, the HIV/HBV, and the HIV/HBV/HCV groups.

## 1.1 JUSTIFICATION

The epidemiology of HBV (hepatitis B virus) and HCV (hepatitis C virus) serotypes and their association with HIV has made management of HIV infection very difficult. In HIV/HBV co-infections, HIV infection causes increased rates of persistent HBV infection, increased cirrhosis and liver-related mortality and increased risk of hepatocellular carcinoma. Similarly in HIV/HCV co-infections, there is a more rapid progression to cirrhosis, end-stage liver disease and hepatocellular carcinoma (Wondimeneh *et al*, 2013). This justifies the quest for determining the sero-prevalence of these hepatitis among HIV patients in Abuja.

## 1.2 RESEARCH QUESTIONS

Five (5) fundamental research questions were raised:

1. Which of the viral infections i.e. HBV or HCV has the highest prevalence within the area of study?
2. What are the prevailing HBV and HCV genotypes in Abuja that has impact on the liver?
3. Which of the diagnostic method is most preferable for the diagnosis of these diseases?
4. What risk factor is prominent in the area under study?

5. What is the level of prevalence of HBV and HCV among HIV positive subjects and non-HIV subjects?

## 1.3 AIM

This research aimed at determining the seroprevalence of hepatitis B and C among HIV patients in 3 district hospitals in Abuja.

## 1.4 OBJECTIVES

Theobjectives of this researchare to;

1. determine the prevalence of HBV and HCV among HIV patients and non HIV volunteers using RTD and PCR
2. compare the accuracy of the diagnostic methods.
3. estimate the prevalence of hepatitis B core antibody (HBcAb), hepatitis B envelop antigens (HBeAg), hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb) and hepatitis B envelop antibody (HBeAb).
4. estimate the liver enzyme profile of the hepatitis positive patients in order to know the impact of these disease to the liver of HIV patients.
5. determinethe Alpha Fetoprotein (AFP) levels in HIV/HBV, HIV/HCV and HIV/HBV/HCV co-infections.
6. determine the hepatitis B and C genotypes that are more prevalent in Abuja;

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 HUMAN IMMUNODEFICIENCY VIRUS

Human immunodeficiency virus (HIV) is a very important global public health problem, infecting about 33 million people worldwide. Human Immunodeficiency Virus (HIV) is an enveloped RNA virus belonging to the lentivirus subgroup of retroviruses, which lead to gradually progressing disease, often with long incubation periods. With the presence of the enzyme reverse transcriptase, retroviruses are able to reverse-transcribe RNA to DNA which normally, RNA is transcribed from DNA. The DNA genome produced (provirus) becomes integrated in the DNA of the infected cell, ensuring permanent infection and replication of the virus (Brooks *et al.,* 2009).

The amount of HIV in the blood (viral load) is of significant prognostic value. There are continual rounds of viral replication and cell killing in each patient, and the steady-state level of viruses in the blood varies from individual to individual. This level reflects the total number of productivity infected cells and their average burst size. It turns out that a single measurement of plasma viral load that is about 6months after infection is able to predict the subsequent risk of development of AIDS in men several years later. However, more recent data suggest a gender difference in this parameter in women the viral load may be less predictive of progression to AIDS. Plasma viral load measurements are a critical element in assessing the effectiveness of antiretroviral drug therapy. It will also determine the extent other analytes will be effected in the body (Brooks *et al*., 2009).

Acute HIV infection is usually accompanied by transient non-specific illness characterized by fever, malaise, myalgia, lymphadenopathy, pharyngitis and rash. Most of these conditions are,

however, subclinical. In two to six weeks, antibodies to the core and surface proteins are usually detected by enzyme immunoassay (EIA) and confirmed by immunofluerescence or Western-blot technique. A chronic infection of AIDS that follows is asymptomatic in early stages. In some patients, a persistent generalized lymphadenopathy in the form of nodes of 1cm or more in diameter in two or more non-contiguous extrainguinal sites, commonly in the cervical and the auxiliary lymph nodes, usually develops. In the later stages of the illness, there may be development of symptoms such as fever, night sweats, diarrhea and weight loss. Patients may also suffer from 'minor' opportunistic infections such as oral candidasis, oral hairy leucoplakia, herpes zoster, recurrent oral or genital herpes simplex, impetigo and tinea infections. These conditions which may be signs of major opportunistic infections to come are now collectively known as symptomatic non-AIDS; they were previously referred to as AIDS-related complex (ARC). The incubation period of HIV infection is long, lasting up to 10 years before the symptoms develop. Classical complications of AIDS are very severe (Ochei and Kolhatkar, 2009).

HIV leads to progressive impairment of body's cellular immune system, causing increased susceptibility to infections and tumors, and the fatal condition AIDS (acquired immunodeficiency syndrome). After primary infection, there is a 4 to 11 day period between mucosal infection and initial viremia; the viremia is detectable for about 8- 12 weeks. Virus is widely disseminated throughout the body during this time, and the lymphoid organs become seeded. An acute mononucleosis-like syndrome develops in many patients (50-75%) 3-6 weeks after primary infection. There is a significant drop in numbers of circulating CD4 T cells at this early time. An immune response to HIV occurs 1 week to 3 months after infection, plasma viremia drops, and levels of CD4 cells rebound. However, the immune

response is unable to clear the infection completely, and HIV-infected cells persist in the lymph nodes (Ochei and Kolhatkar, 2008).

This period of clinical latency may last for as long as 10 years. During this time, there is a high level of ongoing viral replication. It is estimated that 10 billion particles are produced and destroyed each day. Eventually, the patient will develop constitutional symptoms and clinically apparent disease, such as opportunistic infections or neoplasms. Higher levels of virus are readily detectable in the plasma during the advanced stages of infection. HIV found in patients with late-stage disease is usually much more virulent and cytopathic than the strains of virus found early in infection (Brooks *et al.,* 2009).

HIV is found in the semen, vagina/cervical secretions and blood, and these are the main vehicles by which virus are transmitted. It is transmitted sexually, in blood or blood products and prenatally. The most at risk of acquiring HIV infection are homosexuals, injecting drug misusers and those bisexuals orientation. Others are the individuals receiving unscreened blood or blood products and infants born of infected women. The virus may also be present in the saliva, breast milk, tears, urine, infected discharges and cerebrospinal fluid (Ochei and Kolhatkar, 2008).

## 2.2 HEPATITIS VIRUSES

Redmond (2014) defined hepatitis as the inflammation of the liver which may be caused by exposure to autoimmune diseases, bacterial infections or by exposure to certain chemicals but is person, sharing of infected needles or objects that breaks the skin. Viral hepatitis infection often caused by one of several viruses. This virus lives in the body fluids and blood

which can be transmitted through unprotected sexual intercourse with an infected is an important health problem worldwide of which 350-400 million estimated people are chronically infected with hepatitis B virus and 190 million infected with hepatitis c virus. It is estimated that complications of HBV or HCV infection has led to nearly 1.4 million deaths in the year 2010. About 30% of the disease burden due to viral hepatitis is located in the WHO South-East Asia Region, with estimated 100 million and 30 million people infected with HBV and HCV, respectively. In addition nearly half of the global burden of HEV occurs in this region, with amounts to nearly 12 million cases annually (WHO, 2012; CDC, 2016).

There are different types of hepatitis namely hepatitis A –G. Hepatitis A is caused by hepatitis A virus (HAV), an estimated 2,500 new infection was reported in 2014. The route of transmission is by ingesting fecal matter, contact with infected persons and ingestion of contaminated food or drinks. Persons at risk for this infection are travelers who travel to regions with intermediate or high rates of hepatitis A, sex contacts with infected persons, household members or caregivers of infected persons, homosexuals, users of illegal drugs and persons with clotting-factor disorders. The incubation period for this viral infection is 15 to 50 days i.e. an average of 28days. Most persons with acute disease recover with no lasting liver damage; rarely fatal and the serological test for acute infection is IgM anti-HAV. No medication is available, it is best addressed through supportive treatment but hepatitis A vaccine is recommended for children at age 1year, travelers, homosexuals, users of illegal drugs, persons with clotting factor disorder, persons with chronic liver disease and anyone else seeking long term protection. The schedule for the vaccination is 2 doses given 6 months apart (CDC, 2016).

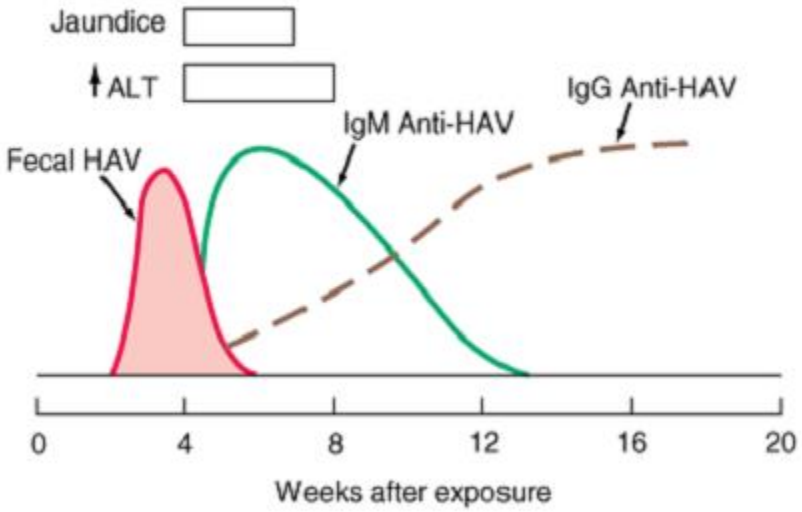



**Fig. 1: Scheme of typical clinical and laboratory features of acute Hepatitis A**

Fauci *et al*, 2008

### 2.2.1 HEPATITIS B VIRUSES (HBV)

It is often referred to as a dane particle. It carries hepatitis B core antigen (HBcAg), hepatitis B surface antigen (HBsAg), and viral DNA and is infective. The virus is DNA, double stranded (with single stranded regions), enveloped hepadnavirus. It can stay up to 7days outside human body unlike the HIV which cannot stay more than hours outside human body, for this reason, HBV is rated as being far more infectious than HIV. Dane particles are released into the blood stream together with small spherical and rod-like particles of envelop origin during infection (Brooks *et al*., 2009). The spherical and rod-like particles carry HBsAg and are non-infective. Hepatitis B e antigen (HBeAg) is part of the core protein of Dane particles and its presence in the blood is associated with infectivity. It is secreted from

infected liver cells during the acute stage of the disease and in some carriers, when there is active virus replication.

Hepatitis B is caused by the hepatitis B virus (HBV). As at 2014, about 19,200 new infections have been estimated and 850,000-2.2 million people with chronic HBV infection. The route of transmission is contact with infectious blood, semen and other body fluids primarily through; birth to an infected mother, sexual contact with infected person, sharing of contaminated needled, syringes or injectable drug equipment (CDC, 2016). The persons at risk includes; infants born to infected mothers, Sex partners of infected persons, persons with multiple sex partners, persons with a sexually transmitted disease (STD), men who have sex with men Injection drug users, household contacts of infected persons, healthcare and public safety workers exposed to blood on the job, hemodialysis patients, residents and staff of facilities for developmentally disabled persons, travelers to regions with intermediate or high rates of Hepatitis B (HBsAg prevalence of ≥2%). The incubation time is between 45 to 160 days, with an average of 120days. Symptoms appear in 5 to 15% of newly infected adults who are immunosuppressed with a high chance for chronic infection to occur. Among unimmunized persons, chronic infection occurs in greater than 90% of infants, 25-50% of children aged 1-5years and 6-10% of older children and adults. Most persons with acute disease recover with no lasting liver damage; acute illness is rarely fatal, 15%–25% of chronically infected persons develop, chronic liver disease, including cirrhosis, liver failure, or liver cancer (WHO, 2013). HBsAg is the serological test conducted in both acute and chronic infection and in acute infection, IgM anti-HBC is positive. Screening is recommended for; all pregnant women, persons born in regions with intermediate or high, rates of Hepatitis B (HBsAg prevalence of ≥2%), infants born to HBsAg-positive mothers, household, needle-sharing, or sex contacts of

HBsAg-positive persons, men who have sex with men, Injection drug users, patients with elevated liver enzymes (ALT/AST) of unknown etiology, hemodialysis patients, persons needing immunosuppressive or cytotoxic therapy, HIV-infected persons, donors of blood, plasma, organs, tissues or semen. This infection has no medication but best addressed through supportive treatment with some patients been treated with antiviral drugs in the case of chronic infection and requires regular monitoring for sign of liver disease progression. Vaccination schedule for infants and children is 3 to 4 doses given over a 6- to 18-month period depending on the vaccine type and schedule, Adults require 3 doses given over a 6-month period (most common schedule) (Kumar *et al*, 2007).

High HBV carrier rates are found in sub-Saharan Africa, Southeast Asia, China, Pacific islands and the Amazon basin. Most people become infected at birth or during childhood, or by sexual contact, and up to 20% become chronic carriers depending on the age at when they are infected. HBV causes the most serious form of viral hepatitis. Persistence of the virus in chronic carriers can lead to the development of liver cirrhosis and liver cancer in years later. This virus causes 60-80% of all primary liver cancer and is a major cause of death in East and Southeast Asia, the Pacific Basin and sub-Saharan Africa, Vaccination is an effective way of preventing HBV transmission and children becoming carriers providing this is done early in life (Ochei and Kolhatkar, 2008).

HBV has a DNA genome of about 3.2 kb, which contains four open reading frames, namely S (surface antigen), P (polymerase), C (core protein) and X (regulatory protein). Generally, the development of antibodies against hepatitis B surface antigen (HBsAg) leads to the viral clearance. However, the concurrent presence of HBsAg and anti-HBs has been reported occasionally. Studies performed with HBV carriers testing positive for both serological

markers have demonstrated that mutations in the pre-S and S, regions of the genome may lead to changes in the immunogenicity of the viral particles, thus influencing the viral behavior and clinical course of the liver disease (Lago *at al,* 2014).

Hepatitis B Virus (HBV) can cause a self-limiting acute infection or a chronic hepatitis, depending on the interaction between the host's immune system and the virus.Typically, the sign of HBV infection is the presence of Hepatitis B Surface Antigen (HBsAg) in the blood. On the other hand, the appearance of the neutralizing antibodies against HBsAg (HBsAb) usually indicates resolution of infection, both spontaneously and after therapy.In this simple virological scenario, some reports have documented the coexistence of HBsAg and HBsAb in some patients with chronic hepatitis B (CHB), often in the absence of amino acid substitutions in the HBsAg sequence able to explain the escape of HBV from the HBsAb immune control. HBV genome has a very compact coding organization, with four partially overlapping open reading frames (ORFs). Because the reverse transcriptase (rt) region of HBV polymerase overlaps the HBsAg ORF, it is possible that mutations in the HBsAg region correspond to mutations in the rt ORF, conferring resistance to nucleos(t)ide analogues (NAs). In addition, due to the quasispecies nature of the HBV genome in each infected individual, some mutations may be present in minor variants of viral population, being not detected by classical population sequencing. The powerful ultra-deep pyro-sequencing (UDPS) approach, based on next generation sequencing (NGS), has been recently used to obtain a complete description of HBV quasispecies, highlighting possible minor populations carrying mutations in the two overlapping ORFs (Galati *et al*, 2014).

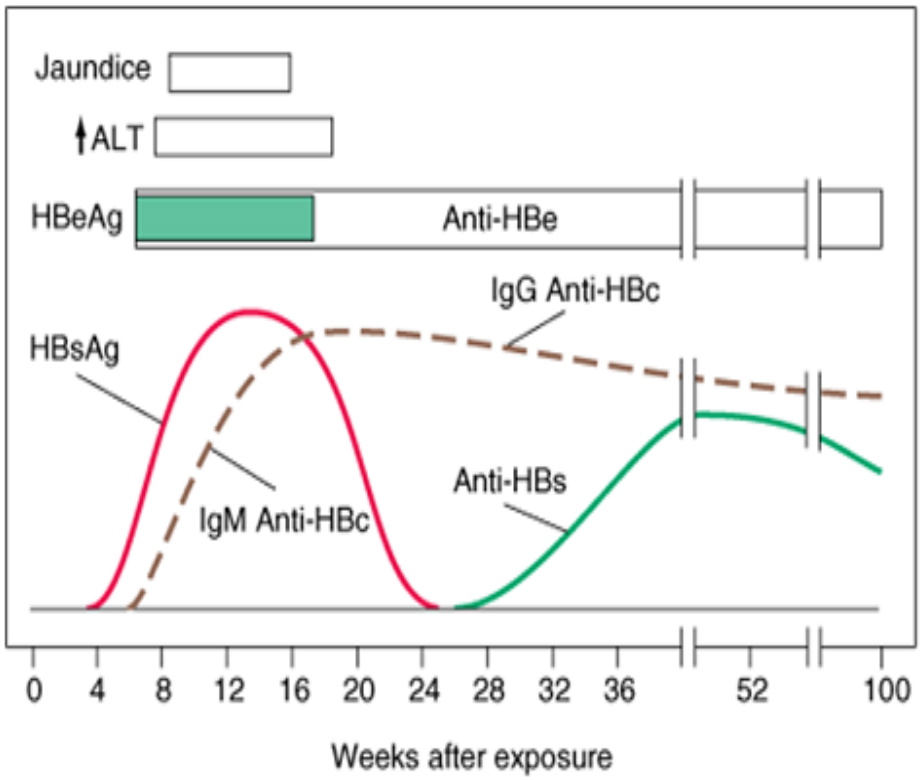



**Fig. 2: Scheme of typical clinical and laboratory features of acute Hepatitis B**

Fauci *et al*, 2008

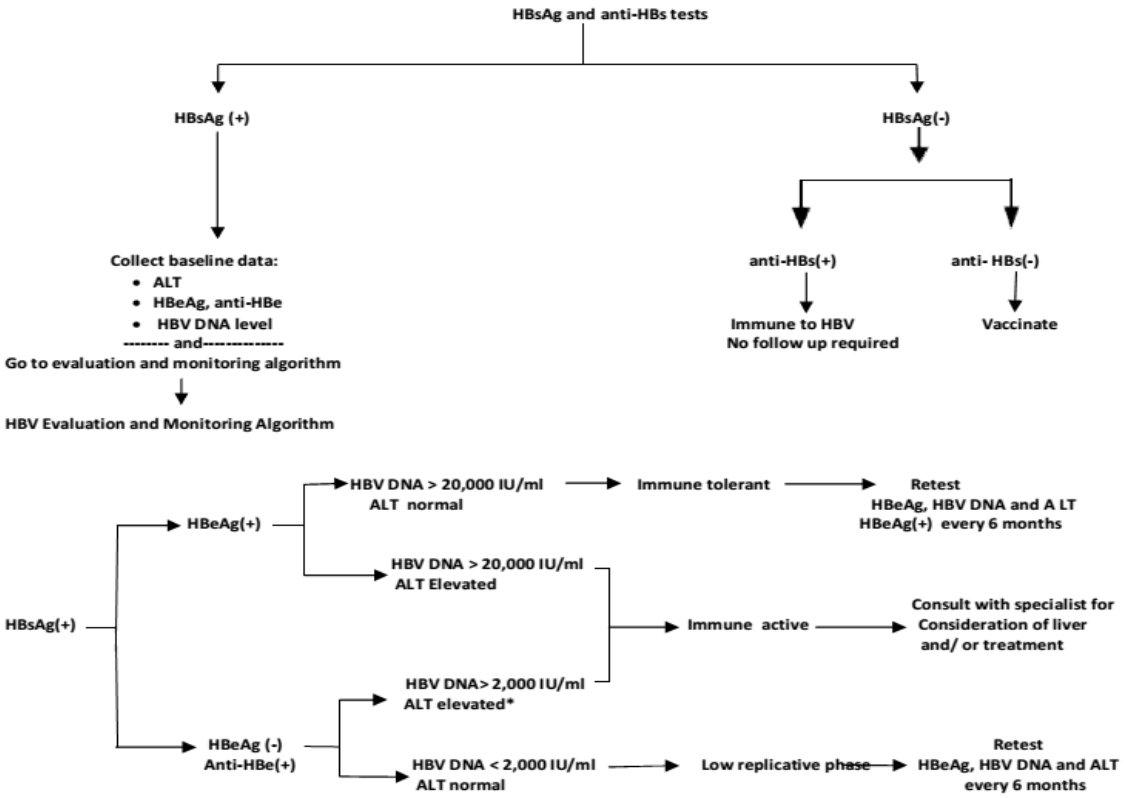



**Fig 3: Algorithm for screening of hepatitis B**

Fauci *et al*, 2008

### 2.2.2 EPIDEMIOLOGY

The prevalence of chronic HBV infection varies greatly in different part of the world. The prevalence of chronic HBV infection worldwide could be categorized as high, intermediate and low endemicity. The age at the time of infection is associated with the endemicity of HBV infection.

#### 2.2.2.1 High Endemicity

The prevalence of HBV infection varies markedly throughout regions of the world. Hepatitis B is highly endemic in developing regions with large population such as South East Asia, China, sub-Saharan Africa and the Amazon Basin, where at least 8% of the population are HBV chronic carrier. Approximately 75% of chronic carriers live in Asia and the Western Pacific (Biswas*et al,* 2013). It was reported that 15-40% of HBV infected patients would develop cirrhosis, liver failure, or HCC, and 500, 000 to 1.2 million people die of HBV infection annually (Teo and Lok 2017). On the high HBV-related morbidity and mortality, the global disease burden of HBV is substantial (Teo and Lok 2017). Africa is globally classified as a high HBV prevalence area, although hepatitis B endemicity may vary greatly from a region to another. Based on HBsAg positivity, HBV is hyperendemic (>8%) in some sub-Saharan countries such as Nigeria, Namibia, Gabon and Cameroon (Lago *et al,* 2014).

In these areas, 70–95% of the population shows past or present serological evidence of HBV infection. Most infections occur during infancy or childhood. Since most infections in children are asymptomatic, there is little evidence of acute disease related to HBV, but the rates of chronic liver disease and liver cancer in adults are high (Alter, 2009).

#### 2.2.2.2 Intermediate Endemicity

In part of Eastern and Southern Europe, the Middle East, Japan, and part of South America, and other countries like; Kenya, Zambia, Côte d'Ivoire, Liberia, Sierra Leone and Senegal are considered areas of intermediate endemicity (2–8%), hepatitis B is moderately endemic. Between 10–60% of the population have evidence of the infection, and 2-7% are chronic carriers. Acute disease related to HBV is common in these areas because many infections occur in adolescents and adults; however, the high rates of chronic infection are maintained mostly by infections occurring in infants and children. In these areas, mixed patterns of transmission exist, including infant, early childhood and adult transmission (Lago *et al*, 2014).

#### 2.2.2.3 Low Endemicity

In most developed areas, the endemicity of HBV is low, such as North America, Northern and Western Europe, Australia, Egypt, Tunisia, Algeria and Morocco, located at the North of the continent, are low endemicity (<2%) regions. Prevalence of hepatitis B core antibody (anti-HBc), a serological marker for previous HBV exposure, is extremely high (>80%) in various African populations (Lago*et al*, 2014). HBV infects 5–7% of the population in these regions, and only 0.5–2% of the populationare chronic carriers. Most HBV infections in these areas occur in adolescents and young adults in relatively well-defined high-risk groups, including injection drug user, homosexual males, health care workers, patients who require regular blood transfusion or hemodialysis (Lago*et al*, 2014).

### 2.2.3 HBV TRANSMISSION

HBV is spread through contact with infected body fluids and the only natural host is human. Blood is the most important vehicle for transmission, but other body fluids have also been implicated, including semen and saliva currently, three modes of HBV transmission have been

recognized: perinatal, sexual and parenteral/percutaneous transmission. There is no reliable evidence that airborne infections occur and feces are not a source of infection. HBV is not transmitted by contaminated food or water, insects or other vectors. In developing countries the main route of transmission include;

- reuse of HBV contaminated needles, syringes, lancets, and instruments including those used in tribal ceremonies,
- neonatal with an HBV carrier mother infecting her infant, usually during birth or soon after birth and close contact,
- transfer of HBV through cuts and grazes example young children,
- sexually transmission,
- transfer of infected blood or blood product,
- needle stick injury,
- contamination of eye,
- Possibly blood-sucking insects and bed bugs (Ochei and Kolhatkar, 2008).

#### 2.2.3.1 Perinatal Transmission

Transmission of HBV from carrier mothers to their babies can occur during the perinatal period, and appears to be the most important factor in determining the prevalence of the infection in high endemicity areas, particularly in China and Southeast Asia. Before HBV vaccine was integrated into the routine immunization program, the proportion of babies that become HBV carriers is about 10-30% for mothers who are HBsAg-positive but HBeAg-negative. However, the incidence of perinatal infection is even greater, around 70-90%, when the mother is both HBsAg-positive and HBeAg-positive. There are three possible routes of

transmission of HBV from infected mothers to infants: transplacental transmission of HBV in uterus; natal transmission during delivery; or postnatal transmission during care or through breast milk. Since transplacental transmission occurs antenatally, hepatitis B vaccine and HBIG cannot block this route. Epidemiological studies on HBV intrauterine infection in China showed that intrauterine infection occurs in 3.7-9.9% pregnant women with positive HBsAg and in 9.8-17.39% with positive HBsAg/HbeAg. The studies on transplacental transmission of HBV suggested two possible mechanisms (1) hemagenous route: a certain of factors, such as threaten abortion, can make the placental microvascular broken, thus the high-titer HBV maternal blood leak into fetus' circulation (Wang*et al,* 2009) (2) cellular transfer: the placental tissue is infected by high-titer of HBV in maternal blood from mother's side to fetus' step by step, and finally, HBV reach fetus' circulation through the villous capillary endothelial cells (Wang*et al,* 2009).

#### 2.2.3.2 Sexual Transmission

The major source of HBV infection in all areas of the world is the sexual transmission, especially in the low endemic areas, such as North America. Hepatitis B is considered to be a sexually transmitted disease (STD). Homosexual men have been considered to be at the highest risk of infection due to sexual contact for a long time;however, heterosexual transmission accounts for an increasing proportion of HBV infections, therefore both the homosexuals and the heterosexuals are not safe from these viral infections. In heterosexuals, factors associated with increased risk of HBV infection include duration of sexual activity, number of sexual partners, history of sexual transmitted disease, and positive serology for syphilis. Sexual partners of injection drug users, prostitutes, and clients of prostitutes are at particularly high risk for infection (Alter, 2009).

#### 2.2.3.3 Parenteral/percutaneous Transmission

This includes injection drug use, transfusions and dialysis, acupuncture, working in a health-care setting, tattooing and household contact. In the United States and Western Europe, injection drug use remains a very important mode of HBV transmission (23% of all patients). Risk of acquiring infection increases with duration of injection drug use. Although the risk for transfusion-associate HBV infection has been greatly reduced since the screening of blood for HBV markers and the exclusion of donors who engage in high-risk activities, the transmission is still possible when the blood donors are asymptomatic carrier with HBsAg negative. Obvious sources of infection include HBV-contaminated blood and blood products, with contaminated surgical instruments and utensils being other possible hazards. Parenteral/percutaneous transmission can occur during surgery, after needle-stick injuries, intravenous drug use, and following procedures such as ear piercing, tattooing, acupuncture, circumcision and scarification. The nosocomial spread of HBV infection in the hospital, particularly in dialysis units, as well as in dental units, has been well described, even when infection control practices are followed (Ochie and Kolhatkar, 2008).

### 2.2.4 HBV GENOTYPE AND ITS CLINICAL SIGNIFICANCE

Phylogenetic analysis has led to the classification of hepatitis B virus into eight genotypes, designated A to H based on based on a sequence divergence >7.5% in the entire genome or complete HBV genomes (Lago*et al*, 2014). Genotypes A–D and F have been divided into sub-genotypes based on an intergroup divergence of about 4%. Genotypes A, D and E are the most frequently found in Africa and show a characteristic distribution, with sub-genotype A1 being prevalent in Southern and Eastern coastal regions, HBV/E spread in West Africa and

genotype D prevailing at the North of the continent. The evolutionary history of HBV/E is still unclear. This genotype is largely spread in West Africa but shows a restricted genetic variability. Indeed, the mean divergence over the HBV/E whole genome does not exceed 1.75%, in comparison to 4% among HBV/A African isolate. Despite the slave trade that lasted from the 16th to the 19th century, HBV/E has not been reported in the New World, except in people who maintained relations with Africa. These findings support the hypothesis of a recent (<200 years) origin of HBV/E.

Genotype E is predominant to Africa, genotype F is more common; in South America and Polynesia, and genotype G is more predominant in the United States and France (Chu *et al*, 2009). However, a new genotype (H), which is closely related to genotype F, has been identified in Central America recently (Lago *et al*, 2014).

### 2.2.5 Relationship between HBV genotypes and specific patterns of HBV-induced disease and response to treatment

Genotype C has been associated with more severe chronic liver disease compared with genotype B in Asia, and with lower response rates to interferon therapy in Taiwan. Genotype B has been associated with a significantly lower prevalence of hepatitis B envelope antigen (HBeAg) positivity at presentation and a higher rate of spontaneous sero-conversion in Chinese and Japanese patients, while genotype C predominates in chronic hepatitis B surface antigen (HBsAg) carriers who develop HCC in Japan (Wang *et al*, 2009). In Europe, genotypes A and D are common in patients with chronic hepatitis with higher rates of biochemical remission and HBsAg clearance reported in genotype A patients compared with genotype D patients. Therefore, the long-term outcome of chronic HBV infection may be different in patients infected with different HBV genotypes.

### 2.2.6 Different types of antigen, antibody and DNA of HBV used to diagnose HBV among ELISA and PCR.

| | |
|---|---|
| HBsAg | Surface protein contained within the lipoprotein coat |
| HBsAb | Antibody to HBsAg, indicator of recovery/immunity to HBV infection |
| HBeAg | Viral product secreted in blood, marker of infectivity, active replication (though absent in precore mutants) |
| HBeAb | Antibody to HBeAg, denoting decreased infectivity |
| HBcAg | Core antigen (viral capsid), intracellular and not detected in serum |
| HBcAb IgM | Antibody denoting recent HBV infection or exacerbation |
| HBcAb IgG | Contamination marker, positive after HBV contact |
| HBV DNA | Quantitative indicator of virus in blood |

(Mumtaz *et al*. 2011).

### 2.2.7 SIGNS AND SYMPTOMS

Viral hepatitis goes with the following symptoms; nausea, loss of appetite, gastrointestinal upset, malaise and an enlarged painful liver. With severe hepatitis, symptoms are more acute, with jaundice, fever, headache, pain in the muscles and joints and often a skin rash. The patient will notice that the urine is dark and the faeces pale. Recovery from viral hepatitis is usually slow. Chronic hepatitis and carrier state may follow infection with HBV, HDV, and

HCV. Chronic hepatitis B and C can lead to cirrhosis of the liver and liver cancer (Ochei and Kolhatkar, 2008).

Acute infection with hepatitis B virus is associated with acute viral "Hepatitis" an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, and dark urine, and then progresses to development of jaundice. It has been noted that itchy skin has been an indication as a possible symptom of all hepatitis virus types. The illness lasts for a few weeks and then gradually improves in most affected people. A few people may have more severe liver disease (fulminant hepatic failure), and may die as a result. The infection may be entirely asymptomatic and may go unrecognized (Brooks*et al.*, 2009).

Chronic infection with hepatitis B virus either may be asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of "Hepatocellular carcinoma" (liver cancer). Across Europe hepatitis B and C cause approximately 50% of hepatocellular carcinomas. Chronic carriers are encouraged to avoid consuming alcohol as it increases their risk for "Cirrhosis" and liver cancer. Hepatitis B virus has been linked to the development of membranous glomerulonephritis(MGN) (Ochei and Kolhatkar, 2008).

Symptoms outside of the liver are present in 1–10% of HBV-infected people and include Serum-sickness–like syndrome "Acute necrotizing vasculitis". (polyarteritisnodosa, membranous glomerulonephritis, and Papularacrodermatitis of childhood"). The serum-sickness–like syndrome occurs in the setting of acute hepatitis B, often preceding the onset of jaundice (Brooks*et al.*, 2009). The clinical features are fever,polyarteritis. The symptoms often subside shortly after the onset of jaundice, but can persist throughout the duration of

acute hepatitis B (Brooks*et al.*, 2009). About 30–50% of people with acute necrotizing vasculitis (polyarteritisnodosa) are HBV carriers. HBV-associated nephropathy has been described in adults but is more common in children (Ochei and Kolhatkar, 2008). Membranous glomerulonephritis is the most common form. Other immune-mediated disorders, such as essential mixed "Cryoglobulinemia" and "Aplastic anemia" (Ochei and Kolhatkar, 2008). In Nigeria, the prevalence of HBV is 12.2% and that of HCV is 7% (Adebola*et al*, 2016).

### 2.2.8 TREATMENT

People with acute hepatitis B do not require treatment. Taking bed rest, drinking a lot of fluids and taking over-the-counter pain relievers (products containing ibuprofen, such as Motrin and Advil, are considered to be safer than products containing acetaminophen, such as Tylenol, in people with acute hepatitis) are usually all that is needed for someone who is experiencing acute hepatitis B symptoms. Treatment is only recommended for people with chronic hepatitis B, notably those with abnormal aminotransferase levels, positive HBV DNA findings, positive or negative hepatitis B e antigen (HBeAg). The goal of therapy is to prevent cirrhosis, liver failure and liver cancer by reducing HBV viral load and the loss of HBeAg (either with or without detection of anti-HBe) while improving liver enzyme levels.

A synergistic approach of suppressing viral load and boosting the patient's immune response with immunotherapeutic interventions is needed for the best prognosis. The prevention of HCC often includes the use of antiviral treatment using pegylated interferon (PEG-IFN) or nucleoside analogues. Pegylated interferon boosts a more convenient dosing schedule (once weekly versus daily) than standard interferon. In addition, patients using either form of

interferon should be monitored closely while on treatment. Combining two or more drugs has worked well for hepatitis B and HIV and is considered a logical approach to treating hepatitis B. However, none of the combination regimens tested to date have proved superior to single-agent treatment (Pyrsopoulos, 2014). Various algorithms have been proposed, such as that by the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver (EASL), the Asian Pacific Association for the Study of the Liver (APASL), the Canadian Association for the Study of the Liver (CASL), the National Institute for Health and Clinical Excellence (NICE), Kuo and Gish, and Keeffe*et al*. in (Pyrsopoulos, 2014).

The National Institutes of Health (NIH) recommends nucleoside therapy for the treatment of patients with acute liver failure, as well as cirrhotic patients who are HBV DNA positive and those with clinical complications, cirrhosis or advanced fibrosis with positive serum HBV DNA, or reactivation of chronic HBV during or after chemotherapy or immunosuppression. In addition, immunoglobulin and vaccination should be administered to newborns born to women positive for hepatitis B surface antigen (HBsAg). In general, for HBeAg-positive patients with evidence of chronic HBV disease, treatment is advised when the HBV DNA level is at or above 20,000 IU/mL ($10^5$ copies/mL) (or, per the EASL, >2,000 IU/mL) and when serum alanine aminotransferase (ALT) is elevated for 3-6 months. For HBeAg-negative patients with chronic hepatitis B disease, treatment can be administered when the HBV DNA is at or above 2000 IU/mL ($10^4$ copies/mL) and the serum ALT is elevated (ALT levels >20 U/L for females; 30 U/L for males) for 3-6 months. In patients co-infected with HBV and HIV, initiate therapy against HBV and administer antiretroviral therapy as well (Pyrsopoulos,

2014). The NIH also indicates that immediate therapy is not routinely indicated for patients who have the following:

- Chronic hepatitis B with high levels of serum HBV DNA but normal serum ALT levels or little activity on liver biopsy (immune-tolerant phase).
- Low levels of or no detectable serum HBV DNA and normal serum ALT levels (inactive chronically infected/low replicative phase)
- Positive serum HBV DNA but not HBsAg (latent HBV infection), unless the patient is undergoing immunosuppression

It is also very important that people with chronic hepatitis B take their medications exactly as prescribed. Missing doses can cause HBV to become resistant to HBV medications. Prematurely stopping HBV medications can also cause HBV viral load and liver enzymes to quickly increase, which can damage the liver and cause severe symptoms. This can also happen in people who have HBV that develops resistance to their medications. In turn, for people with chronic hepatitis B who are receiving treatment, it is very important to be monitored frequently and carefully by a health care provider (Pyrsopoulos, 2014).

### 2.2.9 CARE OF IN PATIENTS

Patients with hepatitis B disease and fulminant hepatic failure should be hospitalized in the intensive care unit (ICU) and be considered as liver transplant candidates in the event that they do not recover. Any patient with acute HBV disease needs to be treated with first-line oral therapy, such as Tenofovir Disoproxil Fumarate (TDF) or entecavir (ETV). Patients with acute hepatitis should be monitored with blood tests in order to document biochemical

improvement. Patients with acute or chronic hepatitis without cirrhosis have no dietary restrictions. For individuals with decompensated cirrhosis (prominent signs of portal hypertension or encephalopathy), the following dietary limitations are indicated:

- A low-sodium diet (1.5 g/day)
- High-protein diet (ie, white-meat protein, such as pork, turkey, or fish)
- Fluid restriction (1.5 L/day) in cases of hyponatremia
- In adults, infection resolves in >95% with loss of serum HBsAg and the appearance of anti-HBs. Chronic infection is characterized by the persistence of HBsAg for more than 6 months. Acute hepatitis B usually results in complete recovery with little if any risk of HCC. In cases with persistent HBV infection, HBV is one of the most important risk factors for HCC.

Chronic HBV infection presents as one of three potentially successive phases: *immune tolerant, immune active and low- or non-replicative*. In the immune tolerant phase, serum HBsAg and HBeAg are detectable, serum HBV DNA levels are high, serum aminotransferases are normal or minimally elevated. In the *immune active phase*, serum HBV DNA levels decrease and serum aminotransferase levels increase. Flares of aminotransferases may be observed, in some patients these flares are followed by HBeAg-anti-HBe seroconversion. Following this conversion, in the *low- or non-replicative phase* the HBV replication persists but at a very low level suppressed by the host immune response. HBV DNA in serum is undetectable by conventional, non-PCR based techniques. This phase is also called the '*inactive carrier state*'. It may lead to resolution of HBV infection where HBsAg becomes undetectable and anti-HBs is detected, anti-HBc staying positive as sign of contact

with the virus. Recently it has been reported that HBV DNA can persist in the serum and liver tissue even after negativation of HBsAg (Michielsen*et al*, 2005). Recent advances in molecular technology have allowed the isolation of HBV variants that either cannot produce HBeAg or produce it less efficiently, based on precore stop codon mutation and mutations in the core promoter region respectively. In patients with HBV variants, progressive liver damage occurs in parallel with relatively high levels of viremia. In perinatally infected people, the immunotolerant phase lasts till the age of 15–35 years, after which hepatitis flares may occur, leading eventually to viral remission. In patients infected during later childhood or adulthood, there is no immunotolerant phase (Michielsen*et al*, 2005).

## 2.3 HEPATITIS C VIRUSES (HCV)

Hepatitis C virus (HCV) is an RNA virus known to infect humans and chimpanzees, causing similar disease in these 2 species. HCV is most often transmitted parenterally but is also transmitted vertically and sexually. HCV is up to 4 times more infectious than Human Immunodeficiency Virus (HIV). It also requires less exposure to cause infection than HIV (Te and Jensen, 2010).HCV was identified in 1989, is an RNA single stranded, enveloped flavivirus. It was formerly called the non-A, non-B hepatitis virus. It shows considerable genomic variation. Six genotypes are recognized. The virus infects liver cells and is also thought to infect mononuclear cells in peripheral blood just like the HIV (Ochei and Kolhatkar, 2008). It has similar transmission route like the HIV and HBV but it is not easily transmittable as HBV. Only small numbers of the virus are excreted and circulate in the blood. Information on the epidemiology in developing countries is incomplete. In some tropical countries, HCV may spread by the re-use of contaminated instruments in tribal ceremonies. The prevalence of HCV seropositivity in various populations of Africa ranges from 0.2%-

40%. In Egypt and Cameroon where genotype 4 is present, seropositivity rates of 30-40% have been reported (Brooks *et al.,* 2009).

HCV infection is often asymptomatic and does not seem to have a major impact on the natural history of HIV infection. In studies before the HAART era, HCV co-infection had no effect on HIV progression. Only about 10% of individuals become jaundiced. WHO, however, estimates that worldwide there are about 170 million chronic carriers of HCV at risk of developing liver cirrhosis and liver cirrhosis and liver cancer. Chronic hepatitis C following acute infection develops in 70-80% of individuals. Co-infection with HCV increases the severity. Auto-antibodies and cryoglobulins are often found in patients. As yet there is no vaccine to protect against hepatitis C (Ochei and Kolhatkar, 2008).For hepatitis C, there are no serological markers for acute infection. Screening assay is used for the serological tests for chronic infection such as EIA and verified by an additional and more specific assay such as nucleic acid testing (NAT) for HCV RNA (Kumar and Sarin, 2007). Testing is recommended for persons who currently inject drugs or who have injected drugs in the past, even if once or many years ago, recipients of clotting factor concentrates before, recipients of blood transfusions or donated organs, long-term hemodialysis patients, persons with known exposures to HCV (e.g., healthcare workers after needle sticks, recipients of blood or organs from a donor who later tested positive for HCV), HIV-infected persons, children born to infected mothers, patients with signs or symptoms of liver disease (e.g., abnormal liver enzyme tests), donors of blood, plasma, organs, tissues or semen (Jasuja *et al.,* 2009). Antivirals and supportive treatment is recommended for chronic infection. Chronic infection requires regular monitoring for signs of liver disease progression, new direct acting antiviral medications offer shorter durations of treatment and increased effectiveness, including over

90% of patients who complete treatment are cured. Vaccines are not available for hepatitis C infection. All these type of hepatitis has similar symptoms such as loss of appetite, vomiting, Nausea, abdominal pain, grey coloured bowel movement, joint pain fever, fatigue and jaundice (CDC, 2016).

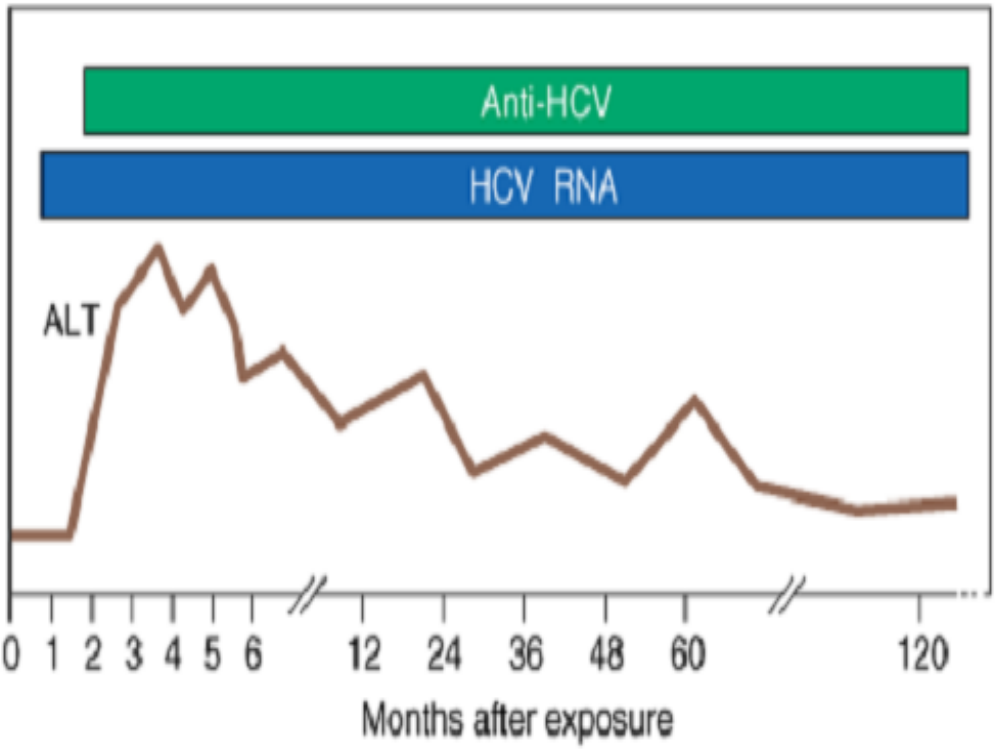



**Fig. 4: Scheme of typical clinical and laboratory features of acute Hepatitis C**

Fauci*et al*, 2008

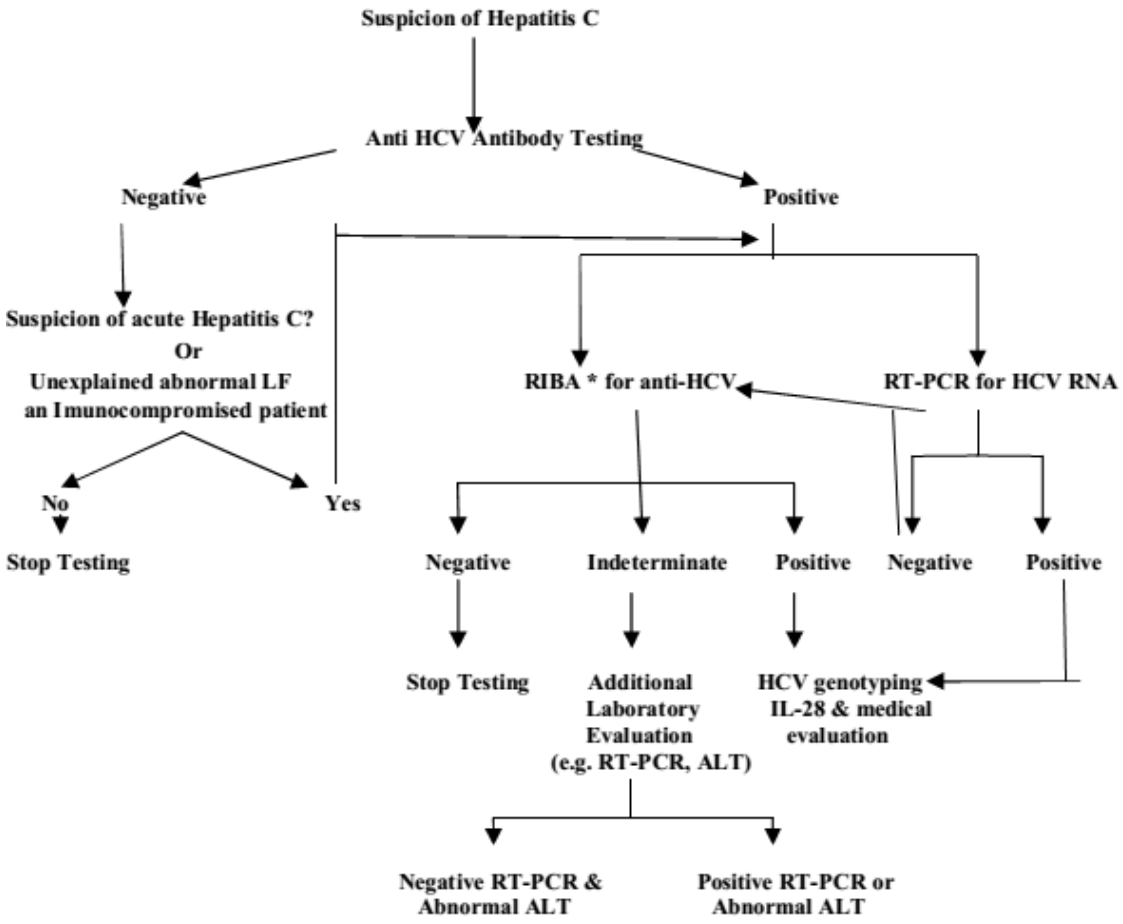



**Fig. 5: Algorithm for screening of hepatitis C**

Fauci*et al*, 2008

### 2.3.1 SIGNS AND SYMPTOMS OF HEPATITIS C

There are two types of Hepatitis C – acute (brief and severe) and chronic (having long duration). Individuals with acute Hepatitis C usually do not manifest symptoms and the small percentage that do (25 to 35 percent) often experience symptoms similar to the other cases of acute hepatitis, including flu-like symptoms, joint aches or mild skin rash. Individuals that are

particularly likely to experience a severe course of Hepatitis C are those individuals that already have Hepatitis B and become infected with acute Hepatitis C (Brooks*et al.,* (2007); Ochei and Kolhatkar, 2008). Other symptoms which may be experienced by individuals with acute Hepatitis C are: loss of appetite, abdominal pain, dark urine, grey colored stool, jaundice (yellowing of the skin and whites of the eyes)

As is the case for acute Hepatitis C, most people who have chronic Hepatitis C do not experience symptoms in the early stages or even in the advanced stages of the disease. Therefore, it is not uncommon to find out, by surprise, that one has the virus when donating blood or during a routine blood examination. It is possible to have Hepatitis C for many years and yet not knowing the reason why the disease has been referred to as a silent killer. If symptoms exists, they are likely exhibited as: Pain and tenderness in the area of the liver, fever, joint and muscle pain, decreased appetite, weight loss, depression, jaundice (yellowing of the skin and whites of the eyes), fatigue (Ochei and Kolhatkar, 2008). In patients who develop symptoms, the average time period from exposure to symptom onset is 4–12 weeks (range: 2–24 weeks).

Chronic Hepatitis C may cause signs and symptoms which manifest in other organs beside the liver as the result of the immune system's effort to fight off the Hepatitis C infection. In some cases of Hepatitis C, the kidneys can be damaged because of a condition known as cryoglobulinemia(Brooks*et al.,* 2007; Ochei and Kolhatkar, 2008). Cryoglobulinemia is the presence of abnormal proteins in the blood called cryoglobulins. Cryoglobulin is a term for proteins in the blood that become solid at low temperatures. When cryoblobulins thicken or become gel-like, they block blood vessels throughout the body which may lead to

complications ranging from skin rashes to kidney failure (Brooks*et al.*, 2007; Ochei and Kolhatkar, 2008).

### 2.3.2 TREATMENT

The primary objective of HCV therapy is permanent eradication of the virus. The secondary potential benefit of eradication is a reduction in the risk of liver failure and liver cancer. Peginterferon alfa-2a plus ribavirin was the only FDA approved treatment for HIV–HCV co-infected patients. Interferon bind to specific cell surface receptors of virus-infected cells, which induces a complex cascade of protein-protein interactions and a rapid activation of gene transcription. The antiviral effects of interferons are mediated through inhibition of viral penetration or uncoating, inhibiting viral replication or translation of viral proteins, and/or viral assembly and release. The difference between peginterferon and interferon is the addition of a "Polyethylene glycol" "Polymer". The addition of PEG decreases plasma clearance considerably, protects the molecule from proteolytic degradation and reduces its immunogenicity. Peak concentrations are approximately 1.5-2 folds higher than trough concentrations and the half-life is 80 hours (compared to 5.1 hours for interferon alpha-2a). Ribavirin is a synthetic nucleoside analogue, but its mechanism of action is not clearly established. Ribavirin inhibits the replication of a wide range of "RNA" and "DNA"viruses.

The duration of standard interferon plus ribavirin therapy has been based on the viral genotype and the pretreatment viral load. The SVR rates for patients infected with genotype 2 or 3 are essentially the same for 24 and 48 weeks of therapy, showing no benefit for the longer course of therapy. For patients infected with genotype 1 isolates, 48 weeks of interferon plus ribavirin therapy is recommended for those with a high viral load (>800,000 IU/ml) and only

24 weeks of therapy for patients with those with a low pretreatment viral load (<800,000 IU/ml) (Poordad *et al,* 2011).

Spontaneous resolution of hepatitis C virus is common and waiting 2-4 months before initiation of therapy is recommended. Efficacy of treatment is assessed by measuring Hepatitis C RNA viral load. The goal is to achieve a Sustained Virological Rate (SVR), defined by the continued absence of hepatitis C RNA 6 months after the completion of treatment. Treatment for chronic HCV infection has evolved from interferon monotherapy, which results in an SVR of 10 to 20% to combination therapy with interferon plus ribavirin, which is associated with a higher SVR rate of nearly 40%. A pretreatment liver biopsy is not mandatory but may be helpful in patients with normal transaminase levels, particularly those with a history of alcohol dependence, in whom little correlation may exist between liver enzyme levels and histologic findings. Liver transplant is the only therapeutic option for patients with end stage liver disease. The drugs used to treat Hepatitis C cost approximately $30,000 for 48 weeks. The cost of treating side effects of these drugs further increase the cost of treating hepatitis C. Future hepatitis C drugs are expected to be more expensive (Daw and Dau, 2012).

Pharmacokinetics is similar in patients with HIV co-infection compared with HCV mono-infection (Falconer*et al.,* 2010). In 2013, the FDA approved these new medications for HCV:

- Sofosbuvir (Sovaldi) is a new drug that can be used in therapies without the addition of pegylated interferon. The cost of this breakthrough drug is extremely high. This places it out of reach for many people throughout the world. There's a compassion program called SupportPath, which can partially or fully subsidize the drug for qualified individuals.

- Sofosbuvir (Sovaldi) combined with ribavirin provides similarly effective treatment without pegylated interferon.

Simeprevir (Olysio)is an effective new drug that's also used without pegylated interferon. It offers many of the same benefits as sofosbuvir.

#### 2.3.2.1 TREATMENT INDICATIONS

All patients with chronic hepatitis C infection should be considered potential candidates for drug therapy. Treatment is recommended for patients who are at risk of developing cirrhosis, generally defined by a measurable hepatitis C RNA level and liver biopsy showing portal or ridging fibrosis along with moderate inflammation and necrosis. Treatment is also recommended for patients with elevated serum ALT levels who meet the following criteria (Sandeep and Dhawan 2012; Dhawan, 2018):

1. Age >18 years
2. Positive HCV antibody and serum HCV RNA test results
3. Compensated liver disease (e.g., no hepatic encephalopathy or ascites)
4. Acceptable hematologic and biochemical indices (hemoglobin at least 13 g/dL for men and 12 g/dL for women; neutrophil count >1500/mm3, serum creatinine < 1.5 mg/dL)
5. Willingness to be treated and to adhere to treatment requirements
6. No contraindications for treatment.

### 2.3.3 HEPATITIS C GENOTYPES

There are six major strains (genotypes) of the hepatitis C virus (HCV) which cause infection. One may be infected with more than one genotype at a time. These include;

- Genotype 1 which is the most common strain in the United States.
- Genotypes 1, 2, and 3 are found worldwide.
- Genotype 4 is found throughout northern Africa.
- Genotype 5 commonly is found in South Africa.
- Genotype 6 is common in Asia

#### 2.3.3.1 HOW GENOTYPE AFFECTS TREATMENT

Knowing the HCV genotype helps a physician in choosing the best treatment plan. The antiviral medicines peginterferon and ribavirin are more likely to work for people who have genotype 2 or 3. These medicines also are used to treat people who have genotypes 5 and 6.A combination of sofosbuvir and simeprevir or a single pill containing ledipasvir and sofosbuvir can be used to treat hepatitis C in people who have genotype 1. If the virus is not detected in your blood after 6 months, treatment may be:

- Continued for another 6 months, if the patient is infected with genotype 1.

- Stopped, if the patient is infected with genotype 2 or 3. Prolonging treatment does not seem to provide any more benefit.

The genotype of HCV does not appear to have any effect on the severity of HCV infection or to affect the risk of developing cancer of the liver.Depending on the drugs used and the genotype of the HCV, these drugs vary in effectiveness. For example, one study found that sofosbuvir (Sovaldi) combined with ribavirin cured 95 percentof people with genotype 2. In addition to higher cure rates, all three drugs offer a far shorter length of treatment and fewer side effects. They are available in pill form (WHO, 2017).

### 2.3.4 PREVALENCE

The prevalence of HCV in the general population in Africa ranges between 0.1% and 17.5%, depending on the country. The countries with the highest prevalence include Egypt (17.5%), Cameroon (13.8%) and Burundi (11.3%). The countries with the lowest prevalence include Zambia, Kenya, Malawi and South Africa (all with a prevalence <1%) (Karoney and Siika, 2013).

### 2.3.5 RISK GROUPS

A recent study by Karoney and Siika, (2013) documented high risk populations in; intravenous drug users; HIV-infected; patients on hemodialysis; patients with history of blood transfusions or organ transplantation; health care workers after needle stick injuries; children born to HCV infected mothers. Also, sexually active adults with multiple partners have higher prevalence rates. Available data on HCV reveal high prevalence in patients with hepatocellular carcinoma or chronic liver disease: (Burundi; 55%, Rwanda; 45.7%) and sexually transmitted diseases (Ethiopia; 38.2%). Countries with low HCV prevalence in high-risk groups include Zimbabwe (1.3%) and Kenya (1.7%).

### 2.3.6 DISEASE BURDEN AND DISTRIBUTION

The estimated prevalence of HCV in Africa is 5.3%. Egypt has the highest worldwide prevalence (17.5%). Egypt unusually high prevalence is attributable to the history of unsterile injection equipment use for mass treatment of the general population with parenteral antischistosomal therapy (PAT) from the 1920s to the 1980s. The prevalence of HCV increases with age, with the highest rate being reported in the age group older than 40 years. No data were available on HCV morbidity and mortality in Africa. However, based on the

general trends for most other diseases, it is possible that these indicators may be worse than the WHO reports of 75% of HCV-infected individuals developing chronic liver disease. Of those HCV-infected patients who develop chronic liver disease 1.6% progress to Hepatocellular carcinoma (HCC), a condition with a mortality rate >80% (Maheshwari and Thuluvath, 2010).

### 2.3.7 PREVENTION STRATEGIES

Primary prevention activities include: screening and testing of blood, plasma, tissue, organ and semen donors; virus inactivation of plasma derived products; risk reduction counseling services and implementation of infection-control practices to include enlightenment campaign of risk factors and epidemiology. Secondary prevention activities includes identification and testing of persons at risk and management of infected persons (Karoney and Siika, 2013).

### 2.3.8 OTHER RELATED STUDIES ON HIV CO-INFECTIONS WITH HBV AND HCV

Studies by Tremeau-Bravard, (2012) documented the prevalence of Hepatitis in Gede Foundation clinic in Abuja, Nigeria to be7.9% for HIV/HBV co-infection, 2.3% for HIV/HCV co-infection, and 0.7% for HBV/HCV/HIV triple infections and the overall prevalence of HIV/hepatitis co-infection in his studied population is 10.8%. A recent study in Ethiopia by Abera *et al* (2014) demonstrated the prevailing seroprevalence of HBV in HIV infected children to be 2.0%. In Thailand, Tsuchiya*et al*, (2012), reported 3.3% prevalence of HIV/HBV infection. In Democratic Republic of Congo 1.6% prevalence of HIV/HBV was reported by Katabuka *et al*, (2013). In Owerri, Imo State, Nwolisa *et al*, (2013) reported

5.8%.In Benue State, 7.8% prevalence of HIV/HBV was reported among children (Angilaje and Olutola, 2013), in Benin, 7.7% was reported by Sadoh and Sadoh, (2011). In Uyo, Ikpeme *et al,* (2013) reported HIV/HBV prevalence of 6.02%. Jobarteh *et al,* (2010) reported 12.2% prevalence in Gambia. In Bahir Dar city, Northwest Ethiopia the prevalence of HIV/HBV was reported to be 19% (Zenebe *et al,*2014). Abera *et al,* 2014 documented the seroprevalence of HIV/HCV in Ethiopia as 5.5. In Africa Karoney and Siika, (2013) estimated prevalence of HCV in Africa to be 5.3%, which is the highest in the world.Eze *et al,* (2014) reported the prevalence of HIV/HCV co-infection in Lagos Nigeria to be 6.8%. Study in Abuja shows that 9% of men and 7% of women are co-infected with HIV/HBV, 3.5% of men and 1.2% of women are co-infected with HIV/HCV, 0.5% of men and 0.8% of women have triple infection of HIV/HBV/HCV.There are no significant differences in the mean age of the HIV, the HIV/HBV, and the HIV/HBV/HCV groups (p=0.91 and 0.60, respectively). The mean age for these groups is 38.7 years.

## 2.4 HEPATITIS D

Hepatitis D which is also called Delta virus is the most serious and rarest form of viral hepatitis which infects people who have hepatitis B (WHO, 2017). Blood andblood products transmit hepatitis D virus infection and the risk factor is similar to that of hepatitis B infection. Hepatitis D virus mostly affects intravenous drug users and presence of the infection should be suspected in patients with chronic hepatitis B infection, whose condition suddenly deteriorates. This viral infection is diagnosed by the presence of antibodies against the hepatitis D virus. Interferon-alpha is used to treat patients with chronic hepatitis B and D infection but with a dose higher than that given to hepatitis B patient.

## 2.5 HEPATITIS E

Hepatitis E virus (HEV) is a major etiological agent for the transmission of non-A and non-B hepatitis. It is a spherical non enveloped single stranded RNA virus, approximately 32-34nm in diameter and can be linked to poor hygiene and contaminated water which can lead to serious illness in pregnant women. The incubation period after exposure to the virus is from 15 to 60 days. The symptoms include; jaundice, malaise, anorexia, fever, dark urine, nausea, hepatomegaly and vomiting. There are other less common symptoms which comprise of arthralgia, diarrhea, pruritus, and urticarial rash. The primary route of transmission is the fecal-oral route and fecally contaminated drinking water is the commonly documented vehicle of transmission (WHO, 2017).

The last type of hepatitis virus is the G hepatitis which is an RNA virus which belongs to the flavivirus group and distinctly related to hepatitis C. Hepatitis G (HGV) is found in 1-2% of blood donors and causes persistent infection in most patients with viraemia persisting for several years. People infected with this virus do not have abnormal liver function tests and evidence of liver cell infection is lacking and so it is diagnosed by seroconversion or detection of RNA in a person previously RNA negative. In the United State, studies have found 10%–20% of HGV patients to be co-infected with HCV and evidence has also shown a protective role of HGV in HIV patients (WHO, 2017).

## 2.6 HEPATITIS AND LIVER ENZYMES

The liver enzymes include alanine aminotransferases (ALT), aspartate aminotransferases (AST), Ý-glutamyltransferase (GGT), alkaline phosphatase (ALP),5 nucleotidase (NTP), serum cholinestarate (CHE) and glutamate dehydrogenase(GLD). The AST, ALT and ALP

are widely used. They have long been mistakenly called, as a group, "liver function tests." Despite the effective decline of the mortality and morbidity rate from HIV/AIDS as the result of highly active antiretroviral therapy (HAART), liver diseases due to chronic HBV and HCV infections become a leading cause of death. Although the direct impact of HCV upon HIV disease progression remains controversial in many reports (Sulkowski *et al*, 2008) the complex interactions between HIV-HBV/HCV co-infection and HAART are increasingly apparent in HIV disease progression.

### 2.6.1 AMINOTRANSFERASES

The aminotransferases constitute a group of enzymes that catalyze the interconversion of amino acids to 2-oxo-acids by transfer of amino groups. Aspartate aminotransferase (l-aspartate:2-oxoglutarate aminotransferase) and alalnine transferase (l-alanine: 2-oxoglutarate aminotransferase; ALT). Transaminases are widely distributed AST is found primarily in the heart, liver, muscle and kidney. ALT is found primarily in the liver and kidney. It is exclusively cytoplasmic in the mitochondria and cytoplasmic forms of AST are found (Burtis *et al*, 2008).

Disease is the most important cause of increased transaminases transaminase, ALT is higher than AST. Exceptionally be seen in alcohol hepatitis, hepatic cirrhosis, liver neoplasia. In viral hepatitis, AST and ALT concentrations are elevated even before physical signs and symptoms of disease (such as jaundice) is seen. Activities for both enzymes may reach values as high as 10 times the upper reference limit. The existence of increased ALT for more than 6months after an episode of acute hepatitis is used to diagnose chronic hepatitis. Most patients with chronic hepatitis have maximum ALT even seven times the upper reference limit. ALT may

be patiently normal in 15% to 50% of patients with chronic hepatitis C, but the likelihood of continuously normal ALTincreases with an increasing number of measurements. In patients with acute hepatitis C, ALT should be measured periodically over the following 1 to 2 years to determine if its activity returns to normal (Burtis *et al*, 2008).

The aminotransferase activities observed in cirrhosis vary with the status of the cirrhotic process and range from the upper reference limit to four to five times higher, with an AST/ALT greater ratio than 1. The ratio's elevation can reflect the grade of fibrosis in these patients. This appears to be attributable to a reduction of ALT production in a damaged liver. Two to four fold elevations of both enzymes occur in patients with primary or metastatic carcinoma of the liver, with AST usually being higher than ALT, but activities are normal in the early stages of malignant infiltration of liver (Burtis *et al*, 2008). In HIV/HBV co-infections, HIV infection causes increased rates of persistent HBV infection, increased cirrhosis and liver-related mortality and increased risk of hepatocellular carcinoma at lower CD4T cell counts (Thio *et al*, 2009). Similarly in HIV/HCV co-infections, there is a more rapid progress to cirrhosis, end-stage liver disease and hepatocellular carcinoma (Burtis *et al*, 2008).

The impact of HBV and HCV could not limited in causing liver hepatotoxicity but also results in failure in immunological recovery in HIV positive patients. For example, a study in Tanzania reported slow rate of immunologic recovery after initiation of HAART treatment and higher risk of hepatotoxicity among HIV/HBV and HIV/HCV co-infected patients (Christian *et al*, 2010). Thus the management of HBV and HCV in HIV infection is complicated and bring high burden in particular where HIV is rampant. As the result, globally

HIV, HBV and HCV become the major public health concerns (Modi *et al*, 2007; Leeratanapetch *et al,* 2008). In some countries, screening of HIV-infected individuals for HBV and HCV is highly recommended before initiation of antiviral treatment (Chung, 2006)

Muhammad *et al.*, (2016) compared the mean levels of AST, ALT and bilirubin in pateints co-infected with HBV and HCV and patients infected with only HCV and discovered that there mean levels were found to be 61 IU/L, 84 IU/L, and 1.6 mg/dL respectively in case of co-infection with HBV and HCV for AST, ALT, and bilirubin and 76 IU/L, 91 IU/L, and 1.9 mg/dL, respectively for AST, ALT and bilirubin for patients with only HCV infection.

### 2.6.2 ALKALINE PHOSPHATASE

Alkaline phosphatase (ALP) catalyzes the alkaline hydrolysis of a large variety of naturally occurring and synthetic substrates. Divalent ions such as $Mg^{2+}$, $Ca^{2+}$, and $Mn^{2+}$, are activators of the enzyme in which $Zn^{2+}$ is a constituentmetal ion. Phosphate, borate, oxalate, and cyanide ions are inhibitors of ALP activity. The type of buffer present in catalytic reaction may affect the rate of activity. ALP activity is presentin most organs of the body and is especially associated with membranes and cell surfaces located in the mucosa of the small intestine and proximal convoluted tubules of the kidney, in the bone, in the liver and placenta. Although the exact metabolic function of theenzyme is not understood, it appears that ALP is associated with lipid transport in the intestine and with the calcification process in bone.

Elevations of the serum ALP activity commonly originate from the liver and bone. Consequently, serum ALP measurements are of particular interest in the investigation of hepatobiliary disease associated with increased osteoblastic activity. The response of the liver

to any form of biliary tree obstruction induces the synthesis of ALP by hepatocytes. In patients with advance primary liver cancer or wide spread secondary hepatic metastes, serum enzyme activity may reach 10 to 12 times the upper reference limit and usually return to normal on removal of the obstruction. Liver diseases that principally affect paranchyma cells, such as infectious hepatitis typically show only moderate (less than threefold) increase or even normal serum ALP activities (Burtis *et al,* 2008).

## 2.7 ALPHA-FETOPROTEIN

The alpha-fetoprotein (AFP) is one of the first α-globulins to react in mammalian sera during development of the embryo and is the dominant serum protein in early embryonic life. It appears in the adult serum during certain pathological cases. The alpha-fetoprotein (AFP) is a glycoprotein of 591 amino acidswith a molecular weight of approximately 70000 daltons and a carbohydrate moiety.Normally, AFP is produced by the liver, yolk sac, and in small concentration by the gastrointestinal tract during fetal and neonatal development. After birth the serum concentration of AFP decrease rapidly and by the second year of life and thereafter only trace amount are normally detected in serum (Brooks*et al.,* 2007; Ochei and Kolhatkar, 2008).

AFP is the most abundant plasma protein found in the human fetus. Plasma levels decrease rapidly. Elevated maternal serum of amniotic fluid AFP indicates the possibility of an open neural tube or abdominal wall defect in fetus. AFP also increases in the presence of many forms of hepatocellular and germ cell carcinomas in children and adult. Normal adult levels are usually achieved by the age of 8 to 12 months. The concentration of ALP is normally less

than 10µg/l. Most patients with hepatitis and liver cirrhosis (95%) have AFP concentrations lower than 200µ/l. AFP concentrations greater than 1000µ/l except in the pregnant patient is indicative of cancer. At this concentration of AFP, about half of hepatocellular carcinomas (HCCs) may be detected. The function of AFP in adult humans is not known;but, in rodents it binds estradiol to prevent the transport of this hormone across the placenta to the fetus. As human AFP does not bind estrogen, its function in humans is not too clear (Burtis *et al*, 2008; Le Tao, 2013).

Alfa-fetoprotein (AFP) is measured generally in two clinical contexts which are as follows;

- It is measured in pregnant women through the analysis of maternal blood or amniotic fluid as a screening test for certain developmental abnormalities.
- Elevated serum level of Alfa-fetoprotein is also seen in people with certain tumors, and so it is used as a biomarker to follow these diseases. Some of these diseases are:

- *developmental birth defects associated with elevated AFP* example Neural tube defects: in this case, there is increased level of α-fetoprotein in amniotic fluid and maternal serum(Burtis *et al*, 2008; Ertle*et al.*, 2013).
- *tumors associated with elevated AFP*
    - Hepatocellular carcinoma
    - Metastatic disease affecting the liver
    - Nonseminomatous germ cell tumors
    - Yolk sac tumor

In addition, increased serum AFP concentrations have been measured in patients with other noncancerous diseases, including ataxia telangiectasia, hereditary tyrosinemia, neonatal hyperbilirubinemia, acute viral hepatitis, chronic active hepatitis, and cirrhosis. In pregnant women, increased serum AFP concentrations are also seen (Burtis *et al*, (2008); Ertle*et al.*, 2013).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 STUDY CENTRE

This research population was drawn from HIV patients attending Heart to Heart clinic in Asokoro, Wuse and Maitama district hospitals in Abuja. The research practical was carried out in Gilead-Balm Medical Laboratory and Diagnostic/research centre Abuja, the Medical Laboratory in the 3 district hospitals, Ralpha Biomedical Laboratory and Diagnostics Ltd Gwagwalada, Abuja and Prof. Tatfeng Molecular Laboratory, Niger Delta University Wilberforce Island, Bayelsa. Heart to Heart clinic is a referral center saddled with the responsibility of counseling, testing and providing care and support for the HIV infected individuals.

The Federal Capital Territory Abujais located in the centre of Nigeria. Abuja's geography is defined by Aso Rock, a 400meter(1,300 ft) monolith left by water erosion. It lies within the Guinean forest-Savanna mosaic zone of the West African sub-region.Abuja is the capital of Nigeria. It is located at latitude 9.072264$^{o}$N and longitude 7.491302$^{o}$E, in the north hemisphere. It is a planned city and was built in the 1980s. It has a population of about 780,000 (LatLong.net, online). It occupies a land area of 7,753.9.sq km (Adepoju, 2017).

It is divided into six (6) area councils namely; Abuja municipal, Gwagwalada, Abaji, Kuje, Bwari and Kwali. There are different districts in Abuja with some district hospitals. These districts have a large industrial and agricultural base and many from other places reside there for job and business. Maitama district hospital is located in the north of the city with the Wuse district hospital and Asokoro district hospital lying to its southern part of the Abuja Municipal city (Adepoju, 2017).

The FCT experiences two weather conditions annually. This includes a dry season and rainy season. In between the two, there is a brief interlude of harmattan occasioned by the northeast trade wind, with the main feature of dust haze and dryness. The rainy season begins from April and ends in October, when daytime temperatures reach 28$^{o}$C (82.4$^{o}$F) to 30$^{o}$C (86.0$^{o}$F) and night time lows hover around 22$^{o}$C (71.6$^{o}$F) to 23$^{o}$C (73.44$^{o}$F). The dry season begins from November and ends in March. In the dry season, day time temperatures can soar as high as 40$^{o}$C (104.0$^{o}$F) and night time temperatures can dip to 12$^{o}$C (53.6$^{o}$F). Even the chilliest nights can be followed by daytime temperature well above 30$^{o}$C (86.0$^{o}$F).The major religions are Christianity and Islamic religion. Majority of the occupants are civil servants and business men (Adepoju, 2017).

## 3.2 ETHICAL APPROVAL

Letter was written to the ethical committee of Hospital Management Board, Federal Capital Territory Administration Authority Abuja (FCTA), seekingtheir approval to carryout the research in the district hospitals. The approval letter received from the ethical committee FCTA was submitted to the Chief Medical Director of each of the district hospitals for permission and other approval letters were issued by the Chief Medical Director of each of the three (3) district hospital. See appendix for copy of the questionnaire.

## 3.3 STUDY SUBJECT

Two hundred HIV patients attending heart to heart clinic in each of the district hospitals in Abuja between the ages of 15-75years with CD4 count of 200 ± 490 from January 2015 to January 2016 were recruited into this study. Those on Antiretroviral Therapy (ART) were separated from those who have not started the Antiretroviral Therapy (ART). Control samples

were drawn from two hundred non HIV infected individuals within the same age limit living around the vicinity.Those control individuals that tested HBsAg positive and who were on Abraka herbs were also noted.

Standardized questionnaires were administered to the patients after obtaining their informed consent. The questionnaire included information concerning their socio-demographic characteristics and risk factors associated with HBV and HCV seropositivity in HIV patients. These information concerned their age, gender, marital status, educational background, nationality, tribe, tribal mark, occupation, parental history, ear piercing, tattooing, alcoholism, smoking habit, chewing tobacco, blood transfusion, previous surgery, STD awareness, family history of jaundice, period of sex experience, number of sex partners, and the use of condom.

### 3.4 SAMPLE SIZE

The specimen for this study was blood specimen from HIV infected patients. Since there are different district hospitals in Abuja, the Simple Random SamplingWithout Replacement (SRSWOR) method by Shalabh (2016) was used to select 3 different hospitals which are Asokoro, Maitama and Wuse District hospitals. The total sample size for this study is eight hundred (800) samples. Six hundred(600) samples from HIV patients (200 each from Asokoro, Maitama and Wuse hospitals) and 200 samples from non HIV patients, collected randomly within Abuja territory to serve as control. The populations of patients in these hospitals are not the same. The hospitals selected are the n, the total number of district hospitals is N. The formular for the Simple Sampling Without Replacement (SRSWOR) Method is as shown below;

$$\frac{N}{n}$$

Where N = the total number of District Hospitals

n = the District Hospital selected

## 3.5 SAMPLING METHOD

The sampling method used was the Stratified Random Sampling (SRS) method.The prevalence of HCV, HBsAg and anti-HBc was determined from the proposition of the seropositive individuals among the HIV positive population and the control population studied and was expressed as a percentage. Descriptive statistics of socio-demographic variables and other characteristics of sampled population were computed. Ninety five percent confidence interval (CI) was used to describe the prevalence of Hepatitis. Odd ratio (OR) and 95% CI were calculated for each association. A p-value less than 0.05 were calculated to be statistically significant.From each hospital, a sample size proportion to the population of each hospital was selected using the proportional allocation in stratified sample as follows;

$n_h$ = $\frac{n}{N}.Nh$

Where nh = Sample size allocated in hospital h

n = the total sample size

$n_h$ = Population of HIV patients in hospital h

N = Total population of all the HIV patients in the hospitals under study

## 3.6 LIMITATIONS OF THE STUDY

Due to lack of sponsorship and finance, this research work took a longer time to be accomplished inorder to meet up with the financial responsibilities. The phylogenetic analysis of the HCV in FCT was not completed because the HCV template submitted to Inqaba South

Africa failed may be due to the template concentration. PCR was done for only 200 the specimens due to the high cost of the test.

## 3.7 RESOURCES

All the equipment used for this research was available in the various laboratories where this research was carried out. Other materials like, reagents, computer software and hardware, that were used were provided by the researcher.

## 3.8 INCLUSION CRITERIA

Male and female HIV patients both those on ART (Anti-Retroviral Therapy) and those who are not on HAART who had attained 15 years to 75 years were tested and confirmed for HIV using the HIV determine kit, HIV Startpack kit and HIV Immunocomb kit and non HIV patients in the same age range who are on local herbs and those that are not on local herbs from Abraka served as control.

## 3.9 EXCLUSION CRITERIA

Male and female HIV patients below 15years and above 75 years, HIV negative individuals below 15 years and above 75 years were excluded from the study, including HIV patients on HAART.

## 3.10 COLLECTION OF SPECIMENS

Five (5) ml of human whole blood was collected aseptically from each individual in such a way that hemolysis was avoided using 5ml disposable syringe, and were transferred separately to different sterile test tube. The whole blood was allowed to clot and the serum was separated by centrifugation at 3000 rpm for 5 minutes. The serum specimens were transferred separately to different vials and were preserved at -2$^{o}$C until when they were used (Ochie and Kolhatkar,

2008).The whole blood were preserved in anticoagulant containers at $4^{o}$C and used within 7days. During the period of sample collection, the general laboratory precautions were observed. All the required Standard Operating Procedures were strictly observed in processing of the specimens to ensure quality performance(Ochie and Kolhatkar, 2008).

## 3.11 LABORATORY ANALYSIS OF SPECIMENS

### 3.11.1 ALERE DETERMINE HIV 1/2

Alere Determine HIV 1/2 is an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2. Specimen was added to the specimen pad. As the specimen migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site. If antibodies to HIV-1 and/or HIV-2 are present in the specimen, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window, forming a red line at the patient window site. If antibodies to HIV-1 and/or HIV-2 are absent, the antigen-selenium colloid flows past the patient window and no red line is formed at the patient window site (Alere Medical, 2012).The protective foil cover from each test cassette was removed. Fifty (50) µl of each serum specimens wereapplied separately to the specimen pad marked by the arrow symbol of the test strip. The test was allowed to stay for 60minutes.The results were read (Alere Medical, 2012).

### 3.11.2 HIV 1/2 STAT-PAK ASSAY

The chembio HIV 1/2 STAT-PAK assay is a single-use immunochromatographic, rapid screening test for the detection of antibodies to Human Immunodeficiency Virus Types 1 and 2(HIV 1/2) in serum specimens. It is intended for use in as a point-of-care test to aid in the diagnosis of infection with HIV1 and HIV 2. It employs a unique combination of a specific antibody binding protein, which is conjugated to colloidal gold dye particles, and HIV 1/2antigens, which are bound to the membrane solid phase. The specimen was added to the SPECIMEN (S) well after which a running buffer was added. The buffer facilitates the lateral flow of the released product and promotes the binding of antibodies to the antigens. The antibodies bind to the gold conjugated antibody binding protein, if it is present. The dye conjugated-immune complex migrates on the nitrocellulose membrane in a reactive specimen and is captured by the antigens immobilized in the TEST (T) area producing a pink/purple line. In the absence of antibodies to HIV, no pink/purple line in the TEST (T) area is observed. There is continuous migration along the membrane and production of a pink/purple line in the CONTROL (C) area containing immunoglobulin G antigens. This procedural control indicates that specimen and reagents have been properly applied and have migrated through the device (Chembio, 2011).

## 3.12 HEPATITIS B SURFACE ANTIGEN TEST (HBsAg) ONE STEP HEPATITIS

The HBsAg One Step Hepatitis B Surface Antigen Test Strip (serum) is a qualitative lateral flow immunoassay for the detection of HBsAg in serum. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the strip. During testing the serum specimen reacted with the particle coated with anti-HBsAg antibody. The mixture migrated upward on

the membrane chromatographically by capillary action and reacted with anti-HBsAg antibodies on the membrane and generated a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result (Blumberg, 2012). All serum specimens, controls and test strip were allowed to equilibrate to room temperature of about 25$^{o}$C prior to testing. The test strips were removed from the sealed pouch and were used immediately. The tests strips were immersed vertically in the serum with the arrows pointing toward the serum for at least 15seconds. It was ensured that the maximum lines on the test strips were not exceeded when immersing the strips. The test strips were placed on a non-absorbent flat surface, the timer started and waited for the red line to appear. The results were read after 15minutes.

### 3.12.1 HBV COMBO RAPID TEST CASSETTE 5 PANEL HBV

This is a rapid test for the qualitative detection of Hepatitis B Surface Antigen (HBsAg), Hepatitis B Surface Antibody (HBsAb), Hepatitis B Envelop Antigen (HBeAg), Hepatitis B Envelop Antibody (HBeAb) and Hepatitis Core Antibody (HBcAb) in serum or plasma (Chizzali-Bonfadin*et al.*, 2013).

### 3.12.2 HBsAg and HBeAg

During testing, the serum specimen reacted with the particle coated with anti-HBsAg or anti-HBeAg antibodies. The mixture migrated upward on the membrane chromatographically by capillary action to react with anti-HBsAg or anti-HBeAg antibodies on the membrane and generated a colored line. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result.

### 3.12.3 HBsAb

Hepatitis B surface Antibody (HBsAb) is also known as anti-Hepatitis B surface Antigen (anti-HBs). This test is a qualitative, lateral flow immunoassay for the detection of HBsAb in serum. The membrane is pre-coated with HBsAg on the test line region of the strip. During testing, the serum specimen reacted with the particle coated with HBsAg. The mixture migrated upward on the membrane chromatographically by capillary action to react with HBsAg on the membrane to generate a colored line. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result.

### 3.12.4 HBeAb and HBcAb

The membrane is pre-coated with HBeAg or HBcAg on the test line region of the strip. During testing, the mixture migrates upward on the membrane chromatographically by capillary action. As the mixture migrates, anti-HBe antibody or anti-HBc antibody, if present in the specimen, will compete with particle coated with anti-HBeAg or anti-HBcAg for limited amount of positive result. A visible colored line will form in the test line region if there is no anti-HBe antibody or anti-HBc antibody in the specimen because all the antibody coated particles will be captured by the antigen coated in the test line region.To serve as a procedural control, a colored line appeared in the control line of the region indicating that proper volume of specimen has been added and membrane wicking has occurred.The test cassettes were removed from the sealed foil pouch and were used within 1 hour. The test

cassettes were placed on clean and leveled surface. The droppers were held vertically and 3 full drops of each of the serum specimens were transferred to each sample well of the cassette respectively. The timer was set on. The results were red at 15 minutes (Chizzali-Bonfadin*et al.*, 2013).

## 3.13 ONE STEP STRIP STYLE ANTI-HCV RAPID SCREEN TESTPRINCIPLE

One step Anti – HCV Rapid Screen Test is a lateral flow, immunochromatographic screening test. Two purified recombinant antigens of HCV are used in test band as capture materials and gold conjugates. If the antibody of Anti-HCV is present in the specimen in concentration above the labeled, complex will be formed. This complex is then captured by antigen immobilized in the test zone of the membrane, producing a visible pink-rose color band on the membrane. The color intensity will depend on the concentration of the anti-HCV present in the specimen. This one step test is very sensitive and only takes about 10-15 minutes. Test results were read without any instrument.The sealed pouch were opened by tearing along the notch, the test were removed from the pouch. The strips were immersed into the sample with the arrow end pointing towards the sample. The strips were brought out after 10seconds and were laid flat on a clean, dry, non-absorbent surface. The results were read within 15minutes after colored bands have appeared (Alter *et al.,* 2009; Aria, 2014).

## 3.14 BILIRUBIN ESTIMATION BY RANDOX COLORIMETRIC METHOD

### 3.14.1 TOTAL BILIRUBIN

The bilirubin method of Jendrassik and Grof (1938); and Sharlock (1951) modified by Randox was used. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid. Two hundred micro

liter (200) ul of reagent 1 (R1) was pipette into different tubes and the tubes well arranged on a tube rack and were labeled accordingly (specimen blank and specimen). Fifty micro liter (50) ul of R2 was added to all tubes labeled specimen. One thousand micro liter (1000ul) each of R3 was added to all the test tubes. Two hundred micro liter (200) ul of each of the specimen was added to the tubes labeled specimen blank and sample respectively. The preparation was mixed and incubated for 10minutes at 25°C. One thousand micro liter (1000) ul each of reagent 4 (R4) was added to all the tubes. The preparation was mixed, incubated for a further 30minutes at 25°C, and the absorbance read at 578nm wavelength against the specimen blank for each specimen respectively (Randox, 2013).

#### 3.14.1.1 MANUAL CALCULATION

Total bilirubin (mg/dl) = 10.8 x ATB (578nm)

### 3.14.2 DIRECT BILIRUBIN

The bilirubin method of Jendrassik and Grof (1938); and Sharlock (1951) modified by Randox was used. Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue colored complex. Two hundred micro liter (200) µl of reagent 1 (R1) was pipette into different tubes and the tubes well arranged on a tube rack and were labeled accordingly (specimen blank and specimen). Fifty micro liters (50) µl each of reagents (R2) was added to all tubes labeled sample. Two thousand micro liter (2000) ul each of 0.9% NaCl was added to all the test tubes. Two hundred micro liters (200) µl of each of the specimen was added to the tubes labeled specimen blank and specimen respectively. The preparation was mixed and incubated for 10minutes at 25°C, and the absorbance read at

546nm wavelength against the specimen blank for each specimen respectively (Randox, 2013).

### 3.14.2.1 MANUAL CALCULATION

Direct bilirubin = 14.4 x ADB (546nm)

### 3.14.2.2 NORMAL VALUES IN SERUM

Total Bilirubin = up to 1mg/dl

Direct bilirubin = up to 0.25mg/dl

## 3.15 ASPARTATE AMINOTRANSFERASE (AST) USING RANDOX METHOD

α-oxoglutarate + L-aspartate → L-glutamate + Oxaloacetate

Aspartate Aminotransferase (AST) was measured in vitro by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine. Test tubes were set on a tube rack placed on a work bench and were labeled accordingly. Zero point one ml (0.1ml) of each of the HBV and HCV positive specimen was transferred into various test tubes and was labeled accordingly. Zero point five (0.5) ml of reagent 1 (R1) was transferred to all the tubes. Zero point one (0.1) ml of distilled water was transferred to all the tubes labeled blank. The reaction was mixed and incubated at exactly thirty (30) minutes at 37°C. Zero point five (0.5) ml of reagent two (R2) was transferred to all the test tubes the reactions mixed and allowed to stand for exactly 20minutes at 20 to 25°C. Five (5) ml of 0.4M Sodium hydroxide, the reactions mixed and the absorbance of the specimen read against the reagent blank after 5minutes (Jendrassik and Grof, 1938; Sharlock, 1951; in: Randox, 2013).

### 3.14.2.1 Manual Calculation

Direct bilirubin = 14.4 x ADB (546nm)

### 3.14.2.2 Normal Values in Serum

Total Bilirubin = up to 1mg/dl

Direct bilirubin = up to 0.25mg/dl

## 3.15 ASPARTATE AMINOTRANSFERASE (AST) ESTIMATION USING RANDOX KIT

α-oxoglutarate + L-aspartate → L-glutamate + Oxaloacetate

Aspartate Aminotransferase (AST) was measured in vitro by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine. Test tubes were set on a tube rack placed on a work bench and were labeled accordingly. Zero point one ml (0.1ml) of each of the HBV and HCV positive specimen was transferred into various test tubes and was labeled accordingly. Zero point five (0.5) ml of reagent 1 (R1) was transferred to all the tubes. Zero point one (0.1) ml of distilled water was transferred to all the tubes labeled blank. The reaction was mixed and incubated at exactly thirty (30) minutes at 37°C. Zero point five (0.5) ml of reagent two (R2) was transferred to all the test tubes the reactions mixed and allowed to stand for exactly 20minutes at 20 to 25°C. Five (5) ml of 0.4M Sodium hydroxide, the reactions mixed and the absorbance of the specimen read against the reagent blank after 5minutes (Reitman and Frankel, 1957; Schmidt and Schmidt, 1963; in: Randox, 2013).

### 3.15.1 Normal Values

Serum up to 12iu/l

## 3.16 ALANINE AMINOTRANSFERASE (ALT) ESTIMATION USING RANDOX KIT

α-oxoglutarate + L-alanine →L-glutamate + Pyruvate

Alanine Aminotransferase was measured in vitro by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine. Test tubes were set on a tube rack placed on a work bench and were labeled accordingly. Zero point one (0.1) ml of each of the HBV and HCV positive specimens was transferred into various test tubes and was labeled accordingly. Zero point five (0.5) ml of reagent 1 (R1) was transferred to all the tubes. Zero point one (0.1) ml of distilled water was transferred to all the tubes labeled blank. The reaction was mixed and incubated at exactly 30 minutes at 37°C. Zero point five (0.5) of reagent two (R2) was transferred to all the test tubes the reactions mixed and allowed to stand for exactly 20minutes at 25°C. Five (5) ml of 0.4M Sodium hydroxide was added to the tubes, the reactions mixed and the absorbance of the specimen read against the reagent blank after 5minutes (Reitman and Frankel, 1957; Schmidt and Schmidt, 1963; in: Randox, 2013).

### 3.16.1 Normal Values

Serum up to 12iu/l

## 3.17 ALKALINE PHOSPHATASE ESTIMATION USING RANDOX KIT

P-nitrophenylphosphate + water + phosphate + p-nitrophenol. Zero point zero two milliters (0.02) ml of each of the HBV and HCV specimen was pipette into cuvette. One (1) ml of the reagent was added to the specimen. The preparation was mixed, initial absorbance read and timer set simultaneously. The absorbance was read again after 1, 2 and 3 minutes respectively (Englehardt *et al.,* 1970; Rec 1972; in: Randox, 2013).

### 3.17.1 Calculation

The alkaline phosphatase was calculated using the following formular:

iu/l = 2760 X Absorbance at 405/minutes

### 3.17.2 Normal Values

Men/women 73-207U/l

## 3.18 ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF ALFA-FETOPROTEIN IN HUMAN SERUM

This Alfa-fetoprotein (AFP) quantitative test is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-AFP antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-AFP antibody in the antibody enzyme (horseadish peroxidase) conjugate solution. The specimen (serum) was added to the AFP antibody coated microtiter wells and incubated with zero buffer. If human AFP is present in the specimen, it will combine with the antibody on the well. The well was then washed to remove any residual test specimen, and AFP antibody labeled with horseadish peroxide (conjugate) is added. The conjugate will bind immunologically to the AFP on the well, resulting in the AFP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubating at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB was added and incubated for 20minutes, resulting in the development of a blue color. The color development was stopped with the addition of 2N HCl, and the color was changed to yellow and was measured spectrophotometrically at 450nm. The concentration of AFP is directly proportional to the

color intensity of the test specimen (Burtis *et al.*,2008).

The desired number of coated wells was secured in the holder. Twenty (20ul), of standard, specimen and controls were dispensed into their appropriate well. One hundred (100ul) of zero specimen was dispensed into the wells. It was thoroughly mixed for 10seconds for complete mixing. It was incubated at room temperature for 30minutes. The incubation mixture was removed by flicking the plate content into a wash container. The microtiter wells were rinsed and flick 5 times with washing buffer 1X. The wells were strike sharply into an absorbent paper to remove all residual water droplets. One hundred and fifty (150µl) of enzyme conjugate reagent was dispensed into each well and mixed gently for 5seconds. The test was incubated at room temperature for 30 minutes. The incubation mixture was removed and flicking plate content into a waste container. The microtiter wells were rinsed and flicked 5 times with washing buffer 1X. The wells were strike sharply into an absorbent paper to remove all residual water droplets. One hundred (100µl) TMB substrate was dispensed into each well and gently mix for 5 seconds and incubated for 20minutes, resulting in the development of a blue color. The color development was stopped with the addition of one hundred (100µl) of TMB. It was mixed for 30seconds to ensure that the blue color has been changed from blue to yellow. The optical density was read at 450nm with a micro titer plate. The concentration of AFP is directly proportional to the color intensity of the test specimen (Burtis *et al.,* 2008; Chan, *et al.,*1986. In Perfemed group, 2013).

### 3.18.1 REFERENCE VALUES

In high risk patients, Alfa-fetoprotein values between 100 and 350ng/ml suggest a diagnosis of hepatocellular carcinoma, and levels over 350ng/ml usually indicates the disease.

## 3.19 DNA EXTRACTION FROM SERUM SAMPLES

DNA extraction was performed using Zymo extraction kit manufactured by Larry Jia (South Africa). The extraction was done according to the guidelines of the manufacturer. DNA was extracted from HBsAg negative/positive specimens of both HIV positive and HIV negative subjects and then PCR was used for detection of HBV-DNA. The protocol is as follows; Four hundred micro liter (400µl) of genomic lysis buffer was added to 100$\mu l$ of serum (4:1) and was mixed completely by vortexing for 6 seconds, it was allowed to stand for 10 minutes at room temperature. The mixture was transferred to a zymo-spin IIC column in a collection tube. It was centrifuge at 10,000×g for 1 minute. The collection tube with the flow through was discarded. The zymo-spin IIC column was transferred to a new collection tube. Two hundred micro liter (200µl) of DNA pre-wash buffer was added to the spin column and centrifuged at 10,000×g for 1 minute. Five hundred micro liter (500µl) of DNA wash buffer was added to the spin column and centrifuged at 10,000×g for 1 minute. The spin column was transferred to a clean microcentrifuge tube. Less than fifty micro liter (>50µl) of DNA elution buffer or water was added to the spin column. It was incubated for 5 minutes at room temperature and then centrifuged at top speed for 30 seconds to elute the DNA. The eluted DNA was used for molecular based application of HBV and sequencing. This is the extracted DNA for PCR assay.

## 3.20 DNA QUANTIFICATION

After the HBV DNA extraction was done, the purity of DNA present was quantified using Nanodrop 1000 with the nanodrop 1000 software preinstalled on a computer system. Two microliters (2µl) of viral genomic DNA was placed on the lower pedestal and the higher pedestal was dropped and the purity was read off the computer.

## 3.21 AMPLIFICATION OF HBV

A portion of the HBV gene was amplified by polymerase chain reaction using primers that flank the HBV sequence (Forward Primer used: →

5′-TCACCATATTCT TGG GAACAACA-3′and Reverse Primer used: 5′-ACC ACT GAA CAA ATG GC-3′←). The PCR components used are; the master mix supplied by Inqaba South Africa (taq polymerase, DNTPs, MgCl) at 1X concentration and volume of 10µl. The forward and reverse primers at concentration of 0.5µM and 0.4ul volume. Template at 2µl volume and water at 4.2µl volume. The final volume was 20µl. Each PCR amplicon was done in 35cycles as follows; Initial denaturation 95$^{o}$C at 5minutes, denaturation 95$^{o}$C at 30seconds; annealing 53$^{o}$C at 30seconds; extension 72$^{o}$C at 30seconds, final extension 72$^{o}$C at 2minutes. These processes were carried out in a 9700 Applied Biosystem Thermo cycler.

## 3.22 AGAROSE GEL ELECTROPHORESIS

In preparing 300ml of 1.5% agarose gel, the following was done; 4.5g of the agarose powder was dissolved in 300ml of Trisboris EDTA (TBE)-running buffer in a cornical flask, the cornical flask containing the gel was heated for 5minutes in a microwave to dissolve the powder, it was allowed to cool to 50$^{o}$C, 2µl of ethidium bromide was added and the gel solution was cast in the gel electrophoretic cast in which the gel comb has been appropriately inserted. It was allowed to polymerize.

The HBV amplicons were resolved using the prepared gel and were visualized using the Ultraviolet Transilluminator. The sizes of the DNA were determined using a quick load molecular ladder.

## 3.23 RNA EXTRACTION FROM PLASMA SPECIMENS

RNA extraction was performed using Zymo extraction kit manufactured by Larry Jia (South Africa). The extraction was done according to the guidelines of the manufacturer. RNA was extracted from HIV and HCV negative/positive specimens of both HIV positive and negative subjects and then PCR was used for detection of HCV RNA and HIV RNA. The RNA extraction protocol is as follows: Three hundred micro liters (300µl) of viral RNA buffer was added to 100µl of plasma specimens and mixed briefly by vortexing. The specimens were transferred to the Zymo-spin IC Column in a collection tube and centrifuged at 10000g for 2 minutes. The flow through was discarded. Five hundred micro liter (500µl) of Viral Wash buffer was added to the column and then centrifuged for 2 minutes at 10000g. The column was carefully transfered into DNase/RNase-free tube. Fifteen (15) ul of DNase/RNase free water directly to the column matrix and centrifuge for 30 seconds to elute. The eluted RNA can be used immediately or stored at -70$^{o}$C for HIV/HCV detection.

## 3.24 AMPLIFICATION OF HIV V3 REGION (NESTED PCR)

### 3.24.1 Primary amplification

A portion of the extracted RNA was amplified by polymerase chain reaction using primers that flank the HIV sequence (Forward Primer used: →5′-GGCATCAAACAGCTCCAGGCAAG-3′and Reverse Primer used: 5′-AGCAAAGCCCTTTCTAAGCCCTGTCT-3′←). The PCR components used are; the master mix supplied by Inqaba South Africa (taq polymerase, DNTPs, MgCl) at 1X concentration and volume of 10ul. The forward and reverse primers at concentration of 0.2uM and 0.16ul volume. Template at 1ul volume and water at 8.68ul volume. The final volume was 20ul.

Each PCR amplicon was done in 25cycles as follows; Initial denaturation 95$^{o}$C at 5minutes, denaturation 95 at 30seconds;Annealing55$^{o}$C at 30seconds; extension 72$^{o}$C at 30seconds, final extension 72$^{o}$C at 2minutes.

### 3.24.2 Secondary amplification

A portion of the HIV primary amplicon was amplified by polymerase chain reaction using primers that flank the HIV sequence (Forward Primer used: → 5′-TCCTGGCTGTGGAAAGATACCTA-3′and Reverse Primer used: 5′-GTCCCCTCGGGGCTGGGAGG-3′←). The PCR components used are; the master mix supplied by Inqaba South Africa (taq polymerase, DNTPs, MgCl) at 1X concentration and volume of 10µl. The forward and reverse primers at concentration of 0.2µM and 0.16µl volume. Template at 0.5µl volume and water at 9.18µl volume. The final volume was 20µl. Each PCR amplicon was done in 35cycles as follows; Initial denaturation 95$^{o}$C at 5minutes, denaturation 95$^{o}$C at 30seconds; annealing 58$^{o}$C at 30seconds; extension 72$^{o}$C at 30seconds, final extension 72$^{o}$C at 2minutes.

### 3.25. AMPLIFICATION OF HCV

A portion of the extracted RNA was amplified by polymerase chain reaction using primers that flank the HCV sequence (Forward Primer used: →5′-ACTGTCTTCACGCAGAAAGCGTCTAGCCAT-3′and Reverse Primer used: 5′-CGAGACCTCCCGG GGCACTCGCAAGCACCC-3′←). The PCR components used are; the master mix supplied by Inqaba South Africa (taq polymerase, DNTPs, MgCl) at 1X concentration and volume of 10ul. The forward and reverse primers at concentration of 0.2uM and 0.16ul volume.Template at 1ul volume and water at 8.68ul volume. The final volume was

20ul.Each PCR amplicon was done in 35cycles as follows; Initial denaturation 95$^{o}$C at 5minutes, denaturation 95$^{o}$C at 30seconds; annealing 50$^{o}$C at 30seconds; extension 72$^{o}$C at 30seconds, final extension 72$^{o}$C at 2minutes.

### 3.26 SEQUENCING

Sequencing was done for HBV, HCV and HIV templates using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa.

### 3.27 PHYLOGENETIC ANALYSIS

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using Clustal X. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969;in: Malin and Lasse, 2017).

### 3.28 STATISTICAL ANALYSIS

Data obtained were statistically analyzed using Graphpad Prism version 7. The P values were calculated and the significant difference was also obtained.

## CHAPTER FOUR

# 4.0 RESULT

The Simple Random Sampling Without Replacement (SRSWOR) method was used to select three district hospitals within Abuja municipal that were used for the collection of specimens and data namely; Asokoro, Maitama and Wuse. A total of 800 specimens were collected, 200 each from HIV positive patients from the three district hospitals and non-HIV volunteers within Asokoro, Maitama and Wuse districts using the Stratified Random Sampling method. Table 1 and Table 2 show the prevalence of hepatitis B and C virus among HIV/non-HIV patients attending Asokoro, Maitama and Wuse hospitals using rapid test strip (RTD). Among the 200 HIV positive patients from each district hospital, 20(10%), 8(4%), 18(9%) for positive HBsAg and 180(90%), 192(96%), 182(91%) for negative HBsAg were recorded in Asokoro, Maitama and Wuse respectively. Patients positive to Hepatitis C Virus were 16(8%), 12(6%) and 10(5%), while patients negative for the Hepatitis C Virus were 184(92%), 188(94%) and 190(95%) for Asokoro, Maitama and Wuse hospitals correspondingly. These tests were also carried out on the non-HIV volunteers, they were however seen to have higher prevalence of hepatitis B virus when compared to the number of individuals positive for the virus among HIV patients. This results of the HBV and HCV is statistically not significant at $p>0.05$ when compared to whether the patient is HIV positive or not, it can affect any body. A total of 22(11%) of the 200 volunteers from Asokoro, Maitama and Wuse districts turned out positive for hepatitis B virus while 8(4%) was positive for hepatitis C virus. The total percentage of the subjects positive to HBV and HCV was 8.5% (68/800) and 5.7% (46/800) respectively. The prevalence of HBV and HCV were not significantly dependent on the hospital at 95% confidence interval (CI) and $p>0.05$.

**TABLE 1: Prevalence of Hepatitis B Virus among HIV patients attending the 3 District Hospitals using Rapid test Strip RTD**

| Districts | No of pts Examined | No Positive for HBsAg | No Negative for HBsAg |
|---|---|---|---|
| Asokoro | 200 | 20(10%) | 180(90%) |
| Maitama | 200 | 8(4%) | 192(96%) |
| Wuse | 200 | 18(9%) | 182(91%) |
| Non HIV Volunteers | 200 | 22(11%) | 178(89%) |
| Total | 800 | 68(8.5%) | 732(91.5%) |

| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 7.457 | 8 | NA | P>0.05 |

**TABLE 2: Prevalence of Hepatitis C Virus among HIV patients attending the 3 District Hospitals using Rapid Test Device Strip (RTD)**

| Districts | No Examined | No Positive for HCV | No Negative for HCV |
|---|---|---|---|
| Asokoro | 200 | 16(8%) | 184(92%) |
| Maitama | 200 | 12(6%) | 188(94%) |
| Wuse | 200 | 10(5%) | 190(95%) |
| Non HIV Volunteers | 200 | 8(4%) | 192(96%) |
| Total | 800 | 46(5.7%) | 754(94.3%) |

| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 3.229 | 8 | NA | P>0.05 |

The Prevalence of Hepatitis B Virus among HIV/non-HIV patients attending Asokoro, Maitama and Wuse using 5 panels RTD test with ELISA Principle is represented on Table 3. The presence of surface antigen (HBsAg), presence of surface antibodies (HBsAb), presence of viral protein (HBeAg), occurrence of viral replication (HBeAb) and presence of active or acute infection (HBcAb) were all determined across the subjects. 30/200 (15%) of patients attending Asokoro had hepatitis B surface antigen, 20/200 (10%) in Maitama and 22/200 (11%) in Wuse hospitals, while 30/200 (15%) were found to have the surface antigen among the non-HIV volunteers. Hepatitis B surface antibody (presence of protective antibodies) was recorded for just 5/200 (2.5%) patients in Asokoro, 25/200 (12.5%) in Maitama, Wuse 35/200 (17.5%) and 20/200 (10%) for non-HIV volunteers. Viral protein on the other hand was recorded to be present in 7/200 (3.5%) from Asokoro, none was recorded for patients in Maitama and Wuse hospitals while 9/200 (4.5%) of the non-HIV volunteers had the viral protein. To determine if there is viral replication or not, HbeAb was determined and it was found out that 70/200 (35%), 5/200(2.5%), 85/200 (42.5%) and 60/200 (30%) of the patients attending Asokoro, Maitama, Wuse and non-HIV volunteers had it respectively. HbcAb which is usually produced by the immune system after infection by hepatitis B virus was detected and it was found to be present in 68/200 (34%), 25/200 (12.5%), 100/200 (50%) of patients in Asokoro, Maitama, Wuse and non-HIV volunteers. The total percentage of the 800 patients studied, was observed to be 102/800 (12.75%), 85/800 (10.6%), 16/800 (2%), 220/800 (27.5%), 248/800 (31%) for HBsAg, HBsAb, HBeAg, HbeAb and HbcAb correspondingly and the result is statistically significant at $P<0.05$.

**TABLE 3: Prevalence of Hepatitis B Virus among HIV patients attending the 3 District Hospitals using 5 panels RTD with ELISA Principle**

| Districts | No of subjects | +ve HbsAg | +ve HBsAb | +ve HBeAg | +ve HbeAb | +ve HbcAb |
|---|---|---|---|---|---|---|
| Asokoro | 200 | 30(15%) | 5(2.5%) | 7(3.5%) | 70(35%) | 68(34%) |
| Maitama | 200 | 20(10%) | 25(12.5%) | 0(0%) | 5(2.5%) | 25(12.5%) |
| Wuse | 200 | 22(11%) | 35(17.5%) | 0(0%) | 85(42.5%) | 100(50%) |
| Non HIV volunteers | 200 | 30(15%) | 20(10%) | 9(4.5%) | 60(30%) | 55(27.5%) |
| Total | 800 | 102(12.75%) | 85(10.6%) | 16(2%) | 220(27.5%) | 248(31%) |

| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 125.7 | 20 | NA | P<0.05 |

Hepatitis B and C virus prevalence was also determined using PCR technique for patients who were not on Anti-Retroviral Therapy (ART) as represented in Table 4. One hundred of the two hundred HIV/non HIV subjects each from each district hospital who were not on ART, were randomly picked and HBV and HCV test were conducted using PCR technique. It was found out that, 40/100 (40%) subjects were positive to HBsAg in Asokoro hospital, 20/100 (20%) in Maitama and 45/100 (45%) in Wuse hospital. HCV positivity was found in 26/100 (26%), 20/100(20%) and 19/100(19%) for Asokoro, Maitama and Wuse hospitals respectively. The volunteers on the other hand numbered 20/100(20%) and 8/100(8%) for HBV and HCV correspondingly. Comparing the outcome with that obtained using RTD, PCR was recorded to detect the virus more prominently as the values from Asokoro was 40(40%) as compared to 20(10%) recorded for RTD, Maitama 20(20%) as against 8(4%) for RTD, Wuse 45(45%) against 18 (9%) and 22(20%) for volunteers as against 20(11%) recorded for RTD. The same trend was observed for HCV, Asokoro 26(26%), Maitama 20(20%), 19(19%) non-HIV volunteers 8(8%) for PCR method of detection as against 16(8%), 12(6%), 10(5%) and 8(4%) respectively using RTD method. The PCR and ELISA results were statistically significant at $p<0.05$.

**TABLE 4: Prevalence of Hepatitis B and C Virus among HIV patients attending the 3 District Hospitals Who were not on ART using PCR**

| Districts | No of subjects | +ve HBsAg | -ve HBsAg | +ve HCV | -ve HCV |
|---|---|---|---|---|---|
| Asokoro | 100 | 40(40%) | 60(60%) | 26(26%) | 74(74%) |
| Maitama | 100 | 20(20%) | 80(90%) | 20(20%) | 80(80%) |
| Wuse | 100 | 45(45%) | 55(55%) | 19(19%) | 8(81%) |
| HIV Non Volunteers | 100 | 20(20%) | 80(89%) | 8(8%) | 92(92%) |
| Total | 400 | 125(31.25%) | 275(68.75%) | 73(18.25%) | 327(92%) |

| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 99.11 | 16 | NA | P<0.05 |

Prevalence of Hepatitis B Virus among HIV patients who were on ART and HIV negative Subjects who were on herbs positive for HBsAg, HBeAg, HBeAb, HbcAb and HBsAb with RTD and ELISA using PCR was determined as shown on Tables 5 - 9. Two categories of patients were examined after the presence of the markers has been detected in them; HIV positive patients on ART and non-HIV patients not on ART but on herbs. PCR technique was used to further ascertain the authenticity of the markers detected and also to see if the ART therapy is having any significant effect on patients on the drug and also the effect of herbs on patients using it. A random selection of 100 subjects positive to the above mentioned markers were picked. The outcome of the result is thus: 38(76%)/50(100%) of the HIV positive patients on ART positive to HBsAg were found to be carriers of the HBV DNA while 42(84%)/50(100%) of the volunteers were also found to be positive to HBV DNA. Out of the total number of HBeAg positive patients documented in this research (Table 3), seven (7(3.5%)) HIV positive patient on ART and 7 non-HIV patients on herbs were analyzed. Six out of seven (6(86%)/7(100%)) of the HIV positive individuals, positive for HBeAg were on ART, none was on herbs and one (1(14%)/7(100%)) individual was not on any medication at all. Four out of seven (4(57%)/7(100%)) of the subjects were seen to have HBV DNA. The non-HIV patients on the other hand were not on ART, two (2(29%)/7(100%)) were on herbs and five (5(71%)/7(100%)) were not on medication. 5(71%)/7(100%) of this particular sets of individuals were found to have HBV DNA. For patients positive to HBeAb on ART and some on herbs, 50 positive and 50 negative HIV subjects were randomly picked. The whole 50 subjects positive to HIV were on ART, 8(16%)/50 of this individuals were positive to HBV DNA. For the non-HIV patients, 10(20%)/50 of the patients were on herbs, 40(80%)/50 were without medication while a total of 34(68%)/50 of them were seen to have HBV DNA.

Patients positive to HbcAb were also examined and 20(40%)/50 HIV positive patients had HBV DNA while 30(60%)/50 volunteers had the DNA for HBV. The total number of patients positive for HBsAb randomly picked was 20 each for positive and negative HIV patients. In this category, HBV DNA was not detected in any of the subjects (both HIV and non-HIV patients). The prevalence of Hepatitis B and C virus among HIV patients attending the 3 district hospitals using PCR was determined over one-hundred HIV positive patients and non-HIV volunteers each randomly selected from the samples collected. Among the HIV positive patients from the randomly selected samples, 80(80%) were positive to HBsAg, 20(20%) to HCV while the volunteers had 40(40%) to HBsAg and 8(8%) to HCV as present on Table 10.

**TABLE 5: Prevalence of HBV among HIV pts on ART and HIV negative Subjects on herbs positive for HBsAg with RTD and ELISA using PCR**

| Status | No of Patients | No of pts on ART | No of pts on herbs | No of pts without herbs | HBV DNA |
|---|---|---|---|---|---|
| HIV +ve | 50 | 50(50%) | 0(0%) | 0(0%) | 38(76%) |
| HIV –ve | 50 | 0(0%) | 10(20%) | 40(80%) | 42(84%) |
| Total | 100 | 46(46%) | 10(20%) | 44(44%) | 80(80%) |

| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 100.8 | 8 | NA | P<0.05 |

**TABLE 6: Prevalence of HBV among HIV pts on ART and HIV negative Subjects on Herbs positive for HBeAg with RTD and ELISA using PCR**

| Districts | Total No of Pts | No of pt on ART | No of pt on herbal | No of pt without medication | HBV DNA |
|---|---|---|---|---|---|
| HIV +ve | 50 | 50(100%) | 0(0%) | 0(0%) | 8(16%) |
| HIV -ve | 50 | 0(0%) | 10(20%) | 40(80%) | 34(68%) |
| Total | 100 | 50(50%) | 10(10%) | 40(40%) | 42(84%) |

| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 10.76 | 8 | NA | P>0.05 |

**TABLE 7: Prevalence of HBV among HIV patients on ART and HIV negative Subjects on Herbs positive for HBeAb with RTD using PCR**

| Districts | Total No of Pts | No of pt on ART | No of pt on herbal | No of pt without medication | HBV DNA |
|---|---|---|---|---|---|
| HIV +ve | 50 | 50(100%) | 0(0%) | 0(0%) | 8(16%) |
| HIV -ve | 50 | 0(0%) | 10(20%) | 40(80%) | 34(68%) |
| Total | 100 | 50(50%) | 10(10%) | 40(40%) | 42(84%) |

| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 114.6 | 8 | NA | P<0.05 |

**TABLE 8: Prevalence of HBV among HIV patients on ART and HIV negative Subjects on Herbs but positive for HBcAb with RTD using PCR**

| Status | No of patients | No of pt on ART | No of pt on herbs | No of pt without herbs | HBV DNA |
|---|---|---|---|---|---|
| HIV +ve | 50 | 50(100%) | 0(0%) | 0(0%) | 20(40%) |
| HIV -ve | 50 | 0(0%) | 8(16%) | 42(84%) | 30(60%) |
| Total | 100 | 50(0%) | 8(16%) | 42(84%) | 50(50%) |

| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 101.8 | 8 | NA | P<0.05 |

**TABLE 9: Prevalence of Hepatitis B Virus among HIV patients on ART and HIV negative Subjects positive for HBsAb using PCR**

| Status | No of subjects | HBV +ve | HBV -ve |
|---|---|---|---|
| HIV +ve | 20 | 0(0%) | 20(100%) |
| HIV -ve | 20 | 0(0%) | 20(100%) |
| Total | 40 | 0(0%) | 40(100%) |

**TABLE 10: Prevalence of Hepatitis B and C Virus among HIV patients attending the 3 District Hospitals using PCR**

| Districts | No of subjects | +ve HBsAg | -ve HBsAg | +ve HCV | -ve HCV |
|---|---|---|---|---|---|
| HIV Pts | 100 | 80(80%) | 20(20%) | 20(20%) | 80(80%) |
| Non HIV volunteers | 100 | 40(40%) | 60(60%) | 8(8%) | 92(92%) |
| Total | 200 | 120(60%) | 80(40%) | 28(14%) | 172(86%) |

| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 39.31 | 3 | NA | P<0.05 |

Determination of prevalence of hepatitis B and C virus among HIV patients attending Asokoro, Maitama and Wuse hospitals with respect to age is represented on Tables 11–13. The Two hundred subjects under study were divided into age groups ranging between 15 to 75 and within this age range, 36(18%)/200, 32(16%)/200 and 12(6%)/200 of the individuals in age brackets 25-35, 35-45 and 45-55 respectively were positive to HBV, while 32(16%)200 and 10(5%)/200 patients were positive to HCV in the age group of 25-35 and 35-45 respectively. A total of 14(7%)/200 individuals were recorded in age bracket 15-25 and none was positive for HBV. Within the age group 55-65, fifteen (15(7.5%))/200 patients were screened and none of them was found with HBV whereas 10(5%) of them were found to be HCV positive. No patients were recorded to be positive to both viral infections within age bracket 65-75 for Asokoro hospital. Maitama hospital on the other hand recorded the following number of positive individuals to HBV and HCV respectively; 30(15%)/200 for age range 25-35, 5(2.5%)/200 for age range 35-45 and 45-55, 15(7.5%)/200 for age range 25-35, 22(11%)/200 for age range 45-55 and 3(1.5%) for the age range 55-65. Wuse hospital recorded 36(18%)/200 for age range 25-35, 30(15%)/200 for age range 35-45, 12(6%)/200 for age range 45-55, 3(1.5%)/200 for the age range 65-75 to HBV. Eight(8) each were recorded for subjects in the age bracket 15-25, 25-35, 35-45 to be positive to HCV, fourteen (14) were positive to HCV within the age bracket 45-55. Age range of 25-35 had the highest prevalence of 18%, 15% and 18% in Asokoro, Maitama and Wuse hospitals respectively for HBV. The least prevalence rate of 6% was recorded for age range of 45-55 for Asokoro, Maitama recorded 35-45 and 45-55 to have the least prevalence of 2.5% and for Wuse, 65-75 was recorded to have the least prevalence of 1.5% for HBV. HCV contrariwise had highest prevalence of 16% at age range 25-35 in Asokoro hospital. Maitama and Wuse had age bracket 45-55 as age range with highest prevalence of 11% and 7% respectively for HCV. The least prevalence rate of 5% and 3% HCV was recorded for the age range 55-65 in Asokoro and maitama respectively, and 4% was recorded for the ages 15-25, 25-35 and 35-45 in Wuse hospital.

**Table 11: Prevalence of hepatitis B and C Virus among HIV patients attending Asokoro Hospital with respect to Age**

| Status | HBV +ve | HBV-ve | Total | HCV+ve | HCV –ve | Total |
|---|---|---|---|---|---|---|
| 15-25 | 0(0%) | 14(7%) | 14(7%) | 0(0%) | 14(7%) | 14(7%) |
| 25-35 | 36(18%) | 46(23%) | 82(41%) | 32(16%) | 50(25%) | 82(41% |
| 35-45 | 32(16%) | 23(11.5%) | 55(27.5%) | 10(5%) | 45(22.5%) | 55(27.5%) |
| 45-55 | 12(6%) | 22(11%) | 34(17%) | 0(0%) | 34(17%) | 34(17%) |
| 55-65 | 0(0%) | 15(7.5%) | 15(7.5%) | 10(5%) | 5(2.5%) | 15(7.5%) |
| 65-75 | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| Total | 80(40%) | 120(60%) | 200(100%) | 52(26%) | 148(74%) | 200(100%) |

**Table 12: Prevalence of hepatitis B and C Virus among HIV patients attending Maitama Hospital with respect to Age**

| Status | HBV +ve | HBV-ve | Total | HCV+ve | HCV –ve | Total |
|---|---|---|---|---|---|---|
| 15-25 | 0(0%) | 12(6%) | 12(6%) | 0(0%) | 12( 6%) | 12(6%) |
| 25-35 | 30(15%) | 58(29%) | 88(44%) | 15(7.5%) | 73(36.5%) | 88(44%) |
| 35-45 | 5(2.5%) | 60(30%) | 65(32.5) | 0(0%) | 65(32.5%) | 65(32.5%) |
| 45-55 | 5(2.5%) | 20(10%) | 25(12.5%) | 22(11%) | 3(1.5%) | 25(12.5%) |
| 55-65 | 0(0%) | 10(5%) | 10(5%) | 3(1.5%) | 7(3.5%) | 10(5%) |
| 65-75 | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| Total | 40(20%) | 160(80%) | 200(100%) | 40(20%) | 160(80%) | 200(100%) |

**Table 13: Prevalence of hepatitis B and C Virus among HIV patients attending Wuse Hospital with respect to Age**

| Status | HBV +ve | HBV-ve | Total | HCV+ve | HCV –ve | Total |
|---|---|---|---|---|---|---|
| 15-25 | 9(4.5%) | 12(6%) | 21(10.5%) | 8(4%) | 13(6.5%) | 21(10.5%) |
| 25-35 | 36(18%) | 48(24%) | 84(42%) | 8(4%) | 76(38%) | 84(42%) |
| 35-45 | 30(15%) | 30(15%) | 60(30%) | 8(4%) | 52(26%) | 60(30%) |
| 45-55 | 12(6%) | 15(7.5%) | 27(13.5%) | 14(7%) | 13(6.5%) | 27(13.5%) |
| 55-65 | 0(0%) | 5(2.5%) | 5(2.5%) | 0(0%) | 5(2.5%) | 5(2.5%) |
| 65-75 | 3(1.5%) | 0(0%) | 3(1.5%) | 0(0%) | 3(1.5%) | 3(1.5%) |
| Total | 90(45%) | 110(55%) | 200(100%) | 38(19%) | 162(81%) | 200(100%) |

The resultant effect of nationality/ethnicity/culture as a prevalence factor of hepatitis B and C virus among HIV patients attending Asokoro, Maitama and Wuse was determined (Table 14-16). All the subjects were Nigerians by nationality, belonging to Igbo, Hausa or Yoruba tribes and presence of tribal incision was also considered. All subjects across the district hospitals positive for HBV and HCV were Nigerians, with 80(40%)/200, 40(20%)/200, 90(45%)/200 of them positive to HBV; 52(26%)/200, 40(20%)/200, 38(19%)/200 individuals positive to HCV attending Asokoro, Maitama and Wuse hospitals respectively. The ratio of Igbo: Hausa: Yoruba in Asokoro, Maitama and Wuse hospitals positive to HBV and HCV was 16(8%)/200: 20(10%)/200: 0(0%)/200, 20(10%)/200:10(5%)/200: 0(0%)/200; 0(0%)/200:25(12.5%)/200:5(2.5%)/200, 0/200:10(5%)/200:0(0%)/200 and 21(11.5%)/200:24(12%)/200:3(1.5%)/200, 7(3.5%)/200:7(3.5%)/200:0(0%)/200 respectively. Thirty-two (32(16%)/200) of those attending Asokoro hospital had tribal mark, fifteen (15(7.5%)/200) had in Maitama hospital and Wuse hospital recorded 32(16%)/200 of the patients. The Hausas and others (i.e. other tribes) were observed to have the highest prevalence and the percentage of those without tribal marks were more than those with tribal mark.

**Table 14: Prevalence of hepatitis B and C Virus among HIV patients attending Asokoro Hospital with respect to Ethnicity**

| Status | HBV +ve | HBV-ve | HCV+ve | HCV –ve |
|---|---|---|---|---|
| **Nationality** | | | | |
| Nigerian | 80(40%) | 120(60%) | 52(26%) | 148(74%) |
| Non-Nigerian | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| Total | 80(40%) | 120(60%) | 52(26%) | 148(74%) |
| **Tribe** | | | | |
| Igbo | 16(8%) | 30(15%) | 20(10%) | 26(13%) |
| Hausa | 20(10%) | 22(11%) | 10(5%) | 32(16%) |
| Yoruba | 0(0%) | 18(9%) | 0(0%) | 18(9%) |
| Others | 44(22%) | 50(25%) | 20(10%0 | 72(36%) |
| Total | 80(40%) | 120(60%) | 52(26%) | 148(74%) |
| **Tribal Mark** | | | | |
| Yes | 32(16%) | 56(28%) | 2(1%) | 86(43%) |
| No | 48(24%) | 64(32%) | 50(25%) | 62(31%) |
| Total | 80(40%) | 120(60%) | 52(26%) | 148(74%) |

**Table 15: Prevalence of hepatitis B and C Virus among HIV patients attending Maitama Hospital with respect to Ethnicity**

| Status | HBV +ve | HBV-ve | HCV+ve | HCV –ve |
|---|---|---|---|---|
| **Nationality** | | | | |
| Nigerian | 40(20%) | 160(80%) | 40(20%) | 160(80%) |
| Non-Nigerian | 0(0%) | 0(0%) | 0(%) | 0(%) |
| Total | 40(20%) | 160(80%) | 40(20%) | 160(80%) |
| **Tribe** | | | | |
| Igbo | 0(0%) | 54(27%) | 0(0%) | 54(27%) |
| Hausa | 25(12.5%) | 32(16%) | 10(5%) | 47(23.5%) |
| Yoruba | 5(2.5%) | 6(3%) | 0(0%) | 11(5.5%) |
| Others | 10(5%) | 68(34%) | 30(15%) | 48(12%) |
| Total | 40(20%) | 160(80%) | 40(20%) | 160(80%) |
| **Tribal Mark** | | | | |
| Yes | 15(7.5%) | 43(21.5%) | 0(0%) | 58(9%) |
| No | 25(12.5%) | 117(58.5%) | 40(20%) | 102(51%) |
| Total | 40(20%) | 160(80%) | 40(20%) | 160(80%) |

**Table 16: Prevalence of hepatitis B and C Virus among HIV patients attending Wuse Hospital with respect to Ethnicity**

| Status | HBV +ve | HBV-ve | HCV+ve | HCV –ve |
|---|---|---|---|---|
| **Nationality** | | | | |
| Nigerian | 90(45%) | 110(55%) | 38(19%) | 162(81%) |
| Non-Nigerian | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| Total | 90(45%) | 110(55%) | 38(18%) | 162(81%) |
| **Tribe** | | | | |
| Igbo | 21(10.5% | 25(12.5%) | 7(3.5%) | 39(19.5%) |
| Hausa | 24(12%) | 18(9%) | 7(3.5%) | 35(18.5%) |
| Yoruba | 3(1.5%) | 15(7.5%) | 0(0%) | 18(9%) |
| Others | 42(21%) | 52(26%) | 24(12%) | 70(35%) |
| Total | 90(45%) | 110(55%) | 38(19%) | 162(81%) |
| **Tribal Mark** | | | | |
| Yes | 32(16%) | 31(15.5%) | 14(7%) | 49(24.5%) |
| No | 58(14%) | 79(39.5%) | 24(12%) | 113(56%) |
| Total | 90(45%) | 110(55%) | 38(19%) | 162(81%) |

In respect to medical history, the prevalence of hepatitis B and C virus among HIV patients attending Asokoro, Maitama and Wuse hospitals was determined as denoted on Tables 17-19. Parameters considered include; history of blood transfusion, surgery, drug abuse, knowledge of STD and family history of HBV. Most of the patient positive to HBV and HCV had no medical record for history of blood transfusion and surgery. An equal percentage of them positive to HBV had used injectable drug (IDU). In Asokoro the number of individuals who do not have an idea of sexually transmitted diseases (STDs) are quit high numbering 40(20%)/200 when compared with the number with an idea about the sexual disease. Only 22(11%)/200 of them were fully informed on what sexually transmitted diseases (STDs) was. It was found out also that a large proportion of the patients had no family history of the viral infections. Maitama and Wuse hospitals also recorded larger number of individuals with no history of blood transfusion and surgery. 25(12.5%)/200; 20(10%)/200 of them positive to HBV correspondingly had used injectable drugs. Individuals positive to HBV and HCV with no idea of sexually transmitted diseases (STDs) were 18(9%)/200 and 30(15%)/200. Family history for HBV was seen to be low across the hospitals. Use of injectable drugs generally had the highest prevalence of all the parameters considered.

**Table 17: Prevalence of hepatitis B and C Virus among HIV patients attending Asokoro District Hospital with respect to Medical history**

| Status | HBV +ve | HBV-ve | HCV+ve | HCV –ve |
|---|---|---|---|---|
| **History of Blood transfusion** | | | | |
| Yes | 20(10%) | 39(19.5%) | 11(5.5%) | 48(24%) |
| No | 60(30%) | 81(40.5%) | 41(20.5%) | 100(50%) |
| Total | 80(40%) | 120(60%) | 52(26%) | 148(74%) |
| **History of Surgery** | | | | |
| Yes | 8(4%) | 44(22%) | 21(10.5%) | 31(15.5%) |
| No | 72(36%) | 76(38%) | 31(15.5%) | 117(58.5%) |
| Total | 80(40%) | 120(60%) | 52(26%) | 148(74%) |
| **History of Injecting drug use** | | | | |
| Yes | 40(20%) | 35(17.5%) | 20(10%) | 55(27.5%) |
| No | 40(20%) | 85(42.5%) | 32(16%) | 93(46.5) |
| Total | 80(40%) | 120(60%) | 52(26%) | 148(74%) |
| **Knowledge of STD** | | | | |
| Fully informed | 32(16%) | 40(20%) | 22(11%) | 30(15%) |
| Little knowledge | 8(4%) | 57((28.5%) | 10(5%) | 47(23.5%) |
| No idea | 40(20%) | 23(11.5%) | 20(10%) | 71(35.5% |
| Total | 80(40%) | 120(60%) | 52(26%) | 148(74%) |
| **Family History of HBV** | | | | |
| Yes | 12(6%) | 47(23.5%) | 0(0%) | 59(29.5%) |
| No | 68(34%) | 73(36.5%) | 52(26%) | 89(44.5%) |
| Total | 80(40%) | 120(60%) | 52(26%) | 148(74%) |

**Table 18: Prevalence of hepatitis B and C Virus among HIV patients attending Maitama District Hospital with respect to Medical history**

| Status | HBV +ve | HBV-ve | HCV+ve | HCV –ve |
|---|---|---|---|---|
| **History of Blood transfusion** | | | | |
| Yes | 10(5%) | 26(13%) | 0(0%) | 36(18%) |
| No | 30(15%) | 134(77%) | 40(20%) | 124(62%) |
| Total | 40(20%) | 160(80%) | 40(20%) | 160(80%) |
| **History of Surgery** | | | | |
| Yes | 15(7.5%) | 47(23.5%) | 0(0%) | 62(31%) |
| No | 25(12.5%) | 113(56.5%) | 40(20%) | 98(49%) |
| Total | 40(20%) | 160(80%) | 40(20%) | 160(80%) |
| **History of Injecting drug use** | | | | |
| Yes | 25(12.5%) | 69(34.5%) | 20(10%) | 74(37%) |
| No | 15(7.5%) | 91(45.5%) | 20(10%) | 86(43%) |
| Total | 40(20%) | 160(80%) | 40(20%) | 160(80%) |
| **Knowledge of STD** | | | | |
| Fully informed | 5(2.5%) | 64(32%) | 0(0%) | 69(34.5%) |
| Little knowledge | 17(8.5%) | 34(17%) | 10(5%) | 41(20.5%) |
| No idea | 18(9%) | (31%) | 30(15%) | 50(25%) |
| Total | 40(20%) | 160(80%) | 40(20%) | 160(80%) |
| **Family History of HBV** | | | | |
| Yes | 10(5%) | 24(12%) | 0(0%) | 34(17%) |
| No | 30(15%) | 136(78%) | 40(20%) | 124(62%) |
| Total | 40(20%) | 160(80%) | 40(20%) | 160(80%) |

**Table 19: Prevalence of hepatitis B and C Virus among HIV patients attending Wuse District Hospital with respect to Medical history**

| Status | HBV +ve | HBV-ve | HCV+ve | HCV –ve |
|---|---|---|---|---|
| **History of Blood transfusion** | | | | |
| Yes | 24(12%) | 26(13%) | 0(0%) | 36(18%) |
| No | 66(33%) | 84(42%) | 38(19%) | 126(63%) |
| Total | 90(45%) | 110(55%) | 38(19%) | 162(81%) |
| **History of Surgery** | | | | |
| Yes | 30(15%) | 47(23.5%) | 9(4.5%) | 62(31%) |
| No | 60(30%) | 63(31.5%) | 27(13.5%) | 100(50%) |
| Total | 90(45%) | 110(55%) | 38(19%) | 162(81%) |
| **History of Injecting drug use** | | | | |
| Yes | 45(22.5%) | 29(14.5%) | 9(4.5%) | 76(38%) |
| No | 45(22.5%) | 81(40.5%) | 27(13.5%) | 86(43%) |
| Total | 90(45%) | 110(55%) | 38(19%) | 162(81%) |
| **Knowledge of STD** | | | | |
| Fully informed | 24(12%) | 34(17%) | 9(4.5%) | 69(34.5%) |
| Little knowledge | 20(10%) | 34(17%) | 9(4.5%) | 43(21.5%) |
| No idea | 46(23%) | 42(21%) | 18(9%) | (25%) |
| Total | 90(45%) | 110(55%) | 38(19%) | 162(81%) |
| **Family History of HBV** | | | | |
| Yes | 30(15%) | 24(12%) | 9(4.5%) | 38(19%) |
| No | 60(30%) | 86(43%) | 27(13.5%) | 124(62%) |
| Total | 90(45%) | 110(55%) | 38(19%) | 162(81%) |

Occupational exposure as a cause of prevalence of hepatitis B and C virus among HIV patients attending the three district hospitals was studied as presented on Tables 20-22. Putting into consideration the nature of their jobs in terms of being a civil servant, public servant, unemployed, traders, international businessmen and apprentice. The highest prevalence of HBV and HCV was recorded for unemployed, petty traders, civil servants across the hospitals, except for Maitama hospital where unemployed individuals and apprentice were negative to both viral infection. Unemployed individuals in Asokoro hospital were 36(18%)/200 positive to HBV, 10(5%)/200 was positive for HCV, public servant had 20(10%)/200 subjects positive to both HBV and HCV. Least prevalence was recorded for public servants, petty traders and business men with 8(4%)/200 individuals each for HBV. HCV had least prevalence of 2(1%)/200 for petty traders and no prevalence for business man and apprentice. In Maitama, civil servants and public servants had prevalence of 20(10%)/200 for HBV and HCV respectively. No prevalence was documented for unemployed persons, apprentice and business man for both viral infection. Wuse hospitals accounted for 33(16.5%)/200 unemployed persons positive to HBV and 24(12%)/200 petty trader while the least prevalence for HBV was recorded for business man 3(1.5%)/200. Civil servants accounted for 16(8%)/200, business man and apprentice 6(3%)/200 each for HCV. No prevalence was recorded for public servant and unemployed persons. The highest prevalence was thus recorded for unemployed persons, public and civil servants.

**Table 20: Prevalence of hepatitis B and C Virus among HIV patients attending Asokoro Hospital with respect to Occupational exposure**

| Status | HBV +ve | HBV-ve | HCV+ve | HCV–ve |
|---|---|---|---|---|
| Civil servant | 8(4%) | 25(12.5%) | 10(5%) | 23(12.5%) |
| Public Servant | 20(10%) | 27(13.5%) | 20(10%) | 27(13.5%) |
| Unemployed | 36(18%) | 29(14.5%) | 10(5%) | 55(27.5%) |
| Petty trader | 8(4%) | 35(17.5%) | 2(1%) | 41(20.5%) |
| International businessman | 8(4%) | 2(1%) | 0(0%) | 10(5%) |
| Apprentice | 0(0%) | 2(1%) | 0(0%) | 2(1%) |
| Total | 80(40%) | 120(60%) | 52(26%) | 158(79%) |

| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 51.75 | 18 | NA | P<0.05 |

**Table 21: Prevalence of hepatitis B and C Virus among HIV patients attending Maitama Hospital with respect to Occupational exposure**

| Status | HBV +ve | HBV-ve | HCV+ve | HCV –ve |
|---|---|---|---|---|
| Civil servant | 20(10%) | 18(9%) | 10(5%) | 28(14%) |
| Public Servant | 10(5%) | 23(11.5%) | 20(10%) | 13(6.5%) |
| Unemployed | 0(0%) | 56(28%) | 0(0%) | 56(28%) |
| Petty trader | 5(2.5%) | 53(26.5%) | 10(5%) | 48(24%) |
| International businessman | 5(2.5%) | 0(0%) | 0(0%) | 5(2.5%) |
| Apprentice | 0(0%) | 6(3%) | 0(0%) | 10(5%) |
| Total | 40(20%) | 160(80%) | 40(20%) | 160(80%) |

**Table 22: Prevalence of hepatitis B and C Virus among HIV patients attending Wuse Hospital with respect to Occupational exposure**

| Status | HBV +ve | HBV-ve | HCV+ve | HCV –ve |
|---|---|---|---|---|
| Civil servant | 20(10%) | 6(3%) | 16(8%) | 10(5%) |
| Public Servant | 6(3%) | 26(13%) | 0(0%) | 32(16%) |
| Unemployed | 33(16.5%) | 18(9%) | 0(0%) | 50(25%) |
| Petty trader | 24(12%) | 56(23%) | 8(4%) | 62(31%) |
| International businessman | 3(1.5%) | 3(1.5%) | 6(3%) | 0(0%) |
| Apprentice | 4(2%) | 10(5%) | 6(3%) | 8(4%) |
| Total | 90(45%) | 119(54.5%) | 36(18.0%) | 162(81%) |

**KEY**

-ve = Negative

+ve = Positive

ART = Antiretroviral Therapy

RTD = Rapid Test Device

No of pts = Number of patients

NA = Not applicable

Liver function test and Alfa fetoprotein was determined in 200 patients randomly selected from the 3 district hospitals, 100 patients positive to HIV/HBV and HIV/HCV, that is 50(100%) with HIV/HBV and 50(100%) with HIV/HCV, then the other 100 patients 50(100%) with HIV mono-infection and 50(100%) with HIV/HBV/HCV co-infections were determined respectively as shown in Table 23. The test included; serum bilirubin (total and direct bilirubin), AST (Aspartate transaminase) ALT (Alanine Aminotransferase) and ALP (Alkaline phosphatase). Both the total and direct bilirubin tested were normal in the HIV, HIV/HBV, HIV/HCV and HIV/HBV/HCV positive patients. They have total and direct bilirubin result between 0.1-0.9mg/dl and 0.1-0.25mg/dl mean of ± 0.6 for total bilirubin and 0.2mg/dl for direct bilirubin (normal range is 0.1-1.0mg/dl and 0.1-0.25mg/dl) correspondingly. Sixty-five percent (65%) out of the first 100 patients sampled had AST result of 4-12 iu/l, which is in the normal range (up to 12 iu/l is normal range). However, fifteen (15/50) 30% of the HIV/HBV positive patients and twenty (20/50) 40% of the HIV/HCV positive patients had results above the normal range (13-25unit/l and 25-45unit/l) for HIV/HBV and HIV/HCV respectively). In the case of ALT, 70/100(70%) of the patients were normal for the test, result ranged between 4-12iu/l (4-12iu/l is a normal range). Fourteen (14/50), 28% HIV/HBV positive patients had result ranging between 13-25iu/l, while 16/50(32%) HIV/HCV patients had readings between 25-50iu/l. The range of normal readings for ALP is between 73-203iu/l. Out of the first 100 patients screened, 90 of them were normal (between 73-203iu/l), 6/50(12%) of the HIV/HBV positive and 4/50(8%) of the HIV/HCV positive patients had high ALP levels (between 204-214iu/l and 215-224iu/l for HIV/HBV and HIV/HCV respectively). Alpha fetoprotein was found to be present in 2(4%)/50 HIV/HBV and 4(8%) HIV/HCV patients and the remaining 94 patients had normal levels of

the alpha fetoprotein. Sixty-eight percent (68%) out of the second 100 patients sampled had AST result of 4-12 iu/l, which is in the normal range (up to 12 iu/l is normal range). However, seven (7/50) 14% of the HIV mono infected patients and twenty five (25/50) 50% of the HIV/HBV/HCV positive patients had results above the normal range (13-25unit/l and 25-54unit/l) for HIV mono-infected patient and HIV//HBV/HCV respectively). In the case of ALT, 73/100(73%) of the patients were normal to the test, result ranged between 4-12iu/l (4-12iu/l is a normal range). Seven (7/50)14% HIV mono-infected patients had result ranging between 13-47iu/l, while 20/50(40%) HIV/HBV/HCV patients had readings between 25-54iu/l. The range of normal readings for ALP is between 73-203iu/l. Out of the second 100 patients screened, 92 of them were normal (between 73-203iu/l), 3/50(6%) of the HIV mono-infected patients and 5/50(10%) of the HIV/HBV/HCV positive patients had high ALP levels (between 150-260iu/l and 190-300iu/l for HIV mono-infected and HIV/HBV/HCV respectively). Alpha-fetoprotein was found to be present in 1(2%)/50 HIV mono-infected patients and 5(10%) HIV/HBV/HCV patients and the remaining 95 patients had normal levels of the alpha-fetoprotein.

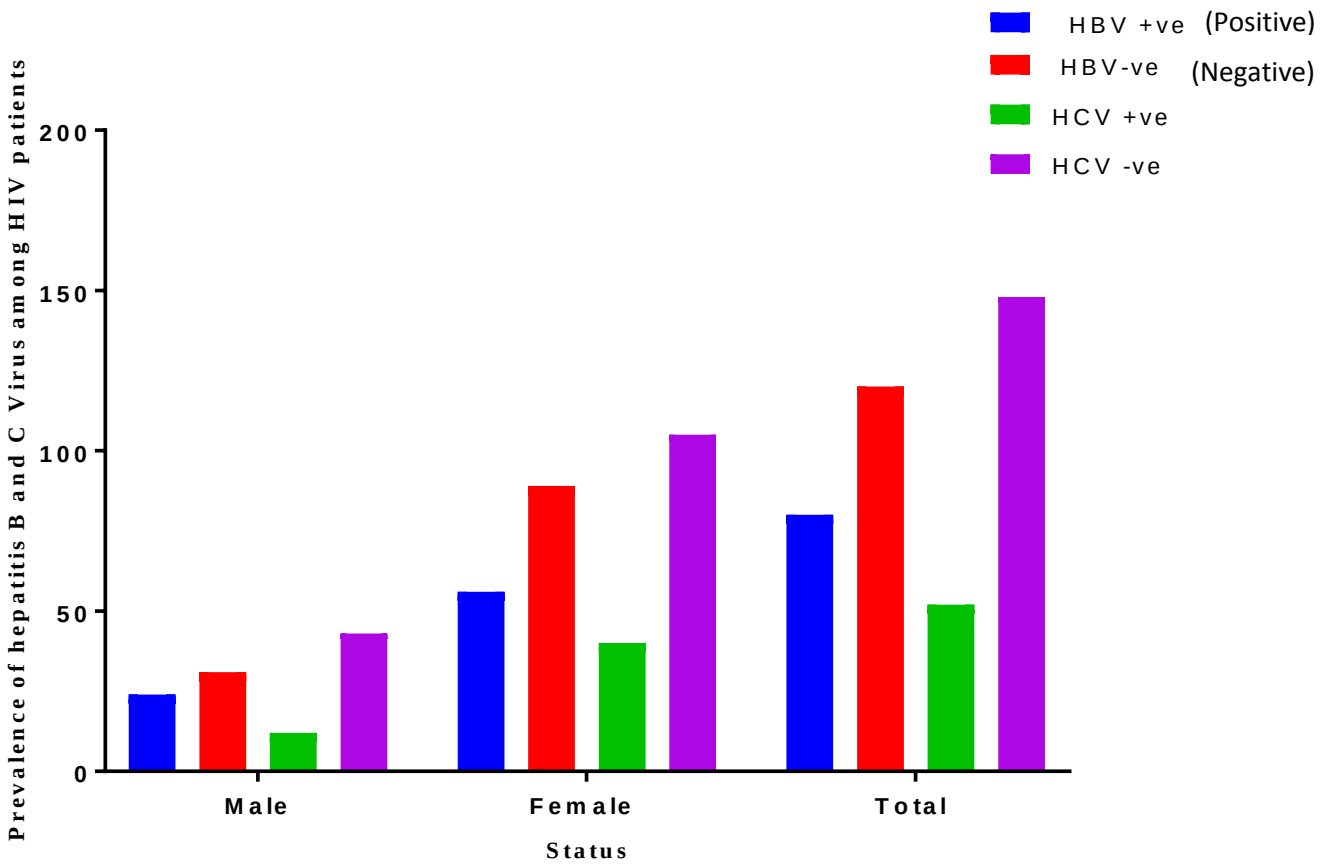
The mean serum levels; AST, ALT, ALP and AFP in HIV mono-infected study participants were 29 (14%) international units (IU), 27 IU(14%) and 150 IU (6%), 50mg/dl(2%) respectively. However, in HIV/HBV co-infected study participants, the levels of AST, ALT, ALP and AFP were non-significantly raised (34 IU, 31 IU, 165 IU and 180mg/dl respectively). Similarly, in HIV/HCV co-infected study participants, the mean levels of ALT, AST, ALP and AFP were 36IU, 29 IU,190 IU and 210mg/dl respectively. Statistically non-significant raised mean serum ALT, AST, ALP and AFP were found in HIV/HBV/HCV triple infected study participants (45iu/l, 38iu/l, 220iu/l and 216mg/dl in Table 23).

**Table 23**: **Liver enzyme levels in HIV/HBV, HIV/HCV and HIV/HBV/HCV co-infection**

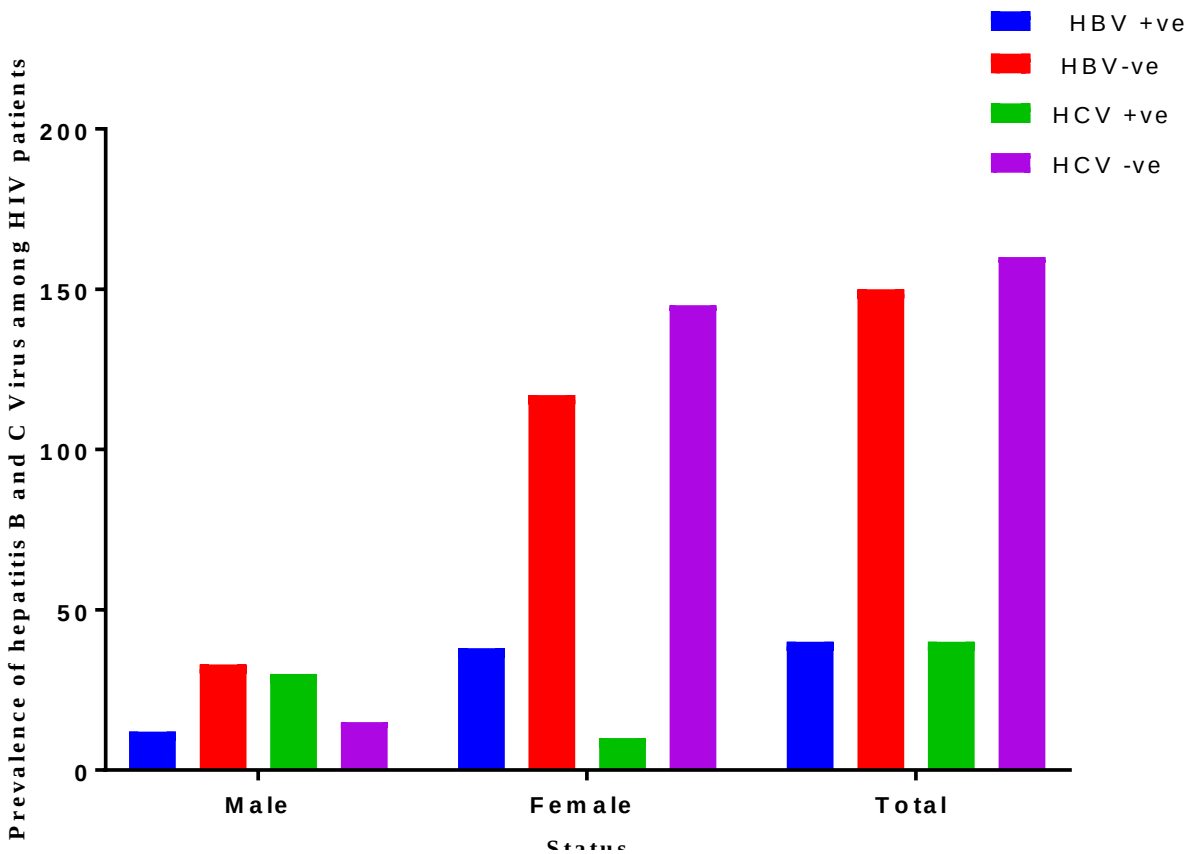
Mean liver enzyme levels and their association with HBV and HCV co-infection in Asokoro, Maitama and Wuse District Hospitals

| Liver biomarkers | HIV | HIV/HBV | HIV/HCV | HIV/HBV/HBCV |
|---|---|---|---|---|
| AST mean±SD | 29±21 | 34±20 | 36±18 | 45±9 |
| Normal Value | 0-12 | 0-12 | 0-12 | 0-12 |
| Abnormal High(%) | 14% | 30% | 40% | 50% |
| ALT mean±SD | 27±20 | 31±19 | 29±18 | 38±14 |
| Normal Value | 0-12 | 0-12 | 0-12 | 0-12 |
| Abnormal High (%) | 14% | 26% | 32% | 40% |
| ALP mean±SD | 150±110 | 165±95 | 190±130 | 220±190 |
| Normal Value | 73-203 | 73-203 | 73-203 | 73-203 |
| Abnormal High (%) | 6% | 12% | 8% | 10% |
| Alfa Fetoprotein | | | | |
| mean±SD | 50±12 | 180±15 | 210±15 | 216±20 |
| Normal Value | >50ng/ml | >50ng/ml | >50ng/ml | >50ng/ml |
| Abnormal High (%) | 2% | 4% | 8% | 10% |

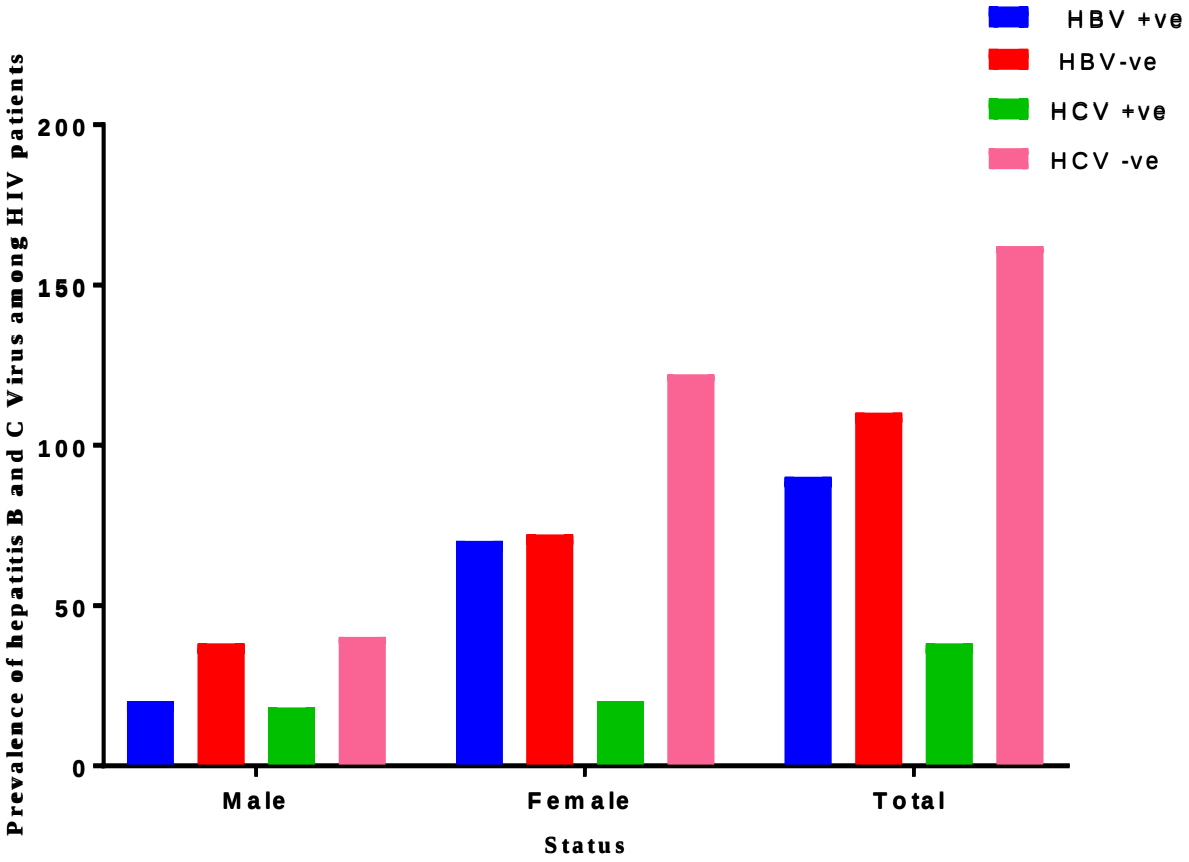
Fig 6-8 show the prevalence of hepatitis B and C virus among HIV patients attending Asokoro, Maitama, Wuse hospitals with respect to gender. Of the two hundred (200) subjects tested from each hospital, a total of 55(27.5%)/200 males and 145(72.5%)/200 females were screened for HBV and HCV in Asokoro hospital. For the detection of the presence of HBV, 24/200(12%) males were positive while 56/200(28%) females were positive. 12/200(6%) positive males, and 40/200(20%) positive females for HCV infection was recorded. In Maitama hospital, 12/200(6%) and 30/200(15%) males were positive to HBV and HCV while 38(19%) and 10(5%) were positive for HBV and HCV in females respectively. Wuse hospital on the other hand recorded 20/200(10%), 18/200(9%) for males, 70/200(35%), 20/200(10%) for females who were positive for HBV and HCV correspondingly. In the three district hospitals, the number of females with HBV and HCV was high as compared with their male counterparts, except in Maitama where the prevalence of HCV among male was higher than that of the females. Generally, female prevalence of both viral infection was higher in this study.




| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 1.107 | 6 | NA | P>0.05 |

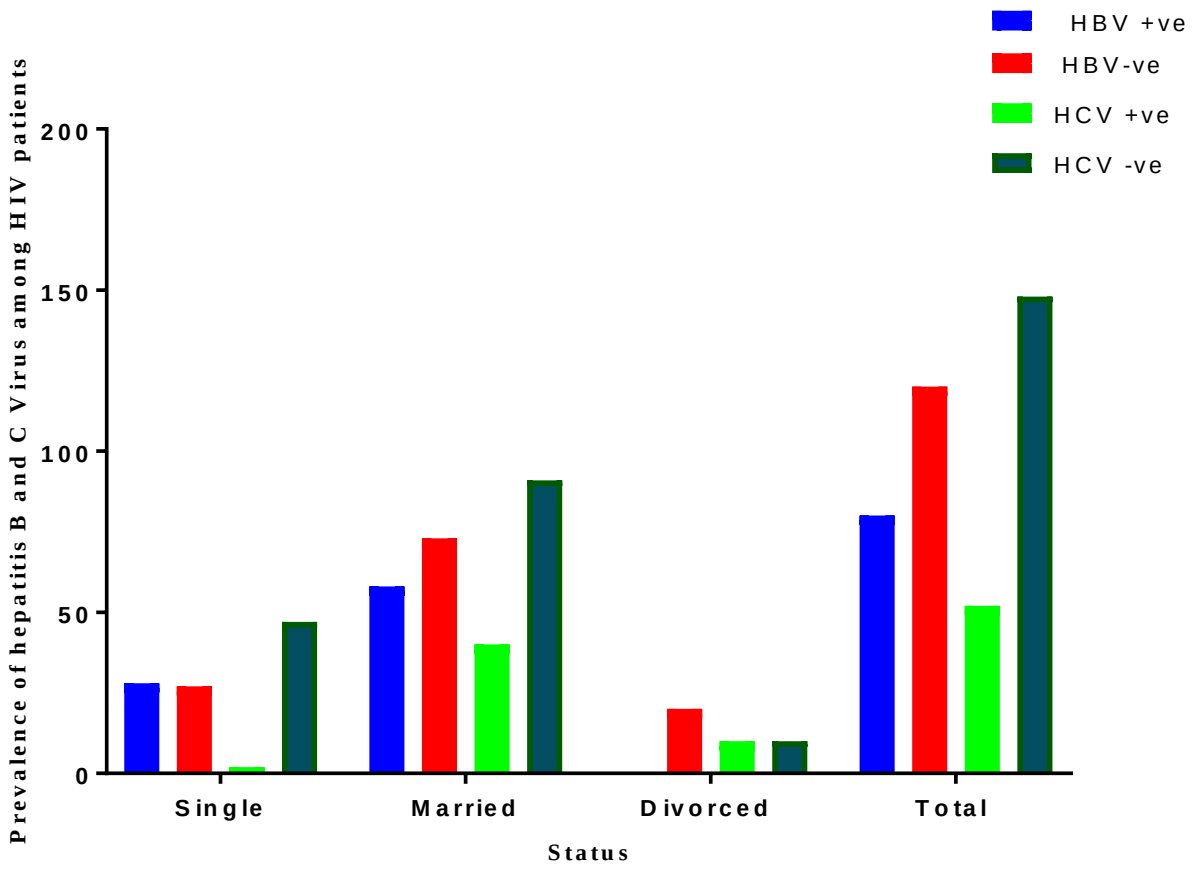



| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 79.12 | 6 | NA | P<0.05 |

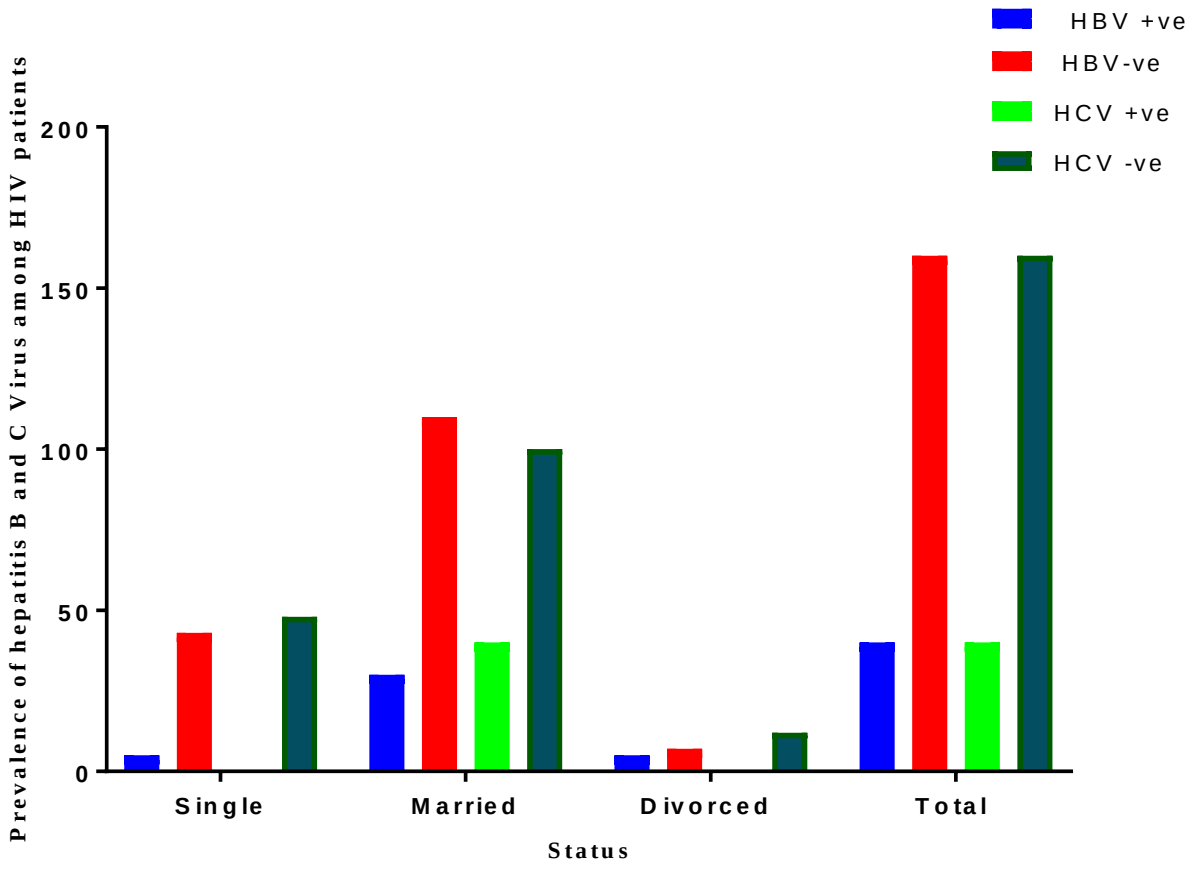



| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 11.34 | 6 | NA | P>0.05 |

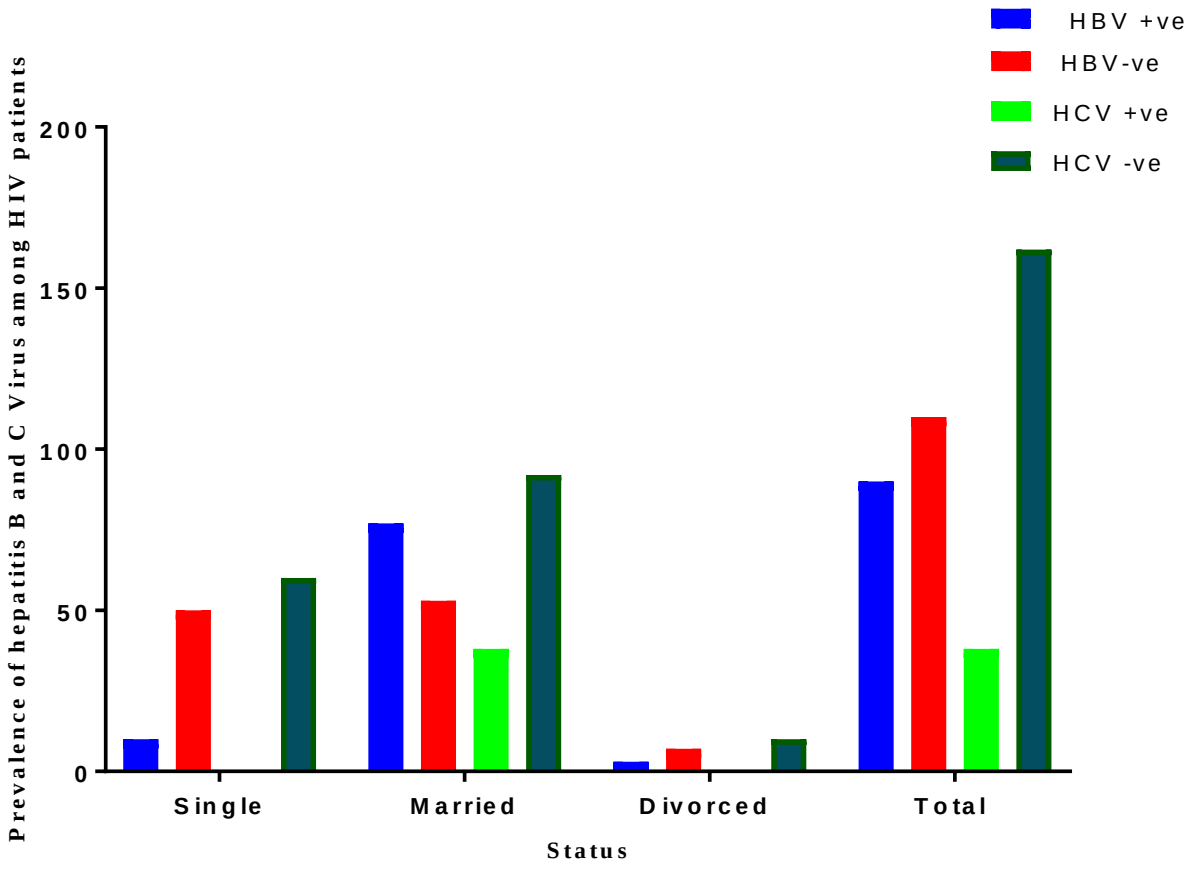
The prevalence of Hepatitis B and C virus was determined among HIV patients attending the three district hospitals based on their marital status (Fig 9-11.). The subjects were divided into singles, married and divorced; it was observed for Asokoro hospital that, twenty-eight (28) 14% of the two hundred (200) subjects studied were positive to HBV virus and two (2) 1% of them to HCV virus among the singles. The married however recorded fifty-eight (58) 29% and forty (40) 20% positives to HBV and HCV virus respectively. The divorced only recorded ten (10) 5% subjects to be positive to HCV. Maitama hospital recorded five (5) 2.5% singles to be positive to HBV but none was seen for HCV. Thirty (30) 15% subjects among the married were found to have HBV virus and forty (40) 20% positive individuals to HCV. The highest prevalence was recorded for Wuse hospital among the married, were subjects positive to HBV and HCV numbered up to seventy-seven (77) 38.5% and thirty-eight (38) 19% respectively, while singles and divorced numbered ten (10) 5% and three (3) 1.5%. In all the three hospitals, the married individuals were all seen to have the highest prevalence to HBV and HCV, tag on by the singles and then the divorced.




| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 36.17 | 9 | NA | P<0.05 |

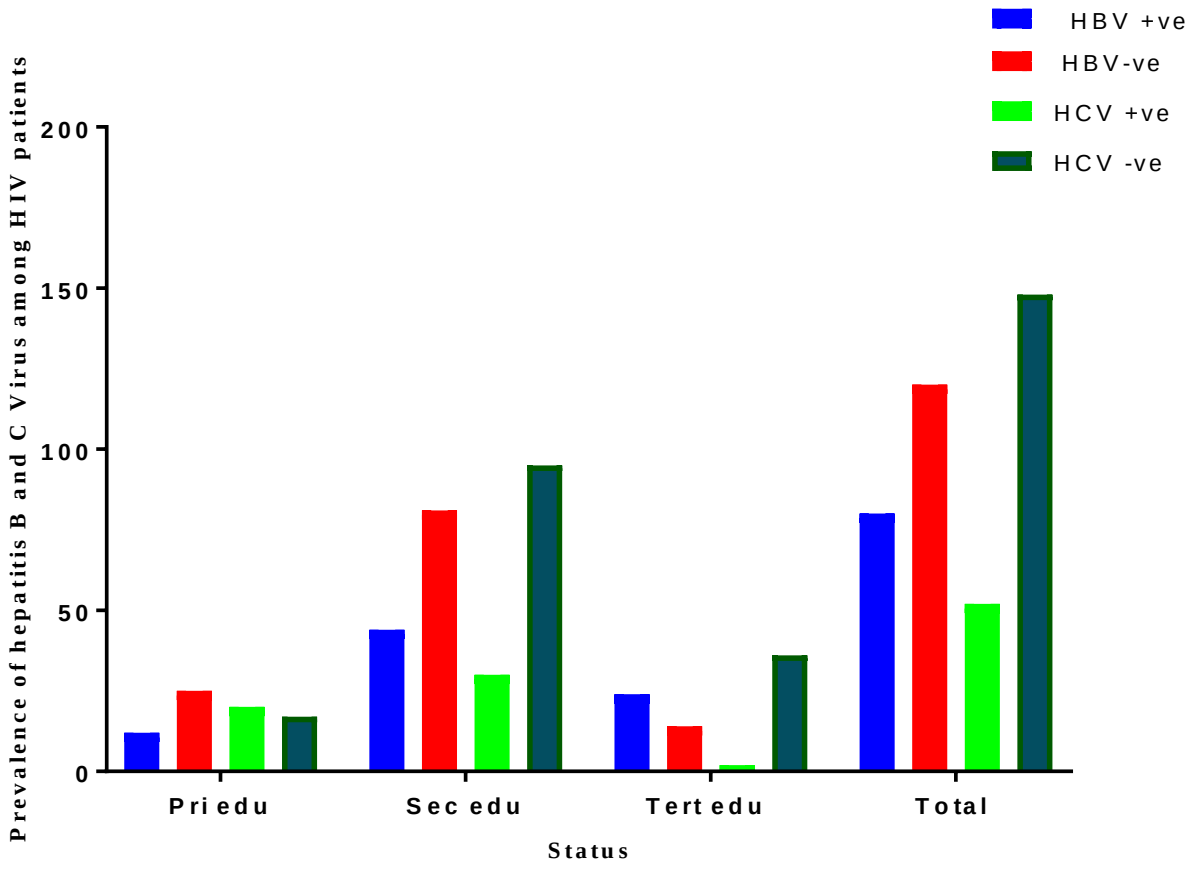



| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 27.88 | 9 | NA | P<0.05 |

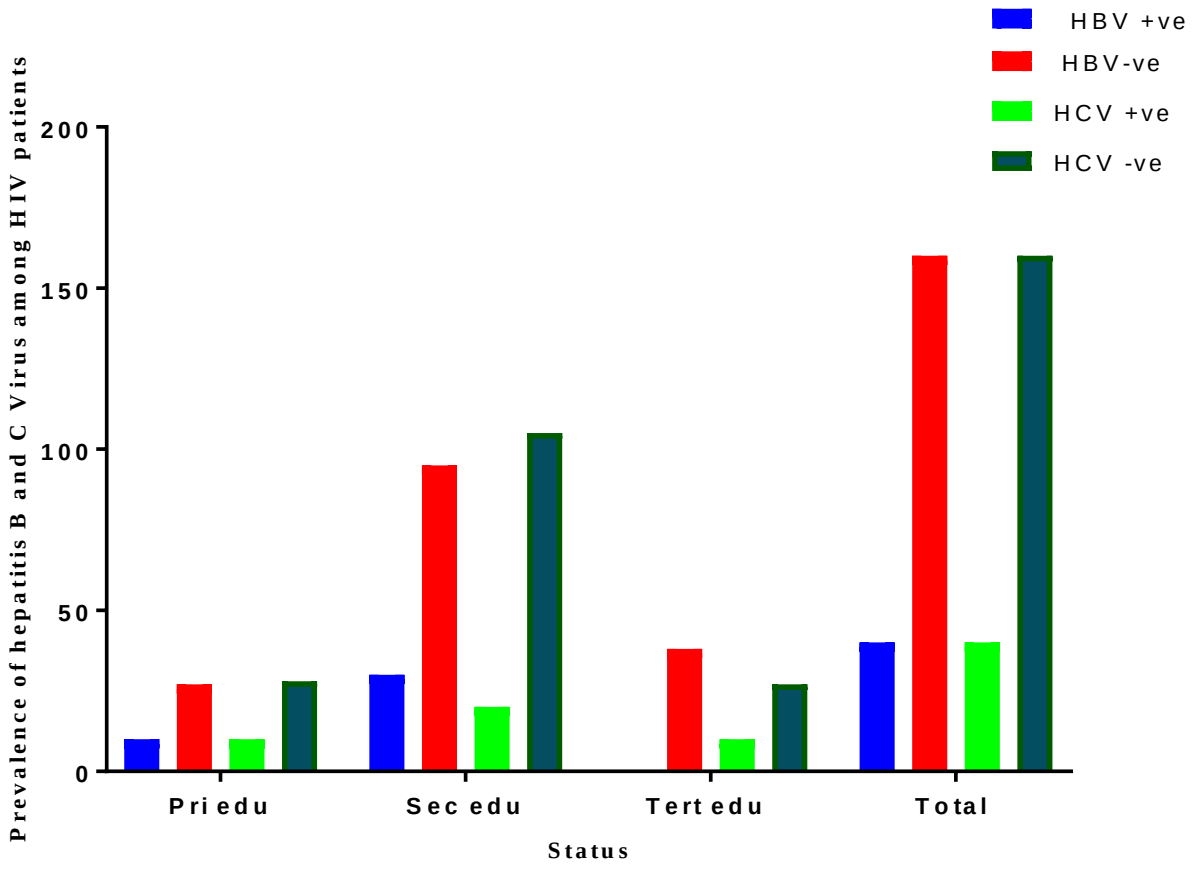



| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 56.27 | 9 | NA | P<0.05 |

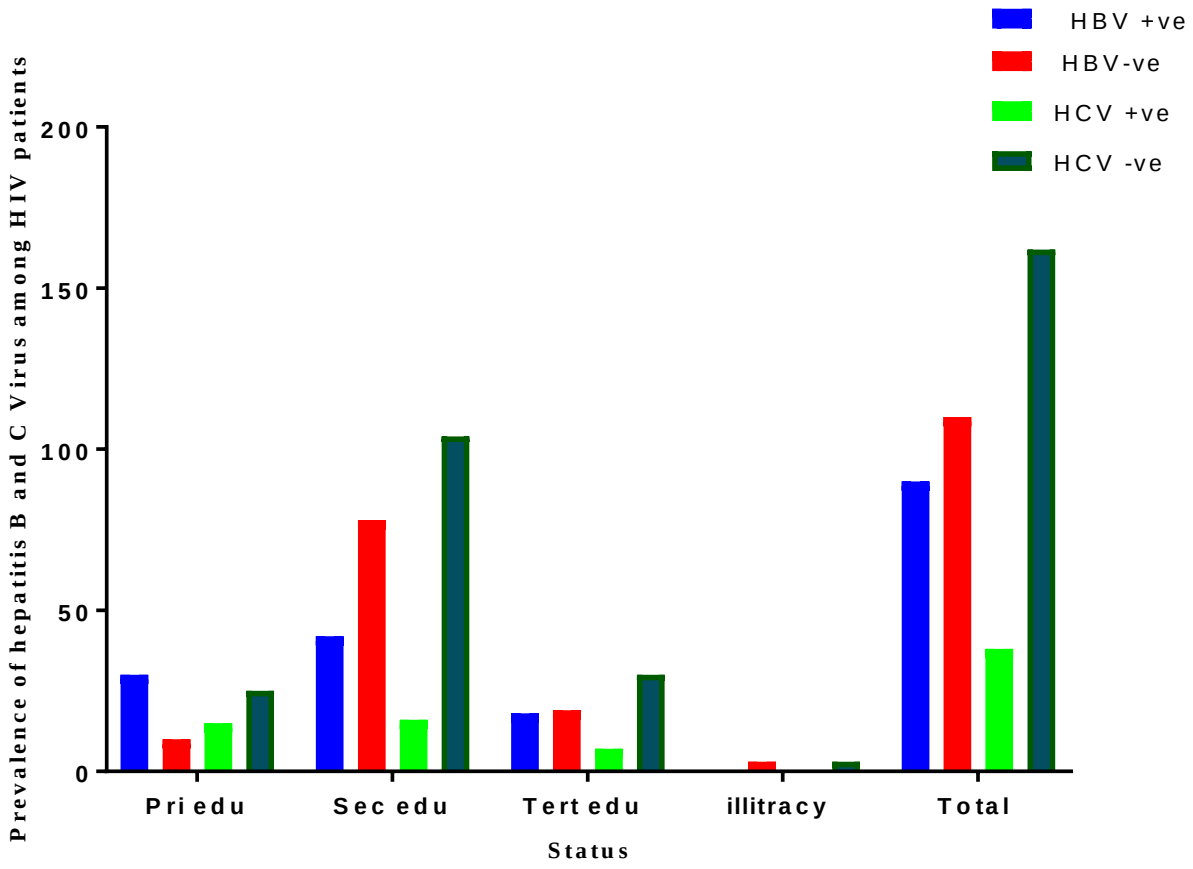
The effect of educational background on the prevalence of hepatitis B and C virus among HIV patients attending Asokoro, Maitama and Wuse was determined, and the outcome presented in Figure 12-14. Based on this parameter, individuals who were opportune to attain only secondary education had the highest prevalence to HBV of which, 44(22%)/200, 30(15%)/200, 42(20%)/200 of the 200 hundred subjects under study in Asokoro, Maitama and Wuse hospitals were positive and 30(15%)/200, 20(10%)/200, 16(8%)/200 were positive to HCV respectively. Subjects who attended tertiary education had 24(12%)/200, 0(0%)/200, 18(9%)/200 individuals positive to HBV and 2(1%)/200, 10(5%)/200, 7(3.5%)/200 to HCV while individuals in primary education category positive to HBV and HCV numbered 12(6%)/200, 10(5%)/200, 30(15%)/200 and 20(10%)/200, 15(7.5%)/200, 10(5%)/200 respectively. Wuse hospital recorded 3(1.5%)/200 illiterates but none of them were positive to either of the viruses under study, while Asokoro and Maitama hospitals recorded none. Generally, across the hospitals, patients with secondary education had the highest prevalence followed by individuals with primary education and then subjects with tertiary education.




| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 34.46 | 9 | NA | P<0.05 |

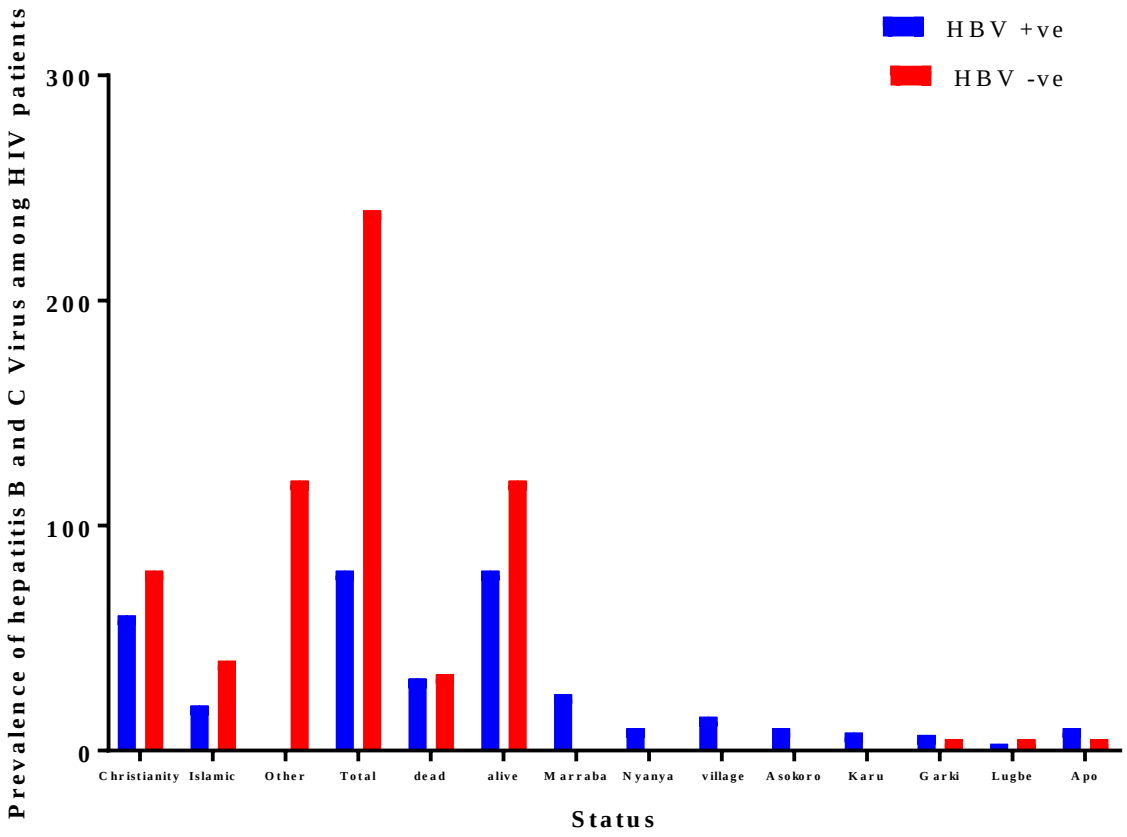



| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 15.37 | 9 | NA | P>0.05 |

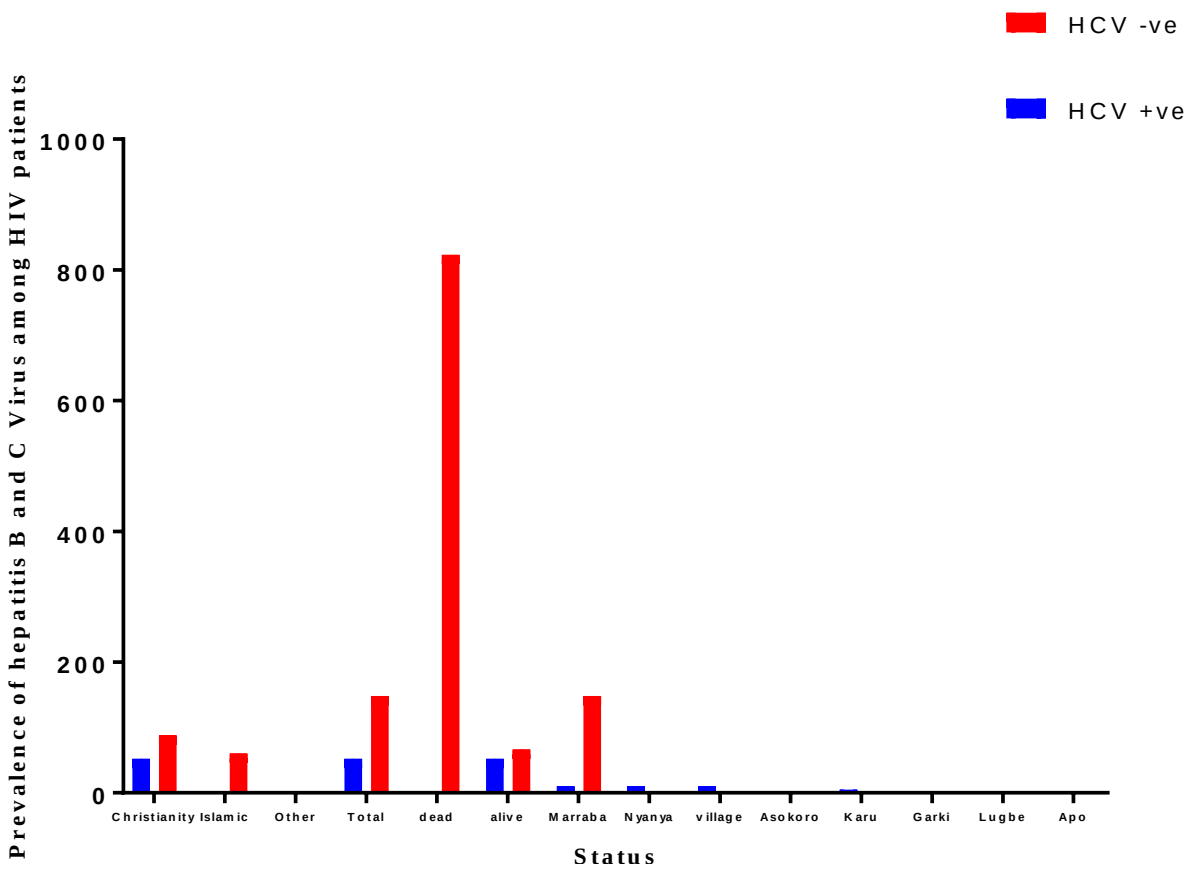


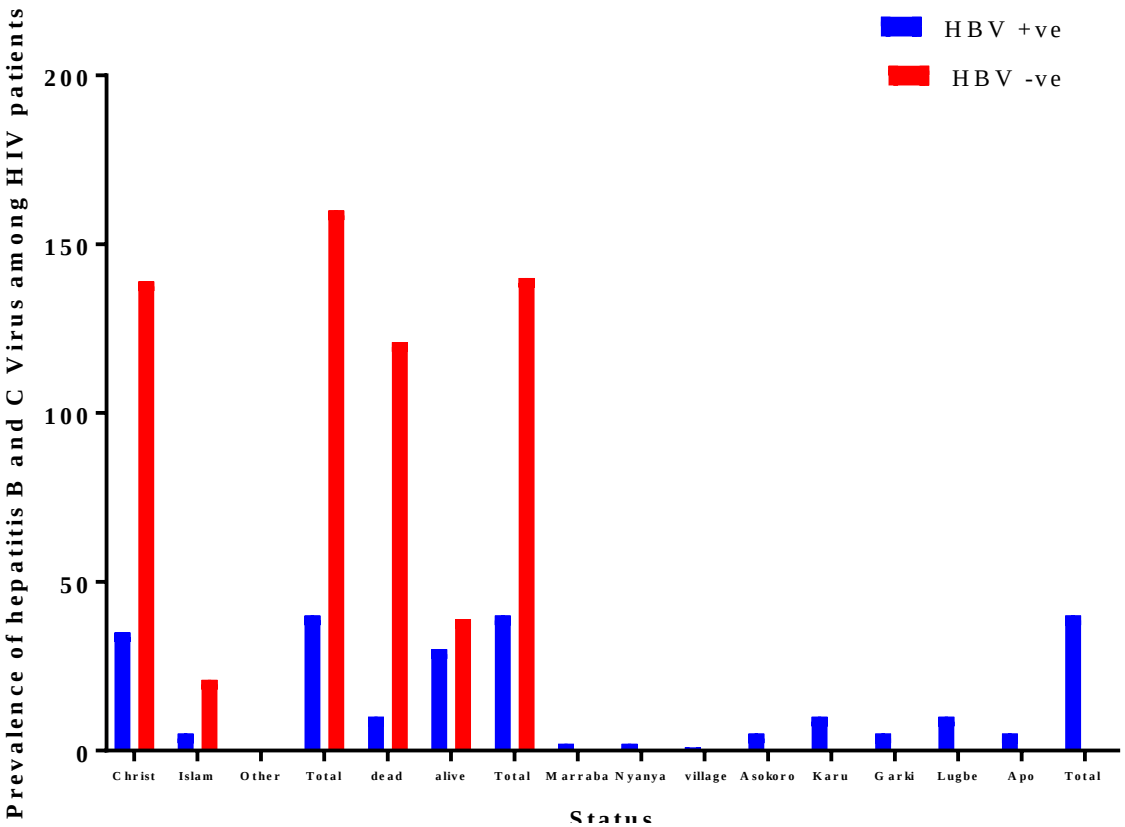

| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 34.15 | 12 | NA | P<0.05 |

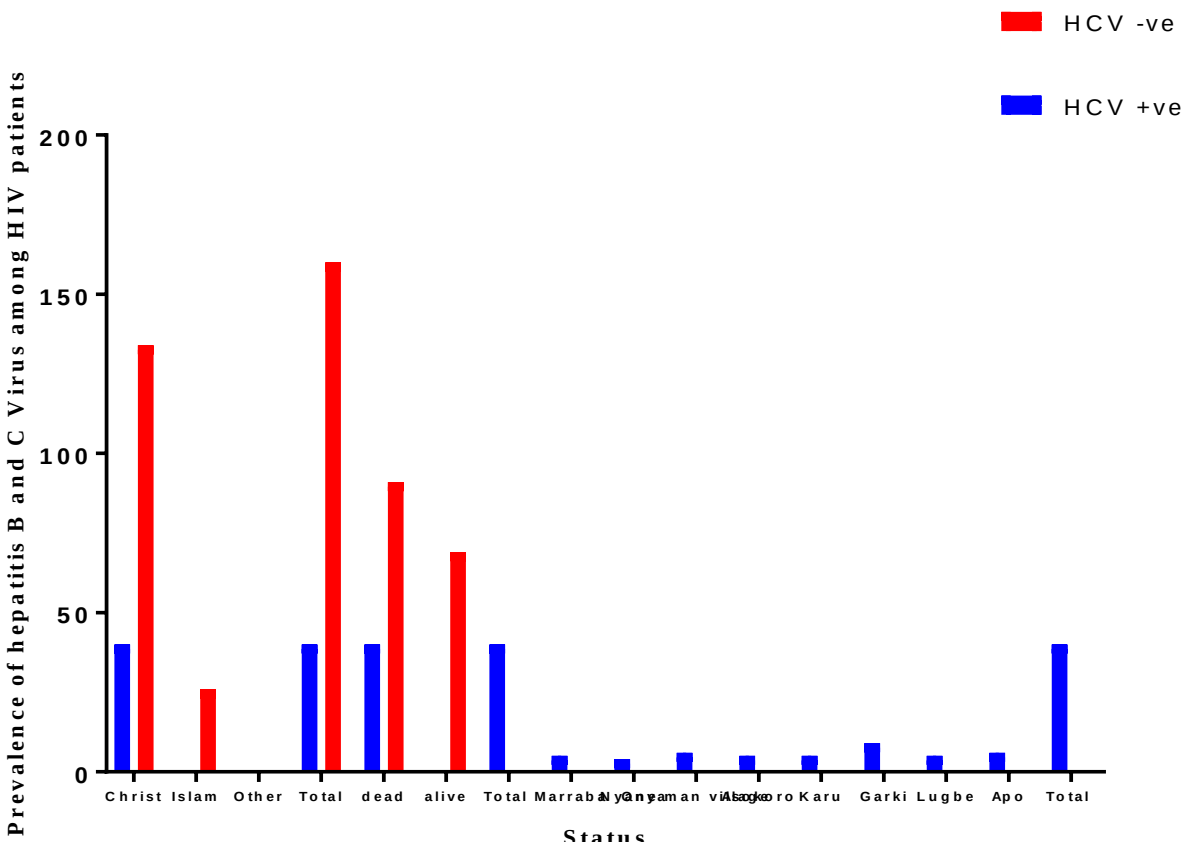
Based on parental origin/residence, the prevalence of hepatitis B and C among HIV patients attending Asokoro, Maitama and Wuse hospitals represented in Fig 15-17. The religion patients belonged to (Christianity, Islam, others), parental status (dead, alive) and residence of the patients (Marraba, Nyanya, one man's village, Asokoro, Karu, Garki, Lugbe, Apo) were put into consideration as risk factors for prevalence of the viruses. Out of the 200 subject, 60(30%)/200 Christians were positive to HBV and 52(52%)/200 positive to HCV in Asokoro hospital. Muslim patients positive to HBV and HCV numbered 20(10%)/200 and 0(0%)/200 accordingly. In Maitama hospital 35(17.5%)/200; 40(20%)/200 Christians and 5(2.5%)/200; 0/200 Muslims were recorded to be positive to HBV and HCV respectively. Meanwhile in Wuse hospital, Christian and Muslim patient positive to HBV and HCV numbered 35/200; 38(19%)/200 and 55(27.5%)/200; 0(0%)/200 respectively. Marraba, Karu, Lugbe and Asokoro had highest numbers of positive subjects to HBV across the three district hospitals. Nyanya, One man's village, Garki, Marraba had highest number of subjects positive to HCV across the hospitals. More of the patients who are Christian and other religion were seen to have higher prevalence to both viral infections.

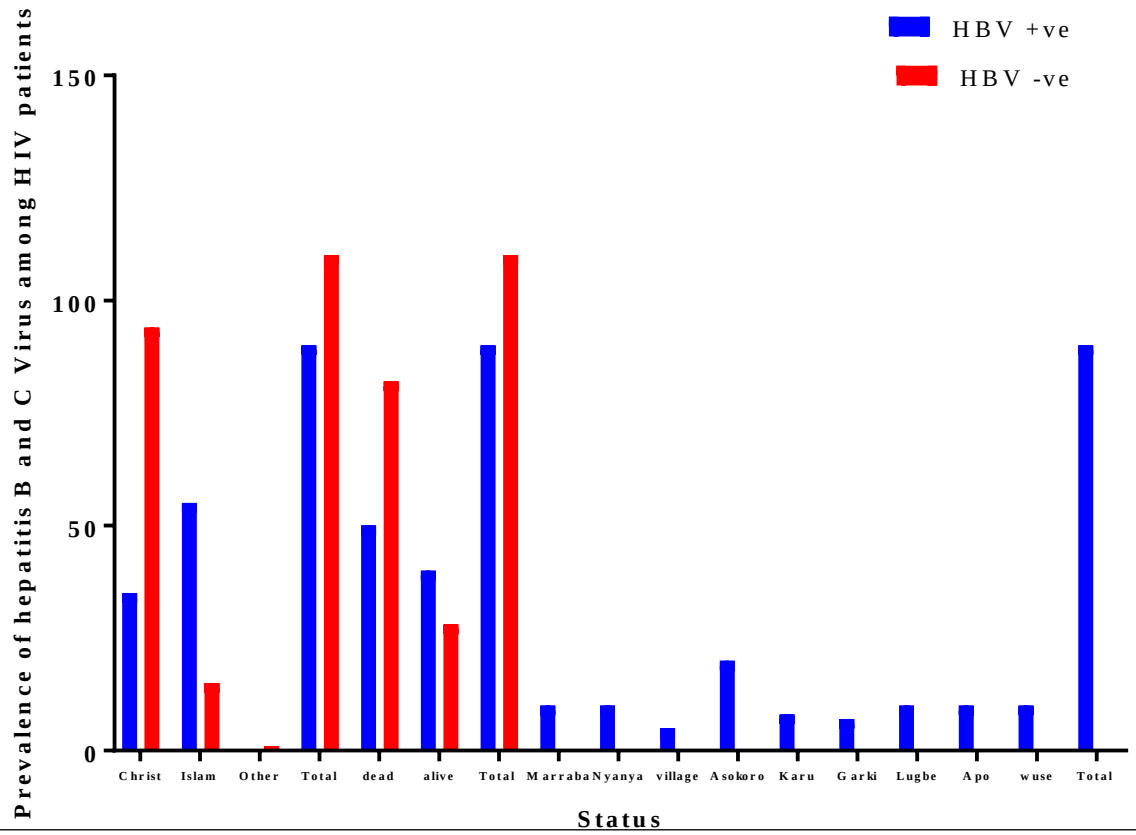



| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 223.7 | 13 | NA | P<0.05 |

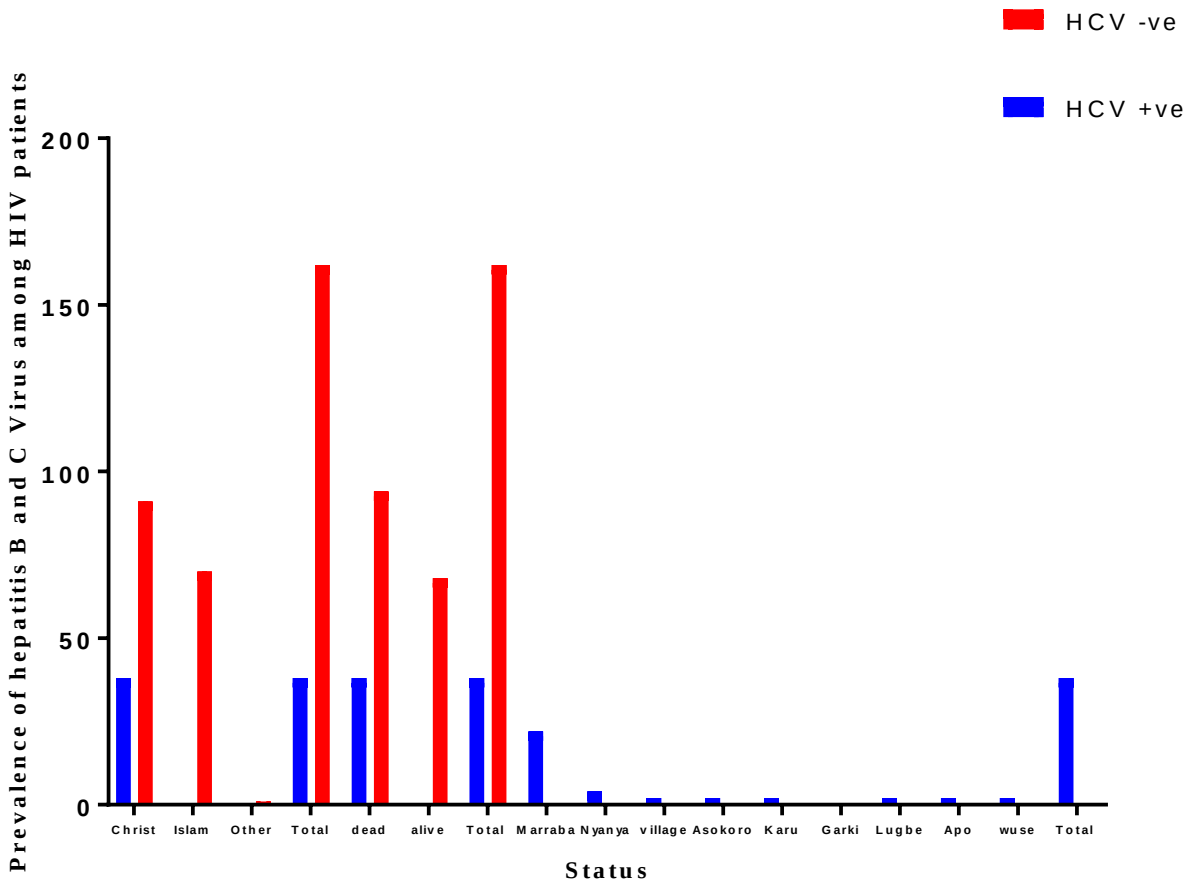

HCV -ve
HCV +ve
Prevalence of hepatitis B and C Virus among HIV patients
1000
800
600
400
200
0
Christianity
Islamic
Other
Total
dead
alive
Marraba
Nyanya
village
Asokoro
Karu
Garki
Lugbe
Apo
Status


HBV +ve
HBV -ve
Prevalence of hepatitis B and C Virus among HIV patients
200
150
100
50
0
Christ
Islam
Other
Total
dead
alive
Total
Marraba
Nyanya
village
Asokoro
Karu
Garki
Lugbe
Apo
Total
Status


HCV -ve
HCV +ve
Prevalence of hepatitis B and C Virus among HIV patients
200
150
100
50
0
Christ
Islam
Other
Total
dead
alive
Total
Marraba
Nyanya
Karaman
Karu
Garki
Lugbe
Apo
Total
Status

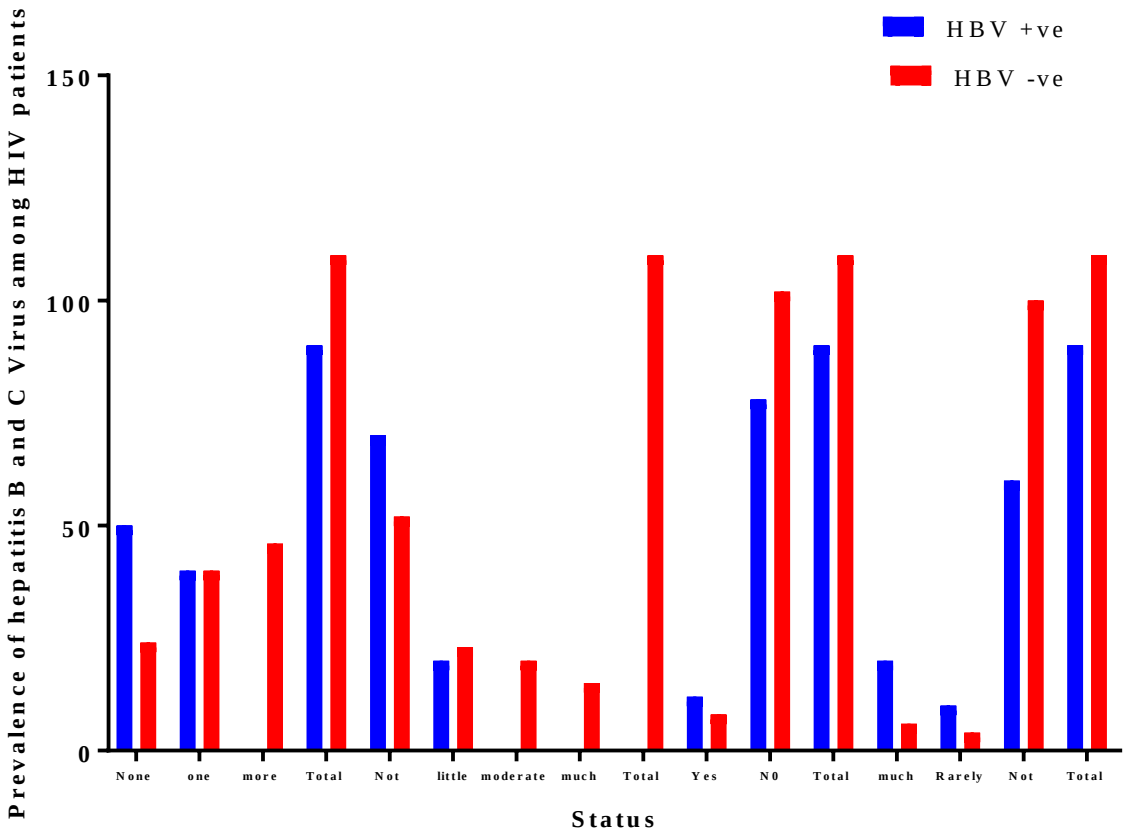



| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 197.1 | 15 | NA | P<0.05 |

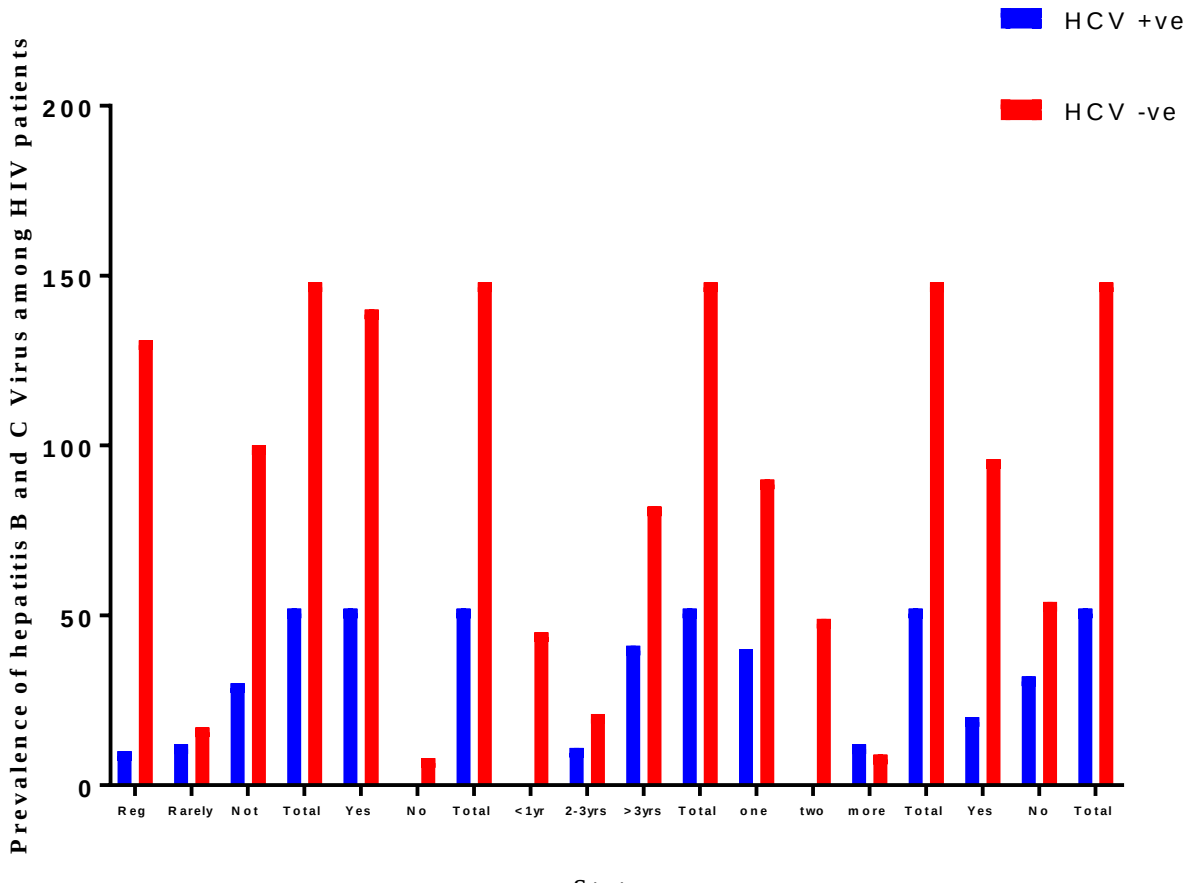

HCV -ve
HCV +ve
Prevalence of hepatitis B and C Virus among HIV patients
200
150
100
50
0
Christ
Islam
Other
Total
dead
alive
Total
Marraba
Nyanya
village
Asokoro
Karu
Garki
Lugbe
Apo
wuse
Total
Status

Prevalence of hepatitis B and C virus among HIV patients attending the three district hospitals in respect to behavioral life style was monitored. The behavioral life style studied include; ear piercing, alcohol intake, tattooing, tobacco, visit to barber, sexual experience, number of sex partners, use of condom. Considering ear piercing, 50(25%)/200 individuals without pierced ear were positive to HBV as compared to those with one or more than one piercing on their ears in Asokoro hospital which was 40(20%)/200 and 0(0%)/200 respectively. Those without ear piercing, those with one ear piercing on both ears and those with more than one ear piercing on both ears positive to HCV numbers 10(5%)/200 and 20(10%)/200 respectively. Those with more than one piercing on both ears positive to HCV number 22(11%)/200. Patients without ear piercing in Maitama hospital positive to HBV were recorded to be 16(8%)/200 as compared to none positive for HCV. Patients with one piercing on both ears positive to HBV were recorded to be 19(9.5%)/200 as against 40(20%)/200 positive to HCV in Maitama hospital while in Wuse hospital, 48(12%)/200 and 30(15%)/200 patients with one piercing on both ears were positive to HBV and HCV respectively. Patients with more than one ear piercing on both ears positive to HBV in Maitama hospital were recorded to be 5(2.5%)/200 as against none positive for HCV, while in Wuse hospital, 12(6%)/200 as against none was recorded. The proportion of individual not taking alcohol as compared to those taking alcohol in Wuse hospital was high. Those not taking alcohol at all in Wuse hospital numbers 50(25%)/200 and 22(11%)/200 to HBV and HCV, but even a higher number of the patients in Asokoro hospital was to be positive to HBV numbering 70(35%)/200 and 40(20%)/200 to HCV. In Maitama hospital, those not taking alcohol at all have the least population of 18(9%)/200 and 10(5%)/200 for HBV and HCV respectively. Fewer patients having tattoo, smoking and visiting barbers were positive to HBV and HCV as compared to

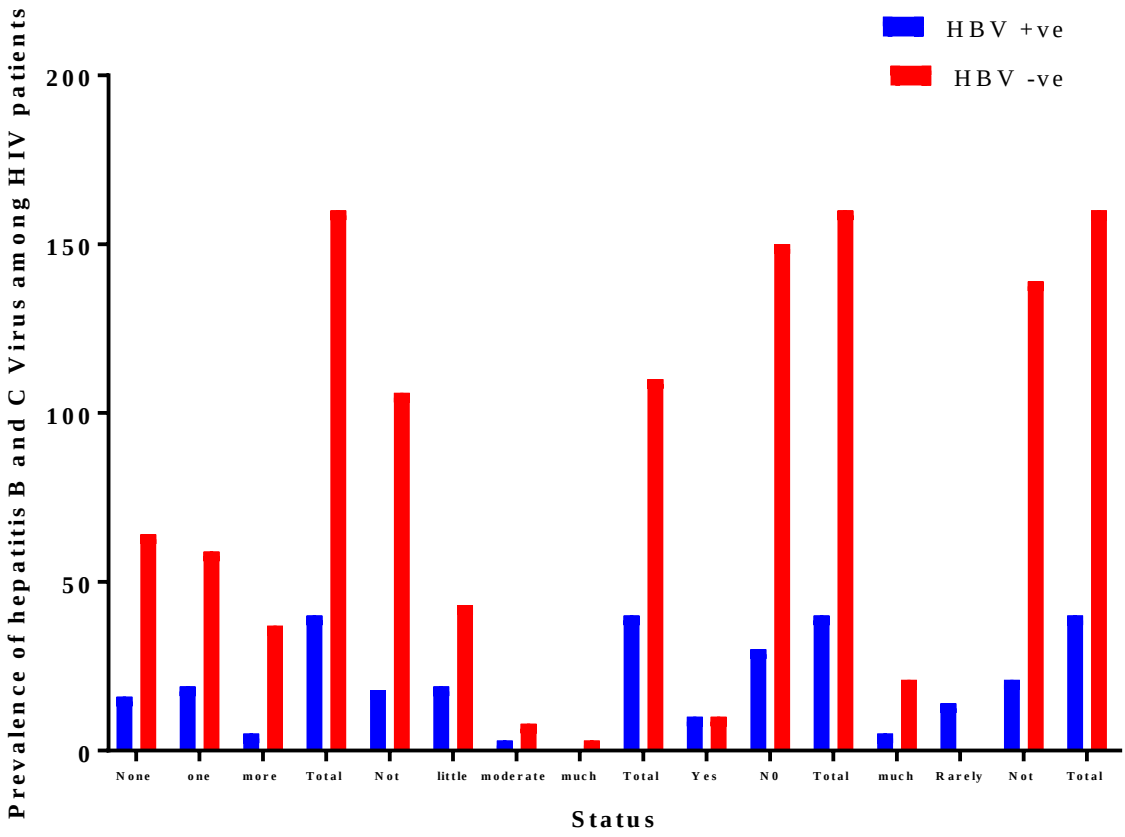
the number of individuals without tattoo, not smoking or visit barbers shop across the hospitals. Also, patients in the three district hospitals have all had sexual experience and a large proportion of them had their first intercourse more than three years as represented in Fig 18-20. At Asokoro hospital, 70(35%)/200 of patients positive to HBV have more than one sex partner and 40(20%)/200 positive to HCV had one sex partner. The use of condom was low as number of HBV and HCV positive individuals who do not use condoms numbered 80(40%)/200 and 32(16%)/200 respectively at Asokoro hospital. The number of individuals positive to HBV and HCV at Maitama and Wuse hospitals were 25(12.5%)/200; 20(10%)/200 and 45(22.5%)/200; 30(15%)/200 respectively. Putting into consideration the various lifestyles of the patients, sexual experience, under use of condom and multiple sex partners were seen as predisposing factors that can increase the prevalence of the viral infection.



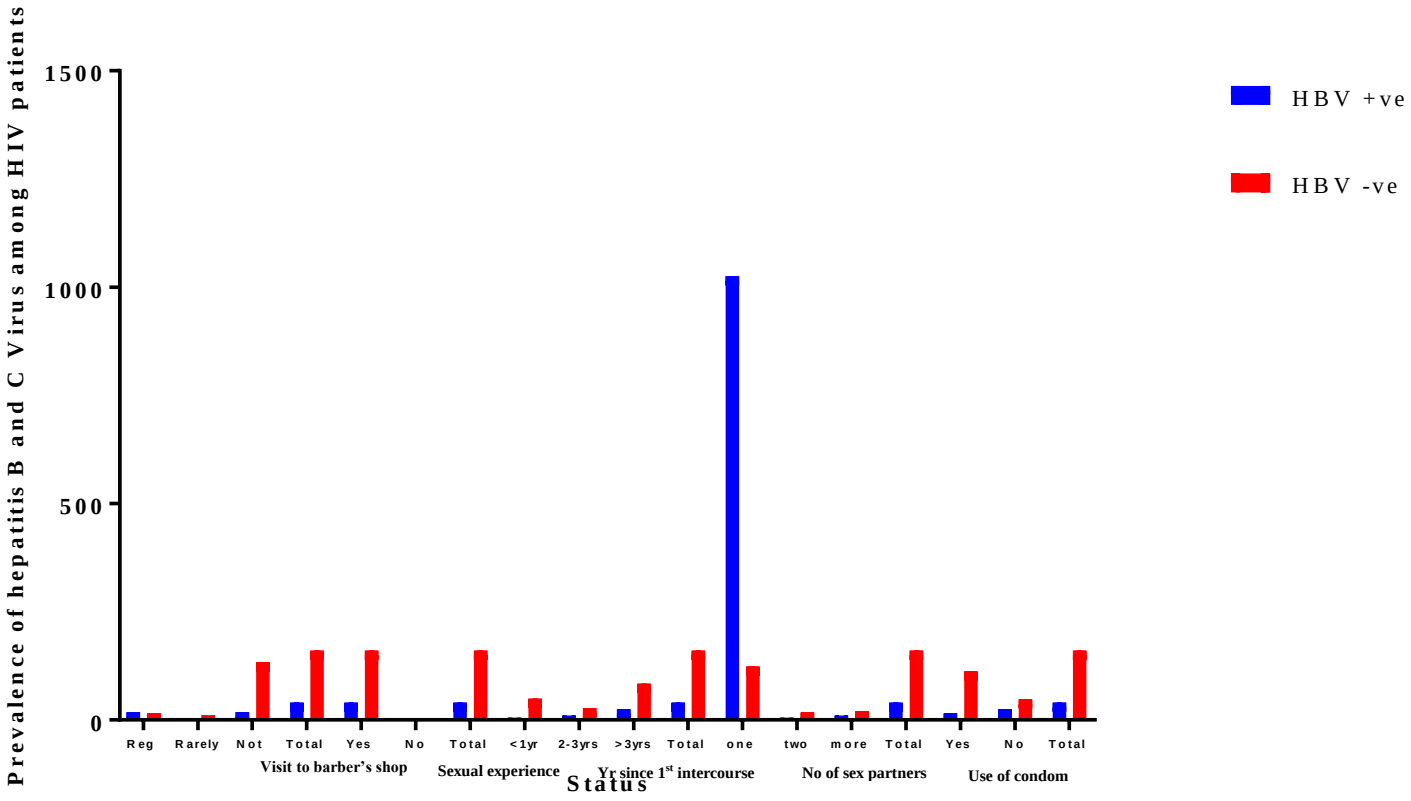

| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 197.1 | 15 | NA | P<0.05 |

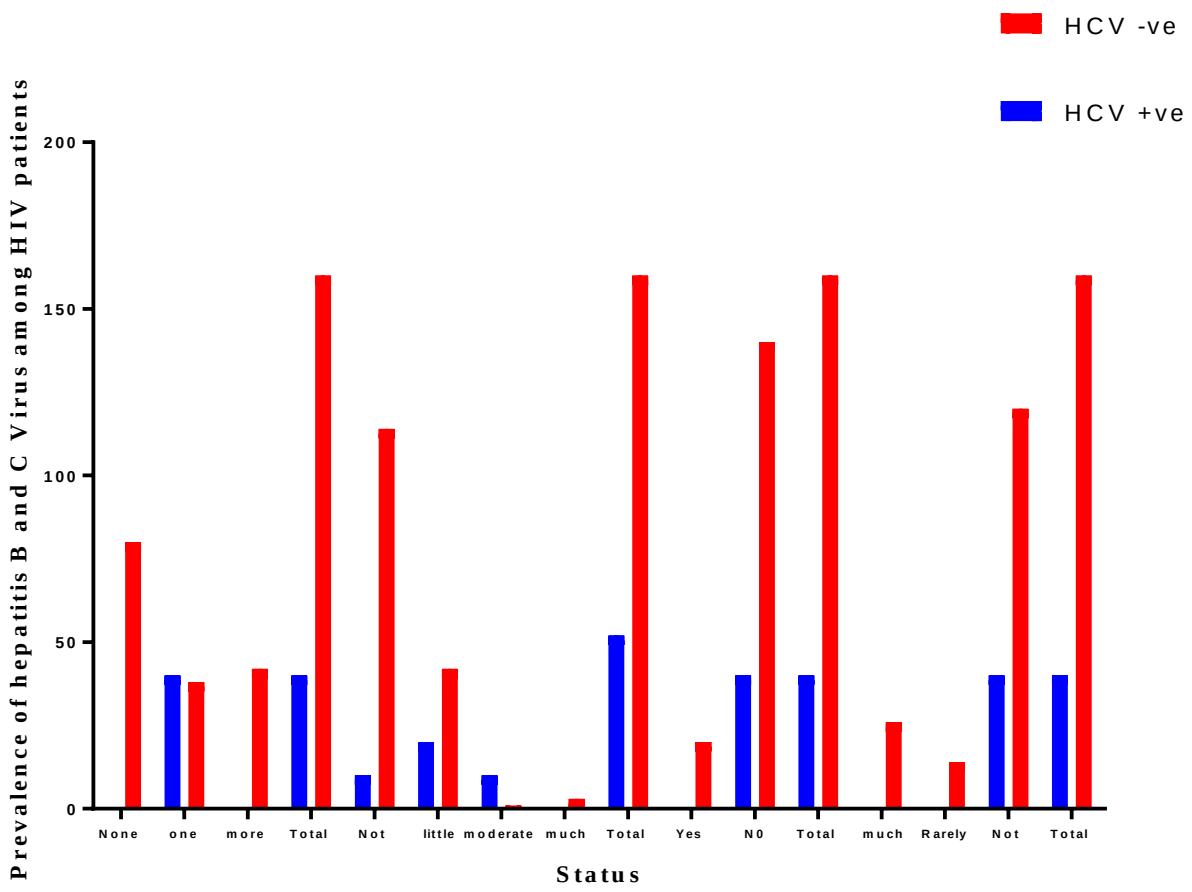



| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 94.58 | 17 | NA | P<0.05 |

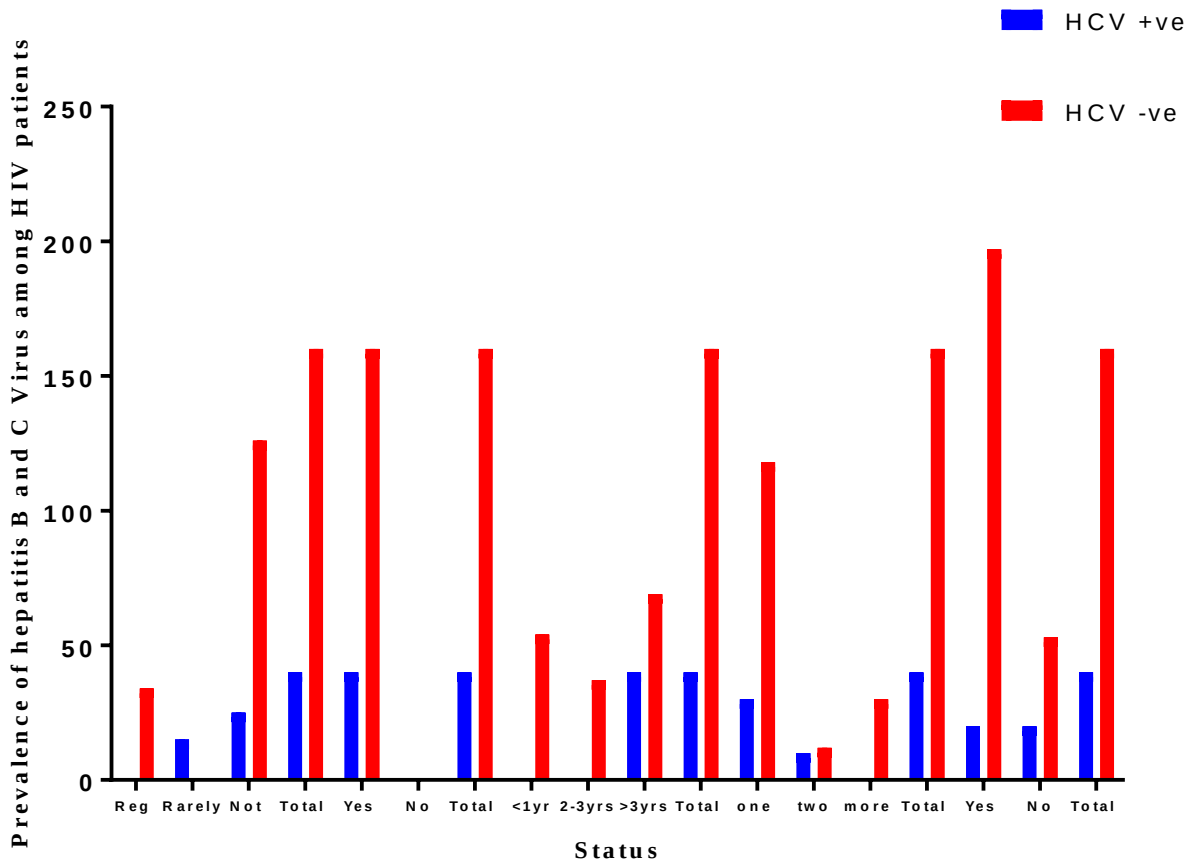



| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 85.44 | 15 | NA | P<0.05 |


Prevalence of hepatitis B and C Virus among HIV patients
1500
1000
500
0
HBV +ve
HBV -ve
Reg
Rarely
Not
Total
Yes
No
Total
<1yr
2-3yrs
>3yrs
Total
one
two
more
Total
Yes
No
Total
Visit to barber's shop
Sexual experience
Yr since 1st intercourse
No of sex partners
Use of condom
Status




| $X^2$ | df | Asymp. Significance | P-value |
|---|---|---|---|
| 147.5 | 15 | NA | P<0.05 |




**KEY**

-ve = Negative

+ve = Positiv e

NA = Not applicable

None = Patients without ear piercing on both ears

One = Patients with one ear piercing on both ears

More = More than one ear piercing on both ears

Df = Difference

# HIV PHYLOGENETIC TREE

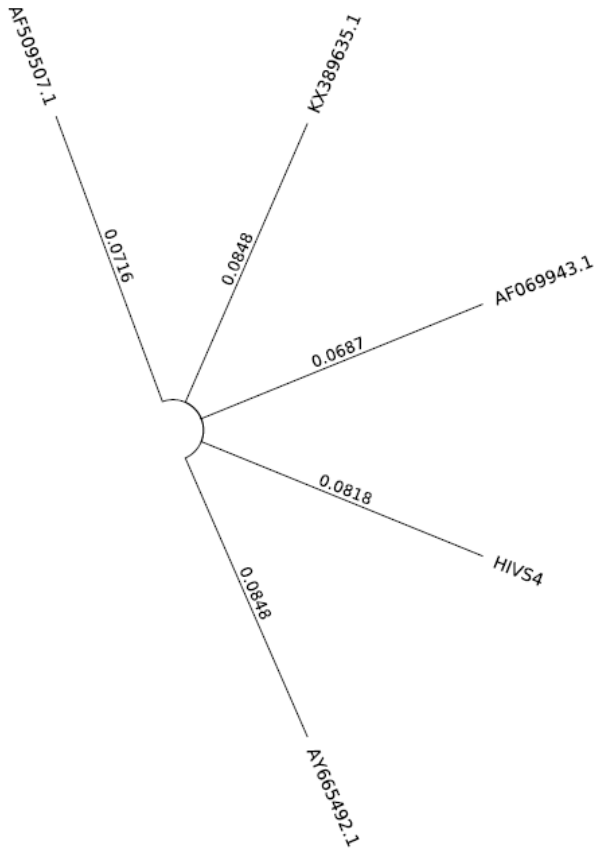



**Fig 21: Phylogenetic tree showing evolutionary relationship between HIVS4 and other HIV viruses**

AF509507.1 HIV-1 isolate 00CMNYU1261 from Cameroon

AF069943.1 HIV-1 isolate NG1929 from Nigeria

KX389635.1 HIV-1 isolate 09NG010105 from Nigeria

AY665492.1 HIV-1 strain 31149 from Democratic Republic of Congo

The template HIVs4 is closely related to AF069943.1 HIV-1 isolate with 2,538 bp genomic DNA obtained in 1995 from a hospitalized individual from Maiduguri, Borno state, Nigeria.

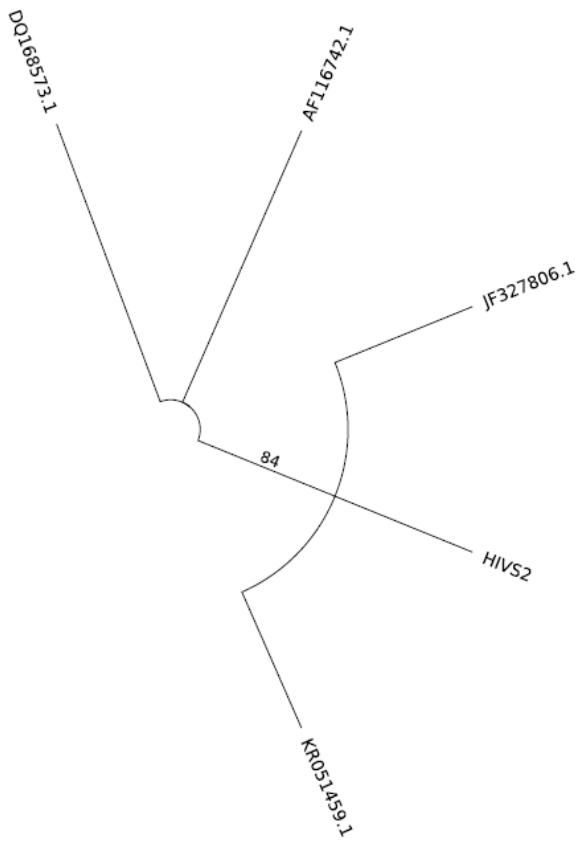



**Fig 22: Phylogenetic tree showing evolutionary relationship between HIVS2 and other HIV viruses**

JF327806.1 HIV-1 isolate MFU54_D1_3 from Spain

DQ168573.1 HIV-1 isolate 01NGPL0567 from Nigeria

AF116742.1 HIV-1 isolate TWG1.4 from Taiwan

KR051459.1 HIV-1 isolate BS51_B6_26082011 from Cameroon

The isolate is closely related to HIV-1 isolate KR051459.1GI with 2,583bp genomc DNA, subtype G, isolate B obtained from Homo sapiens in Cameroon in 2007. Gene cds,vpu protein, rev protein ant tat protein particles partial cds

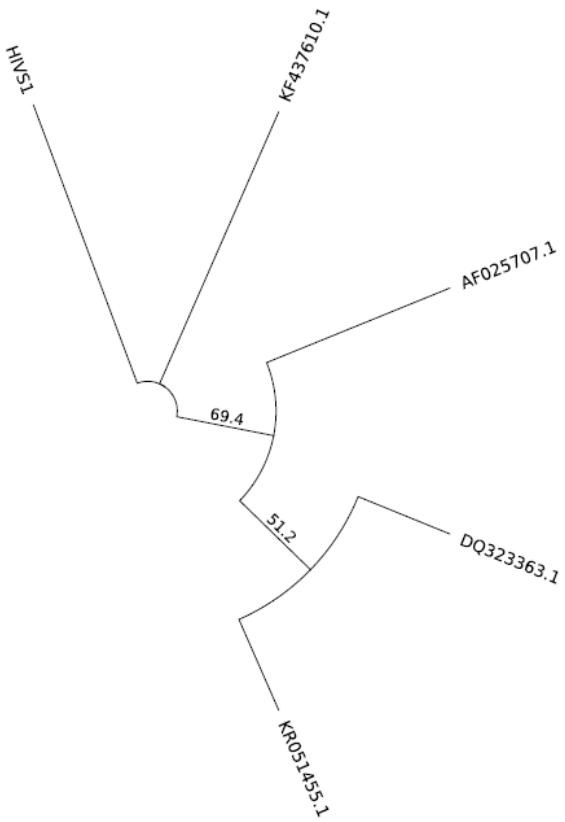



**Fig 23: Phylogenetic tree showing evolutionary relationship between HIVS1 and other HIV viruses**

DQ323363.1 HIV-1 isolate P3599 from Senegal

KF437610.1 HIV-1 isolate NGIB.04__009 from Nigeria

AF025707.1 HIV-1 isolate LE24 from Lebanon1Z

KR051455.1 HIV-1 isolate BS48_F5_23062011 from Cameroon

The isolate HIVs1 is closely related to HIV-1 isolate KF437610.1GI, subtype G, Group M with 350 bp genomic DNA. Obtained from blood source in Nigeria in 2004.

## HBV PHYLOGENETIC TREE

The obtained sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The sequence of HB1 in figure 9 showed a percentage similarity to other species at 99%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the isolates within the Hepatitis viruses and revealed a closely relatedness to Hepatitis B Virus isolate SDAC_059 (gb: KF170780.1) from Sudan obtained in 2008 than other Hepatitis viruses. It is serotype ayw4 and the gene is Hepatitis genotype E. It has 3,200 bp genomic DNA (circular).

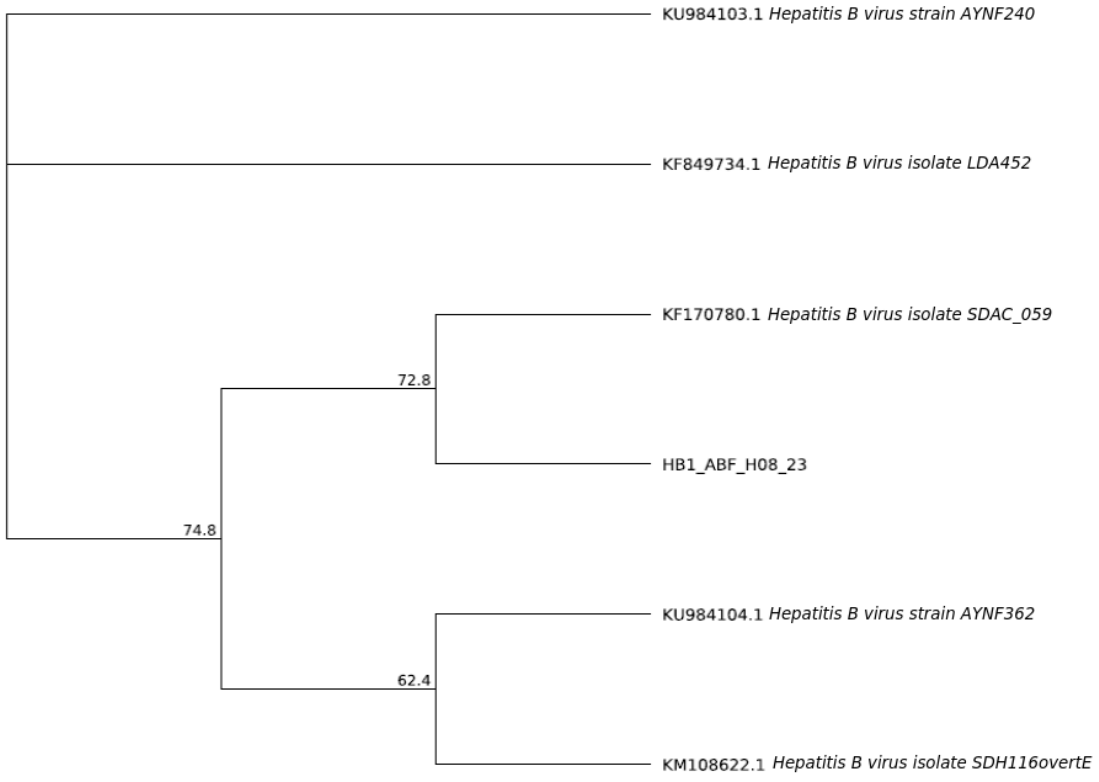



**Fig 24: Phylogenetic tree showing relationship between HB1 and other Hepatitis viruses.**

The sequence of HB3 showed a percentage similarity to other species at 99%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the isolates within the Hepatitis viruses and revealed a closely relatedness to Hepatitis B virus strain SDAC_059 (gb: KF170780.1) than other Hepatitis viruses(Fig.10).

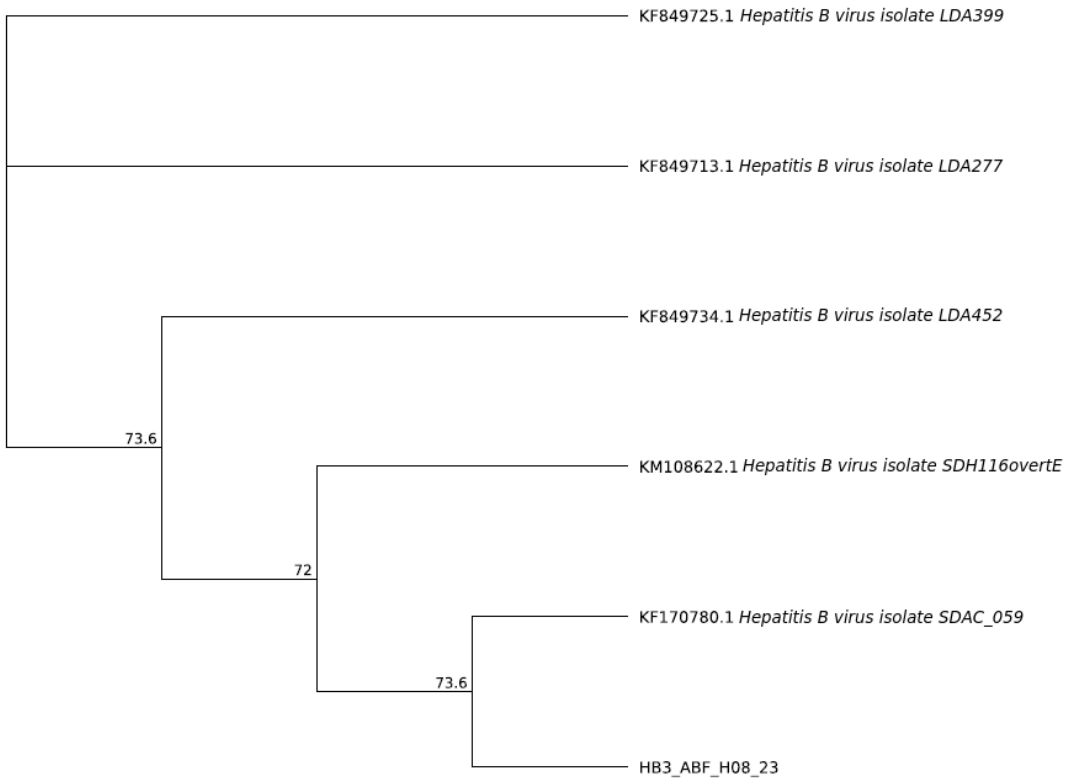



**Fig 25: Phylogenetic tree showing relationship between HB3 and other Hepatitis viruses.**

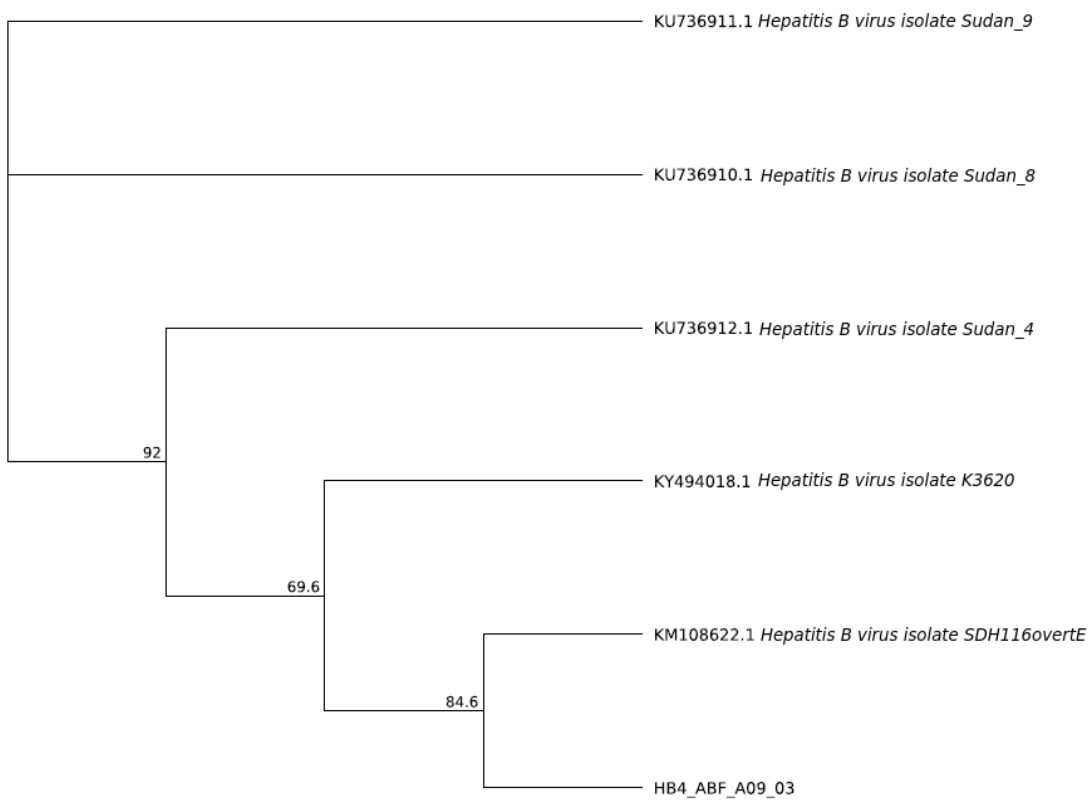



**Fig 26: Phylogenetic tree showing relationship between HB4 and other Hepatitis viruses.**

Sample in figure 4.11 is closely related to Hepatitis B virus isolate SDH116overtE, partial genome3,212 bp genomic DNA (circular), originated from Homosapiens in Sudan in 2011. It is hepatitis genotype E.

## HCV PHYLOGENETIC TREE

HCV Phylogenetic tree for the templates submitted failed probably due to;

- The template concentration
- The viral load

## LIST OF PLATES

### Molecular Screening of Samples

A total of 600 known HIV samples, were subjected to PCR-based detection for viral RNA, all the 600 samples were proved as HIV positive, with a band size of approx. 300 bp in PCR, as shown in plate 1.

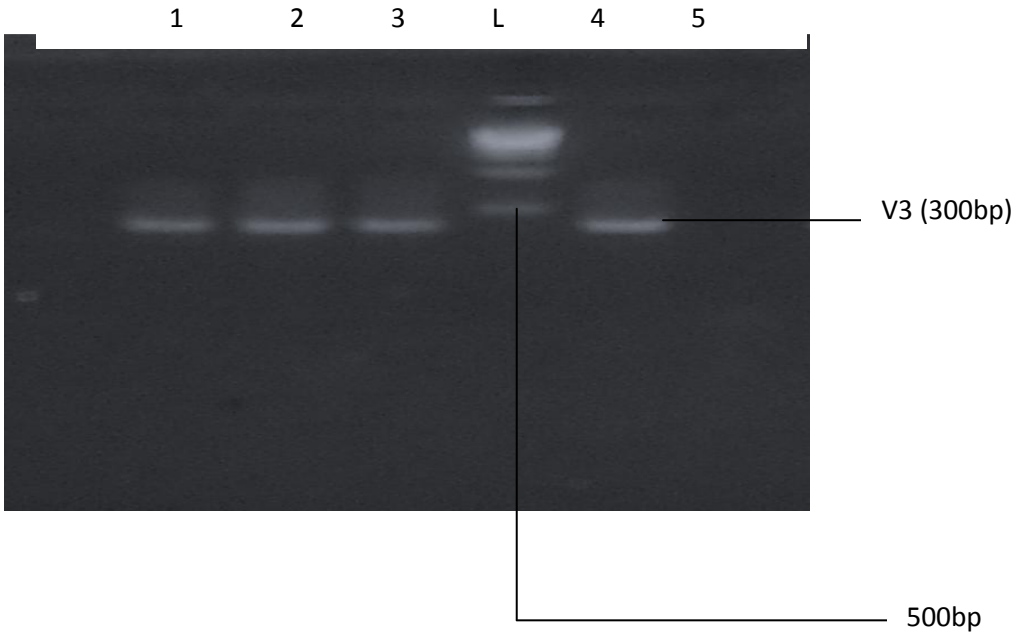



**Plate 1:Agarose gel electrophoresis showing the amplified HIV V3 gene bands Lanes 1-4 represents the V3 bands Lane L represent the 1kb ladder while lane 5 shows no band.**

A total of 100 HIV samples and 100 non HIV samples positive to Hepatitis B infection, were randomly selected and were subjected to PCR-based detection for viral RNA, out of which 40(40%) samples from HIV positive patients and 20(20%) non HIV volunteers were proved as HBV positive, with a band size of approx. 600 bp in PCR, as shown in figure 2.

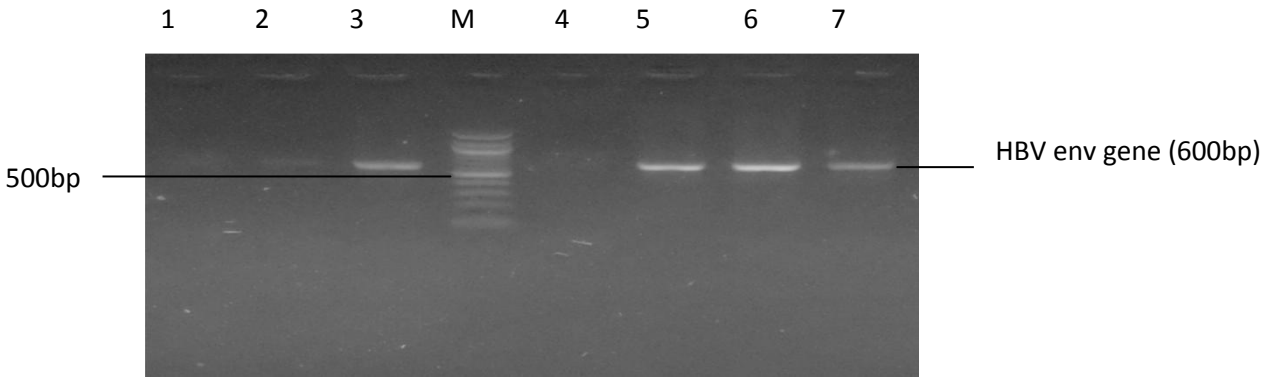



**Plate 2: Agarose gel Electrophoresis of the HBV env gene. Lanes 3, 5, 6, 7 showing the amplified HBV env gene. Lane M represents the 100 bp ladder.**

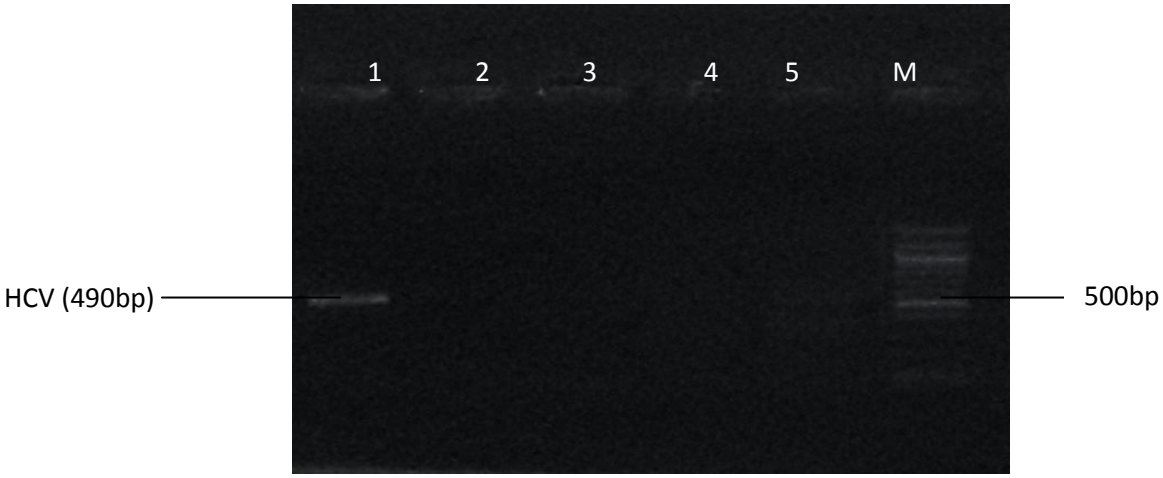



**Plate 3: Agarose gel electrophoresis showing the amplified HCV bands, L represents a 100bp ladder, lanes 1 represents HCV bands while 2-5 shows no bands**

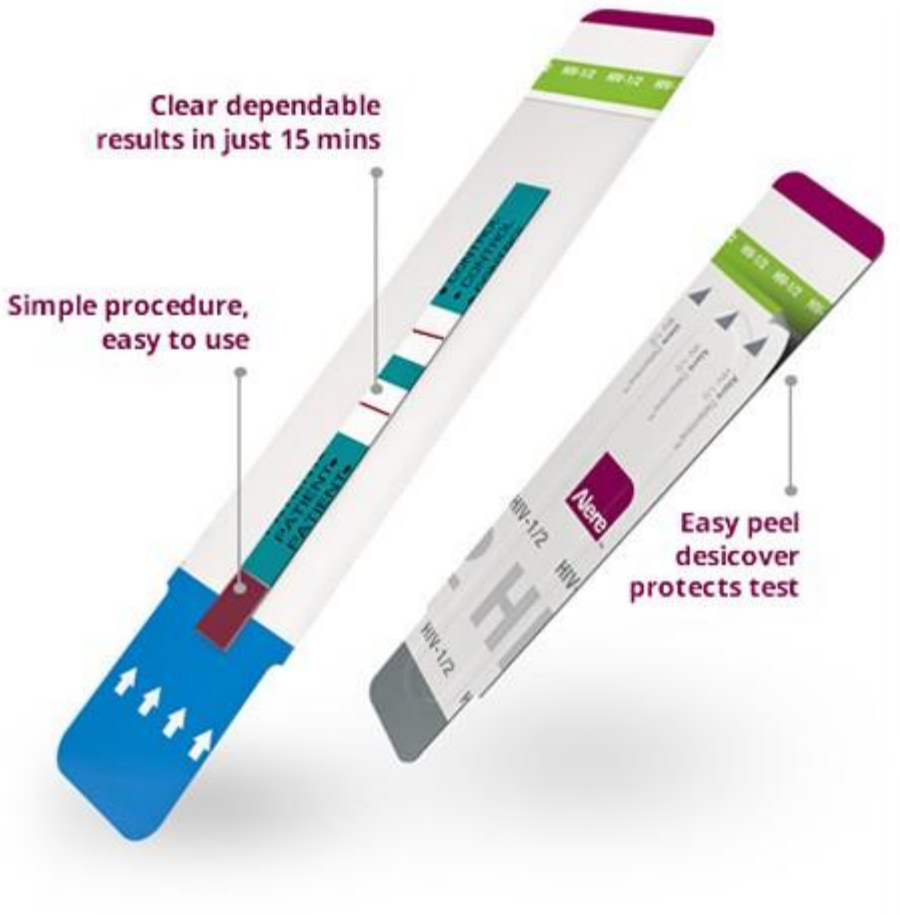



**Plate 4: Indicating positive Alere HIV Determine Strip**


**Plate 5: Plain Alere HIV determine Strip**


**Plate 6: HIV ½ Stat-Pak Cassette With Double Line Indicating a Positive HIV result**


**Plate 7: Hepatitis B Strips with Double Lines Indicating a Positive Result**

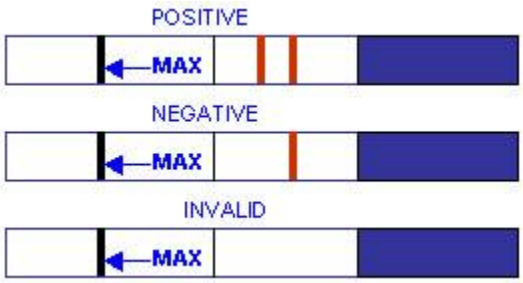



**Plate 8: Hepatitis C Strips Indicating positive, Negative and Invalid Results**

# CHAPTER FIVE

## 5.0 DISCUSSION

In Nigeria, Hepatitis B, hepatitis C and HIV are common blood-borne infections that share common routes of transmission, which is unevenly distributed across different regions (Okonkwo *et al.,* 2017). A few population based prevalence studies have been carried out to determine the prevalence of these infections and risk factors for infection with the viruses. In this study, the seroprevalence of hepatitis B and C among HumanImmunodeficiency Virus (HIV) infected individuals and various predisposing risk factors were studied. The prevalence of Hepatitis B and C Virus among HIV patients attending the 3 District Hospitals using Rapid test Strip RTD (Table1), surprisingly showed larger percentage of the non-HIV volunteers 11% to be positive to Hepatitis B Virus (HBV) as compared to the Human Immunodeficiency Virus (HIV) patients from individual district hospitals under study. The result is statistically insignificant at P-value (P>0.05). Although, more of the HIV positive patients were positive to Hepatitis C virus (HCV). The study of Thompson *et al.,* (2015) revealed that hepatitis B infection is commoner than hepatitis C infection among the study group and its prevalence was considerably high even though the co-infection is at the minimal. This discovery is comparable to the result obtained in this work.

Looking at the proportion of individuals under study with the viral infection from the different hospitals, it was observed to still be on the high side. A report by Atefeh *et al.,* (2015) indicated a wide range of HBV infection prevalence rate of 1.2 and 9.7% while that obtained in this research was 8.5% for HIV patients and 11% for non-HIV patients with RTD. The overall prevalence of HBsAg in this study population with ELISA increased to 12.75% and with PCR it increased to 31.25% when the samples were selected at random and run with PCR

the prevalence increased to 80% with a sex specific prevalence of female higher than that of the male. This result shows that ELISA and PCR methods gives higher prevalence and more reliable than the RTD method.However, the rate for HCV-Ab (5.7% with RTD, 18.25% with PCR and when samples were randomly selected and run with PCR 20%) was lower than the rate for HBsAg. The rates of HBsAg and HCV-Ab co-infections with HIV in this study are comparable with findings in other studies. For instance, results of an earlier study in Nigeria conducted on 1779 HIV-positive patients revealed that the rates for HBsAg, HCV, and HBV/HCV co-infections were 11.9, 4.8, and 1%, respectively (Otegbayo *et al.*, 2008).

The prevalence rate of hepatitis B reported in; Maiduguri by Thompson *et al.,* (2015) was 11.6%; Ilorin by Agbede *et al.,* (2007) was 5.7%; Port Harcourt by Akanni *et al.,* (2005) was 4.3%; Zaria by Luka *et al.,* (2008) was 8.3%, which are relatively low when compared with the values obtained with PCR in this study. Krunal *et al.,* (2013) reported 2.9% for HBV, also quite low compared with HBV and HCV obtained in this study, which shows that the prevalence of HBV is higher in Abuja. A close percentage of individuals (17.3%) with similar number of patients with HBV were accounted by Collenberg and colleagues in Burkina Faso.The study of Udeze *et al.,* 2015 showed that 32% of the HIV patients studied were HBsAg positive although, the value is slightly higher than that obtained with RTD but corresponds to that obtained with PCR in this work. The values obtained are quite considerable but indicates that HBV is a threat to HIV patients. This result is on a par with the findings of Agbaji *et al*., (2005): 14.8%; Uneke *et al*., (2005): 25.9% and Forbi et al., (2007): 20.6%. The co-infection of HIV and HBV is significant and confirms that HBV is a major threat to HIV patients in Nigeria, as also reported in other parts of the world.

In Africa Karoney and Siika, (2013) estimated prevalence of HCV in Africa to be 5.3%, which recorded as the highest in the world. Eze *et al.,* (2014) reported the prevalence of HIV/HCV co-infection in Lagos Nigeria to be 6.8%. Belay *et al.,* (2010) reported 0.7% for HCV which is relatively on the low side when compared with that of this study. A study also carried out in an urban center in Nigeria showed the prevalence of hepatitis B to be 11.5% (Olanisun *et al.,* 2009). In New York, 25% of HIV patients were co-infected with HCV and 4.4% for HBV (Diwe *et al.,* 2013), which in this case projects HCV as most prevalent infection as opposed to what was observed in this study.

The prevalence of HBV and HCV are considerably high and requires that the patients be adequately attended to in order to reduce the transmission of the viral infection to other individuals and also reduce the endemicity of the infection. Another report by Bui *et al.,* (2014) showed HBV (8.4%) to be more prevalent than HCV which is close to the report of this study. The prevalence rates of HBV obtained in this study using the RTD method of diagnosis is however not comparable with previously reported prevalence in different parts of Nigeria; keffi (20.6%) (Forbi *et al.,* 2007, Jos (28.7%) (Irisena *et al.,* 2002), Illorin (30.4%) (Olatunji *et al.,* 2008), Kano (70.5%) (Nwokedi *et al.,* 2006). It is thus comparable with results obtained in Lagos 9.2% (Lesi *et al.,* 2007 and Niger-Delta 9.7% (Akyala *et al.,* 2013). A report on similar study conducted in Abuja (Adewole *et al.,* 2009) indicated a prevalence rate of 11.5%. The prevalence rate of HCV in the patients in the three district hospitals was found to be on the high side when measured up with what was obtained by Karoney and Siika, (2013) and Eze *et al.,* (2014) who recorded a prevalence rate of 5.3% and 6.8% respectively.That notwithstanding, the prevalence rate of HBV using ELISA 12.75% and PCR 31.25% is comparable with the previously reported prevalence in different part of Nigeria;

keffi (20.6%) (Forbi *et al.,* 2007, Jos (28.7%) (Irisena *et al.,* 2002), Illorin (30.4%) (Olatunji *et al.,* 2008), Kano (70.5%) (Nwokedi *et al.,* 2006). These result together with those obtained in other similar studies by other researchers in other regions of Nigeria, 12.5% in Kano (Hamza *et al.,* 2013), 11.9% in Ibadan (Otegbayo *et al.,* 2008)and 11.8% in Jos (Lar *et al.,* 2013), gives a picture of the endemic nature of HIV/HBV or HCV co-infections in Nigeria. An estimated prevalence of HCV among HIV individuals in sub-Saharan Africa is about 3 to 7% as reported by Nagu *et al.,* (2008). Similar studies in East Africa (10%), West Africa (1.6%), and South Africa (13.4%) showed varying levels of prevalence of HCV.

The total percentage of subjects positive to HBV and HCV across the three district hospitals both HIV positive and non-HIV positive volunteers in this present study however was 8.5%and 5.7% respectively with RTD. A similar prevalence was obtained in the work of Okonkwo *et al.,* (2017) of 8.8% prevalence for HBV, which was rated high when compared with 5.6% reported among hospital staffs and volunteers in Calabar. Viral hepatitis has been reported to be a major public health concern as hepatitis B virus afflicts an estimated 350million people and hepatitis C affects 150 million people worldwide. It is therefore a matter of concern as it is reported that 3.6% of Nigerian populace have been estimated to be leaving with HIV as at 2009 (Tremeau-Bravard *et al.,* 2013). This therefore means that this proportion of individuals diagnosed with HIV can easily be predisposed to hepatitis viral infection. There is need for enhanced interest in HIV/HBV co-infection. Around 5% to 10% of HIV patients harbor persistent serum HBsAg and therefore suffer from chronic hepatitis B (Alter, 2009). Progression to end-stage liver disease occurs more quickly in HIV/HBV co-infected patients; this is characteristic in the absence of significant elevations in liver

enzymes, as inflammatory phenomena in the liver are ameliorated in HIV infection although paradoxically fibrogenesis is enhanced.

The interesting factor is that hepatitis B virus infection is preventable. This can be achieved by similar measures taken to prevent the transmission of HIV infection such as safe sex practice, safe handling of sharps, and avoidance of sharing of intravenous drug paraphernalia, among others. In addition, safe and effective vaccines with probably lifelong immunity against all hepatitis B virus serotypes and genotypes are currently available (WHO, 2017).The findings of this research confirm that HBV is a major co-morbid infection and a threat to HIV patients. A clear picture of HIV, HBV and HCV prevalence in Nigeria is necessary in order to better educate the populace and control these epidemics. The prevalence of HBV, HCV and HIV infection is high in Nigeria as reported by Okonkwo *et al.,* 2017 which indicated that there are urban and rural disparity with the possibility of HCV posing more of a public health concern than HBV in some communities. It is thus important to have a population based study that will provide vital data which can give optimal national control strategy information.

Three different assay methods were used for the detection of hepatitis B and C viral infection in HIV positive and non-positive volunteer patients studied, namely; RTD (Rapid Test Detection), ELISA (Enzyme Linked Immunosorbent Assay) and PCR (Polymerase Chain Reaction). There were variations in the number of patients with the viral infections with PCR, ELISA and RTD producing highest number of patients in descending order. This might be due to the accuracy of the qualitative measurement tools. Natalia *et al.,* (2015) reported that negative result using molecular test does not rule out the presence of the virus due to the fact that viremia may be undetectable or temporarily suppressed due to antiretroviral drugs. The

findings in this research supports the findings of Natalia *et al* (2015), as the positivity of HBV for both HIV positive patients on ART reduced to 76% in table five (5). A guideline by WHO recommended the use of single quality assured serological invitro diagnostic test, either laboratory-bases immunoassay (Enzyme Immunoassay) or rapid diagnostic test (RTD) to detect HBsAg and HCV antibodies. RTD used should meet minimum performance standards and be delivered at the point of care to improve access and linkage to care and treatment (WHO, 2017). The selection of assay format to best test for HBsAg for example in a particular setting should depend on the performance characteristics of the assay and on key operational considerations such as, accessibility, cost, ease of use (technical complexity of test procedure) and specimen collection methods. It was reported by WHO in the 2017 guideline that in HIV-infected individuals, clinical sensitivity of RTD is poor and the key challenge to the use of RTDs include; limited availability of quality-assured RTDs, reduced analytical sensitivity compared to laboratory-based methods and few RTDs for measuring HBsAg for example meet the analytical sensitivity of 0.0130IU/ml. EIA (Enzyme Immunoassay) is another type of assay for detecting the presence of hepatitis which has a high analytical sensitivity. Relating the result of the outcome of using the different assay methods, PCR (Polymerase Chain Reaction) is still the best method because it will be able to detect the presence of the viral DNA or RNA as the case may be. The limitation of using PCR assay method is the cost implication, the government should however fund the government/public hospitals with such equipment for easy detection and management of the infection.

Based on the results obtained in this study, Nigeria can be classified as high endemic area for HBV since the prevalence is more than 8%. This agrees with the work reported earlier by other researchers that Nigeria has been classified as a hyper endemic nation for HBV with a

prevalence of 12% but a relatively lower prevalence for HCV of 0.5-4% and prevalence for HIV is 3.1% (Lee, 2008; Lago*et al.,* 2014). Moderate endemicity given by WHO in 2009 for the presence of hepatitis B surface antigen (HBsAg) for HBV infection was given as 3.1%. Brunetto *et al.,* 2010 classified high endemicity from HBV infection and defined it as HBsAg greater than 7% in an adult population which supports the report of WHO, reporting Nigeria as a highly endemic area with prevalence greater than 8% (Itode *et al.,* 2016). Now, let's put into consideration the population of Nigerians using the statistics of 170million of the 2006 census, it means therefore that about 23million of the total population is living with HBV, approximately 6million lives with HCV and 3.5 million lives with HIV (Global AIDS, 2016; Malu *et al.,* 2015). This population is considerably high and thus requires the prompt action of the government in acquiring necessary machinery and also individuals should be self-aware of the risk factors associated with these viral infections. This is because individuals infected with these viruses may be asymptomatic for many years and remain as reservoir host of infection during this period.

Reflecting on the difference in prevalence obtained in different study on HIV/HBV/HCV around the same region and different regions of Nigeria and the world at large, the difference could be as a result of differences in sample technique, sample size, and sample population. The disparity in prevalence could also be due to differences in social behaviors and cultural differences (Ayele *et al.,* 2012). Again, most studies on prevalence in Nigeria are mostly conducted among high-risk subjects such as patients with liver diseases in HBV infection for example or other chronic infections, which may lead to over estimation of rates. This findings is not comparable in this study as there is no much difference in prevalence when compared with HIV/HBV, HIV/HCV and non HIV volunteers using RTD but with the use of ELISA

and PCR, the determined prevalence of HBV and HCV became higher than that of the non HIV volunteers.

The presence of hepatitis B markers (antibodies, antigens, viral proteins, active or acute infection) in the patients (Table 3), showed the presence of both Hepatitis B surface Antigen (HBsAg) and antibodies (HBsAb). Surface antibodies are formed in response to hepatitis B virus which is possible when, the patients have been vaccinated or recovering from hepatitis B infection. The positivity of patients to this test indicates that the immune system has successfully developed a protective antibody against hepatitis B virus(Christine and Heather, 2015). The number of patients positive to HBsAb was considerable low in patients attending Asokoro hospital which poses a concern on the level of their immune system. A number of reports have been documented for the co-existence of Hepatitis B surface Antigen (HBsAg) and anti-HBsAg antibodies HBsAb in patients with chronic hepatitis B, which has been associated with the non-appearance of amino acid substitutes in the HBsAg sequences of the hepatitis B Virus (HBV) genome able to escape an immunological variant. Concurrent HBsAb positivity in active chronic hepatitis B infection is a clinical and virological tight spot (Galati *et al.,* 2014). We however did not identify any individual that was co-infected with HBsAg/HBsAb in this study that to say there was none with any of such co-infection.

In Taiwan, the latest sero-epidemiologic survey that was conducted 25 years after implementation of the universal HBV vaccination program to evaluate the effectiveness of program in the general population has shown that the prevalence of HBsAg positivity was 0.9%, and that of anti-HBs and anti-HBc positivity was 55.9% and 10.0%, respectively, in the subjects born during the vaccination era (Ni *et al.,* 2012). This is contrary to the result obtained in this work, in table 3 result obtained shows a prevalence of 12.75% HBsAg, 10.6%

HBsAb (anti-HBs), and 31% HBcAb(anti-HBc) which means that only (10.6%) of the population have been vaccinated or immune to HBV that is why the spread of HBV is increasing. About 1/3 of our participants showed evidence of previous or current HBV infection, and there was corresponding increase in seropositivity to anti-HBc. The government and health sector should create more awareness and make available the vaccine so that individuals will be vaccinated. Ochei and Kolhatkar (2008) reported that vaccination is an effective way of preventing HBV transmission and children becoming carriers providing this is done early in life. HIV/HBV co-infected patients in Asokoro hospital shows low active infectivity having HBeAg 3.5% whereas patients in Maitama and Wuse show no percentage of infectivity. The infectivity rate is higher in non HIV volunteers who show HBeAg of 4.5%. On the other hand, decreased infectivity is seen more in HIV patients in Asokoro and Wuse hospital having HbeAb 35% and 42.5% respectively and the non HIV volunteers having HBeAb of 30% which shows that HAART also plays a role in reducing infectivity of HBV. This is open for further studies. The prevalence of the populace with recent infection is higher as the overall HBcAb was 31% that was why the prevalence obtained in the ELISA and PCR was higher than that of the RTD because some of the HBcAb cannot be detected with the RTD method, this is also open for further studies. On this note, the findings in this method rated the ELISA and PCR method better for HBV diagnosis than the RTD.

HBeAg is an antigen often present when the virus is reproducing rapidly. The prevalence of HBeAg in this study was considerably low when compared with the finding of Iroezindu *et al.*, (2013) who reported that co-infected patients (i.e. HBV/HIV Patients) had notably higher value than the mono-infected controls (i.e. individuals without HIV infection but HBV). This trend has also been recorded in developed nations and in some sub-Sahara Africa countries as

documented by Ruta *et al.,* 2005. The prevalence of HBeAg seropositivity of between 25-27% documented in other studies in sub-Sahara Africa and 50-78% in European and Asian countries (Elefsiniotis *et al.,* 2006; Geretti *et al.,* 2010; Lesi *et al.,* 2007; Saravanan *et al.,* 2007) are in variance with the outcome of this study. A routine baseline screening for markers in HIV patients majorly which could affect the choice of HAART treatment for the patients positivity to HBeAb means that the immune system has cleared HBeAg from the system, but HBeAb is needed in order to completely vanquish the infection (Christine and Heather, 2015).

Studies have been carried out to evaluate prevalence of HBV and HCV among HIV infected individuals either on retroviral drugs or not. From these various studies, low prevalence of HBV and HCV markers have been found in different parts; Brazil recorded 1% for HBsAg and 1.6% for HCV (Oliveira *et al.,* 2014), India (2.6% for HBsAg and 1.7% for HCV), Columbia 2.1% for HBsAg and 0.8% for HCV) while Nigeria recorded 7.9% for HBsAg and 2.3% for HCV (Tremeau-Bravard *et al.,* 2012; Raizda *et al.,* 2011). But higher prevalence have been recorded also in African countries such as Gambia (12.2%, Cote D'Ivoire (13.4%) (Nagu *et al.,*2008; Attia *et al.,* 2012). The above result is comparable to that obtained in this work as tables 5 to 8 show reduced HBV DNA in the HBV markers although higher than the prevalence documented earlier. This data has only shown that the rate of spread of this infection is increasing as the values obtained in this study is high as compared to those indicated as high in previous studies. The reduction in the ability of the body to eliminate hepatitis B envelop antigen (HBeAg) and reduced immunity in HIV infected patients leads to the reactivation of the latent virus (Alter, 2009).

In all epidemiological studies, age proves to be an important factor; the age of acquiring infection is the major determinant of incidence and prevalence rate. In respect of association

of age with the prevalence of hepatitis, this study has shown that age group between 25-35 and above excluding age bracket 65-75, had higher frequency of HBV and HCV than lesser or higher age groups. This report is in variance with the work of Atefeh *et al.*, (2014) who reported that age group higher than 30 years had higher frequency of HBV and HCV, thus excluding the age range of 25-35. But Khan *et al.*, (2014) on the other hand reported that age groups 21-30 and 40-50 years showed highest frequency of the viral infections, which is in agreement with this study. The likely reason for such trend among this particular age group might be related to the sexual activeness of the said age group Al-Ajlan (2011). Patients in this age group are most likely to be married with fewer exceptions. Ezegbudo *et al.*, (2014) reported that significant infection rates for HIV/HBV co-infection was associated with age group of 16-20 and 21-30 years. The predominance of hepatitis B virus infection in the younger age group could be attributed to the fact that hepatitis B virus infection is frequently acquired at childhood partially due to vertical transmission and partially due to horizontal transmission before the age of 20 years. Prevalence rate for HBsAg studied among students and young adults aged 15-29 was 4.1% as reported by Okonko and his colleagues, Tremeau-Bravard *et al.*, discovered that the prevalence rate for both HBV and HCV was high for patients were under 39years of age (55%). These findings correspond with the findings in this work as the prevalence of HBV and HCV was found to be high (16%) among young adults in the age group between 25-35 and 35-45 years.

For the prevalence of HBV and HCV on the bases of ethnicity, it was observed that the number of Hausas and Igbos with the infection was highest across the hospitals followed by other tribes. This could be explained by the fact that more of the Hausas attend the hospitals under study. Also, it's a custom for this group of people to marry more than one wife, which

could be a resultant effect. A study on population conducted by Hsun-Wen Lai and colleagues on Pin-Jen Taiwan showed prevalence rate of hepatitis B and C to vary within the ethnic groups studied. A population based study carried out in North Jakarta revealed that hepatitis B transmission is associated with low socioeconomic status and Chinese ethnic group with a moderate positive large family size has more risk of developing hepatitis B but hepatitis C was more associated with age (older age group) and medical history (blood transfusion). In Nigeria, limited work has been done to compare the prevalence of hepatitis B and C with ethnicity. However, this work has been able to show the relationship of ethnicity with prevalence of hepatitis. The Hausas and Igbo tribes surveyed were seen to have highest prevalence which could be due to the fact that the study area is predominantly dominated by this group of individuals. Apart from that, their culture and socio-economic status could also be a contributing factor. For collection of data for surveillance therefore, health organizations should carry out a survey to really determine the dependence of hepatitis prevalence on ethnicity.

Tribal mark was not common among the younger agegroup between 15-25, 25-35 and 35-45 years with the highest prevalence rather it was more common among the older age group in this study, therefore, it was not used as a yard stick because it was discovered among the study population that it is phasing out. The findings are not comparable with the work done by Adebola *et al.,* (2016) who revealed that the common risk factors for HBV in their study were presence of tribal marks and sharing of sharp objects. The findings by Maddawa *et al.,* (2002), Otegbayo *et al.,* (2008); Agbede *et al.,* (2007) which demonstrated that unsafe injections from unqualified medical personnel using HBV contaminated needle/syringe and socio-cultural

practices such as tribal marks circumcision and scarification are important routes of HBV transmission that needs public sensitization cannot be comparable to the findings in this work.

Some researchers documented that the main risk factors for both HBV and HCV were exposure to surgery (Edris *et al.,* 2014) in other studies needle-stick injuries have been documented as risk factor for HBV and blood transfusion was not seen as risk factor (Aminu *et al.,* 2013). This is comparable with the findings in this research, most of the patients positive to HBV and HCV had no medical record for history of blood transfusion and surgery. An equal percentage of them positive to HBV had used injectable drug (IDU). In Asokoro the number of individuals who do not have an idea of sexually transmitted diseases (STDs) are quit high numbering 20% when compared with the number with an idea about the sexual disease. Only 11% of them were fully informed on what sexually transmitted diseases (STDs) was. It was found out also that a large proportion of the patients had no family history of the viral infections. Maitama and Wuse hospitals also recorded larger number of individuals with no history of blood transfusion and surgery.

Assessing occupational risk in picking up the infection, this study has pointed out that the highest prevalence of HBV and HCV was recorded for unemployed, petty traders, civil servants across the hospitals which, corresponds with the findings of Bassey *et al.,* (2009) who indicated that traders suffered the highest rate with HIV, HBV and HCV. For the unemployed for example, this could be accounted for by their youthful exorbitance and high sexual activity with likelihood of multiple sex partners. Traders travel long distances away from their spouses and result in illicit unprotected sex which may lead to sexually transmitted diseases. Unemployed persons are within the sexual active stage and so could justify the reason for the high prevalence among this group of individuals some may even engage in sex

hawking. Julius and colleagues also recorded high prevalence of 4.7% in individuals without jobs. The high prevalence in this group of individuals could be due to free indulge in more sexual activity and risky relationship/behavior.

The liver function test carried out showed that all the patients sampled have normal total and direct bilirubin though some of them with deterioration in their liver. This is to show that the major role of HBV and HCV is not to cause jaundice at the onset of the disease but great damage to the liver leading to hepatocellular carcinoma and liver cirrhosis (Michielsen *et al.*, 2011). Documentation by Lacombe and Rockstroh, (2012) revealed that a rapid progression of liver fibrosis to cirrhosis and hepatocellular carcinoma is more common among HIV/HBV or HCV co-infection if the individuals remain untreated. Chronic viral hepatitis is an outplaying cause of liver related deaths among HIV patients. Estimate by WHO, 2013 showed a global burden of HIV, HBV and HCV to be 33.2million, 400million and 170million correspondingly. It has also been estimated that almost one third of HIV deaths are in a way associated with liver disease which is mainly reported in the settings of co-infection with HBV or HCV. Decline of opportunistic infections in HIV patients during the use of very active anti-retroviral therapy has shed light on the morbidity and mortality attributed to HBV/HCV and end stage liver disease (Seyed *et al.*, 2012). About one to two million people die annually from HBV related acute and chronic liver diseases world over and majority of chronic carries of HBV are found in Africa. Chronic liver disease results from inflammatory injury to the liver which persist for six or more months without complete resolution (Laraba *et al.*, 2010). The incidence of liver cell injury among individuals with HBV and HCV was statistically significant. The occurrence of liver injury among patients is higher than 10% documented for development of liver disease. Findings has however shown that individuals on

antiretroviral therapy (ART) were less prone to liver disease because the drug helps in clearing hepatitis B and C virus and therefore reduces the effect of developing liver disease (Yun-Fan and Tung, 2006) reported that fewer HIV/HBV individuals on ART had evidence of liver cell injury. The interaction between HIV and HBV or HCV co-infection have a negative impact on the onset and progression of liver disease caused by these viruses and these infections also increases toxicity to antiretroviral medications. Additionally, HIV has been reported to worsen HCV infection thus leading to serve fibrosis, cirrhosis and death from liver disease. Co-infection of HIV/HCV increases vertical transmission of HCV resulting in HCV incidence in newborn babies which has grave public health consequences (Rockstroh, 2006).

Liver enzymes in this study were found not to be significantly elevated. Despite the absence of statistical significance difference in the mean levels of the liver enzymes between HIV-mono-infected and HIV-viral hepatitis co-infected individuals, raised ALT, AST and ALP were found in HIV/HBV, HIV/HCV and HIV/HBV/HCV co-infected individuals. However, in a study which was conducted in South Africa, 70% of HIV/HBV and HIV/HCV co-infected study participants had significantly elevated AST and ALT, 56% of them had elevated ALP (Lodenyo*et al.*, 2010). Similarly, significantly raised ALT was found in 14% of HIV/HBV co-infections and 20% in HIV/HCV co-infected patients in India (Tripathi, 2007). These liver enzyme levels difference between different studies may be due to difference in study design, duration of the viral hepatitis infection as well as the patient's condition like having chronic alcoholism or other drug induced hepatotoxicity. In addition, HIV can also infect the hepatic or kupffer cells that may farther contribute for the development of liver fibrosis and raised liver enzyme levels.

Alpha-fetoprotein is used to measure maternal serum during the first and second trimesters of pregnancy and it has shown to be useful in screening natural tube defects and adverse pregnancy outcome including fetal death, pre-eclampsia, fetal growth restriction and preterm. It also serves as a tumor marker and early detection of Hepatocellular carcinoma (Beta *et al.*, 2011). As HCC mostly occurs in patients with cirrhosis, or at least advanced fibrosis, most studies have been performed in these patients at risk. The most frequently used tests have been serum alpha-fetoprotein (AFP) and hepatic ultrasound (US) (Michilsen *et al.,* 2011). In this study, 4% of HBV patients have positive Alpha-Fetoprotein test which is in line with what was documented in the work of Teo and Lok (2017) that some of the HBV infected patients would develop cirrhosis, liver failure, or HCC, and 500, 000 to 1.2 million people die of HBV infection annually. HCV positive patients have higher prevalence (8%) of Alpha-Fetoprotein test than HBV positive patients which supports the work of other researchers that HCV plays an important role in the causation of chronic liver disease (Ochei and Kolhatkar, 2008), and has become the leading cause of liver cirrhosis and primary liver cell carcinoma in North America, Southern Europe and Japan (Chen, 2011).

Alpha-fetoprotein is the major fetal serum protein which is not essential for embryonic development but required for female fertility, the biological role of this major embryonic serum protein is unknown although there are speculations. It was observed in this current research that all the nursing mothers among them had HIV negative babies and they breast feed their babies regularly without the babies contacting the virus. Some of the HIV patients about 3% were living with HIV negative partners having unprotected sex for more than 7years yet their partners remain HIV negative. There could be some hormones, antibodies or genes that these individuals possessed which prevents them from contacting the virus or prevents the

proliferation of the virus in their system. This is open for further research. In that case, serum samples from such HIV resistant individuals could be used by our pharmaceutical industries in search for HIV vaccine or medicine.

This study has revealed that females had higher prevalence of HBV and HCV as compared to men in the three district hospitals under study. Some reports have documented that there is no significant association between gender and viral infection even when the prevalence varies between the male and female counterparts. Analysis of gender seroprevalence of HBV and HCV co-infection among HIV and non-HIV patients showed that the females were more infected than the male counterpart which is in variance to the documentation of ECRTD, 2013 reported 12.7% for males and 2.1% for females both of which are low and in variance when compared with the values obtained in this study (male: 9.3%; females: 27.3%). They reported that the reason for higher prevalence in male as compared to females was probably as a result of higher frequency of exposure to risk factors associated with the virus such as injection of drug use, unprotected sex, having multiple sex partners and occupation (ECRTD, 2013). The above mentioned factors could be risk factors but this study has found use of injectable drugs as a low risk factor in the area of study. Unprotected sex and multiple sex partners were more likely reasons for high prevalence of the viral infections in male (Zhou *et al.,* 2007). Another predisposing factor reported was the fact that males have a predilection for aggressive sports and plays that could result in injury with bleeding which may lead to horizontal HBV transmission (Bella *et al.,* 2012) However, observations of Lesi *et al.,* 2007 is in par with this study and some previous studies who reported a higher prevalence among female patients (37.5%) as compared to the male patients who numbered 18.8%. Baseke *et al.,* (2015) reported that there was statistical significant difference in the prevalence of HBV between

male and female in their study and the high prevalence among women was said to be possibly due to high exposure to risk factors. HIV test counseling has been reported to be generally low in Nigeria with 23% of males and 29% of females knowing their HIV status (Federal Ministry of Health, 2012). This situation could also be found true for other forms of infection or diseases general to both men and women. This trend could be due to the fact that women are more conscious about their health status than men. In the study of okonkwo and colleagues, the prevalence of HBV and HCV was higher in the males as compared to the females but reverse was the case in this present study. They attributed the higher prevalence of the viral infection in the male counterparts to higher clearance of the virus by females compared with males. Report by Saeed *et al.,* (2005) showed significant higher infection rate in males than in females donating blood, suggesting other routes of exposure other than sex. The trend for such an outcome was attributed to the use of unsterilized barbing razors to sheave which could expose users to facial bleeding. They suggested that health education programs on barber's community could help to reduce HBV transmission.

The prevalence of married individuals with HBV and HCV were seen to be higher as compared to the singles and divorced analyzed in this study. This outcome is in variance with what was obtained in a study by Julius *et al.,* (2016) single participants (10.6%) were more infected as compared to the married individuals. They reported that married couples are less likely to be engaged in acts that might put them at risk of contracting the viral infection or other sexually transmitted infections. A study reported carrier rate for HBV to be higher among singles which is consistent with a report from Jos by Sirisena *et al.,* (2014) and Awka by Ezegbudo *et al.,* (2014) but on the contrary, the findings in this study shows a higher prevalence rate among the married.Usually, married individuals are exempted when it comes

to sexual promiscuity which should not be. This study revealed individuals infected with the virus with more than one sexual partner, meaning that most of the married individuals are not faithful to their spouses. This acts can lead to the transmission and the eventual spread of the infections to a large group of the population. Nigeria as a country has different culture and believes which allows a man to marry more than one wife which is dangerous and encouraging spread of infection. The irony however is that individuals practicing such do not have enough resources to take care of the wives in case of eventualities such as ill health. Another reason for which the prevalence of hepatitis was high among married individuals could be attributed to societal discrimination mostly among the women folk. Instead of speaking up, they rather keep the infection to themselves even when they see the signs and the symptoms. On the other hand, some of the married HIV/HBV, HIV/HCV co-infected patients could have contacted the HBV or HCV from their spouses as HIV patients are encouraged to marry themselves, most of them will go ahead and marry without screening for HBV or HCV. On this note, HIV patients who intend marrying themselves should screen for HBV and HCV before marriage to avoid co-infections.

Study on educational background as a probable risk factor was determined in this study and it was found out generally across the hospitals that, patients with secondary education had the highest prevalence while individuals with tertiary education had the least prevalence for HBV and HCV. Unlike the report of Okonkwo and colleagues who stated that educated subjects had the highest prevalence rates, thus faulting the influence of education and public enlightenment on the carrier rates of these infections. It could be true to an extent but not justifiable because education is paramount for advancement of a country. Individuals with tertiary education are more aware of the transmission mode of these infections because they most have been

exposed to it in one way or the other, either in school or while reading science articles. This privilege is not accorded to the less educated; they will have to be sensitized by NGO's and government agencies on the effect of these viral infections and other diseases. Another report which deviated from what is reported in this study is that of Ezegbudo and colleagues whose report showed prevalence rate among academic and non-academic staffs to be high. They concluded by saying that the findings collaborated with other reports, that even though educated individuals are knowledgeable about contraction route for the infections, especially HIV and HBV, it did not deter them from engaging in unprotected sexual intercourse. At this junction, the education board should be hinted on these issues and should be advised on adding a course such as disease and infection awareness to the curriculum at the secondary and tertiary levels in a bid to capture individuals not in the medical line. If a survey is carried out among science students and non-science students on matters relating to health, it will be surprising to find out that non-science students will have little or no knowledge about the health matter been surveyed. This thus calls for general orientation of all individuals in the urban and rural areas of the country.

The prevalence of hepatitis with respect to parental origin/residence was studied and found out thatparental origin/residence has a role it plays in the transmission and spontaneous progression of hepatitis viral infection in individuals.

The prevalence of hepatitis with respect to behavioral lifestyle was studied and found out the behavioral lifestyle has a role it plays in the transmission and spontaneous progression of hepatitis viral infection in individuals. Less than 3% of patients reported a history of injectable drug use, but in studies conducted in Brazil, from 20 to 100% of patients were users of injectable drug which was associated with positivity to HCV (Wolff *et al.*, 2010). About 5%

was recorded in the work of Natalia *et al.*, (2015). Blood transfusion, use of injectable drug, homosexuality and tattooing have been described as important factors most especially in HIV and HCV transmission. A study by Ogbodo *et al.,* (2015) on the other hand said there was no correlation between HIV and HCV infection and blood transfusion, tattooing and use of injectable drugs in their study. The number of individuals to which the above mentioned factors applied to in this study is minimal as compared to other factors of infection such as sex, which could be because most of the infections are largely heterosexual activity. Although, Ogunro *et al.*, (2007) documented association of sanctification marks on some individuals with infection like hepatitis infection and that the presence of tattoo did not notably influence HCV prevalence among the study population. The report of Reddy *et al.*, (2005) on the other hand observed that the risk of two-fold infection with HBV-HCV was greater among chronic renal failure patients which was reported to be due to the frequency in blood exposure from transfusion and extracorporeal circulation during hemodialysis. This thus calls for scrutiny during blood screening and blood products from donors in blood banks and health facilities in Nigeria before transfusion is been done. In the work of Geane *et al.,* 2016 it was reported that some risk factors such as history of intravenous medicine administration, dental procedures, piercing of ears, pedicure and manicure were put into consideration during study and they were found to be risk factors for HIV acquisition in HBV and HCV positive individuals. Sharing unsterilized instruments was described recently as a possible route of transmission of infection (Matsuda *et al.,* 2014). Although, consumption of alcohol was reported to be more frequent among HBV positive individuals but use of illicit narcotic substances was more common among HCV positive persons. Regarding sexual behavior, a large percentage of the patients were heterosexual and had regular sex (some with more than

one partner) mostly without condoms. This risky sexual behavior observed in some persons could contribute to the risk of infection in this group. As demonstrated in the study of (Távora *et al.,* 2013), unprotected sex is related to high frequency of infection. Some findings have shown that the major route of HBV transmission in some population are the tribal marks, scarification, circumcision which accounted for 8%.This factors were not seen as risk factors in this study, as the number of individuals with tribal marks positive to the viral infections were fewer as compared with the number of individuals positive to the viral infections with no tribal marks. Because this sources of infection are not the cause of prevalence in this study does not make them insignificant or less of a risk factor.

The phylogenetic analysis of the HIV samples confirmed that the patients were all HIV patients indeed some of them with gene closely relatedness to AF069943.1 HIV-1 isolate with 2,538 bp genomic DNA obtained in 1995 from a hospitalized individual from Maiduguri, Borno state, Nigeria. Others closely related to HIV-1 isolate KR051459.1GI with 2,583bp genomc DNA, subtype G, isolate B obtained from Homo sapiens in Cameroon in 2007. Gene cds,vpu protein, rev protein ant tat protein particles partial cds.

Genotype E is more predominant in Africa, genotype F is more common in South America and Polynesia, and genotype G is more predominant in the United States and France (Chu *et al.,* 2012). Studies made by Andernach *et al.,* 2013 documented that one of the three genotypes that are predominates in Africa depending on the region includes; E, A and D. While genotype D is the most prevalent variant in Northern Africa, genotype A prevails in East and South Africa. Except for Cameroon, where genotype A is dominant, genotype E is highly endemic in most of sub-Saharan Africa. HBV genotype E has a high prevalence and

wide geographic spread throughout large parts of Africa. This is in line with findings made in this research, all the HBV isolated from the three district hospitals are of genotype E.

## 5.1 CONCLUSION

Hepatitis is an infection which affects both HIV positive and non-HIV individuals alike. The proportion of non-HIV patients with hepatitis infection was high as compared to the HIV positive patients in this study. The prevalence of Hepatitis among HIV and non-HIV individuals studied is quite high, which therefore requires constant monitoring to evaluate control schemes/measures. The results classified Abuja as high endemic area for HBV. Both the ART and the locally prepared herbs have the tendency of reducing the HBV DNA butthe effect of ART on HBV DNA is higher than that of the locally made herbs. The HBcAb has the highest prevalence than other HBV antibodies and the HBsAg has a higher prevalence than other HBV antigen.The best assay method for the detection of the viral infection is the Polymerase Chain Reaction (PCR), which is more effective and sensitive but expensive. The prevalence of HBV and HCV was higher among young adult between the ages of 25-35 and 35-45years. The possibility that ethnicity and religion could be a contributing factor to the prevalence of hepatitis is high, as individuals of a particular ethnicity (Hausas) and religion (Christians).Behavioral lifestyle observed to likely increase risk of hepatitis in people in this research work includes; having more than one sexual partner, unprotected sex and use of injectable drugs. Similarly, highest prevalence of the viral infection was seen in unemployed persons, petty traders and civil servants of the studied population across the hospitals.HBV and HCV patients have normal serum bilirubin and non-significantly raised AST, ALT and ALP. HIV/HCV patients have higher Alpha-fetoprotein than HIV/HBV patients showing that HIV/HCV patients are more prone to developing Hepatocellular carcinoma, liver cirrhosis and

liver cancer.The Hepatitis B was more prevalent than hepatitis C viral infection among the female respondents, married persons and younger age group.The less educated subjects, that is, individuals who only have primary and secondary education, had the highest prevalence to the infections. Greater percentage of positive patients come from the out sketch of Abuja to the hospital, places like; Marraba, Nyanya, Karu, One man's village and Lugbe. Preventive measures are important to curb spread of the viral infection, after knowing the highest risk factor for infection in each area/region of the country. Awareness on the health implication of the viral infections should be made to the general public both educated and non-educated. Sensitization on safe sex practices, safe injection usage, proper sterilization of instruments, use of sensitive laboratory test kits, immunization of people and strict blood donor selection should make up an important package of a prevention program. The prevailing HBV genotype in Abuja is the HBV genotype E. The prevailing HCV in Abuja was not identified as the templates submitted failed. More studies are needed to identify the particular gene possessed by those healthy individuals living with and having unprotected sex for more than 7 years with any of the HIV, HBV and HCV infected individual and yet remain uninfected.

## 5.2 RECOMMENDATION

To offer an opportunity to identify individuals with diagnosed and undiagnosed infection, population based screening should be conducted in the urban and rural areas of the country for HIV, HBV, HCV and other viral infection known to be endemic in the country. This will help reduce the national burden of complications resulting from these infections among HIV positive and non-HIV positive individuals alike. The government should invest in provision of molecular laboratories in all corners of the country to help in the molecular diagnosis of HIV,

HBV, HCV and other diseases at early stage when other methods may not detect it so to help in disease control. HIV patients who intend to marry themselves should also screen for HBV and HCV to avoid co-infection. More research should be made on healthy spouses living either HIV, HBV and HCV positive partners for years and still remain negative to know the particular gene they possess. This could help in the discovery of the medications or vaccines for either of these ailment. More studies are needed to generate data on hepatitis infection prevalence in relation to ethnicity.

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# APPENDIX 1:

# SOME REAGENTS USED AND THE PRINCIPLES BEHIND THEM

## REAGENTS FOR HBV

The test cassette contains anti-HBsAg particles, HBsAb particles, anti-HBeAg particles, anti-HBeAb particles, HBcAb particles respectively.

### HBsAg and HBeAg

The HBsAg and the HBeAg tests are qualitative, two-site sandwich immunoassays for the detection of HBsAg or HBeAg in serum or plasma. The membrane is pre-coated with anti-HBsAg and anti-HBeAg antibodies on the test line region of the strip.

### HBsAb

Hepatitis B surface Antibody (HBsAb) is also known as anti-Hepatitis B surface Antigen (anti-HBs). This test is a qualitative, lateral flow immunoassay for the detection of HBsAb in serum or plasma. The membrane is pre-coated with HBsAg on the test line region of the strip. During testing, the serum or plasma specimen reacts with the particle coated with HBsAg. The mixture migrates upward on the membrane chromatographically by capillary action to react with HBsAg on the membrane to generate a colored line.

### HBeAb and HBcAb

Hepatitis B envelop Antibody (HBeAb) is also known as anti-Hepatits B envelop Antigen (anti-HBe). Hepatitis B core Antibody (HBcAb) is also known as anti-Hepatitis B core Antigen (anti-HBc). These tests are immunoassays based on the principle of competitive binding. During testing, the mixture migrates upward on the membrane chromatographically

by capillary action. The membrane is pre-coated with HBeAg or HBcAg on the test line region of the strip. During testing, anti-HBe antibody or anti-HBc antibody, if present in the specimen, will compete with particle coated with anti-HBeAg or anti-HBcAg for limited amount of positive result. A visible a visible colored line will form in the test line region if there is no anti-HBe antibody or anti-HBc antibody in the specimen because all the antibody coated particles will be captured by the antigen coated in the test line region. To serve as a procedural control, a colored line always appeared in the control line of the region indicating that proper volume of specimen has been added and membrane wicking has occurred. The test cassettes were removed from the sealed foil pouch and were used within 1 hour (Chizzali-Bonfadin *et al.*, 2013).

**REAGENTS FOR HCV**

The test strip consists of the following;

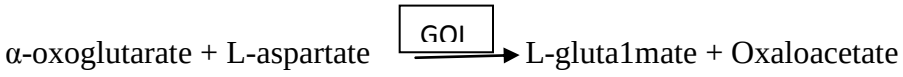
- A burgundy colored conjugate pad containing recombinant HCV infusion antigen (cor, NS3, NS4 and NS5) conjugated with colloidal gold.
- A nitrocellulose membrane strip containing a test line (T) and a control line (C line). The T line is precoated with recombinant HCV fusion antigen (core, NS3, NS4 and NS5), and C line is precoated with a control line antbody.

**MATERIALS**

Test cassettes, droppers, centrifuge, timer.

**REAGENT COMPOSITION FOR ASPARTATE TRANSFERASE (AST):**

**PRINCIPLE:**




AST is measured by monitoring the concentration of oxaloacetate hydrazine formed with 2, 4-dinitrophenylhydrazine.

| Content | Initial Concentration of Solution |
|---|---|
| **R1 Buffer** | |
| Phosphate buffer | 100mmo/l, pH 7.4, |
| L- aspartate | 100mmol/l |
| α-oxoglutarate | 2mmol/l |
| **R2. 2,4-dinitropphenylhydrazine** | 2mmol/l |

**MATERIALS REQUIRED:** Buffer,2,4-dinitropphenylhydrazine, Water bath, Timer spectrophotometer, automatic pipettes, Sodium Hydroxide(0.4mol/l).

| | |
|---|---|
| Wavelength | Hg 546nm |
| Cuvette | 1cm light path |
| Incubation Temperature | 37°C |

**Measurement against reagent black**

**REAGENT COMPOSITION FOR ALANINE AMINOTRANSFERASE:**

**PRINCIPLE:**

α-oxoglutarate + L-alanine $\xrightarrow{\text{GPT}}$ L-glutamate + Pyruvate

Alanine Aminotransferase is measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenylhydrazine.Content Initial Concentrations of Solutions

**R1 Buffer**

| | |
|---|---|
| Phosphate buffer | 100 mmo/l, pH 7.4, |
| L- alanine | 200 mmol/l |
| α-oxoglutarate | 2.0 mmol/l |
| **R2. 2,4-dinitropphenylhydrazine** | 2.0 mmol/l |

**MATERIALS REQUIRED:** Buffer 2,4-dinitropphenylhydrazine, Water bath, Timer spectrophotometer, automatic pipettes, Sodium Hydroxide(0.4mol/l).

| | |
|---|---|
| Wavelength | Hg 546nm |
| Cuvette | 1cm light path |
| Incubation Temperature | 37°C |

Measurement against reagent black

**REAGENT COMPOSITION FOR ALKALINE PHOSPHATASE (ALP)**

**PRINCIPLE:**


| Content | Concentrations in the test |
|---|---|
| **R1a Buffer** | |
| Diethanolamine buffer | 1mol/l, pH 9.8 |
| MgCl2 | 0.5mmol/l |
| **R1b Substrate** | |
| p-nitrophenylphosphate | 10mmo/l |

**MATERIALS REQUIRED:** Diethanolamine buffer, spectrophotometer, automatic pipettes, Substrate.

| | |
|---|---|
| Wavelength: | Hg 405nm |
| Cuvette: | 1cm light path |
| Temperature: | 30°C |
| Measurement: | against air |

**SAMPLE:** Serum and EDTA plasma samples were used.

**REAGENT COMPOSITION FOR TOTAL BILIRUBIN**

**PRINCIPLE:**

Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue colored complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

| Content | Initial Concentration of Solution |
|---|---|
| **R1. Sulphanilic acid** | 29mmol/l |
| Hydrochloric acid | 0.17N |
| **R2. Sodium Nitrite** | 38.5mmol/l |
| **R3. Caffeine** | 0.26mol/l |
| Sodium benzoate | 0.52mol/l |
| **R4. Tartrate** | 0.93mol/l |
| Sodium Hydroxide | 1.9N |

**MATERIALS REQUIRED:** Sulphanilic acid, Nitrite, Caffeine, Tartrate, spectrophotometer, automatic pipettes, 0.9% Sodium Chloride, Water bath and Timer.

| | |
|---|---|
| Wavelength | Total bilirubin: 578nm (560-600nm) |
| Cuvette: | 1cm light path |
| Reaction Temperature: | 25°C |
| Measurement: | against sample blank |

**DIRECT BILIRUBIN**

| | |
|---|---|
| Wavelength | Total bilirubin: 578nm (560-600nm) |
| Cuvette: | 1cm light path |
| Reaction Temperature | 25°C |
| Measurement: | against sample blank |

**APPENDIX 2:**

**DNA EXTRACTION FROM PLASMA SPECIMEN**

1. 200ul of sample was added to a microcentrifuge and 200ul Biofluid and cell buffer(Red) with 20ul proteinase k was added
2. The preparation was mixed thouroughly and the tube incubated at $55^{o}$C for 10minutes.
3. 1 volume of Genomic Binding Buffer was added to the digested sample and mixed thoroughly.
4. The mixture was transferred to a zymo-spin™IIC-XL column in a collection tube. The transferred mixture was centrifuged at 12,000xg for 1minute. The collection tube was discarded with the flow through.
5. 400ul DNA pre-wash buffer was added to the column in a new collection tube and centrifuged for 1 minute. The collection tube was emptied.
6. 700ul g-DNA wash buffer was added and centrifuged for 1 minute. The collection tube was emptied.
7. 200ul g-DNA was buffer was added and centrifuged for 1 minute.The collection tube was discarded with the flow through.
8. To elute the DNA, the processed sample was transferred to a clean microcentrifuge tube. ≥ 50ul DNA Elution Buffer was added and incubated for 5minutes and then centrifuged for 1 minute.

**APPENDIX 3:**

**POLYMERASE CHAIN REACTION FOR HBV AMPLIFICATION**

| PCR components | Concentration | Volume |
|---|---|---|
| Master mix | 1X | 10ul |
| Forward Primer | 0.5uM | 0.4ul |
| Reverse Primer | 0.5uM | 0.4ul |
| Template | - | 2ul |
| H2O | - | 4.2ul |
| Final volume | | 20ul |

Initial denaturation 95$^{o}$C at 5minutes, denaturation 95$^{o}$C at 30seconds;

annealing 53$^{o}$C at 30seconds; extension 72$^{o}$C at 30seconds,

final extension 72$^{o}$C at 2minutes.

Number of cycle: 35.

Forward Primer used: .TCA CCA TAT TCT TGG GAA CAA CA

Reverse Primer used: CGA ACC ACT GAA CAA ATG GC (Inquaba, 2017)

**APPENDIX 4:**

**RNA EXTRACTION FROM PLASMA SPECIMENS**

RNA extraction was performed using Zymo extraction kit manufactured by Zymo Research Inc. and marketed by Ingaba Biotech Ltd Pretoria, South Africa. The extraction was done according to the guidelines of the manufacturer. RNA was extracted from HIV and HCV negative/positive samples and then PCR was used for detection of HCV RNA and HIV RNA. The RNA extraction protocol is as follows:

**AMPLIFICATION OF HIV V3 REGION (NESTED PCR)**

**Primary or Nest 1**

Forward Primer: GGCATCAAACAGCTCCAGGCAAG

Reverse Primer: AGCAAAGCCCTTTCTAAGCCCTGTCT

| PCR components | Concentration | Volume |
|---|---|---|
| Master mix | 1X | 10ul |
| Forward Primer | 0.2uM | 0.16ul |
| Reverse Primer | 0.2uM | 0.16ul |
| Template | - | 1ul |
| H2O | - | 8.68ul |
| Final volume | | 20ul |

Conditions:

Initial denaturation 95$^{o}$C at 5 minutes, Denaturation 95$^{o}$C at 30seconds

Annealing 55$^{o}$C at 30 seconds, Extension 72$^{o}$C at 30 seconds, Final Extension 72$^{o}$C at 2 minutes

Number of cycle: 25

**Secondary of Nest 2**

Forward Primer: TCCTGGCTGTGGAAAGATACCTA

Reverse Primer: GTCCCCTCGGGGCTGGGAGG

| PCR components | Concentration | Volume |
|---|---|---|
| Master mix | 1X | 10ul |
| Forward Primer | 0.2uM | 0.16ul |
| Reverse Primer | 0.2uM | 0.16ul |
| Template | - | 0.5ul |
| H2O | - | 9.18ul |
| Final volume | | 20ul |

Conditions:

Initial denaturation 95$^{o}$C at 5 minutes, Denaturation 95$^{o}$C at 30seconds

Annealing 58$^{o}$C at 30 seconds, Extension 72$^{o}$C at 30seconds,

Final Extension 72$^{o}$C at 2minutes

Number of cycle: 35

**AMPLIFICATION OF HCV**

Forward Primer: ACTGTCTTCACGCAGAAAGCGTCTAGCCAT

Reverse Primer: CGAGACCTCCCGG GGCACTCGCAAGCACCC

| PCR components | Concentration | Volume |
|---|---|---|
| Master mix | 1X | 10ul |
| Forward Primer | 0.2uM | 0.16ul |
| Reverse Primer | 0.2uM | 0.16ul |
| Template | - | 1ul |
| H2O | - | 8.68ul |
| Final volume | | 20ul |

Conditions:

Initial denaturation 95$^{o}$C at 5 minutes, Denaturation 95$^{o}$C at 30 seconds

Annealing 50$^{o}$C at 30 seconds,Extension 72$^{o}$C at 30 seconds

Final Extension 72$^{o}$C at 2 minutes

Number of cycle: 35

**APPENDIX 5:**

**AGAROSE GEL ELECTROPHORESIS**

To prepare 1.5% agarose gel;

1. 1.5g agarose powder was weighed
2. 100ml of running buffer was added (TAE)
3. The mixture was heated in a microwave for 5 minutes.
4. It was allowed to cool for $50^{o}C$
5. 2ul ethidium bromide was added
6. The gel solution was cast in gel electrophoretic cast in which the gel comb has been appropriately inserted.
7. The agarose gel was allowed to polymerize

**APPENDIX 6:**

**MATERIALS AND COMPONENTS FOR ALPHA-FETOPROTEIN (AFP)**

Antibody-coated microtiter plate with 96 wells, Microtitre reader

Zero buffer, 12ml

Reference standard set containing 0, 5, 20, 50, 150 and 300ng/ml (WHO, 72/225)AFP, in liquid form (ready to use) or lyophilized form.

Enzyme conjugate reagent, 18ml

TMB substrate, 12ml

Wash buffer concentrate (50X), 15ml

Pipettes: 5-40ul, 50-200ul and 1.0ml, disposable pipette tips, distilled water, vortex mixer or equivalent, absorbent paper or paper towel, graph paper, microtiter plate reader, micotiter wells.

**REAGENT PREPARATION FOR AFP**

All reagent were brought to room temperature before use. All lyophilized reference standards were reconstituted each with 0.5ml distilled water and were allowed to stand for at least 20minutes. The reconstituted standards were sealed and stored at 2-8$^{o}$C. 1 volume of wash buffer concentrate (50X) was diluted with 49 volumes of distilled water to get 1X. The dilution was well mixed before use.

## APPENDIX 7:

**Table 1: Prevalence of Hepatitis B Virus among HIV patients attending the 3 District Hospitals using Rapid Test (RTD)**

Test statistics

| | Prevalence difference in HBV in the 3 district hospitals |
|---|---|
| Chi-square | 7.457 |
| df | 8 |
| p-value | 0.4882 |

**Table2: Prevalence of Hepatitis C Virus among HIV patients attending the 3 District Hospitals using Rapid Test Strip (RTD)**

Test statistics

| | Prevalence difference in HCV in the 3 district hospitals |
|---|---|
| Chi-square | 3.229 |
| df | 8 |
| p-value | 0.9192 |

**Table 3: Prevalence of Hepatitis B Virus among HIV patients attending the 3 District Hospitals using 5 panels RTD with ELISA Principle**

Test statistics

| | Prevalence difference in 5 panel RTD with ELISA principle in the 3 district hospitals |
|---|---|
| Chi-square | 125.7 |
| df | 20 |
| p-value | Less than 0.0001 |
| p-value summary | **** |

**Table 4: Prevalence of Hepatitis B and C Virus among HIV patients attending the 3 District Hospitals Who are not on ART using PCR**

Test statistics

| | Prevalence difference among those who are not on ART using PCR |
|---|---|
| Chi-square | 99.11 |
| df | 16 |
| p-value | Less than 0.0001 |
| p-value summary | **** |

**Table5 : Prevalence of Hepatitis B Virus among HIV pts on ART and HIV negative Subjects who are on herbs positive for HBsAg with RTD and ELISA using PCR**

| | Prevalence difference among those on ART using PCR |
|---|---|
| Chi-square | 100.8 |
| df | 8 |
| p-value | Less than 0.0001 |
| p-value summary | **** |

**Table 6: Prevalence of Hepatitis B Virus among HIV pts on ART and HIV negative Subjects on herbs positive for HBeAg with RTD using PCR**

Test statistics

| | Prevalence difference among patients on ART and herbs, positive for HBeAg using PCR |
|---|---|
| Chi-square | 10.76 |
| df | 8 |
| p-value | 0.2158 |
| p-value summary | Ns |

**Table7: Prevalence of Hepatitis B Virus among HIV pts on ART and HIV negative Subjects on Herbs positive for HBeAb with RTD using PCR**

Test Statistics

| | Prevalence difference among patients on ART and herbs, positive for HBeAb using PCR |
|---|---|
| Chi-square | 114.6 |
| df | 8 |
| p-value | Less than 0.0001 |
| p-value summary | **** |

**Table8: Prevalence of Hepatitis B Virus among HIV pts on ART and HIV negative Subjects on Herbs but positive for HBcAb with RTD using PCR**

Test Statistics

| | Prevalence difference among Patients on ART and herbs positive for HBcAb using PCR |
|---|---|
| Chi-square | 101.8 |
| df | 8 |
| p-value | Less than 0.0001 |
| p-value summary | **** |

**Table9: Prevalence of Hepatitis B Virus among HIV patients on ART and HIV negative Subjects on Herbs but positive for HBsAb using PCR**

Test Statistics

| | Prevalence difference among Patients on ART and herbs positive for HBsAb using PCR |
|---|---|
| Chi-square | Nil |
| df | nil |
| p-value | nil |
| p-value summary | nil |

**Table10: Prevalence of Hepatitis B and C Virus among HIV patients attending the 3 District Hospitals using PCR**

Test Statistics

| | Prevalence difference of HBV and HCV in the 3 District Hospital using PCR |
|---|---|
| Chi-square | 39.31 |
| df | 3 |
| p-value | Less than 0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to Age |
|---|---|
| Chi-square | Nil |
| df | Nil |
| p-value | Nil |
| p-value summary | Nil |

**Table 12: Prevalence of hepatitis B and C Virus among HIV patients attending Wuse Hospital with respect to Age**

Test Statistics

| | Prevalence difference in Maitama Hospital with respect to Age |
|---|---|
| Chi-square | Nil |
| df | Nil |
| p-value | Nil |
| p-value summary | Nil |

**Table 13: Prevalence of hepatitis B and C Virus among HIV patients attending Wuse Hospital with respect to Age**

Test Statistics

| | Prevalence difference in Maitama Hospital with respect to Age |
|---|---|
| Chi-square | 40.38 |
| df | 18 |
| p-value | 0.0018 |
| p-value summary | ** |

**Table 14: Prevalence of hepatitis B and C Virus among HIV patients attending Asokoro Hospital with respect to Ethnicity**

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to Ethnicity |
|---|---|
| Chi-square | Nil |
| df | Nil |
| p-value | Nil |
| p-value summary | Nil |

Test Statistics

| | Prevalence difference in Maitama Hospital with respect to Ethnicity |
|---|---|
| Chi-square | Nil |
| df | Nil |
| p-value | Nil |
| p-value summary | Nil |

Test Statistics

| | Prevalence difference in Wuse Hospital with respect to Ethnicity |
|---|---|
| Chi-square | Nil |
| df | Nil |
| p-value | Nil |
| p-value summary | Nil |

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to Occupational exposure |
|---|---|
| Chi-square | 51.75 |
| df | 18 |
| p-value | 0.0006 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Maitama Hospital with respect to Occupational exposure |
|---|---|
| Chi-square | 118.3 |
| df | 18 |
| p-value | 0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Wuse Hospital with respect to Occupational exposure |
|---|---|
| Chi-square | Nil |
| df | Nil |
| p-value | Nil |
| p-value summary | Nil |

**Table 23**: **Liver enzyme levels in HIV/HBV, HIV/HCV and HIV/HBV/HCV co-infection**

$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n}}$$

$\sigma$ = **lower case sigma**
$\sum$ = **capital sigma**
$\bar{x}$ = **x bar**

s means 'standard deviation'.
S means 'the sum of'.
$\bar{x}$ means 'the mean'

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to sex |
|---|---|
| Chi-square | 1.107 |
| df | 6 |
| p-value | 0.9812 |
| p-value summary | Ns |

Test Statistics

| | Prevalence difference in Maitama Hospital with respect to sex |
|---|---|
| Chi-square | 79.12 |
| df | 6 |
| p-value | Less than 0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Wuse Hospital with respect to sex |
|---|---|
| Chi-square | 11.34 |
| df | 6 |
| p-value | 0.0785 |
| p-value summary | Ns |

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to Marital Status |
|---|---|
| Chi-square | 36.17 |
| df | 9 |
| p-value | < 0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Maitama Hospital with respect to Marital Status |
|---|---|
| Chi-square | 27.88 |
| df | 9 |
| p-value | 0.0010 |
| p-value summary | *** |

Test Statistics

| | Prevalence difference in Wuse Hospital with respect to Marital Status |
|---|---|
| Chi-square | 56.27 |
| df | 9 |
| p-value | <0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to Educational Background |
|---|---|
| Chi-square | 34.49 |
| df | 9 |
| p-value | 0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Maitama Hospital with respect to Educational Background |
|---|---|
| Chi-square | 15.37 |
| df | 9 |
| p-value | 0.0813 |
| p-value summary | Ns |

Test Statistics

| | Prevalence difference in Wuse Hospital with respect to Educational Background |
|---|---|
| Chi-square | 34.15 |
| df | 12 |
| p-value | 0.0006 |
| p-value summary | *** |

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to Ethnicity |
|---|---|
| Chi-square | 223.7 |
| df | 13 |
| p-value | <0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to Parental Origin/Residence |
|---|---|
| Chi-square | Nil |
| df | Nil |
| p-value | Nil |
| p-value summary | Nil |

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to Ethnicity |
|---|---|
| Chi-square | 223.7 |
| df | 13 |
| p-value | <0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to Parental Origin/Residence |
|---|---|
| Chi-square | Nil |
| df | Nil |
| p-value | Nil |
| p-value summary | Nil |

Test Statistics

| | Prevalence difference in Maitama Hospital with respect to Parental Origin/Residence |
|---|---|
| Chi-square | Nil |
| df | Nil |
| p-value | Nil |
| p-value summary | Nil |

Test Statistics

| | Prevalence difference in Maitama Hospital with respect to Parental Origin/Residence |
|---|---|
| Chi-square | Nil |
| df | Nil |
| p-value | Nil |
| p-value summary | Nil |

Test Statistics

| | Prevalence difference in Wuse Hospital with respect to Parental Origin/Residence |
|---|---|
| Chi-square | 237 |
| df | 16 |
| p-value | <0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Wuse Hospital with respect to Parental Origin/Residence |
|---|---|
| Chi-square | Nil |
| df | Nil |
| p-value | Nil |
| p-value summary | Nil |

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to Behavioural life style |
|---|---|
| Chi-square | 197.1 |
| df | 15 |
| p-value | <0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to Behavioural life style |
|---|---|
| Chi-square | 197.1 |
| df | 15 |
| p-value | <0.0001 |
| p-value summary | **** |

Test statistics

| | Prevalence difference in Asokoro Hospital with respect to Behavioural life style |
|---|---|
| Chi-square | 60.41 |
| df | 15 |
| p-value | <0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Asokoro Hospital with respect to Behavioural life style |
|---|---|
| Chi-square | 94.58 |
| df | 17 |
| p-value | <0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Maitama Hospital with respect to Behavioural life style |
|---|---|
| Chi-square | 85.44 |
| df | 15 |
| p-value | <0.0001 |
| p-value summary | **** |

Test Statistics

| | Prevalence difference in Maitama Hospital with respect to Behavioural life style |
|---|---|
| Chi-square | Nil |
| df | Nil |
| p-value | Nil |
| p-value summary | Nil |

Test Statistics

| | Prevalence difference in Maitama Hospital with respect to Behavioural life style |
|---|---|
| Chi-square | 147.5 |
| df | 15 |
| p-value | <0.0001 |
| p-value summary | **** |

**APPENDIX 8:**

**ALFA-FETOPROTEIN RESULTS FOR HBV PATIENTS**

| LAB ID | PATIENTS' ID | ABSORBANCE @ 450nm & 630nm | RESULT |
|---|---|---|---|
| Calibrator 1 | 0.0ng/ml | 0.060 | 0.0ng/ml |
| Calibrator 2 | 5.0ng/ml | 0.256 | 5.0ng/ml |
| Calibrator 3 | 20.0ng/ml | 0.780 | 20.0ng/ml |
| Calibrator 4 | 50.0ng/ml | 1.459 | 50.0ng/ml |
| Calibrator 5 | 150.0ng/ml | 2.919 | 150.0ng/ml |
| Calibrator 6 | 300.0ng/ml | >3.000 | 300.0ng/ml |
| 1. | 001 | 0.401 | 9.0ng/ml |
| 2. | 002 | 0.139 | 2.70ng/ml |
| 3. | 003 | 0.233 | 4.6ng/ml |
| 4. | 004 | 0.142 | 2.8ng/ml |
| 5. | 005 | 0.124 | 2.4ng/ml |
| 6. | 006 | 0.273 | 5.3ng/ml |
| 7. | 007 | 0.254 | 4.9ng/ml |
| 8. | 008 | 0.214 | 4.2ng/ml |
| 9. | 009 | 0.134 | 2.6ng/ml |
| 10. | 010 | 0.265 | 5.2ng/ml |
| 11. | 011 | 2.919 | 150ng/ml |
| 12. | 012 | 1.458 | 50ng/ml |

| | | | |
|---|---|---|---|
| 13. | 013 | 0.139 | 2.70ng/ml |
| 14. | 014 | 0.233 | 4.6ng/ml |
| 15. | 015 | 0.142 | 2.8ng/ml |
| 16. | 016 | 0.124 | 2.4ng/ml |
| 17. | 017 | 0.273 | 5.3ng/ml |
| 18. | 018 | 0.254 | 4.9ng/ml |
| 19. | 019 | 0.214 | 4.2ng/ml |
| 20. | 020 | 0.134 | 2.6ng/ml |
| 21. | 021 | 0.265 | 5.2ng/ml |
| 22. | 022 | 0.142 | 2.8ng/ml |
| 23. | 023 | 0.139 | 2.70ng/ml |
| 24. | 024 | 0.233 | 4.6ng/ml |
| 25. | 025 | 0.142 | 2.8ng/ml |
| 26. | 026 | 0.124 | 2.4ng/ml |
| 27. | 027 | 0.273 | 5.3ng/ml |
| 28. | 028 | 0.254 | 4.9ng/ml |
| 29. | 029 | 0.214 | 4.2ng/ml |
| 30. | 030 | 0.134 | 2.6ng/ml |
| 31. | 031 | 0.265 | 5.2ng/ml |
| 32. | 032 | 0.239 | 2.70ng/ml |
| 33. | 033 | 0.139 | 2.70ng/ml |
| 34. | 034 | 0.233 | 4.6ng/ml |
| 35. | 035 | 0.142 | 2.8ng/ml |

| | | | |
|---|---|---|---|
| 36. | 036 | 0.130 | 2.8ng/ml |
| 37. | 037 | 0.273 | 5.3ng/ml |
| 38. | 038 | 0.258 | 45.0ng/ml |
| 39. | 039 | 0.214 | 4.2ng/ml |
| 40. | 040 | 0.138 | 2.7ng/ml |
| 41. | 041 | 0.269 | 5.6ng/ml |
| 42. | 042 | 0.305 | 300ng/ml |
| 43. | 043 | 0.139 | 2.70ng/ml |
| 44. | 044 | 0.233 | 4.6ng/ml |
| 45. | 045 | 0.142 | 2.8ng/ml |
| 46. | 046 | 0.124 | 2.4ng/ml |
| 47. | 047 | 0.276 | 5.4ng/ml |
| 48. | 048 | 0.254 | 4.9ng/ml |
| 49. | 049 | 0.214 | 4.2ng/ml |
| 50. | 050 | 0.134 | 2.6ng/ml |

**APPENDIX 9:**

**ALFA-FETOPROTEIN RESULTS FOR HCV PATIENTS**

| LAB ID | PATIENTS' ID | ABSORBANCE @ 450nm & 630nm | RESULT |
|---|---|---|---|
| Calibrator 1 | 0.0ng/ml | 0.060 | 0.0ng/ml |
| Calibrator 2 | 5.0ng/ml | 0.256 | 5.0ng/ml |
| Calibrator 3 | 20.0ng/ml | 0.780 | 20.0ng/ml |
| Calibrator 4 | 50.0ng/ml | 1.459 | 50.0ng/ml |
| Calibrator 5 | 150.0ng/ml | 2.919 | 150.0ng/ml |
| Calibrator 6 | 300.0ng/ml | >3.000 | 300.0ng/ml |
| 1. | 001 | 0.401 | 9.0ng/ml |
| 2. | 002 | 0.249 | 5.0ng/ml |
| 3. | 003 | 0.233 | 4.6ng/ml |
| 4. | 004 | 0.142 | 2.8ng/ml |
| 5. | 005 | 0.124 | 2.4ng/ml |
| 6. | 006 | 0.273 | 5.3ng/ml |
| 7. | 007 | 0.254 | 4.9ng/ml |
| 8. | 008 | 0.214 | 4.2ng/ml |
| 9. | 009 | 0.134 | 2.6ng/ml |
| 10. | 010 | 0.265 | 5.2ng/ml |
| 11. | 011 | 0.124 | 2.4ng/ml |
| 12. | 012 | 0.139 | 2.70ng/ml |

| | | | |
|---|---|---|---|
| 13. | 013 | 0.139 | 2.70ng/ml |
| 14. | 014 | 0.233 | 4.6ng/ml |
| 15. | 015 | 0.142 | 2.8ng/ml |
| 16. | 016 | 0.124 | 2.4ng/ml |
| 17. | 017 | 0.273 | 5.3ng/ml |
| 18. | 018 | 0.254 | 4.9ng/ml |
| 19. | 019 | 0.214 | 4.2ng/ml |
| 20. | 020 | 0.134 | 2.6ng/ml |
| 21. | 021 | 3.100 | 301ng/ml |
| 22. | 022 | 0.142 | 2.8ng/ml |
| 23. | 023 | 0.139 | 2.70ng/ml |
| 24. | 024 | 0.233 | 4.6ng/ml |
| 25. | 025 | 0.142 | 2.8ng/ml |
| 26. | 026 | 0.124 | 2.4ng/ml |
| 27. | 027 | 0.273 | 5.3ng/ml |
| 28. | 028 | 2.919 | 150ng/ml |
| 29. | 029 | 0.214 | 4.2ng/ml |
| 30. | 030 | 0.134 | 2.6ng/ml |
| 31. | 031 | 0.265 | 5.2ng/ml |
| 32. | 032 | 0.239 | 2.70ng/ml |
| 33. | 033 | 0.139 | 2.70ng/ml |
| 34. | 034 | 0.233 | 4.6ng/ml |
| 35. | 035 | 0.142 | 2.8ng/ml |

| | | | |
|---|---|---|---|
| 36. | 036 | 0.130 | 2.8ng/ml |
| 37. | 037 | 0.273 | 5.3ng/ml |
| 38. | 038 | 0.258 | 45.0ng/ml |
| 39. | 039 | 0.214 | 4.2ng/ml |
| 40. | 040 | 3.00 | 300ng/ml |
| 41. | 041 | 0.269 | 5.6ng/ml |
| 42. | 042 | 0.305 | 302ng/ml |
| 43. | 043 | 0.139 | 2.70ng/ml |
| 44. | 044 | 0.233 | 4.6ng/ml |
| 45. | 045 | 0.142 | 2.8ng/ml |
| 46. | 046 | 0.124 | 2.4ng/ml |
| 47. | 047 | 0.276 | 5.4ng/ml |
| 48. | 048 | 0.254 | 4.9ng/ml |
| 49. | 049 | 0.214 | 4.2ng/ml |
| 50. | 050 | 0.134 | 2.6ng/m |

## APPENDIX 10:

## APPROVAL

**AMECHI OYEKA** Ph.D
*Professor of Microbiology*
Nnamdi Azikiwe University
PMB 5025 Awka,
Anambra State, Nigeria.

01-09-2014

**TO WHOM IT MAY CONCERN**

**PERMISSION TO SCREEN HIV PATIENTS FOR HEPATITIES.**

We are carrying out a research on Seroprevalence of Hepatitis among HIV patients in District hospitals in Abuja.

Kindly authorize the bearer Ezeji Charity who is currently being supervised by me to screen the HIV patients in your hospital.

We count on your cooperation to enable us carry out this very important research. The results of which will be made available to you at the end of the research.

Thank you very much.

Yours Faithfully,
Oyeka
**Prof. Amechi Oyeka**

Supervisor.

E-mail: silveramechi@yahoo.com

# FEDERAL CAPITAL TERRITORY
# HEALTH RESEARCH ETHICS COMMITTEE

Research Unit, Room 10, Block A Annex, HHSS
FCT Secretariat No. 1 Kapital Street Area 11, Garki, Abuja - Nigeria

| | |
|---|---|
| Name of Principal Investigator | Ezeji, Charity Ndidi |
| Address of Principal Investigator: | All Saints Anglican Church, Kpaduma II, Asokoro Extension, Asokoro - Abuja |
| Date of receipt of valid application: | 26/09/2014 |

**NOTICE OF RESEARCH APPROVAL**

**Protocol Approval Number: FHREC/2014/01/77/16-12-14**

**Study Title: Seroprevalence of Hepatitis B and C among HIV Patients in Abuja District Hospitals**

This is to certify that the FCT Health Research Ethics Committee (FCT HREC) has fully approved the research described in the above stated protocol.

**Approval Date:** - **16/12/2014**
**Expiration Date:** - **15/12/2015**

Note that no activity related to this research may be conducted outside of these dates. Only the FCT HREC approved informed consent forms may be used when written informed consent is required. They must carry FCT HREC assigned protocol approval number and duration of approval of the study.

The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code. The FCT HREC reserves the right to conduct compliance visit to your research site without previous notification.

**Modifications:** Subsequent changes are not permitted in this research without prior approval by the FCT HREC.

**Problems:** All adverse events or unexpected side effects arising from this project must be reported promptly to FCT HREC.

**Renewal:** This approval is valid until the expiration date. If you are continuing your project beyond the expiration date, endeavor to submit your annual report to FCT HREC early, and request for renewal of your approval to avoid disruption of your project.

**Closure of Study:** At the end of the project, a copy of the final report of the research should be forwarded to FCT HREC for record purposes, and to enable us close the project.

Ikwubiela S. Adem
Secretary, FCT HREC
December 16, 2014

# ASOKORO DISTRICT HOSPITAL

FEDERAL CAPITAL TERRITORY ADMINISTRATION

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+234(0)80 661 73328
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E-mail: fctasokorodh@yahoo.com

31 Julius Nyerere Crescent,
Asokoro District,
Abuja
NIGERIA.


11th June, 2015

## ETHICAL CLEARANCE

**Name of Investigator**: *Ezeji Charity Ndidi

**Title of project:** *Sero-prevalence of Hepatitis among HIV patients in Abuja District Hospitals.

**Documents Reviewed**:
* Research Proposal Containing
* Methodology, Consent Form and
* Questionnaire.

The Medical Ethics Committee of Asokoro District Hospital, FCTA, Abuja has concluded the review of your application to carry out a study in this facility.

By this medium, we wish to communicate to you that **permission** has been granted you to carry out the study. However, be informed that you are bound to comply with the rules and guidelines governing Health Research in this Institution and the National code for Health Research Ethics.

The Medical Ethics committee reserves the right to ensure compliance with the agreed protocol. Any breech on your part may result in the withdrawal of this approval without prior notification.

A copy of your completed work should be submitted to the committee for records.

Please accept our support for a successful study.

Best wishes.

**Dr Udofia I.Oscar** MBBCh, FWACP,
Chairman, Medical Ethics Committee.

HEALTH AND HUMAN SERVICES SECRETARIAT (FCTA)

**WUSE DISTRICT HOSPITAL**

**Our Ref:** FCTA/HHSS/HMB/WDH/GEN/T **Date:** 01/09/2015

Mrs. Ezeji Charity Ndidi
All Saints Anglican Church,
Kpaduma II Asokoro Extension,
Asokoro – Abuja.

**LETTER OF APPROVAL**

With reference to your letter dated 15th June, 2015, I am directed to inform you that approval has been granted for you to carry on with your research work in Wuse District Hospital on "Seroprevalence of Hepatitis B
And C among HIV patients in Abuja District Hospitals"

2. You are therefore expected to report to the Head, Clinical Services Wuse District Hospital for further instructions, please.

**Aguowo Cordelia N. (Mrs)**
***For: Hospital Secretary (WDH)***

Drs Adebayo/Egbuson/Udeani
Kindly assist fee bearer, please
thank you
Dr. Kanagbagbe

15 CONAKRY STREET, WUSE ZONE 3 P.M.B 24 FCTA, ABUJA.
Tel: 0809 286 8157 E-mail: fctwuse_hospital@yahoo.com

HEALTH AND HUMAN SERVICES SECRETARIAT (FCTA)
**MAITAMA DISTRICT HOSPITAL**
61 Aguiyi Ironsi Street, Maitama
E-mail: fctmaitamadh@yahoo.com P.M.B. 66, FCTA, Abuja
08020871867
08125603937

Our Ref: FCTA/HHSS/HMB/GEN/0381/I

Date: 19th November, 2015

**Mrs. Ezeji Charity Ndidi**
All Saint Anglican Church
Kpaduma II Asokoro Extension,
Asokoro-Abuja.

**LETTER OF APPROVAL**

With reference to your application letter dated 19th June, 2015, I am directed to inform you that approval has been granted for you to carry on with your research work in Maitama District Hospital on Seroprevaience of Hepatitis among HIV patients in Abuja District Hospital.

2. You are therefore expected to report to the Head, Clinical Services Maitama District Hospital for further instructions, please.

**Dr. S.Y. Mohammed**
*Chairman, Ethics & Research Committee*

## APPENDIX 11:

## QUESTIONNAIRE

I will like to invite you to take part in this research study titled "Sero-prevalence of Hepatitis among HIV patients in Abuja District Hospitals", being conducted by me Ezeji, Charity Ndidi under the supervision of Prof. (Mrs) C. A. Oyeka lecturer Medical Microbiology in the department of Microbiology, Faculty of Sciences, Nnamdi Azikiwe Univesity Awka, Anambra State. This questionnaire will include information concerning the socio-demographic characteristics and risk factors associated with Hepatitis B and Hepatitis C sero-positivity in HIV patients. Please I will like you to fully participate in answering these questions so as to enable me get the appropriate information needed to carry out the research.

**Please tick where appropriate**

Age: 18-25 years ☐ 25-35 years ☐ 35-45years ☐ 45-55years ☐

55-65years ☐

Gender: ✓ Male ☐ Female ☐

Marital Status: Single ☐ Married ☐ Divorced ☐

Educational background: Primary education ☐ Public Secondary School ☐

Private Secondary School ☐ University ☐

Religion: Christian ☐ Islamic ☐ Others ☐

Nationality: Nigerian ☐ Non-Nigerian ☐

Residencial Area: Marraba ☐ Nyanya ☐ One man village ☐ Asokoro ☐

Karu ☐ Garki ☐ Lugbe ☐ Apo ☐

Tribe: Igbo ☐ Hausa ☐ Yoruba ☐

Any Tribal mark? YES ☐ NO ☐

Are you on any of these drugs? Please tick ART ☐ Herb ☐ None ☐

Occupation: Civil servant ☐ Public servant ☐ Unemployed ☐

Petty trader ☐ International businessman ☐ Apprentice ☐

Parents: One or two are dead, ☐ are alive ☐

Ear piercing: None ☐ One on both ears ☐ more than one on both ears ☐

Do you take alcohol: Not at all ☐ Very little ☐ Moderate ☐ Very much

Tattooing: YES ☐ NO ☐

Do you chew tobacco? Very much ☐ Not at all ☐

How often do you visit barbers shop without using your personal clipper? Regularly

Rarely ☐ Not at all ☐

Any history of Blood Transfusion? YES ☐ NO ☐

Any previous Surgery? YES ☐ NO ☐

Injecting Drug Use: YES ☐ NO ☐

Knowledge of STD: Fully informed ☐ little knowledge ☐ No idea ☐

Have you any family history of HBV or HCV? YES ☐ NO ☐

Any Sexual experience: YES ☐ NO ☐

Years since first intercourse: ≤ 1 year ☐ 2 – 3 years ☐ >3 years ☐

Number of sex partners: One ☐ Two ☐ More than two ☐

Use of Condoms: YES ☐ NO ☐

**Thank you so much.**

**INFORMED CONSENT FORM FOR RESEARCH STUDIES**

**TITLE OF RESEARCH PROJECT:**

"Seroprevalence of Hepatitis among HIV Patients in Abuja District Hospitals".

**Student Researcher:** Ezeji, Charity Ndidi

**Please tick the box**

1 I confirm that I have understood the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2 I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my rights being affected. ☐

3 I understand that I will not be identified or identifiable in any report subsequently produced by the researcher. ☐

4 I accept that taking part in this study is voluntary and confirm that any risks associated with this have been explained to me. ☐

5 I agree to take part in the above study. ☐

6 I hereby authorize you to take my blood sample. ☐

| _____ | _____ | _____ |
|---|---|---|
| **Participant's Name/ Taking Consent** | **Date** | **Signature** |

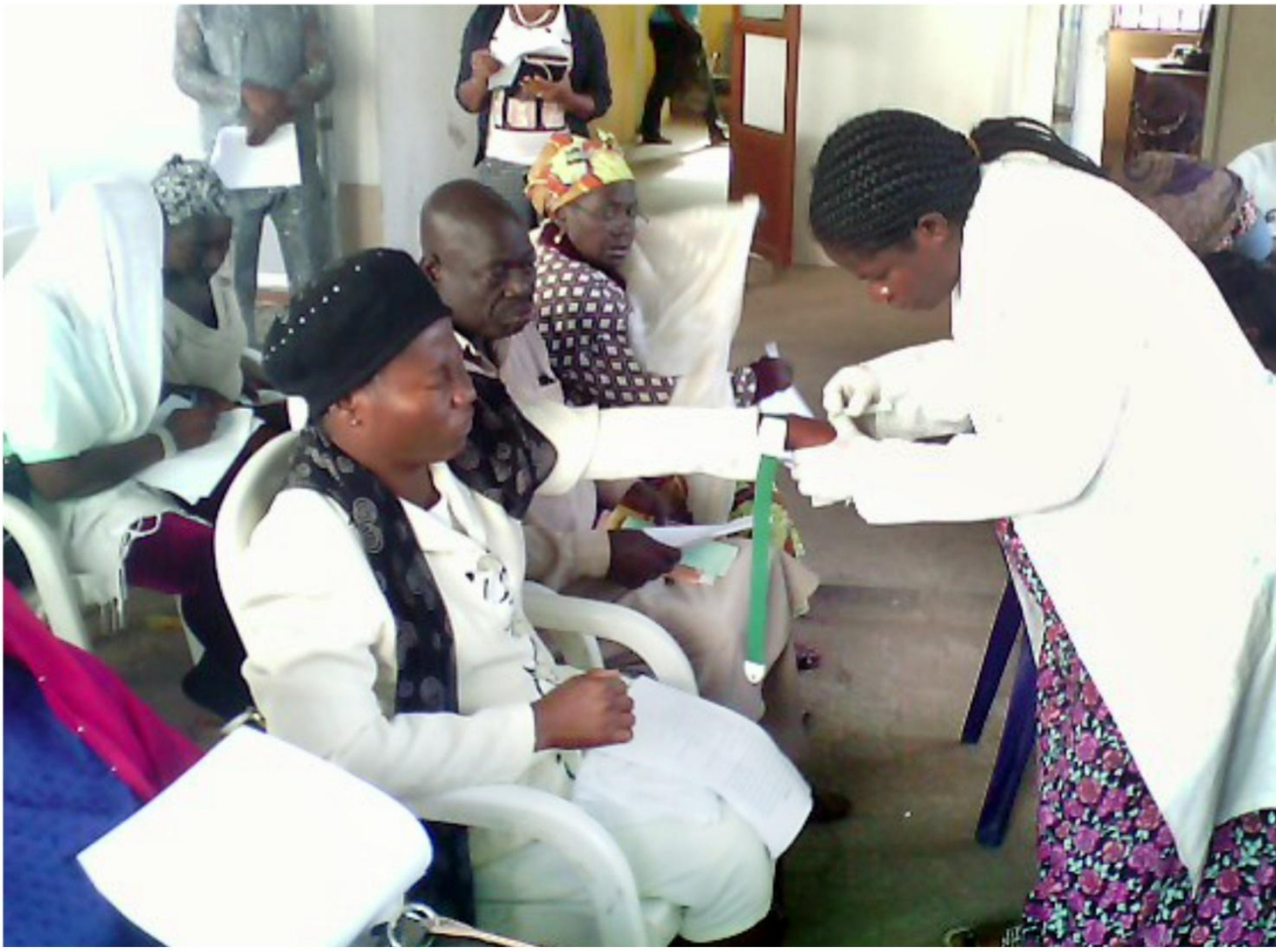
**Name of Person Contact details of the student/researcher: Ezeji, Charity Ndidi**

All Saints' Anglican Church, 22 Douala Street Wuse Zone 5, FCT Abuja. Phone Number: 08037362882, email: n_charitez@yahoo.co.uk.

**APPENDIX 12:**

**PLATES SHOWING SAMPLE COLLECTION IN THE THREE (3) DISTRICT HOSPITAL**

**HIV 1/2 STAT-PAK® Assay**



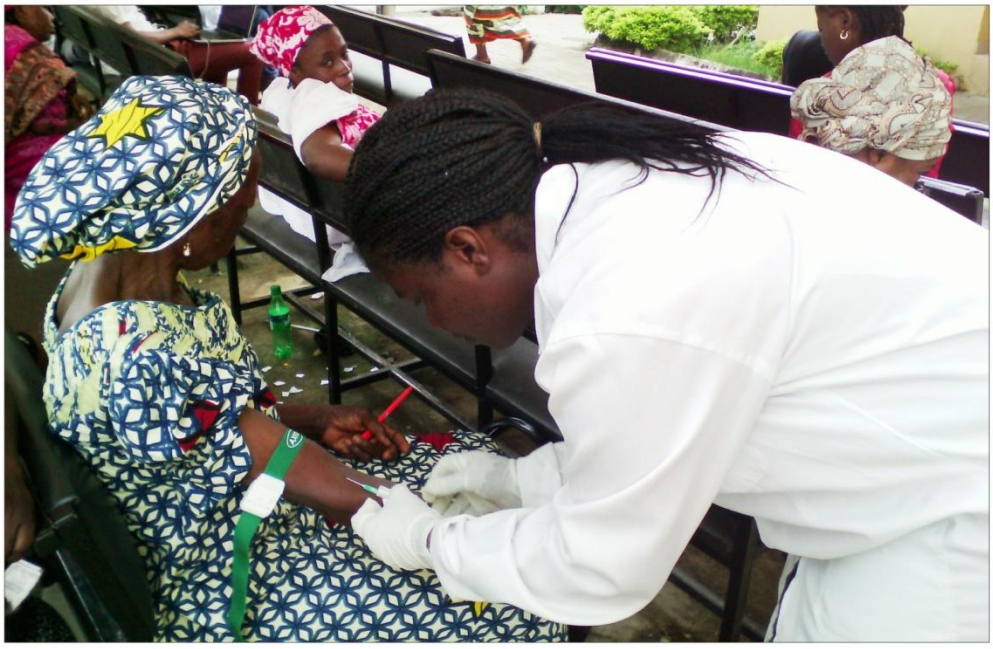


Plate 2: Sample collection at Asokoro District Hospital, Federal Capital Territory, Abuja.

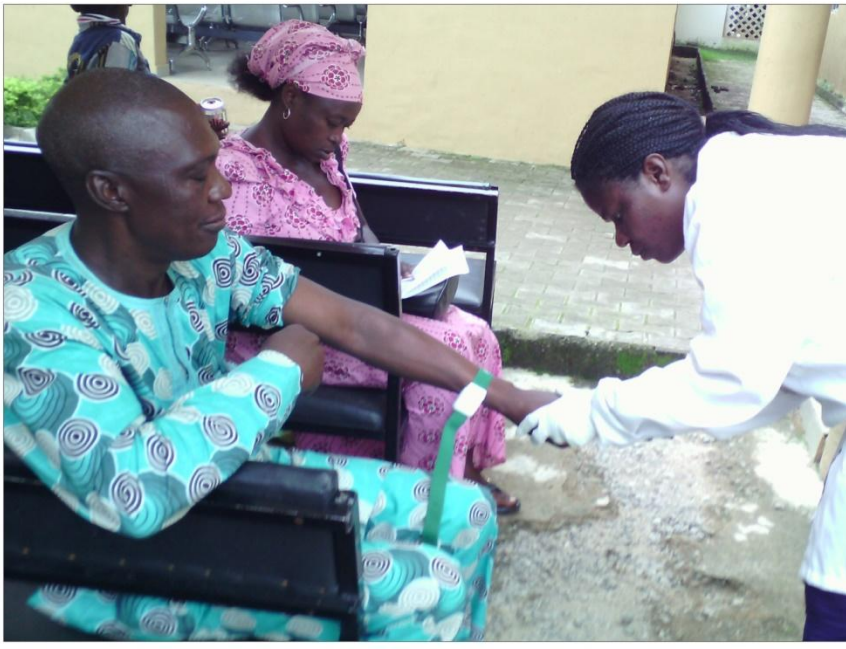


Plate 3: Sample collection at Asokoro District Hospital, Federal Capital Territory, Abuja

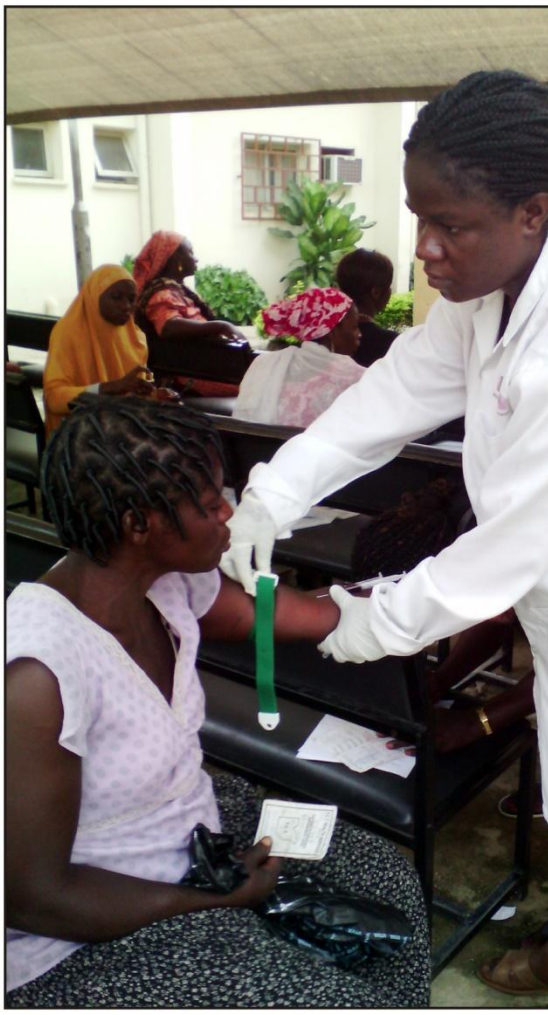


Plate 4: Sample collection at Asokoro District Hospital, Federal Capital Territory, Abuja

Plate 5: Sample collection at Maitama District Hospital, Federal Capital Territory, Abuja

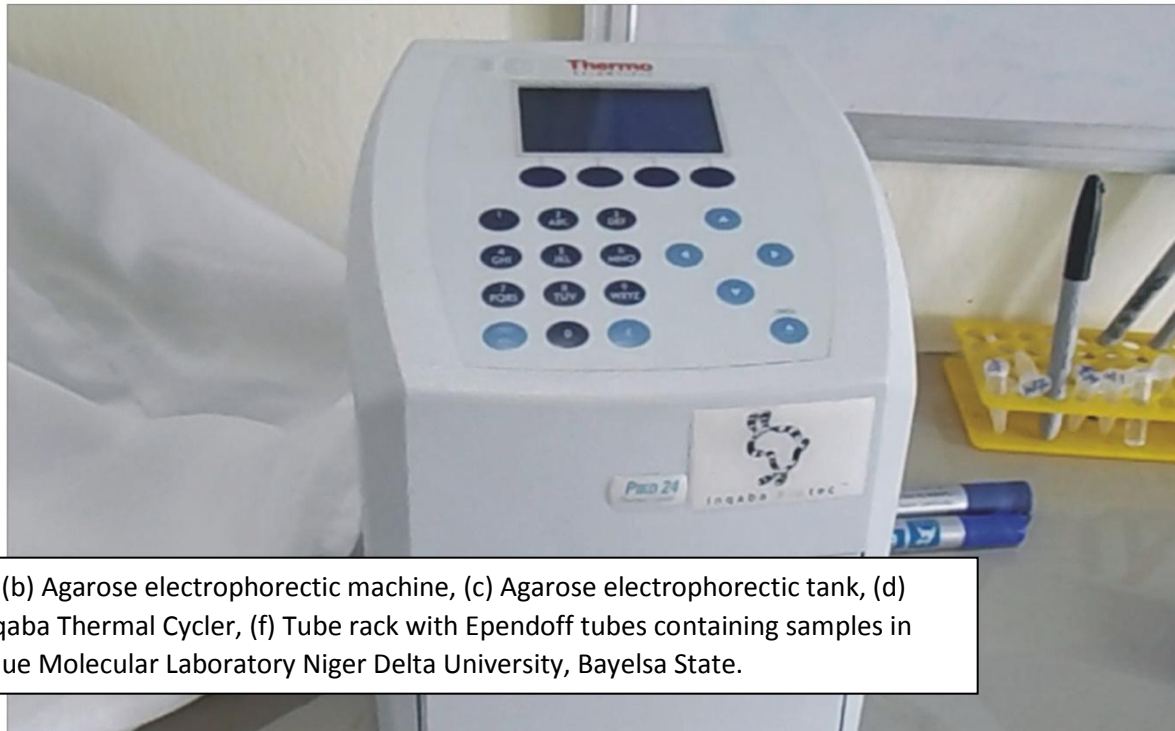
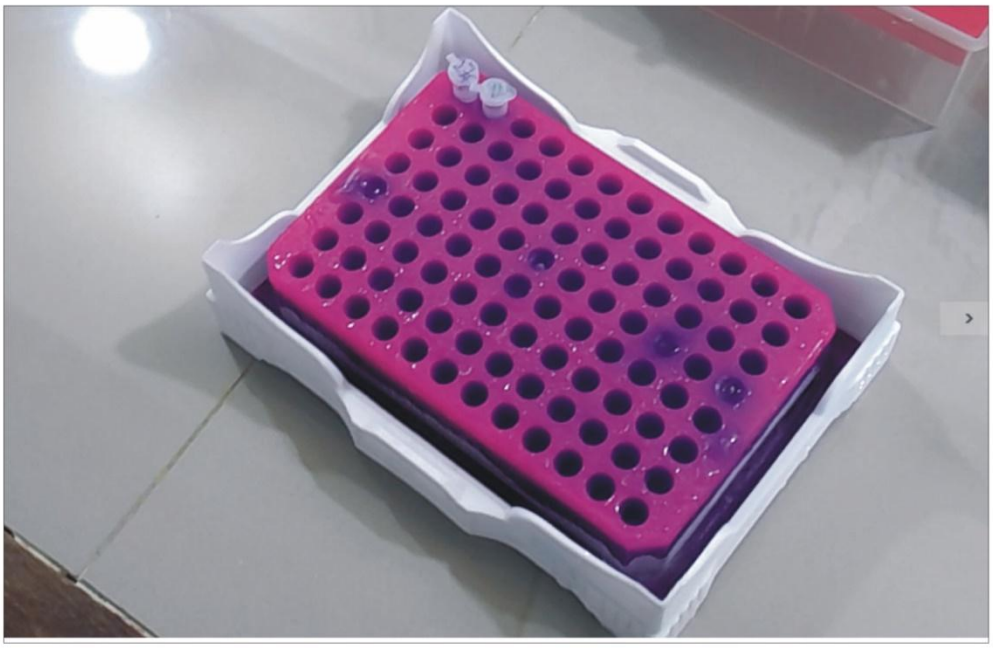
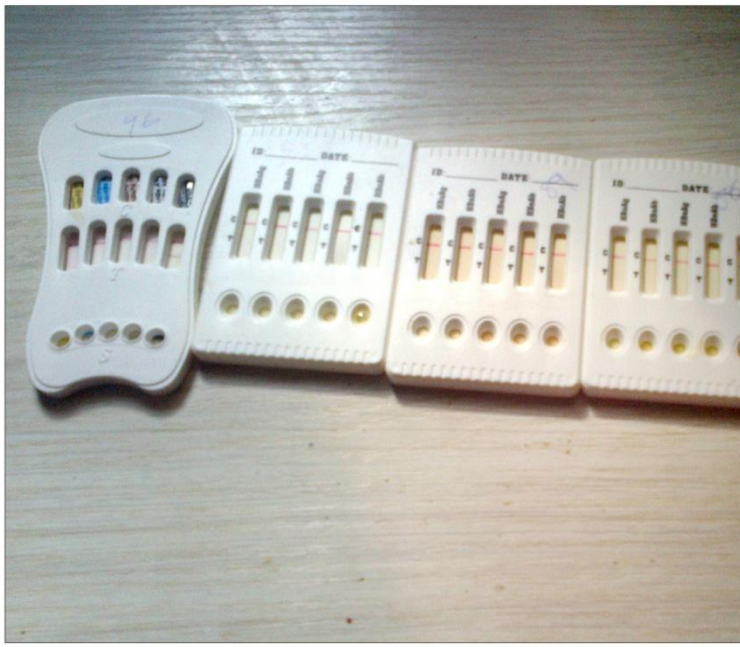



Plate 6: (g) Ice rack, (b) Agarose electrophorectic machine, (c) Agarose electrophorectic tank, (d) Micropipettes (e)Inqaba Thermal Cycler, (f) Tube rack with Ependoff tubes containing samples in Prof Tatfeng Mirabaue Molecular Laboratory Niger Delta University, Bayelsa State.





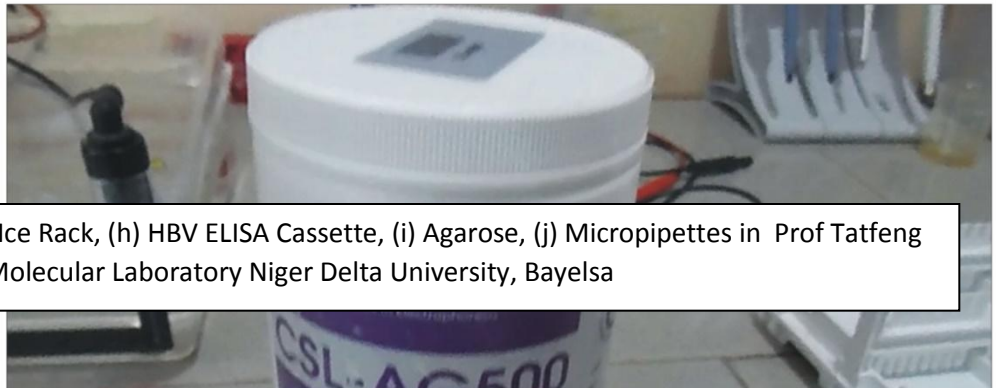


Plate 7: (g) Ice Rack, (h) HBV ELISA Cassette, (i) Agarose, (j) Micropipettes in Prof Tatfeng Mirabaue Molecular Laboratory Niger Delta University, Bayelsa


Plate 8: (k) Charity Operating the Thermal cycler in Prof Tatfeng Mirabaue Molecular Laboratory Niger Delta University, Bayelsa State.

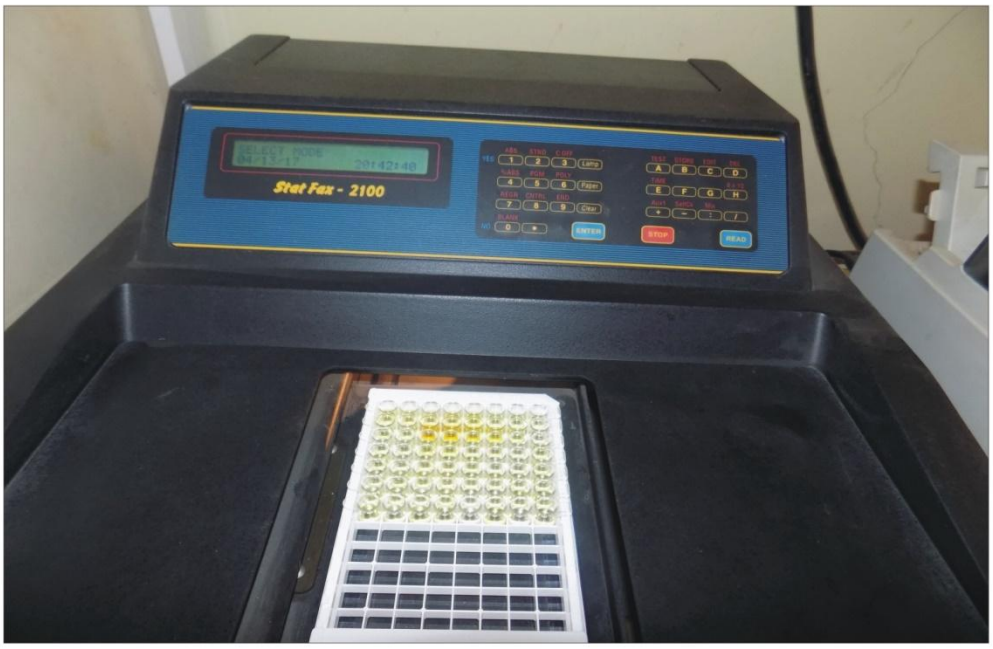
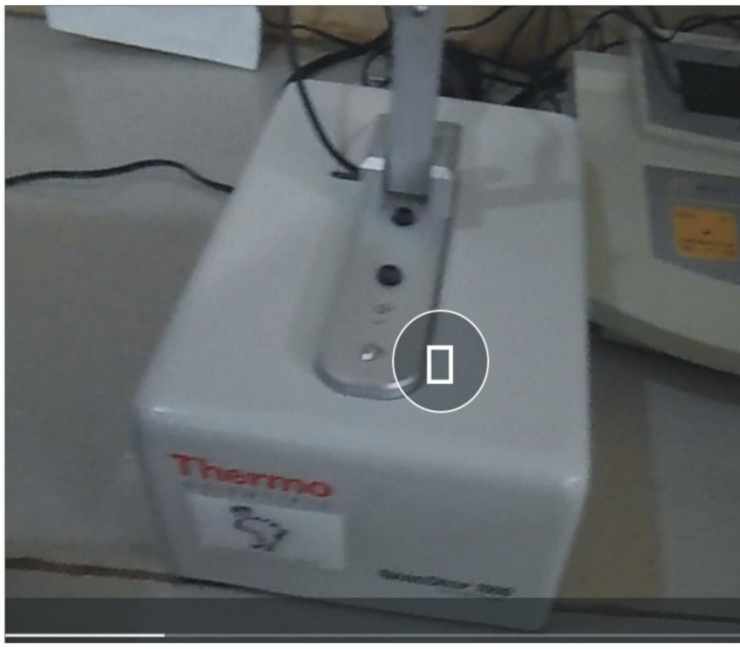




Plate 9: (m) Stat Fax 2100 ELISA plate Reader, (n) Printer in Rapha Biomedical Laboratory and Diagnostics Ltd Gwagwalada, Abuja

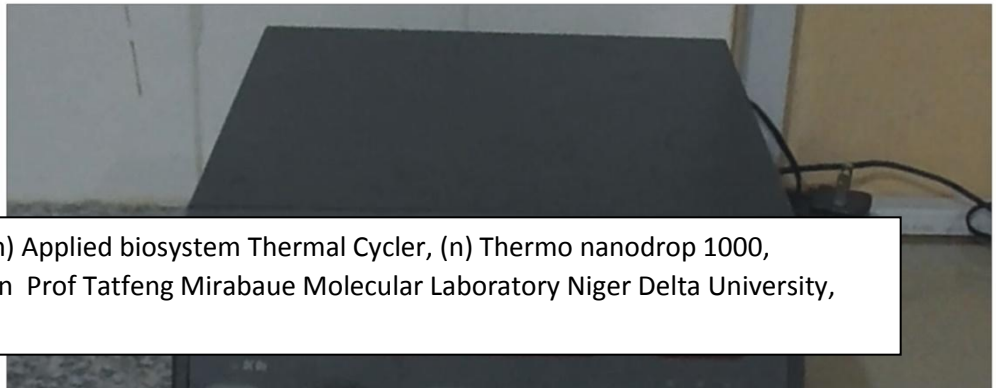
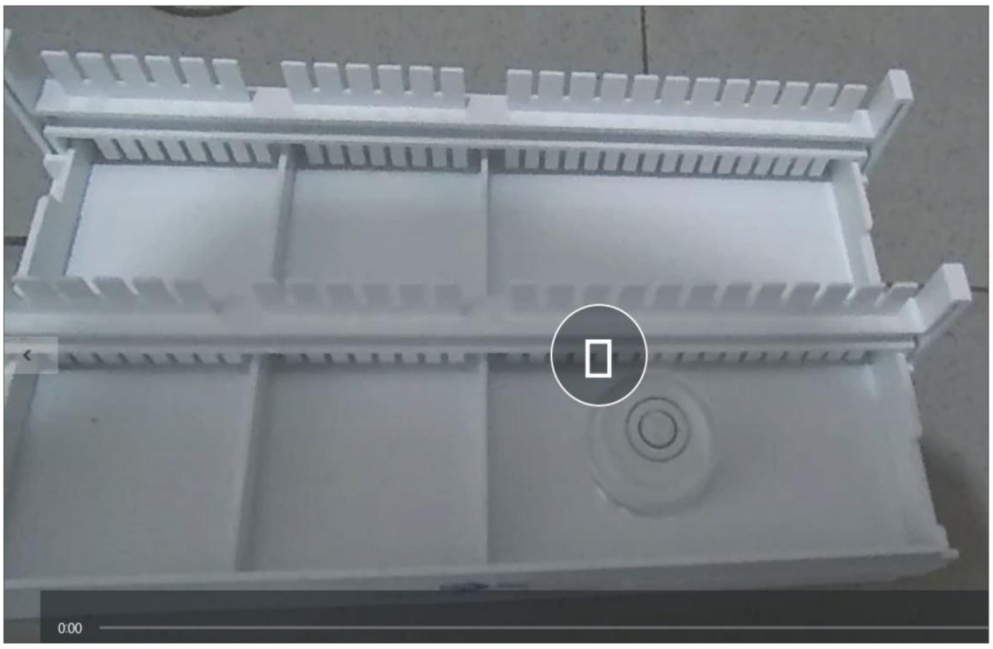



Plate 10: (m) Applied biosystem Thermal Cycler, (n) Thermo nanodrop 1000, centrifuge in Prof Tatfeng Mirabaue Molecular Laboratory Niger Delta University, Bayelsa.



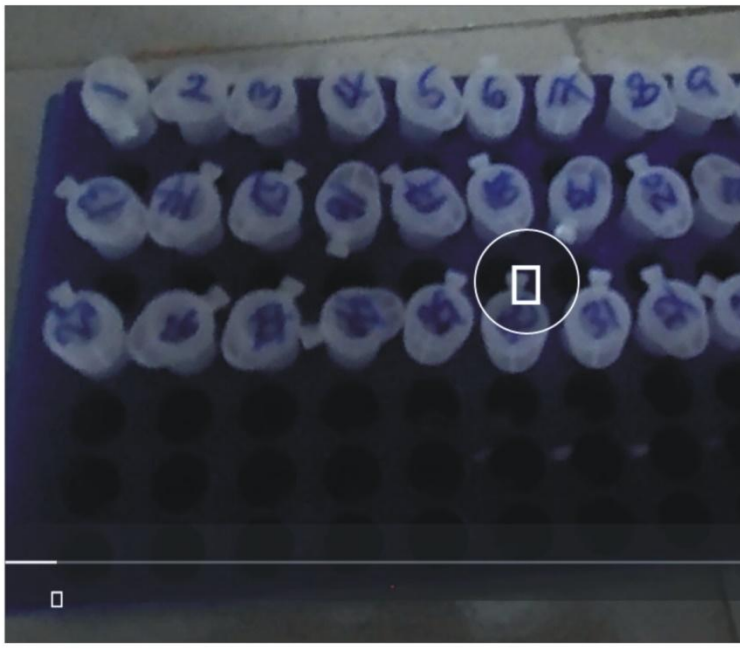
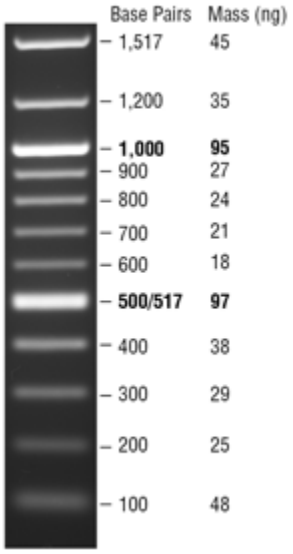
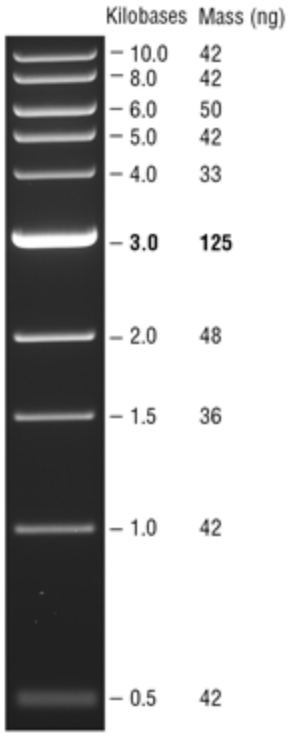


Plate 11: (o) Gel electrophoretic cast with appropriately fixed gel comb and leveler, (p) Eppendoff's tubes containing extracted HBV and HCV DNA samples, (q) Gel electrophoretic cast with appropriately fixed gel comb and agarose gelin Prof Tatfeng Mirabaue Molecular Laboratory Niger Delta University, Bayelsa.




One hundred (100) bp DNA Ladder visualized by ethidium bromide staining on a 1.3% TAE agarose gel. Mass values are for 0.5 µg/lane (Sambrook, *et al*, 1989)




One (1) kbp DNA Ladder visualized by ethidium bromide staining on a 0.8 % TAE agarose gel. Mass values are for 0.5 µg/lane (Sambrook, *et al,* 1989)