CHAPTER ONE
INTRODUCTION

Human Immuno Deficiency Virus (HIV), a non-oncogenic retrovirus, is the causative agent of Acquired Immuno Deficiency Syndrome (AIDS) and AIDS related complexes. HIV belongs to the family retroviridae and the subfamily lentiviridae known as "slow viruses" having a long incubation period with gradual onset and progressive cause of disease that invariably ends in death. AIDS was first described in 1981 and the responsible virus was isolated by the end of 1983. Since then, HIV/ AIDS have become pandemic, expanding in scope and magnitude as HIV infections have affected different population and geographic regions. Millions are now infected worldwide; once infected, individuals remain infected for life, as there has been no cure for HIV infection and AIDS (Barles, 2003).

HIV is the acronym for Human Immuno-deficiency virus, a virus transmitted from one person to another by exchanging body fluids through sex, sharing needles or sharps and from mother to infant. There is currently no cure or vaccine for the HIV virus, although several research companies are currently working toward a vaccine. Specifically, AIDS is diagnosed when a certain number of specific illnesses, referred to as AIDS defining illness, occur and/or when the T4 helper cells drop below a given limit (less than 200cells/mm^3). T4 helper cells, or CD_4^+ cells, are components of white blood cells. White blood cells are immune cells and a lower amount indicates the weakening of the immune system. The onset of AIDS is different for specific individuals living with HIV and depends on a number of factors (Ahaneku, 2010).

Within a period of about 10 years, if left untreated, the vast majority of HIV-infected individuals develop HIV-induced deficiency in the immune system, resulting in AIDS. An individual is said to have AIDS when he/she starts having opportunistic infections or when the CD4 cell count is below 200/μl of blood in the presence of HIV infection. The time it takes for HIV infection to become a full blown AIDS also depends on the strain of the virus, and host/genetic factors. The period could be shorter than 10 years in developing countries (Thomas, 2006).

Currently, there is no cure for HIV/AIDS or vaccine to successfully prevent HIV infection. However, some therapies can be used to treat or even heal some of the opportunistic

infections and relieve the symptoms associated with HIV/AIDS, which include fever, coughing, itching, poor appetite, difficulties in breathing and swallowing and chronic diarrhoea. Another group of drugs known as antiretroviral drugs (ARVs) directly attack the HIV and significantly reduce the rate of replication of the virus in the body of the infected persons thus decreasing the viral load and slowing down the progression of HIV disease.

There are two types of HIV, HIV-1 and HIV-2. HIV-1 is more prevalent and potent than HIV-2. The HIV-1 has spread throughout the world. Although HIV-2 was initially endemic primarily in areas of West Africa, infection has now been documented all over the world, but at a significantly lower prevalence than HIV-1. HIV-1 is responsible for the devastating HIV pandemic that exists today and has been used as the protype in the majority of the studies on HIV pathogenesis and treatment. Both HIV-1 and 2 are closely related to the Simian Immuno deficiency Viruses (SIVs), which were distributed among and derived from various species of ancestral primates (Gallo, 2005).

Precisely, there are about 3 ways by which HIV can be transmitted from infected individuals to healthy persons. They include

a. Sexual contact with infected people.
b. Transmission through transfusion of contaminated blood and it's products and use of non-sterilized instruments, such as needles, razors and other medical implements which may be contaminated (Tefler *et al*., 2004).
c. Vertical transmission from infected mother to her child during pregnancy, birth or breast feeding.

Hepatitis B virus (HBV), hepatitis C virus (HCV) and Syphilis infections are common among patients with HIV infection because of the shared routes of viral transmission. Liver disease due to chronic HBV & HCV infection is becoming the leading cause of death among persons living with HIV worldwide. Hepatitis B (HBV) and C (HCV) are disease causing viruses that attack the liver. The viruses can cause lifelong infection, cirrhosis (scarring) of the liver, liver cancer, liver failure and death (CDC, 2002).

Hepatitis B virus and HIV can be transmitted in similar ways but hepatitis B is more infectious. Both are spread by contact with infected body fluids such as blood, semen and vaginal fluid, or from mother to her baby during pregnancy or delivery. Coinfection with HBV and HIV is common, with 70-90% of HIV infected individuals having evidence of

past or active infection with HBV. HIV infected person are half as likely as HIV uninfected person to spontaneously clear HBV. Therefore chronic HBV infection occurs in 5-10% of HIV infected individuals who are exposed to HBV, a rate 10 times higher than that for the general population. HIV/HBV coinfection rates are highest among men having sex with men (MSM) and injection drug users (Chung *et al*., 2001).

The course of acute HBV may be modified in the presence of HIV infection with a lower incidence of icteric illness and lower rates of spontaneous clearance of HBV. Persons with HIV and chronic HBV coinfection have higher level of HBV DNA and lower rates of clearance of the hepatitis B antigen (HBeAg). Serum transaminase levels may be lower in HIV/HBV coinfected patients than in HBV-monoinfected patients, but normal transaminase level should not be interpreted to mean that there is no underlying hepatic fibrosis. HIV increases the risk of cirrhosis and end stage liver disease in HBV coinfection (Schanschmidt *et al.,* 1992).

The majority of patients coinfected with HIV and HCV are multiple transfused subjects or pervious/current intravenous drug abusers or sexually contacted (Barreiro *et al*., 2006). Following infection with HCV, profound defects occur in cell mediated immunity, specifically impaired CD_4 (cluster of differentiation T lymphocyte class 4) function develop. HIV infection causes immune suppression by destroying CD_4 cells and may also affect HCV replication (Esyter *et al.* 2004).

Studies in hemophiliacs have suggested that liver-related mortality is higher in those patients coinfected with HCV and HIV than in those patients with hepatitis C infection alone, thereby implying that HIV infection adversely affects the natural history of hepatitis C infection (Alemayehu *et al*., 2011). Hepatic failure from HIV-related infections, such as fungal sepsis and mycobacterial infections, is well recognized but in those coinfected with HCV, the increased mortality from liver disease appears to be caused by hepatitis C (Tefler *et al.,* 2004).

HCV infection has a high propensity to persist in the host, in fact, acute infected patients fail to eradicate the virus in about 80% of cases and subsequently develop chronic infection. This condition leads to both extra hepatic and hepatic disorders, mainly chronic liver inflammation, liver cancer and liver cirrhosis. To persist in the host, HCV uses

different strategies aimed at subverting both the innate and adaptive immune responses (Lanucer and Walker, 2001).

The immune system, in an attempt to clear the virus, induces continuous and extensive cytolytic activity on the infected hepatocyctes resulting in chronic inflammation, possibly evolving to severe liver disorders. The immune mediated damage although considered the main mechanism for HCV-related liver injury, is not exclusive, and a direct viral cytopathic effect has been suggested (Rockstroh *et al*., 2005). This effect actively participate in the pathogenic mechanism by killing infected cells, and less directly, by the production of chemokines and cytokines with anti viral and pro-inflammatory activity as well as by shaping the adaptive immune response. In HCV infection, a strong inhibition of natural killer (NK) cells response has been documented and the mechanism of this impairment could be related to the effects of E2 protein on the CD81 molecule in NK cells (Ahmad and Alvarez, 2004).

Epidemiologic studies demonstrate that sexually transmitted diseases (STDs) including syphilis, and particularly genital ulcers associated with primary syphilis, are associated with an increased risk of HIV acquisition. Usually multiple or deeper chancres and overlap of primary and secondary stage features of syphilis occur in HIV and syphilis coinfected patients, Initial serologic responses to early syphilis have been shown to be generally equivalent in HIV-negative and HIV-positive individuals (Kim *et al*., 2002).

Medical Experts now also recommend screening HIV patients at higher risk for syphilis every 3 to 6 months, depending on the level of risk; factors include multiple anonymous partners, concomitant recreational drug use, or frequenting of commercial sex venues or internet sex partner sites. Because serum antibody to *Treponema palladium* (the causative agent of syphilis) is not protective, reinfection may occur if risk is ongoing (Winston *et al*., 2003).

Like many acute infections in HIV-infected patients, early syphilis may decrease CD_4 T-cell counts (CD_4 cell counts) and increase HIV RNA in plasma and semen. Epidemiologic studies demonstrate that history of an STD, including syphilis is associated with an increased risk of HIV disease among both gay men and heterosexuals because sexual behaviours that increased the risk of acquiring STDs also increase the risk of acquiring HIV. Furthermore, genital ulcerations and inflammation caused by STDs are implicated as factors for acquiring or transmitting HIV infection. Individuals already having other STDs

are 3 to 5 times more likely to acquire HIV if exposed to the virus through sexual contact (Stamm *et al*., 1988).

Most HIV-infected patients with *T. pallidium* infection present with typical dermatologic clinical features of primary and secondary disease, such as chancres and diffuse maculopapular rashes. The rash of secondary syphilis can mimic many dermatologic conditions, such as *Tinea versicolor*, *Pityriasis rosea*, Scabies, fixed drug eruptions and erythema multiforme; in HIV-infected individuals taking ART (Anti-retroviral therapy), it has been misdiagnosed as an antiretroviral drug reaction. Like primary lesions, secondary symptoms and signs will resolve without therapy; such resolution marks the beginning of the latent period of the disease (Hutchinson *et al*., 1994).

Liver disease among HIV-infected individuals is a common and important cause of non-AIDS related morbidity and mortality. The liver is of major importance to HIV-positive people as it is responsible for making new proteins needed by the immune system, helps the body to resist infection, and processes many of the drugs used to treat HIV and AIDS- related opportunistic infections. Unfortunately the same medications can also damage the liver, which can prevent the liver from performing all of its necessary tasks and can eventually cause damage to the liver. "Hepatotoxicity" is the official term for liver damage caused by medications and other chemicals (Thomas, 2006).

Liver enzyme elevations are common in HIV-infected patients. The early occurrence of liver enzyme elevations after the recent introduction of antiretroviral drug mainly corresponds with the development of drug-related hepatitis that resolves after discontinuation of the causative drug. Liver enzyme abnormalities may also reflect hepatitis B (HBV) or hepatitis C (HCV) infection, which each have their own risks for chronic immune-mediated liver disease and of direct cytotoxicity (Greub *et al.,* 2000).

The best indicator of hepatotoxicity is an increase in certain liver enzymes that circulate in the blood stream. The most important enzymes are aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). These three enzymes are normally checked as a part of a "Chem screen", a panel of tests each time blood is drawn from an HIV-positive individual for CD_4 count and viral load (Koziel & Peters, 2007).

Other factors that can cause more severe liver disease, include alcohol misuse, drug-associated hepatotoxicity, and fatty liver (steatosis), are also common in infected population (Crum-Cianflone *et al*., 2010).

1.1 Statement of Problem

i. Opportunistic infections contribute to the high morbidity and mortality rates recorded among people living with HIV/AIDS. Hepatitis B,C and Syphilis having common routes of infection with HIV are some of the commonest opportunistic infections in people living with HIV/AIDS.

ii. Organs failure are common among HIV/AIDS infected patients. Liver being one of the important organs in the body if affected leads to elevation in liver enzymes levels in the body such as alanine transferase (ALT/GPT) and alkaline phosphatase (ALP). Coinfection of HIV with hepatitis B or hepatitis C viruses may lead to liver impairment in people living with HIV/AIDS, leading to elevated liver enzymes level.

iii. The use of antiretroviral therapy (ART) especially highly active antiretroviral therapy (HAART) has been implicated as the cause of liver problems experienced by people living with HIV/AIDS.

iv. There are other diseases including other viral diseases called opportunistic infections which might be contributing to the complications experienced by people living with HIV/AIDS. If these other diseases can be promptly diagnosed and treated, will help alleviate the health problems faced by HIV/AIDS patients.

v. Also increase in hepatic enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are common in people living with HIV/AIDS. This may lead to more serious liver diseases and may be as a result of intake of certain ART drug or presence of viral hepatitis. If the hepatic enzymes elevation should be detected early will lead to the withdrawal or substitution of that particular ART drug before it causes a serious damage to the liver.

1.2 Aim of the Study

The aim of this study was to determine the clinical effects of syphilis, hepatitis B & C and hepatic enzymes activities in people living with HIV/AIDS (PLWHA).

1.3 Objectives of the Study

1. To determine the socio-demographic characteristics of PLWHA in Anambra state.
2. To determine the seroprevalence of HIV only, hepatitis B and C co-infections among people living with HIV/AIDS.
3. To determine the seroprevalence of syphilis co-infection among people living with HIV/AIDS.
4. To determine the prevalence of HIV/AIDS patients with raised hepatic enzymes.
5. To determine the effect of hepatitis B and C coinfection on the CD_4 level of the HIV/AIDS patients.
6. To determine the effect of ART on the hepatic enzymes.
7. To determine the effect of raised hepatic enzymes on the CD_4 level of the HIV/AIDS patients.

1.4 Significance of the Study

The study was meant to investigate seroprevalence of Hepatitis B, C, syphilis co-infections and liver enzymes levels and the impact of the co-infections and raised liver enzymes on HIV disease progression in people living with HIV-AIDS.Recommendations will be made on how to prevent or combact those effects in PLWHA if they eventually occur.

CHAPTER TWO
LITERATURE REVIEW

2.1.1 Epidemiology of HIV

Throughout the world, AIDS is the major cause of death for entire generations of people. As the number of infections and deaths reach untold heights, children are left orphans, the elderly are left without care. Some nations face the potential collapse of their society and economy as their people are decimated (Hooshyar *et al.*, 2007).

Sub-Saharan Africa has continued to bear the greatest burden of HIV and AIDS epidemic, with approximately 67.6% of the total number of people living with HIV; 69.2% of the 2.6 million total new infections and 72.2% of the 1.8 million deaths in 2009. Over the decades, the epidemic, once dominated by infected males has become progressively feminized and in sub-Saharan Africa approximately 57% of adults living with the HIV are women. Over 90% of infection in children is acquired through mother-to-child transmission (MTCT) and as more women contract the virus, the number of children infected has been growing (Ahaneku, 2010).

Since the first case of AIDS was reported in a 13-year old girl in Nigeria in 1986, the epidemic has persisted with national HIV sero-prevalence rate of 1.8% in 1991, 5.8% in 2001, 4% in 2005 and 4.6% in 2008. It is currently at 4.1% in 2010 antenatal survey. The prevalence rate in the states of Nigeria ranges from 1.0% to 12.7%. Seventeen states has sero prevalence rates above 5%. The HIV prevalence rate was generally higher in the urban than rural areas. Among young persons, the highest prevalence rate of 5.4% was in the age group of 25 to 29 years old. By the end of 2008, it was estimated that there were 2.955 million Nigerians living with HIV. New infections in adults were 323,000 while 57,000 infants were born with HIV. Heterosexual transmission accounts for 80-95% of all HIV infections, Mother To Child Transmission [MTCT] accounts for 10%, while another 10% is transmitted through the use of unsterilized sharps, infected blood and blood products (Ahaneku, 2010)

2.1.2 Laboratory Diagnosis of HIV

Evidence of infection by HIV can be detected in three ways:

1. Serologic determination of antiviral antibodies
2. Virus isolation

3. Measurement of viral nucleic acid or antigens

Serologic determination of antiviral antibodies

Two common types of test currently being used for HIV detection are the Enzyme Immune Assay (EIA) and the Enzyme Linked Immunosorbent Assay (ELISA). If the initial EIA test result shows a reaction, the same blood is tested again. If reactive twice, a confirmation test such as the Western Blot is used and an HIV positive result is returned. All HIV screening tests must be confirmed by another, more specific test. Other tests include: The Radio Immuno Precipitation Assay (RIPA), which is another follow-up test used when antibody levels are low or difficult to detect; the Dot-blot immunobinding assay, which is a rapid screening blood test; and Polymerase Chain Reaction (PCR), a blood test capable of detecting the virus in a recently infected person by scanning the genetic information of HIV.

Easily confused are the laboratory tests and the devices (sometimes called kits) used to collect specimens, which can involve blood, urine, saliva, and vaginal secretions. Example of testing kits include the confide HIV home test. The orasure saliva/cheek swab test, and the recent approved 20-minute "rapid test". There are advantages and disadvantages associated with each test relating to confidentiality, ease of collection, required personnel and reporting time. A positive rapid test result is confirmed either by a different rapid test or by another laboratory tests. If the result of the two tests differ, a third test is generally done using a third kit which is known as the "Tie breaker" (Mellor *et al*., 1996).

Since the approval of ELISA in 1985, detection methods have improved and their associated costs have lessened. As the majority of HIV tests detect antibodies, the major factor impacting the ability for any test to detect infection is time. Due to the window period between infection and the development of detectable antibodies, recently infected individuals are often missed by even the most advanced testing methods, which can impact projections. According to the CDC (2002) it takes at least 3 months for the detection of antibodies, and they recommend testing every 6 months. In addition to the time that it takes for antibodies to reach detectable levels, there is also a problem of the time between testing and results. Many people who are tested often fail to retrieve results. This is the problem that the rapid tests was developed to counter.

From the beginning of the AIDS epidemic to the early 90s, AIDS is considered to be a disease that affected many untold populations, and was invariably quickly fatal. Evidence today, however, shows that HIV and AIDS are managed more as chronic diseases. With the advent of highly active antiretroviral therapy (HAART) many people with HIV are living greatly increased life spans. People with AIDS historically have died from immune failure and the onset of the opportunistic diseases, but today die of many of the life threatening chronic diseases found in HIV negative people, such as diabetes, heart disease, and more common forms of cancer (CDC, 1999).

Virus Isolation

HIV can be cultured from lymphocytes in peripherial blood and occasionally from specimens from other sites. The numbers of circulating infected cells vary with the stage of infection. Higher titers of virus are found in the plasma and in peripherial blood cells of patients with AIDS as compared with asymptomatic individuals. The magnitude of plasma viremia appears to be a better correlate of the clinical stage of HIV infection than the presence of any antibodies. The most sensitive virus isolation technique is to cocultivate the test sample with uninfected, mitogen stimulated peripherial blood mononuclear cells (Koziel & Peters, 2007). Primary isolates of HIV grow very slowly compared with the laboratory adopted strains. Viral growth is detected by testing culture supernatant fluids after about 7-14days for viral reverse tanscriptase activity or for virus specific antigen (P24).

The vast majority of HIV-1 antibody-positive persons will have virus that can be cultured from their blood cells. However, virus isolation techniques are time-consuming and laborious and are limited to research studies. PCR amplification techniques are commonly used for detection of virus in clinical specimens (Jawetz *et al*., 2010).

Detection of Viral Nucleic Acid or Antigens

Amplification assays such as the RT-PCR, DNA PCR, and bDNA tests are commonly used to detect viral RNA in clinical specimens. The RT-PCR assay uses an enzyematic method to amplify HIV RNA; the bDNA assay amplifies viral RNA by sequential oligonulceotide hybridization steps. The tests can be quantitative when reference standards are used; appropriate positive and negative controls must be included with each test (Greub *et al*., 2000). These molecular-based tests are very sensitive and form the basis for plasma viral

load determinations. HIV sequence heterogeneity may limit the sensitivity of these assays to detect HIV infections. The HIV RNA levels are important predictive markers of disease progression and valuable tools with which to monitor the effectiveness of antiviral therapies.

Early diagnosis of HIV infection in infants born to infected mothers can be accomplished using plasma HIV-1 RNA tests. The presence of maternal antibodies makes serologic tests uninformative.

Low levels of circulating HIV-1 p24 antigen can be detected in the plasma by EIA soon after infection. The antigen often becomes undetectable after antibodies develop because the P24 protein is complexed with p24 antibodies but may appear late in the course of infection, indicating a poor prognosis (Jawetz *et al.,* 2010).

2.1.3 CD_4 T Cells

Cellular component of blood comprises red blood cells and white blood cells. Two populations of leucocytes constitute the later- the granulocytes and non-granulocytes including the lymphocytes. Surface receptors of the lymphocytes provide identity to sub population of lymphocytes which differentiate into unique clusters. This property gives the subtypes of lymphocytes a nomenclature of clusters of differentiation followed by the number of the unique subtypes (CD_1 CD_2, CD_3, CD_4....). CD_4 stands for cluster of differentiation. CD numbers are now used to identify cell surface antigen that can be distinguished by monoclonal antibodies. CD_4 T-cells are also known as helper cells and play a vital role in managing the integrity of human immune system. CD_4 cells are the primary target of HIV which is preferentially depleted during the course of HIV disease. CD_4 cells send signals to activate the body immune response when they dictate intruders like viruses and bacteria (WHO, 2007).

Once a person is infected with HIV, the virus begins to attack and destroy the CD_4 cells of the person's immune system. HIV uses the machinery of CD_4 cells to multiply (make copies of itself) and spread throughout the body (Bernard *et al.,* 1984).

2.1.4 CD_4 Cell Count

CD4 cell count is the most significant immunological tests for monitoring HIV infected individuals. The utility of CD_4 T cell measurements involves clinical considerations for HIV disease classification and AIDS definition, assessment of prognosis, and the design of

clinical trials. It is well recognized that accurate and reliable enumeration of CD_4 T cell count is very crucial for monitoring the rate of progression to AIDS, both for initiating prophylaxis for opportunistic infections as well as monitoring the impact of antiretroviral therapy (Wondimeneh *et al.*, 2013). The CD_4 count test in addition to plasma viral RNA levels (viral load) and clinical conditions of the patient facilitates decisions regarding the initiation or change of antiretroviral therapy (Foti *et al*., 2002).

2.1.5 Methods of Enumeration of CD_4 T Cells

The major available technique, flow cytometry, in combination with automated blood cell analyzers have been used to quantitate directly various T-lymphocytes subtypes such as CD_4 and CD_8. Due to the high cost of flow cytometry, manufacturers have introduced manual non-cytofluoremetric method of CD_4 and CD_8 estimation. Available methods are based on immunological resetting and enzyme immune assay technique. CD_4 cells can be expressed in absolute count, percentage or ratios (Johnson & Kuritzikes, 1997).

CD_4 depletion causes the characteristic immune deficiency. The decline in CD_4 T lymphocytes count may be influenced by various factors such as plasma virus level, opportunistic infections, nutritional factors etc. Based on the speed of disease progression, HIV-1 infected individuals can be categorized as typical progressors, rapid progressors and long term non-progressors. Typical progressors show latent period of 5 to 8 years before appearance of clinical AIDS. About 10% of HIV-infected individuals rapidly progress to AIDS within 2 to 3 years after infection that is the rapid progressors where as less than 5% do not show clinical symptoms even after 10 years of infection in absence of antiretroviral therapy. They are the long term non-progressors (CDC, 1999).

Normal values of CD_4 cell counts are 400-1200 cell/μl for men and 500-1500 cells/μl for women. In general, treatment should be given to HIV patients with CD_4 cell count less than 350cells/μl (UNAIDS, 2004).

2.1.6 Monocytes and Macrophages

Monocytes and macrophages play a major role in the dissemination and pathogenesis of HIV infection. Certain subsets of monocytes express the CD_4 surface antigen and therefore bind to the envelope of HIV. The HIV coreceptor on monocytes and macrophages is CCRS chemokine receptor. In the brain, the major cell types infected with HIV appear to be the monocytes and macrophages and this may have important consequences for the

development of the neuropsychiatric manifestation associated with HIV infection. Infected pulmonary alveolar macrophages may play a role in the interstitial pneumonitis seen in certain patients with AIDS Bernard *et al*., 1984).

Macrophage – tropic strains of HIV predominate early after infection, and these strains are responsible for initial infections even when the transmitting source contains both M-tropic and T-tropic viruses.

It is believed that monocytes and macrophages serve as major reservoirs for HIV in the body. Unlike the CD_4 T lymphocyte, the monocyte is relatively refractory to the cytopathic effects of HIV, so that the virus not only can survive in this cell but can be transported to various organs in the body such as the lungs and brain. Infected macrophages may continue to produce virus for a long period of time (Wondimeneh *et al*. 2013).

2.1.7 Lymphoid Organs

Lymphoid organs play a central role in HIV infection. Lymphocytes in the peripherial blood represent only about 2% of the total lymphocyte pool, the remainder being located chiefly in the lymphoid organs. It is in the lymphoid organs that specific immune responses are generated. The network of follicular dendritic cells in the germinal centres of lymph nodes traps antigens and stimulates an immune response. Throughout the course of untreated infection – even during the stage of clinical latency – HIV is actively replicating in lymphoid tissues. The microenvironment of the lymph node is ideal for the establishment and spread of HIV infection. Cytokines are released, activating a large pool of CD4 T cells that are highly susceptible to HIV infection. As the late stages of HIV disease progress, the architecture of the lymph nodes becomes disrupted (Foti *et al*. 2002).

2.1.8 Neural Cells

Neurological abnormalities are common in late stages of infection and are an AIDS – defining condition. Central nervous system disease occurs to varying degrees in 40-90% of patients. These include HIV encephalopathy, peripherial neuropathies and most serious – AIDS dementia complex. Both direct and indirect pathogenic mechanisms might explain the neuropsychiatric manifestations of HIV infection. The predominant cells types in the brain that are infected with HIV are monocytes and macrophages. Vines may enter the brain through infected monocytes and release cytokines that are toxic to neurons as well as

chemotactic factors that lead to infiltration of the brain with inflammatory cells. HIV is present rarely if at all, in neuron, oligodendrocytes and astrocytes (Jawetz *et al.*, 2010).

2.1.9 Viral Coinfections

Activation signals are required for the establishment of a productive HIV infection. In the HIV-infected individual, a wide range of in vivo antigenic stimuli seem to serve as cellular activators. For example, active infection by *Mycobacterium tuberculosis* substantially increases plasma viremia. The damaging effects of HIV on the immune system leave patients vulnerable to many types of infection. The World Health Organization reports that infection with HIV increases the risk of getting tuberculosis as much as 20-fold. Of the 9 million new tuberculosis cases worldwide in 2006, it is estimated that 15% occurred in persons infected with HIV (WHO, 2007).

Other concomitant viral infections like Epstein-Barr virus, cytomegalovirus, herpes simplex virus, or hepatitis B virus may serve as cofactors of AIDS. Hepatitis C virus coinfection, which occurs in 15-30% of HIV cases in the United States and often result in liver disease, is a leading cause of morbidity and mortality in HIV-infected persons. There is also a high prevalence of cytomegalovirus infection in HIV positive individuals.

Coinfections with two different strains of HIV can occur. There are documented cases of Super infection with a second strain in an HIV-infected individual, even in the presence of a strong CD_8 T cell response to the first strain. HIV super infection is considered to be a rare event (Jawetz *et al.*, 2010).

2.1.10 Replication Cycle of HIV

The life of HIV is generally programmed to highjack the reproductive machinery of human cells, and then trick it into churning out as many copies of the virus as it can before the cell dies. The stages involved in the replication cycle include Binding, Fussion,- Reverse transcriptase, Integration, Replication, Assembly and Budding (Alemayehu *et al*., 2011).

Binding (also called attachment): HIV envelope structure of gp120 envelope protein binds to CD_4 receptors on the surface of some T-lymphocytes, macrophages and microglial cells. The binding of CD_4 with gp120 of the virus imitates a conformational change in the CD_4 molecule to align gp120 with co receptors that facilitate HIV attachment and fussion.

Fussion- After binding, the viral envelope gp 120 fuses with the membrane of the target cell and the contents of the virus is subsequently taken up into the cell cytoplasm.

Reverse transcription - Inside the host cell, the genetic information is transcribed (transferred from RNA to DNA) in a process known as transcription, but is called reverse transcription in the case of retrovirus such as HIV. This process is made possible by an enzyme called reverse transcriptase. Reverse transcriptase then directs the synthesis of complementary strand of DNA on the RNA template from the virus, after which the enzyme also directs the synthesis of a second strand of DNA complementary to the initial DNA strand (Levy, 1998).

Integration:- The DNA now bearing the viral genetic information is inserted into the host cell DNA through the activity of an enzyme called integrase to produce for provirus. The provirus may remain inactive for several years, producing few or no new copies of HIV.

Replication: This is the transcription of the genetic information in the proviral DNA into messenger RNA and the mRNA is subsequently translated into viral proteins. These protein chains are the building blocks for more HIV.

Assembly: These various products are then assembled to produce new viral particles or progeny viral particles. Each progeny particle also contains new RNA and enzymes, along with some cellular components. These are immature non-infectious HIV.

Budding: New formed immature HIV pushes itself out of the CD_4 cell. The new HIV releases protease (an HIV enzyme). Protease acts to break up the long protein chains that form the immature HIV virus. The smaller HIV proteins combined to form mature (infectious) HIV (Fields *et al*., 1996).

2.1.11 Stages of HIV/AIDS

World Health Organization (WHO, 1995) categorizes HIV infection and AIDS into four stages. This classification is designed to be used with confirmed HIV infection. Along with the measurement of the CD_4 T lymphocytes count, where available, the staging system is used to guide decisions on when to start prophylaxis for opportunistic infections and when to start and switch antiretroviral therapy.

Stage 1 Asymptomatic period: This stage is the acute initial stage of virus entry into it's host. Most people newly infected with the virus show no symptoms. A small number of newly infected individuals may show few symptoms for a few years (Ahaneku, 2010). The

asymptomatic period brings about the general saying that ‘HIV no dey show for face’. In this stage, the virus is actively multiplying, infecting and killing cells in the immune system and one can transmit the virus to uninfected persons. As the immune system weakens, symptoms begin to emerge.

Stage 2 Early (Minor) symptoms of HIV: Some HIV infected people develop minor symptoms within a month or two of exposure to HIV. The symptoms may include fever, rash, headache, loss of appetite, swollen glands, achy muscles and joints. These early symptoms usually disappear within a week to a month. Most HIV-infected people who experience these early symptoms will not experience more signs of infection for at least a few years and even, some may remain asymptomatic for life (WHO, 1995).

Stage 3 Moderate symptoms of HIV: It usually takes about 8 to 9 years from the time of infection to the appearance of later symptoms, although this varies from person to person, and depends on so many factors. These symptoms are signals that the immune system function is deteriorating due to declining CD_4 + T-cells. The symptoms associated with this stage are persistent, enlarged lymph nodes, excess fatigue, weight loss, frequent fever, night sweet, genital sores, thrush, skin rash, bone pain and blurred vision.

As the CD_4 + T-cells drops and the immune system deteriorates further, infected individuals may continue to experience symptoms and also develop new ones. This stage if not properly managed, it will result to AIDS manifestations.

Stage 4 AIDS stage with major symptoms: The term AIDS stand for Acquired Immuno Deficiency Syndrome. AIDS is an advanced stage of HIV infection, when the immune system has sustained substantial damage that make the individual highly vulnerable to life-threatening infections and diseases like tuberculosis and various types of cancer. Not every one who has HIV infection progresses to AIDS. It takes about 10 years for HIV to develop to AIDS, though this varies greatly from person to person and depends on many factors including a person health status and health related behaviours. AIDS associated conditions include opportunistic infections by bacteria, fungi and virus; development of certain cancers including cervical cancers and lymphomas; and certain autoimmune disorders. Symptoms of the opportunistic infections may include coughing and shortness of breath, seizure and lack of coordination, difficult or painful swallowing, mental retardation, severe

and persistent diarrhea, fever, vision loss, nausea, vomiting, extreme weight loss and fatigue, severe headache and coma (WHO (1995).

2.1.12 Clinical Findings

Symptoms of acute HIV infection are non specific and include fatigue, rash, headache, nausea, and night sweets. AIDS is characterized by pronounce suppression of the immune system and development of a wide variety of severe opportunistic infections or unusual neoplasms especially Kaposi sarcoma. The more serious symptoms in adults are often preceded by a prodrome ("diarrhoea and dwindling") that can include fatigue, malaise, weight loss, fever, shortness of breath, chronic diarrhoea, white patches on the tongue (hairy leukoplakia, oral candidiasis), and lymphadenopathy. Disease symptoms in the gastrointestinal tract from the oesophagus to the colon are a major cause of debility. With no treatment, the interval between primary infection with HIV and the first appearance of clinical disease is usually long in adults, average of about 8-10 years. Death occurs about 2 years later (UNAIDS, 2004).

2.1.12a Plasma Viral Load

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 virus in the blood (viral set point) varies from individual to individual during the asymptomatic period. This level reflects the total number of productively infected cells and their average burst size. It turns out that a single measurement of plasma viral load about 6 months after infection is able to predict the subsequent risk of development of AIDS in men several years later. High set points tends to correlate with rapid disease progression and poorer responses to treatment. 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 HIV RNA levels can be determined using a variety of commercially available assays. The plasma viral load appears to be the best predicator of long-term clinical outcome, where as CD4 lymphocytes counts are the best predictor of short-term risk of developing an opportunistic disease. Plasma viral load measurements are a critical element in assessing the effectiveness of antiretroviral drug therapy (Fields *et al.*, 1996).

2.1.12b Pediatric AIDS

The responses of infected neonates are different from those observed in HIV-infected adults, Pediatric AIDS – acquired from infected mothers – usually presents with clinical symptoms by 2 years of age; death follows in another 2 years. The neonate is particularly susceptible to the devastating effects of HIV because the immune system has not developed at the time of primary infection. Clinical findings may include lymphoid interstitial pneumonitis, pneumonia, severe oral candidiasis, encephalopathy, wasting, generalized lymphadenopathy, bacterial sepsis, hepatosplenomegaly, diarrhoea and growth retardation (Levy, 1998).

Children with perinatally acquired HIV-1 infection, if untreated, have a very poor prognosis. A high rate of disease progression occurs in the first few years of life. High levels of plasma HIV-1 load appear to predict infants at risk of rapid progression of disease. The pattern of viral replication in infants differs from that in adults viral RNA load levels are generally low at birth, suggesting infection acquired close to that time, RNA levels then rise rapidly within the first 2 months of life and are followed by a slow decline until the age of 24 months, suggesting that the immature immune system has difficulty containing the infection. A small percentage of infants (about 5%) display transient HIV infections, suggesting that some infants can clear the virus (Jawetz *et al.*, 2010).

2.1.12c Neurologic Disease

Neurologic dysfunction occurs frequently in HIV-infected persons. Forty to ninety percent of patients have neurologic symptoms, and many are found during autopsy to have neuropathologic abnormalities.

Several distinct neurologic syndromes that occur frequently include subacute encephalitis, vacuolar myelopathy, aspetic meningitis, and peripheral neuropathy. AIDS dementia complex, the commonest neurologic syndrome, occurs as a late manifestation in 25-65% of AIDS patients and are characterized by poor memory, inability to concentrate, apathy, psychomotor retardation, and behavioural changes. Other neurologic diseases associated with HIV infection include toxoplasmosis, cryptococcosis, primary lymphoma of the central nervous system and JC virus induced progressive multifocal leuko-encephalopathy. Mean survival time from onset of severe dementia is usually less than 6 months.

Pediatric AIDS patients also display neurologic abnormalities. These include seizure disorders, progressive loss of behavioral developmental milestones, encephalopathy, attention deficit disorders and developmental delays. HIV encephalopathy may occur in as many as 12% of children, usually accompanied by profound immune deficiency. Bacterial pathogens predominate in pediatric AIDS as the most common cause of meningitis.

As children born with HIV infection are living to adolescence due to antiretroviral therapy, many appear to be at high risk for psychiatric disorder. The most common problems are anxiety disorders (Pratt *et al.,* 2000).

2.1.12d Opportunistic Infections

The predominant causes of morbidity and mortality among patients with late-stage HIV infections are opportunistic infections, that is, severe infections induced by agents that rarely cause serious disease in immune-competent individuals. Opportunistic infections usually do not occur in HIV-infected patients until CD_4 T cell counts have dropped from the normal level of about 1000 cells/μl to less than 200 cells/μl. As treatments are developed for some common opportunistic pathogens and management of AIDS patients permits longer survivals, the spectrum of opportunistic infection changes (UNAIDS, 2004).

The most common opportunistic infections in untreated AIDS patients include the following:

1. Protozoa*: Toxoplasma gondli, Isospora belli, Cryptosporidium species.*
2. *Fungi: Candida albicans, Cryptococcus neoforrmans, Coccidioides immitis, Histoplasma capsulatum, Pneumocystis jirovec*i.
3. Bacteria; *Mycobacterium avium* – intracellular, *M. tuberculosis*, *Listeria monocytogenes*, *Norcadia asteroids*, *Salmonella species*, *Streptococcus species.*
4. Viruses: Cytomegalovirus, herpes simplex virus, varicella-zoster virus, adenovirus, ploymavirus, JC virus, hepatitis B Virus and hepatitis C virus.

Herpes virus infections are common in AIDS patients, and multiple herpesviruses are frequently detected being shed in saliva. Cytomegalovirus retinitis is the most common severe ocular complication of AIDS (Jawetz *et al*., 2010).

2.1.12e Cancer

AIDS Patients exhibit a marked predisposition to the development of cancer, another consequence of immune suppression. AIDS-associated cancers tend to be those with a viral cofactor and include non-Hodgkin lymphoma, Kaposi sarcoma, cervical cancer, and anogenital cancers. Epstein-Barr viral DNA is found in the majority of B cell malignancies classified as Burkitt lymphoma and those of the central nervous system but not found in most systemic lymphoma. Polymavirus SV40 has been detected in some non-Hodgkin lymphomas. Burkitt lymphoma occurs 1000 times more commonly in AIDS patients than in the general population (WHO, 2007).

Kaposi sarcoma is a vascular tumor thought to be of endothelial origin that appears in skin, mucous membranes, lymph nodes, and visceral organs. Kaposi sarcoma is 20,000 times more common in untreated AIDS patients than in the general population. Cervical cancer is caused by high-risk papillomaviruses; the anogenital cancer also arise as a result of coinfections with human papillomaviruses.

Effective antiretroviral drug therapy has resulted in a marked reduction in the occurrence of Kaposi sarcomas but has had less of an effect on the incidence of non-Hodgkin lymphomas in HIV-infected individuals.

As HIV infected persons live longer lives due to effective antiretroviral therapy, they are developing a broad spectrum of cancers at higher frequencies than the noninfected population. These HIV associated malignancies include head and neck cancer, lung cancer, Hodgkin lymphoma, liver cancer, melanoma, and oral cancer. There does not appear to be an increase risk of breast, colon or prostate cancer (Jawetz *et al*., 2010).

2.1.13 Immunity

HIV-infected persons develop both humoral and cellular mediated responses against HIV-related antigens. Antibodies to a number of viral antigens develop soon after infection.

Most infected individuals make neutralizing antibodies against HIV, directed against the envelop glycoprotein. However, the levels of neutralizing activity are low; many anti-envelop antibodies are non-neutralizing. It is believed that the dense glycosylation may inhibit binding of neutralizing antibody to the envelop protein. The envelope glycoprotein shows great sequence variability. This natural variation may allow the

evolution of successive populations of resistant virus that escape recognition by existing neutralizing antibodies.

The neutralizing antibodies can be measured in vitro by inhibiting HIV infection of susceptible lymphocyte cell lines. Viral infection is quantified by:

1. Reverse transcriptase assay which measures the enzyme activity of realized HIV particles.
2. Indirect immunofluorescence assay, which measures the percentage of infected cells; and
3. Reverse transcriptase-polymerase chain reaction (RT-PCR) or branched-chain DNA (bDNA) amplification assays that measure HIV nucleic acids (Ochei and Kolhatkar, 2007).

Cellular responses develop that are directed against HIV proteins. Cytotoxic T lymphocytes (CTLs) recognize *env, pol, gag* and *nef* gene products; this reactivity is mediated by major histocomatibility complex-restricted CD3-CD8 lymphocytes. The *env*. specific reactivity occurs in nearly all infected people and decreases with progression of disease. Natural killer (NK) cell activity has also been detected against HIV-1 gp120. A problem confronting AIDS vaccine research is that the correlates of protective immunity are not known, including the relative importance of humoral and cell mediated immune responses (Foti *et al*., 2004).

2.1.14 Prevention Treatment and Control

A. Antiviral Drugs

HIV anti-retroviral therapy is comprised of four classes of drugs. The first class of HIV drugs is compromised of Nucleoside (or nucleotide) Reverse Transcriptase Inhibitors (NRTIS), which- includes abacavir (Ziagen), abacavir/lamivudine/zidovudine (Trizivir), didanosine, ddl (videx, videx EC, lamivudine, ZTC (Epivir), lamivudine/zidovudine (combivir), ddc (HIVID), tenofovir (Viread), stavudine, d4t (Zerit), and Zidovudine, AZT (Retrovir) (FMOH, 2010).

Nucleoside analogs mimic nucleotides, the basic building blocks of generic materials. The reverse transcriptase enzyme converts HIV RNA into DNA by building new chains of nucleotides. If a nucleoside analog is added to the chain instead of a real nucleotide, the chain cannot be continued. The second class, Non-nucleoside Reverse Transcriptase

Inhibitors (NnRTIs), includes delavirdine (Rescriptor), efavirenz (sustiva), and nevirapine (viramune). NnRTIs interfere with the action of HIV reverse transcriptase enzyme. However they work by binding to the enzyme and preventing it from working (CDC, 2002).

The third, Protease Inhibitors (PIs), contains amprenavir (Agenerase), indinavir (crixivan), iopinavir/ritonvir (kaletra), nelfinavir (viracept), ritonavir (norvir), saquinavir (fortavase), and saquinavir (invirase). Protease inhibitors drugs interfere with the action of the protease enzyme, which cuts newly formed HIV polyproteins (long protein chains) into usable pieces. With the recent inclusion of the fusion inhibitors, Fuzeon, or T-20, there are four main classes of HIV drugs. Fusion inhibitors prevent HIV from attaching or fussing with a host cell. If HIV cannot get into the cell, it cannot replicate and cause disease (NCSL, 2003).

B. Vaccines Against HIV

A safe and effective vaccine offers the best hope of controlling the worldwide AIDS epidemic. Viral vaccines are typically preventive; given to uninfected individuals to prevent either infection or disease. However, all candidate HIV vaccines tested as of 2009 proved ineffective at preventing infection.

Vaccine development is difficult because HIV mutates rapidly, is not expressed in all cells that are infected; and is not completely cleared by the host immune response after primary infection. Because of the safety concerns, vaccines based on attenuated or inactivated HIV or on simian isolates are viewed with apprehension. Recombinant viral proteins – especially those of the envelop glycoproteins – are likely candidates, whether delivered with adjuvants or with heterologous viral vectors. Many novel vaccination methods are also under investigation. Gene therapy approaches are being developed that are designed to achieve “intracellular immunization”; to genetically alter target cells in such a way as to make them resistant to HIV.

A large hurdle of vaccine development is the lack of an appropriate animal model of HIV. Chimpanzees are the only animals that are susceptible to HIV. Not only is the supply scarce, but chimpanzees develop only viremia and antibodies, they do not develop immunodeficiency. The SIV-macaque model of simian AIDS does develop disease and is useful for vaccine development studies (Greub *et al.,* 2000).

C. Topical Microbicides

In many countries in the world, women make up at least 50% of those living with HIV/AIDS, and the majority of those became infected through heterosexual contact. Efforts are underway to develop safe and effective topical microbicides to prevent sexual transmission of HIV. To date, none of the candidates compounds tested has proved effective in clinical trials (Mabala, 2006).

D. Control Measures

Without control by drugs or vaccines, the only way to avoid epidemic spread of HIV is to maintain a lifestyle that minimizes or eliminates the high risk factors. No cases have been documented to result from such common exposures as sneezing, coughing, sharing meals or other casual contacts. Because HIV may be transmitted in blood, all blood donors should be tested for antibody. Properly conducted antibody tests appear to detect almost all HIV-1 and HIV-2 carriers. In settings with widespread screening of blood donors for viral exposure and the rejection of contaminated blood, transmission by blood transfusion has virtually disappeared (Ochei and Kolhatkar, 2007).

Public health authorities have recommended that since HIV infected persons will remain infected for life, they should avoid spreading the infection by refraining from donating blood, always use condom for sexual intercourse and avoid sharing sharps like toothbrushes, razors, needles and other implements that could be contaminated with blood.

E. Health Education

Without a vaccine or treatment, the prevention of cases of AIDS relies on the success of education projects involving behavioral changes. The health education messages for the general public have been summarized as follows:

1. Any sexual intercourse (outside of mutually monogamous HIV antibody-negative relationships) should be protected by a condom.
2. Do not share unsterile needles or syringes.
3. All women who have been potentially exposed should seek HIV antibody testing before becoming pregnant and, if the test is positive, should consider avoiding pregnancy.

4. HIV-infected mothers should avoid breast feeding to reduce transmission of the virus to their children if safe alternative feeding options are available. (Jawetz *et al*., 2010).

2.2 LIVER

The liver is one of the largest and most important organs in the human body. It is located behind the lower right section of the ribs and carries out numerous functions that the body requires to remain healthy. These are just a few of the liver's many functions:

a. Storing important nutrients from the food we eat.
b. Building necessary chemicals that the body needs to stay healthy.
c. Breaking down harmful substances, like alcohol and other toxic chemicals.
d. Removing waste products from the body (Pratt *et al*., 2000).

2.2.1 Liver Enzymes

Enzymes are proteins found in the body that speed up certain chemical reactions. Liver enzymes perform these jobs within the liver. Two of the common ones are known as "AST" and "ALT" (Aminotransferases).

If the liver is damaged, AST, and ALT pass into the bloodstream. AST and ALT values are higher than normal if the liver is damaged. The damage to the liver can come from viruses, such as the hepatitis C virus, over-the-counter drugs, and prescription, street drugs and even antiretroviral drugs (Megan, 2012).

Liver enzymes or liver (hepatic) function tests are common blood tests used to determine if the liver is functioning normally or if it has an injury or disease. The two main liver enzymes that can be routinely checked are the alkaline phosphates (ALP) and the Aminotransferases (Boyer, 2001).

Serum or Plasma Alkaline Phosphatase

Alkaline phosphatase is one of the enzymes present in the serum that are capable of transporting phosphate groups across cell membrane. It is most active between PH 8 and 10. It is also found in bone, liver, kidney and other tissues. The serum or plasma ALP is estimated to investigate diseases of the liver or none especially in cases of biliary obstruction, tumours, hydatid cysts, abscesses and HIV (Foti *et al*., 2004).

Principle: The enzyme alkaline phosphatase hydrolyses the substrate, disodium phenyl phosphate to release phenol. The quantity of phenol released under standardized conditions

of time, temperature and pH, is measured by the absorbance of the red colour it assumes in alkaline solution. The phenol reacts with 4-aminophenazone in the presence of alkaline potassium fericyanide to produce the red colour.

Conditions affecting serum Alkline phosphatase levels include:

1. Marked increased (more than 5 times normal levels) occurs in conditions such as biliary cirrhosis, bile duct obstruction, osteogenic sarcoma, hyper-parathyroidism and AIDS.
2. Moderate increase (3-5 times more than normal) is usually expected in cases of infectious mononucleosis, metastatic tumour in bone, metabolic bone diseases and some diseases of the liver.
3. Small increase (up to 3 times normal) is encountered in viral hepatitis, healing fractures, pregnancy, HIV and in normal growth period in children (Ochei and Kolhatkar, 2007).

Aminotransferases

The two enzymes concerned with amino acid metabolism are the aspartate aminotransferase (AST) which was previously referred to as glutamate oxaloacetate transminase (GOT), and the alamine aminotransferase also formerly called glutamate pyruvate transaminase (GPT). AST is found in the liver, kidneys, cardiac muscles and skeletal muscles in large amounts while small amounts can be found in brain, pancreas and lungs. ALT on the other hand is found mainly in the liver with only very small amount in other organs.

Measurement of serum or plasma AST (SGOT) and ALT (SGPT) (The colorimetric method of Reitman and Frankel Method for AST (GOT).

$$\text{Aspartate + ketoglutarate} \xrightleftharpoons{\text{AST}} \text{Glutamate + oxalacetate}$$

$$\text{Oxalacetate + 2,4-dinitrophenylhydrazine} \xrightarrow{\text{Alkaline pH}} \text{2,4-dinitrophenly hydrazone (Red broad)}$$

When AST is incubated at 37^{o}c for exactly 60 minutes in pH 7.5 buffered subtract containing aspartate and 2-ketoglutarate, it catalyses the transfer of amino acid group from aspartate to ketoglutarate forming oxaloacetate and glutamate. The oxaloacete 2,4 dinitrophenlyhydrazone which in alkaline pH is red brown. The absorbance of the colour

produced is measured in a colorimeter at 505nm (blue-green filter) (Ochei and Kolhatkar, 2007).

For abnormally high results, repeat the test using a 10 minute incubation time instead of 60 minutes and then multiply the result by six.

Interpretation

Very high level of AST is associated with hepatocellular damage, myocardial infarction, circulatory collapse(shock) and infectious mononucleosis. Moderately high results are obtained in cases of biliary tract obstruction, congestive heart failure, tumour in the liver, while mildly high values are seen in cases of severe bacterial infections, malaria, HIV, liver cirrhosis, pericarditis, cerebrovascula injury and some muscle disorders.

Tissue such as liver, kidney, heart, skeletal muscle and pancreas are rich in ALT, very high results are obtained in cases of liver necrosis and acute hepatitis. Moderately high results are seen in myocardial infarction, obstructive jaundice, and chronic hepatitis. Liver cirrhosis and infectious mononucleosis; and mildly high values are obtained in pancreatitis, alcoholic fatty liver and neoplastic liver diseases (Ochei and Kolhatkar, 2007).

Test Principles

GPT (ALT)

Glutamate pyruvate transaminase belongs to the group of transaminases which catalyses the conversion of amino acids to the corresponding α-keto acids and vice versa by transfer of amino groups. After the application to the test strip, the sample flows into, the reaction zone. In the presence of GPT, α-ketoglutarate and alanine are converted to glutamate and pyruvate oxidase. The resulting pyruvate is cleaved into acetyl phosphate, carbondioxide and hydrogen peroxide. In the presence of peroxidase the hydrogen peroxide converts an indicator into its oxidized blue form:

α-ketoglutarate + alanine $\xrightarrow{\text{GPT}}$ glutamate + pyruvate

Pyruvate + $PO^{3}\text{-}_{4}$ + O_2 + H_2O $\xrightarrow{\text{PyOD}}$ actetyl phosphate + H_2O_2 + CO_2

H_2O_2 + indicator $\xrightarrow[\text{(reduction)}]{\text{POD}}$ $\underset{\text{(oxidation)}}{\text{indicator}}$ + H_2O

Endogenous pyruvate is eliminated in a preliminary reaction. At a temperature of 30°C, the formation of the dye is measured kinetically at 567nm as a measure of the enzymes activity of GPT and the result displayed after 140 seconds (Tefler *et al*., 2004).

Glutamate oxaloacetate transaminase belongs to the group of transaminases which catalyses the conversion of amino acids to the corresponding α-keto acids and vice versa by transfer of amino groups. After application of the serum sample to the test strips, the sample flows into the reaction zone. In the presence of GOT, α-ketogluterate and alanine sulfinate are converted to pyruvate and glutamate. In a second reaction step, catalysed by pyruvate oxidase the resulting pyruvate is cleaved into acetyl phosphate, carbondioxide and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide converts an indicator into its oxidized blue form.

α-ketoglutarate + alanine sulfinate $\xrightarrow{GOT}$ glutamate + pyruvate + SO_3^{2-}

Pyruvate + PO_4^{3-} + O_2 + H_2O $\xrightarrow{PyOD}$ actelyphosphate + H_2O_2 + CO_2

H_2O_2 + indicator $\xrightarrow[\text{(reduction)}]{POD}$ $\underset{\text{(oxidation)}}{\text{indicator}}$ + H_2O

Endogenous pyruvate is eliminated in a preliminary reaction. At a temperature of 30°C, the formation of the dye is measured kinetically at 567nm wavelength as a measure of the enzyme activity of GOT and the result displayed after 124 seconds. Normal range GOT (AST) - men 5-40 U/L, women 5-33U/L.

ALP

Alkaline Phosphatase

Alkaline phosphatase is present in liver, bone, intestine and placenta; and catalyses the conversion of o-cresolphthalein phosphate to o-cresolphthalein and vice versa. This enzyme when measured with ALT and AST permits the differential diagnostic identification of cholestatic disorders (Mabala, 2006). After the application to the test strip, the sample flows into the reaction zone. ALP hydrolyses O-cresolphthalein phosphate to O-cresolphthalein and transfers the phosphate group to the acceptor molecule methylglucamine. The colour hydrolysis product o-cresolphthalein that is produced per unit of time under alkaline condition is directly proportional to alkaline phosphatase activity.

O-cresolphthalein phosphate + methylglucamine ALP→
O-cresolphthalein + methylglucamine phosphate

Dye formation is determined kinetically at 30°C as a measure of the enzyme activity of ALP. The result is displayed after approximately 135 seconds in *U*/L at 567nm wavelength. Normal range ALP - men 40-129 U/L, women 35-104U/L

2.2.2 Viral Hepatitis in HIV Infections

Hepatitis C virus (HCV) and hepatitis B virus (HBV) infections are common among patients with human immunodeficiency virus (HIV) infection because of shared routes of viral transmission. Liver disease due to chronic HBV and HCV infection is becoming a leading cause of death among persons with HIV infection nationwide, and the risk of death related to liver disease is inversely related to the CD_4 cell count. There is also an increase in the incidence of hepatocellular carcinoma and hepatotoxic effects associated with antiretroviral drugs in patients with HCV and HBV coinfection. New treatments for both HCV and HBV infections have increased the opportunities to manage these infections and potentially prevent complications of liver disease (Alter, 2006).

In most early studies, hepatotropic HBV or HCV infections did not appear to change the natural course of HIV infection. More recently, it has been suggested that genotype Ib HCV may worsen the spontaneous evolution of HIV to both AIDS and death in patients with haemophilia and in illicit drug users. HCV infection was shown to be an important factor in the morbidity and mortality of HIV-I-infected patients, possibly through impaired recovery of CD_4 cells levels in HCV-positive patients receiving potent anti-retroviral therapy (Greub *et al*., 2000).

By contrast, HIV significantly modifies the natural history of HBV or HCV infection. HIV infection increases the level of HBV or HCV viremia, especially at the time of HIV seroconversion. This 2-8 fold increase in the level of viremia results in a significant increased risk of mother-to-child or sexual transmission (from a mean rate of 6% to 20% and from 0% to 3% respectively) (Jobareth *et al*., 2010).

HIV coinfection worsens the histological course of HBV or HCV infection by increasing and accelerating the risk of cirrhosis. Rarely, it leads to the lethal disease fibrosing cholestatic hepatitis, which is clearly related to the direct cytotoxicity of HCV; high levels

of viremia lead to an accumulation of viral proteins in the endoplasmic reticulum and to hepatocyte death. Indeed, the rate of cirrhosis is increased 2-5 fold among HIV/HBV or HIV/HCV coinfected patients, compared with HCV-infected patients, and the mean elapsed time between transmission and the onset of cirrhosis is significantly reduced. This increased rate of cirrhosis and shorter disease progression is explained by a significantly increased yearly progression of the fibrosis scare in coinfected subjects that seems to depend on the CD_4 cell count (< 200cells/μl) (Tolan *et al.*, 2001).

Liver enzyme abnormalities are frequent in HIV-infected patients, especially those receiving Highly active antiretroviral therapy (HAART), and are influenced by several nonexclusive factors including drug-related haptatotoxicity, hepatotrophic viral coinfections, other causes of liver disease including steatosis, which is associated with both HAART and abnormal metabolic syndromes, and hepatotoxicity related to the consumption of alcohol, drugs and other medications (Megan *et al*., 2012).

2.2.3 Hepatitis - B

Hepatitis B (serum hepatitis) is caused by the hepatitis B virus (HBV), an enveloped, double-stranded circular DNA virus of complex structure. HBV is classified as an orthohepadnavirus within the family hepadnaviridae. Serum from individuals infected with hepatitis B contains three distinct antigenic particles: a spherical 22nm particle, a 42nm spherical particle (containing DNA and DNA polymerase) called the Dane particle, and tubular or filamentous particles that vary in length. The viral genome is 3.2kb in length, consisting of four partially over lapping, open reading frames that encode viral proteins. Viral replication takes place predominantly in the hepatocytes. The infecting virus encases its double-shelled Dane particles within membrane envelopes coated with hepatitis surface antigen (HBsAg). The inner nucleocapsid core antigen (HBcAg) encloses a single molecule of double-stranded HBV DNA and an active DNA plolymerase. HBsAg in body fluids is

a. An indicator of hepatitis B infection
b. Used in the large scale screening of blood for the hepatitis B virus.
c. The basis for the first vaccine for human used developed by recombinant DNA technology (Modi and Field, 2007).

Diagnosis of HBV is made by detection of HBsAg in unimmunized individuals or HBcAg antibody, or detection of HBV nucleic acid by polymerase chain reaction (PCR).

The hepatitis B virus is normally transmitted through blood or other body fluids (saliva, sweat, semen, breast milk, urine, faeces) and body fluid contaminated equipment (including shared intra venous needles). The virus can also pass through the placenta to the foetus of an infected mother. Worldwide, hepatitis B infects over 200 million people.

The clinical signs of hepatitis B vary widely (Foti, *et al*., 2002). Most cases are asymptomatic. However, sometimes fever, loss of appetite, abdominal discomfort, nausea, fatigue and other symptoms gradually appear following an incubation period of 1 to 3 months. The virus infects liver hepatic cells and causes liver tissue degeneration and the release of liver-associated enzyme (transaminases) into the blood stream. This is followed by jaundice, the accumulation of bilirubin (a breakdown product of hemoglobin) in the skin and other tissues with a resulting yellow appearance. Chronic hepatitis B infection also causes development of primary liver cancer, known as hepatocellular carcinoma (Nagu *et al*., 2008).

2.2.4 Stages of HBV Infection

There are four stages of hepatitis B infection

Stage 1: Immune tolerance: During this stage HBV is able to reproduce freely in the body but does not cause any symptoms or liver damage because the immune system is not responding to the infection. In adults, this stage is very short-lived, if it happens at all. In babies and young children, it can last for several years.

Stage 2: Immune response: During this stage the immune system attacks HBV - infected cells in the liver and starts to clear the infection from the body. In some people, this phase may last for just a few weeks. But if the immune system cannot clear the virus, it can last for years. As the immune system attacks infected cells in the liver, this leads to liver inflammation and liver damage that can worsen over time. Some people with hepatitis B develop symptoms and become unwell at this stage.

Stage 3: Viral clearance: This stage is also known as 'seroconversion'. The body produces antibodies in response to pieces of the virus known as antigens. During this stage, the immune system stops HBV from reproducing. Most adults will naturally clear hepatitis B without treatment.

Stage 4: Immunity to hepatitis B: This is when the immune system produces a full antibody response against hepatitis B and clears the body of the virus. Hepatitis B genetic material (DNA) may remain inside liver cells, however, and on rare occasions it reactivates at a later time, especially if the immune system becomes weakened.

Most adults infected with hepatitis B recover fully and develop lifelong immunity. But up to 10% of people infected with HBV as adults will become chronic carriers of the virus. This means that they can pass HBV on to others and may develop serious, long-term liver damage. Babies and young children who acquire HBV are much more likely to become chronic carries. People living with HIV are also less likely to clear HBV than HIV-negative people (Fenton, 2007).

General measures for prevention and control involve:

- Excluding contact with HBV infected blood and secretions and minimizing accidental needle stick.
- Passive prophylaxis with intramuscular injection of hepatitis B immune globulin within 7 days of exposure.
- Active prophylaxis with combinant vaccines: Energix-B, Recombivax HB, Pediatrix and Twinrix

2.2.5 Reactivation of Hepatitis - B

Several factors have been shown to contribute to hepatitis-B reactivation. Hepatitis B reactivation is the consequence of changing dynamic between the host and viral factors due to immunesuppression, which is induced by pharmacology agents. Screening for the evidence of hepatitis B infection at risk population is the key element to manage patients receiving anti retroviral therapy. It is has been observed that patients with HBsAg positive with detectable DNA have more frequent reactivation than the other entities. However, isolated HBcAb positive patients can still have disease reactivation, especially in the setting of lymphoma (CDC, 2002).

These vaccines are widely used and are recommended for routine prevention of HBV in infants to 18 year-olds, and risk groups of all ages (for example, household contacts of HBV carriers, healthcare and public safety professionals, men who have sex with other men, international travelers, hemodialysis patients.) Recommended treatments for HBV include Adefovir dipivoxil, alpha-interferon, and lamivudine (Dieterich, 2007).

2.2.6 Clinical Study of Hepatitis -B

DNA polymersase activity, HBV DNA, and HBeAg, which are representative of the viremic stage of hepatitis B, occur early in the incubation period, concurrently or shortly after the first appearance of HBsAg. High concentrations of HBV particles may be present in the blood (up to 10^{10} particles/ml) during the initial phase of infection, communicability is highest at this time. HBsAg is usually detectable 2-6 wks in advance of clinical and biochemical evidence of hepatitis and persists throughout the clinical course of the disease but typically disappears by the sixth month after exposure (Fields *et al*., 1996).

High levels of IgM specific anti-HBc are frequently detected at the onset of clinical illness. Because this antibody is directed against the 27nm internal core component of HBV, its appearance in the serum is indicative of viral replication. Antibody to HBsAg is first detected at a variable period after the disappearance of HBsAg. It is present in low concentrations. Before HBsAg disappears, HbeAg is replaced by anti HBe, signaling the start of resolution of the disease. Anti-HBe level often are no longer detectable after 6 months.

By definition, HBV chronic carriers are those in whom HBsAg persist for more than 6 months in the presence of HBeAg or anti-HBe. HBsAg may persist for years after loss of HBeAg. In contrast to the high titers of 1gM specific anti-HBc observed in acute disease, low titers of 1gM anti HBc are found in the sera of most chronic HBsAg carriers. Small amount of HBV DNA are usually detectable in the serum as long as HBsAg is present. The most useful detection methods are ELISA for HBV antigens and antibodies; and PCR for viral DNA (Jawetz *et al.*, 2010).

2.2.7 Structure and Composition of HBV

Electron microscopy of HBsAg positive serum reveals three morphologic forms. The most numerous are spherical parties measuring 22nm in diameter. These small particles are made up exclusively of HB_sAg - as are tubular of filamentous forms, which have the same diameter but may be over 200nm long and result from overproduction of HB_sAg. Larger, 42nm spherical virions (originally referred to as Dame particles) are less frequently observed. The outer surface, or envelope, contains HBsAg and surrounds a 27-nm inner nucleocapsid core that contains HB_cAg. The variable length of a single stranded region of

the circular DNA genome results in genetically heterogenous particles with a wide range of buoyant densities (Jawetz *et al.*, 2010).

The viral genome consists of partially double- stranded circular DNA, 3200bp in length. Different HBV isolates share 90-98% nucleotide sequence homology. The full length DNA minus strand (L or long strand) is complementary to all HBV mRNAs. The positive strand (S or short strand) is variable and between 50% and 80% of unit length.

There are four open reading frames that encode seven polypeptides. These include structural proteins of the virion surface and core, a small transcriptional transactivator (x) and a large polymerase (p) protein that includes DNA polymerase, reverse transcriptase, and RNase H activities. The S gene has three in-frame initiation codons and encodes the major HB_sAg, as well as polypeptides containing in addition pre-S2 or pre-S_1 and Pre- S_2 sequences. The C gene has two in-frame initiation codons and encodes HB_cAg plus the HB_e protein, which is processed to produce soluble HB_eAg (Pratt *et al.,* 2002).

The particles containing HB_sAg are antigenically complex. Each contains a group-specific antigen, a, in addition to two pairs of mutually exclusives subdeterminants, d/y and w/r. Thus four phenotypes of HB_sAg have been observed adw, ayw, adr and ayr. These virus-specific markers are useful in epidemiologic investigations, as secondary cases have the same subtype as the index case.

The stability of HBsAg does not always coincide with that of the infectious agent. However, both stable at -20°C for over 20 years and stable to repeated freezing and thawing. The virus also is stable at 37^oC for 60mins and remains viable after being dried and stored at 25°C for at least 1 week. HBV (but not HBsAg) is sensitive to higher temperatures (100°C for 1 minutes) or to longer incubation periods 60°c for 10 hours). HBsAg is stable at Ph 2.4 for up to 6 hours, but HBV infectivity is lost. Sodium hypochlorite, 0.5%, e.g. 1:10 chlorine bleach, destroys antigenecity within 3 minutes at low protein concentrations, but undiluted serum specimens require high concentrations (5%). HBsAg is not destroyed by ultraviolet irradiation of plasma or other blood products, and viral infectivity may also resist such treatment (Jawetz *et al.,* 2010).

2.2.8 Replication of Hepatitis B Virus

The infectious virion attaches to cells and becomes uncoated. In the nucleus, the partially double stranded viral genome is converted to covalently closed circular double-stranded DNA (CCCDNA). The CCCDNA serves as template for all viral transcripts, including a 3.5-kb pregenome RNA. The pre-genome RNA becomes encapsidated with newly synthesized HBcAg. Within the cores, the viral polymerase is synthesized by reverse transcription, a polymerase starts to synthesize the positive DNA strand but the process is not completed. Cores bud from the pre-Golgi membranes, acquiring HBsAg containing envelopes, and may exit the cell. Alternatively, cores may be reimported into the nucleus and initiates another round of replication in the same cells (Jawetz *et al.,* 2010).

2.3 HEPATITIS-C

Clinical and epidemiologic studies in Chimpanzees in the past had suggested that there were several non- A, non-B (NANB) hepatitis agents which, based on serologic tests, were not related to HAV or HBV. The major agent was identified as hepatitis C virus (HCV). HCV is a positive-stranded RNA virus, classified as family flaviviridae, genus Hepacivirus.

Hepatitis C is caused by the envelope hepatitis C virus (HCV), which has an 80nm diameter, a lipid coat, contains a single strand of linear RNA. The hepatitis C virus is a member of the family flaviviridae. HCV is classified into multiple genotypes (Agwale *et al*., 2004). This virus is transmitted by contact with virus contaminated blood, by the faecal-oral route, by in utero transmission from mother to foetus, sexually, or through organ transplantation. Diagnosis is made by enzyme-linked immunosorbent assay (ELISA), which detects serum antibody to a recombinant antigen of HCV, and nucleic acid detection by PCR. HCV is found worldwide. Prior to routine screening, HCV accounted for more than 90% of hepatitis cases developed after a blood transfusion. Worldwide, hepatitis C has reached epidemic proportions, with more than 1 million new cases reported annually. Treatment is with Ribavirin and Pegylated (coupled to polyethylene glycol) recombinant interferon-alpha (intron - A and Referon) (Koike *et al.,* 2007). This combination therapy can rid the virus in 50% of those infected with genotype 1 and in 80% of those infected with genotype 2 or 3 (Prescott *et al*., 2008).

Various viruses can be differentiated by RNA sequence analysis into at least six major genotypes (clades) and more than 100 subtypes. Clades differ from each other by 25-35% at the nucleotide level; subtypes differ from each other by 15-25%. The genome is 9.4kb in size and encodes a core protein, two envelope glycoproteins, and several non-structural protein. The expression of cDNA clones of HCV in yeast led to the development of serologic tests for antibodies to HCV. Most cases of post transfusion NANB hepatitis were caused by HCV (Wasley & Alter, 2000).

Most new infections with HCV are subclinical. The majority (70-90%) of HCV patients develop chronic hepatitis and many are at risk of progressing to chronic active hepatitis and cirrhosis (10-20%). HCV displaces genomic diversity with different genotypes (clades) predominating in different parts of the world. The viruses undergo sequence variation during chronic infections. This complex viral population in a host is referred to as "quasi-species", This genetic diversity is not correlated with differences in clinical disease, although differences do exist in response to anti-viral therapy according to viral genotype (Balagopal *et al*., 2008).

Most primary infections with HCV are asymptomatic or clinically mild (20-30% have jaundice, 10-20% have only nonspecific symptoms such as anorexia, malaise, and abdominal pain). Serologic assays are available for diagnosis of HCV infection. Enzyme immune assays (EIA) detect antibodies to HCV but do not distinguish between acute, chronic, or resolved infection. Anti-HCV antibodies can be detected in 50-70% of patients at non-set of symptoms, whereas in others, antibody appearance is delayed 3-6 weeks. Antibodies are directed against core, envelope and NS3 and NS4 proteins and tend to be relatively low in titre (Agwale *et al*., 2004).

Nucleic acid-based assay (e.g. reverse transcription polymerase chain reaction) detect the presence of circulating HCV RNA and are useful for monitoring patients on anti-retroviral therapy. Nucleic acid assays also are used to genotype HCV isolates.

Occult HBV infection occur frequently (about 33%) in patients with chronic HCV liver disease. Occult infections are those in which the patients lack detectable HBsAg but HBV DNA can be identified in liver or serum samples. These unrecognized HBV coinfections may be clinically significant. Clearance of HBV-DNA is expected to occur following loss

of HBsAg and seroconversion to anti-HBs; anti HBsAg is considered the classical marker of past resolved infection (Mphahlele *et al*., 2006).

2.3.1 Other Liver Diseases in HIV-Infected Individuals

Chronic liver disease is common among HIV-infected patients, and is increasingly a cause of mortality and morbidity as effective ART allows persons with HIV to live longer. HIV infection may accelerate damage caused by HCV or HBV infection. HCV infection is particularly common among HIV-infected patients, especially those who acquired HIV through injection drug use (IDU). Long-term complications of HBV and HCV infection include cirrhosis, end-stage liver disease (ESLD), and hepatocellular carcinoma (HCC). Long term management of cirrhosis is important to providing optimal prevention and treatment of complications (Prescott *et al.,* 2008).

Since the advent of effective antiretroviral therapy (ART) for human immunodeficiency virus (HIV), there has been a substantial decrease in deaths related to AIDS. However, in the ART-era, liver disease is now the most common non-AIDS related cause of death among HIV-infected patients accounting for 14-18% of all deaths among hospitalized HIV-infected patients (Boyer, 2001). Just as the burden of non-AIDS morbidity and mortality has changed in the ART-era, the types of liver disease the clinician is likely to encounter among these patients have changed as well. Managing liver disease is an increasingly important component to the care of individuals infected with HIV. Prior to ART, the most common causes of liver dysfunction in HIV-infected patients were opportunistic infections, including cytomegalo virus (CMV) and mycobacterium infections, and AIDS-related neoplasms such as lymphoma and Kaposi's sarcoma. Since the ART-era, however, the spectrum of liver disease among HIV-infected individuals has shifted to concomitant infection with chronic HCV, chronic HBV, medication-related hepatoxicity, alcohol abuse and nonalcoholic fatty liver disease. (NAFLD) (Thio *et al*., 2002).

Liver enzyme elevations are common in HIV infected patients, and their diagnosis or management may be difficult because of the intricacies of the pathogenic mechanisms involved. These include hepatotoxicity related to the highly active antiretroviral therapy (HAART) regimen, idiosyncratic or immuno allergic mechanisms, and direct cytotoxicity enhanced by an underlying liver disease. Other factors that may affect liver deterioration,

include alcohol-related liver disease, non-alcoholic steatohepatitis associated with metabolic syndromes (e.g. hyperlipidemia, diabetes, or being overweight) that are potentially HAART related, and use of medication or illicit drugs (e.g. methamphetamine) (Wondimeneh *et al*., 2013).

The development of HAART regimens composed of nucleoside reverse-transcriptase inhibitors (NRTIS), protease inhibitors (PIS) and/or nonnucleoside reverse-transcriptase inhibitors (NNRTIS) have resulted in a significant decrease in morbidity and mortality among HIV-infected patients. HAART has completely modified the pattern of hepatic events in HIV infection, and the liver is now one of the most important organs to consider when treating HIV-infected patients (Sulkowski *et al*., 2000).

The early recognition and diagnosis of hepatic events will facilitate the safe and effective use of HAART and enhance the survival of HIV-infected patients. Nevertheless, the intricacies of the various pathogenic mechanisms may result in difficulties in the diagnosis, as well as in management of such patients with their liver abnormalities (Soriano *et al*., 1999).

2.3.2 Medication Toxicity

Liver toxicity is one of the most common serious adverse events associated with ART. The clinical presentation can range from mild asymptomatic increases in serum transminases to adverse liver failure. In retrospective studies, the incidence of ART related several hepatotoxicity is approximately 10%, and life threatening events occur at a rate of 2.6 per 100 persons.

Even though HIV drugs are intended for good health, the liver recognizes these medications as toxic compounds. They are not naturally produced by the body and do contain some chemicals that could potentially cause damage to the body. Working with the kidneys and other organs, the liver processes these drugs to render them safer. In the process, the liver can become “overworked”, which can lead to liver damage (Puoti *et al*., 2009).

There are two ways that HIV medications can lead to liver damage.

1. Direct damage to liver cells:

Liver cells called hepatocytes, play a vital role in the functioning of the liver. If these cells begin to work too hard to remove chemicals from the blood, or if they are harmed by other

infections, abnormal chemical reactions can occur that can damage these cells. There are several ways in which this can happen.

a. Taking a very high dose of a drug. When a high dose, many pills of ARV drug or another medication is taken when one or two pills of the drug is suppose to be taken, can cause immediate and sometimes damage to liver cells. Almost every drug, when an overdose is taken, can cause this type of liver damage.
b. Taking standard doses of medication for a long period of time. If a medication is taken on a regular basis for a long period of time, there is also a risk of damage to these liver cells. This usually occurs after several months or years of taking certain medications. Protease inhibitors have the ability to cause damage to liver cells if they are used for long periods of time (Lebovics *et al*., 1998).
c. An Allergic reaction: Being allergic to a particular drug, the immune system can cause the liver to become inflamed as a result of interactions between key liver proteins and the drug. If the drug is not stopped, the inflammation can worsen and can cause serious damage to the liver. Two HIV drugs known to cause such allergic reactions (hypersensitivity) in HIV-positive people are ziagen and viramune. Allergic reactions such as these usually occur within a few weeks or months after the drug is started and either may or may not be accompanied by other allergy related symptoms (eg fever or rash).
d. Non-allergic liver damage: some drugs can cause liver damage without an allergic reaction or use at high doses. Two particular HIV drugs that can cause serious damage, though in relatively small numbers of people, are Aptivus, Tipranavir and Prezista (darunavir).

2. Lactic Aciodisis: Nucleoside reverse transcriptase inhibitors (NRTIS) are not processed by the liver; they are removed from the blood stream and from the body by the kidneys. These drugs can damage "cellular mitochondria" the "powerhouses" inside cells that convert nutrients into energy.

This can cause levels of lactate, a cellular waste products, to become elevated. If these levels become too high, a condition called lactic acidosis can occur, which can result in liver problems, including a buildup of fat in and around the liver and liver inflammation. (Lebovics *et al.*, 1988).

2.3.3 How to Find out Liver Damage Caused by HIV Medications

The best indicator of hepatotoxicity is an increase in certain liver enzymes that circulate in the bloodstream. The most important enzymes are AST (aspartate aminotransferase), ALT (alanine aminotransferase), alkaline phosphatase and bilirubin. These four enzymes are normally checked as a part of a "chem screen," a panel of tests that the doctor probably orders every time blood is drawn to check CD_4 cells and viral loads of HIV-patients (Ahaneku, 2010).

It is always best to detect hepatotoxicity in its early stages so that steps can be taken to prevent it from getting worse and to allow the liver to heal. Most of the time, hepatotoxicity takes several months or years to develop and usually begins with mild increase in either AST or ALT that progresses to more serious increase. If The AST or ALT levels are elevated but are not higher than five times the normal range (AST above 43 IU/L but below 215 IU/L or ALT above 60 IU/L but below 300 IU/L), it is mild-to-moderate hepatotoxicity. If the AST is higher than 215 IU/L or ALT is above 300 IU/L, it is severe hepatotoxicity, which can lead to permanent liver damage and serious problems. Increased liver enzymes can rarely be felt, i.e. there may not be any physical symptoms when the liver enzymes are elevated (Pol *et al*., 2004).

Non-ART Related Medication Toxicity

HIV-infected patients are often prescribed a number of non-ART medications that can have adverse liver effects either alone or in combination.

Alcoholic Liver Disease

Active alcohol intake is known to be associated with faster liver disease progression in HCV monoinfection. In one study of HIV-HCV coinfected patients, excessive alcohol use was associated with elevated HCV RNA levels. Some other studies suggest that alcohol abuse is prevalent among HIV-infected individuals and can independently contribute to liver disease progression. As a modifiable risk factor for liver disease, it is important that physicians provide counseling regarding alcohol consumption to this population (Bernard *et al*., 1984).

Non-Alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) refers to fat deposition in hepatocytes, or steatosis, in individuals with little or no alcohol use. When accompanied by inflammation and fibrosis, it is referred to as non-alcoholic steatohepatitis (NASH).

Metabolic abnormalities are extremely common in HIV-infected persons on ART, especially NRTI-PI combinations. These include insulin resistance, dyslipidemia, hypertriglyceridemia, and lipidystrophy, a disorder of peripheral fat distribution resulting in lipatrophy and visceral adiposity. NRTI's can also lead to hepatic steatosia via inhibition of mitochondrial DNA replication, resulting in triglyceride accumulation in the liver. These metabolic abnormalities have been associated with the development of NASH in HIV-infected patients (Reid, 2001).

Nodular Regenerative Hyperplasia

Nodular regenerative hyperplasia (NRH) is a rare condition characterized by multiple, small regenerative nodules in the liver parenchyma. NRH has recently become increasingly recognized in HIV-infected patients with cryptogenic liver disease (Bonacini, 1992),

AIDS-Related Liver Disease

AIDS cholangiopathy - AIDS cholangiopathy occurs when infected-related strictures in the biliary tract lead to biliary obstruction. It typically presents with right upper quadrant pains (RUQ) and a markedly increased alkaline phosphatase with a less elevated bilirubin and normal or slightly increased transminases. Patients may also have fever, nausea, vomiting and diarrhoea, jaundice is uncommon. It is usually seen in low CD_4 counts ($<100/mm^3$). The most common infection associated with AIDS cholangiopathy is *Cryptosporidium parvum* followed by cytomegalovirus (CMV) (Saves *et al.*, 1999).

Acalculous Cholecystitis

Acalculous cholecystitis has been usually associated with CMV or Cryptosporidium, although other infections including *Isospora* and *Microsporidia* have been implicated. Patients typically present with RUQ abdominal pain and fever with cholestasis; leukocytosis is often not present. Cholecystectomy is the treatment of choice Ahamad & Alvarez, 2004).

Aids-Related Neoplasms

The AIDS-defining malignancies non-Hodgkin lymphoma (NHL) and kaposi's sarcoma (KS), involve the liver in 33% and 9% of cases respectively. Hepatic involvement of NHL may present with asymptomatic liver function test abnormalities but patients may develop abdominal pain or jaundice. Hepatic involvement of KS rarely causes symptoms or mortality (Keaveny and Karasik, 1998).

Hepatic Steatosis/Fatty Liver

Hepatic steatosis is a medical term for fatty liver. This can develop from alcohol use, hepatitis, obesity and drug toxicity with the family of HIV drugs called NRTs (nukes). This build-up of fat in the liver can affect the way it processes fats. Hepatic steatosis often also leads to lactic acidosis. People who weigh over 70kg, especially women, may be more at risk of developing hepatic steatosis and lactic acidosis (Adinolfi *et al.,* 2001).

Ultrasonography is a sensitive, accurate, non-invasive screening tool to detect steatosis as this is not always shown in liver function tests. Steatosis is also common in HIV-positive children. It has no impact on disease, testing or management.

2.3.4 Liver Related side Effects with Some ART

A few HIV drugs have been linked to liver problems. Nevirapine is particularly associated with liver toxicity and the information leaflet that comes with the medicine includes a 'black box' warning. Liver toxicity has also been reported with efavirenz. Ritonavir and tipranavir (due to the higher ritonavir dose) are also linked to liver toxicity (Hooshyar *et al*., 2007).

The following factors can increase the risk of liver complications from HIV treatment.

a. Viral hepatitis: HBV or HCV
b. Increased alcohol consumption
c. Use of other drugs, including recreational drugs, toxic to the liver.
d. Gender: women are more prone to liver problems with HIV drugs.

Nevirapine related liver toxicity is different between men and women. The risk is related to CD_4 count when starting treatment. Women starting treatment for the first time should not use nevirapine if their CD_4 count is over 250cells/mm^3 and men should not use nevirapine if their CD4 count is over 400cells/mm^3 (Koziel & Peters, 2007).

Efavirenz can also cause hepatotoxicity but does so less frequently than nevirapine or etravirine. Stavudine, didanosine (ddl), and zidovudine which are nucleoside reverse transcriptase inhibitors (NRTI'S) are associated with mitochondirial toxicity due to their ability to inhibit mitochondrial polymerase. Clinically this presents with hepatic steatosis and lactic acidosis from weeks to months after initiation (Martinez *et al.,* 2001).

2.4 SYPHILIS

Veneral syphilis is a contagious, sexually transmitted disease caused by the *Spirochete, Treponema pallidium.* Congenital syphilis is the disease acquired in the uterus from the mother. *T. pallidium* enters the body through mucus membrane or minor breaks or abrasions of the skin. It migrates to the regional lymph nodes and rapidly spreads throughout the body. The disease is not highly contagious, and there is only about a 1 in 10 chance of acquiring it from a single exposure to an infected sex partner.

T. *pallidium* is a thin, elongated (0.10- 0.l8μm) bacterium that cannot be readily visualized by light microscopy, instead requiring visualization by dark field microscopy, which uses obliquely applied light. The organism displays 6 to 14 regularly wound coils, has a characteristics corkscrew motility, and flexes centrally at 90-degree angles. Sustained in vitro cultivation of *T. pallidium* is not currently possible for the diagnostic purposes. Investigators have propagated *T. pallidium* in rabbits or guinea pigs to provide organisms for scientific study, to evaluate new antimicrobial agents or candidate vaccines, or to demonstrate the presence of treponemes in clinical specimens (Jawetz *et al.*, 2010).

2.4.1 Sexually Transmitted Syphilis

This form of syphilis is transmitted by sexual intercourse. The disease occurs in three stages. Primary, secondary and latent (tertiary) stages.

Primary Syphilis

The spirochete reaches the subepithelial tissues through inapparent breaks in the skin or possibly by passage between the epithelial cells of a mucus membrane. It multiplies locally with a generation time of about 30hrs; although the primary lesion is local, the organism also disseminates rapidly to local lymph nodes and then to other organs by way of the bloodstream. The primary lesion develops 2 to 10 weeks after infection as an indurated swelling at the site of infection. The surface necroses yield a hard-based ulcerated lesion, termed the chancre, which is teeming with spirochetes and is highly infectious Pratt *et al*.,

2000). The chancre fluid is rich in treponemes, and the regional lymph nodes are enlarged. The lesion may spontaneously heal up without treatment in 10-40 days. The basic pathologic lesion is an endarteritis. The small arterioles show swelling and proliferation of their endothelial cells. This reduces or obstructs local blood supply and probably accounts for the necrotic ulceration. Dense, granulomatous cuffs of lymphocytes, monocytes, and plasma cells surround the vessels. The primary lesion is not always apparent.

Secondary Syphilis

About 2-8 weeks after the primary lesion has healed, skin eruptions may occur, resulting in red rashes with papules. This is due to widespread multiplication of the spirochetes and dissemination in the blood. Lesions are heavily infected with *T. pallidium.* In most areas around the vulva or anus, hypertrophic popular lesions (*Condyloma lata*) can occur. The patient is highly infectious and can transmit *T. pallidium* in his blood. Generalized lymphadenopathy, fever, malaise, splenomegaly, sore throat, headache, and arthralgia can be present. Immune complexes of antibody, spirochetal components and complement are present in arteriolar walls and account for some of the clinical manifestations. About 30% of patients develop flat genital lesions called condyloma (Stamm *et al*., 1998). This stage may last from 10 days to one year. Relapses may occur between two and four years.

Early Latent Syphilis

This is the inactive period following the secondary stage. About 70% of patients will show no signs of disease. This stage is generally regarded as a non-infectious stage, though the foetus of an infected pregnant woman can be infected. This period is about one to two years after the secondary stage (Jawetz *et al.*, 2010).

Late (Tertiary) Syphilis

This stage may appear at least three years after the secondary stage in an untreated patient. Progressive inflammatory lesion of any organ or tissue may develop, but the central nervous system and the cardiovascular system are involved. This stage characteristically occurs after 15 to 20 years. Other complications of the late syphilis include gummas or granulomas of skin or bone, liver damage, eye impairment or ear dysfunction. Neurosyphilis may appear as acute meningitis or meningovascular involvement of the CNS. This condition is generally referred to as ‘general paralysis of the insane’. The meningovascular syphilis involves vascular changes of the meningis associated with

increased cells and protein in the cerebrospinal fluid and focal neurologic changes. In general paresis, there is extensive cortical degeneration of the brain, with mental changes ranging from decreased memory to hallucinations or frank psychosis. *Tabbes dorsalis* involves demyelination of the posterior columns and dorsal roots and damage to dorsal root ganglia. The latter produces ataxia, wide-based gait, foot slap, and loss of the sensation of position, pain, and temperature (Jawetz *et al.,* 2010).

Not all patients with central nervous system involvement have symptomatic disease. The most characteristics lesion of late cardiovascular syphilis is the development of an aneurysm of the ascending and transverse segments of the aortic arch as a result of gummatous changes in the middle coat of the oarta and loss of elasticity. This aneurysm can lead to aortic valve incompetence, pressure necrosis of structures adjacent to the aorta, or rupture of the aorta. The isolated gumma is a granulomatous reaction to *T. Pallidium* infection. It occurs most often in skin, bones, or joints, but may involve any organ. Clinical manifestations of gumma are similar to other mass-producing lesions in the tissues, such as tumors. Too few spirochetes are in the lesions to be demonstrated by microscopic techniques, except in general paresis, when large numbers are found in the cerebral cortex. The late stage is non-infectious except to the foetus of infected pregnant mother (Hutchinson *et al*., 1994).

2.4.2 Congenitally Acquired Syphilis

A pregnant mother with untreated syphilis may infect her unborn baby. This is by transplacental transmission. Congenital syphilis causes foetal death in about 40% of cases. If the infection of the foetus occurs early in the pregnancy, abortion may result. Clinical symptoms in live born babies may not appear until about one year after birth. The most common signs are skin eruptions (rashes), jaundice, painful limbs, ascitis, anaemia and the infant may develop 'saddle' nose. Other symptoms include osteitis, pneumonitis, mucocutaneous lesions and hemorrhage. Late manifestations of congenital syphilis can involve the central nervous system, bones, teeth, eyes, skin and/or cartilage (Ochei and Kolhatkar, 2007).

2.4.3 Immunity to Infection

In early stages of syphilis, the patient rapidly becomes immune to reinfection, but immunity is short-lived if the patient is successfully treated. In the later stages, immunity

to reinfection is more solid and continues after treatment. Syphilis in immunocompromised patients such as those suffering from the acquired immunodeficiency syndrome may present with unusually aggressive or atypical manifestations.

2.4.4 Method of Diagnosis of Syphilis

Definitive diagnosis is achieved by identifying spirochetes by microscopic dark field examination or direct florescent antibody tests of lesion exudates or tissue. Most cases of syphilis are diagnosed serologically by presumptive diagnosis is possible using two types of serological tests i.e.

a. Non treponemal tests and treponemal tests

Non-treponemal test for syphilis include:

i. The VDRL slide test (Veneral Disease Research Laboratory)
ii. The rapid plasma reagin (RPR) test
iii. The automated reagin test (ART)

b. Treponemal tests involve direct detection of antibody to *T. Pallidium* using

i. The fluorescent treponemal antibody absorption test (FTA -ABS).
ii. Increasingly the microhemagglutination tests for *T. Pallidium* antibody (MHA-TP).

Penicillin remains the best studied and the preferred therapy for syphilis (Ochei and Kolhatkar, 2007).

2.4.5 HIV/Syphilis Coinfection

The interrelationship between genital ulcer disease (GUO); including syphilis and the risk for HIV infection is well documented. Syphilis may increase the rate of HIV acquisition between two and four fold and the risk for transmitting HIV between two and nine fold. Explanations include behavioral factors as well as pathogenic mechanisms, such as facilitation of HIV transmission caused by syphilis - associated ulceration and inflammation (Stamm *et al.*, 1988).

The resurgence of syphilis has been linked to increase in the number of anonymous sex partners, decrease in condom use, use of the internet for meeting sex partners, and more widespread use of methamphetamine and sildenafil (Viagra), among other drugs. Moreover, the indirect contribution of highly active antiretroviral therapy (HAART) to

higher syphilis transmission rates among those infected with HIV has been well described (Katz *et al*., 2002).

2.4.6 Screening for Syphilis in HIV-infected Patients

Recognition of a sexually transmitted disease (STD) in an HIV-infected patient is a sentinel clinical event that should trigger the provider's exploration of underlying risk behaviour, the patient's interpersonal HIV disclosure capacity, and his or her appreciation of risks posed to others. Sexual health assessment should be a routine part of care for patients with HIV. For the patient with multiple sex partners or with a partner who has multiple partners, screening for STDs such as syphilis, gonorrhea, and Chlamydia should be done annually. Experts now also recommend screening patients at higher risk for syphilis every 3 to 6 months, depending on the level of risk; factors include multiple anonymous partners, concomitant recreational drug use, or frequenting of commercial sex venues or internet sex partner sites. Because serum antibody to *T. Pallidium* is not protective, reinfection may occur if risk is ongoing (Klausner and Wong, 2003).

2.4.7 Clinical Features of Syphilis in HIV-infected Patients

Syphilis is well-known for its protein presentations, reflecting systemic dissemination of the treponeme. Aside from more common mucocutaneous and systemic features in the primary and secondary stages of the disease, widespread involvement in early and late syphilis may result in ocular, otologic, neurologic, gastrointestinal, hepatic, renal and osseus manifestations, albeit rarely (Kin *et al*., 2000).

2.4.8 Syphilis Features by Stage

Conventional staging of syphilis is unaltered by HIV coinfection. Symptoms may appear within several days of exposure; mean- 21 days; range- 14-90 days. (Golden *et al*., 2003), The rash of secondary syphilis can mimic many dermatologic conditions, such as *Tinea versicolor*, *Pityriasis rosea,* scabies, fixed drug eruptions, and erythema multiforme. In HIV-infected patients receiving HAART, it has been misdiagnosed as an antiretroviral drug reaction. In general, infectious syphilis refers to the primary and secondary stages because syphilitic chancres, mucous patches, and condylomata lata are highly infectious lesions.

Tertiary syphilis describes disease with late manifestations, encompassing cardiovascular features such as aortitis with aneurysm formation, late neurologic sequelae (Stamm *et al*.,

1988) and formation of gummas (indolent albeit destructive granulomatous lesions that may occur in any organ but chiefly involve skin, bone and liver). Neurosyphilis is not a stage rather a site of infection, where symptoms may manifest earlier or later in the course of infection.

2.4.9 Modified Presentation in the HIV/T. *Pallidium* coinfected Patients

With minor exceptions, syphilis was not found to present or progress atypically in an important, large scale, prospective trial of 101 HIV-infected patients that evaluated treatment outcomes in early-stage infection as reported by Kim *et al*., (2002). Numerous early case reports and more recent reviews have sought to characterize the extent to which HIV infection alters the clinical presentation of syphilis, with most minimizing its impact.

Seventy percent of HIV-infected patients in one study presented initially with multiple chancres rather than a single ulcer, compared to 34% of HIV-negative patients. Others have suggested that primary lesions may be more severe and/or slow to resolve, although these data are less convincing. However, in a study of 253 patients with secondary syphilis, 25% of those with HIV presented with persistent genital ulcers, compared to only 14% of HIV-uninfected patients. Therefore, presentation involving a combination of primary and secondary features should not cause one to doubt syphilis as the underlying Diagnosis. Furthermore, in evaluating possible secondary syphilis, careful genital and rectal examinations remain worthwhile for identification of resolving primary lesions (Musher *et al.*, 1990).

2.4.10 Ophthalmologic and Otologic Sequelae

Clinicians should be aware of ocular and otologic sequelae observed in HIV/T. *pallidium* coinfected patients, ocular manifestations include uveitis, chorioretinitis, and retrobulbar neuritis. Among these, uveitis is the most common and was found in about 10% of patients with a positive cerebrospinal fluid (CSF) treponemal infection. In rare cases, retinal detachment and blinding may occur. Otologic involvement is infrequent and may present with asymmetric hearing loss, tinnitus, or vestibular disturbances. Whether incidence of these manifestations is increased in HIV-infected patients is uncertain, and reliable clinical predictors have not been identified (Shalaby *et al*., 1997).

2.4.11 Treatment of Syphilis

General Treatment Considerations

Long-acting, injectable Benzanthine penicillin -G (BPG) remains the treatment of choice for all stages of infection. In early syphilis (primary, secondary, and early latent), a single IM injection of BPG (2.4mg) should be administered. In late latent syphilis or infection of unknown duration 7.2MU of BPG in three weekly divided doses should be administered, after the patient has had lumber puncture (LP) for examination of the CSF to rule out neurosyphilis. In such a patient who is sexually active, it is impossible to rule out recent infection with the potential of symptom development and further transmission of infection; before examination of CSF if indicated, must be timed to avoid delay in treatment.

In HIV-negative nonpregnant adults, particularly those with an allergy to penicillin, doxycyline and tetracycline are alternatives for treating early syphilis and have been used for decades with anecdotal success. Because dosing over 14 days is required, efficacy is compromised by potential non-adherence in addition to pharmacologic properties diminishing their activity compared to BPG. Azithromycin, an azalide with high tissue penetration and a long half-life, garners continues interest for the treatment of early syphilis, but the long-term efficacy of this antimicrobial has not been clearly established (Rapp, 1998).

Treatment of Neurosyphilis

When syphilis involves the CNS, BPG is considered inadequate therapy because it does not cross the blood-brain barrier. The established treatment remains IV Aqueous crystalline penicillin-G (ACPG), 18 to 24MU per day in divided doses for 10 to 14 days. A regimen of daily 1M procaine penicillin G, in combination with oral probenecid, is an acceptable substitute. Most experts recommend the addition of 7.2MU of BPG in three weekly divided doses as follow-up to these regimens, although the benefit of this practice has not been evaluated in a prospective manner (Mohr *et al*., 1976).

The only alternative that has been studied is ceftriaxone 2g 1M for 14 days. The small sample size of this study precludes its recommendation as standard therapy, although the drug can be considered in special circumstances in which neither ACPG nor procaine penicillin is feasible. For the penicillin-allergic HIV-infected patient with neurosyphilis, and when concern for cephalosporin allergy also exists, desensitization to penicillin is the

only option. Evaluation of penicillin allergy with preliminary skin testing may obviate the need for desensitization (Marra *et al*., 2000).

Treatment of Sex Partners

The sex partners of patients with syphilis should be treated presumptively with BPG (2.4MU) or an alternative such as doxycycline or azithromycin if exposed within 90 days of the diagnosis of the partner. Those exposed more than 90 days before diagnosis should be tested and treated presumptively if results of serologic tests are unavailable or if follow-up is uncertain (CDC, 2002).

2.4.12 Follow-up of HIV-infected Patients Treated for Syphilis

Treatment failure can occur with any regimen in patients with or without HIV infection. Because the possibility of clinical relapse after syphilis therapy may be slightly higher in HIV-infected patients, the importance of closely following HIV-infected patients with syphilis cannot be overstated. Clinicians should monitor all patients carefully for persistent or recurrent symptoms, for any signs of neurologic involvement, and for increasing serologic titres (Shalaby *et al.,* 1997).

After treatment for syphilis, patients should be reexamined clinically at 1 to 2 weeks and retested with a quantitative nontreponemal tests at 3, 6, 9, 12 and 24 months after treatment for primary, secondary and early latent syphilis and at 6, 12, 18 and 24 months after treatment for late latent syphilis or syphilis of unknown duration.

In all patients experiencing treatment failure as defined by rising titers or development of signs or symptoms of syphilis in the absence of reinfection, and in cases of possible treatment failure as defined by inadequate serologic response, especially if treated for primary, secondary, or early latent syphilis without an initial CSF examination, experts recommend CSF examination and repeat treatment with 3 weekly injections of BPG (2.4MU 1M weekly) for a total of 7.2MU, unless CSF examination indicates that neurosyphilis is present. Such patients should be monitored with repeat serologic testing every 6 to 12 months. Those patients with persistent nontreponemal antibodies of unknown clinical significance should be monitored annually for evidence of clinical or serologic relapse (CDC, 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA

Anambra State is located in the South Eastern part of Nigeria. It is located at latitude of $6°20^{1}00^{11}$N and longitude of $7°00^{1}00^{1}$E (see the scaled map on plate 3). Anambra State has an estimated population of 7,821,850 million people and covers a total area of 4,844km^3 (l,870sqml), with a population density of 840/km^3 (2,200/sqm). Anambra State has a relative humidity of about 75% and pressure at 0.9934 Atm (Igboegbunam, 2009).

The inhabitants of Anambra State include civil servants, business men and women, students, artisans and farmers from Anambra state, other neighbouring states like Imo, Abia, Enugu, Delta, Kogi and other states of the federation. Anambra State has many tertiary, secondary and primary health facilities.

3.2 STUDY POPULATION

The study population includes people living with HIV/AIDS that attend clinic at General Hospital Onitsha, General Hospital Ekwulobia and General Hospital Enugwu-ukwu. Prior to commencement of the study, permission was obtained from the Management of the General Hospitals and the research was endorsed by the Ethical Committee of Anambra State University Teaching Hospital, Amaku, Awka (Appendix III). The study sample of 502 HIV/AIDS clients were selected among those that gave their consent. The parent or relation of the underaged participants and those who could not write signed and filled the consent form and questionnaire on behalf of the participants. Their selection was done by simple random sampling.Their socio-demographic data were collected through the laboratory request & result form and questionnaire (Appendix II & IV) given to each of the participants to be filled and submitted. Five hundred and two of the people living with HIV/AIDS were selected from the three general hospitals after a non-coercive informed consent was obtained.

SAMPLE SIZE DETERMINATION

The sample size was determined statistically using the formular:

$$SS = \frac{(Z - score^2)\,(P)\,(1-P)}{(D)^2}$$

Where SS – Sample size

D - Desired level of significance (0.05)

Z – Score = 1.96 for 95% confidence interval;

P – Population proportion (expressed as decimal assumed to 0.5).

Adjusted sample size (SSA) for finite population = $\frac{SS}{1 + \left(\frac{SS - 1}{PS}\right)}$ (Daniel, 1999)

(Detailed calculation in appendix vi)

PS is the known study population of HIV/AIDS out-patients from which the sample size was obtained.

3.3 ETHICAL CONSIDERATION

The study was conducted after obtaining ethical clearance from Ethical Committee of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka, Anambra State with Ref. No: COOUTH/AA/Vol. XI/1108. Informed consent was also obtained from each study participant.

3.4 SCOPE OF THE STUDY

This study involved randomly selected PLWHA who attend ART clinic in any of the three General Hospitals chosen for the study that gave their consent to participate by signing the consent form. Only those that visited the clinic during the study period, regardless of age, gender, ethnincity or tribe participated. Their blood specimen were be collected for the tests, questionnaire was used to ascertain their socio-demographic characters and the results statistically analysed to determine the significance of the effects on the immune system of PLWHA.

3.5 SPECIMEN COLLECTION

Veinous blood samples were collected from consenting 502 HIV infected individuals attending General Hospital Onitsha, General Hospital Enugwu-Ukwu and General Hospital Ekwulobia between September, 2015 and December, 2016. Two hundred samples were collected from General Hospital Onitsha, 200 from Ekwulobia General Hospital and 102 samples from Enugwu-Ukwu General Hospital.

Containers for blood sample collection were appropriately labeled using the laboratory number and identification number. Laboratory form was filled for each participant by

name to avoid collecting sample twice or more from a participant (Giuseppe *et al*., 2010). The site of sample collection was chosen, after tying the tourniquet to make the vein more prominent, alcohol swab was used to sterilize the site. Six mililitres of the veinous blood was drawn from each patient using 10ml syringe and 23guage needle (Alstrom, *et al*.,1993).

Two mililitres of each drawn blood sample was put separately into ethylenediamine tetra-acetic acid (EDTA) container. The EDTA serves as an anticoagulant. The sample was gently mixed to avoid clotting or lysing of the blood sample. The remaining 4ml of the blood was placed into a dry tube without EDTA and the sample was allowed to clot (Lippi *et al*., 2006). After 2hours, the clotted blood was carefully retracted from the wall of the tube to avoid lysing the blood cells, centrifuged at 1500rpm for 5mins, and the serum was separated into another labeled clean dry cryo tubes and stored at -4^{o}C (Meyer and Harvey, 2001).

A structural questionnaire administered to each participants captured socio-demographic data such as age, gender, marital status, educational level, occupation and risk factors associated with HIV such as intravenous drug users, smokers, alcohol abusers, those that have undergone surgery or blood transfusion.

3.6 HIV TESTING OF ALL THE PARTICIPANTS

Prior to the testing procedures, all the participants were screened to confirm they are HIV positive using the National approved serial algorithm for HIV screening with Alere Determine and Uni-Gold kits.

Testing procedure with Alere Determine HIV Kit

i. The protective foil covering the test strip was removed.
ii. With automatic pipette, 50μl of whole blood sample in the EDTA container was applied to the sample pad.
iii. After one minute, one drop of chase buffer was added to the sample pad.
iv. The result was read after 15mins.
v. Double line on the test strip indicates a positive result while a single line indicates a negative result (WHO, 1993).

Testing Procedure with Uni-Gold Kit

i. The pouch containing the kit was properly examined to make sure the pouch was still intact.
ii. The pouch was opened after reaching room temperature.
iii. With the inclusive pipette, 2 drops of whole blood sample in EDTA container was applied to the sample pad.
iv. Two drops of the wash solution in a dropper was added to the sample pad.
v. The result was read after 10mins.
vi. Double line indicates a positive result while a single line indicates a negative result (Schoenbaum *et al*., 1989).

3.7 CD_4 COUNT

The CD_4 count test was performed using Partec Cyflow machine. The sample used was the fresh anticoagulated whole blood in the EDTA containers.

3.7.1 Principle

The Cyflow machine is a flow cytometer which operates by introducing cells stained with fluorescence conjugated antibody or absorption dyes in a fluid stream under a slight pressure to pass through a nozzle into the beam light, usually generated by a laser. Light that is scattered and emitted by cells is then separated into constituent wavelength by a series of optical filters and mirrors. This separated light falls on individual photo detectors and then is translated into electrical pulse, or analog signals, proportional to the amount of incident light detected by the detectors. Each analog signal is finally converted into a digital signal, is then processed by the data processing and analysis unit. The numbers are proportional to the amount of light emitted from or scattered by individual stained cells. Cells to be measured must be suspended in a liquid. (Fryland *et al*., 2004).

The Cyflow machine was properly cleaned, waste bottles were emptied and sheath fluid bottle filled.

3.7.2 Processing of the Sample

i. With an automatic pipette, $20\mu l$ of properly mixed whole blood sample was carefully dropped on the bottom of a clean dry cyflow cuvette tube.

ii. Twenty microlitre of the absolute fluorescent CD_4 monoclonal antibody (Partec CD_4 easy count kit) which recognizes the T-lymphocytes CD_4 surface antigen was added to the blood sample in the cuvette.

iii. The content of the cuvette was properly mixed and incubated in a dark cupboard for 15 minutes.

During the incubation, the cyflow machine was switched on and set ready for the test by running 1.6ml of sheath fluid.

i. Eight hundred and fifty microlitre of countcheck bead was ran, and the value of it fell within the range.

ii. After the 15mins incubation, 800μl of buffer '1' was added to the cuvette content and properly mixed to react.

iii. Then the 840μl content of the cuvette was run on the cyflow cytometer which gives the CD count in cells/μl of blood. The test result was displayed on the machine screen after 3mins and result was recorded against the patient laboratory number and identification number (Mandy *et al*., 2003).

3.8 TEST FOR THE LIVER ENZYMES

The liver enzymes tested were Glutamate pyruvate transaminase (alanine aminotransferase, (i.e. GPT or ALT), Glutamate oxaloacetate transaminase (aspartate amino-transferase, (i.e. GOT or AST) and Alkaline phosphatase [ALP] .These three tests were performed quantitatively using Reflotron chemistry autoanalyser (dry chemistry) with Roche Diagnostic kits.

3.8.1 Procedure Involved in Running the Liver Enzymes; GPT Test using Roche Reflotron Plus Machine

The machine was properly cleaned and the flap opened and cleaned with alcohol swab, after which the flap was left open for 2mins to dry very well.

i. The machine was switched on, the check strip was inserted into the strip column and the flap closed. The machine was started to check the optical system and the value gotten was within the range for the check strip.

ii. The GPT strip to be tested was placed on the strip holder, with automatic pipette, 30μl of the clear serum sample was carefully applied to the application zone on the strip.

iii. The flap was carefully closed after the machine displayed the test (GPT) and the test confirmed.

iv. The strip was properly inserted in the strip column, when the machine displayed "Ready" within 15secs of dropping the serum.

v. At a temperature of 30^{o}C, the formation of dye is measured kinetically at 567nm as a measure of the enzyme activity of GPT and the result displayed in 140 seconds (Amin *et al*., 2002).

3.8.2 Procedure Involved in Running the Liver Enzymes; GOT Test using Roche Reflotron Plus Machine

The machine was properly cleaned and the flap opened and cleaned with alcohol swab, after which the flap was left open for 2mins to dry very well.

i. The machine was switched on, the check strip was inserted into the strip column and the flap closed. The machine was started to check the optical system and the value gotten was within the range for the check strip.

ii. The GOT strip to be tested was placed on the strip holder, with automatic pipette, 30μl of the clear serum sample was carefully applied to the application zone on the strip.

iii. The flap was carefully closed after the machine displayed the test (GPT) and the test confirmed.

iv. The GOT strip was properly inserted in the strip column, when the machine displayed "Ready" within 15secs of dropping the serum.

v. At a temperature of 30^{o}C, the formation of dye is measured kinetically at 567nm as a measure of the enzyme activity of GOT and the result displayed in 124 seconds (Amin *et al*., 2002).

3.8.3 Procedure Involved in Running the Liver Enzymes; ALP Test using Roche Reflotron Plus Machine

The machine was properly cleaned and the flap opened and cleaned with alcohol swab, after which the flap was left open for 2mins to dry very well.

i. The machine was switched on, the check strip was inserted into the strip column and the flap closed. The machine was started to check the optical system and the value gotten was within the range for the check strip.

ii. The ALP strip to be tested was placed on the strip holder, with automatic pipette, 30μl of the clear serum sample was carefully applied to the application zone on the strip.

iii. The flap was carefully closed after the machine displayed the test (GPT) and the test confirmed.

iv. The ALP strip was properly inserted in the strip column, when the machine displayed "Ready" within 15secs of dropping the serum.

v. At a temperature of 30^{o}C, the formation of dye is measured kinetically at 567nm as a measure of the enzyme activity of ALP and the result displayed in 135 seconds (Amin *et al*., 2002).

3.9 Tests for Hepatitis-B, Hepatitis C and Syphilis

The tests for the viral hepatitis and syphilis were carried out using "Rapid Test Dipstick",

3.9.1 Hepatitis-B

The hepatitis-B-surface antigen (HBsAg) kit was used. The HBsAg Rapid Test Dipstick is a rapid chromatographic immunoassay for the "qualitative detection of Hepatitis B surface Antigen in serum/plasma. The test dipstick contains anti-HBsAg particles and anti-HBsAg coated on the membrane.

Principle of the Test

The HBsAg Rapid Test Dipstick is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg in serum/plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the Dipstick. During testing, the serum specimen reacts with the particle coated with anti-HBsAg antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a coloured line. The presence of the coloured line in the test region indicates a positive result while its absence indicates a negative result. To serve as a procedural control, a coloured line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred (Blumberg, 1971).

3.9.2 Hepatitis- C

The Hepatitis C virus (HCV) kit used is a rapid chromatographic immunoassay for the qualitative detection of antibody to Hepatitis C virus in serum/plasma. The test Dipstick

contains recombinant HCV antigen conjugated colloid gold and HCV antigen coated on the membrane.

Principle

The HCV Rapid Test Dipstick is a qualitative, membrane based immunoassay for the detection of antibody to HCV in serum/plasma. The membrane is pre-coated with recombinant HCV antigen on the test line region of the Dipstick. During testing, the serum specimen reacts with recombinant HCV antigen conjugated colloid gold. The mixture migrates upward on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a coloured line. Presence of this coloured line indicates a positive result, while its absence indicates a negative result. A control line will always appear in the control line region indicating that proper volume of the specimen has been added (Vander Poel *et al*., 1991).

3.10 SYPHILIS

The syphilis Rapid Test Dipstick was used. It is a rapid chromatographic immunoassay for the qualitative detection of antibodies (IgG and IgM) to *Treponema pallidium* in serum/plasma to aid in the diagnosis of syphilis. The Dipstick contains syphilis antigen coated particle and syphilis antigen coated on the membrane.

3.10.1 Principle

The syphilis rapid test Dipstick is a qualitative membrane based immuoassay for the detection of *T. pallidium* antibodies (IgG and IgM) in serum/plasma. In this test procedure, recombinant syphilis antigen is immobilized in the test line region of the test. After specimen is added to the specimen well of the test Dipstick, it reacts with syphilis antigen coated particles in the test. The mixture migrates chromatographically along the length of the test and interacts with the immobilized syphilis antigen. The double antigen test format can detect both IgG and IgM in specimens. If the specimen contain *T. pallidium* antibodies, a coloured line will appear in the test region, indicating a positive result. If the specimen does not contain *T. pallidium* antibodies, a coloured line will not appear in the region indicating a negative result. The control line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred (CDC, 1999).

3.10.2 Testing Procedure for HBsAg using HBsAg Rapid Test Dipstick

i. The pouch containing the HBsAg dipsticks were properly examined to make sure the sealing of the pouch was still intact.
ii. The pouch was allowed to reach room temperature.
iii. The test dipsticks were used immediately after opening the foil pouch.
iv. One hundred and fifty microlitre of clear serum sample was carefully transferred to the bottom of the test tube.
v. With the arrows pointing towards serum specimen, the HBsAg dipstick was vertically immersed in the serum for 15 seconds making sure the serum did not pass the maximum line (MAX).
vi. The dipstick was placed on non absorbent flat surface and the timer started.
vii. After l5mins, the result were taken and recorded against each patient.
viii. Double line on the test strip indicates a positive result while a single line indicates a negative result (Johnson, 1994).

3.10.3 Testing Procedure for HCV using HCV Rapid Test Dipstick

i. The pouch containing the HCV dipsticks were properly examined to make sure the sealing of the pouch was still intact.
ii. The HCV pouch was allowed to reach room temperature.
iii. The test dipsticks were used immediately after opening the foil pouch.
iv. The HCV pouch was opened and pasted on the test card provided.
v. Twenty five microlitre of the clear serum was carefully dropped on the sample region.
vi. One hundred and twenty microlitre of the buffer was also added on the sample region and the timer started.
vii. After 10mins, the result of HCV was taken and recorded against each patient. (Wilber, 1993)

3.10.4 Testing Procedure for Syphilis using Syphilis Rapid Test Dipstick

i. The pouch containing the Syhilis dipsticks were properly examined to make sure the sealing of the pouch was still intact.
ii. The Syphilis pouch was allowed to reach room temperature.
iii. The test dipsticks were used immediately after opening the foil pouch.

iv. Carefully 150μl of clear serum sample was transferred to the bottom of a clean test tube.

v. With the arrows pointing towards serum specimen, the Syphilis dipstick was vertically immersed in the serum for making sure the serum did not pass the maximum line (MAX).

vi. The syphilis dipstick was left in the tube until the coloured line appeared.

vii. The result of the syphilis rapid test dipstick was read after 5mins (Johnson, 1994).

3.11 QUALITY CONTROL

The standard operational procedures were strictly followed for the quality control issues. The hepatitis B, C and syphilis kits were checked by using known HB_sAg, anti-HCV and anti-VDRL antibody positive and negative control samples. Similarly the quality of both CD_4 and liver enzyme reagents were regularly monitored by running control materials each morning before the actual work was done.

3.12 PRECAUTIONARY MEASURES

Proper and adequate precautions were taken during sample collection for safety. Personal protective equipments (PPE) like lab coat and hand gloves were worn to avoid direct blood stains on the body.

3.13 ASSESSMENT OF RISK FACTORS

The impact of risk factors on the participants and their health management were assessed using the data collected from the questionnaires and the participants' hospital records. These risk factors include Intravenous drug users (IDU), Alcohol abuser, Smokers and those that have undergone a surgery case. Other factors are the Homosexuals, Lesbians and those that have ever taken blood from another person through blood transfusion.

3.14 DATA ANALYSIS

Data obtained were subsequently entered and analysed using the statistical package for social science (SPSS) software, version 21.0 (Appendix VIII- XXXVIII). Mean, frequencies and percentages were used to summarize descriptive statistics of the data. Chi-square (x^2) test was used to assess relationships between selected and/or qualitative variables namely Sex, ART & Non-ART, $CD_4 \geq 200$ & $CD_4 < 200$, Occupation, Education and exposure to risk factors. Pearson correlation was used to determine the relationship between the biochemical parameters and CD_4 cells count. The significant difference was set at the threshold $P \leq 0.05$ (95% confidence interval).

CHAPTER FOUR

RESULTS

4.1 Study Population

The results show that out of the 502 people living with HIV that participated in the study, 156 (31.1%) were males while 346 (68.9%) were females, in the ration 1:2.2. Three hundred and ninety nine (79.5%) were on ART and 103 (20.5%) were not on ART. Ninety two (18.3%) participants had CD_4 count less than 200cells/μl while 410 (81.7%) had CD_4 count up to 200cells/μl and above. Coinfection of HIV/HBV and HIV/HCV were found in 26(5.2%) and 8 (1.6%) participants respectively (Table 1).

4.2 Age Distribution

The 502 people living with HIV/AIDS that participated in this study were grouped in to five age groups. The mean age of the participants was 32 and their ages ranged from 1½ to 78 years. Table 2 show that age group 31-45 had the highest number of participants, 246 (49.0%) while age group 61 and above had the least number of participants, 15(3.0%). Age group 16-30 had the least percentage of male participants (18.5%) while age group 61 and above had the least percentage of female participants (26.7%).

From Table 3, the statistical analysis showed that the difference between participants on ART and those not on ART is not statistically significant ($P > 0.05$). Table 3 indicates that age group 61 and above had the highest percentage of participants on ART and age group 16-30 had the highest percentage of their participants not on ART (Non-ART).

From Table 4, Age does not significantly affect the CD_4 count of the people living with HIV/AIDS ($P > 0.05$).

From Table 4, all the age groups had higher percentages of their members having CD_4 count 200 cells/μl of blood and above. When the CD4cell count of a person living with HIV drops below 200cells/μl due to advanced HIV disease, the individual is diagnosed with AIDS, hence the division of CD4 count <200 and $\geq$ 200cells/μl.

The geographical prevalence of HIV/AIDS and viral hepatitis within the state could not really be ascertained from this study since the participants come from diverse areas due to HIV/AIDS stigmatization and discrimination.

Table 1: ART, CD_4^+, HBV & HCV and Syphilis status of PLWHA studied

Characteristics	**Parameters**	**Result Frequency (%)**
Gender	Male	156 (31.1)
	Female	346 (68.9)
	Total	502
ART/Non-ART	ART	399 (79.5)
	Non-ART	103 (20.5)
	Total	502
CD_4^+ cell count /μl	<200	92 (18.3)
	≥200	410(81.7)
	Total	502
Positive to viral hepatitis	HBV	26 (5.2)
	HCV	8(1.6)
	Syphilis	0 (0.0)
Elevated Liver enzymes	GPT/ALT	24 (4.8)
	GOT/AST	30 (6.0)
	ALP	16 (3.2)

Table 2: Gender distribution of the participants

Age group	**Female N(%)**	**Male N(%)**	**Total N (%)**
0-15	14 (53.8)	12(46.2)	26 (5.2)
16-30	97 (81.5)	22 (18.5)	119 (23.7)
31-45	177 (72.0)	69 (28.0)	246 (49.0)
46-60	54 (56.3)	42 (43.7)	96 (19.1)
61 & above	4 (26.7)	11 (73.3)	15 (3.0)
	346	156	502

Table 3: Distribution of participants based on anti-retroviral therapy (ART) or Not (Non-ART)

Age group	**ART N (%)**	**Non -ART N(%)**	**Total (%)**
0-15	20 (76.9)	6 (23.1)	26 (5.2)
16-30	85 (71.4)	34 (28.6)	119 (23.7)
31-45	202 (82.1)	44 (17.9)	246 (49.0)
46-60	79 (82.3)	17 (17.7)	96 (19.1)
61 & above	13 (86.7)	2(13.3)	15 (3.0)
	399	103	502 (100)

$P = 0.146$ $(P > 0.05)$

Table 4: Distribution of participants based on CD4 cell count

Age group	**<200 (%)**	**>200 (%)**	**Total (%)**
0-15	2(7.7)	24(92.3)	26 (5.2)
16-30	16 (13.4)	103 (86. 6)	119 (23.7)
31-45	50 (20.3)	196 (79.7)	246 (49.0)
46-60	21 (21.9)	75 (78.1)	96 (19.1)
61 & above	3 (20.0)	12 (80.0)	15 (3.0)

$P = 0.253$ ($P > 0.05$)

Table 5 contains the reference ranges for the liver enzymes as written in Reflotron Roche's Diagnostic inclusion manual. Based on these normal values, participants with liver enzyme values above these were determined. The values in the table are the upper limit values.

Table 6 showed the level of liver enzyme elevations as obtained from the results. The level of elevation of the liver enzymes of most of the participants as shown in the table is mild to moderate. For GPT and GOT, elevations ranging from 60 to 100 IU/L and ALP ranging from 140 to 200 IU/L, the elevation are said to be moderate but GPT and GOT above 100 IU/L and ALP above 200 IU/L are considered to be severe.

From Table 7,for HIV and viral Hepatitis,

This implies that age is not a factor for persons living with HIV/AIDS to be coinfected with viral hepatitis ($P > 0.05$; $P = 0.139$)

For HIV and HBsAg: The analysis showed that age significantly affected the coinfection with hepatitis B in patients living with HIV/AIDS ($P< 0.05$; $P = 0.024$)

For HIV and HCV: Age was not a determinant factor for coinfection with HIV/HCV ($P > 0.05$; $P = 0.414$)

4.3 Prevalence of elevated liver enzymes

According to the normal ranges given in the inclusion manual of the Reflotron Roche's Diagnostic kits used in this study as shown in table 5, 24(4.8%) participants had elevated GPT while 30 (6.0%) and 16 (3.2%) had elevated GOT and ALP respectively. The Table 8 showed that age group 46-60 had the highest percentages of participants with elevated GPT, GOT and ALP while age group 16-30 had lowest percentages of participants with elevated GPT and GOT; and age group 61 and above had the lowest for ALP. The levels of elevation of the liver enzymes as obtained in the results were shown in Table 6.

4.4 Seroprevalance of HIV/HBV and HIV/HCV Coinfection

In this study, the seroprevalence of HIV/HBV and HIV/HCV were 5.2% and 1.6% respectively. Table 7 showed that age group 0-15 had the highest percentage of members positive to HBsAg though age group 31-45 had the highest number of participants. Age group 60 and above had the highest percentage of participants that tested positive to HCV, while age group 0-15 had the least. Coinfection of HIV/HBV/HCV was 0.0%

This study unlike many other researches on HBV and HCV coinfection in PLWHA involved children 15years and below. HIV prevalence in the children was (26) 5.2% of the whole participants which comprises 14(2.8%) females and 12(2.4%) males. Among the children, 3 (0.6%) were coinfected with HBV but coinfection with HCV was 0.0%

Table 5: Normal values of liver enzymes and CD_4 cell count

Parameters	**Male**	**Female**
GPT/ALT	up to 41 IU/L	up to 32 IU/L
GOT/AST	Up to 40 IU/L	up to 33 IU/L
ALP	up to 129 IU/L	up to 104 IU/L
CD_4 cell count	400 – 1,200 cells/μl	500 – 1,500 cells/μl

(Heil *et al*., 2000)

Table 6: Elevation ranges of the participants with elevated liver enzymes

Parameters	**Ranges (IU/L)**	**Male**	**Female**
GPT/ALT	60-100	11	12
	101 & above	0	1
GOT/AST	60-100	12	17
	101 & above	0	1
ALP	140-200	7	8
	201 & above	0	1

Table 7: Distribution of the studied population for viral hepatitis (HB_sAg and HCV) according to age

Age group	HIV (%) only	HIV/HBsAg (%)	HIV/HCV(%)	Total
0-15	23 (88.5)	3(11.5)	0 (0.0)	26 (5.2)
16-30	116 (97.5)	0 (0.0)	3 (2.5)	119 (23.7)
31-45	225 (91.5)	18 (7.3)	3(1.2)	246 (49.0)
46-60	91 (94.8)	4 (4.2)	1 (1.0)	96 (19.1)
61 & above	13 (86.7)	1 (6.7)	1 (6.7)	15 (3.0)
	468	26	8	502 (100)

For HIV/ HBsAg; $P = 0.024$ ($P < 0.05$)

For HIV/HCV; $P = 0.414$ ($P > 0.05$)

From Table 8, for HIV/GPT, the analysis showed that an individual living with HIV/AIDS having elevated GPT is not determined by the age of the individual ($P > 0.05$).

For HIV/GOT: It implies that age of a person living with HIV/AIDS does not affect the person GOT level ($P > 0.05$).

For HIV/ALP; In this case, the analysis showed that age does not in any way affect the ALP level of an individual living with HIV/AIDS ($P > 0.05$).

N.B some of the participants have only one enzyme elevated.

Table 9,for HIV/GPT/GOT; The analysis showed that age does not significantly affect the GPT and GOT levels of individuals living with HIV/AIDS ($P> 0.05$).

For HIV/GPT/ALP: This showed that age does not contribute to the GPT and ALP of individuals living with HIV/AIDS being elevated ($P > 0.05$).

For HIV/GOT/ALP: It means that age is not a factor in raising the GOT arid ALP of persons living with HIV/AIDS ($P > 0.05$).

For HIV/GPT/GOT/ALP: The analysis showed that age does not significantly affect the level of liver enzymes in people living with HIV/AIDS ($P > 0.05$).

From the Table 9, age group 61 and above had none of it's participants with more than one liver enzymes elevated, while age groups 0-15 and 16-30 had one participant each with more than one liver enzymes elevated.

Table 8: Distribution of the studied population according to elevated liver enzyme values

Age group	HIV (%) only	HIV/GPT (%)	HIV/ GOT (%)	HIV/ ALP (%)
0-15	23 (88.5)	2 (7.7)	2 (7.7)	1 (3.8)
16-30	116 (97.5)	1 (0.8)	3 (2.5)	3 (2.5)
31-45	225 (91.5)	12 (4.9)	14 (5.7)	8 (3.2)
46-60	91 (94.8)	8 (8.3)	10 (10.4)	4(4.1)
6 1 & above	13 (86.7)	1 (6.7)	1 (6.7)	0 (0.0)
	468	24	30	16

For HIV/GPT; P = 0.115 (P > 0.05)

For HIV/GOT; P = 0.185 (P > 0.05)

For HIV/ALP; P = 0.906 (P > 0.05)

Table 9: Distribution of the studied population with more than one liver enzymes elevated

Age group	HIV (%) only	HIV/GPT/GOT	HIV/GPT/ALP	HIV/GOT/ALP	HIV/GPT/GOT/ALP
0-15	23 (88.5)	1	0	0	0
16-30	116 (97.5)	0	0	1	0
31-45	225 (91.5)	1	0	3	3
46-60	91 (94.8)	2	1	1	1
61 and above	13 (86.7)	0	0	0	0
	468	4	1	5	4

For HIV/GPT/GOT; P = 0.155 (P > 0.05)

For HIV/GPT/ALT; P = 0.369 (P > 0.05)

For HIV/GOT/ALP; P = 0.967 (P > 0.05)

For HIV/GPT/GOT/ALP; P = 0.751 (P > 0.05)

For Table 10, For CD_4, The analysis showed that the CD_4 count level of people living with HIV/AIDS is not significantly affected by the gender of the person ($P > 0.05$).

For HIV/HBsAg, This analysis means that coinfection with HIV/HBV is significantly influenced by the gender of the individual ($P < 0.05$).

For HIV/HCV, It implies that gender is not a factor for a person living HIV/AIDS to be coinfected with HCV ($P > 0.05$).

For HIV/GPT, This showed that gender does not significantly affect the GPT level of people living with HIV ($P > 0.05$).

For HIV/GOT, This implies that gender does not significantly affect the level of GOT in people living with HIV/AIDS ($P > 0.05$).

For HIV/ALP, It means that gender is not a factor that determines the level of ALP in individuals living with HIV/AIDS ($P > 0.05$).

Table 10 showed that out of the 92 participants with CD_4 count less than 200; 57 (62.0%) were females while 35(38.0%) were males. Amongst the 26 participants that tested positive to HB_sAg, 11(42-3%) were females and 15(57.7%) were males.

4.5 Gender Effects on Coinfection of HIV/HBV, HIV/HCV and CD_4 count

Coinfection of HIV/HBV occurred more in male participants, 15(57.7%) than in females 11(42.3%) while coinfection of HIV/HCV was more in females 7(87.5%) than in the males counterparts (Table 10). The difference in the HIV/HBV coinfection between the males and females participants was statistically significant ($P < 0.05$) but not significant for HIV/HCV coinfection ($p>0.05$). Thirty five (22.4%) of the male participants had CD_4 count less than 200cells/μl of blood while 51 (16.5%) of the females had CD_4 count less than 200 (Table 10). The mean CD_4 count of the female participants was higher (546.8 ± 18.84) (Appendix XXXIX) than that of the males, (480.0 ± 30.13), though the difference was not statistically significant ($P > 0.05$).

Table 10: Gender distribution of participants with elevated liver enzymes and viral hepatitis

Parameters	**Female (%)**	**Male (%)**
CD_4 < 200	57 (62.0)	35 (38.0)
>200	289 (70.5)	121 (29.5)
HbsAg	11 (42.3)	15 (57.7)
HCV	7 (87.5)	1(12.5)
GPT	13(54.2)	11(45.8)
GOT	18(60.0)	12 (40.0)
ALP	9(56.3)	7 (43.7)

For CD_4; P = 0.110 (P > 0.05)

For HIV/HBsAg; P = 0.003 (P < 0.05)

For HIV/HCV; P = 0.252 (P > 0.05)

For HIV/GPT; P = 0.106 (P > 0.05)

For HIV/GOT; P = 0.268 (P > 0.05)

For HIV/ALP; P = 0.260 (P > 0.05)

From Table 11, for HIV/HBsAg, This analysis means that CD_4 count does not significantly influence the person living with HIV/AIDS being exposed to HBV infection ($P > 0.05$).

For HIV/HCV, In this case CD_4 count is not a predisposing factor for coinfection with HIV/HCV($P > 0.05$).

For HIV/GPT, This showed that the CD_4 count of people living with HIV/AIDS does not significantly affect the level of GPT ($P > 0.05$).

For HIV/GOT, This means that the level of CD_4 count of people living with HIV/AIDS is significantly determined by the level of GOT ($P < 0.05$).

For HIV/ALP, The analysis in this case showed that elevated ALP in people living with HIV/AIDS significantly influenced the CD_4 count of the individual ($P < 0.05$).

From the Table 11 , out of the 26 participants that tested positive to HBsAg, 5(19.2%) had CD_4 count less than 200 and 21 (80-8%) had CD_4 count 200 and more. 7 (29.2%) of the participants with elevated GPT had CD_4 count lower than 200 while 17 (70,8%) had CD_4 count up to 200 and above.

4.6 The Impact of HBV/HCV on CD_4 Count

Out of the 26 participants that tested positive to HBsAg, 5(19.2%) had CD_4 count less than 200 and 21 (80.8%) had CD_4 count 200 and more. The mean CD_4 of participants coinfected with HBsAg was 494.9 ± 81.3 cells//μl of blood which was lower than CD_4 count of those with only HIV (531. 0±163). One person (12.5%) of the 8 people coinfected with HIV/HCV had CD_4 less than 200 while 7 (87.5%) had CD_4 count 200 and above. The mean CD_4 count of participants coinfected with HIV/HCV was 673.4± 136.7, higher than that for those not coinfected (526.8+16.14). The differences in the CD_4 counts were not statistically significant ($P > 0.05$).

4.7 The Effects of elevated Liver Enzymes on CD_4 count

The participants with elevated GPT, 7(29.2%) out of 24 had CD_4 count less than 200, 13(43.3%) out of 30 people that had elevated GOT, had CD_4 count less than 200 while 9 (56.3%) out of 16 people with elevated ALP also had their CD_4 count less than 200cells/μl of blood (Table 11). Statistically analysed, the difference in the CD_4 counts of those with elevated GPT and those with normal GPT was not significant ($P > 0.05$), but the difference in the CD_4 counts of those that had elevated GOT and ALP, and those with normal GOT and ALP were statistically significant ($P < 0.05$). The mean CD_4 count of those participants with elevated GPT, GOT and ALP were 382.0 $\pm$ 18.1, 328.7 $\pm$ 13.4 and 256.5 $\pm$ 12.8 respectively. Those participants without liver enzyme elevation have high mean CD_4 count of 561.0 $\pm$ 11.7.

Table 11: Distribution of participants with viral hepatitis and elevated liver enzymes based on CD4 count

Parameters	**CD_4 count < 200(%)**	**≥200 (%)**	**Total**
HBsAg	5 (19.2)	21 (80.8)	26
HCV	1 (12.5)	7(87.5)	8
GPT	7 (29.2)	17 (70.8)	24
GOT	13 (43.3)	17 (56.7)	30
ALP	9 (56.3)	7(43.7)	16

For HIV/HBsAg; P = 0.903 (P > 0.05)

For HIV/HCV; P = 0.668 (P > 0.05)

For HIV/GPT; P = 0.161 (P > 0.05)

For HIV/GOT; P = 0.000 (P < 0.05)

For HIV/ALP; P = 0.000 (P < 0.05)

From Table 12, for CD_4, This analysis showed that ART significantly affected the CD_4 level of people living with HIV/AIDS ($P < 0.05$).

For HIV/HBsAg, It showed that ART does not influence the presence of HBV in individuals living with HIV/AIDS ($P > 0.05$).

For HIV/HCV, This means that coinfection with HIV/HCV is not significantly based on the use of ART in people living with HIV/AIDS ($P > 0.05$).

For HIV/GPT, This showed that ART does not significantly influence the level of GPT on people living with HIV/AIDS ($P > 0.05$).

For HIV/GOT, The analysis showed that ART does not significantly affect the level of GOT in people living with HIV/AIDS ($P > 0.05$).

For HIV/ALP, It implied that ART does not determine the level of ALP in people living with HIV/AIDS ($P > 0.05$).

From the Table 12, among the 410 participants with CD_4 count 200 and above, 336 (82.0%) were on ART while 74 (18.0%) were Non-ART. Higher percentages of the participants that tested positive to viral hepatitis and elevated liver enzymes were on ART.

4.8 The Effect of ART on HBV/HCV

Twenty four people representing 92.3% of people coinfected with HIV/HBV use ART and 2(7.7%) do not use ART while 7(87.5%) of people coinfected with HIV/HCV use ART and 1(12.5%) does not use ART (Table 12). Although majority of the participants coinfected with either HIV/HBV or HIV/HCV were on ART, the differences were not statistically significant ($P > 0.05$).

Table 12: Distribution of participants with viral hepatitis and elevated liver enzymes based on either ART or Non –ART

Parameters	**ART (%)**	**Non - ART (%)**	**Total**
CD_4<200	63 (68.5)	29 (31.5)	92
≥200	336 (82.0)	74 (18.0)	410
HB_sAg	24 (92.3)	2 (7.7)	26
HCV	7 (87.5)	1 (12.5)	8
GPT	20 (83.3)	4 (16.7)	24
GOT	21 (70.0)	9 (30.0)	30
ALP	13 (81.3)	3 (18.7)	16

For CD_4; P = 0.004 (P < 0.05)

For HIV/HBsAg; P = 0.096 (P > 0.05)

For HIV/HCV; P = 0.571 (P > 0.05)

For HIV/GPT; P = 0.629 (P > 0.05)

For HIV/GOT, P = 0.187 (P > 0.05)

For HIV/ALP, P = 0.856 (P > 0.05)

4.9 The Effect of ART on Liver Enzymes

Twenty (83.3%) people among the 24 with elevated GPT were on ART, 21(70.0%) of 30 people that had elevated GOT were on ART and also 13(81.3%) of 16 that had elevated ALP use ART. This implied that greater percentages of the participants that had elevated levels of the liver enzymes use ART.

This study recorded 1.6% prevalence of HCV among people living with HIV/AIDs, which was exactly the percentage reported in Kano by Hanza et al., (2013) but the 5.2% prevalence of HBV recorded in this study was lower than 12.3% reported by Hanza et al., (2013) in their own research.

From Table 13, higher percentage of the participants (58.4%) were married, 32.3% were single while 7.7% and 1.6% were widowed and divorced respectively. In educational status, 224(44.6%) of the participants had secondary school certificate, while 196(39.0%) had Tertiary Institution certificate. This really showed high literacy among the participants. Majority of the participants were students, 157(31.3%) or in business, 146(29.1%) while unemployed group had the least members, 34 (6.8%). About 159 (31.7%) of the participants were exposed to risk factors (NB: 45 were exposed to more than one risk factor). Those that were exposed to blood transfusion were the highest followed by smokers, IV drug users and homosexual/lesbians have the least number.

From Table 14, for HIV/Viral hepatitis, the analyses show that the marital status of the participant does not affect the coinfection of viral hepatitis with HIV ($P > 0.05$).

For HIV/HBV, The coinfection of HIV with HBsAg is not determined by the marital status of the individual ($P > 0.05$).

For HIV/HCV, Marital status is not a determinant factor for coinfection with HIV/HCV ($P > 0.05$).

From Table 15, for HIV/GPT, The analysis showed that marital status affected the level of GPT of an individual living with HIV/AIDS ($P < 0.05$).

For HIV/GOT, Marital status does not affect the GOT level of a person living with HIV/AIDS ($P > 0.05$).

For HIV/ALP, The analysis showed that marital status of an individual does not in any way affect the ALP of a person living with HIV/AIDS ($P > 0.05$).

Table 13: Socio-demographic characteristics of the participants

Characteristics	**Groups**	**Frequency (%)**
Marital status	Single	162(32.3)
	Married	293(58.4)
	Widowed	39(7.7)
	Divorced	8(1.6)
	Total	502
Educational level	Non-formal Edu.	50(10.0)
	Primary	32(6.4)
	Secondary	224(44.6)
	Tertiary Edu.	196(39.0)
	Total	502
Occupation	Civil servants	63(12.5)
	Artisans	102(20.3)
	Business	146 (29.1)
	Students	157(31.8)
	Unemployed	34(6.8)
	Total	502
Risk factors	IV drug users	11(2.2)
	Blood transfusion	78(15.5)
	Surgery	32(6.4)
	Alcohol abuse	25(5.0)
	Smokers	48(9.6)
	Homosexual/lesbians	10(2.0)
	Total	204

Table 14: Marital status of participants that tested positive to viral hepatitis

Marital status	**HIV only (%)**	**HIV/HBsAg(%)**	**HIV/HCV (%)**	**Total (%)**
Single	150(92.6)	9(5.3)	3(1.9)	162 (32.3)
Married	276 (94.2)	13(4.4)	4(1.2)	293 (58.4)
Widowed	35(89.7)	3(7.7)	1(2.6)	39 (7.7)
Divorced	7(87.5)	1(12.5)	0(0.0)	8 (1.6)
	468	26	8	502 (100)

For HIV/HBV; P = 0.626 (P > 0.05)

For HIV/HCV; P = 0.912 (P > 0.05)

Table 15: Marital Status of participants with elevated GPT or GOT or ALP

Marital status	**HIV only (%)**	**HIV/GPT (%)**	**HIV/GOT (%)**	**HIV/ALP(%)**
Single	150(92.6)	8(4.9)	10(6.2)	5(3.1)
Married	276 (94.2)	11(3.8)	19(6.5)	11(3.8)
Widowed	35(89.7)	3(7.7)	1(2.6)	0(0.0)
Divorced	7(87.5)	2(12.5)	0(0.0)	0(0.0)
	468	24	30	16

For HIV/GPT; P = 0.035 (P < 0.05)

For HIV/GOT; P = 0.691 (P > 0.05)

HIV/ALP; P = 0.602 (P > 0.05)

4.10: Marital Status of the Participants

The 502 participants were grouped into 4 groups; single, married, widowed and divorced. Two hundred and ninety-three representing 58.4% of the participants were married while the divorced group had least participants, 8 representing 1.6%. Among the male participants, 68 representing 44% of them were single while 94 female participants (27%) were single. The female participants had higher percentages of participants that were married and widowed than the male counterparts. From Fig. 6, General Hospital Ekwulobia had highest percentage of married participants while General Hospital Onitsha had highest percentage of single participants.

From Table 16,for HIV/HBV

The above analysis showed that educational level of a person living with HIV/AIDS does not affect coinfection of HIV/HBV ($P > 0.05$).

For HIV/HCV,

This implied that coinfection of HIV/HCV was not significantly affected by the educational level of the individual ($P > 0.05$).

From Table 17,for HIV/GPT, This means that the level of education of an individual living with HIV/AIDS does not affect GPT level ($P > 0.05$).

For HIV/GOT, This showed that the GOT level of a person living with HIV/AIDS is not dependent on his/her level of education ($P > 0.05$).

For HIV/ALP, From the analysis, ALP level of an individual living with HIV/AIDS is not affected by the educational level of the individual ($P > 0.05$).

From Table 18, For HIV/HBsAg, This showed that the occupation of a person living with HIV/AIDS does not determine coinfection of HIV/HBsAg ($P > 0.05$).

For HIV/HCV, This implies that coinfection of HIV/HCV is not affected in any way by the occupation of the individual ($P > 0.05$).

From Table 19, for HIV/GPT, The above analysis showed that the GPT level of an individual living with HIV/AIDS is independent on the occupation of the individual ($P > 0.05$).

For HIV/GOT, This showed that the occupation of a person living with HIV/AIDS does not affect his/her GOT level ($P > 0.05$).

For HIV/ALP, This implies that the ALP level is not determined by the occupation of a person living with HIV/AIDS ($P > 0.05$).

Table 16: Educational status of participants that tested positive to viral hepatitis

Educational level	**HIV only (%)**	**HIV/HBsAg(%)**	**HIV/HCV (%)**	**Total**
Non-formal Edu.	45(9.0)	4(8.0)	1(0.2)	50(1.0)
Primary	30(6.0)	2(0.4)	0(0.0)	32(6.4)
Secondary School	208 (41.4)	11(2.2)	5(1.0)	224(44.6)
Tertiary Edu.	185(36.9)	9(1.8)	2(0.4)	196(39.0)
	468 (93.2)	26 (5.2)	8 (1.6)	502(100)

For HIV/HBsAg; P = 0.788 (P > 0.05)

For HIV/HCV; P = 0.668 (P > 0.05)

Table 17: Educational status of participants with elevated GPT or GOT or ALP

Educational level	**HIV only (%)**	**HIV/GPT(%)**	**HIV/GOT(%)**	**HIV/ALP(%)**
Non- formal Edu.	45(9.0)	3(0.6)	1(0.2)	0(0.0)
Primary	30(6.0)	3(0.6)	2(0.4)	1(0.2)
Secondary School	208 (41.4)	12(2.4)	16(3.2)	10(2.0)
Tertiary Edu.	185(36.9)	6(1.2)	11(2.2)	5(1.0)
	468 (93.2)	24(4.8)	30 (6.0)	16(3.2)

For HIV/GPT; P = 0.379 (P > 0.05)

For HIV/GOT; P = 0. 572 (P > 0.05)

For HIV/ALP; P = 0.378 (P > 0.05)

Table 18: Occupation of participants that tested positive to viral hepatitis

Occupation	**HIV only (%)**	**HIV/HBsAg(%)**	**HIV/HCV(%)**	**Total**
Civil servant	57(11.4)	4(0.8)	2(0.4)	63 (12.5)
Artisans	96(19.1)	5(1.0)	1(0.2)	102(20.3)
Traders	136(27.1)	8(1.6)	2(0.4)	146(29.1)
Students	148(29.5)	7(1.4)	2(0.4)	157(31.3)
Unemployed	31(6.2)	2(0.4)	1(0.2)	34(6.8)
	468 (93.2)	26 (5.2)	8(1.6)	502(100)

For HIV/HBsAg; P = 0.981 (P > 0.05)

For HIV/HCV; P = 0.774 (P > 0.05)

Table 19: Occupation of participants with elevated GPT, or GOT or ALP

Occupation	**HIV only (%)**	**HIV/GPT(%)**	**HIV/GOT(%)**	**HIV/ALP(%)**
Civil servant	57(11.4)	3(0.6)	3(0.6)	2(0.4)
Artisans	96(19.1)	4(0.8)	7(1.4)	3(0.6)
Traders	136(27.1)	6(1.2)	11(2.2)	5(1.0)
Students	148(29.5)	8(1.6)	8(1.6)	6(1.2)
Unemployed	31(6.2)	3(0.6)	1(0.2)	0(0.0)
	468 (93.2)	24(4.8)	30(6.0)	16(3.2)

For HIV/GPT; P = 0.774 (P > 0.05)

For HIV/GOT; P= 0.788 (P > 0.05)

For HIV/ALP; P = 0.849 (P > 0.05)

From Table 20, for HIV/HBsAg, This showed that coinfetcion of HIV/HBsAg is significantly affected by exposure to risk factors for people living with HIV/AIDS ($P < 0.05$).

For HIV/HCV, This implies that exposure to risk factors does not necessarily determine coinfection of HIV/HCV ($P > 0.05$).

From Table 21,for HIV/GPT, The above showed that GPT level of a person living with HIV/AIDS is not affected by exposure to any risk factor ($P > 0.05$).

For HIV/GOT, This implies that GOT level of an individual living with HIV/AIDS is not affected by the individual being exposed to any risk factor ($P > 0.05$).

For HIV/ALP, This means that the ALP level is not dependent on exposure to risk factors in people living with HIV/AIDS ($P > 0.05$).

From Table 22, for ART, From the above table, participant being on ART or Non-ART is not determined by the marital status of the participants. Just as greater percentage of the whole participants were on ART, greater percentage of each marital group were on ART ($P > 0.05$).

From Table 22 , for CD_4 From the table above, marital status significantly affected the CD_4 level of a person living with HIV/AIDS. All marital groups have greater percentages of their members with CD_4 count 200cells/μl and above ($P < 0.05$).

From Table 23, for Gender, Gender does no significantly affect the educational level of the participant ($P > 0.05$).

From this table, a person living with HIV/AIDS being on ART is not dependent on the educational level of the individual. All the educational levels have greater percentages of their members on ART.

From the Table 23, educational level significantly affected the CD_4 level of a person living with HIV/AIDS, also the illiterate and elementary groups have higher percentages of those with CD_4 less than 200cells/μl of blood ($P < 0.05$).

4.11 Educational level of the participants

The whole participants were allocated into 4 groups namely; illiterate, elementary, secondary and OND cert. & above based on the highest educational level attained by each of the participants. Majority of the participants, 420 (83.6%) had at least secondary school certificate. Those with Non-formal education among the participants were very few, 50(10.0%). From Table 24, low literacy level was found more among the male participants

than the female participants. This confirms the common saying that Easterners train their girls more in schools while the boys will rush into one business or the other. From Fig. 7, General Hospital Onitsha had the highest percentages of participants with high school certificate and OND or above while General Hospital Ekwulobia had highest percentages of participants with elementary school certificate and illiterate. This implied that the illiteracy level among the participants were found more in rural or semi urban of Ekwulobia than Onitsha which is an urban city.

From Table 24, for Gender, The Table showed that gender does not significantly affect the occupation of the participants ($P > 0.05$).

From Table 24, for ART, The Table indicated that the occupation of an individual living with HIV/AIDS is significantly affected the individual being on ART or Non-ART ($P < 0.05$).

From Table 24, for CD_4, From the Table, occupation of a person living with HIV/AIDS significantly influenced the CD_4 level of the person ($P < 0.05$).

From Table 25, for Gender, The above analysis showed that gender of the participants significantly influenced the rate at which they were exposed to risk factors ($P < 0.05$). Higher percentages of male were exposed to most of the risk factors in the Table 26.

From Table 25, for ART, The analysis implied that exposure to risk factors did not significantly affect the participants being on ART or Non-ART ($P > 0.05$). Just as in overall participants, there were higher percentages on ART than non-ART, the same applied to the participants that were exposed to risk factors.

From Table 25, For CD_4, from the analysis, the CD_4 level of the participants was not influenced by the participant being exposed to risk factors or not ($P > 0.05$).

4.12: Participants exposure to risk factors

Some of the participants, 159(31.7%) were exposed to certain risk factors while 45(9.0%) of the participants exposed to risk factors were exposed to more than one risk factor. The most common among those exposed to more than one risk factor, were those exposed to smoking and alcohol abuse, and also those who had undergone surgery and blood transfusion. Those exposed to blood transfusion had highest participants 78 (15.5%), while those for IV drug users and homosexual/lesbianism had the least participants of 11 (2.2%) and 10 (2.0%) respectively. In all the risk factors, General Hospital Onitsha had the highest number of participants exposed as observed from Fig. 9. From the figure also, it was noticed that none of the participants from General Hospital Enugwu-ukwu was involved in IV drug usage. Table 25 showed that all the IV drug users were males and that males had highest percentage of participants exposed to most of the risk factors than the female participants.

Table 20: Participants exposed to risk factors that tested positive to viral hepatitis

Risk factors	**HIV only (%)**	**HIV/HBsAg(%)**	**HIV/HCV(%)**	**Total(%)**
IV Drug users	9(4.4)	2(0.9)	0(0.0)	11(5.4)
Blood transfusion	76(37.3)	1(0.5)	1(0.5)	78(38.2)
Surgery	29(14.2)	2(0.9)	1(0.5)	32(15.7)
Alcohol abuse	20(9.8)	5(1.5)	0(0.0)	25(12.3)
Smokers	45(22.1)	2(0.9)	1(0.5)	48(23.5)
Homosexual lesbians	8(3.9)	2(0.9)	0(0.0)	10(4.9)
	187(91.7)	14(6.9)	3(1.5)	204(100)

For HIV/HBsAg; $P = 0.007$ ($P < 0.05$)

For HIV/HCV; $P = 0.920$ ($P > 0.05$)

Table 21: Frequency of Participants exposed to risk factors that had elevated GPT, or GOT or ALP

Risk factors	**HIV only (%)**	**HIV/GPT(%)**	**HIV/GOT(%)**	**HIV/ALP(%)**	**Total(%)**
IV Drug users	9(4.4)	1(0.5)	1(0.5)	0(0.0)	12(5.7)
Blood transfusion	76(37.3)	2(0.9)	3(1.4)	1(0.5)	80(37.9)
Surgery	29(14.2)	0(0.0)	2(0.9)	1(0.5)	34(16.1)
Alcohol abuse	20(9.8)	3(1.4)	3(1.4)	0(0.0)	26(12.3)
Smokers	45(22.1)	1(0.5)	4(1.9)	0(0.0)	49(23.3)
Homosexual/lesbians	8(3.9)	1(0.5)	0(0.0)	1(0.5)	10(4.7)
	187(91.7)	8(3.8)	13(6.2)	3(1.4)	211(100)

For HIV/GPT; P = 0.145 (P > 0.05)
For HIV/GOT; P = 0.656 (P > 0.05)
For HIV/ALP; P = 0.228 (P > 0.05)

Table 22: Marital status of participants based on sex, ART/Non ART and CD_4 count

Marital Status	**Male (%)**	**Female (%)**	**ART (%)**	**Non-ART(%)**	**CD_4 <200 (%)**	**CD_4≥200 (%)**
Single	68(13.5)	94(18.7)	125(31.3)	37(35.9)	42(8.4)	120(29.3)
Married	82(16.3)	211(42.0)	235(58.9)	58(56.3)	39(7.8)	254(61.9)
Widowed	4(0.8)	35(7.0)	32(8.0)	7(6.8)	11(2.2)	28(6.8)
Divorced	2(0.4)	6(1.2)	7(1.8)	1(1.0)	0(0.0)	8(1.9)
	156(31.1)	346(68.9)	399(79.5)	103(20.5)	92(18.3)	410(81.7)

For ART; P = 0.776 (P > 0.05)

For CD_4 ; P = 0.001 (P < 0.05)

For Sex ; P = 0.241 (P > 0.05)

Table 23: Educational status of participants based on sex, ART/Non ART and CD_4 count

Educational level	**Male (%)**	**Female (%)**	**ART (%)**	**Non-ART(%)**	**CD_4 <200 (%)**	**$CD_4 \geq 200$ (%)**
Non-formal Edu.	18(3.6)	32(6.4.)	43(8.6)	7(1.4)	20(4.0)	30(6.0)
Primary	15(3.0)	17(3.4)	26(5.2)	6(1.2)	13(2.6)	19(3.8)
Secondary school	66(13.1)	158(31.5)	168(33.4)	56(11.2)	34(6.8)	190(37.8)
Tertiary Edu.	57(11.4)	139(27.6)	162(32.3)	34(6.7)	25(5.0)	171(24.0)
	156(31.1)	346(68.9)	399(79.5)	103(20.5)	92(18.4)	410(81.6)

For Gender; $P = 0.031$ ($P < 0.05$)

For ART; $P = 0.149$ ($P > 0.05$)

For CD_4; $P = 0.000$ ($P < 0.05$)

Table 24: Occupation of participants based on sex, ART/Non ART and CD_4 count

Occupation	**Male (%)**	**Female (%)**	**ART (%)**	**Non-ART(%)**	**CD_4 <200(%)**	**CD_4 ≥200(%)**
Civil servant	18(3.6)	45(9.0)	59(11.8)	4(0.8)	9(1.8)	54(10.8)
Artisans	28(5.6)	74(14.7)	88(17.5)	14(2.8)	25(5.0)	77(15.3)
Traders	49(9.8)	97(19.3)	108(21.5)	38(7.6)	11(2.2)	135(26.9)
Students	53(10.6)	104(20.7)	114(22.7)	43(8.6)	35(7.0)	122(24.3)
Unemployed	8(1.6)	26(5.2)	30(6.0)	4(0.8)	12(2.4)	22(4.4)
	156(31.1)	346(68.9)	399(79.5)	103(20.5)	92(18.4)	410(81.6)

For Sex; $P = 0.325$ ($P > 0.05$)

For ART; $P = 0.001$ ($P < 0.05$)

For CD_4 ; $P = 0.000$ ($P < 0.05$)

Table 25: Distribution of participants exposed to risk factors based on Gender, ART/Non ART and CD_4 count

Risk Factors	**Male (%)**	**Female (%)**	**ART(%)**	**Non-ART (%)**	**CD_4 <200(%)**	**CD_4 ≥200(%)**
IV Drug users	11(5.4)	0(0.0)	8(3.9)	3(1.5)	2(1.0)	9(4.4)
Blood transfusion	32(15.7)	46(22.5)	58(28.4)	20(9.8)	17(8.3)	61(29.9)
Surgery	12(5.9)	20(9.8)	27(13.2)	5(2.5)	7(3.4)	25(12.3)
Alcohol abuse	21(10.3)	4(2.0)	18(8.8)	7(3.4)	6(2.9)	19(9.3)
Smokers	45(22.1)	3(1.5)	35(17.2)	13(6.4)	16(7.9)	32(15.7)
Homosexual/Lesbianism	8(3.9)	2(1.0)	7(3.4)	3(1.5)	2(1.0)	8(3.9)
	129 (63.2)	75(36.8)	153(75.0)	51(25.0)	50(24.5)	154(75.5)

For Sex; $P = 0.024$ ($P < 0.05$)

For ART; $P = 0.861$ ($P > 0.05$)

For CD_4; $P = 0.730$ ($P > 0.05$)

From Table 26, the above analysis showed that the age of the participants significantly influenced the marital status of the participants ($P < 0.05$).

From Table 27, the analysis implied that the educational status of the participants was significantly affected by their ages ($P < 0.05$). For instance, ages 45 years and below have least percentages of participants with Non-formal education while 46years and above have higher percentages.

From Table 28, the analysis implied that age of the participants greatly and significantly influenced the occupation of the participants ($P < 0.05$). Just as most of the participants below 30 years of age were students, most participants above 30 years were not students but were engaged in business or were employed.

From Table 29, $p > 0.05$

The analysis showed that the age of the participants had a significant effect on their exposure to risk factors. It can be seen from the Table 39 that participants under the age of 15 years were not exposed to most of the risk factors, unlike those between the ages of 31 and 40 years who were more exposed to risk factors.

4.13: Occupation of the participants

Out of the 502 people living with HIV/AIDS that participated in this study, 63(12.5%) were civil servants, 157 (31.3%) were students while only 34 (6.8%) were unemployed. Artisans and those in business made up 102 (20.3%) and 146 (29.1%) of the participants respectively. Almost equal percentages of males 18(12%) and females 45(13%) participants were civil servants while 49 representing 34% of male participants were in business and 97 which is 28% of the female participants were in business. From Fig. 8, General Hospital Onitsha had the highest participants as civil servants, artisans, business people and students while the highest participants that were unemployed were found in General Hospital Ekwulobia.

Table 26: Marital status of the participants based on age groups

Age group	Single	Married	Widowed	Divorce	Total(%)
0-15	26(5.2)	0(0.0)	0(0.0)	0(0.)	26(5.2)
16-20	64(12.7)	47(9.4)	7(1.4)	1(0.2)	119(23.7)
31-45	61(12.1)	162(32.3)	20(4.0)	3(0.6)	246(49.0)
46-60	11(2.2)	73(14.5)	9(1.8)	3(0.6)	96(19.1)
61& above	0(0.0)	11(2.2)	3(0.6.)	1(0.2)	15(3.0)
	162(32.3)	293(58.4)	39(7.8)	8 (1.6)	502(100)

$P = 0.003$ ($P < 0.05$)

Table 27: Educational status of the participants based on age groups

Age group	Non-formal Edu.	Primary	Secondary	Tertiary Edu.	Total
0-15	2(0.2)	18(3.6)	6(1.2)	0(0.0)	26(5.2)
16-30	6(1.2)	2(0.4)	82(16.3)	29(5.8)	119(23.7)
31-45	15(3.0)	3(0.6)	80(16.0)	148(29.5)	246(49.0)
46-60	19(3.8)	4(0.8)	54(10.7)	19(3.8)	96(19.1)
61& above	8(1.6)	5(1.0)	2(0.4)	0(0.0)	15(3.0)
	50(10.0)	32(6.4)	224(44.6)	196(39.0)	502(100)

$P = 0.014$ ($P < 0.05$)

Table 28: Occupation of the participants based on age groups

Age group	**Civil servants**	**Artisans**	**Traders**	**Students**	**Unemployed**	**Total**
0-15	0(0.0)	0(0.0)	0(0.0)	24(4.8)	2(0.4)	26(5.2)
16-30	12(2.4)	20(4.0)	24(4.8)	51(10.1)	12(2.4)	119(23.7)
31-45	33(6.6)	45(9.0)	79(15.7)	79(15.7)	10(2.0)	246(49.0)
46-60	18(3.6)	33(6.6)	38(7.6)	3(0.6)	4(0.8)	96(19.1)
61 & above	0(0.0)	4(0.8)	5(1.0)	0.(0.0)	6(1.2)	15(3.0)
	63(12.5)	102(20.3)	146(29.1)	157(31.3)	34(6.8)	502(100)

$P = 0.011$ ($P < 0.05$)

Table 29: Distribution of participants exposed to risk factors according to their age group

Age group	IV Drug users	Blood transfusion	Surgery	Alcohol abuse	Smokers	Homosexual lesbians
0-15	0(0.0)	6(2.9)	2(0.9)	0(0.0)	0(0.0)	0(0.0)
16-30	3(1.5)	18(8.8)	12(5.9)	3(1.5)	4(2.0)	4(2.0)
31-45	6(2.9)	30(14.7)	14(6.9)	11(5.4)	20(9.8)	4(2.0)
46-60	2(0.9)	22(10.8)	3(1.5)	7(3.4)	22(10.8)	2(0.9)
61 & above	0(0.0)	2(2.6)	1(0.5)	4(2.0)	2(0.9)	0(0.0)
	11(5.4)	78(38.2)	32(15.7)	25(12.3)	48(23.5)	10(4.9)

$P = 0.143$ ($P > 0.05$)

Figure 1 showed the distribution of the participants based on the General Hospital they attend. Out of the 502 participants, 200 (134 female and 66 male) were from General Hospital Onitsha, 200 (128 female and 72 male) from General Hospital Ekwulobia and 102 (84 female and 18 male) from General Hospital Enugwu-ukwu. From General Hospital Onitsha, 155 (77.5%) people were on ART while 45 (22.5%) were not on ART. In General Hospital Ekwulobia, 164 (82.0%) were on ART and 36 (18.0%) were not. Eighty (78.4%) people were on ART while 22 (21.6%) were not on ART at General Hospital Enugwu-ukwu. Among the 200 participants from General Hospital Onitsha, 165 (82.5%) had CD_4 count $\geq$200, 35 (17.5%) had CD_4 < 200 while at General Hospital Ekwulobia, 156 (78.0%) had CD_4 count $\geq$200 and 44 (22.0%) had CD_4 count < 200. At General Hospital Enugwu-ukwu, 89 (87.3%) had CD_4 count $\geq$200 while 13 (12.7%) had CD_4 count < 200.

Figure 2 show that 10 (5%) people tested positive to Hepatitis B in both General Hospital Onitsha and Ekwulobia while 6 (5.9%) tested positive to Hepatitis B in Enugwu-ukwu. Five people had HCV in General Hospital Onitsha, 2 in Ekwulobia and 1 in Enugwu-ukwu. General Hospital Enugwu-ukwu had the highest coinfection of HIV/HBV while Onitsha General Hospital had the highest coinfection of HIV/HCV. For elevated GPT, General Hospital Onitsha had 8 people, 10 people at General Hospital Ekwulobia and 6 for G.H Enugwu-ukwu while 12, 11 and 7 participants had elevated GOT in General Hospital Onitsha, General Hospital Ekwulobia and General Hospital Enugwu-ukwu respectively. Fewer participants had elevated ALP compared to GPT and GOT; 6, 7, and 3 for G.H. Onitsha, G.H Ekwulobia and General Hospital Enugwu-ukwu respectively.

Figure 3 show that 1 person had high ALP and HBsAg, 3 had both HBsAg and high GOT while 5 had high GPT and HBsAg.

Figure 4 show that one person had high GOT and HCV and nobody had either both elevated ALP and HCV or high GPT and HCV.

4.14 The Effect of HBV/HCV on Liver enzymes

One (12.5%) participant coinfected with HIV/HCV had elevated GOT, while 9(34.6%) of people coinfected with HIV/HBV also had elevated liver enzymes (Fig. 3). Five (19.2%) had elevated GPT while 3 (11.5%) and 1 (3.8%) had elevated GOT and ALP respectively. This result in other words implied that GPT activity was significantly higher among HIV/HBV coinfected participants compared to GOT and ALP activities, while coinfection with HCV has little or no effect on the liver enzymes activities. This might the reason why the mean CD_4 count of the participants coinfected with HCV was not affected.

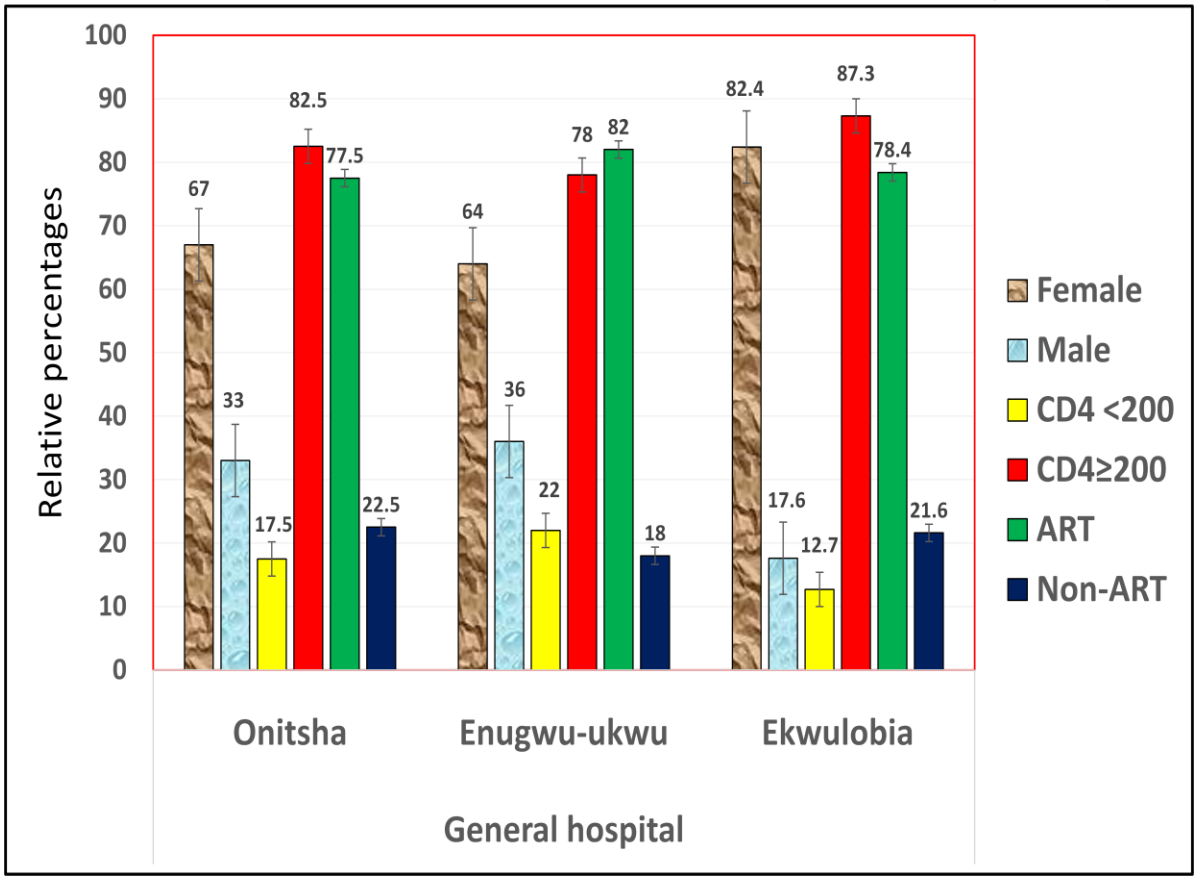

Fig. 1: Gender, CD_4 and ART distribution of participants according to General Hospital they attend

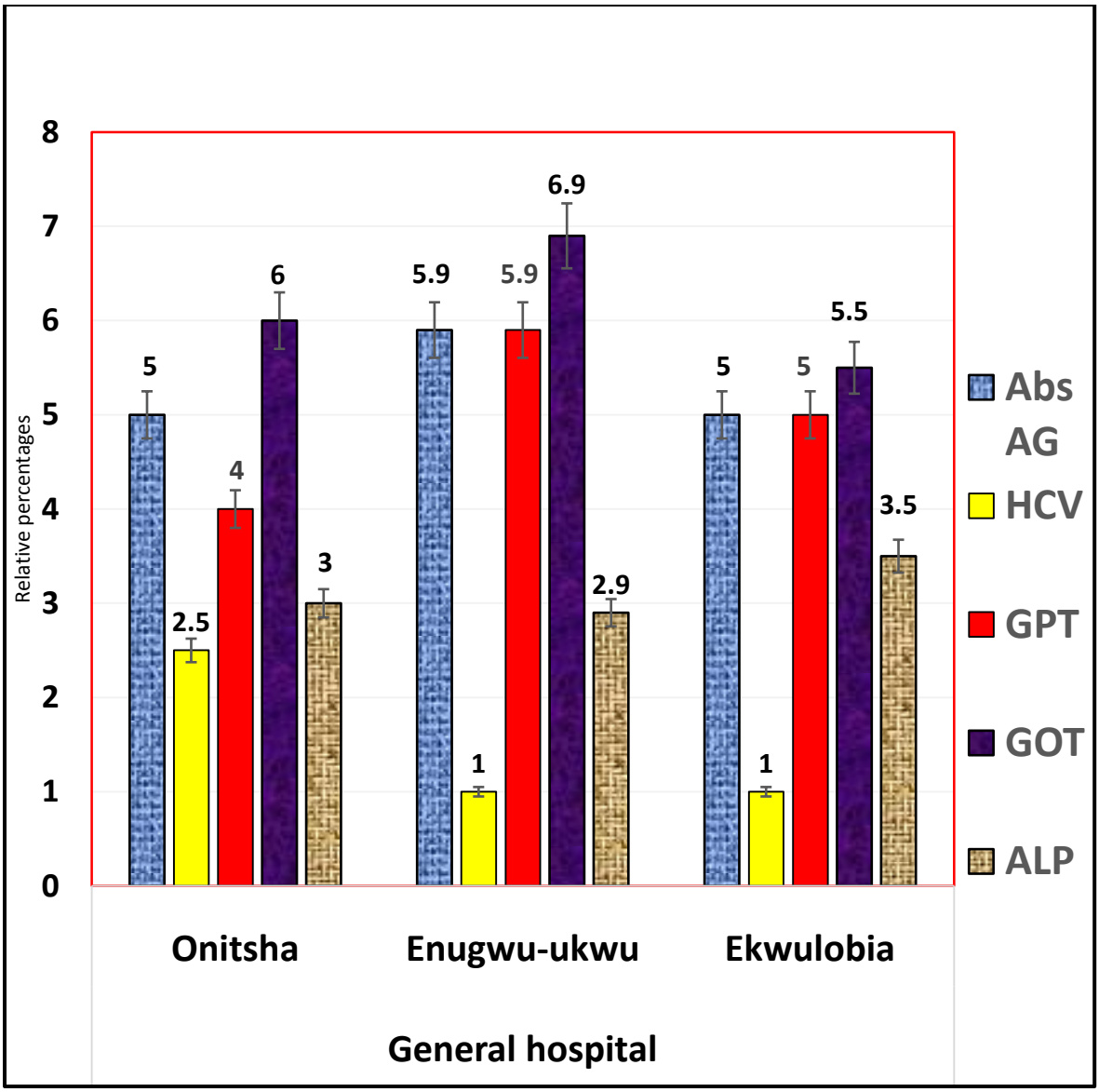

Figure 2: Hospital distribution of participants positive to HBsAg, HCV and elevated liver enzymes.

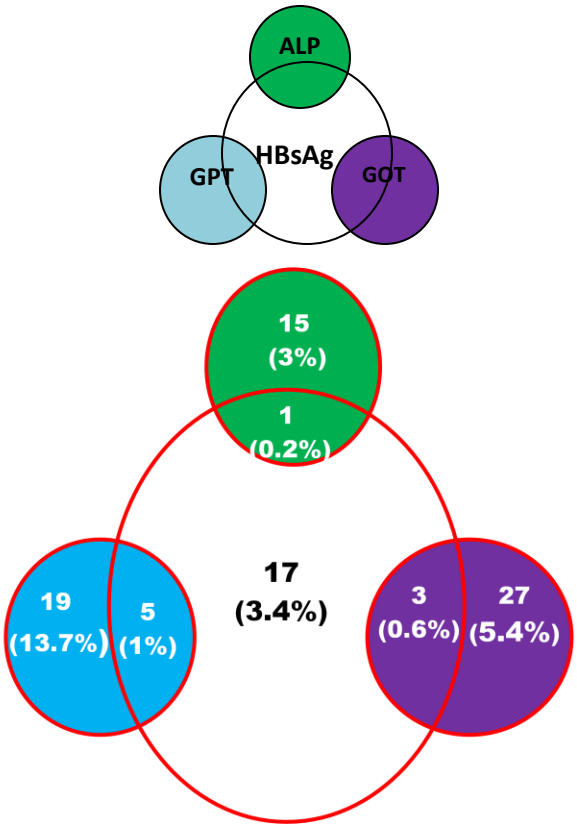

Figure 3: Distribution showing participants with HBsAg and high liver enzymes

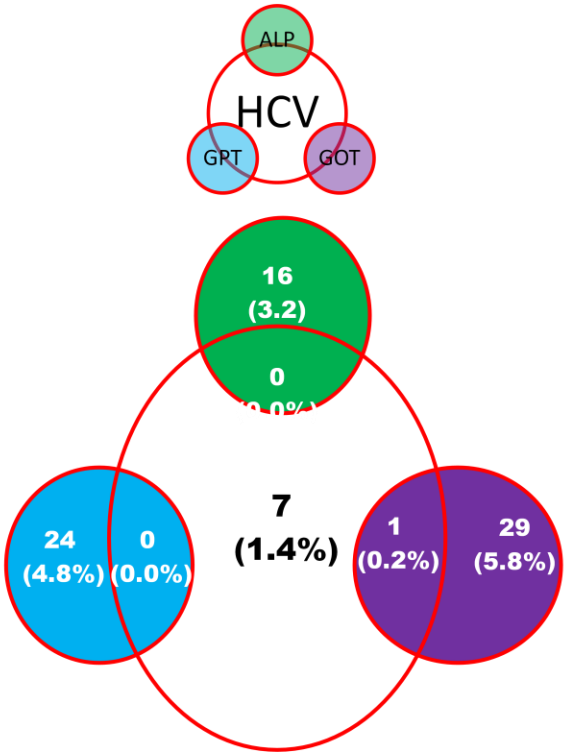

Figure 4: Distribution showing participants with HCV and elevated liver enzymes

Figure 5, the three General Hospitals had higher number of their participants married though Ekwulobia General Hospital had the highest. Divorced group had the lowest percentage, followed by widowed in all the General Hospitals.

Figure 6 showed that lower number of participants from the 3 General Hospitals had none or elementary certificate while greater number had minimum of secondary school certificate.

Figure 7 showed that greater number of the participants from the 3 General Hospitals were either students or business men/women while civil servants, self employed and unemployed were in lower percentages.

Figure 8 showed the number of participants from each General Hospital that were exposed to risk factors Onitsha General Hospital had the highest followed by Ekwulobia General Hospital and Enugwu-ukwu had the least (though it had least number of participants). These exposed to blood transfusion had the highest, followed by smokers, then those that had undergone surgery, while the IV drug users and homosexual/lesbians had the least number of participants.

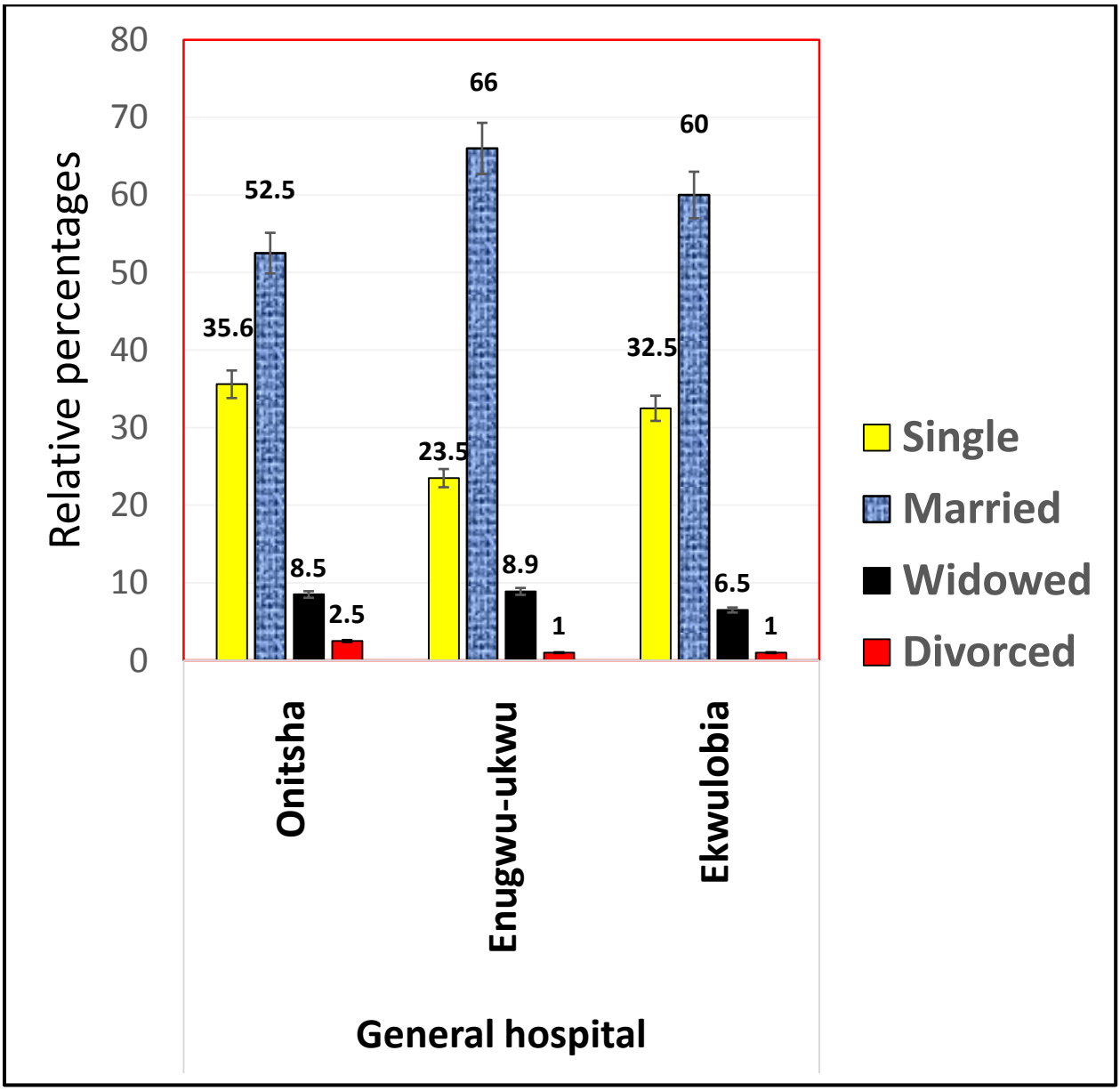

Fig. 5: Marital Status of participants based on General Hospitals.

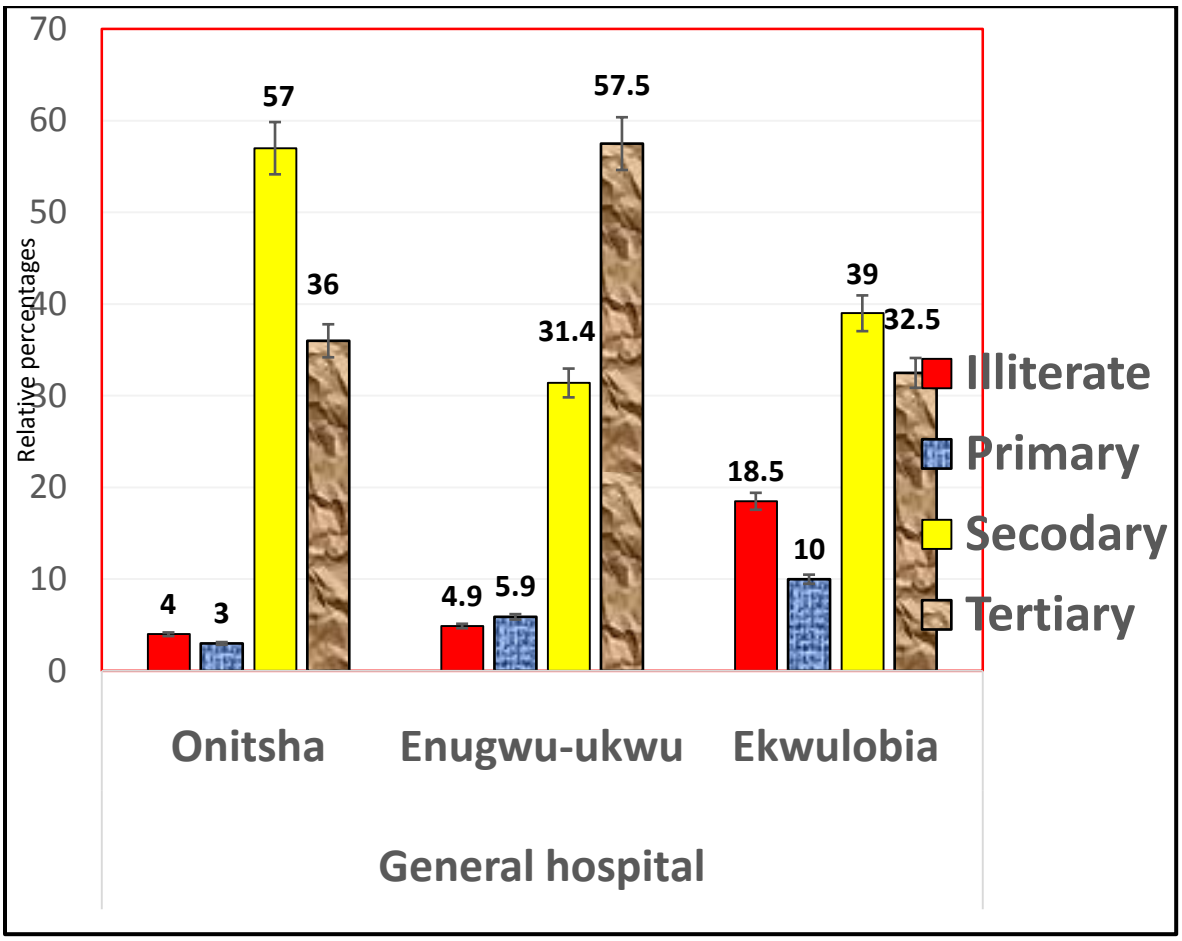

Fig. 6: Educational status of participants based on General Hospitals.

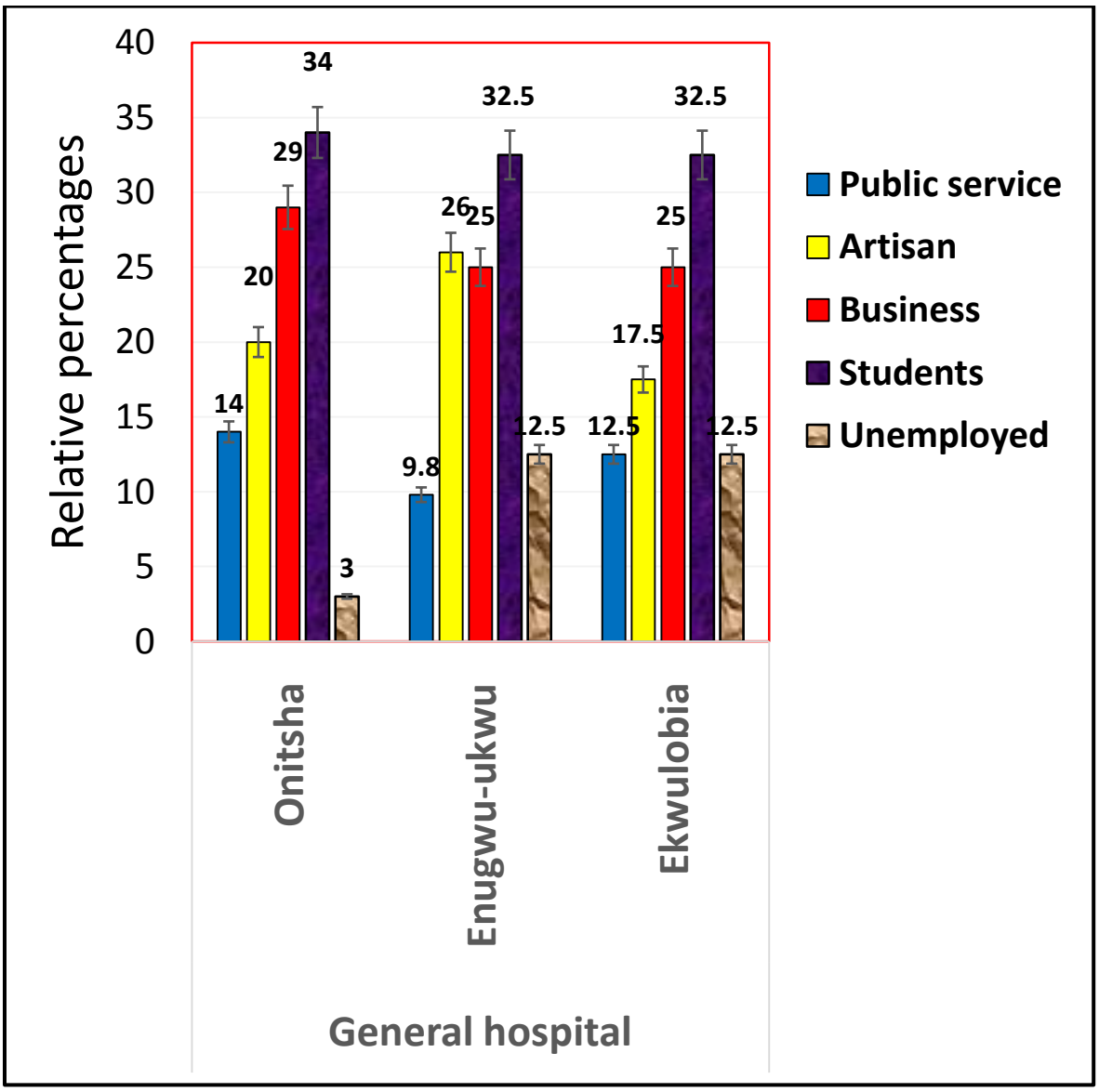

Fig. 7: Occupation of participants based on General Hospitals.

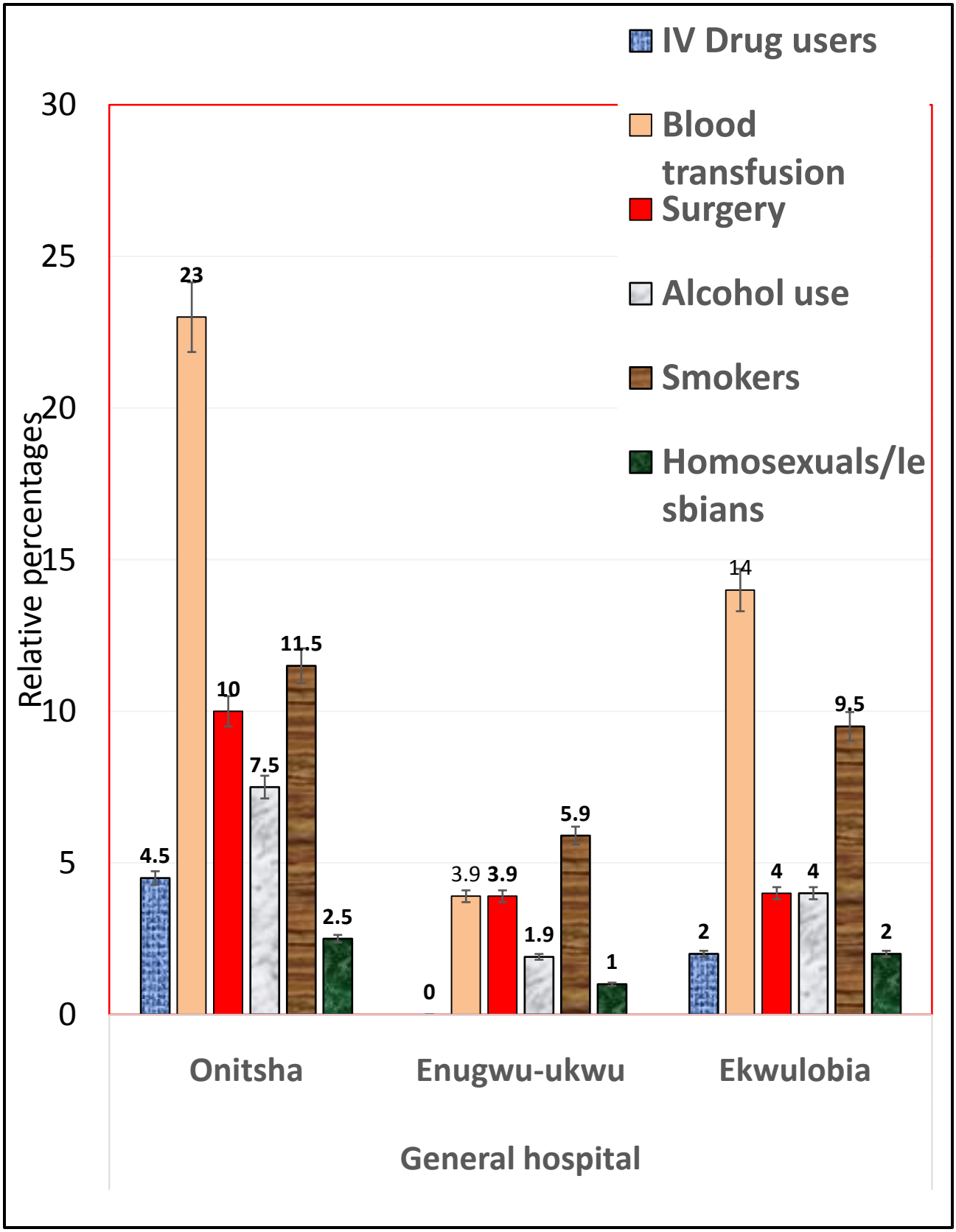

Fig. 8: Distribution of participants exposed to risk factor based on General Hospitals.

CHAPTER FIVE

DISCUSSION

This study showed that out of 502 people living with HIV/AIDS that participated in the research, 346 (68.9%) were females and 156 (31.1%) were males. In other words more females than males in the ratio of 1:2.2 for males to females were involved. This agrees with Ola *et al*., (2005) where they also documented more females than males in the ratio of 2:1 for people living with HIV/AIDS attending clinic at UCH Ibadan. This however does not necessarily imply in absolute terms that more women are infected with HIV in our population, though the anatomy of female genital system may be a contributing factor for having more female participants than male in this study. It has also been discovered that the semen carry more viruses than the vaginal fluid (Mabala, 2006).

In contrast to this report, higher percentages of males was recorded in Italy (Sukowski *et al*., 2008) and Spain (Larsen *et al*., 2008), 72% and 84% respectively. Among the participants, 399 (79.5%) were on ART while 103 (20.5%) were not on ART. The CD_4 count of 92 (18.3%) of the participants were less than 200 cells/μ1 and 410 (81.7%) had CD_4 count up to 200 and above. Twenty-six people representing 5.2% tested positive to hepatitis B. This result was low when compared with Pouti *et al*., (2009) where they recorded that approximately 10% of HIV infected individuals worldwide are also chronically infected with hepatitis B virus, though they also recognized that the prevalence of HIV-HBV coinfection varies by geographical location. In comparison with earlier studies carried out in Nigeria, this figure was lower than 11.9% reported in Ibadan, 6.6% in Nasarawa but higher than 2.7% in Ado Ekiti. Up to 20.6% has been documented in North central Nigeria, 25.7% in Jos (Agwele *et al.,* 2004) and 28.4% in Lagos (Otegbajo *et al.,* 2008). In this study, it was observed that the mean CD_4 count of participants coinfected with HBV was lower (459cell/μl) than those negative to HBV (531 cell/μ1) while the mean CD_4 for those positive to HCV was higher (673 cells/μl) than 526 cells/μl for those negative to HCV. This result is comparable with that of Vitayih *et al*., (2013) that reported higher CD_4 mean in patients coinfected with HCV than those not coinfected in his research at University Teaching Hospital Gondar, North West Ethiopia. This result also agrees with that of Adewole *et al*., (2009) that recorded highest mean CD_4 count among those

coinfected with HIV/HCV (260cellsμl) than those with HIV/HBV (121 cellsμl) and with HIV only (171 cellsμl). HCV by itself has not been shown conclusively to be an independent risk factor for more rapid CD_4 decline, although it has been associated with increased occurrence of AIDS defining events (Stebbing *et al*., 2005).

Only 8 (1.6%) tested positive to hepatitis C virus. This is lower than the results recorded by Otegbayo *et al*., (2008) which was 4.8% and Adewole *et al*., (2009) that was 3.0% positive to hepatitis C virus, among those living with HIV/AIDS, but higher than 1.1% record by Hamza *et al*., (2013). Sulkowski, (2008) reported that the prevalence rates of HIV-HCV coinfection vary depending on the route of HIV transmission. Out of the 502 participants, none (0%) tested positive to syphilis. This is rare considering the shared routes of transmission it has with HIV. The zero percent recorded in syphilis can be attributed to the availability and efficacy of Penicillin G as drug of choice for the treatment of syphilis which is a bacterial disease and not a viral disease (Landep *et al*., 2008).

The 502 participants were grouped into various age groups. Those of 31-45yrs of age had the highest number of participants, 246 (49.0%). This should be due to sexual activeness of the people within this age group. Ojide *et al*., (2015) also recorded highest participants among the age group 31-50yrs in their research on people living with HIV/AIDS in Benin City. Davis *et al*., (2007) in their own research reported highest HIV prevalence among people aged 45yrs and younger, which agreed with the result of this study. All the age groups had more females than male except for the last age group 61 and above that had more males.

It was observed, that all the age groups had higher percentage of their participants on ART. In this part of the world and in most developing countries, due to their belief and culture, HIV infected individuals do not go for test early enough for early diagnosis. In that case, most of the people infected with HIV are not detected until the infection is advanced in stage in which case the patient will be needed to be placed on Anti retroviral therapy (ART) immediately after diagnosed because the CD_4 count will be low (Otegbayo *et al*., 2008).

All the groups had most of their participants having their CD_4 cells count up to 200 and above while lower percentages had CD4 count of less than 200cells/ μl of blood. Since majority were on ART and also majority had CD_4 cells count 200cells/ μl and above, this

indicates their responsiveness to the anti-retroviral drugs they were taking. The female participants had a higher CD_4 count mean of 547cells/ μ1 than the male counterparts that had 480cells/ μl CD4 count mean.

Age group 16-30 had the lowest percentage (0%) of participants that tested positive to HBsAg, while age group 0-15 had the highest percentage (11.5%) of participants that tested positive to HBsAg. For HCV, age group 0-15 had 0% participants positive while age group 61 and above had highest percentage (6.7%) of participants that tested positive to HCV. This result agrees with that of Davis *et al*., (2007) that reported highest prevalence of HIV/HBV coinfections among age group 40 and below but does not agree that HIV/HCV coinfection prevalence was also highest among the same age group. In this study none of the participants tested positive to both HBsAg and HCV. This is more or less comparable to the reports from Senegal (0.5%), Kenya (0.26%) and Egypt (0.44%) as reported Kazem *et al.,* (2004) and Diop-Nidiaye *et al.,* (2008) respectively.

The research showed that 24 (4.7%), 30 (6.0%) and 16 (3.2%) of the participants had elevated levels of GPT (ALT), GOT (AST) and ALP respectively according to the normal values on the manual (Appendix V). Pol *et al.,* (2004) also stated that ALT and AST above 60 IU/L but below 100 IU/L is mild to moderate hepatotoxicity, and if higher than 100 is severe hepatotoxicity. Also Lefkowitch, 2006, recognizes any liver enzyme level up to 2.5 times the upper limit of normal value as high. According to Pol *et al.,* (2004) and Lefkowitch, (2006) ranges, almost all the participants with elevated liver enzymes in this study, had mild to moderate hepatotoxicity and except only one female participant that had severe hepatotoxicity. The female participant was from General Hospital Enugwu-ukwu, on ART, had 124 IU/L, 117 IU/L and 215 IU/L for GPT, GOT and ALP respectively, but not coinfected with viral hepatitis. This could be due to drug effect.

Age group 16-30 had the lowest percentage of participants, 0.8% and 2.5% with elevated GPT and GOT respectively while the age group 46-60 had the highest percentage of it's participants having elevated GPT, GOT and ALP; 8.3%, 10.4% and 4.1% respectively. This is similar to that of Djuidje *et al*., (2017) where he recorded lowest percentage of GOT and GPT elevation among age group 21-30 and highest percentage among age group 41-60. This may be due to fact that the organs of the body including the liver weakens as ones gets older.

Based on the gender of participants, out of the 92 with CD_4 count less than 200, 57 (62.0%) were females while 35 (38.0%) were males (table 10). Among the 26 participants that tested positive to HB_sAg, 15 (57.7%) were males while 11 (42.3%) were females, but for hepatitis C virus, higher percentages of the participants that tested positive to HCV were females. Davis *et al*., (2007), also reported higher prevalence of HIV/HBV coinfection in males; (16.9% vs 9.2%) for males against females. This finding could be linked with the earlier observation that a high proportion of HBV infection in sub-Saharan Africa is acquired vertically or horizontally from family members to other children before the age of 5 years (Drew *et al*., 1998). This could be the reason why this study recorded high percentage HIV/HBV coinfection in the age group 0-15.

Also since boys tend to participate more in aggressive sports which may result in injury and bleeding when compared to girls, boys are at higher risk of contacting the virus. Societal acceptance of multiple sexual partners for men may contribute to higher HBV prevalence among HIV infected men than women. Also practice of unprotected sex in our polygamous setting may be a contributing factor (Obi *et al.*, 2012).

The above result of more males with HIV/HBV but more females with HIV/HCV is compatible with the report of Taiwo *et al.,* (2012). This higher rate of HIV/HCV coinfection among females may have accounted for by the fact that women of all ages are more likely than men to become infected with HIV and HCV during unprotected vaginal intercourse, due to the nature of their genital organ.

In this study, liver enzymes especially GPT (ALT) were significantly higher in HIV/HBV coinfected patients compared to those not coinfected. This finding highlighted some challenges being encountered in treating patients who were coinfected, especially regarding the choice of HAART regimen, how to prevent further hepatic damage, and when to initiate HAART, particularly in resource limited settings with limited ARV options. (Iwalokan *et al*., 2006).

The serum levels of ALT and AST among mono and co-hepatitis infected patients respectively were significantly higher among male patients than in female patients. This difference was much more significant among the coinfected patients and therefore confirms previous studies (Adewole *et al*., 2009) that HIV infection accelerated the progression to hepatic complications in hepatitis B infected men. Mean serum level of

GPT (ALT), GOT (AST) and ALP of the patients with CD_4 count below 200cells/μl were significantly higher than those with CD_4 count $\geq$ 200cells/μl of blood.

Five (19.2%) out of 26 participants that were positive to HB_sAg had CD_4 count less than 200 while 21 (80.8%) of them had CD_4 count up to 200 and above. Also 1(12.5%) of the participants positive to HCV had CD_4 count less than 200 while higher percentage (87.5%) had CD_4 count 200 and above. In other words, this study is saying that the presence of viral hepatitis had little or no effect on the CD_4 count of HIV/AIDS infected individual.

Among the participants with elevated ALP, 87.5% had low CD_4 count, below 200cells/μl while higher percentages of the participants with elevated GPT (70.8%) and GOT (56.7%) had their CD_4 count up to 200cells/μl of blood. This indicates that the CD_4 count of the participants in this study were affected by raised ALP raised GPT and GOT but GPT was mostly affected.

Based on ART, the study showed that out of 399 on ART, 336 (82.2%) had CD_4 count up to 200 and 63 (15.8%) had CD_4 count less than 200 while 29 (28.2%) of the people not on ART had CD_4 count less than 200 and 74 (71.8%) had CD_4 count 200 and above. This implies that the ART boosted the immune system of people living with HIV/AIDS thereby increasing their CD_4 count. For viral hepatitis, greater percentages of the participants that tested positive to HB_sAg (92.3%) and HCV (87.5%) were on ART, while non ART had 7.7% and 12.5% of HBsAg and HCV respectively. This indicates that ART had no effect in treatment of viral hepatitis but that does not mean that ART was the cause of viral hepatitis since they are caused by viral agents. For liver enzymes, higher percentages of the participants with elevated GPT and GOT were not on ART. This showed that raised levels of liver enzymes were not only caused by intake of drugs in people living with HIV/AIDS. Lefkowitch, (2006), stated that liver enzyme elevations were frequent in HIV-infected patients, especially those treated with HAART. Although reports of liver enzyme elevations are frequent, the analysis of these events is limited, because HIV-infected patients have several risk factors for biochemical abnormalities, and a precise etiology is rarely clearly defined (Laurent *et al.*, 2010).

This study recorded HIV/HBV coinfection of 5.2% which is low when compared with some other studies in Nigeria . The variation noted between this study and other studies in Nigeria may have resulted from the differences in the risk of acquisition of HBV infection

and unmeasured socioeconomic and environmental factors across. In the past, there were practices that increased the risk of HBV infection such as mass childhood circumcision in the North, though, it is still being practiced in some rural areas. Similarly there is usage of local unsterilized blades for commercial barbing services and the use of unsterilized sharps for facial marks and tattoos. (Musa *et al*., 2013).

Hamza *et al*., (2013) recorded 16.5% and 17.3% in Ghana and Tanzania (Nagu *et al*., 2008) respectively for HIV/HBV coinfections which were the highest in the table. The 1.6% recorded in this study for HIV/HCV coinfection is higher than 1.1% and 1.2% reported in Kenya and Coted'ivoire respectively but lower than 4.8%, 2.3% and 8.2% reported in Ibadan (Otegbayo *et al*., 2008), Abuja (Adewole *et al*., 2009) and Ghana (Hamza *et al*., 2013) respectively. This percentage (1.6%) for HIV/HCV coinfection is exactly the same with that reported in Kano by Nwokedi *et al.,* (2006).

This study recorded that 9 participants representing 34.6% of people that tested positive to HBsAg also had elevated liver enzymes. 5(19.2%) had elevated GPT, 3 (11.5%) elevated GOT and only 1 (3.8%) had elevated ALP. In other words GPT activity is significantly higher among HIV/HBV coinfected participants compared with GOT and ALP. This report is at par with the findings resulting from other investigations in Cameroon by Zoufaly *et al.,* (2012) and Djuidje *et al.,* (2017). It has already been demonstrated that high GPT (ALT) serum level activity principally reflects direct hepatocellular damage or liver dysfunction. Consequently, both HIV and HBV create pressure on liver, leading to elevation of liver transaminase, alanine amino-transferase (Pratt and Kaplan, 2000).

However in a study which was conducted in South Africa, 70% of HIV/HBV and HIV/HCV coinfected study participants had significantly elevated GPT and GOT, 56% of them had elevated ALP (Stern *et al.*, 2013). Similarly, significantly raised GPT was found in 14% of HIV/HBV coinfections and 20% in HIV/HCV coinfected patients in India (Ksirsagar *et al*., 2012). These liver enzyme levels difference between different studies may be due to difference in the study design, duration of the viral hepatitis infection, as well as the patient's condition like having chronic alcoholism or other drug induced hepatotoxicity (Monforte *et al*., 2001).

In this study, there was also difference on the CD_4 values in relation to gender. The mean CD4 values in the male was lower than females (489cells/μl versus 546cell/μl). Similar

findings were reported in studies which have been conducted in Nigeria (Akinsegun *et al*., 2012), Uganda (Tugume *et al*., 1995) and also Otegbayo *et al*., (2008) (204.7 cells/μl versus 259.9 cells/μl for male vs female). This lower CD_4 count in males may be associated with their daily activities. In addition, males are more muscular and may not be ready to accept their HIV, HBV and /or HCV results and they may develop mental stress that further contribute to the impairment of their immune system or lower CD_4 (Nyirenda *et al.,* 2008). After initial vriral infection, women are more likely to clear the virus spontaneously. Women also have slower rates of liver disease progression than men if they become chronically infected. Estradiol and ostrogen receptors in the liver protect hepatocytes from oxidative stress, inflammatory injury, and cell death, which all contribute to fibrosis. As a consequence of the overall slower liver disease progression and increased viral clearance in women, the disease burden from viral infection is found predominatly in men. Post menopausal women have increased rates of fibrosis compared with women of reproductive age because they have lost the protective effects of ostregen hormone. Furthermore, males may spend most of their time with hard works for a long period of time and this may contribute to lower CD4 count (Saravanan *et al.,* 2007).

In this study, 32.3% of the participants were single while 58.4% were married. These are close to 27.0% and 57.7% reported by Adewole *et al.,* (2009) for single and married respectively. The higher prevalence of HIV/AIDS recorded amongst married people may be attributed to the high level of infidelity among married people, male and female inclusive. Again the poor economy of the country makes it very difficult for many parents to provide for their families as a result some women may choose to adopt prostitution as an alternative means of making the two ends meet (Nwokedi *et al*., 2006). Among the participants in this study, 10.0% and 6.4% had no formal education and primary school certificate respectively while Dijuidje *et al*., (2017) recorded a lower percentage of 4.0% for those with non-formal edcation and a higher percentage of 17.3% for those that had primary school certificate as their highest educational level. This study show highest percentage 44.6% for the participants with secondary school certificate just as Dijuidje *et al*., (2017) recorded highest percentage of 61.3%. In this study, civil servants formed 12.5% and unemployed 6.8% while Dijuidje *et al*., (2017) reported 10.6% and 28% for civil servants and unemployed respectively. Adewole in his own study at Abuja, (2009),

reported 27.0% and 30.8% for smokers and alcohol abusers respectively which is relatively high compared with 9.6% and 5.0% recorded in this study for smokers and alcohol abusers respectively. The discrepancy may be attributed to the difference in the social life in the cities used for the studies.

In this study, higher percentage of the singles (5.3%) were coinfected with HBV than the married(4.4%).This result agreed with that of Djuidje *et al*.,(2017) which recorded higher percentage of HIV/HBV coinfectin among the singles(9.3%) but does not agree with the report of Adewole *et al*., (2009) that coinfection of HIV/HBV was more among the married than the singles. This study recorded more HIV/HBV and HIV/HCV coinfection among the civil servants which is in contrast to the report of Otegbayo *et al*., (2008) where the Artisans were more coinfected. Also this study recorded highest coinfection of HIV/HBV among those with non-formal education and primary school certificate holders while Otegbayo *et al*., (2008) reported highest HIV/HBV among people with secondary school certificates.

The result of this study, that alcohol abusers had the highest percentage of those coinfected with HIV/HBV (20.0%) agrees with that of Adewole *et al*., 2009 that had highest coinfection of HIV/HBV among people that consume alcohol (32%). This study show low coinfection of both HIV/HBV and HIV/HCV among those that had blood transfusion (1.3% for both HIV/HBV and HIV/HCV) while Otegbayo *et al*., (2008), in their work recorded highest coinfection of both HIV/HBV and HIV/HCV among those that had blood transfusion.

EVALUATION

This study had really investigated the clinical effects of co-infection of HBV and HCV and also elevated hepatic enzymes on PLWHA. If the Federal government and the Non-governmental Organizations involved in HIV-AIDS care should embibe and embrass the recommendations made below by providing laboratory kits for free baseline and routine check for HBV, HCV and liver enzymes levels, it will go a long way in improving the health conditions of PLWHA not only in Anambra state but the nation at large.

CONTRIBUTION TO KNOWLEDGE

1. The physicians, especially hepatologists, internists and other infectious-disease specialists, should be aware of the frequency of liver enzyme abnormalities that require diagnostic procedures, mainly on the basis of the history of the patients, biochemical (drug level monitoring), immunological (CD_4 cell count) and virological (HBV and HCV) evaluations.
2. In some cases, liver biopsy will also be required to distinguish between a potential symptomatic liver diseases in HIV/HBV or HIV/HCV coinfected patients receiving HAART who are experiencing immune restoration or those who have a high HCV load, potential drug-related hepatitis, and other common causes of liver disease, including use of alcohol drugs and concomitant medications.
3. It should be noted that these conditions may occur concurrently, thus complicating the diagnostic process, therefore screening of HBV and HCV before initiation of antiretroviral treatment is mandatory for strict monitoring and a regular evaluation of liver enzymes levels and CD_4 status in order to minimize the complication of the liver and for effective HIV management.

RECOMMENDATIONS

In view of the high frequency of HBsAg and modest frequency of anti-HCV in HIV patients, the study recommends that;

1. A routine baseline screening for these markers in HIV infected patients, as this could affect the choice of highly active anti-re troviral therapy (HAART) regimen for the patients.

2. Hepatitis B virus and hepatitis C virus coinfected HIV positive patients should be given first line antiretroviral drugs that are non-hepatotoxic. Patients with CD_4 count below 200cells/μl should also be given non-hepatotoxic antiviral drugs regardless of their HB_sAg and HCV status.
3. Elevated liver enzymes at baseline is an indication that the liver is already compromised and so drugs that are hepatotoxic will have to be avoided in order not to further compromise the liver function.
4. Those with co-infection with HBV or/and HCV are usually given drugs that are also effective for treatment of HBV.
5. .Baseline CD_4 count which used to be a standard requirement for commencement of ART may also be used as a surrogate test for liver function especially in coinfected patients, so that even if the liver enzymes are normal, a low CD_4 count below 200 may be a signal for performing more sensitive or invasive test to assess the function of the liver.

LIMITATIONS

1. The window period of the Australian antigen, time between the disappearance of HBsAg and the appearance of anti-HB_s was not considered in this study.
2. HBV and HCV DNAs would have as well equally helped in this regard but was not tested in the participants due to lack of facility for the test and high cost of sending samples out for such test.
3. A cross sectional study like this could not establish cause and effect relationship on the viral markers detected in the HIV patients.
4. Since the plasma HIV, HBV and HCV viral load were not quantified, this study could not make a distinction of active HBV and HCV infections.

CONCLUSION

Routine estimation of liver enzymes of people living with HIV/AIDS is a means of assessing liver disease as it reflects the activity of hepatotropic viruses and status of liver during therapy with various hepatotoxic drugs. Even though it is well known that liver enzymes may even be normal in the presence of advanced liver disease, in resource limited

countries like Nigeria, it still remains the affordable test in the assessment of liver function in the management of HIV/AIDS patient.

This study has expounded knowledge of adverse effects the presence of hepatitis B, hepatitis C and Syphilis can cause in the health of the people living with HIV/AIDS. This study also revealed the need for the routine check of liver enzymes levels and presence of viral hepatitis and syphilis in HIV infected individuals. The early detection of presence of hepatitis B, C, syphilis and elevated liver enzymes will help in the choice of ART and management of the patient.

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APPENDICES

Appendix 1: Distribution (%) of HIV/AIDs out-patients in three General hospitals studied

Demographic characteristics		General Hospitals							
		Onitsha		Ekwulobia		Enugwu-Ukwu		Total	
		No.	%	No.	%	No.	%	No.	%
Gender	Male	66	13.1	72	14.3	18	3.6	**156**	**31.1**
	Female	134	26.7	128	25.5	84	16.7	**346**	**68.9**
	Total	**200**	**39.8**	**200**	**39.8**	**102**	**20.3**	**502**	**100**
Age group (years)	<15	8	1.6	12	2.4	6	1.2	**26**	**5.2**
	16-30	54	10.7	42	8.4	23	4.6	**119**	**23.7**
	31-45	90	17.9	106	21.1	50	10.0	**246**	**49.0**
	46-60	41	8.2	35	7.0	20	4.0	**96**	**19.1**
	>60	7	1.4	5	1.0	3	0.6	**15**	**3.0**
	Total	**200**	**39.8**	**200**	**39.8**	**102**	**20.3**	**502**	**100**
Marital status	Single	73	14.5	65	12.9	24	4.8	**162**	**32.3**
	Married	105	20.9	120	23.9	68	13.5	**293**	**58.3**
	Widowed	17	3.4	13	2.6	9	1.8	**39**	**7.8**
	Divorced	5	1.0	2	0.4	1	0.2	**8**	**1.6**
	Total	**200**	**39.8**	**200**	**39.8**	**102**	**20.3**	**502**	**100**
Educational level	Illiterate	8	1.6	37	7.4	5	1.0	**50**	**10.0**
	Primary	6	1.2	20	4.0	6	1.2	**32**	**6.4**
	Secondary	114	22.7	78	15.5	32	6.4	**224**	**44.6**
	Tertiary	72	14.3	65	12.9	59	11.7	**196**	**39.0**
	Total	**200**	**39.8**	**200**	**39.8**	**102**	**20.3**	**502**	**100**
Occupation	Civil servant	28	5.6	25	5.0	10	2.0	**63**	**12.5**
	Artisan	40	8.0	35	7.0	27	5.4	**102**	**20.3**
	Traders	58	11.5	50	10.0	38	7.6	**146**	**29.1**
	Student	68	13.5	65	12.9	24	4.7	**157**	**31.3**
	Unemployed	6	1.2	25	4.9	3	0.6	**34**	**6.8**
	Total	**200**	**39.8**	**200**	**39.8**	**102**	**20.3**	**502**	**100**
Risk factors	IV drug user	7	3.4	4	2.0	0	00	**11**	**5.4**
	Blood transfusion	46	22.5	28	13.7	4	2.0	**78**	**38.2**
	Surgery	20	9.8	8	3.9	4	2.0	**32**	**15.7**
	Alcohol use	15	7.3	8	3.9	2	1.0	**25**	**12.3**
	Smokers	23	11.3	19	9.3	6	3.0	**48**	**23.5**
	Homosexual/lesbian	5	2.5	4	2.0	1	0.5	**10**	**4.9**
	Total	**116**	**56.9**	**71**	**34.8**	**17**	**8.3**	**204**	**100**

Appendix 2: Demographic distribution (%) of the HIV/AIDs out-patients by gender

Demographic characteristics		Male		Female		Total	
		No.	%	No.	%	No.	%
Age (y)	<15	12	2.4	14	2.8	**26**	**5.2**
	16-30	22	4.4	97	19.3	**119**	**23.7**
	31-45	69	13.7	177	35.3	**246**	**49.0**
	46-60	42	8.4	54	10.7	**96**	**19.1**
	>60	11	2.2	4	0.8	**15**	**3.0**
	Total	**156**	**31.1**	**346**	**68.9**	**502**	**100.0**
Marital status	Single	68	13.6	94	18.7	**162**	**32.3**
	Married	82	16.3	211	42.0	**293**	**58.3**
	Widowed	4	0.8	35	7.0	**39**	**7.8**
	Divorced	2	0.4	6	1.2	**8**	**1.6**
	Total	**156**	**31.1**	**346**	**68.9**	**502**	**100.0**
Educational level	Illiterate	18	3.6	32	6.4	**50**	**10.0**
	Primary	15	3.0	17	3.4	**32**	**6.4**
	Secondary	66	13.1	158	31.5	**224**	**44.6**
	Tertiary	57	11.4	139	27.6	**196**	**39.0**
	Total	**156**	**31.1**	**346**	**68.9**	**502**	**100.0**
Occupation	Civil servant	18	3.5	45	9.0	**63**	**12.5**
	Artisan	28	5.6	74	14.7	**102**	**20.3**
	Traders	49	9.8	97	19.3	**146**	**29.1**
	Student	53	10.6	104	20.7	**157**	**31.3**
	Unemployed	8	1.6	26	5.2	**34**	**6.8**
	Total	**156**	**31.1**	**346**	**68.9**	**502**	**100.0**
Risk factors	IV drug user	11	5.4	0	0.0	**11**	**5.4**
	Blood transfusion	32	15.7	46	22.5	**78**	**38.2**
	Surgery	12	5.9	20	9.8	**32**	**15.7**
	Alcohol use	21	10.3	4	2.0	**25**	**12.3**
	Smokers	45	22.0	3	1.5	**48**	**23.5**
	Homosexual/lesbian	8	3.9	2	1.0	**10**	**4.9**
	***Total**	**129**	**63.2**	**75**	**36.8**	**204**	**100.0**

Appendix 3: Demographic distribution (%) of the HIV/AIDs out-patients by age groups

Demographics	<15y		16-30y		31-45y		46-60y		≥60y		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	**No.**	**%**
Gender												
Male	12	2.4	22	4.4	69	13.7	42	8.4	11	2.2	**156**	**31.1**
Female	14	2.8	97	19.3	177	35.2	54	10.8	4	0.8	**346**	**68.9**
Total	**26**	**5.2**	**119**	**23.7**	**246**	**48.9**	**96**	**19.2**	**15**	**3.0**	**502**	**100**
Marital status												
Single	26	5.2	64	12.7	61	12.1	11	2.2	0	0.0	**162**	**32.3**
Married	0	0.0	47	9.4	162	32.2	73	14.6	11	2.2	**293**	**58.4**
Widowed	0	0.0	7	1.4	20	4.0	9	1.8	3	0.6	**39**	**7.7**
Divorced	0	0.0	1	0.2	3	0.6	3	0.6	1	0.2	**8**	**1.6**
Total	**26**	**5.2**	**119**	**23.7**	**246**	**48.9**	**96**	**19.2**	**15**	**3.0**	**502**	**100**
Educational level												
Illiterate	2	0.4	6	1.2	15	3.0	19	3.8	8	1.6	**50**	**10.0**
Primary	18	3.6	2	0.4	3	0.6	4	0.8	5	1.0	**32**	**6.4**
Secondary	6	1.2	82	16.3	80	15.9	54	10.8	2	0.4	**224**	**44.6**
Tertiary	0	0.0	29	5.8	148	29.4	19	3.8	0	0.0	**196**	**39.0**
Total	**26**	**5.2**	**119**	**23.7**	**246**	**48.9**	**96**	**19.2**	**15**	**3.0**	**502**	**100**
Occupation												
Civil servant	0	0.0	12	2.4	33	6.6	18	3.6	0	0.0	**63**	**12.6**
Artisan	0	0.0	20	4.0	45	8.9	33	6.6	4	0.8	**102**	**20.3**
Traders	0	0.0	24	4.8	79	15.7	38	7.6	5	1.0	**146**	**29.1**
Student	24	4.8	51	10.1	79	15.7	3	0.6	0	0.0	**157**	**31.2**
Unemployed	2	0.4	12	2.4	10	2.0	4	0.8	6	1.2	**34**	**6.8**
Total	**26**	**5.2**	**119**	**23.7**	**246**	**48.9**	**96**	**19.2**	**15**	**3.0**	**502**	**100**
Risk factors												
IV drug user	0	0.0	3	1.5	6	2.9	2	1.0	0	0.0	**11**	**5.4**
Blood transfusion	6	2.9	18	8.8	30	14.7	22	10.7	2	1.0	**78**	**38.1**
Surgery	2	1.0	12	5.9	14	6.8	3	1.5	1	0.5	**32**	**15.7**
Alcohol use	0	0.0	3	1.5	11	5.4	7	3.4	4	2.0	**25**	**12.3**
Smokers	0	0.0	4	2.0	20	9.8	22	10.7	2	1.0	**48**	**23.5**
Homosexual/lesbian	0	0.0	4	2.0	4	2.0	2	1.0	0	0.0	**10**	**5.0**
Total	**8**	**3.9**	**44**	**21.7**	**85**	**41.6**	**58**	**28.3**	**9**	**4.5**	**204**	**100**

Appendix4: Demographic distribution (%) of the HIV/AIDs out-patients by marital status

Demographics	Single		Married		Widowed		Divorced		Total	
	No.	%	No.	%	No.	%	No.	%	**No.**	**%**
Gender										
Male	68	13.6	82	16.3	4	0.8	2	0.4	156	31.1
Female	94	18.7	211	42.0	35	7.0	6	1.2	346	68.9
Total	**162**	**32.3**	**293**	**58.3**	**39**	**7.8**	**8**	**1.6**	**502**	**100.0**
Age group (y)										
<15	26	5.2	0	0.0	0	0.0	0	0.0	**26**	**5.2**
16-30	64	12.7	47	9.4	7	1.4	1	0.2	**119**	**23.7**
31-45	61	12.2	162	32.2	20	4.0	3	0.6	**246**	**49.0**
46-60	11	2.2	73	14.5	9	1.8	3	0.6	**96**	**19.1**
>60	0	0.0	11	2.2	3	0.6	1	0.2	**15**	**3.0**
Total	**162**	**32.3**	**293**	**58.4**	**39**	**7.8**	**8**	**1.6**	**502**	**100.0**
Educational level										
Illiterate	22	4.4	21	4.2	6	1.2	1	0.2	**50**	**10.0**
Primary	18	3.6	8	1.6	4	0.8	2	0.4	**32**	**6.4**
Secondary	66	13.1	137	27.3	18	3.6	3	0.6	**224**	**44.6**
Tertiary	56	11.2	127	25.3	11	2.2	2	0.4	**196**	**39.0**
Total	**162**	**32.3**	**293**	**58.4**	**39**	**7.8**	**8**	**1.6**	**502**	**100.0**
Occupation										
Civil servant	12	2.4	41	8.2	8	1.6	2	0.4	**63**	**12.5**
Artisan	11	2.2	74	14.7	15	3.0	2	0.4	**102**	**20.3**
Traders	16	3.2	118	23.5	9	1.8	3	0.6	**146**	**29.1**
Student	112	22.3	42	8.4	3	0.6	0	0.0	**157**	**31.3**
Unemployed	11	2.2	18	3.6	4	0.8	1	0.2	**34**	**6.8**
Total	**162**	**32.3**	**293**	**58.4**	**39**	**7.8**	**8**	**1.6**	**502**	**100.0**
Risk factors										
IV drug user	6	2.9	3	1.5	1	0.5	1	0.5	**11**	**5.4**
Blood transfusion	22	15.7	42	20.6	4	2.0	0	0.0	**78**	**38.2**
Surgery	11	5.4	17	8.3	3	1.5	1	0.5	**32**	**15.7**
Alcohol use	9	4.4	13	6.4	2	1.0	1	0.5	**25**	**12.3**
Smokers	18	8.8	26	12.7	4	2.0	0	0.0	**48**	**23.5**
Homosexual/lesbian	8	4.9	1	0.5	0	0.0	1	0.5	**10**	**4.9**
Total	**84**	**41.1**	**102**	**50.0**	**14**	**7.0**	**4**	**2.0**	**204**	**100.0**

Appendix 5: Demographic distribution (%) of the HIV/AIDs out-patients by education

Demographics	Illiterate		Primary		Secondary		Tertiary		Total	
	No.	%	No.	%	No.	%	No.	%	**No.**	**%**
Gender										
Male	18	3.6	15	3.0	66	13.1	57	11.4	**156**	**31.1**
Female	32	6.4	17	3.4	158	31.5	139	27.6	**346**	**68.9**
Total	**50**	**10.0**	**32**	**6.4**	**224**	**44.6**	**196**	**39.0**	**502**	**100.0**
Age group (y)										
<15	2	0.4	18	3.6	6	1.2	0	0.0	**26**	**5.2**
16-30	6	1.2	2	0.4	82	16.3	29	5.7	**119**	**23.7**
31-45	15	3.0	3	0.6	80	15.9	148	29.5	**246**	**49.0**
46-60	19	3.8	4	0.8	54	10.8	19	3.8	**96**	**19.1**
>60	8	1.6	5	1.0	2	0.4	0	0.0	**15**	**3.0**
Total	**50**	**10.0**	**32**	**6.4**	**224**	**44.6**	**196**	**39.0**	**502**	**100.0**
Marital status										
Single	22	4.4	18	3.6	66	13.1	56	11.2	**162**	**32.3**
Married	21	4.2	8	1.6	137	27.3	127	25.3	**293**	**58.4**
Widowed	6	1.2	4	0.8	18	3.6	11	2.2	**39**	**7.7**
Divorced	1	0.2	2	0.4	3	0.6	2	0.4	**8**	**1.6**
Total	**50**	**10.0**	**32**	**6.4**	**224**	**44.6**	**196**	**39.0**	**502**	**100.0**
Occupation										
Civil servant	4	0.8	6	1.2	23	4.6	30	0.6	**63**	**12.5**
Artisan	18	3.6	4	0.8	54	10.8	26	5.2	**102**	**20.3**
Traders	14	2.8	5	1.0	51	10.2	76	15.1	**146**	**29.1**
Student	0	0.0	12	2.4	86	17.1	59	11.7	**157**	**31.3**
Unemployed	14	2.8	5	1.0	10	2.0	5	1.0	**34**	**6.8**
Total	**50**	**10.0**	**32**	**6.4**	**224**	**44.6**	**196**	**39.0**	**502**	**100.0**
Risk factors										
IV drug user	0	0.0	2	1.0	4	2.0	5	2.4	**11**	**5.4**
Blood transfusion	6	2.9	4	2.0	42	20.5	26	12.7	**78**	**38.2**
Surgery	4	2.0	3	1.5	16	7.8	9	4.4	**32**	**15.7**
Alcohol use	2	1.0	3	1.5	12	5.8	8	3.9	**25**	**12.3**
Smokers	5	2.4	8	3.9	21	10.3	14	6.9	**48**	**23.5**
Homosexual/lesbian	0	0.0	1	0.5	3	1.5	6	2.9	**10**	**4.9**
Total	**17**	**8.3**	**21**	**10.4**	**98**	**48.0**	**68**	**33.3**	**204**	**100.0**

Appendix 6: Demographic distribution (%) of the HIV/AIDs out-patients by occupation

Demographics	Civil servant		Artisan		Traders		Student		Unemployed		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	**No.**	**%**
Gender												
Male	18	3.6	28	5.5	49	9.8	53	10.6	8	1.6	**156**	**31.1**
Female	45	9.0	74	14.7	97	19.3	104	20.7	26	5.2	**346**	**68.9**
Total	**63**	**12.6**	**102**	**20.2**	**146**	**29.1**	**157**	**31.3**	**34**	**6.8**	**502**	**100.0**
Age group (y)												
<15	0	0.0	0	0.0	0	0.0	24	4.8	2	0.4	**26**	**5.2**
16-30	12	2.4	20	4.0	24	4.8	51	10.2	12	2.4	**119**	**23.7**
31-45	33	6.6	45	8.9	79	15.7	79	15.7	10	2.0	**246**	**49.0**
46-60	18	3.6	33	6.5	38	7.6	3	0.6	4	0.8	**96**	**19.1**
>60	0	0.0	4	0.8	5	1.0	0	0.0	6	1.2	**15**	**3.0**
Total	**63**	**12.6**	**102**	**20.2**	**146**	**29.1**	**157**	**31.3**	**34**	**6.8**	**502**	**100.0**
Marital status												
Single	12	2.4	11	2.2	16	3.2	122	22.3	11	2.2	**162**	**32.3**
Married	41	8.2	74	14.7	118	23.5	42	8.4	18	3.6	**293**	**58.4**
Widowed	8	1.6	15	3.0	9	1.8	3	0.6	4	0.8	**39**	**7.7**
Divorced	2	0.4	2	0.4	3	0.6	0	0.0	1	0.2	**8**	**1.6**
Total	**63**	**12.5**	**102**	**20.3**	**146**	**29.1**	**157**	**31.3**	**34**	**6.8**	**502**	**100.0**
Educational level												
Illiterate	4	0.8	18	3.6	14	2.8	0	0.0	14	2.8	**50**	**10.0**
Primary	6	1.2	4	0.8	5	1.0	12	2.4	5	1.0	**32**	**6.4**
Secondary	23	4.6	54	10.8	51	10.2	86	17.1	10	2.0	**224**	**44.6**
Tertiary	30	0.6	26	5.2	76	15.1	59	11.7	5	1.0	**196**	**39.0**
Total	**63**	**12.5**	**102**	**20.3**	**146**	**29.1**	**157**	**31.3**	**34**	**6.8**	**502**	**100.0**
Risk factors												
IV drug user	2	1.0	3	1.5	1	0.5	3	1.5	2	1.0	**11**	**5.4**
Blood transfusion	14	6.9	17	8.3	24	11.7	18	8.8	5	**2.4**	**78**	**38.2**
Surgery	8	3.9	4	2.0	10	5.0	8	3.9	2	1.0	**32**	**15.7**
Alcohol use	3	1.5	8	4.0	6	2.9	2	1.0	16	2.9	**25**	**12.3**
Smokers	5	2.4	16	7.8	15	7.4	9	4.4	3	1.5	**48**	**23.5**
Homosexual/lesbian	1	0.5	2	1.0	1	0.5	4	2.0	2	**1.0**	**10**	**4.9**
Total	**33**	**16.2**	**50**	**24.5**	**57**	**28**	**44**	**21.6**	**20**	**9.8**	**204**	**100.0**

Appendix7: Demographic distribution (%) of the HIV/AIDs out-patients by risk factors

Demographics	IV drug		Blood transfusion		Surgery		Alcohol		Smoker		Homosexual Lesbian		Total	
	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	**no.**	**%**
Gender														
Male	11	5.4	32	15.7	12	5.9	21	10.3	45	22.0	8	3.9	**129**	**63.2**
Female	0	0.0	46	22.5	20	9.8	4	2.0	3	1.5	2	1.0	**75**	**36.8**
Total	**11**	**5.4**	**78**	**38.2**	**32**	**15.7**	**25**	**12.3**	**48**	**23.5**	**10**	**4.9**	**204**	**100**
Age group (y)														
<15	0	0.0	6	2.9	2	1.0	0	0.0	0	0.0	0	0.0	**8**	**3.9**
16-30	3	1.5	18	8.8	12	5.8	3	1.5	4	2.0	4	2.0	**44**	**21.6**
31-45	6	2.9	30	14.7	14	6.9	11	5.4	20	9.8	4	2.0	**85**	**41.7**
46-60	2	1.0	22	10.7	3	1.5	7	3.4	22	10.7	2	1.0	**58**	**28.4**
>60	0	0.0	2	1.0	1	0.5	4	2.0	2	1.0	0	0.0	**9**	**4.4**
Total	**11**	**5.4**	**78**	**38.1**	**32**	**15.7**	**25**	**12.3**	**48**	**23.5**	**10**	**5.0**	**204**	**100**
Marital status														
Single	6	2.9	32	15.7	11	5.4	9	4.4	18	8.8	8	3.9	**84**	**41.2**
Married	3	1.5	42	20.4	17	8.3	13	6.4	26	12.7	1	0.5	**102**	**50.0**
Widowed	1	0.5	4	2.0	3	1.5	2	1.0	4	2.0	0	0.0	**14**	**6.8**
Divorced	1	0.5	0	0.0	1	0.5	1	0.5	0	0.0	1	0.5	**4**	**2.0**
Total	**11**	**5.4**	**78**	**38.1**	**32**	**15.7**	**25**	**12.3**	**48**	**23.5**	**10**	**5.0**	**204**	**100**
Educ. level														
Illiterate	0	0.0	6	2.9	4	2.0	2	1.0	5	2.4	0	0.0	**17**	**8.3**
Primary	2	1.0	4	2.0	3	1.5	3	1.5	8	3.9	1	0.5	**21**	**10.4**
Secondary	4	2.0	42	20.5	16	7.8	12	5.8	21	10.3	3	1.5	**98**	**48.0**
Tertiary	5	2.4	26	12.7	9	4.4	8	4.0	14	6.9	6	2.9	**68**	**33.3**
Total	**11**	**5.4**	**78**	**38.1**	**32**	**15.7**	**25**	**12.3**	**48**	**23.5**	**10**	**5.0**	**204**	**100**
Occupation														
Civil servant	2	1.0	14	6.9	8	3.9	3	1.5	5	2.4	1	0.5	**33**	**16.2**
Artisan	3	1.5	17	8.3	4	2.0	8	4.0	16	7.8	2	1.0	**50**	**24.5**
Traders	1	0.5	24	11.7	10	5.0	6	2.9	15	7.4	1	0.5	**57**	**27.9**
Student	3	1.5	18	8.8	8	3.9	2	1.0	9	4.4	4	2.0	**44**	**21.6**
Unemployed	2	1.0	5	2.4	2	1.0	6	2.9	3	1.5	2	1.0	**20**	**9.8**
Total	**11**	**5.4**	**78**	**38.1**	**32**	**15.7**	**25**	**12.3**	**48**	**23.5**	**10**	**5.0**	**204**	**100**

SUMMARY OF RESULTS

Table 1: Hospital-based distribution (%) of ART, CD_4^+, viral hepatitis+, and elevated liver enzymes among the HIV/AIDs out-patients studied

Parameters	General Hospital Onitsha		General Hospital Ekwulobia		General Hospital Enugwu-Ukwu		Total	
	No.	%	No.	%	No.	%	**No.**	**%**
Males	66	13.1	72	14.3	18	3.6	**156**	**31.1**
Females	134	26.7	128	25.5	84	16.7	**346**	**68.9**
Total	**200**	**39.8**	**200**	**39.8**	**102**	**20.3**	**502**	**100.0**
ART	155	30.9	164	32.6	80	15.9	**399**	**79.5**
Non ART	45	8.9	36	7.2	22	4.4	**103**	**20.5**
Total	**200**	**39.8**	**200**	**39.8**	**102**	**20.3**	**502**	**100.0**
CD_4^+<200 cell/µl	35	7.0	44	8.8	13	2.6	**92**	**18.3**
CD_4^+≥200 cell/µl	165	32.9	156	31.0	89	17.7	**410**	**81.7**
Total	**200**	**39.9**	**200**	**39.8**	**102**	**20.3**	**502**	**100.0**
HIV only	185	36.8	188	37.4	95	18.9	**468**	**93.2**
HBV +ve	10	2.0	10	2.0	6	1.2	**26**	**5.2**
HCV +ve	5	1.0	2	0.4	1	0.2	**8**	**1.6**
Total	**200**	**39.8**	**200**	**39.8**	**102**	**20.3**	**502**	**100.0**
GPT/ALT	8	1.6	10	2.0	6	1.2	**24**	**4.8**
GOT/AST	12	2.4	11	2.2	7	1.4	**30**	**6.0**
ALP	6	1.2	7	1.4	3	0.6	**16**	**3.2**
HIV/GPT/GOT	2	0.4	1	0.2	1	0.2	**4**	**0.8**
HIV/GPT/ALP	0	0.0	1	0.2	0	0.0	**1**	**0.2**
HIV/GOT/ALP	2	0.4	2	0.4	1	0.2	**5**	**1.0**
HIV/GPT/GOT/ALP	2	0.4	1	0.2	1	0.2	**4**	**0.8**

Table 2: Gender-based distribution (%) of ART, CD_4^+, viral hepatitis+, and elevated liver enzymes among the HIV/AIDs out-patients studied

Parameters	Male		Female		Total	
	No.	**%**	**No.**	**%**	**No.**	**%**
ART	125	24.9	274	54.6	**399**	**79.5**
Non ART	31	6.2	72	14.3	**103**	**20.5**
Total	**156**	**31.1**	**346**	**68.9**	**502**	**100.0**
CD_4^+<200 cell/µl	35	7.0	57	11.4	**92**	**18.4**
CD_4^+≥200 cell/µl	121	24.1	289	57.5	**410**	**81.6**
Total	**156**	**31.1**	**346**	**68.9**	**502**	**100.0**
HIV only	140	27.9	128	25.4	**468**	**93.3**
HBV +ve	15	3.0	11	2.2	**26**	**5.2**
HCV +ve	1	0.2	7	1.4	**8**	**1.6**
Total	**156**	**31.1**	**346**	**68.9**	**502**	**6.8**
GPT/ALT	11	2.2	13	2.6	**24**	**4.8**
GOT/AST	12	2.4	18	3.6	**30**	**6.0**
ALP	7	1.4	9	1.8	**16**	**3.2**
HIV/GPT/GOT	2	0.4	2	0.4	**4**	**0.8**
HIV/GPT/ALP	1	0.2	0	0.0	**1**	**0.2**
HIV/GOT/ALP	2	0.4	3	0.6	**5**	**1.0**
HIV/GPT/GOT/ALP	2	0.4	2	0.4	**4**	**0.8**

Table 3: Age group-based distribution (%) of ART, CD_4^+, viral hepatitis+, and elevated liver enzymes among the HIV/AIDs out-patients studied

Parameters	**<15y**		**16-30y**		**31-45y**		**46-60y**		**≥60y**		**Total**	
	No.	%	No.	%	No.	%	No.	%	No.	%	**No.**	**%**
Male	12	2.4	22	4.4	69	13.7	42	8.4	11	2.2	**156**	**31.1**
Female	14	2.8	97	19.3	177	35.3	54	10.7	4	0.8	**346**	**68.9**
Total	**26**	**5.2**	**119**	**23.7**	**246**	**49.0**	**96**	**19.1**	**15**	**3.0**	**502**	**100.0**
ART	20	4.0	85	16.9	202	40.3	79	15.7	13	2.6	**399**	**79.5**
Non ART	6	1.2	34	6.8	44	8.7	17	3.4	2	0.4	**103**	**20.5**
Total	**26**	**5.2**	**119**	**23.7**	**246**	**49.0**	**96**	**19.1**	**15**	**3.0**	**502**	**100.0**
CD_4^+<200cell/µl	2	0.4	16	3.2	50	10.0	21	4.2	3	0.6	**92**	**18.4**
CD_4^+≥200cell/µl	24	4.8	103	20.5	196	39.0	75	14.9	12	2.4	**410**	**81.6**
	26	**5.2**	**119**	**23.7**	**246**	**49.0**	**96**	**19.1**	**15**	**3.0**	**502**	**100.0**
HIV only	23	4.6	116	23.1	225	44.8	91	18.1	13	2.6	**468**	**93.2**
HIV/HBsAg	3	0.6	0	0.0	18	3.6	4	0.8	1	0.2	**26**	**5.2**
HIV/HCV	0	0.0	3	0.6	3	0.6	1	0.2	1	0.2	**8**	**1.6**
Total	**26**	**5.2**	**119**	**23.7**	**246**	**49.0**	**96**	**19.1**	**15**	**3.0**	**502**	**100.0**
HIV/GPT	2	0.4	1	0.2	12	2.4	8	1.6	1	0.2	**24**	**4.8**
HIV/GOT	2	0.4	3	0.6	14	2.8	10	2.0	1	0.2	**30**	**6.0**
HIV/ALP	1	0.2	3	0.6	8	1.6	4	0.8	0	0.0	**16**	**3.2**
HIV/GPT/GOT	1	0.2	0	0.0	1	0.2	2	0.4	0	0.0	**4**	**0.8**
HIV/GPT/ALP	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	**1**	**0.2**
HIV/GOT/ALP	0	0.0	1	0.2	3	0.6	1	0.2	0	0.0	**5**	**1.0**
HIV/GPT/GOT/ALP	0	0.0	0	0.0	3	0.6	1	0.2	0	0.0	**4**	**0.8**

Table 4: Marital status-based distribution (%) of ART, CD_4^+, viral hepatitis+, and elevated liver enzymes among the HIV/AIDs out-patients studied

Parameters	**Single**		**Married**		**Widowed**		**Divorced**		**Total**	
	No.	%	No.	%	No.	%	No.	%	No.	%
Male	68	13.5	82	16.3	4	0.8	2	0.4	**156**	**31.1**
Female	94	18.7	211	42.0	35	7.0	6	1.2	**346**	**68.9**
Total	**162**	**32.2**	**293**	**58.3**	**39**	**7.8**	**8**	**1.6**	**502**	**100.0**
ART	125	24.9	235	46.8	32	6.4	7	1.4	**399**	**79.5**
Non ART	37	7.4	58	11.5	7	1.4	1	0.2	**103**	**20.5**
Total	**162**	**32.3**	**293**	**58.3**	**39**	**7.8**	**8**	**1.6**	**502**	**100.0**
CD_4^+<200cell/µl	42	8.4	39	7.8	11	2.2	0	0.0	**92**	**18.4**
CD_4^+≥200cell/µl	120	23.9	254	50.5	28	5.6	8	1.6	**410**	**81.6**
Total	**162**	**32.3**	**293**	**58.3**	**39**	**7.8**	**8**	**1.6**	**502**	**100.0**
HIV only	150	29.9	276	54.9.0	35	7.0	7	1.4	**468**	**93.2**
HBV +ve	9	1.8	13	2.6	3	0.6	1	0.2	**26**	**5.2**
HCV +ve	3	0.6	4	0.8	1	0.2	0	0.0	**8**	**1.6**
Total	**162**	**32.3**	**293**	**58.3**	**39**	**7.8**	**8**	**1.6**	**502**	**100.0**
GPT/ALT	8	1.6	11	2.2	3	0.6	2	0.4	**24**	**4.8**
GOT/AST	10	2.0	19	3.8	1	0.2	0	0.0	**30**	**6.0**
ALP	5	1.0	11	2.2	0	0.0	0	0.0	**16**	**3.2**
HIV/GPT/GOT only	1	0.2	2	0.4	1	0.2	0	0.0	**4**	**0.8**
HIV/GPT/ALP only	0	0.0	1	0.2	0	0.0	0	0.0	**1**	**0.2**
HIV/GOT/ALP only	2	0.4	3	0.6	0	0.0	0	0.0	**5**	**1.0**
HIV/GPT/GOT/ALP only	2	0.4	2	0.4	0	0.0	0	0.0	**4**	**0.8**

Table 5: Educational level-based distribution (%) of ART, CD_4^+, viral hepatitis+, and elevated liver enzymes among the HIV/AIDs out-patients studied

Parameters	**Illiterate**		**Primary**		**Secondary**		**Tertiary**		**Total**	
	No.	%	No.	%	No.	%	No.	%	No.	%
Male	18	3.6	15	3.0	66	13.1	57	11.4	**156**	**31.1**
Female	32	6.4	17	3.4	158	31.5	139	27.6	**346**	**68.9**
Total	**50**	**10.0**	**32**	**6.4**	**224**	**44.6**	**196**	**39.1**	**502**	**100.0**
ART	43	8.6	26	5.2	168	33.4	162	32.3	**399**	**79.5**
Non ART	7	1.4	6	1.2	56	11.2	34	6.7	**103**	**20.5**
Total	**50**	**10.0**	**32**	**6.4**	**224**	**44.6**	**196**	**39.0**	**502**	**100.0**
CD_4^+<200cell/µl	20	4.0	13	2.6	34	6.8	25	5.0	**92**	**18.4**
CD_4^+≥200cell/µl	30	6.0	19	3.8	190	37.8	171	34.0	**410**	**81.6**
Total	**50**	**10.0**	**32**	**6.4**	**224**	**44.6**	**196**	**39.0**	**502**	**100.0**
HIV only	**45**	**9.0**	**30**	**6.0**	**208**	**41.4**	**185**	**36.8**	**468**	**93.2**
HBV +ve	4	0.8	2	0.4	11	2.2	9	1.8	**26**	**5.2**
HCV +ve	1	0.2	0	0.0	5	1.0	2	0.4	**8**	**1.6**
Total	**50**	**10.0**	**32**	**6.4**	**224**	**44.6**	**196**	**39.0**	**502**	**100.0**
GPT/ALT	3	0.6	3	0.6	12	2.4	6	1.2	**24**	**4.8**
GOT/AST	1	0.2	2	0.4	16	3.2	11	2.2	**30**	**6.0**
ALP	0	0.0	1	0.2	10	2.0	5	1.0	**16**	**3.2**
HIV/GPT/GOT only	0	0.0	0	0.0	2	0.4	2	0.4	**4**	**0.8**
HIV/GPT/ALP only	0	0.0	0	0.0	1	0.2	0	0.0	**1**	**0.2**
HIV/GOT/ALP only	0	0.0	0	0.0	3	0.6	2	0.4	**5**	**1.0**
HIV/GPT/GOT/ALP only	0	0.0	0	0.0	2	0.4	2	0.4	**4**	**0.8**

Table 6: Occupation-based distribution (%) of ART, CD_4^+, viral hepatitis+, and elevated liver enzymes among the HIV/AIDs out-patients studied

Parameters	Civil servants		Artisans		Traders		Students		Unemployed		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Male	18	3.6	28	5.6	49	9.8	53	10.6	8	1.6	**156**	**31.1**
Female	45	9.0	74	14.7	97	19.3	104	20.7	26	5.2	**346**	**68.9**
Total	**63**	**12.6**	**102**	**20.3**	**146**	**29.1**	**157**	**31.3**	**34**	**6.8**	**502**	**100.0**
ART	59	11.8	88	17.5	108	21.5	114	22.7	30	6.0	**399**	**79.5**
Non ART	4	0.8	14	2.8	38	7.6	43	8.6	4	0.8	**103**	**20.5**
Total	**63**	**12.6**	**102**	**20.3**	**146**	**29.1**	**157**	**31.3**	**34**	**6.8**	**502**	**100.0**
CD_4^+<200cell/µl	9	1.8	25	5.0	11	2.2	35	7.0	12	2.4	**92**	**18.4**
CD_4^+≥200cell/µl	54	10.8	77	15.3	135	26.9	122	24.3	22	4.4	**410**	**81.6**
Total	**63**	**12.6**	**102**	**20.3**	**146**	**29.1**	**157**	**31.3**	**34**	**6.8**	**502**	**100.0**
HIV only	57	11.4	96	19.1	136	27.1	148	29.5	31	6.2	**468**	**93.2**
HBV +ve	4	0.8	5	1.0	8	1.6	7	1.4	2	0.4	**26**	**5.2**
HCV +ve	2	0.4	1	0.2	2	0.4	2	0.4	1	0.2	**8**	**1.6**
Total	**63**	**12.6**	**102**	**20.3**	**146**	**29.1**	**157**	**31.3**	**34**	**6.8**	**502**	**100.0**
GPT/ALT	3	0.6	4	0.8	6	1.2	8	1.6	3	0.6	**24**	**4.8**
GOT/AST	3	0.6	7	1.4	11	2.2	8	1.6	1	0.2	**30**	**6.0**
ALP	2	0.4	3	0.6	5	1.0	6	1.2	0	0.0	**16**	**3.2**
HIV/GPT/GOT	0	0.0	0	0.0	1	0.2	2	0.4	1	0.2	**4**	**0.8**
HIV/GPT/ALP	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0	**1**	**0.2**
HIV/GOT/ALP	0	0.0	1	0.2	2	0.4	3	0.6	0	0.0	**5**	**1.0**
HIV/GPT/GOT/ALP	0	0.0	1	0.2	1	0.2	2	0.4	0	0.0	**4**	**0.8**

Table 7: Risk factor-based distribution (%) of ART, CD_4^+, viral hepatitis+, and elevated liver enzymes among the HIV/AIDs out-patients studied

Parameters	IV drug users		Blood transfusion		Surgery		Alcohol use		Smokers		Homosexual / Lesbian		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Male	11	5.4	32	15.7	12	5.9	21	10.3	45	22.1	8	3.9	**129**	**63.2**
Female	0	0.0	46	22.5	20	9.8	4	2.0	3	1.5	2	1.0	**75**	**36.8**
Total	**11**	**5.4**	**78**	**38.2**	**32**	**15.7**	**25**	**12.3**	**48**	**23.6**	**10**	**4.9**	**204**	**100**
ART	8	3.9	58	28.4	27	13.2	18	8.8	35	17.2	7	**3.4**	**153**	**75.0**
Non ART	3	1.5	20	9.8	5	2.5	7	3.4	13	6.4	3	**1.5**	**51**	**25.0**
Total	**11**	**5.4**	**78**	**38.2**	**32**	**15.7**	**25**	**12.2**	**48**	**23.6**	**10**	**4.9**	**204**	**100.0**
CD_4^+<200cell/µl	2	1.0	17	8.3	7	3.4	6	2.9	16	7.9	2	**1.0**	**50**	**24.5**
CD_4^+≥200cell/µl	9	4.4	61	29.9	25	12.3	19	9.3	32	15.7	8	**3.9**	**154**	**75.5**
Total	**11**	**5.4**	**78**	**38.2**	**32**	**15.7**	**25**	**12.2**	**48**	**23.6**	**10**	**4.9**	**204**	**100.0**
HIV only	9	4.4	76	37.2	29	14.2	20	9.8	45	22.1	8	**3.9**	**187**	**91.6**
HBV +ve	2	1.0	1	0.5	2	1.0	5	2.5	2	1.0	2	**1.0**	**14**	**6.9**
HCV +ve	0	0.0	1	0.5	1	0.5	0	0.0	1	0.5	0	**0.0**	**3**	**1.5**
Total	**11**	**5.4**	**78**	**38.2**	**32**	**15.7**	**25**	**12.3**	**48**	**23.6**	**10**	**4.9**	**204**	**100.0**
GPT/ALT	1	0.5	2	1.0	0	0.0	3	1.5	1	0.5	1	0.5	**8**	**3.9**
GOT/AST	1	0.5	3	1.5	2	1.0	3	1.5	4	2.0	0	0.0	**13**	**6.4**
ALP	0	1.0	1	0.5	1	0.5	0	0.0	0	0.0	1	0.5	**3**	**1.5**
HIV/GPT/GOT	1	0.2	1	0.2	0	0.0	0	0.0	1	0.2	0	0.0	**3**	**0.6**
HIV/GPT/ALP	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	**1**	**0.2**
HIV/GOT/ALP	0	0.0	0	0.0	1	0.2	1	0.2	1	0.2	0	0.0	**3**	**0.6**
HIV/GPT/GOT/ALP	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	**1**	**0.1**

Table 8: The CD_4^+ cell count-based distribution (%) of viral hepatitis+ and elevated liver enzymes among the HIV/AIDs out-patients studied

Parameters	**CD_4^+<200cell/µl**(n=92)		**CD_4^+≥200cell/µl**(n=410)		**Total** (N=502)	
	No.	%	No.	%	No.	%
HBs Ag	5	1.0	21	4.2	**26**	**5.2**
HCV	1	0.2	7	1.4	**8**	**1.6**
GPT	7	1.4	17	3.4	**24**	**4.8**
GOT	13	2.6	17	3.4	**30**	**6.0**
ALP	9	1.8	7	1.4	**16**	**3.2**

Table 9: The ART-based distribution (%) of viral hepatitis+ and elevated liver enzymes among the HIV/AIDs out-patients studied

Parameters	**ART**		**Non-ART**		**Total**	
	No.	%	No.	%	No.	%
CD_4^+<200cell/µl	63	12.6	29	5.8	**92**	**18.4**
CD_4^+≥200cell/µl	336	66.9	74	14.7	**410**	**81.6**
Total	**399**	**79.5**	**103**	**20.5**	**502**	**100**
HBs Ag	24	4.8	2	0.4	**26**	**5.2**
HCV	7	1.4	1	0.2	**8**	**1.6**
GPT	20	4.0	4	0.8	**24**	**4.8**
GOT	21	4.2	9	1.8	**30**	**6.0**
ALP	13	2.6	3	0.6	**16**	**3.2**

APPENDIX I

CONSENT FOR COLLECTION OF VENOUS BLOOD SAMPLE

I, Nweke Rita Ngozi; a post graduate student of the Department of Applied Microbiology & Brewing, Nnamdi Azikiwe University, Awka, hereby seek your consent for the collection of venous blood sample from you. These samples will be used to determine CD_4 count, Hepatitis B & C, Syphilis and liver enzymes level. I assure you of the confidentiality of the results and also, that the samples will only be used for the purpose stated above.

_____	_____
Researcher	**Respondent**
_____	_____
Date	**Date**

APPENDIX II
LABORATORY REQUEST AND RESULT FORM

Non-ART ☐ ART ☐ collection Date----------

LGA----------Facility----------

Name:----------

Surname other names

Sex: Male ☐ Female ☐ Age ☐

Phone Number:----------Client No----------

CD_4 count----------cell/µl ALT /SGPT----------IU/L

HBsAg---------- AST/SGOT----------IU/L

HCV----------ALP----------IU/L

Syphilis/VDRL----------

Med. Lab. Scientist Signature---------- Date----------


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APPEENDIX IV

QUESTIONNAIRE

I, Nweke Rita Ngozi of the department of Applied Microbiology and Brewing of Nnamdi Azikiwe University Awka, Anambra State under the supervision of Prof. (Mrs) C.A. Oyeka humbly invite you to take part in this research titled "Clinical Effects of Hepatitis B, C, Syphilis and Elevated Liver Enzymes on People Living with HIV/AIDS in Anambra State". This questionnaire will help me to get the proper information about the social life and risk factors associated with coinfection of hepatitis B, C and syphilis in people living with HIV/AIDS needed to carry out this research.

[Please tick √ in the appropriate column)

Non-ART ☐ ART ☐ Date: _____

LGA: _____ Facility_____

Sex: Male ☐ Female ☐ Age ☐

Phone Number:_____

Marital Status

Single ☐ Married ☐ Widowed ☐ Divorced ☐

Highest Educational Level

Non-formal ☐ Primary ☐ Secondary ☐ Tertiary ☐

Occupation

Civil servant ☐ Self employed ☐ Trader ☐ Student ☐ Unemployed ☐

Exposure to Risk Factors

Intravenous drug users ☐ Blood transfusion ☐ Surgery ☐ Alcohol abuse ☐

Smokers ☐ Homosexual/lesbianism ☐

_____	_____	_____
Participant's Name	**Signature**	**Date**

Thank you for your participation.

APPENDIX V

Reflotron Roche Diagnostic Inclusion Manual

Reference ranges in IU/L

Parameters	Male	female
GOT/AST	up to 40	up to 33
GPT/ALT	up to 41	up to 32
ALP	up to 129	up to 104

APPENDIX VI

SAMPLE SIZE DETERMINATION

$$SS = \frac{(Z\text{ – score}^2)\,(P)\,(1\text{-}P)}{(D)^2}$$

Where SS – Sample size

D - Desired level of significance (0.05)

Z – Score = 1.96 for 95% confidence interval;

P – Population proportion (expressed as decimal assumed to 0.5).

Adjusted sample size (SSA) for finite population = $\frac{SS}{1 + \left(\frac{SS - 1}{PS}\right)}$ (Daniel, 1999)

PS is the known study population of HIV/AIDS out-patients from which the sample size was obtained.

$$SS = \frac{(Z\text{ – score}^2)\,(P)\,(1\text{-}P)}{(D)^2}$$

$$= \frac{(1.96)^2 x\ (0.5)\,(1\text{-}0.5)}{(0.05)^2}$$

SS = 384.16

Adjusted sample size (SSA) for finite population = $\frac{SS}{1 + \left(\frac{SS - 1}{PS}\right)}$

$PS = P_o + P_e + P_k$

Where P_o - Population of PLWHA at General Hospital Onitsha = 1000

P_e – Population of PLWHA at General Hospital Ekwulobia = 1000

P_k - Population of PLWHA at General Hospital Enugwu-Ukwu = 300

$$SSA = \frac{384.16}{1 + \left(\frac{(384.16\text{-}1)}{2300}\right)} = 329$$

APPENDIX VIII

Analysis for table 3

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *use of Anti Retroviral drug	502	100.0%	0	0.0%	502	100.0%

Age groups of the study participants * use of Anti-Retro viral drug Crosstabulation Count

Count

		Use of Anti-Retroviral drug		Total
		Use ART	Do not use ART	
Age groups of the study participants	0-15 years	20	6	26
	16-30 years	85	34	119
	31-45 years	202	44	246
	46-60 years	79	17	96
	Above 60 years	13	2	15
Total		399	103	502

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	6.821[a]	4	.146
Likelihood Ratio	6.543	4	.162
Linear-by- Linear Association	4.057	1	.044
N of Valid Cases	.502		

a. 1 cells (10.0%) have expected count less than 5. The minimum expected count is 3.08

APPENDIX IX

Analysis for table 4

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *use CD4 count	502	100.0%	0	0.0%	502	100.0%

Age groups of the study participants * use of CD4 Count Crosstabulation

Count

		CD4 count		Total
		Less than 200	Equal to or greater than 200	
Age groups of the study participants	0-15 years	2	24	26
	16-30 years	16	103	119
	31-45 years	50	196	246
	46-60 years	21	75	96
	Above 60 years	3	12	15
Total		92	410	502

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	5.351[a]	4	.253
Likelihood Ratio	5.887	4	.208
Linear-by- Linear Association	4.134	1	.042
N of Valid Cases	502		

a. 2 cells (20.0%) have expected count less than 5. The minimum expected count is 2.75.

APPENDIX X

Analysis for table 7 (for HIV only)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *prevalence of HIV infection only	502	100.0%	0	0.0%	502	100.0%

Age groups of the study participants * Prevalence of HIV infection only Crosstabulation

Count

		Prevalence of HIV infection only		
		HIV/HBV or HCV	HIV only	Total
Age groups of the study participants	0-15 years	3	23	26
	16-30 years	3	116	119
	31-45 years	21	225	246
	46-60 years	5	91	96
	Above 60 years	2	13	15
Total		34	468	502

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	6.949[a]	4	.139
Likelihood Ratio	7.540	4	.110
Linear-by-Linear Association	.392	1	.531
N of Valid Cases	502		

a. 2 cells (20.0%) have expected count less than 5. The minimum expected count is 1.02.

APPENDIX XI

Analysis for table 7 (for HIV/HBV)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *Hepatitis B surface antigen	502	100.0%	0	0.0%	502	100.0%

Age groups of the study participants * Hepatitis B surface antigen Crosstabulation

Count

		Hepatitis B surface antigen		
		Positive	Negative	Total
Age groups of the study participants	0-15 years	3	23	26
	16-30 years	0	119	119
	31-45 years	18	228	246
	46-60 years	4	92	96
	Above 60 years	1	14	15
Total		26	476	502

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	11.198[a]	4	.024
Likelihood Ratio	16.588	4	.002
Linear-by-Linear Association	.294	1	.587
N of Valid Cases	502		

a. 3 cells (30.0%) have expected count less than 5. The minimum expected count is 78.

APPENDIX XII

Analysis for table 7 (for HIV/HCV)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *Hepatitis C virus	502	100.0%	0	0.0%	502	100.0%

Age groups of the study participants * Hepatitis C virus Crosstabulation

Count

		Hepatitis C virus		
		Positive	Negative	Total
Age groups of the study participants	0-15 years	0	26	26
	16-30 years	3	116	119
	31-45 years	3	243	246
	46-60 years	1	95	96
	Above 60 years	1	14	15
Total		8	494	502

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	3.941[a]	4	.414
Likelihood Ratio	3.222	4	.521
Linear-by-Linear Association	.087	1	.768
N of Valid Cases	502		

a. 5 cells (50.0%) have expected count less than 5. The minimum expected count is 24.

APPENDIX XIII

Analysis for table 8 (for HIV only)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *HIV infection only	502	100.0%	0	0.0%	502	100.0%

Age groups of the study participants * Hepatitis infection only Crosstabulation

Count

		Hepatitis infection only		Total
		Normal values of enzymes	Elevated values of enzymes	
Age groups of the study participants	0-15 years	22	4	26
	16-30 years	113	6	119
	31-45 years	222	24	246
	46-60 years	80	16	96
	Above 60 years	13	2	15
Total		450	52	502

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	8.683[a]	4	.070
Likelihood Ratio	8.781	4	.067
Linear-by-Linear Association	3^.258	1	.071
N of Valid Cases	502		

a. 2 cells (20.0%) have expected count less than 5. The minimum expected count is 1.55.

APPENDIX XIV

Analysis for table 8 (for HIV/GPT)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *Pyruvate transaminase	502	100.0%	0	0.0%	502	100.0%

Age groups of the study participants * Pyruvate transaminase Crosstabulation

Count

		Pyruvate transaminase		Total
		Normal enzymes	Elevated enzymes	
Age groups of the study participants	0-15 years	24	2	26
	16-30 years	118	1	119
	31-45 years	234	12	246
	46-60 years	87	8	95
	Above 60 years	14	1	15
Total		477	24	501

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	7.417[a]	4	.115
Likelihood Ratio	8.890	4	.064
Linear-by-Linear Association	3.038	1	.081
N of Valid Cases	501		

a. 3 cells (30.0%) have expected count less than 5. The minimum expected count is 72.

APPENDIX XV

Analysis for table 8 (for HIV/GOT)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *Oxalate transaminase	501	99.8%	1	0.2%	502	100.0%

Age groups of the study participants * Oxalate transaminase Crosstabulation

Count

		Oxalate transaminase		Total
		Normal enzyme	Elevated enzyme	
Age groups of the study participants	0-15 years	24	2	26
	16-30 years	116	3	119
	31-45 years	232	14	246
	46-60 years	85	10	95
	Above 60 years	14	1	15
Total		471	30	501

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	6.202[a]	4	.185
Likelihood Ratio	6.257	4	.181
Linear-by-Linear Association	2.852	1	.091
N of Valid Cases	501		

a. 2 cells (20.0%) have expected count less than 5. The minimum expected count is 90.

APPENDIX XVI

Analysis for table 8 (for HIV/ALP)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *Alkaline Phosphatase	501	99.8%	1	0.2%	502	100.0%

Age groups of the study participants * Alkaline Phosphatase Crosstabulation

Count

		Alkaline Phosphatase		Total
		Normal enzyme	Elevated enzyme	
Age groups of the study participants	0-15 years	25	1	26
	16-30 years	116	3	119
	31-45 years	238	8	246
	46-60 years	91	4	95
	Above 60 years	15	0	15
Total		485	16	501

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	1.025[a]	4	.906
Likelihood Ratio	1.487	4	.829
Linear-by- Linear Association	.019	1	.890
N of Valid Cases	501		

a. 4 cells (40.0%) have expected count less than 5. The minimum expected count is 48.

APPENDIX XVII

Analysis for table 9 (for HIV only)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *No enzyme elevated	501	99.8%	1	0.2%	502	100.0%

Age groups of the study participants * No enzyme elevated Crosstabulation

Count

		No enzyme elevated		
		1.00	2.00	Total
Age groups of the study participants	0-15 years	22	4	26
	16-30 years	113	6	119
	31-45 years	222	24	246
	46-60 years	79	16	95
	Above 60 years	13	2	15
Total		449	52	501

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi- Square	8.854[a]	4	.065
Likelihood Ratio	8.929	4	.063
Linear-by- Linear Association	3.332	1	.068
N of Valid Cases	.501		

a. 2 cells (20.0%) have expected count less than 5. The minimum expected count is 1.56.

APPENDIX XVIII

Analysis for table 9 (for HIV/GPT/GOT)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *Elevated GPT and GOT	501	99.8%	1	0.2%	502	100.0%

Age groups of the study participants * Elevated GPT and GOT Crosstabulation

Count

		Elevated GPT and GOT Only		
		No	Yes	Total
Age groups of the study participants	0-15 years	25	1	26
	16-30 years	119	0	119
	31-45 years	245	1	246
	46-60 years	93	2	95
	Above 60 years	15	0	15
Total		497	4	501

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	6.653*	4	.155
Likelihood Ratio	5.726	4	.221
Linear-by-Linear Association	.045	1	.831
N of Valid Cases	-50 1		

a. 5 cells (50.0%) have expected count less than 5. The minimum expected count is 12.

APPENDIX XIX

Analysis for table 9 (for HIV/GPT/ALP)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *Elevated GPT and ALP	501	99.8%	1	0.2%	502	100.0%

Age groups of the study participants * Elevated GPT and ALP Crosstabulation

Count

		Elevated GPT and ALP Only		
		No	Yes	Total
Age groups of the study participants	0-15 years	26	0	26
	16-30 years	119	0	119
	31-45 years	246	0	246
	46-60 years	94	1	95
	Above 60 years	15	0	15
Total		500	1	501

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	4.282[a]	4	.369
Likelihood Ratio	3.334	4	.504
Linear-by-Linear Association	1.598	1	.206
N of Valid Cases	501		

a. 5 cells (50.0%) have expected count less than 5. The minimum expected count is 03.

APPENDIX XX

Analysis for table 9 (for HIV/GOT/ALP)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants *Elevated GOT and ALP	501	99.8%	1	0.2%	502	100.0%

Age groups of the study participants * Elevated GOT and ALP Crosstabulation

Count

		Elevated GOT and ALP Only		
		No	Yes	Total
Age groups of the study participants	0-15 years	26	0	26
	16-30 years	118	1	119
	31-45 years	243	3	246
	46-60 years	94	1	95
	Above 60 years	15	0	15
Total		496	5	501

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	.568[a]	4	.967
Likelihood Ratio	.971	4	.914
Linear-by-Linear Association	.057	1	.811
N of Valid Cases	501		

a. 5 cells (50.0%) have expected count less than 5. The minimum expected count is 15.

APPENDIX XXI

Analysis for table 9 (for HIV/GPT/GOT/ALP)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants * Elevated	501	99.8%	1	0.2%	502	100.0%

Age groups of the study participants * Elevated Crosstabulation

Count

		Elevated GOT and ALP Only		
		No	Yes	Total
Age groups of the study participants	0-15 years	26	0	26
	16-30 years	119	0	119
	31-45 years	243	3	246
	46-60 years	94	1	95
	Above 60 years	15	0	15
Total		497	4	501

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)
Pearson Chi-Square	1.916[a]	4	.751
Likelihood Ratio	3.110	4	.540
Linear-by- Linear Association	.630	1	.427
N of Valid Cases	501		

a. 5 cells (50.0%) have expected count less than 5. The minimum expected count is 12.

APPENDIX XXII

Analysis for table 10 (for CD_4 count)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Gender of the study participants *CD_4 count	502	100.0%	0	0.0%	502	100.0%

Gender of the study participants *CD_4 count Crosstabulation

Count

		CD4 count		Total
		Less than 200	Equal greater than 200	
Gender of the study Participants	Male	35	121	156
	Female	57	289	346
Total		92	410	502

Chi-Square Tests

	Value	Df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	2.553[a]	1	.110		
Continuity Correction[13]	2.171	1	.141		
Likelihood Ratio	2.484	1	.115		
Fisher's Exact Test				.134	.072
Linear-by-Linear Association	2.548	1	.110		
N of Valid Cases	502				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 28.59.
b. Computed only for a 2x2 table

APPENDIX XXIII

Analysis for table 10 (for HIV/HBV)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Gender of the study participants *Hepatitis B surface antigen	502	100.0%	0	0.0%	502	100.0%

Gender of the study participants *Hepatitis B surface antigen Crosstabulation

Count

		Hepatitis B surface antigen		Total
		Positive	Negative	
Gender of the study Participants	Male	15	141	156
	Female	11	335	346
Total		26	476	502

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi- Square	9.069[a]	1	.003		
Continuity Correction[13]	7.806	1	.005		
Likelihood Ratio	8.298	1	.004		
Fisher's Exact Test				.004	.004
Linear-by-Linear Association	9.501	1	.003		
N of Valid Cases	502				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 8.08.
b. Computed only for a 2x2 table

APPENDIX XXIV

Analysis for table 10 (for HIV/HCV)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Gender of the study participants *Hepatitis C virus	502	100.0%	0	0.0%	502	100.0%

Gender of the study participants *Hepatitis C virus Crosstabulation

Count

		Hepatitis C virus		Total
		Positive	Negative	
Gender of the study Participants	Male	1	155	156
	Female	7	339	346
Total		8	494	502

Chi-square Tests

	Value	Df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi- Square	1.310[a]	1	.252		
Continuity Correction[13]	.577	1	.448		
Likelihood Ratio	1.540	1	.215		
Fisher's Exact Test				.445	.232
Linear- by- Linear Association	1.307	1	.253		
N of Valid Cases	502				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.49.
b. Computed only for a 2x2 table

APPENDIX XXV

Analysis for table 10 (for HIV/GPT)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Gender of the study participants *Pyruvate transaminase	501	99.8%	1	0.2%	502	100.0%

Gender of the study participants *Pyruvate transaminase Crosstabulation

Count

		Pyruvate transaminase		Total
		Normal enzyme	Elevated enzyme	
Gender of the study Participants	Male	144	11	155
	Female	333	13	346
Total		477	24	501

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	2.618[a]	1	.106		
Continuity Correction[13]	1.937	1	.164		
Likelihood Ratio	2.456	1	.117		
Fisher's Exact Test				.116	.085
Linear-by-Linear Association	2.612	1	.106		
N of Valid Cases	501				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 7.43.
b. Computed only for a 2x2 table

APPENDIX XXVI

Analysis for table 10 (for HIV/GOT)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Gender of the study participants *Oxalate transaminase	501	99.8%	1	0.2%	502	100.0%

Gender of the study participants *Oxalate transaminase Crosstabulation

Count

		Oxalate transaminase		Total
		Normal enzyme	Elevated enzyme	
Gender of the study Participants	Male	143	12	155
	Female	328	18	346
Total		471	30	501

Chi-square Tests

	Value	df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi- Square	1.226[a]	1	.268		
Continuity Correction[13]	.817	1	.366		
Likelihood Ratio	1.175	1	.278		
Fisher's Exact Test				.309	.182
Linear-by-Linear Association	1.224	1	.269		
N of Valid Cases	501				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 9.28.
b. Computed only for a 2x2 table

APPENDIX XXVII

Analysis for table 10 (for HIV/ALP)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Gender of the study participants *Akaline Phosphatase	501	99.8%	1	0.2%	502	100.0%

Gender of the study participants * Akaline Phosphatase Crosstabulation

Count

		Akaline Phosphatase		Total
		Normal enzyme	Elevated enzyme	
Gender of the study Participants	Male	148	7	155
	Female	337	9	349
Total		485	16	501

Chi-square Tests

	Value	Df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	1.270[a]	1	.260		
Continuity Correction[5]	.726	1	.394		
Likelihood Ratio	1.198	1	.274		
Fisher's Exact Test				.278	.195
Linear-by-Linear Association	1.267	1	.260		
N of Valid Cases	501				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.95.

b. Computed only for a 2x2 table

APPENDIX XXVIII

Analysis for table 11 (for HIV/HBV)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
CD4 count *Hepatitis B surface antigen	502	100.0%	0	0.0%	502	100.0%

CD4 count * Hepatitis B surface antigen Crosstabulation

Count

		Hepatitis B surface antigen		Total
		Positive	Negative	
CD4	less than 200	5	87	92
	equal to or greater than 200	21	389	410
Total		26	476	502

Chi-square Tests

	Value	Df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	.015[a]	1	.903		
Continuity Correction[13]	.000	1	1.000		
Likelihood Ratio	.015	1	.903		
Fisher's Exact Test				.800	.535
Linear-by-Linear Association	.015	1	.903		
N of Valid Cases	502				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.76

b. Computed only for a 2x2 table

APPENDIX XXIX

Analysis for table 11 (for HIV/HCV)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
CD4 count *Hepatitis C virus	502	100.0%	0	0.0%	502	100.0%

CD4 count * Hepatitis C virus Crosstabulation

Count

		Hepatitis C virus		Total
		Positive	Negative	
CD4	less than 200	1	91	92
	equal to or greater than 200	7	403	410
Total		8	494	502

Chi-square Tests

	Value	Df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	.184[a]	1	.668		
Continuity Correction[13]	.000	1	1.000		
Likelihood Ratio	.202	1	.653		
Fisher's Exact Test				1.000	.553
Linear-by-Linear Association	.184	1	.668		
N of Valid Cases	502				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 1.47.
b. Computed only for a 2x2 table

APPENDIX XXX

Analysis for table 11 (for HIV/GPT)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
CD4 count *Pyruvate transaminase	501	99.8%	1	0.2%	502	100.0%

CD4 count * Pyruvate transaminase Crosstabulation

Count

		Pyruvate transaminase		Total
		Normal enzyme	Elevated enzyme	
CD4	less than 200	85	7	92
	equal to or greater than 200	392	17	409
Total		477	24	501

Chi-square Tests

	Value	Df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	1.963[a]	1	.161		
Continuity Correction[b]	1.279	1	.258		
Likelihood Ratio	1.746	1	.186		
Fisher's Exact Test				.176	.131
Linear-by-Linear Association	1.959	1	.162		
N of Valid Cases	501				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.41.
b. Computed only for a 2x2 table

APPENDIX XXXI

Analysis for table 11 (for HIV/GOT)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
CD4 count *Oxalate transaminase	501	99.8%	1	0.2%	502	100.0%

CD_4 count * Oxalate transaminase Crosstabulation

Count

		Oxalate transaminase		Total
		Normal enzyme	Elevated enzyme	
CD4	less than 200	79	13	92
	equal to or greater than 200	392	17	409
Total		471	30	501

Chi-square Tests

	Value	Df	Asymp.Sig.(2 - sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi- Square	13.272	1	.000		
Continuity Correction[13]	11.560	1	.001		
Likelihood Ratio	10.723	1	.001		
Fisher's Exact Test				.001	.001
Linear-by-Linear Association	13.246	1	.000		
N of Valid Cases	501				

a. 1 cells (0.0%) have expected count less than 5. The minimum expected count is 5.51.
b. Computed only for a 2x2 table

APPENDIX XXXII

Analysis for table 11 (for HIV/ALP)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
CD_4 count *Alkaline Phosphatase	501	99.8%	1	0.2%	502	100.0%

CD_4 count * Alkaline Phosphatase Crosstabulation

Count

		Alkaline phosphatase		Total
		Normal enzyme	Elevated enzyme	
CD4	less than 200	83	9	92
	equal to or greater than 200	402	7	409
Total		485	16	501

Chi-square Tests

	Value	Df	Asymp.Sig.(2 - sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi- Square	15.825[a]	1	.000		
Continuity Correction[b]	13.322	1	.000		
Likelihood Ratio	11.932	1	.001		
Fisher's Exact Test				.001	.001
Linear-by- Linear Association	15.794	1	.000		
N of Valid Cases	501				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.94.
b. Computed only for a 2x2 table

APPENDIX XXXIII

Analysis for table 12 (for CD_4 count)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Use of Anti Retroviral drug * CD4 count	502	100.0%	0	0.0%	502	100.0%

Use of Anti Retroviral drug *CD_4 count Crosstabulation

Count

		CD4 count		Total
		Less than 200	Equal to or greater than 200	
Use of Anti Retroviral drug	Use ART	63	336	399
	Do not use ART	29	74	103
Total		92	410	502

Chi-square Tests

	Value	Df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	8.364[a]	1	.004		
Continuity Correction[13]	7.558	1	.006		
Likelihood Ratio	7.710	1	.005		
Fisher's Exact Test				.006	.004
Linear-by-Linear Association	8.347	1	.004		
N of Valid Cases	502				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 18.88.
b. Computed only for a 2x2 table

APPENDIX XXXIV

Analysis for table 12 (for HIV/HBV)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Use of Anti Retroviral drug *Hepatitis B surface antigen	502	100.0%	0	0.0%	502	100.0%

Use of Anti Retroviral drug *Hepatitis B surface antigen Crosstabulation

Count

		Hepatitis B surface antigen		Total
		Positive	Negative	
Use of Anti Retroviral drug	Use ART	24	375	399
	Do not use ART	2	101	103
Total		26	476	502

Chi-square Tests

	Value	Df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	2.766[a]	1	.096		
Continuity Correction[b]	1.999	1	.157		
Likelihood Ratio	3.398	1	.065		
Fisher's Exact Test				.133	.070
Linear-by-Linear Association	2.760	1	.097		
N of Valid Cases	502				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.33
b. Computed only for a 2x2 table

APPENDIX XXXV

Analysis for table 12 (for HIV/HCV)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Use of Anti Retroviral drug *Hepatitis C virus	502	100.0%	0	0.0%	502	100.0%

Use of Anti Retroviral drug * Hepatitis C virus Crosstabulation

Count

		Hepatitis C virus		Total
		Positive	Negative	
Use of Anti Retroviral drug	Use ART	7	392	399
	Do not use ART	1	102	103
Total		8	494	502

Chi-square Tests

	Value	Df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	.320[a]	1	.571		
Continuity Correction[13]	.016	1	.901		
Likelihood Ratio	.359	1	.549		
Fisher's Exact Test				1.000	.487
Linear-by-Linear Association	.320	1	.572		
N of Valid Cases	502				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 1.64.
b. Computed only for a 2x2 table

APPENDIX XXXVI

Analysis for table 12 (for HIV/GPT)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Use of Anti Retroviral drug *Pyruvate transaminase	501	99.8%	1	0.2%	502	100.0%

Use of Anti Retroviral drug * Pyruvate transaminase Crosstabulation

Count

		Pyruvate transaminase		Total
		Normal	Elevated enzyme	
Use of Anti Retroviral drug	Use ART	378	20	398
	Do not use ART	99	4	103
Total		477	24	501

Chi-square Tests

	Value	Df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	.234[a]	1	.629		
Continuity Correction[13]	.051	1	.822		
Likelihood Ratio	.245	1	.620		
Fisher's Exact Test				.798	.429
Linear-by-Linear Association	.233	1	.629		
N of Valid Cases	501				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.93.
b. Computed only for a 2x2 table

APPENDIX XXXVII

Analysis for table 12 (for HIV/GOT)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Use of Anti Retroviral drug *Oxalate transaminase	501	99.8%	1	0.2%	502	100.0%

Use of Anti Retroviral drug * Oxalate transaminase Crosstabulation

Count

		Oxalate transaminase		Total
		Normal enzyme	Elevated enzyme	
Use of Anti Retroviral drug	Use ART	377	21	398
	Do not use ART	94	9	103
Total		471	30	501

Chi-square Tests

	Value	Df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	1.742[a]	1	.187		
Continuity Correction[b]	1.181	1	.277		
Likelihood Ratio	1.593	1	.207		
Fisher's Exact Test				.241	.139
Linear-by-Linear Association	1.738	1	.187		
N of Valid Cases	501				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.17.

b. Computed only for a 2x2 table

APPENDIX XXXVIII

Analysis for table 12 (for HIV/ALP)

Case processing summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Use of Anti Retroviral drug *Alkaline phosphatase	501	99.8%	1	0.2%	502	100.0%

Use of Anti Retroviral drug * Alkaline phosphatase Crosstabulation

Count

		Alkaline phosphatase		Total
		Normal enzyme	Elevated enzyme	
Use of Anti Retroviral drug	Use ART	385	13	398
	Do not use ART	100	3	103
Total		485	16	501

Chi-square Tests

	Value	Df	Asymp.Sig.(2-sided)	Exact Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	.033[a]	1	.856		
Continuity Correction[13]	.000	1	1.000		
Likelihood Ratio	.034	1	.854		
Fisher's Exact Test				1.000	.575
Linear- by- Linear Association	.033	1	.856		
N of Valid Cases	501				

a. 0 cells (25.0%) have expected count less than 5. The minimum expected count is 3.29.

b. Computed only for a 2x2 table

APPENDIX XXXIX

Mean CD_4 counts (Gender, HBV, HCV)

Report CD4

CD_4 count

Gender of the study participants	Mean	N	Std. Deviation	Std. Error of Mean
Male	489.9936	156	376.32377	30.13002
Female	546.8092	346	350.39709	18.83746
Total	529.1534	502	359.24230	16.03377

Report

CD4 count

Hepatitis B surface antigen	Mean	N	Std. Deviation	Std. Error of Mean
Positive	494.8846	26	414.33478	81.25773
Negative	531.0252	476	356.39285	16.33524
Total	529.1534	502	359.24230	16.03377

Report

CD4 count

Hepatitis C virus	Mean	N	Std. Deviation	Std. Error of Mean
Positive	673.3750	8	386.63897	136.69752
Negative	526.8178	494	358.72506	16.13980
Total	529.1534	502	359.24230	16.03377

APPENDIX XL

Mean CD_4 counts (ART, Non-ART, GPT, GOT, ALP)

Report CD4

CD_4 count

ART/Non-ART	Mean	N	Std. Deviation	Std. Error of Mean
ART	552.1254	399	484.23411	10.50632
Non-ART	446.3212	103	328.02562	16.40035
Total	529.1534	502	359.24230	16.03377

Report

CD_4 count

Elevated liver enzymes	Mean	N	Std. Deviation	Std. Error of Mean
GPT	382.0427	24	363.04512	18.12674
GOT	328.7345	30	342.42346	13.44898
ALP	256.5092	16	318.50724	12.80256
HIV only	561.0148	432	397.63418	11.71824
Total	529.1534	502	359.24230	16.03377

PLATE 1: PARTEC CYFLOW MACHINE

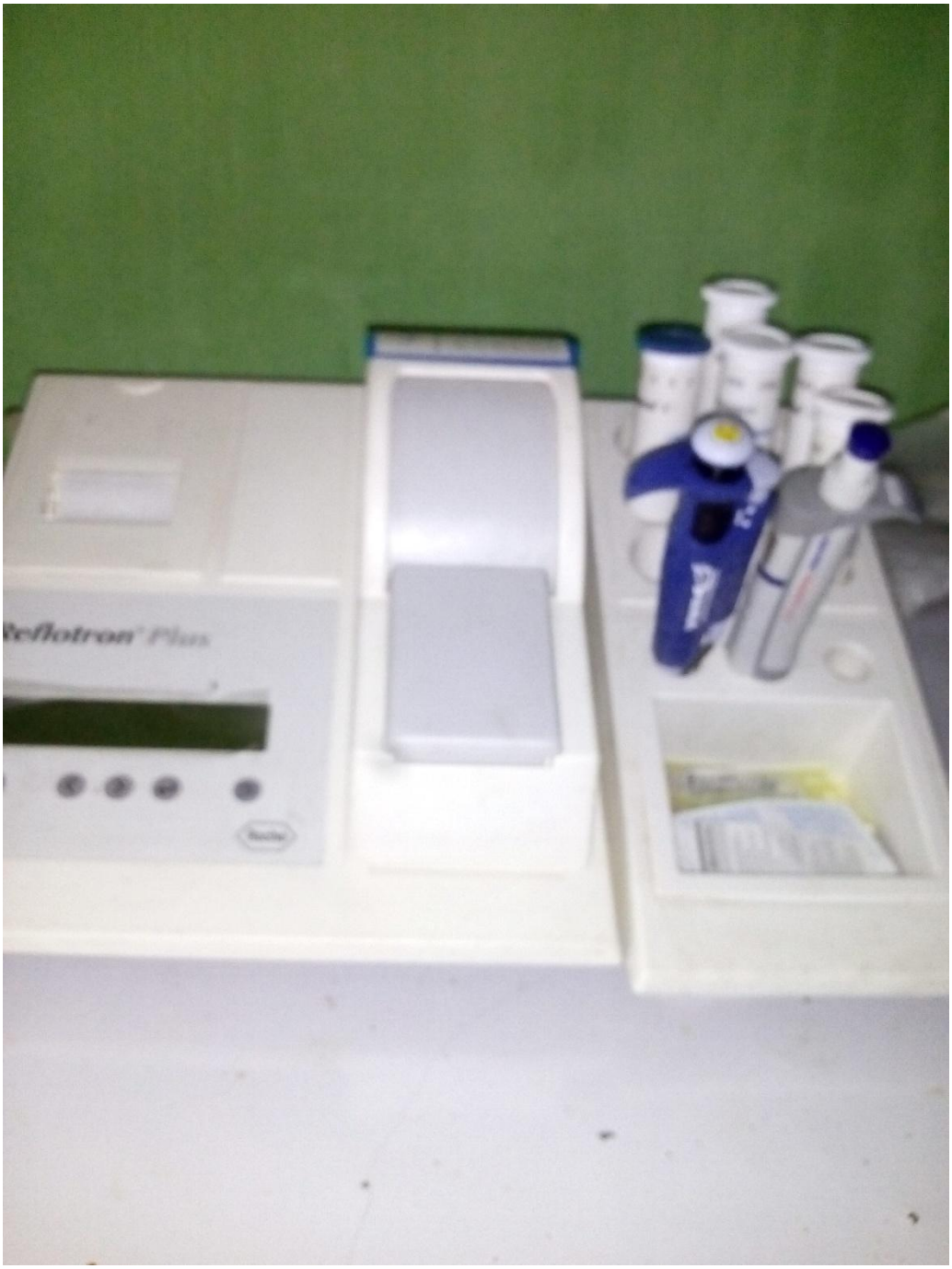

PLATE 2: REFLOTRON PLUS MACHINE