SEROPROFILE, RISK FACTORS OF HEPATITIS B, C, SYPHILIS AND DISEASE STAGE AMONG HIV PATIENTS ATTENDING ENGINEER HOSPITAL, NYANDARUA COUNTY, KENYA

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A thesis submitted in partial fulfillment of the requirements for the award of Degree of Master of Science (Medical Microbiology) in the School of Pure and Applied Sciences of Kenyatta University

NOVEMBER, 2019
DECLARATION

This thesis is my original work and has never been done or presented for the award of degree in any other university or institution of higher learning.

Signature........................................ Date............................ 25/11/2019

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APPROVAL BY SUPERVISORS

We confirm that the work reported in this thesis was carried out by the candidate under our supervision

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DEDICATION

I wish to dedicate this thesis to everyone who supported me during the study period.
ACKNOWLEDGEMENTS

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<th>Full Form</th>
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<tbody>
<tr>
<td>ABC</td>
<td>Abacavir</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>ARV</td>
<td>Anti retroviral</td>
</tr>
<tr>
<td>AZT</td>
<td>Azidothymidine</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control</td>
</tr>
<tr>
<td>CD4</td>
<td>Cluster of differentiation 4</td>
</tr>
<tr>
<td>CTL</td>
<td>Cytotoxic T lymphocytes</td>
</tr>
<tr>
<td>CHB</td>
<td>Chronic Hepatitis B</td>
</tr>
<tr>
<td>CMV</td>
<td>Cytomegalo Virus</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic Cells</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tetra Acetic Acid</td>
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<tr>
<td>EFV</td>
<td>Efavirenz</td>
</tr>
<tr>
<td>FSW</td>
<td>Female Sex Workers</td>
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<td>GBD</td>
<td>Global Burden Disease</td>
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HAART - Highly Acute Antiretroviral Therapy
HBV - Hepatitis B Virus
HCV - Hepatitis C Virus
HBcAg - Hepatitis B Core Antigen
HBeAg - Hepatitis B e Antigen
HCC - Hepatocellular Carcinoma
HIV - Human Immunodeficiency Virus
HLA - Human Leukocyte Antigen
HRPH - Human Immunodeficiency Virus Related Pulmonary Hypertension
IgA - Immunoglobulin Alpha
IgG - Immunoglobulin Gamma
IL - Interleukin
IgM - Immunoglobulin Miu
KAIS - Kenya Aids Indicator Survey
KEMRI - Kenya Medical Research Institute
KDA - Kilo Daltons
LC - Liver Cirrhosis
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>LPV</td>
<td>Lopinavir</td>
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<tr>
<td>LPV/r</td>
<td>Lopinavir/ritonavir</td>
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<tr>
<td>MHC</td>
<td>Major Histocompactibility</td>
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<td>NVP</td>
<td>Nevirapine</td>
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<tr>
<td>NLS</td>
<td>Nuclear Localization Signal</td>
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<td>NS</td>
<td>Non Structural</td>
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<td>NTR</td>
<td>Non Translated Region</td>
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<td>ORF</td>
<td>Open Reading Frames</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<td>PK</td>
<td>Protein Kinase</td>
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<tr>
<td>PWID</td>
<td>People Who Inject Drugs</td>
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<tr>
<td>RdRp</td>
<td>Ribonucleic Acid Dependent Ribonucleic Acid Polymerase</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acids</td>
</tr>
<tr>
<td>STD</td>
<td>Sexually Transmitted Diseases</td>
</tr>
<tr>
<td>STI</td>
<td>Sexually Transmitted Infection</td>
</tr>
<tr>
<td>SVR</td>
<td>Sustained Virological Rate</td>
</tr>
<tr>
<td>TDF</td>
<td>Tenofovir Disoproxil Fumarate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>TMA</td>
<td>Transcription Mediation Amplification</td>
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<tr>
<td>TMD</td>
<td>Transmembrane Domains</td>
</tr>
<tr>
<td>VDRL</td>
<td>Venereal Disease Research Laboratories</td>
</tr>
<tr>
<td>VZV</td>
<td>Varicella Zoster Virus</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Hepatitis, especially B, C and Syphilis co infections are very common among HIV infected individuals and their manifestation is a growing public health concern. These diseases share similar modes of transmission with HIV. However, at earlier stages these infections are myriads that mirrors one another hence they are easily missed. Due to this particular challenge, the infection prevalence of the cases is not fully known in most areas of the country, with rural settings of Nyandarua being one of them. This study was therefore aimed at determining the Seroprevalence, risk factors of hepatitis B, C, Syphilis and disease stage of HIV infected individuals attending HIV care clinic. A hospital based cross sectional study was conducted and socio-demographic data collected using a well-structured questionnaire during the period of between July and September 2018. A total of 385 patients consisting of 263 (68.3%) female and 122 (31.7%) male were consecutively sampled during patients visit. The blood samples were screened for antibodies against HBV (HBsAg), HCV (anti HCV) and Syphilis (anti syphilis) using rapid test strips (CTK Biotech USA test strips) and confirmed by ELISA (cobas analyser). Their CD4 counts were also estimated using CyFlow® Counter technique (Sysmex Partec analyzer machine). From the analysis, the overall prevalence of co infections was HBV (3.9%), HCV (0.78%) and Syphilis (3.1%) among HIV patients attending comprehensive care clinic. The farmers, primary education level, age of 45-54 years, marital status and CD4 levels < 200 count/ul had high exposures of these co infections. However gender ($\chi^2 = 8.822, p = 0.008$) was found to significantly be associated with co infections of HBV. Equally, CD4 levels had a significant relationship with age ($\chi^2 = 35.096, p = 0.028$) and treatment ($\chi^2 = 34.778, p = 0.010$). The overall mean of patients CD4 levels were 227 count/ul and this indicates the advanced stage of immunosuppression. Most of the patients had low detectable levels of HIV viral load (99%) with 1% high detectable level. However, HIV viral load had no significant relationship with CD4 levels ($\chi^2 = 0.878, p = 0.831$). The rates of HBV and HCV infections were found to be lower than the national prevalence while Syphilis was found to be higher. The age, occupation, education level, marital status and CD4 T cells levels influenced the rates of HBV, HCV and Syphilis exposures although they were not significant at $p \leq 0.05$. As the HIV/HBV/HCV/Syphilis co infections are clinically consequential in people living with human immunodeficiency virus/Acquired immunodeficiency syndrome, there is need for constant screening of the population for these particular infections since they are common.
CHAPTER ONE

INTRODUCTION

1.1 Background

1.1.1 Human immunodeficiency virus

Human Immunodeficiency Virus is of genus lentivirus within the family of retroviridae. It is grouped into two types namely; HIV-type 1 and HIV-type 2. Globally the most common type is HIV-1 while HIV-2 is common in certain regions such as Western and Central Africa. The infections caused by HIV are linked with a long period of clinical latency, viral replication and invades the central nervous system. Their genome has two similar copies of single-stranded RNA molecules and has the same structural genes, gag, pol and env (Luciw, 1996). HIV-1 globally has infected an approximate population of 38.6 million people. The infection spreads rapidly with the year 2005 having an estimated figure of 4.1 million new infections and 2.8 million AIDS related deaths (UNAIDS, 2006).

The major mode of transmission is heterosexual and is estimated to facilitate 85% of HIV-1 infection. The highest rates of infections are common in Southern Africa (Hayes and Weiss 2006). The infection rates in India and China are generally low. In Europe and Asia, the commonest mode of transmission is drug injections with an estimated population of 8.8 million people infected (UNAIDS, 2006). Women are the most infected especially in sub-Saharan Africa and this greatly leads to higher mother to child transmissions. The major contributing factor to its rapid spread involves change of
partner, acquisition of sexually transmitted diseases, economic disturbances and drug or alcohol influence (Aral et al., 2005). The East Africa member states such as Tanzania, Kenya and Rwanda, have shown tremendous decline in these infections. Others include Burkina Faso, Zambia, Ghana, and Zimbabwe due to sexual behavior change (UNAIDS, 2006). Viral load levels are usually above 1000 copies/µl of blood at very established (advanced) stages of HIV-1. STDs increases transmissions as they are associated with ulceration making it easier of virus penetration (Wawer et al., 2005). HIV-2 infection is common in West African nations like Guinea-Bissau, Gambia, Senegal, Cape Verde, Cote d'Ivoire, Mali, Sierra Leone and Nigeria. The approximate infected population is 2 million people (Gottlieb et al., 2008). However, co infections of HIV -1 and HIV-2 are rated at 0.3% –1% (Landman et al., 2009). Other nation where HIV -2 has been reported includes Portugal (4.5%) and France 1.8% (Barin et al., 2007).

1.1.2 Implications of HIV

Oral candidiasis could occur as an opportunistic infection among HIV patients due to lowered immunity and could serve as an indicator of increased viral burden in the body. The introduction of Highly Acute Antiretroviral Therapy boosts the immune systems and limits pronounced oral manifestation of the infection (Tappuni and Fleming, 2001). Consequently, the condition may be facilitated by fungal, bacterial, viral, neoplastic and miscellaneous lesions. Oral candidiasis presents as pseudomembranous candidiasis (thrush) which invades the palate, buccal, labial and dorsum of the tongue. Hyperplastic candidiasis (leukoplakia) involves mainly buccal mucosa. Ulcerative lesions could be
drug allergic enhanced or viral infection activated such as herpes simplex, varicella zoster and Cytomegalo Virus (Patton, 2000).

Retinitis facilitated by CMV is actualized when the T cells (CD4+) count are decreased at levels below 100µ/l of blood. The improvement of immunity as a result of HAART may result to intraocular inflammation and is characterized with vitritis and retinal edema. Defectives in the blood-ocular barrier could fail to hinder antigens of CMV leakage from eye, giving it accessibility to the lymphoid organs (Whitcup, 2000). The most common cutaneous presentations are lesions, associated with the initial infections and can present as fungal disease. The serverity increases with the disease progression and reduced immunity (Jensen et al., 2000).

Herpes virus is more pronounced in areas such as perianal, genital, orofacial and digital. It may create resistance to (first) 1st-line therapy in HIV-infected individuals. The lesions of herpes simplex appear as painful erythematous papules, later as vesiculate and ulcerations forming pustules. Varuzell Zoster virus typically appears when the CD4+ T-cell count reduces to less than 200 cell/µl of blood (Zampogna and Flowers, 2000).

HIV-related pulmonary hypertension (HRPH) conditions do occur in HIV infected patients in an approximate ratio of 1:200. Plexiform arteriopathy is often seen in most cases with perivascular inflammation exhibiting in plexiform pulmonary hypertension. The HRPH is not solely associated with the extent of immunosuppression and the T cell count but also genetical manifestations. Small population of HIV patients has pulmonary
hypertension. The pulmonary hypertension reduces survival rates but administration of HAART improves it (Mehta et al., 2000).

Cardiac manifestations involve dilated cardiomyopathy usually seen in late stage of the infection with low levels of CD4+ T-cell. The cause is associated with enhanced TNF-α production, increased production of nitric oxide followed by negative ionotropic effect on cardiac contractility. The Aortic root dilatation has also been linked with left side ventricle dilatation, high load (viral) and reduced T-cell levels. Polypoid masses are also seen and could later develop into pericardial effusion, leading to heart failure. However, the introduction of HAART have shown great improvement (Pugliese et al., 2000), although associated with cardiotoxicity (Fantoni et al., 2001).

The neurological presentaions includes subacute encephalitis (AIDS encephalopathy or AIDS dementia complex), aseptic meningitis, peripheral neuropathy and vacuolar myelopathy. Neuropathy sometimes complicates ART especially on patients taking stavudine/zalcitabine and didanosine. HIV infection facilitates peripheral neuropathy, which presents inform of chronic, distal, symmetric and sensory (WHO, 2005).

The genitourinary manifestations have been associated with sclerosing glomerulopathy in patients infected with HIV. Other conditions such as nephropathy, which enhances end-stage renal disease, are common in patients with low CD4+ T-cell count. Clinical presentation could be nephrotic-range proteinuria or subnephrotic proteinuria. Renal ultrasononographic could reveal enlarged kidneys, glomerulonephropathies including
mesangioproliferative, diffuse proliferative, membranous nephropathy, lupus-like nephropathy and IgA nephropathy (D’Agati and Appel 1998).

Rheumatological cases of polymyositis might show a subacute, progressive and proximal muscle weakness with high levels of creatinine. The organisms that have been associated with the infectious pyomyositis include Staphylococcus aureus, Salmonella enteriditis, Microsporidia species and Toxoplasma gondii. Zidovudine drugs have been associated with myopathy especially within one year of medication. The condition is accompanied by muscle complications such as tenderness and weakness (Dalakas et al., 1990).

The gastrointestinal presentations involve abdominal pain which is actualized by presence of opportunistic infections such as cytomegalo virus and cryptosporidium species. This is linked with acalculous cholecystitis. The clinical manifestations are fever, right upper quadrant pain, loose stool, reduced weight and T-cell count of \( \leq 200/\text{mm}^3 \) (WHO, 2005).

Anemic conditions are noted with reduced hematocrit level. Most often the red cells are normochromic, normocytic and anisocytosis do occur. The erythropoietin response is usually low with increased levels of TNF-\( \alpha \) and IL-1\( \beta \). These are likely to interfere with the development of red blood cells (erythropoiesis). Conditions such as anaemia and Human parvovirus B19 are likely to occur (Krantz, 1994). Likewise, low counts of reticulocytes, platelets, neutrophils and hypergammaglobulinemia do occur (Peltier et al., 1991).
Other implications includes the development of brain cancer, Kaposi sarcoma, CNS lymphoma, aid related lymphoma, Hodgkins disease, multiple myeloma, seminoma and angiosarcoma. These are characterized with low levels of CD4 cells (Jones et al., 2000).

Globally, about 350 million people are chronically infected with hepatitis B virus. This predisposes infected people to conditions such as liver cirrhosis, liver failure and hepatocellular carcinoma. HBV infection attributes to approximately 45% of HCC cases and 30% of liver cirrhosis cases (Vos et al., 2010). The prevalence of HBV infection varies greatly among different regions of the world (Liang et al., 2009). In Africa, most countries have an intermediate prevalence of 2-7% or high above 8% prevalence of chronic HBV infection (WHO 2016).

Annually, more than half a million infected individuals die worldwide from HBV and its effects. Early diagnosis of HBV reduces instances of complications and leads to provision of better treatment. However, most of the infected patients are asymptomatic and this makes the early diagnosis difficult. Subsequently, the diagnosis, provision of drugs and the follow up of such infection may increase the economic burden especially in countries with limited resources. The infection can be controlled by administration of vaccines and educational programs (Zaheer et al., 2014).

HCV infection is common in most countries in the world (Gower et al., 2014). The estimated population affected by chronic HCV infection is 80 million people, and this corresponds to a global prevalence of 1.1%. Annually, approximately 700,000 persons die of chronic HCV infection without receiving treatment. Improvement on the source of
infection needs to be availed in general population to reduce the risks of HCV infections (Cui and Jia, 2013).

HBV and HCV have common modes of transmission such as infective blood, semen, perinatal, blood transfusion and blood products, injections during medical procedures, drug use and sexual transmission (Liu and Hou 2006).

Sexually transmitted diseases including syphilis accounts for approximately 340 million new infections every year (WHO 2001). This infection when acquired in early stages of life especially on adolescence stage, can cause danger on sexual reproductive health of women and their babies later in life. Syphilis is usually asymptomatic and may go unnoticed, untreated and this can cause morbidity. Equally, can contribute to complications such as in pregnancy, cancer, infertility and enhanced HIV transmission (Rompalo et al., 2001). These conditions are preventable if STI testing and treatment is implemented. Moreover, bacterial vaginosis common in the reproductive tract is a risk factor for HIV (Low et al., 2011).

1.2 Study variables

The study variables of age have been identified as a risk factor of the HIV/HCV co infections. The males are affected more than the females among the HIV population. The ages above 20 years has been confirmed to be a risk factor of HBV/HCV in relation to those below age and the very elderly (Manjula et al., 2017). Equally, the study variable of marital status has been found to have a correlation with HCV and not with HBV. Subsequently, the singles had been found in various studies to be at risk of HBV, HCV
than the divorcees, married and widowers (Gyar et al., 2014). The education as a variable had not been found as a risk factor of either HBV or HCV (Manjula et al., 2017).

Syphilis/HIV co infection has been found to be a risk factor in ages above 25 years than in lower ages of below 15 years. Education has been highlighted as the cause of higher prevalence among the non educated women. Overall, the prevalence of syphilis on adults is at 3.4 million new cases per year in Africa (WHO 2012).

1.3 Statement of the Problem

Worldwide, it is estimated that, two billion people are infected with HBV infection with those at chronic stage rated at 150 million. Sub Saharan Africa, prevalence ranges from 9 – 12% (Lavanchy, 2004). HIV/HBV co infections in Kenya are rated at 6% (Muriuki et al., 2013). The WHO recommends that persons who are confirmed to be co infected with HBV and HIV should immediately be initiated on anti-retroviral therapy regardless of their CD4 count. Hepatitis B virus compared to HIV is considered to be more infectious (50-100 times) making its transmissions easier (De Cock et al., 2014).

WHO estimates that 170 million people are infected with HCV globally. Subsequently, various transmissions do occur each year making it a public health problem in the world. The Sub-Saharan Africa burden of the disease is rated high in the world with a prevalence of 5.3% and infected population is estimated at 32 million people (Madhava et al., 2002). HCV is up to 4 times more infectious than HIV and also requires less exposure than HIV to cause an infection. The general Africa prevalence ranges from 0.1 %-17.5%. Kenya’s HCV/HIV prevalence is rated at 0.7% (Kerubo et al., 2015).
Syphilis symptoms go frequently undetected due to length period before it shows after infection. Co infection with HIV can be very dangerous among women and children. Worldwide estimated annual new cases transmission is 12 million. Approximate co infection of Syphilis/HIV prevalence is 10%. Antenatal mothers, co infected with HIV/Syphilis has double chance of infecting their babies with HIV compared to those living with mono infections. Syphilis prevalence in Kenya is high among men having sex with other men 6.4%. However, the national prevalence is rated at 1.8% (KAIS, 2007). The prevalence rate of HIV in Nyandarua County is 3.8% according to the National AIDS and STI Control Programme (2008) with majority of people living in rural areas and a substantial population being aware of HIV transmission according to Kenya HIV County profile 2016. Subsequently, no co infection studies have been done in this particular area and yet there exists a substantial number of HIV infected individuals attending comprehensive HIV care clinics.

1.4 Justification

In Engineer county hospital, a functional comprehensive care for HIV patients does exist with an approximate client population of 700. Nevertheless, screening of HBV, HCV and Syphilis have not been made compulsory especially on HIV population. Likewise, no documented research had been done in these particular area. These prompts need to carry out the research in order to give data support. However, similar research has been carried out in other regions of Kenya and has shown that HBV, HCV (Muriuki et al., 2013) and Syphilis (Kenya Aids Indicator Survey), 2007 among HIV patients are common. Equally, these studies have not been extensively done to give a clear information of their
prevalence especially in rural settings like Nyandarua. These infections share common transmission routes with HIV, however they often undergoes latency and in some cases cleared immunologically with those progressing to disease risking progression to cancer. WHO recommends that persons (HIV positive) co infected with either HBV, HCV or both should immediately be initiated on ART irrespective of their CD4 count.

Studies have shown that HIV infections adversely affect on the progression of HBV and HCV to chronic liver disease due to drug related hepatotoxicity and hepatitis reactivation. Most patients are prone to die compared to those who are not infected with HIV. This demonstrates a correlation between these infections. However, HIV confirmed patients should be screened for HBV, HCV, Syphilis and their disease stage assessed before initiation of highly active antiretroviral therapy. This practice would guide in giving correct choice of drugs for the HIV patients.

1.5 Hypotheses

(i) Hepatitis B, C and Syphilis are present amongst HIV infected individuals attending Engineer hospital.

(ii) There are risk factors associated with Hepatitis B, C and Syphilis co infections amongst HIV infected individuals.

(iii) The disease stage helps in assessing immunosuppression levels of HIV patients.
1.6 General objective

To determine the Seroprofile, risk factors of HBV, HCV, Syphilis and disease stage of HIV infected patients attending Engineer County hospital comprehensive care clinic, Nyandarua County, Kenya.

1.6.1 Specific objectives

(i) To determine the seroprofile of Hepatitis B, C and Syphilis co infections amongst HIV patients attending Engineer County hospital comprehensive care clinics.

(ii) To determine the risk factors associated with Hepatitis B, C and Syphilis co infections among HIV infected patients attending Engineer County hospital comprehensive care clinics.

(iii) To determine the disease stage of HIV infected individuals attending Engineer County hospital.

1.7 Significance of the study

The study findings are useful in policy making and budgetary allocation to procure necessary commodities for testing and treatment of HIV infected patients.
CHAPTER TWO
LITERATURE REVIEW

2.1 HIV/HBV/HCV/Syphilis co infections

The co infections of HIV with HBV and HCV occur at the rate of 7-15% in Sub Saharan Africa (Nyirenda et al., 2008). The co infections prevalence of HIV/ HBV has occasionally been done in various regions of Kenya. A parently varied result has been obtained depending with geographical region. A prevalence of 4.75% (Mabeya et al., 2016) was found in Nairobi especially among the HIV infected individuals. Other studies which have been carried out in the country includes, Muriuki et al., 2013 (6%), Chepkurui et al., 2014 (5%). These studies have also been done in other countries such as Uganda 0.6% (Enzouki et al., 2014) and Rwanda 2.4% (Pirillo et al., 2007).

HIV/HCV co infection was earlier determined at the rate of 1.8% (Mwangi, 1999). However among the subgroup of HIV people it has been rated at 0.76% (Kerubo et al., 2015) and 1% (Harania et al., 2008). In Addis Ababa Ethiopia, HCV prevalence levels of 0.9% has been reported on general population with co infection of HCV/HIV at 4.5% and 0.8% HCV positive among HIV negative individuals (Ayele et al., 2002). Nevertheless, among the antenatal care attenders and sex workers, co infections of HIV/HCV were found to be 2.9% and 5.3% respectively (Ayele et al., 2002). Subsequently, other countries such as Gambia (Mboto et al., 2010) and Zambia (Kapembwa et al., 2011) the rates have been defined at 0.6% and 2.2%. In Asia, China co-infection prevalence of HCV among HIV infected patients is 12% and its co infection rates varies depending on
the mode of primary transmission (Wang et al., 2006). The trio co infections of HIV/HBV/HCV are rare (Shimelis et al., 2008).

HIV/Syphilis co infection have also been noted especially in MSM, their prevalence is 6.4% and the overall co infection is rated at 1.8% in Kenya (KAIS, 2007). The overall burden of syphilis have been reported by WHO to be 12 million cases. Most of these cases are concentrated in Africa and South Asia with 3.5 and 5.8 million cases respectively. Apparently, 4-15% cases have been identified in Africa prenatal clinics and above 10% in young women and men having sex with other men (Tabet et al., 2002). Limited data exists with regards to co infections of Syphilis. Sudan rates Syphilis among FSW at 1.5-8.9% (Elhadi et al., 2012). In United States co infections prevalence of HIV/Syphilis varies with the target group of study. Research have shown military recruits (8.3%), STD-clinics (23%), AIDS patients at (1.5%) and neurosyphilis (lumbar punctures) (7-16%) (Rolfs et al., 1997). Overall, Syphilis/HIV co infection in Europe is rated at 2%-43%, North America (1%-21%) (Kalichman et al., 2011) and Brazil (2.7%) (Adolf et al., 2012). There is no documented evidence of HIV/HBV/HCV/Syphilis co infection occurring together in one patient.

2.2 Hepatitis B virus

The Hepadnaviridae are small, enveloped, hepatotropic, double-stranded virus with relaxed circular (rc) DNA genome (Seeger and Mason, 2000; Ganem and Prince, 2004). They are associated with acute, persistent conditions, viremia, hepatotropism and antigenemia. According to international committee taxonomy of viruses 2012, many
species and genotypes of orthohepadna viruses infects humans, gorillas and chimpanzees. Likewise, HBV have been found to infect even the New World ‘woolly monkey’ species (Lanford et al., 1998). The additional hosts are Sciuridae family which includes woodchuck, ground squirrel, and arctic squirrel. Other hepadnaviruses have been isolated from bats in many parts of the world including Asia, Africa and have similarity with HBV genotype F (He et al., 2013). The HBV virus is spherical in shape and its outer diameter approximates to be 52 nm (Seitz et al., 2007). The icosahedral nucleocapsid core is enclosed with by surface protein and lipids, which binds 240 subunits core protein that measures averagely 36 nm in diameter. The viral DNA and protein enzymes are contained in nucleocapsid. Consequently, the surface proteins (HBsAg) have non viral DNA and they cannot cause any infection. However, they are secreted as subviral particles which are spherical or can be in filamentous shape. The second major viral antigen, core antigen protein, is secreted as ‘e-antigen’ which is infectious and is produced in large quantities (Wittkop et al., 2010).

2.2.1 Hepatitis B Virus genetic diversity

HBV belongs to the Hepadnaviridae family. They are enveloped virus with a double-strand DNA genome (Lavanchy, 2004). HBV was initially classified into ten different serological subtypes depending on the antigenic determinants. However, they were compared with the full nucleotide sequence of 18 HBV strains resulting into four groups, genotype A to D, which varies by more than 8% among the genotypes. Since then, at least 10 genotypes (A to J) have been identified with a divergence of 4%. The genotypes are further classified into sub-genotypes (Okamoto et al., 1988). This approach has
resulted in HBV genotype A (A1-A7), genotype B (B1-B9), genotype C (C1-16), genotype D (D1-D8) and genotype F (F1-F4). HBV genotypes and sub-genotypes have distinct geographical distributions. HBV sub genotype B1 dominates in Japan, B2 dominates in China and Vietnam, B3 is confined to Indonesia and B4 is confined to Vietnam (Norder et al., 2004). Sub genotype B7, B8 and B9 have been found in an island in Southeast Asia (Thedja et al., 2011). HBV/C1 (Cs) is found mainly in Southeast Asia, whereas C2 (Ce) is predominant in East Asia (HUY et al., 2004). HBV/C3 was confined to Oceania, while C4 (Caus) was exclusively found in Australia and regarded as the most divergent sub-genotype within HBV/C (Davies et al., 2013). Sub-genotypes C5 and C7 were found in Philippines, while C6 and C8 to C16 were isolated from Indonesia. This pattern of defined geographical distribution was less evident for D1-D4, where the sub-genotypes were widely spread in Europe, Africa, and Asia (Norder et al., 2004).

Immigration has become an important confounding factor of global HBV distribution and has been substantially changing the geographic pattern of HBV sub-genotypes (Pourkarim et al., 2014). Inter genotyped recombination is favored in particular geographical regions. B/C recombinants are more prevalent in South and East Asia. Other inter genotype recombinants including A/D, A/E, C/D and G/C have variedly been identified globally (Shi et al., 2012). HBV genotypes and sub-genotypes related are also associated with the pathogenesis and outcome of HBV infection. Studies have revealed genotype A infections are associated with a significantly higher rate of sustained
biochemical remission, HBV DNA and HBsAg clearance in patients with chronic HBV infection compared with genotype D infections (Sanchez et al., 2002).

2.2.2 Transmission

Hepatitis B infection is mostly transmitted through sex especially in low endemic regions such as North America. Men who does sex with other men are at increased risk of acquiring the infection (Alter, 2003). Quite a number of heterosexual accounts for transmission of HBV including factors such as number of sexual partners, period of sexual act, history of STD and presence of Syphilis. Others at higher risk of infection include the intravenous drug users, prostitutes and their clients (Alter and Mast, 1994).

Parenteral/percutaneous transmission includes acts such as injection drug use, blood transfusions and renal dialysis, acupuncture, contacts with infected patients, tattooing and household infected persons. In developed countries such as U.S and Western Europe, people injecting themselves are at increased risk of getting HBV (Margolis et al., 1991).

The exposure period determine the chances of acquiring the infection. The asymptomatic carriers are still a possible source of infection especially in cases of blood transfusion. Also while screening blood donors especially those who engage in risky activities (Luo et al., 1993).

The blood products, surgical equipments, utensils and contaminated blood are potential source of infection. Likewise, procedures such as surgery, after needle-prick injury, injection of intravenous drug, ear piercing, tattooing, acupuncture, circumcision, dialysis and dental procedures. Others at risk are healthcare workers, police and laundry workers
(Margolis et al., 1991). Sometimes, infants may acquire HBV from infected mothers perinatally even if they have been given immunoprophylaxis (Xu et al., 2002). Other factors contributing to immunoprophylaxis failure are uterotransmission; perinatal transmission and surface gene escape mutants (Zhu et al., 2003).

These propylaxis is suitable for patient having liver transplant and when higher doses are administered recurrence is reduced. Currently, it’s combined with nucleoside analog in conditions of liver transplantation which have greatly decreased the viral load (Terrault and Vyas, 2003). Vaccination can be used to prevent primary infection thus reducing chances of acquiring the infection after exposure. HBV vaccine which is an inactive plasma-derived vaccine was introduced in 1982 and the DNA recombinant vaccine was made available in 1986. WHO approved these vaccines as safe for use and later in 1991 recommended their use especially in countries with carrier prevalence of more than 8%. This was taken positively and in 2002, many countries had introduced routine infant immunization with hepatitis B vaccine (Lavanchy, 2004). The vaccination program has reduced incidences of acute hepatitis in young age groups especially in Italy and United States (Da Villa, 2000). After vaccination, protection is sustained despite presence of undetectable levels of anti-HBs levels. However, lack of finances has hindered progress and success of immunization against hepatitis B thus more support is required (Ayerbe and Pe´rez, 2000). Changes in behavior such as wearing condoms, screening of blood and wearing of protective equipments while handling infected patients can reduce transmissions.
2.2.3 Pathogenesis

Hepatocellular carcinoma (HCC) associated with HBV is among the leading cancers that causes death in the world. However, the genetical components of HBV that facilitates HCC is unclear with suggestions of HBV DNA interlinks with tumour cells (Feitelson, 1999). Innate immunity is important during infancy since adaptive immunity is still not developed (Koch et al., 2001). The mannose-binding lectin which are the genetic variants molecules of HBV, increases disease susceptibility during childhood (Hibberd et al., 1999). Phagocytosis is facilitated by attachment of surface protein in viral envelope of HBV via lectin pathway of the complement which MBL plays a greater role and modulates cytokines (Chong et al., 2005).

In Cellular immunity the presentation of antigen is accelerated by MHC (Safioleas et al., 2007). HBV pathogenesis is actually supported by the functions of human leucocyte antigen class I restricted T- cytotoxic cells and class II restricted T – helper cells (Chang and Lewin, 2007). Properties are varied due to genetical changes of HLA molecules and direct the T-cell respond to specific HBV antigens (Martin, 2005). The HLA-DR13 allele acts upon T-helper cell (CD4+ T cells) and similarly respond to the HBcAg (Singh et al., 2007). Higher cases of HLA-DR*03 and HLA-DRB1*07 alleles occurs among pregnant women, who later transmits the virus to their newborns via intrauterine infection (Xu et al., 2008). The expression of monocytes (Fraser et al., 2006) and genes that encode greater levels of MBL are associated with virus clearance, viral resistance and continuity of disease (Brown et al., 2006).
HBV infection in adults and children has been associated with polymorphism of cytokine and their receptors. Early infection is revealed by production of interferon type 1 with antiviral and immunomodulatory. The HBV persistence has been associated with class II cytokine receptor. This harbours type 1 interferon receptors and other cytokine receptors (Frodsham et al., 2006). The adaptive immunity is activated by viral replication, IFN-γ and tumor necrosis factor (TNF)-α production (Chang and Lewin, 2007). The TNF-α −238A allele facilitates intrauterine infection. IL-10 −1082G allele have a protective responsibility hence clear indication of different regulation of TNF-α gene in newborns and adults (Zhu et al., 2005). Chemokines facilitates T cells chemo attraction recruited in the liver. The CC chemokine receptor 5 also seems to be important in other perinatally acquired infections for example HIV infection. Genetical polymorphism of the CCR5 gene, may induce protection against HIV (Pedersen et al., 2007).

The monocytes and TH2 cells are activated by active form of vitamin D, inhibiting TH1 cells, and regulating production of interferons, cytokines, and chemokines. HBV infections are affected by TaqI and FokI vitamin D receptor genes. In chronic HBV T-C (TaqI-FokI) haplotype is common. Cyclin D2s (CCND2s) have recently been shown to act as distinct immunomodulatory factors, implicated in cell cycle regulation and altering the proliferation of T and B lymphocytes (Liu et al., 2006).

The adaptive immunity involves the utilization of Kupffer cells especially the DCs, presentation, maturation of HBV-specific T-cells and ensures HBV clearance. Antigens
which are foreign are presented to CD4+ and CD8+ T-cells by APC as they produce cytokines, IL-12 and TNF-α. Moreover there is production of IFN-γ while CD8+ T-cells are proliferated. The differentiation of CD4+ T-cell to T-helper cell type 1 (Th1) subset is made possible by IL-12 (Kimura et al., 2002).

The HBV-specific Th1 CD4+ T-cells when activated is multispecific. Some responses which are strong are experienced against peptides c50 to c 69, present in HBcAg and HBeAg. This could be seen once acute HBV infections are resolved without considering HLA type regardless of the infected persons (Ferrari et al., 1991). During the time of elevated HBV DNA, the HBV specific CD4 are detected in presence of acute infection and stays longer after recovery. There are other HBV gene products which can be detected against CD4+ T-cells, including the envelope and polymerase (Mizukoshi et al., 2004). However, overall CD4+ T-cell responses determined by cytokine production or proliferation are usually sensed in core than in other proteins (Penna et al., 1996).

HBV clearance involves (CD8+) T-cells which are mature. HBV-specific (CD8+) T-cells, NK, and HBV-specific Th1 (CD4+) T-cells facilitates production of IFN-γ (Kakimi et al., 2000). HBV can be cleared by making viral capsid unstable, via NF-κB pathway, degradation of proteins by proteosome and through post-transcription (Biermer et al., 2003). Equally, in transgenic mice, cytokines role is associated with the application of anti-IFN-γ and anti-TNF-α antibodies (Guidotti et al., 2002). The virus are cleared without much destruction of liver cells due to the action of cytokines and cytolytic activity (Murray et al., 2005). Every epitope has its specific receptor as response is
polyclonal and multispecific to protein associated with HBV due to acute infections. These factors enhance identification of the targeted epitope and limits viral ‘escape’ through genetic mutation. The Th1 CD4+ T-cells and IL-12, sustains HBV-specific CD8+ T-cells. These HBV-specific CD8+ T-cell responses have predominantly been assessed in individuals positive with HLA-A2. Their cells respond to core (c18–27), envelope (s183–191, s250–258, and s335–343) and enzyme polymerase epitopes with increased frequency (Maini et al., 2000). The cells are compartmentalized in the liver. Detection of Activated HLA-A2-restricted CD8+ HBV-specific T-cells is revealed in acute hepatitis and is high following recovery (Sprengers et al., 2006).

The humoral response plays an important role in clearance and protection of HBV. Type 2 T-helper cells CD4+ T-cells enhance B-cell production of HBsAb, HBcAb and HBeAb. Detection of HBsAb in an early infection is not possible. This is complicated by envelope antigens when viral replication takes place (Chisari and Ferrari, 1995). HBsAb is also vital in protection against reoccurrence of HBV infections and forms background of protection upon vaccinated persons. The pathogenetic role of antibody to non-envelope protein remains controversial. Generally the virus neutralization is not done by anti-HBcAb, but with the application of anti-HBc/anti-HBe antibodies passively indicates a likely possibility without its defined role (Pignatelli et al., 1987).

The “X protein” also known as HBx, is a product of 154 amino acid and have consistently been associated with oncogenesis. Great interest is in the function of X on development of cancer. More over X transgenic mice have been used in the study with no clear
evidence how X proteins causes cancer (Zhou et al., 1998). Other importance of “X” protein is trans-activation of several cellular gene activities such as viral genes, c-jun, c-fos, c-myc and MHC I (Feitelson, 1999). Equally, it works with other several developed signaling proteins that include PI3K p85 subunit, a product of PI3K- and alerts pathway, p53, proteins involved in DNA damage, repair and degradation (Lee et al., 2001). Other viral proteins roles involve transformation and diseases induced by HBV. Loading of large envelope protein (LHBs) within the ER of liver cells might cause transformation and fulminant hepatitis in human (Chisari et al., 1989). HBV DNA intergration hepatoma cell of human li ne Huh4 encodes a carboxyl reported as MHBs protein, referred as MHBst (Hildt et al., 1995). While, the M protein induces proliferation of hepatocytes (Dragani et al., 1990).

**HBV Life Cycle**

HBV is actually a retrovirus and have been associated with the cause of HCC. The life cycle depends on reverse transcription reffered as pre-genomic (pg) RNA. Usually, in the attachment and entry the interactions between viral envelope proteins and specific receptor cells of liver plasma membrane occurs. The N-terminal point of large envelope protein (pre-S1) play role in inducing receptor binding and igniting an infection (Tai et al., 2002). Pre-S1 region has a peptide that inhibits infection of HepaRG cells. This creates a chance for drug application (Urban and Gripon 2002). Virus enters the cell by endocytosis process and binds to specific receptor cells (Schmitt et al., 1999). Viral capsid is released from endosome into the cytoplasm facilitated by low protein and enveloped protein attached to proteolytic enzymes (Lu et al., 1996).
Translocation of the viral rcDNA to the nucleus, the relaxed circular DNA (rcDNA) is passed on by capsid into nuclear through the pore (Kann et al., 2007). The interaction of cellular microtubules facilitates movement of intra-cytosolic. Movement through nuclear pore is actualized by close interactions between nuclear localization signals (NLS) on the C termini of the viral capsid. Likewise, by nuclear import receptors importin α and β (Kann et al., 1999). The cycle of nuclear localization is dependent on genome maturation which causes change within the structure of the capsid. Subsequently, in the nuclear of NPC there is arrangements of capsid and release of viral DNA into nucleus (Rabe et al., 2003).

The (cccDNA) a covalently closed circular type of the viral chromosome is suitable for the sustainance of the infection. Viral and host activities are necessary in producing cccDNA from the infectious viral DNA material. These transcript products of HBV, with the proteins they specify (for mRNA), encapsidation and replications (for pregenomes). The viral rcDNAs, which are incomplete circles, when they reach in the nucleus, they are transformed into a covalently closed circular (cccDNA). Hepadnavirus rcDNA has several unique structural features. The two strands of viral DNA are asymmetric in length. Consequently, the minus strand DNA (the strand that is complementary to the RNA) is the length of one complete genome (unit length). The plus strand DNA is variable in length and usually constitutes approximately 50% of a full length strand (Summers et al., 1975). These shows the strands should be completed early for effective infection to continue. The viral DNA polymerase protein is covalently bound to the 5’ end of minus strand DNA and a capped RNA oligomer obtained from 5’ end of
approximately 18 nt in length is linked to the 5’ end of plus strand DNA (Lien et al., 1986).

The viral rcDNA can be converted into cccDNA and requires the plus strand DNA synthesis to be completed. The process of terminal elimination of modifications and DNA strands covalent ligation in both ends and the way these reactions occurs have never been known. Cellular DNA repair enzymes could facilitate process of removing RNA primer from 5’ end of plus strand DNA and binds both DNA strands. Reaction leading to deproteinization is activated by nucleolytic attachment of viral DNA near to the 5’ end by an enzyme endonuclease. Moreover, could be facilitated by breakdown of the phosphodiester bond that is usually between amino acid tyrosine of polymerase and the 5’-phosphoryl group of the minus strand in DNA. Merging of HBV DNA into host cellular chromosomes is not necessary since the cccDNA harbours the nucleus of invaded cells as an episomal minichromosome (Newbold et al., 1995). Nevertheless, there is intergration of HBV genome into cellular nucleus which have been used as genetical markers to observe the infected liver cells (Block et al., 2003).

Apparently, cccDNA can live inside the nucleus of hepatocytes that have been infected. The stable source of progeny viral templates is cccDNA. The viral conversions are a problem in the pathogenesis and therapeutic. However cccDNA could be distributed to progeny cells and suitable way of elimination during cell division. It’s for these reason, stopping treatment there is likelihood of viral rebound from the surviving cccDNA making its elimination a great therapeutic challege (Zhang et al., 2003).
The four systems of viral RNA which are transcribed by cccDNA in the nucleus include, 3.5kb pre-C mRNA and pg RNAs, mRNA for large envelope protein, mRNA for middle and major surface proteins and mRNA for the X protein. Pre-C mRNA is converted to produce an end product “pre-C” protein. This is later proteolytically transformed to HBeAg which is secreted by infected cells and its detection reveals higher amount of viral replication. The pre-C gene which is transcribed and obtained from cccDNA produces HBeAg and viremia levels correlates with the amount of HBeAg that is in circulation. Low levels of HBeAg in infected persons with confirmed chronic viral hepatitis is a clear indication of limited viral production. However, genetic changes within the pre C region have contributed to the loss of HBeAg, but these mutations might be more pathogenic than other varied types (Tong et al., 1990). When viral DNA amount are high and there is significant absence of HBeAg these is not favourable and the importance of HBeAg may be for natural infection (Ou, 1997). Moreover, inhibition of DNA synthesis may induce immune response that causes viral suppression (Milich and Liang, 2003).

The pgRNA plays an important role in viral DNA synthesis by reverse transcription, DNA polymerase and act as mRNA which is present in viral capsid protein (Seeger and Mason, 1996). However, in the transfected liver cells which have been invaded, spliced RNA has been identified and their role is not known. Apparently, in the duck, HBV pgRNA act in large enveloped protein as another mRNA suitable in the secretion of viral particle from infected duck hepatocytes (Obert et al., 1996).
The viral replication of DNA takes place inside immature nucleocapsids in protein which are primed through reverse transcription. Pg RNA at its 5’ epsilon stem-loop structure, binds to protein DNA polymerase inducing nucleocapsid assembly and sets viral minus-strand DNA production for three nucleotides. Polymerase and covalent bonded DNA is translocated to the 3’ DR1 and synthesis of minus strand DNA occurs by making copies of pgRNA (Wang and Seeger, 1993). Its degradation is facilitated by RNase H action (Perlman et al., 2005).

Once polymerase arrives at 5’ end point of pgRNA, oligomer of RNA that has DR1 together with 6 - 7 nucleotides at the 5’ end point of DR1, chain remains unbound. The RNA oligomer is however translocated and attached. When it primes with the plus strand DNA, synthesis of relaxed circular DNA occurs (Lien et al., 1986). Nucleocapsids are usually mature, enveloped and released outside the cells on the complete formation of rc DNA (Perlman, 2003). The new virion are enveloped, capsidated and contain rcDNA which are converged in the nucleus. It’s at this area where they are transformed to several molecules of cccDNA in the absence cell re-infection (Wu et al., 1990). In early stage of viral infection, intracellular pathway works efficiently to build a pool of nuclear cccDNA. However, at initial period of infection, due to low levels of L protein, delivery of nuclear capsid is favoured allowing cccDNA accumulation, which will ensure infected cells are controlled (Rabe et al., 2009).

When the Virions are assembled, they bud from endoplasmic reticulum (ER) membrane by engulfing of rc or dsl DNA containing capsids (so-called mature capsids). The ER
membranes are studded with viral envelope proteins. The three envelope proteins that HBV encodes are Large, Medium and Small (HBs) surface antigens. Their synthesis takes place from a similar open reading frame but different starting codons of the viral genome. The LHBs and MHBs proteins contain SHBs plus amino-terminal extensions of pre-S2, pre-S1 and pre-S2 domains (Schmitt et al., 2004).

The monoglycosylated SHBs is the main viral surface protein. Virions contain approximately 100 copies of SHBs for every 5 MHBs and 1 LHBs protein. Usually the infected cells releases thousands polymers of size 22 nm per particle. The SHBs are normally determined as HBsAg and its presence in the circulation beyond six months is a sign of chronic infection. However, unusual it may have been manufactured from viral defective genomes which are integrated in host cellular chromosomes, and in absence of productive viral infection could be released (Patient et al., 2009).

The oligmerization of viral envelopes polpeptides in the ER helps them be less sensitive to cellular proteasomes (Block et al., 2006). Incases of resistance to proteasome degradation, HBV might not be sensitive to immune systems of the host facilitating the development of chronic infection. Attachment of virions to cellular receptor(s) is facilitated by LHBs pre-S1 domain which is necessitated by outside membrane exposure of viral envelope. However, most of the LHBs protein, the pre-S1 domains points to the inner part of viral envelope (Guo and Pugh, 1997). Moreover, pre-S domain plays role as a matrix protein and attachment point of developed viral capsids necessary for assembly
and budding of virions (Bruss and Ganem 1991). The N-terminal myristylation transforms LHBs which is adequate for infectivity (Persing et al., 1987).

2.2.4 Epidemiology of HBV

Chronic hepatitis, liver cirrhosis and hepatocellular carcinoma is caused by hepatitis B virus with at least 2 billion people being infected worldwide. The estimated figure of chronic carriers is 350 million (Lavanchy, 2004). Asia and Western Pacific have a higher population of people who are infected with chronic hepatitis (Lok, 2002). Upto about 1.2 million people are likely to die from HBV infection yearly, 15% develops cirrhosis and liver failure.

2.2.4.1 Endemicity

The prevalence is ranked as high, intermediate and low depending with the region. The high endemic regions have prevalence of more than 8% and differs globally. The countries known of greater infections include South East Asia, China, sub-Saharan Africa and the Amazon Basin. Some infections are known to occur during infancy or childhoods which are asymptomatic and greater fraction of population carries serological evidence of HBV. Adult people are at increased risks of developing chronic liver disease and liver cancer (Alter, 2003). Intermediate endemicity is commonly found in Eastern and Southern Europe, Middle East, Japan, and South America. The chronic infection occurs at 5% and at least 10% of the population have some evidence of infection. Acute diseases are also common among adults and adolescents in these regions with higher infections.
being experienced on children and infants. However, varied way of transmissions does exist among infant, early childhood and adult (Hyams, 1995).

More over in low endemic regions such as North America, Northern and Western Europe and Australia up to 6% of the population is infected with amaximum of 2% developing chronic infection. The infections are known to be accelerated by risk activities such as drug injection, homosexual, failure to observe safety issues in health care settings, blood transfusion and hemodialysis (Lavanchy, 2004).

2.2.5 HBV prevalence

Globally an estimated population of 2 billion people is infected with HBV. This is usually experienced in sub-Saharan Africa and other endemic regions (WHO, 2009). The Sub Saharan Africa prevalence among pregnant women ranges at 9% to 12%. More over low rates are common in countries such as Tanzania 3.9% (Mbaawuaga et al., 2008), Ethiopia 3.7% (Ndams et al., 2008), Sierra Leone 6.2%, Congo and Zambia 6.5% (Awole et al., 2005). Prevalence of HBV co infections among HIV in South Africa 4.8% (DiBisceglie et al., 2010), Nigeria 9.7% (Ejele et al., 2004) and in Kenya is rated at 6% (Muriuki, et al., 2013).

2.3 Hepatitis C Virus

HCV have single-stranded RNA genome which encodes a large polyprotein of 3010 amino acids. Also its small, enveloped and a positive-sense virus. The structural and non-structural proteins are produced after processing the polyptrotein by proteases. The NS protein includes non structural 3 serine-like protease and the RNA-dependent RNA
polymerase (RdRp). These are suitable in viral maturation, replication and participates in the development of small molecule anti-HCV compounds (DeFrancesco et al., 2003).

HCV is formed by an enveloped particle harbouring a plus-strand RNA of 9.6 kb. The genome carries a long open reading frame (ORF) encoding a polyprotein precursor of 3010 amino acids. Translation of the HCV ORF is directed via a 340 nucleotide long 5' non translated region (NTR) functioning as an internal ribosome entry site. It permits the direct binding of ribosomes in close proximity to the start codon of the ORF. The HCV polyprotein is cleaved co- and post-translationally by cellular and viral proteases into ten different products, with the structural proteins (core (C), E1 and E2) located in the N-terminal (Beaulieu and Tsantrizos, 2004).

The HCV core protein constitutes the viral nucleocapsid. It contains 191 amino acids of HCV which are divided into domains based on their hydrophobicity. Domain 1 consists of amino acids 1 to 117. Equally, it has basic residues and two short regions which are hydrophobic while domain 2 consists of amino acids 118 to 174. This is more hydrophobic than basic. Subsequently, domain 3 consists of amino acids 175 to 191. Subsequently, it is more hydrophobic and participates in E1 envelope protein sequence signalling (Bukh et al., 1994). They bind viral RNA in domain 1 in amino acids 1 to 74 (Santolini et al., 1994). However, they are membrane-bound protein which is cytosolic and relates with the endoplasmic reticulum, lipid droplets, mitochondria and the nucleus. These core proteins also plays a major role in hepatocarcinogenesis and steatosis hepatitis (Hope et al., 2002). Further, works together with several proteins (cellular) enhancing
functions such as transcription of gene, metabolism of lipids, apoptosis and signals various pathways (Tellinghuisen and Rice, 2002).

The structural protein E1 and E2 of HCV is necessary in cell entry and are much glycosylated. They have different roles where E1 acts as the fusogenic subunit while E2 is important on receptor binding subunit of the HCV envelope. The 4 to 5 N-linked glycans are contained in E1 with E2 having a total of 11 N-glycosylation sites. However, genotype determines the numbers of glycosylation sites on E1 and E2. The glycosylation sites have combinations of complex, high-mannose side-chains and are much conserved. HCV glycans play an important role in envelope glycoprotein folding. Moreover, it’s involved in the formation of the HCV E1E2 complexes, folding of glycoprotein envelopes, receptor site interactions and antigenic variation (Slater et al., 2004). These envelope proteins recognise cellular membrane receptor proteins and assist in the cell entry. The cellular receptors have been actively recognized by cells releasing infectious HCV pseudotype particles and could be neutralized by anti-E2 monoclonal antibodies (Hsu et al., 2003).

The CD81, scavenger receptor type B class 1 protein and high density lipoprotein binding molecule interacts well with E2 forms which are truncated (Scarselli et al., 2002). Their soluble state interferes with entry of HCV pseudotype particle (pp) into the cells (Hsu et al., 2003). Likewise, CD81 works as a co-receptor since its ectopic expression in negative cells does not permit entry of HCVpp. The low density lipoprotein receptor assists in endocytosis of the virus. Moreover, the anti-LDL monoclonal antibody prevents
viral entry in cells (Agnello et al., 1999). Two hypervariable regions (HVR) are found in E2 which includes hypervariable regions 1 and hypervariable regions 2 and they are aimed for neutralizing antibodies. HVR1 genetic variations may help virus in its action upon immune systems resulting to chronic infection (Boulest et al., 2002).

P7 protein is a 63-amino acid polypeptide situated between HCV E2 and NS2 genes. Commonly found in endoplasmic Reticulum (ER) whose signal peptidases of the host cell facilitates its attachment and is a membrane-spanning protein. The carboxyl-terminal transmembrane domains (TMDs) of P7 enhances signal sequence. Further, it increases translocation of NS2 into the ER lumen for attachment to the host signal peptidases, providing appropriate route of virus to infect (Griffin et al., 2003). These proteins also facilitate virus particle assembly and infectious virions release (Steinmann et al., 2007).

The non structural proteins (NS) 2 is suitable in the accomplishment of the virus replication cycle both in vitro and in vivo (Pietschmann et al., 2006). Equally, it has much of hydrophobic N-terminal residues which forms 3-4 transmembrane helices and protrudes into the ER membrane. Moreover, its C-terminal part is located in the cytoplasm and is appropriate in NS2/3 auto protease activity in conjuction with N-terminal domain of NS3. The attachment occurs on amino acids 827 and 1207 at C-terminus point of NS2. Zinc compound accelerates protease activity and stabilises the NS3 structure at the active site. The chelating agent, (EDTA), inhibit protease activity (Lorenz et al., 2006). The NS3 N, C-terminal has serine protease and NTPase/helicase activity (DeFrancesco et al., 2003). However, the ER membrane, NS4A and NS3 protein
binds together (Wolk et al., 2000). The 185 amino acids contained in HCV NS3 is useful in cleavage between non structural protein 3-4A, 4A-4B, 4B-5A and 5A-5B. Moreover, the rapid chemical reactions of HCV NS3 are facilitated by three amino acid components: His-1083, Asp-1107 and Ser-1165. The His-1083 and Ser-1165 can be replaced with alanine and no effect is noted on protein structure of NS3 (Lorenz et al., 2006). Further, short consensus sequence found in NS3 protein, works together with protein kinase A (PKA) limiting its entrance into the nucleus. The phosphate groups added by PKA alters target protein function. Hence interactions of NS3/PKA contributes to deregulate intracellular signalling (Borowski et al., 1997).

The NS4A is a 54 amino acids protein and works as a cofactor of NS3 protein. This has an N-terminus that is highly hydrophobic and its action aims on NS3 in the endoplasmic reticulum membrane (Wolk et al., 2000). A transmembrane helix is formed which attaches NS3/NS4A complex on the membrane of ER. The interaction between NS4A and NS3 is facilitated by residues found in the core of NS3 and the C-terminus of NS4A allowing protease cleavage. Phosphorylation is also enhanced by NS4A (Asabe et al., 1997).

NS4B being a small hydrophobic protein of 27 kDa protein is suitable in recruitment of other viral proteins. NS4B has 4 transmembrane domains. Cytoplasmic C-terminus and the N-terminus of NS4B has a dual topology which interacts indirectly with NS3 and NS5A (Lundin et al., 2006). This has been found to be an integral membrane protein that targets the ER and localizes non-structural proteins in the ER membrane (Hugle et al.,
2001). However, it has been noted to cause morphological changes in ER forming a membranous web and assist in replication (Egger et al., 2002).

The hydrophilic phosphoprotein NS5A is essential in viral replication, cell modulation, signaling pathways and interferon. Subsequently it does not have transmembrane domains (Macdonald et al., 2004). The N-terminus is amphipathic alpha helix which mediates membrane association of NS5A (Penin et al., 2004). Genetic variations on amphipatic helix disturb membrane association and hinder formation of replicon-harbouring cells (Elazar et al., 2003). Equally, it’s related with lipid droplets or as a section of the polyprotein (Shi et al., 2002). Moreso, it modulates IFN and have sites which give virus the ability to resist treatment with interferon. This region, point interferon-α sensitivity-determining region (ISDR) which can be used to determine IFN-α treatment. Both its sensitivity and resistance of HCV, works closely with an IFN-α stimulated gene product and PKR protein kinase which is catalyzed by attaching to double-stranded RNA stopping of protein synthesis (Moradpour et al., 2005). Other important responsibilities of NS5A are its association with cell signals, thus modulating all MAPK pathways. This activates host cell mitogenic signals, incorporates apoptosis and hinders ROS pathways and phosphatidylinositol 3-kinase resulting to hepatocyte transformation and HCC formation (Macdonald et al., 2004).

NS5B size is 65 kDa and tail anchored protein which facilitates manufacture of new RNA genome and assist in viral interventions. The amino acid motif GDD is important in polymerase activity. A typical 'right hand' is its structural organization with subdomain of
finger, palm, and thumb covering completely encircled active site. The replication occurs through synthesis of a complementary minus-strand RNA (Yamashita et al., 1998).

2.3.1 Hepatitis C Virus Genotypes

There are six main genotypes of HCV namely 1–6 with their multiple subtypes. These genotypes spreads across the world especially genotypes 1–3. The importance of Genotyping is in management of HCV treatment and elimination of the disease (Islam et al., 2015). HCV 4, 5 and 6 are usually found in specific areas around the globe. Subsequently, in Europe a population of 25 million people are infected with HCV (Rockstroh et al., 2015) while in Pakistan 5% (Afridi et al., 2013) and can accelerate liver cancer (Munaf et al., 2014). HCV-4 is common in Middle East, Africa and European countries while genotype 5 (South Africa) and 6 (Hong Kong and Southeast Asia) (Messina et al., 2015). Globally, high prevalence is in Egypt and the disease is the major source of chronic hepatitis, liver cirrhosis, hepatocellular carcinoma and organ transplantation (Rajaguru and Nettleman, 2011). The general overview of HCV genotypes prevalence is that 1a is common in Northern Europe and United State, genotypes 2a, 2b and 2c in America (Simmonds, 2004).

2.3.2 Transmission of HCV

The various modes of HCV transmission include IDU, contact with infectious blood product, blood transfusion, congenital, organ transplant, vaginal, anal and oral sex. Other routes are use of unsterile needle, tattoo, needle pricks, haemodialysis, nose and ear piercing (Lavanchy, 2011). Sexual transmission occurs mainly among high-risk and those
infected with STIs. These mainly exist among men who does sex with other men and women who practices sex for commercial gain. HIV infection is also identified as a contributing element of HCV transmission in absence of other STI. Consequently, having multiple partners and homosexual acts accelerates HCV transmission among HIV infected persons especially in cases of unprotected anal sex (Ghosn et al., 2006).

The disease symptoms appear within ½ - 6 months when one has acquired the infectious virus. These includes yellow colouration of skin due to excess production of bilirubin, feeling tired, stool with grey collocation, joint and belly pain, general body weakness, anorexia, itchness on skin and dark urine production (Chen and Morgan, 2006). However, HCV infections can be asymptomatic (Jara et al., 2003).

2.3.3 Pathogenesis of HCV

The virus is non-cytopathic causing replication inside the liver cell. Various systems such as immune-mediated cytolysis, oxidative stress, hepatic steatosis and insulin resistance facilitate cell necrosis. HCV pathogenesis causation can be enhanced by protein and peptides of varied genomic regions (Irshad and Dhar, 2006).

Innate immunity offers first line defense against HCV infection. The Type 1 IFN is usually produced as HCV infection occurs. This facilitates cells to obscure the infections, oversee viral replication, induce adaptive immunity, activates natural killer cells, DCs and Kupffer cells. The innate immunity action against HCV is actualised by pathogen-associated molecular patterns acting like non-self recognition receptors. These are tolling-like receptors (TLRs) and retinoic acid-inducible gene-I (Saito et al., 2008).
Moreover, the binding of pathogen-associated molecular initiates the activation of interferon regulatory factor-3 leading to expression of IFN-α/β and anti-viral/interferon stimulated genes (Liu and Gale, 2010). T and B cell immunity support is usually aligned with natural killer cells and Kupffer. The release depends on IFN and cytokines (Saito and Gale, 2008). Persistent viral infection arises occasionally when HCV succeeds in destroying innate immunity and counteract the RIG-1 pathway resulting in chronic infections (Schoggins and Rice, 2013). The complex of NS3 and NS4A is formed. During the processes it acts on NS protease domain to facilitate cleavage of IPS-1limiting signals that could activate IRF-3 and NFκB (Loo et al., 2006).

In the initial stages of infection the NK cells which are more in the liver plays a suitable role in eliminating HCV. Other functions of activated NK cells includes recruiting virus-specific T cells, inducing antiviral immunity, cytolytic mechanisms and secretion of cytokines such as IFN-γ and TNF-α which provide antiviral state in host cells. The NK cells inactivates viral invasion (Golden and Rosen, 2013).

The adaptive immunity is also suitable when the virus enters and replicates inside the liver cells. Its relationship with major histocompatibility complex occurs once its molecular components are moved to endoplasmic reticulum and to the cell surface. The T cells meant for immune recognition confirms the molecules on cell surface. The CTLs which destroys virus infected cells are CD8+ and CD4+. However, the CD8+ have a higher portion and identifies antigens on MHC class I molecules. The CD4+ identifies antigens presented on MHC class II molecules. The HCV virus limits expression of MHC
or hinders viral peptide presentation on cell surfaces hence cannot be sensed by CTLs (Neumann and Thimme, 2013). When HCV-infected liver cells are destroyed they release fragments which are taken up by myeloid to lymph nodes highlighting HCV antigens on HLA class II molecules. Moreover, co stimulatory molecules such as CD80 and CD86 are expressed leading to the activation of antigen-specific helper T cells (Malta et al., 2013). The maturation of DCs is enhanced by T helper cells facilitating the showup of CD40 ligand and TNF-α. Once the DCs mature they induces T-cell activation and increases presentation of antigens through HLA-I with cytokines production that stimulates activation of T-cell. The IFN-γ production arising from activated T cells is usually induced by IL-12 (Jaime et al., 2011). The perforin are produced by effector CTLs and leads to direct attack on HCV-infected liver cells (Zhang et al., 2013).

The enzymes 2’-5’ oligoadenylate synthetase and RNA-dependent protein kinase in liver cells leads to viral suppression by production of type I IFNs (Samuel, 2001). HCV markers are recognized by plasmacytoid DC, relating closely with single-stranded RNA (Liu et al., 2001). RNAs are eliminated by oligoadenylate synthetase while PKR inhibits formation of viral mRNA polysomes. The persistent infection occurs when CTL responses are not strong (Samuel, 2001). Th1 immune response acts as a measure in clearing of HCV infection (Fahey et al., 2013). CTLs secretes cytokines such as IFN-γ, IFN-α/β and TNF-α which enhances an antiviral state in host cells, uninfected cells resist to infection and limits viral replication (Irshad et al., 2008). However, CD4+CD25+ regulatory T cells present in the blood inhibit HCV specific CD8+ T cell and CD4+ T cell increase and secretion (Sugimoto et al., 2003). The secretion of IL-10 by Treg cells
and transforming growth factor-β (TGF-β) suppresses virus specific T cell responses (Haseda et al., 2013). CD4+CD25+ Treg cells from chronically HCV infected patients have more suppressive action towards HCV specific CD8+ T cells (Thimme et al., 2006). Other studies have shown presence of CD4+CD25+FoxP3+ Treg cells and their suppressive ability against virus specific T cell responses are greater in HCV recovered chimpanzees as compared to persistently one (Manigold et al., 2006). The various notable symptoms of HCV-infected persons are fatigue, arthralgia, paraesthesia, myalgia, pruritus and sicca syndrome (Chen and Morgan, 2006).

**HCV life cycle**

HCV invades liver through blood circulation hence transmitted by blood and it spread requires factors such as scavenger receptor class B type I, Occludin, Claudin-I and CD81. CLDN1 can be substituted by CLDN6 and CLDN9 as entry factors in human non-liver cells (Haid et al., 2013). The CD81 is a viral receptor that binds with the particle of virus, leading to its liver cell entry. Usually exhibited on surfaces of nucleated cells and forms complex with receptors including CD19 and CD21 especially in B cells passing on signals to the cells. The extracellular loop of CD8 can bind with E2 protein of enveloped virus (Flint et al., 1999). HCV provides varied attachment regions beside binding molecules such as the receptor for low-density lipoprotein, dendritic cell and its liver counterpart (Scarselli et al., 2002). Association of E2 with CD81 is strain-specific. High mutation rate is experienced with the virus by fact that it lacks proof reading ability of its RNA-dependent RNA polymerase and has hypervariable 1 and 2. Hence the virus survives in different forms and are called quasispecies (Roccasecca et al., 2003).
2.3.4 Epidemiology of HCV

Hepatitis C virus is a major disease that gives burden to the entire nations of the world. It is estimated that global prevalence is about 3% with a population of 170 million being affected (WHO, 1999). Studies based on blood donors may not provide the real picture of the disease since these is a highly selected population. Equally, in the United States (1988 to 1994) 3.9 million people were found infected with HCV while 2.7 million people had chronic infection according to Third National Health and Nutrition Examination Survey. However, infection was dominant in ages below 50 years (Alter et al., 1999). However, in 2001-2002, Central and South America, an estimated prevalence of 6.3% was noted in San Juan, Puerto Rico (Perez et al., 2005). More over 1.2% was found in Mexico (Uribe and Mendez, 2002) while in Chile and Brazil among the blood donors prevalence was rated at 0.3% and 1.14% respectively (Munoz et al., 1998). The general prevalence of HCVAb in Europe is at most 1% but varies depending with the country (Touzet et al., 2000). Belgium had a prevalence of 0.87% between 1993 and 1994 with a population of more than 200000 infected in the United Kingdom. Likewise, in Northern Italy (3.2%), (Bellentani and Tiribelli, 2001) Central and Southern Italy (8.4%-22.4%), mostly in elderly people (Raffaele et al., 2001). Consequently in Lyon prevalence was rated at 1.3% which almost resembles that of France (Pradat, 2001) and among Russian army (1.5%) (Ogarkov et al., 2004). In Pakistan (4.57%) were noted positive for HCV Ab (1998-2002), with age 41-50 being most infected (Muhammad and Jan 2005). Egypt has recorded the highest prevalence (28%) with lower prevalences in Saudi Arabia (1.8%) (Al-Faleh et al., 1995) and Yemen (2.1%) (ElGuneid et al., 1993). Average prevalence
has been identified among blood donors in countries such as Japan (0.49%) from year 1995 to 2000, China (1.0%) (Zhang et al., 1992), Malaysia (1.6%) and Singapore (0.54%), Thailand (3.2-5.6%) (Songsivilai et al., 1997) and in New Delhi, India (1.85%) (Panigrahi et al., 1997).

Few studies have been done in Africa with lower rates reported such as in Ethiopia 1.6% (Frommel et al., 1993) and Kenya 0.9% (Ilako et al., 1995) especially among the blood donors. In Australia prevalence is rated at 2.3% with ages below being infected more (Amin et al., 2004). Most of the studies have been studied in other areas in Africa, however in the HCV/HIV co infections limited has been done and this needs to be extensively done in Kenya.

2.3.5 HCV Prevalence

Globally, World Health Organization (WHO) estimates that 170 million people are infected with HCV. However, 3-4 million new transmissions do occur each year, making it one of the leading public health difficulties in the world (Lavanchy, 2004). Due to its high prevalence and easily transmitted in nature, the infection remains under-diagnosed and underreported in Africa (Madhava et al., 2002). The continent of Africa is noted to have a prevalence of 5.3% and an estimated 32 million people with the infectious infection. Sub Saharan Africa has the highest burden of the disease. Other regions noted by WHO with high prevalence of HCV include Eastern Mediterranean (prevalence 4.6%) and Western Pacific (prevalence 3.9%). The prevalence in the general population of Africa ranges between 0.1% and 17.5%, depending on the country. The highest burden of
the disease is in Egypt (17.5%), Cameroon (13.8%) and Burundi (11.3%). The countries with the lowest prevalence include: Kenya 0.7% (Kerubo et al., 2015), Malawi and Zambia (0.1-1%) according to global burden disease 2004.

2.4 Treponema Pallidum (Syphilis)

The *T. pallidum* subsp. pallidum is an obligate microaerophilic spiral-shaped bacteria and belongs to the family Spirochaetaceae (spirochetes). They are differentiated from other pathogenic treponemes by their clinical manifestations and by genetic diversity. Their size ranges from 6 to 15 μm in length and 0.2 μm in diameter. The cytoplasmic membrane is below the outer membrane and it surrounds the spiral shaped part of *T. Pallidum*. Structural stability is provided by a layer of peptidoglycan (thin) which is situated between membranes. They have endoflagella, organelles that allow for the characteristic corkscrew motility and are situated in the periplasmic space (Harman et al., 2013).

2.4.1 Genotypes

Several other genes that encode outer membrane proteins belong to the tpr gene family. There are twelve tpr genes which are divided into three main subfamilies namely; I, II and III. Sub family I consist of genes tprC, tprD, tprF, and tprI while subfamily II consist of genes tprE, tprG, and tprJ. The subfamily III consist of genes tprA, tprB, tprH, tprK, and tprL. Genes tprF, tprI, and tprK encodes proteins that are said to be located in the outer membrane (Cameron, 2003). Cleavable signal sequences are associated with tprK and subfamily I proteins. However, an immune response is induced by proteins which are
encoded by the tpr genes (Leader et al., 2003). Antibody production occurs differently depending on the duration of infection. Subsequently, anti-TprK which is induced after seventeen days postinfection and is reactive after aduration of thirty days (Morgan et al., 2002). Subfamilies I and II antibodies are detectable not later than forty five days and higher infection ratio arrived after a period of sixty days (Sun et al., 2004). The time of development of antibodies to specific Tprs may reveal the timing of expression of the proteins that induced those antibodies. Regulation of expression of related proteins is referred to as the phase variation. This involves controlling protein expression and could be used by T. pallidum to weaken or eliminate Tprs which an immune response has been set. Consequently regulates the expression of new Tprs. Continuity of chronic infection is enhanced by the presence of new proteins that are not easily recognized by the initial immune system with various strains of T. pallidum encoding different repertoires of Tpr proteins (Leader et al., 2003).

Pathogenesis of syphilis is enhanced by TprK protein with the presence of a translational start site downstream induced by the T. pallidum genome sequence (Hazlett et al., 2001). The TprK translational start initiates cleavable signal sequence (N-terminal), introducing much prediction that they are membrane-localized protein (Centurion et al., 2000). Seven discrete variable (V) regions are used to describe the tprK gene and predicted amino acid sequences which are separated by stretches of conserved sequence. More over, in most T. pallidum strains, various tprK sequences are well established (Centurion et al., 2000). Multiple subpopulations of organisms invade the host and tprK sequences are used to identify them. Creation of new diverse sequences by gene conversion is accelerated by
donor sites. This is likely to replace small or large portions of V-region sequences in the tprK genes and the diversity occurs when there is an infection. The variation in tprK is useful in the existence of *T. Pallidum* (Centurion *et al.*, 2004).

Cellular and humoral immunity are initiated by TprK protein in infected animals. Antibodies released by TprK proteins that arise in response to *T. pallidum* infection targets the V regions (Morgan *et al.*, 2003). TprK epitopes are sensed by T cells showing that antibody binding to V regions is highly specific (Morgan *et al.*, 2002). V-region-specific immunity is suitable in protection against progressive infection. Presence of *T. pallidum* strain that is single to immunizing proteins shows alternative defence than those of TprK proteins with varied V regions. When new changes are generated in TprK it may assist the variant *T. pallidum* organisms to be missed by immune response, initiating its survival and continuity of chronic infection (Morgan *et al.*, 2003).

Patients infected with primary and secondary syphilis has shown diversity especially in tprK sequenced (Myint *et al.*, 2004). Diversity is also found among strains of tprC and tprD differently from tprK which occurs within strains. Likewise, the loci location of tprC and tprD genes in *T. pallidum* genome is different but sharing similar sequence in Nichols strains (Centurion *et al.*, 2004). Identical tprC and tprD sequences are also common among the non Nichols *T. pallidum* strains. Gene tprD2 is carried by some strains in the tprD locus and differs in sequence (Centurion *et al.*, 2000). More over tprD2 carried by some strain differs slightly in the tprC locus and are not identical in sequence (Sun *et al.*, 2004). Other pathogenic *T. pallidum* subspecies, such as subspecies pertenue
and endemicum have tprD2 sequence and they accelerate the nonvenereal treponematoses yaws and bejel. Consequently, in tprJ locus Gauthier strain, a subsp. pertenue carries in it sequences of tprG and tprJ. Lipoprotein of TpN15 is encoded by genes that are identical among subsp. pallidum strains, subspecies endemicum and pertenue. Restriction sites in genes encoding TpN15 and Gpd facilitates genetic differentiation of spirochetes from pathogenic ones (Centurion et al., 2004).

2.4.2 Transmission

Sexual activities practiced by female sex workers (FSWs) are known as mode of acquiring and transmitting Syphilis (Minichiello et al., 2013). These infection are said to be high among FSW than in the general population (UNAIDS, 2012). Moreover, the transmission can occur through blood transfusion and organ donation where blood is not well screened. Infections such as Syphilis, HIV, hepatitis B and C virus are likely to be transmitted due to poor screening. Men who does sex with other men (MSM) also do transmit syphilis infection. Countries such as United States primary stages as well as secondary levels of infection are most infectious and common among MSM (Peterman et al., 2005).

Inflammatory genital ulcers and lesions caused by syphilis infection accelerates acquisition of HIV by providing appropriate route for the entry of virus and also influences shedding (Buchacz et al., 2004). Further, it has been associated with drug failure among HIV patient hence contributing to higher viral load (Ghanem et al., 2007). Risky sexual behaviors which includes failure to use condom during anal sex, exchanging
partners for sex and having sex with several partners provide appropriate way of transmission. Likelyhood of transmission depends on factors such as how often sex is done, mode of penetration (penile-vaginal, anal or oral). Others are the stage of syphilis infection, susceptibility of the partner and use preventive measures such as condoms (Peterman and Furness, 2007).

Transmission that involves blood products and organ donation such as liver transplantation can be a source of syphilis. Occupational exposure to syphilis such as accidental injury with a scalpel, needle is also a source of transmission (Salazar et al., 2007).

Vertical transmission occurs between mother and child during delivery. Most often, transmission occurs in utero during pregnancy. Primary and secondary stages of infection may lead to premature birth, neonatal death and still birth. Further, the amniotic fluid and fetus can harbor treponemes during pregnancy. Maternal Syphilis increases chances of HIV acquisition and transmission by breaking the genital wall epithelium (Murray et al., 2002).

2.4.3 Pathogenesis

The abundant lipoproteins in *Treponema Pallidum* activates macrophages, DCs, Toll-like receptor 1 (TLR1) and TLR2-dependent signaling pathways (Wooten et al., 2002). Moreover, the pathogen associated molecular patterns are major pro-inflammatory agonists when spirochetal infection occurs. Apparently, not accessed easily by toll-like receptors due to the fact that they don’t have exposed surface receptors (Salazar et al., 2009).
Innate immune system is not triggered since the organisms divides in the tissues. The organisms are taken up by dendritic cells which drain it in the lymph nodes before presenting it to T and B-cells. Opsonized antibodies would facilitate uptake and breakdown of bacterium in tissues. Further, the spirochetal pathogen associated molecular patterns would enhance phagocytosis and activation (Cervantes et al., 2011). Their cellular immune response is usually provoked by \textit{T. pallidum} and causes the destruction of tissues leading to clinical manifestation of the infections (Baughn and Mushner, 2005).

Biopsy specimens have also demonstrated that syphilitic skin lesions acquired from patients infected with infectious levels of primary and secondary syphilis contains lymphocytes and macrophages. Consequently there is the expression of mRNA for the Th1 cytokines, IL-2, IFN\(\gamma\) and IL-12. The CD8+ T-cells characterize human SS syphilis and facilitates inflammatory infiltrates (Stary et al., 2010). Presence of perforin and granzyme B in human syphilis lesions provides enough evidence that in Tp-infected SS skin tissues cytolytic T-cells participates in bacterial clearance (Van et al., 1996).

However, activation of T-cells (CD8+) is not well established. The lymphocyte subset normally acknowledges antigens presented through MHC class I pathway with no impact on extracellular pathogens. Familiarization of immune systems of syphilitic infections have greatly been intervered by failure to grow it in vitro and no appropriate animal for breeding to enhance immunological studies. The CD4+, CD8+, CD56+ and NK-cells are vital in activation of dermal macrophages by secreting IFN-\(\gamma\) (Cruz et al., 2008). The
Treponema pallium ssp. Pallidum causes Syphilis which is a multistage chronic disease that causes an estimated 8 million new cases of infections annually (Newman et al., 2015). The infections is high among men who have sex with other men (MSM) and could spread through congenital routes thus advanced diagnostic technique is required (Read et al., 2015). The membrane do not have lipopolysaccharide (LPS), however, the outer membrane have low amount of proteins (Liu et al., 2010). Lack of in vitro cultivation of the organism has interfered with genetic manipulation experiments. Rabbit models experiments have facilitated the understanding of the T. Pallidum especially in genome description, transcriptome and proteome (Norris, 1993).

Twelve genes are contained in the tpr gene family and are classified into subfamilies I (tpr C, D, F and I), II (tprE, G and J) and III (tprA, B, H, K and L) which assists in the immune invasion (Palmer et al., 2009). Encoded proteins have variable regions targeted incases of infection by humoral response and TprK assist in the evasion of host immune response (Reid et al., 2014).

**Stages of Syphilis**

The primary Syphilis occurs when T. pallidum gains access through dermal layer microabrasions or intact mucous membranes. At this particular stage it may lead to a single chancre formation on the site of entrance and lymphadenopathy may arise. This chancre is not painful, but might develop, transforms to induration resulting to ulceration with no pus. Ordinarily, in heterosexual men, primary chancre develop on the penis but on homosexual they can develop on other sites such as rectum, anal canal and at oral
cavity (Hourihan et al., 2004). Further, in women, they appear on the labia or cervix and diagnosis is usually delayed until later stage when signs become apparent. Other symptoms of disease involve appearance of non indurated lesions with irregular borders which are painful especially in individuals co infected with HIV (Rompalo et al., 2001). The incubation period ranges from 10-90 days. Consequently, lesions may appear within 3 weeks from time of exposure. Primary chancre might disappear and may reappear at the start of secondary Syphilis. These primary and secondary diseases are common among HIV infected than uninfected persons (Hutchinson et al., 1994).

When secondary Syphilis establishes, *T. pallidum* multiplies very fast and spreads to various tissues. Clinical signs and symptoms shows up within a period of 3 months from the initial infection. Some of the symptoms of this stage include the Sore throat, muscle aches, malaise, weight loss, lymphadenopathy and mucocutaneous rash. Others include Pale and discrete macular lesions which originally shows on the trunk and proximal extremities. Their after appearance of lesions with varied shapes on infected patients. The several secondary syphilis lesions that can be noted in human include maculopapular, papular, macular, annular papular and sometimes they are identified as necrotic (lues maligna) (Baughn and Musher, 2005).

Lesions at this stage are usually inconspicuous and their presence in infected patients might not be noticed. Infection signs extend to the palm of hands (rash), soles of feet, hair follicles (alopecia of the scalp) and result to loss of body hair. Conditions such as condylomata lata establishes in some patients body parts that are moist, warm such as in
perineum and anus. However, the mucous patches are usually accelerated by swelling of tongue, oral cavity and mucous membrane of genitals (Mindel et al., 1989).

More often, conditions such as gastric, renal failure and hepatitis occurs incases of secondary Syphilis (Mullick et al., 2004). Organs such as liver has been found to be invaded by T. Pallidum leading to glomerulonephritis which end up damaging the kidney. There is also the presence of nephrotic conditions and development of neurosyphilis that involve the development of meningitis and ocular disease (Mindel et al., 1989). T. pallidum sometimes enters into the central nervous system in early stages of disease and can be detected in cerebrospinal fluid. This is associated with increased protein and white blood cells. The neurosyphilis is associated with titers equal or above 1:32 with serum rapid plasma reagin without regards to its stage or HIV infection (Marra et al., 2004).

The initial symptoms that are associated with neurosyphilis may appear in primary or secondary syphilis. Further, in cases of acute meningitis: fever, nausea, headache, vomiting and stiff neck symptoms do occur. Others include blurred vision, photophobia, failure to hear and general weakness of the body arising as a result of cranial nerve disturbance. Numbness, pain and ocular inflammation referred as uveitis in the extremities are also noted and these symptoms of neurosyphilis have not been associated with HIV infection (Rompalo et al., 2001).

Latent Syphilitic stage of infection is classified into two upon estimation of when infection was acquired and in period accumulating to one year of infection. There is
likely hood of developing early latent syphilis and secondary conditions. Late latent syphilis is known for not showing symptoms of infection in a period exceeding one year. During this period sexual transmission is not guaranteed although serological tests are positive. The microorganisms do multiply into the blood stream and could invade the fetus incases of pregnancy. The disease ends when treated and when tertiary stage occurs (Poliseli et al., 2008).

The tertiary Syphilis has symptoms such as gumma (late benign syphilis) arising from progressive inflammation. More often, in untreated condition it may lead to the destruction of tissues and bone. After two years of infections conditions such as granulomatous and nodular lesions with varied central necrosis may develop. These situation causes harm to the skin, bones, liver, heart, brain, stomach, and upper respiratory tract. Apparently, lesions don’t heal fast, although, they respond with appropriate drug combinations especially when they have not invaded the major organs of the body. The *T. pallidum* can be found in gummatous lesions and also reffered as late benign Syphilis (Handsfield et al., 1983).

Others such as cardiovascular syphilis is usually associated with the infection and invades the aorta causing uncomplicated, asymptomatic situations. Coronary ostial stenosis and saccular aneurysm complications do occur by detection of *T. pallidum* DNA in an aortic aneurysm with PCR occuring (Jackman et al., 1989). Advanced state of neurological conditions, is enhanced by prolonged and untreated conditions for aperiod of 5-10 years. Subsequently, the infection may spread to CNS progressing to meningovascular syphilis
and presence of vertigo, insomnia, personality changes, Loss of consciousness and seizures is usually noted (Marra et al., 2010).

Late parenchymatous syphilis can also be experienced in decades not exceeding three. The symptoms associated with it includes, general paresis or tabes dorsalis of personality changes, emotional instability, memory impairment, hallucinations and hyperactive reflexes. Tabes dorsalis is facilitated by disturbance upon the spinal cord posterior columns and dorsal root ganglia. Others such as temperature adjustments and great painful feeling in the body can be experienced leading to Charcot's joints which involves trophic lesions in ankles, knees, and hips (Simon, 1985). Equally, in cases of congenital Syphilis, pregnant mothers who have acquired the infection can with easy transmit through blood to their developing fetus. However, higher infection rate is experienced during the first year of infection than the later years (Sheffield et al., 2002). Treatment is quite effective especially during the first two consecutive trimesters. Poor management of the disease can result to damage, death or delivery of an infected fetus. Other prominent features include destruction of immune system, abortion, delivery of stillbirth and premature babies. The infants who are infected are associated with low weight. Likewise, death of the infected neonates after delivery can be accelerated by bacteria and chronic hepatitis infection (Mullick et al., 2004).

The two forms of congenital Syphilis are early and late manifestation. Early manifestations occurs within the first two years of infection while late manifestation occurs after two years of infection. Infectious stage is associated with symptoms such as
snuffles and rhinitis with blood stained discharge appearing within 2-10 weeks after the baby is delivered. Gummatous, skin lesions with desquamation, condylomata lata and mucous patches also occurs. Sometimes conditions such as low levels of blood regardless of age and sex, enlargement of spleen, renal conditions, skin yellow colouration (excess bilirubin) and osteochondritis do occurs. These may result to Parrot's pseudoparalysis. Late manifestations at the duration of 5 - 25 years, malfunction of cornea and iris may occur as a result of damaged interstitial keratitis. Others are the asymptomatic and symptomatic neurosyphilis, arthropathy, Clutton's joints and gummatous periostitis. These conditions may appear on the palate and nasal septum, Hutchinson’s teeth, peg-shaped notched upper incisors, tooth deformities, saddle nose and saber shin (Chawla et al., 1985).

2.4.4 Epidemiology of Syphilis

The outbreaks of syphilis especially in town a mong MSM is linked with HIV co infection that ranges from 20% to 73% worldwide. In New York City (2001) the cases of primary and secondary Syphilis had doubled the ordinary number with a case rate of 6.9 per 100,000 which was higher than what was experienced in 1994. The infection cases were noted to have increased in whites from 24% to 33% and decreased in black population from 47% to 36% in the year 1999 and 2000. Moreover, in the U.S. the non-Hispanic blacks have 70.9% and their nationwide composition was 62.5% of the cases in 2000 and 2001 according to (Morb Mortal Weekly Rep, 2002). Syphilis is treatable and curable although it enhances the spread of HIV by 3 – 5 fold and causes adverse effects on neonatal health (Workowski and Berman 2002).
The development level of a nation and individual variations determines the disease burden and rates are as high upto 47% among sex workers in Africa. WHO (1995) reported 12 million cases of syphilis globally with highest rates in South and Southeast Asia (5.8 million), Africa (3.5 million), Latin America and caribbean (3m). Others are North America (100000), Australia and New Zealand (10000), North Africa and Middle East (370000). Estimated prevalence of syphilis in Africa is at 4% to 15% on prenatal clinics (Singh and Romanowski, 1999). However, various studies have shown rates to be above 10% in young women and in MSM especially in developing countries (Tabet, 2002). Further, in Tanzania active syphilis prevalence is 12.8% (Todd et al., 2001) while in Kenya is 3.8% (Temmerman et al., 1999). Moreover, among blood donors in Ethiopia (1995), Syphilis seroprevalence was 12.8% (Rahlenbeck et al., 1997).

2.4.5 Syphilis prevalence

Globally WHO approximates 12 million people are infected while in Africa the general figure ranges from 3–18% while in the Western world today the infection is rare (Klass et al., 1994). Burkina Faso overall sero prevalence of syphilis among first time blood donors is 1.5% and is relatively low compared to 7.5% and 5.7% cases in Ghana and Cameroon (Nagalo et al., 2011).

The association between syphilis, HBV and HCV has been examined among the blood donors (Balogun et al., 2012). Sexually transmitted diseases such as syphilis increase the risk of acquiring HIV infection (Feller et al., 2011). Equally, in Nigeria the sero prevalence rate of syphilis among pregnant women attending the antenatal clinics is
2.97% (Oyelese et al., 1990). In Tanzania active Syphilis is rated at 12.8% (Todd et al., 2001) while in Kenya the estimated prevalence of Syphilis in women and men is 1.8% and 1.7% respectively (Kenya Aids Indicator Survey, 2007).

Higher rates of infections were experienced in men with HIV (6.4%). However, in the general population prevalence is relatively low. The eradication could be possible but would require intensified prevention and control measures. The ‘Pambazuko’ (dawn or ‘the start of a new day’ in Kiswahili) project held in Kisumu among FSW revealed that 3.4% had active syphilis (Vandenhoudt et al., 2013).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

The study was carried out at Engineer hospital HIV clinic in Nyandarua County. This is a public health facility offering health care services to all at a subsidized cost. The facility is located in Kinangop Sub County and borders Kiambu County to the South, Nakuru County (West) and Nyeri County (East) with a catchment population of 215123 (Nyandarua County HIV/AIDS strategic plan 2016 to 2020).

Figure 3.1: Map showing the location of Engineer hospital, in Nyandarua County, Kenya (Nyandarua County HIV/AIDS strategic plan 2016 to 2020).
3.2 Sample population

The study targeted HIV infected individuals both female and male attending comprehensive care clinic of Engineer hospital, in Nyandarua County.

3.3 Study design

A cross sectional study design was done during the period between July and September 2018 among HIV infected individual seeking comprehensive care in Engineer hospital, Nyandarua County.

3.4 Sampling design

A consecutive sampling technique (Straus et al., 2005) was used where each consecutive and eligible patient who attended clinic were approached for enrollment in the study.

3.4.1 Sampling size calculations

The sample size was based on an estimated prevalence of 50% since these particular study has never been carried out within the County. The (Cochran formula 1963:75) was used to calculate the sample size. \[ n = \frac{Z^2pq}{e^2} \] where: \( e \) = desired level of precision (i.e. the margin of error), \( p \) = (estimated) proportion of the population which has the attribute in question, \( q = (1-p) \) and Z-value was found in a Z table (constant).

\[
N = \frac{1.96^2 \times 0.5\times 0.5}{0.05^2} = 385 \text{ (HIV patients)}
\]
3.4.2 Inclusion criteria

The study included consented HIV positive patients of any gender and age who attended comprehensive care clinic in the month of July to September 2018.

3.4.3 Exclusion criteria

The study excluded participants who were HIV negative, had not consented and attended clinic before and after research period.

3.5 Demographic data

The pretest counselling was done at comprehensive care clinic by the counsellors after consenting to participate in the study. Demographic data of age, sex, marital status, education levels and occupation were captured using a well administered questionnaire (APPENDIX V) before being sent to laboratory for blood collection.

3.6 Dependent and independent variables

The dependent variables in the study were HIV/HBV, HIV/HCV, HIV/Syphilis and the independent variables were age, gender, marital status, occupation, education level, cd4 counts and drug regiments.

3.7 Sample collection

Four mililitre of venous blood samples was collected from consented HIV positive patients into the K$_3$EDTA vacuitainer tubes (BD, New Jersey USA). For anticipated delays, the samples were kept at room temperature but not exceeding 24 hours before analysis of CD4 levels and screening for hepatitis B, C and syphilis.
3.8 Seroprofile determination (rapid)

HBsAg (OnSite) rapid test was based on lateral flow chromatographic immunoassay. The test had a burgundy coloured conjugate pad with anti HBsAg antibody conjugated with colloidal gold and a control antibody conjugated with colloidal gold. The nitrocellulose membrane containing a test line (T) and control line (C). The T line with pre coated non conjugated HBsAg antibody and C line precoated with control line antibody. The test specimen (3-4 drops) was dispensed on the sample pad of the strip and the sample migrates by capillary action. Presence of HBsAg will bind to HBsAg Ab conjugates. The immunocomplex captured by precoated non conjugated HBsAg antibody forming a burgundy colored T line indicating positive test result and absence of T line negative result (Emanuel and John 1994).

The HCV Ab plus (OnSite) was a double antigen lateral flow chromatographic immunoassay. The test had a burgundy colored conjugate pad with recombinant HCV fusion Antigen (core NS3, NS4 and NS5) conjugated with HCV Ag conjugates and control antibody conjugated with colloidal gold. The nitrocellulose membrane consist of a test line (T) and control line (C). The test specimen was applied on sample pad of the strip (1 drop and 1 drop of sample diluent). Presence of antibodies to HCV will bind to HCV conjugates forming a burgundy colored T line (positive) and absence of T line negative (Kuo et al., 1989).

The Syphilis ultra rapid test was a qualitative membrane based immunoassay for detection of TP antibodies (IgM and IgG) in whole blood, serum or plasma. The recombinant syphilis was immobilized in test line of the strip when (2-3 drops of sample and 1 drop of
buffer) was placed on test pad it reacts with syphilis antigen coated particle. The mixture migrated chromatographically and reacted with immobilized syphilis antigen. Presence of TP antibodies a red line appeared on test line (T) indicating positive result and absence of T line negative (Wasserrheit 1992).

### 3.9 Enzyme Linked Immunosorbent Assay (ELISA)

The positive samples for HBV, HCV and Syphilis were confirmed using Immunoanalyser (e 411) at Kikuyu Mission Hospital. The process involved the sandwich principle; 1<sup>st</sup> incubation of sample and reagent containing biotinylated antigen, antigen labelled with a ruthenium complex reacts to form a sandwich complex.

The 2<sup>nd</sup> incubation, involves addition of streptavidin coated microparticle, the complex was bound to the solid phase when it reacts with biotin and streptavidin. The reaction mixture was aspirated into the measuring cells and microparticles were magnetically captured into the surface of the electrode while the unbound substances were removed with aprocell. When voltage was applied to the electrode it induced chemiluminescent emissions which were measured by a photomultiplier.

The results were defined automatically by elecsys software which involves comparing the electrochemiluminescence signal obtained from the sample with the cut at value obtained by ant HCV, anti Syphilis and HBV (Busch et al., 1995).
3.9.1 Procedure of HBV analysis
Total duration of assay was 18 minutes. The samples was placed in the sample zone and registered by entering the sample identification data. positive control: PC HBSAG2/PC HBSAGII2 was run parallel with the samples of confirmation. 1st incubation: 50 μL of sample, two biotinylated monoclonal anti-HBsAg antibodies, and a mixture of monoclonal anti-HBsAg antibody and polyclonal anti-HBsAg antibodies labeled with a ruthenium complexa) to form a sandwich complex. 2nd incubation: After addition of streptavidin-coated microparticles, the complex became bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with ProCell II M. The voltage was applied on the electrode, chemiluminescent emission was induced which was measured by a photomultiplier. Results were automatically determined by the software comparing the electrochemiluminescence signal obtained from the reaction product of the sample and the signal of the cutoff value obtained by calibration. The result of HBV was interpreted as: Reactive with cut off index (COI) ≥1, Nonreactive (COI) ≤ 0.9 (Liaw 2011).

3.9.2 Procedure of HCV analysis
Total duration of assay: 18 minutes. A mixture of 40μL of sample plasma, 60μL of a reagent containing biotinylated HCV antigens and 60 μL of a reagent containing HCV antigens labeled with a ruthenium complex were incubated and reacted to form a
sandwich complex. 2nd incubation: After addition of streptavidin-coated microparticles, the complex became bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with ProCell II. The voltage was applied on the electrode, chemiluminescent emission was induced and measured by a photomultiplier. Results were automatically determined by the software comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value obtained by calibration. For quality control, Elecsys PreciControl Anti-HCV 1 and 2, was run once every 24hrs when the test was to be done and after calibration. The result of HCV was interpreted as: Reactive with cut off index (COI) ≥1, Non reactive (COI) ≤ 0.9 (Courouc4 1998).

3.9.3 Procedure of syphilis analysis

Total duration of assay: 18 minutes. Plasma sample 6 μL, biotinylated TP-specific recombinant antigens and TP-specific recombinant antigens labeled with a ruthenium complexa) were mixed together reacting to form a sandwich complex. After addition of streptavidin-coated microparticles, the complex was bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with ProCell II M. The voltage was applied on the electrode and chemiluminescent emission was induced and measured
by a photomultiplier. Results were automatically determined by the software comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value obtained by calibration. For quality control, PreciControl Syphilis was run once every 24 hours. The result of Syphilis was interpreted as: Reactive with cut off index (COI) ≥1, Non reactive (COI) ≤ 0.9 (Seña et al., 2010).

3.10 Flow cytometry for CD4 counts determination

An aliquot of an EDTA whole blood sample was mixed with two antibodies (CD4 and CD45) each bound to a different fluorochrome for labeling the specific dedicated cell populations. After a specified fixed incubation time, two buffer solutions were added and the sample was ready for analysis on Cyflow Counter flow cytometer. The light source excites the fluorescent dye that was linked with the stained cell and the emitted light detected when a given volume of blood sample was run through the instrument. The integrated software calculated the concentration of the dedicated cell population (Cassens et al., 2004).

3.10.1 Procedure of cd4 analysis

20µl of whole blood was put in a tube.

Add 10µl of cd4 Macpe and mix.

Add 10 µl of cd45 mabcys and mix.

Incubate at room temperature, dark environment for 15 minutes.

Add 400 µl buffer 1 and Prior to measurement, add 400 µl buffer 2.
Put sample mixture on machine for aspiration and analysis (Kohreanudom and Dettraira 2012).

3.11 The HIV Viral load determination

The blood samples was collected and DBS prepared and left to stand overnight before being triple packed and sent to National HIV Reference Laboratories in Nairobi for viral load analysis. A real time polymerase chain reaction (PCR) monitors amplification of a specific DNA molecule. Usually used quatitatively (quantitative real time PCR). The process involved the transcription of RNA into complementary DNA (cDNA) by reverse transcriptase from total RNA or messanger RNA (mRNA). The (cDNA) was used as a template for the qPCR reaction and was primed using random primers, oligo (dT) and gene specific primers. The temperature that was used for cDNA depended on RT enzyme selected and an estimated 10% of the cDNA was transferred to a separate tube for real time PCR reaction.

The reactions were generally run for fourty cycles and it involved three major processes. The denaturation where a high temperature was used to melt the double stranded DNA to single strands loosening the secondary structures in it. The annealing was meant to hybridize complementary sequences and an appropriate temperature was used 5° below the temperature of the primer. When the activity of DNA was optimal at extension stage, and primers extended upto 100 bases per second (Joseph et al., 2001).
3.12 Data analysis

The data was collected, entered into a register, cleaned and analyzed using SPSS software version 22. The variables of the study were age, sex, marital status, education level, occupation and CD4 levels. The data was analysed based on confirmed result against each specific variable.

3.13 Ethical considerations

Permission to carry out research was obtained from Engineer County Hospital (APPENDIX IV) and approval from Kenyatta university ethical review committee (Ref: KU/ERC/APPROVAL/VOL.1 (166) (APPENDIX III) before research started.
CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of the studied population

Out of the 385 respondents, the total number of females and males involved in the study were 263 (68.3%) and 122 (31.7%) respectively. Majority of the respondents (37.7%) were in the ages of 35 – 44 years and 20.0% were in the ages of 45 – 54 years. Most (50.0%) of the respondents were married while 35.1% were single. Highest education level obtained among this population was established as college. However, most of the respondents (49.4%) had primary level of education. Only 12.5% had college education. The main occupation of the respondents was farming. One hundred and sixty four (42.6%) of the respondents were farmers, 23.4% were involved in business, 13.8% were casual workers whereas only 6.0% were employed. Table 4.1.
Table 4.1: Demographic characteristics of studied subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency (n = 385)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>122</td>
<td>31.7</td>
</tr>
<tr>
<td>Females</td>
<td>263</td>
<td>68.3</td>
</tr>
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<td><strong>Ages (years)</strong></td>
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<td></td>
</tr>
<tr>
<td>Less than 4</td>
<td>3</td>
<td>0.8</td>
</tr>
<tr>
<td>5 – 14</td>
<td>35</td>
<td>9.1</td>
</tr>
<tr>
<td>15 - 24</td>
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<td>6.5</td>
</tr>
<tr>
<td>25 – 34</td>
<td>76</td>
<td>19.7</td>
</tr>
<tr>
<td>35 – 44</td>
<td>145</td>
<td>37.7</td>
</tr>
<tr>
<td>45 – 54</td>
<td>77</td>
<td>20.0</td>
</tr>
<tr>
<td>55 – 64</td>
<td>19</td>
<td>4.9</td>
</tr>
<tr>
<td>65 +</td>
<td>5</td>
<td>1.3</td>
</tr>
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<td><strong>Marital status</strong></td>
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</tr>
<tr>
<td>Married</td>
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<tr>
<td>Single</td>
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<tr>
<td>Divorced</td>
<td>57</td>
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<tr>
<td>Widowed</td>
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<td>9.4</td>
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<td><strong>Education</strong></td>
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<td>Primary</td>
<td>190</td>
<td>49.4</td>
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<tr>
<td>Secondary</td>
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<tr>
<td>College</td>
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<td>12.5</td>
</tr>
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<td>Never went to school</td>
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<td>0.3</td>
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<tr>
<td><strong>Occupation</strong></td>
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<tr>
<td>Employed</td>
<td>23</td>
<td>6.0</td>
</tr>
<tr>
<td>Not employed</td>
<td>55</td>
<td>14.3</td>
</tr>
<tr>
<td>Casual</td>
<td>53</td>
<td>13.8</td>
</tr>
<tr>
<td>Business</td>
<td>90</td>
<td>23.4</td>
</tr>
<tr>
<td>Farmer</td>
<td>164</td>
<td>42.6</td>
</tr>
</tbody>
</table>

4.1.2 HIV viral load of the patients

Out of the 385 patients attending Engineer county hospital comprehensive care unit, 4 patients (1.0%) had high detectable levels, > 1000 viral load while 381 patients (99.0%) had low detectable levels, < 1000 viral copies.
4.1.2.1 HIV viral load in patients of different ages

High detectable viral load levels were recorded in patients 35 – 44 years (50.0%), 45 – 54 years (25.0%) and those in 55 – 64 years (25.0%). Other ages had viral load < 1000 viral copies. Among the respective ages, All the patients < 4 years of age, 5 – 14 yrs, 15 – 24 yrs, 25 – 34 yrs, and above 65 yrs of age had low detectable levels < 1000 viral Copies.

![Image](image.png)

**Figure 4.2:** HIV viral load among the ages

4.2 To determine the sero profile of Hepatitis B, C and Syphilis co infections amongst HIV patients attending Engineer county hospital comprehensive care clinics.

4.2.1 Confirmed HBV positive patients

Patients’ HBV statuses was screened using rapid tests and confirmed with ELISA technique. Out of the sampled 385 population, 15 (3.9%) were positive to HBV while 370 (96.1%) were negative. The HBV prevalence among the male patients (8.2%) while among the female patients, it was 1.9%. This shows that HBV was significantly higher
(χ² = 8.822, P = 0.008) among the male patients than the female patients with an odds ratio estimate of 4.607 (lower limit of 1.539 and upper limit of 13.788).

Among the patients’ ages, HBV prevalence was more among the 65 year old patients (20.0%) and among those who were 45 – 54 years (7.8%). However, the difference in HBV prevalence was not significant among the ages (χ² = 9.636, P = 0.210). Similar non significance prevalence of HBV was recorded in the patients education levels (χ² = 2.363, P = 0.501). The prevalence was higher among the patients having primary education (4.1%) than the other levels of education. Prevalence did not vary significantly among the marital status of the patients (χ² = 6.107, P = 0.106). Widowed patients had a prevalence of 8.3% which was higher than other marital status (Table 4.2).
Table 4.2: Hepatitis B exposure rates in relation to Social demographic of HIV patients attending Engineer county hospital.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Positive N (%)</th>
<th>Negative N (%)</th>
<th>Chi value</th>
<th>P value</th>
<th>OR</th>
<th>OR 95% CI</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10 (8.2)</td>
<td>112 (91.8)</td>
<td>8.822</td>
<td>0.008</td>
<td>4.607</td>
<td>1.54–13.788</td>
</tr>
<tr>
<td>Female</td>
<td>5 (1.9)</td>
<td>258 (98.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Age (years)</strong></td>
<td></td>
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<tr>
<td>&lt; 4</td>
<td>0 (0.0)</td>
<td>3 (100)</td>
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<tr>
<td>5-14</td>
<td>0 (0.0)</td>
<td>35 (100)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>15-24</td>
<td>0 (0.0)</td>
<td>25 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-34</td>
<td>2 (2.6%)</td>
<td>74 (97.4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-44</td>
<td>5 (3.4%)</td>
<td>140 (96.6%)</td>
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<td></td>
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</tr>
<tr>
<td>45-54</td>
<td>6 (7.8%)</td>
<td>71 (92.2%)</td>
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<tr>
<td>55-64</td>
<td>1 (5.3%)</td>
<td>18 (94.7%)</td>
<td></td>
<td></td>
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<tr>
<td>65&gt;</td>
<td>1 (20.0%)</td>
<td>4 (80.0%)</td>
<td>9.636</td>
<td>0.210</td>
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<tr>
<td>Married</td>
<td>9 (5.7)</td>
<td>148 (94.3)</td>
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<tr>
<td>Single</td>
<td>2 (1.5)</td>
<td>133 (98.5)</td>
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<td>Divorced</td>
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<td>56 (98.2)</td>
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<td>Widowed</td>
<td>3 (8.3)</td>
<td>33 (91.7)</td>
<td>6.107</td>
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<tr>
<td>Casuals</td>
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<td>51 (96.2)</td>
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<td>Business</td>
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<td>88 (97.8)</td>
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<tr>
<td>Farmers</td>
<td>11 (6.7)</td>
<td>153 (93.3)</td>
<td>7.299</td>
<td>0.121</td>
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<td>9 (4.7)</td>
<td>181 (95.3)</td>
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<td>College</td>
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<tr>
<td>Never went school</td>
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<td>1 (100)</td>
<td>2.363</td>
<td>0.501</td>
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</table>

4.2.1.1 Patients co infected with HBV and HIV treatment they undergo

Patients HBV status was evaluated against their HIV treatments. The HIV treatment the patients were undergoing include; TDF/3TC/EFV, ABC/3TC/NVP, AZT/3TC/NVP, TDF/3TC/NVP, AZT/3TC/LPV/r, TDF/3TC/LPV/r and ABC/3TC/LPV/r. Out of the 15
HBV positive patients, the highest number of patients, (66.7%) were those under treatment with TDF/3TC/EFV (Table 4.3).

Table 4.3: A cross tabulation of the patients HBV status and the HIV treatment

<table>
<thead>
<tr>
<th>HBV status</th>
<th>TDF/3TC/EFP</th>
<th>ABC/3TC/NVP</th>
<th>AZT/3TC/NVP</th>
<th>TDF/3TC/C/LPV/r</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve</td>
<td>10 (66.7%)</td>
<td>0 (0.0%)</td>
<td>2 (13.3%)</td>
<td>3 (20.0%)</td>
<td>15</td>
</tr>
<tr>
<td>-ve</td>
<td>189 (51.1%)</td>
<td>31 (8.4%)</td>
<td>48 (13.0%)</td>
<td>95 (25.7%)</td>
<td>370</td>
</tr>
</tbody>
</table>

Key: TDF-tenofovir disoproxil fumarate, 3TC- Lamivudine, EFV- efavirenz, ABC - Abacavir, NVP-Nevirapine, AZT-Azidothymidine, LPV-Lopinavir, LPV/r-lopinavir/ritonavir

4.2.2 Confirmed HCV positive patients

Patients’ Hepatitis C status was confirmed using ELISA technique. Out of the sampled 385 population, three (0.8%) were positive to HCV while 382 (99.2%) were negative. The HCV prevalence among the male and the female patients was 0.8%. This shows that HCV was not significantly different in the gender ($\chi^2 = 0.004, P = 1.00$) with an odds ratio estimate of 1.079 (lower limit of 0.097 and upper limit of 12.009).

Among the various patients’ ages, HCV prevalence was more among those in the ages of 25 – 34 years, 45 – 54 and 55 – 64 years old patients (5.3%). The difference among the ages was however not significant ($\chi^2 = 7.165, P = 0.412$). Similarly, there was no significant difference in HCV prevalence recorded in the patients marital status ($\chi^2 = $).
7.011, $P = 0.072$), employment status ($\chi^2 = 1.643, P = 0.801$) and education levels ($\chi^2 = 1.201, P = 0.753$). The prevalence was higher among the patients having secondary education (1.4%) than the other levels of education. Among the marital status of the patients, divorced patients had a prevalence of 3.5% which was higher than the single patients (0.7%) Table 4.4.

**Table 4.4**: Hepatitis C exposure rates in relation to Social demographic of HIV patients attending Engineer county hospital.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Positive N (%)</th>
<th>Negative N (%)</th>
<th>Chi value</th>
<th>P value</th>
<th>OR</th>
<th>OR 95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1 (0.8)</td>
<td>121 (99.2)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>Female</td>
<td>2 (0.8)</td>
<td>261 (9.2)</td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
<td>0.097-12.009</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4</td>
<td>0 (0.0)</td>
<td>3 (100)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>5-14</td>
<td>0 (0.0)</td>
<td>35 (100)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>15-24</td>
<td>0 (0.0)</td>
<td>25 (100)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>25-34</td>
<td>1 (1.3)</td>
<td>75 (98.7)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>35-44</td>
<td>0 (0.0)</td>
<td>145 (100)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>45-54</td>
<td>1 (1.3)</td>
<td>76 (98.7)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>55-64</td>
<td>1 (5.3)</td>
<td>18 (94.7)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>65+</td>
<td>0 (0.0)</td>
<td>5 (100)</td>
<td>7.165</td>
<td>0.412</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Marital s.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>0 (0.0)</td>
<td>157 (100)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>Single</td>
<td>1 (0.7)</td>
<td>134 (99.3)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>Divorced</td>
<td>2 (3.5)</td>
<td>55 (96.5)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>Widowed</td>
<td>0 (0.0)</td>
<td>36 (100)</td>
<td>7.011</td>
<td>0.072</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>0 (0.0)</td>
<td>23 (100)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>Not employed</td>
<td>0 (0.0)</td>
<td>55 (100)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>Casuals</td>
<td>1 (1.9)</td>
<td>52 (98.1)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>Business</td>
<td>1 (1.1)</td>
<td>89 (98.9)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>Farmers</td>
<td>1 (0.6)</td>
<td>163 (99.4)</td>
<td>1.643</td>
<td>0.801</td>
<td>-</td>
<td>--</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>1 (0.5)</td>
<td>189 (99.5)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>Secondary</td>
<td>2 (1.4)</td>
<td>144 (98.6)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>College</td>
<td>0 (0.0)</td>
<td>48 (100)</td>
<td></td>
<td>0.004</td>
<td>0.951</td>
<td>1.079</td>
</tr>
<tr>
<td>Never w. sch</td>
<td>0 (0.0)</td>
<td>1 (100)</td>
<td>1.201</td>
<td>0.753</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
4.2.2.1 Patients co-infected with HCV and the HIV treatment they undergo

The patients HCV status was evaluated against their HIV treatments. This study found out that all the three patients who were coinfected with HCV were on treatment. Two were TDF/3TC/EFV while one was TDF/3TC/NVP (Table 4.5).

Table 4.5: A cross tabulation of the patients HCV status and the HIV treatment

<table>
<thead>
<tr>
<th>HBV status</th>
<th>HIV treatment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TDF/3TC/EFV</td>
<td>ABC/3TC/NVP</td>
</tr>
<tr>
<td>+ve</td>
<td>2 (66.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>-ive</td>
<td>197 (51.6%)</td>
<td>31 (8.1%)</td>
</tr>
</tbody>
</table>

Key: TDF-tenofovir disoproxil fumarate, 3TC- Lamivudine, EFV- efavirenz, ABC- Abacavir, NVP- Nevirapine, AZT-Azidothymidine, LPV-Lopinavir, LPV/r-lopinavir/ritonavir

4.2.3 Syphilis profiling

Syphilis infection statuses of the patients were confirmed using ELISA technique. Out of the sampled 385 population, twelve (3.1%) were confirmed positive to syphilis infection while 373 (96.9%) were negative. Syphilis prevalence among the male and the female patients was 4.1% and 2.7% respectively. This showed that there was no significant difference in infection by gender ($\chi^2 = 570$, $P = 530$) with an odds ratio estimate of 1.563 (lower limit of 0.486 and upper limit of 5.027).
In the various patients’ ages, Syphilis had a higher prevalence among the patients of above 65 years (20.0%) and those 15 - 24 years old patients (4.0%). The difference in syphilis infection among the ages was however not significant ($\chi^2 = 5.681, P = 0.577$). Similarly, there was no significant difference in syphilis prevalence recorded in the patients marital status ($\chi^2 = 0.633, P = 0.889$), employment status ($\chi^2 = 2.960, P = 0.565$) and education Levels ($\chi^2 = 1.809, P = 0.613$). The prevalence was higher among the patients having college education (6.3%) than the other levels of education. Among the marital status of the patients, married patients had a prevalence of 3.8% which was higher than the single patients (3.0%) Table 4.6.
Table 4.6: Syphilis exposure rates in relation to Social demographic of HIV patients attending Engineer county hospital.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Positive (n-12) (%)</th>
<th>Negative N (%)</th>
<th>Chi value</th>
<th>P value</th>
<th>OR</th>
<th>OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (4.1)</td>
<td>117 (95.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7 (2.7)</td>
<td>256 (97.3)</td>
<td>0.570</td>
<td>0.530</td>
<td>1.563</td>
<td>0.486-5.027</td>
</tr>
<tr>
<td><strong>Age(years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4</td>
<td>0 (0.0)</td>
<td>3 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-14</td>
<td>1 (2.9)</td>
<td>34 (97.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-24</td>
<td>1 (4.0)</td>
<td>24 (96.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-34</td>
<td>2 (2.6)</td>
<td>74 (97.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-44</td>
<td>5 (3.4)</td>
<td>140 (96.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-54</td>
<td>2 (2.6)</td>
<td>75 (97.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55-64</td>
<td>0 (0.0)</td>
<td>19 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65&gt;</td>
<td>1 (20.0)</td>
<td>4 (80.0)</td>
<td>5.681</td>
<td>0.577</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>6 (3.8)</td>
<td>151 (96.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>4 (3.0)</td>
<td>131 (97.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>1 (1.8)</td>
<td>56 (98.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>1 (2.8)</td>
<td>35 (97.2)</td>
<td>0.633</td>
<td>0.889</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>2 (8.7)</td>
<td>21 (91.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not employed</td>
<td>1 (1.8)</td>
<td>54 (98.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casuals</td>
<td>1 (1.9)</td>
<td>52 (98.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Business</td>
<td>3 (3.3)</td>
<td>87 (96.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmers</td>
<td>5 (3.0)</td>
<td>159 (97.0)</td>
<td>2.960</td>
<td>0.565</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>5 (2.6)</td>
<td>185 (97.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>4 (2.7)</td>
<td>142 (97.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>College</td>
<td>3 (6.3)</td>
<td>45 (93.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never w.sch</td>
<td>0 (0.0)</td>
<td>1 (100)</td>
<td>1.809</td>
<td>0.613</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
4.2.3.1 Patients co-infected with Syphilis and the HIV treatment they undergo

The patients Syphilis status was similarly evaluated against the patients HIV treatments. The result of this study established that out of the 12 confirmed Syphilis positive cases, the highest number, (58.3%) were patients under treatment with TDF/3TC/EFV. The other Syphilis positive patients were under treatment with TDF/3TC/NVP (41.7%) Table 4.7.

**Table 4.7:** A cross tabulation of the patients Syphilis status and the HIV treatment

<table>
<thead>
<tr>
<th>Syphilis status</th>
<th>HIV treatment</th>
<th>TDF/3TC/EFV</th>
<th>ABC/3TC/NVP</th>
<th>AZT/3TC/NVP</th>
<th>TDF/3TC/NVP</th>
<th>AZT/3TC/LP V/r</th>
<th>TDF/3TC/LP V/r</th>
<th>ABC/3TC/LP V/r</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve</td>
<td></td>
<td>7 (58.3%)</td>
<td>0 (0.0%)</td>
<td>5 (41.7%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>12</td>
</tr>
<tr>
<td>-ive</td>
<td></td>
<td>192 (51.5%)</td>
<td>31 (8.3%)</td>
<td>50 (13.0%)</td>
<td>93 (24.9%)</td>
<td>2 (0.5%)</td>
<td>1 (0.3%)</td>
<td>4 (1.1%)</td>
<td>373</td>
</tr>
</tbody>
</table>

Key: TDF-tenofovir disoproxil fumarate, 3TC- Lamivudine, EFV- efavirenz, ABC - Abacavir, NVP- Nevirapine, AZT-Azidothymidine, LPV-Lopinavir, LPV/r- lopinavir/ritonavir

4.3 To determine the risk factors associated with Hepatitis B, C and Syphilis co-infections among HIV infected patients attending Engineer county hospital comprehensive care clinics

4.3.1 Risk factors associated with Hepatitis B

Demographic risk factors associated with Hepatitis B include gender, age, marital status, education levels and occupation of the patients. Male patients (66.7%) are more at risk of
getting HBV than the female patients (33.3%). Patients in the ages of 45 – 54 years are more at risk of getting HBV (40.0%) than other ages. When considering the marital status of the patients, married patients are more at risk (60.0%) of getting HBV than single persons (13.2%) and other marital status. In this study, farmers were more at risk 73.3% than other occupations. Patients who had primary levels of education were established to be more at risk of getting HBV (60.0%) than other education levels (Table 4.8).

Table 4.8: Risk factors associated with Hepatitis

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Positive</th>
<th>Negative</th>
<th>χ² value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10 (66.7%)</td>
<td>112 (30.3%)</td>
<td></td>
<td>8.822</td>
</tr>
<tr>
<td>Female</td>
<td>5 (33.3%)</td>
<td>258 (69.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age(year)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>0 (0.0%)</td>
<td>4 (0.8%)</td>
<td></td>
<td>9.636</td>
</tr>
<tr>
<td>5 – 14</td>
<td>0 (0.0%)</td>
<td>35 (9.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 – 24</td>
<td>0 (0.0%)</td>
<td>25 (6.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 – 34</td>
<td>2 (13.3%)</td>
<td>74 (6.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 – 44</td>
<td>5 (33.3%)</td>
<td>140 (37.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 – 54</td>
<td>6 (40.0%)</td>
<td>71 (19.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55 – 64</td>
<td>1 (6.7%)</td>
<td>18 (4.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65+</td>
<td>1 (6.7%)</td>
<td>4 (1.1%)</td>
<td></td>
<td>6.107</td>
</tr>
</tbody>
</table>

| **Marital status** |          |          |          |         |
| Married            | 9 (60.0%) | 148 (40.0%) |          |        |       |
| Single             | 2 (13.2%) | 133 (35.9%) |          |        |       |
| Divorced           | 1 (6.7%) | 56 (15.1%) |          |        |       |
| Widowed            | 3 (20.0%) | 33 (8.9%) |          | 7.299 | 0.121 |

| **Occupation**     |          |          |          |         |
| Employed           | 0 (0.0%) | 23 (6.2%) |          |        |       |
| Not employed       | 0 (0.0%) | 55 (14.9%) |          |        |       |
| Casuals            | 2 (13.3%) | 51 (13.8%) |          |        |       |
| Business           | 2 (13.3%) | 88 (23.8%) |          |        |       |
| Farmers            | 11 (73.3%) | 153 (41.4%) |          |        |       |

| **Education**      |          |          |          |         |
| Primary            | 9 (60.0%) | 181 (48.9%) |          |        |       |
| Secondary          | 6 (40.0%) | 140 (37.8%) |          |        |       |
| College            | 0 (0.0%) | 48 (13.0%) |          |        |       |
| Never went to school | 0 (0.0%) | 1 (0.3%) |          | 2.363 | 0.501 |
4.3.1.1 Relationship between the patients’ demographic information and the HBV status
To establish the effect of gender, ages and education levels of the patients on HBV status of the patients, a Spearman rank correlation was done. The finding in this study showed that, male patients were significantly infected by HBV than female patients ($r = 0.151$, $P = 0.003$). Similarly, age of the patients was a significant factor in HBV infection of a patients ($r = -0.133$, $P = .009$). The negative correlation value indicates that the risk of HIV infection increase with advancedment in age. Education level of a patient did not have any significant effect on HBV infection ($r = 0.060$, $P = 0.236$). Correlation Table 4.9.

**Table 4.9: Correlation matrix showing factors affecting HBV infection**

<table>
<thead>
<tr>
<th></th>
<th>Confirmed HBV Elisa</th>
<th>Gender of respondent</th>
<th>Age of respondent</th>
<th>Education level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spearman's rho</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confirmed HBV Elisa r-value</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender of respondent r-value</td>
<td>.151**</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of respondent r-value</td>
<td>-.133**</td>
<td>.014</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.009</td>
<td>.790</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education level r-value</td>
<td>.060</td>
<td>.109*</td>
<td>-.201**</td>
<td>1.000</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.236</td>
<td>.033</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>385</td>
<td>385</td>
<td>385</td>
<td>385</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).
The effect of the patients gender and the ages (years) was therefore subjected to a linear regression analysis to fit in a model; \( Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \epsilon \)

Where: \( Y \) = Infection by HBV, \( \beta_0 \) is the constant, \( X_1 \) = effect of gender, \( X_2 \) = effect of age, \( \beta \) = beta coefficient, \( \epsilon \) = error term.

Using unstandardized beta coefficient therefore the effect of gender and age of the patients on HBV status was; \( Y = 1.945 + 0.066X_1 - 0.020X_2 \)

4.3.2 Risk factors associated with Hepatitis C

Patients’ risk factors associated with Hepatitis C were tested. These were gender, age, marital status, education levels and occupation of the patients. Female patients (66.7%) were more at risk of getting HCV than the male patients (33.3%). Patients in the ages of 25 – 34, 45 – 54 and 55 - 64 years were found to be more at risk of getting HCV (33.3%) and not in other ages. Divorced patients were more at risk (66.7%) of getting HBV than single persons (33.3%). The findings showed that farmers, casuals and business persons (33.3%) were at risk of getting HCV. Patients who had secondary levels of education were established to be more at risk of getting HCV (66.7%) than those having primary (33.3%) Table 4.10.
Table 4.10: Risk factors associated with Hepatitis C

<table>
<thead>
<tr>
<th>Gender</th>
<th>Positive</th>
<th>Negative</th>
<th>( \chi^2 ) value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1 (33.3%)</td>
<td>121 (31.7%)</td>
<td>0.004</td>
<td>1.000</td>
</tr>
<tr>
<td>Female</td>
<td>2 (66.7%)</td>
<td>261 (68.3%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age(years)</th>
<th>Positive</th>
<th>Negative</th>
<th>( \chi^2 ) value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4</td>
<td>0 (0.0%)</td>
<td>3 (0.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 – 14</td>
<td>0 (0.0%)</td>
<td>35 (9.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 – 24</td>
<td>0 (0.0%)</td>
<td>25 (6.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 – 34</td>
<td>1 (33.3%)</td>
<td>75 (19.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 – 44</td>
<td>0 (0.0%)</td>
<td>145 (38.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 – 54</td>
<td>1 (33.3%)</td>
<td>76 (19.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55 – 64</td>
<td>1 (33.3%)</td>
<td>18 (4.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65+</td>
<td>0 (0.0%)</td>
<td>5 (1.3%)</td>
<td>7.165</td>
<td>0.412</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Marital status</th>
<th>Positive</th>
<th>Negative</th>
<th>( \chi^2 ) value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>0 (0.0%)</td>
<td>157 (41.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>1 (33.3%)</td>
<td>134 (35.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>2 (66.7%)</td>
<td>55 (14.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>0 (0.0%)</td>
<td>36 (9.4%)</td>
<td>7.011</td>
<td>0.072</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Positive</th>
<th>Negative</th>
<th>( \chi^2 ) value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employed</td>
<td>0 (0.0%)</td>
<td>23 (6.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not employed</td>
<td>0 (0.0%)</td>
<td>55 (14.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casuals</td>
<td>1 (33.3%)</td>
<td>52 (13.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Business</td>
<td>1 (33.3%)</td>
<td>89 (23.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmers</td>
<td>1 (33.3%)</td>
<td>163 (42.7%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education</th>
<th>Positive</th>
<th>Negative</th>
<th>( \chi^2 ) value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>1 (33.3%)</td>
<td>189 (49.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>2 (66.7%)</td>
<td>144 (37.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>College</td>
<td>0 (0.0%)</td>
<td>48 (12.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never went to school</td>
<td>0 (0.0%)</td>
<td>1 (0.3%)</td>
<td>1.201</td>
<td>0.753</td>
</tr>
</tbody>
</table>

4.3.3 Risk factors associated with Syphilis

Female patients (58.3%) were more at risk of getting syphilis than the male patients (41.7%). Those in the ages of 35 – 44 years were found to be more at risk of getting syphilis (41.7%) than other ages. Married patients were more at risk (50.0%) of getting syphilis than single persons (33.3%). Farmers (41.7%) were found to be at risk of getting
syphilis more than patients who were doing business (25.0%) and those who were employed (16.7%). More of the patients who had primary education (41.7%) had a higher risk of getting syphilis (Table 4.11).

Table 4.11: Risk factors associated with Syphilis

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Positive</th>
<th>Negative</th>
<th>$\chi^2$ value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (41.7%)</td>
<td>117 (31.4%)</td>
<td>0.670</td>
<td>0.530</td>
</tr>
<tr>
<td>Female</td>
<td>7 (58.3%)</td>
<td>256 (68.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>0 (0.0%)</td>
<td>3 (0.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5–14</td>
<td>1 (8.3%)</td>
<td>34 (9.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15–24</td>
<td>1 (8.3%)</td>
<td>24 (6.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25–34</td>
<td>2 (16.7%)</td>
<td>74 (19.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35–44</td>
<td>5 (41.7%)</td>
<td>140 (37.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45–54</td>
<td>2 (16.7%)</td>
<td>75 (20.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55–64</td>
<td>0 (0.0%)</td>
<td>19 (5.1%)</td>
<td>5.681</td>
<td>0.577</td>
</tr>
<tr>
<td>65+</td>
<td>1 (8.3%)</td>
<td>4 (1.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>6 (50.0%)</td>
<td>151 (40.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>4 (33.3%)</td>
<td>131 (35.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>1 (8.3%)</td>
<td>56 (15.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>1 (8.3%)</td>
<td>35 (9.4%)</td>
<td>0.633</td>
<td>0.889</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>2 (16.7%)</td>
<td>21 (5.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not employed</td>
<td>1 (8.3%)</td>
<td>54 (14.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casuals</td>
<td>1 (8.3%)</td>
<td>52 (13.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Business</td>
<td>3 (25.0%)</td>
<td>87 (23.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmers</td>
<td>5 (41.7%)</td>
<td>159 (42.6%)</td>
<td>2.960</td>
<td>0.565</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>5 (41.7%)</td>
<td>185 (49.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>4 (33.3%)</td>
<td>142 (38.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>College</td>
<td>3 (25.0%)</td>
<td>45 (12.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never went to school</td>
<td>0 (0.0%)</td>
<td>1 (0.3%)</td>
<td>1.809</td>
<td>0.613</td>
</tr>
</tbody>
</table>
4.4 To determine the disease stage of HIV infected individuals attending Engineer county hospital.

In this study, the result from the patients, based on the WHO 2005, showed majority of the patients, 40.0% had severe immunosuppression 38.4%, advanced immunosuppression and 14.5%, mild immunosuppression while 7.0% had not significant immunosuppression.

![Figure 4.3: Patients levels of CD4 according to WHO (2005)](image)

4.4.1 Distribution of CD4 in the patients ages (years)

Among the patients who were less than 4 years old, 66.7% had severe immunosuppression while 33.3% were having advanced immunosuppression. Patients in the ages of 5 – 14 mainly had advanced immunosuppression (40.0%) same case to those in the ages of 15 – 24 years (48.0%) and those who were in the ages of 35 – 44 years (42.1%). Patients in the ages of 45 – 54 years and those in the ages of 55 – 64 years
mainly had severe immunosuppression (50.6% and 57.9% respectively). All the 5 patients who were older than 65 years had severe immunosuppression. This result showed a significant association of the patients’ ages and their CD4 levels ($\chi^2 = 35.096, 0.028$) Table 4.12.

**Table 4.12:** CD4 levels of different patient’s ages

<table>
<thead>
<tr>
<th>CD 4 Counts</th>
<th>&lt;4 (%)</th>
<th>5 – 14 (%)</th>
<th>15 – 24 (%)</th>
<th>25 – 34 (%)</th>
<th>35 – 44 (%)</th>
<th>45 – 54 (%)</th>
<th>55 – 64 (%)</th>
<th>65 &gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;500</td>
<td>0 (0.0%)</td>
<td>4 (11.4%)</td>
<td>2 (11.4%)</td>
<td>7 (9.2%)</td>
<td>8 (5.5%)</td>
<td>5 (6.5%)</td>
<td>1 (5.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>350 – 499</td>
<td>0 (0.0%)</td>
<td>10 (28.6%)</td>
<td>5 (20.0%)</td>
<td>16 (21.1%)</td>
<td>16 (11.0%)</td>
<td>9 (11.7%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>200 – 349</td>
<td>1 (33.3%)</td>
<td>14 (40.0%)</td>
<td>12 (48.0%)</td>
<td>29 (38.2%)</td>
<td>61 (42.1%)</td>
<td>24 (31.2%)</td>
<td>7 (36.8%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>&lt;200</td>
<td>2 (66.7%)</td>
<td>7 (20.0%)</td>
<td>6 (24.0%)</td>
<td>24 (31.6%)</td>
<td>60 (41.4%)</td>
<td>39 (50.6%)</td>
<td>11 (57.9%)</td>
<td>5 (100%)</td>
</tr>
</tbody>
</table>

$\chi^2$ value 35.096
P value 0.028

Progress of advanced immunosuppression and severe immunosuppression among the ages of the patients showed a significant increase in the percentage of the patients with an increase in age. Advanced immunosuppression cases among the different ages showed an increase with a gradient of $Y = 6.0976x + 21.586$ where $Y =$ percentage patients having advanced immunosuppression in the age group, $x =$ age group in years. On the contrary is the trend of the patients having severe immunosuppression where the result showed a decrease in the percentage of patients having severe immunosuppression in the ages, $Y =$
-3.519 + 49.536, where Y = percentage patients having severe immunosuppression in the age group, x = age group in years.

**Figure 4.4:** Advanced immunosuppression and severe immunosuppression cases among the individual ages of the patients

### 4.4.2 Distribution of CD4 levels among the patients’ gender

The levels of CD4 was significantly different ($\chi^2 = 12.319$, $P = 0.006$) in the gender of the patients. Among the female patients, 6.8% recorded a CD4 of $> 500$ and 44.1% recorded CD4 levels of 200 – 349. These levels were lower among the males where 7.4% recorded CD4 levels of $> 500$ and 26.2% recorded CD4 levels of 200 – 349 (Table 4.13).
Table 4.13: Levels of CD4 in the patients of different gender

<table>
<thead>
<tr>
<th>CD 4 levels</th>
<th>Male n = 122</th>
<th>Female n = 263</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;500</td>
<td>9 (7.4%)</td>
<td>18 (6.8%)</td>
</tr>
<tr>
<td>350 – 499</td>
<td>19 (15.6%)</td>
<td>37 (14.1%)</td>
</tr>
<tr>
<td>200 – 349</td>
<td>32 (26.2%)</td>
<td>116 (44.1%)</td>
</tr>
<tr>
<td>&lt;200</td>
<td>62 (50.8%)</td>
<td>92 (35.0%)</td>
</tr>
</tbody>
</table>

χ² value 12.319  P value 0.006

4.4.3 Correlation analysis on treatment and CD4 counts

From the study findings, majority of the patients were on first-line drugs with most of them being on TDF/3TC/EFV (55.6%) with seven patients on secondline. From the CD4 counts, equally majority had CD4 counts < 200 with least on >500 CD4 counts. The study evaluated whether treatment had any impact on CD4 counts. From the analysis, treatment had significant influence on the levels of CD4 counts χ²= 34.778, p- value 0.010 (Table 4.14).
Table 4.14: Correlation analysis on treatment and CD4 counts

<table>
<thead>
<tr>
<th>CD4</th>
<th>TDF/3TC/EFV</th>
<th>ABC/3TC/NVP</th>
<th>AZT/3TC/NVP</th>
<th>TDF/3TC/NVP</th>
<th>AZT/3TC/LPV/r</th>
<th>TDF/3TC/LPV/r</th>
<th>ABC/3TC/LPV/r</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 500</td>
<td>15 (55.6%)</td>
<td>3 (11.1%)</td>
<td>7 (25.9%)</td>
<td>1 (3.7%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>1 (3.7%)</td>
</tr>
<tr>
<td>350-499</td>
<td>38 (67.9%)</td>
<td>7 (12.5%)</td>
<td>5 (8.9%)</td>
<td>5 (8.9%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>200-349</td>
<td>68 (45.9%)</td>
<td>10 (6.8%)</td>
<td>23 (15.5%)</td>
<td>44 (29.7%)</td>
<td>0 (0.0%)</td>
<td>1 (0.7%)</td>
<td>2 (1.4%)</td>
</tr>
<tr>
<td>&lt; 200</td>
<td>78 (50.6%)</td>
<td>11 (7.1%)</td>
<td>15 (9.7%)</td>
<td>48 (31.2%)</td>
<td>2 (1.3%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

\( \chi^2 \) value 34.778

p-value 0.010

**Key:** TDF - tenofovir disoproxil fumarate, 3TC - Lamivudine, EFV - efavirenz, ABC - Abacavir, NVP - Nevirapine, AZT - Azidothymidine, LPV - Lopinavir, LPV/r - lopinavir/ritonavir, > 500 - not immunosuppressed, (350-499) - Mild immunosuppression, (200-349) - Advanced immunosuppression, < 200 - Severe immunosuppression

**4.4.4 Co infection prevalence by age and CD4 counts**

Most of the co infections were mostly in ages 25-34 (1.3%), 35-44 (2.6%) and 45-54 years (2.3%) and least was found on ages below 25 years and above 55 years. The overall
mean of CD4 was 227 counts per µl of blood and Cd4 was found to decline with advancement of age.

Table 4.15: Co infection prevalence of HBV, HCV, Syphilis among HIV patients by age and CD4 counts.

<table>
<thead>
<tr>
<th>Age group (yrs)</th>
<th>N=385</th>
<th>HBV</th>
<th>HCV</th>
<th>Syphilis</th>
<th>CD4 counts average</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>185.66</td>
</tr>
<tr>
<td>5 – 14</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>1 (0.26%)</td>
<td>349.9</td>
</tr>
<tr>
<td>15 – 24</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>1 (0.26%)</td>
<td>273.53</td>
</tr>
<tr>
<td>25 – 34</td>
<td>76</td>
<td>2 (0.52%)</td>
<td>1 (0.26%)</td>
<td>2 (0.52%)</td>
<td>276.64</td>
</tr>
<tr>
<td>35 – 44</td>
<td>145</td>
<td>5 (1.3%)</td>
<td>0</td>
<td>5 (1.3%)</td>
<td>241.35</td>
</tr>
<tr>
<td>45 – 54</td>
<td>77</td>
<td>6 (1.56%)</td>
<td>1 (0.26%)</td>
<td>2 (0.52%)</td>
<td>223.06</td>
</tr>
<tr>
<td>55 – 64</td>
<td>19</td>
<td>1 (0.26%)</td>
<td>1 (0.26%)</td>
<td>0</td>
<td>197.3</td>
</tr>
<tr>
<td>&gt;65</td>
<td>5</td>
<td>1 (0.26%)</td>
<td>0</td>
<td>1 (0.26%)</td>
<td>74.3</td>
</tr>
<tr>
<td>Total</td>
<td>385</td>
<td>15 (3.9%)</td>
<td>3 (0.78%)</td>
<td>12 (3.1%)</td>
<td></td>
</tr>
</tbody>
</table>

4.4.5 Correlation analysis on HIV viral load and CD4 counts

The study findings revealed that patients who had CD4 >500 had low undetectable levels of viral load with those with high detectable viral load being detected with low CD4 counts <200. Those detected with high viral load, it implied that they had > 1000 viral particled per microliter of blood (virological failiure) with those with LDL being equal or less than 20 µl of blood (total viral supression). Therefore, from these findings, two patients had a both immunological and virological failure in response to treatment. Nevertheless, two patients were detected with high viral load but with good immunological response (Table 4.15) Viral load was determined to find out if it had any
impact on the levels of CD4 counts. However, from the analysis, there was no any significant influence of viral load on levels of CD4 count $\chi^2 = 0.878$, $p$ value = 0.831.

**Table 4.16:** Correlation analysis on HIV viral load and CD4 counts

<table>
<thead>
<tr>
<th>CD4</th>
<th>LDL $&lt; 20 \mu l$ blood</th>
<th>HDL $&gt; 1000 \mu l$ blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>$&gt; 500$</td>
<td>27 (100.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>350-499</td>
<td>55 (98.2%)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>200-349</td>
<td>147 (99.3%)</td>
<td>1 (0.7%)</td>
</tr>
<tr>
<td>$&lt; 200$</td>
<td>152 (98.7%)</td>
<td>2 (1.3%)</td>
</tr>
</tbody>
</table>

$\chi^2$ value 0.878  
$P$-value 0.831

**Key:** LDL – Low detectable levels, HDL: High detectable levels, CD4 – Cluster of differentiation 4.
CHAPTER FIVE

5.1 DISCUSSION

5.1.1 Seroprofile

The prevalence of HIV/HBV co infection was found to be 3.9%, with males 2.6% and females 1.3%. These findings were similar to the research carried out in Ethiopia, (3.9%) (Shimeli et al., 2008) but higher than the levels detected among children attending HIV clinic in Tanzania, (1.2%) (Telatela et al., 2007) and Rwanda, (2.4%) (Pirillo et al., 2007) among HIV infected pregnant women. However, this finding was lower compared with other similar studies conducted in Kenya and other regions previously, such as (6.0%) (Muriuki et al., 2013), (8.8%) (Mutuma et al., 2011), (5.0%) (Chepkurui et al., 2015) and in Nigeria (19.8%) (Otegbayo et al., 2003), South Africa, (4.8%) (Firnhaber et al., 2008), (4.7%) (Belay et al., 2010) and in Tanzania, (17.3%) (Nagu et al., 2008).

The low levels of HBV/HIV co infections detected in this study, could be associated with its rural settings where farming such as dairy farming, horticulture is done. This makes people retreat on their farms according to Nyandarua HIV/AIDS strategic plan 2016 to 2020 keeping them busy compared to urban settings where most people are exposed to risk behaviors such as prostitution for financial favours. Equally, according to Kenya HIV County profile 2016, Nyandarua region has low population of individuals who engage in high risk behaviours like prostitution or sharing of needles by IDUs compared to urban settings such as Nairobi.
From this study, it was found that the risk of HBV infections was high among males than females. Males are known to visit social areas such as bars and are likely to engage with multiple partners and unprotected sex. Female were consistent with the levels being attributed to protective factor and resistance of hepatic cells to chronic liver disease (Baig, 2009).

By the location of the study site, the participants were mainly indigenous/ local inhabitants and therefore they could not engage in risk sexual behaviours and sharing of needles or sharp objects that could predispose them to HBV infection. The study engaged all including the health workers and other educated groups who could have been vaccinated against HBV. Equally, due to the culture and the conservative nature of the region, some practices such as sharing partners, homosexuality and intravenous drug use have been reported to be lower in Nyandarua (according to Kenya HIV County profile 2016) and this could have contributed to low detected prevalence of HBV. Nevertheless, the limited nature of sampled population of those who visited the facility, despite it serving a wide area of the Nyandarua County, may not be sufficient to present the sampled county.

HIV/HCV co infection prevalence was found to be 0.78% (males 0.26%, females 0.52%) which was closely similar to other studies that has been carried out in Zimbabwe, (0.8%) (Kallestrup et al., 2003), Kenya, (0.76%) (Kerubo et al., 2015), Ethiopia, (0.7%) (Belay et al., 2010), Gambia (0.6%) (Mboto et al., 2010) and Uganda, (0.6%) (Pirillo et al., 2007). Equally, the study findings was found to be consistent with the general prevalence on drug users in Kenya of (0.2-0.9%) (Muasya et al., 2008). However, the detected
prevalence was lower than those that have been done in Kenya previously (10.3%) (Muriuki et al., 2013), South Africa (1.0%), (Lodenyo et al., 2000), Nigeria (8.4%) (Ampofo et al., 2002), Malawi (4.5%) (Nyirenda et al., 2008) and Senegal, (1.6%) (Diop-Niaye et al., 2008). The lower prevalence could be associated with its rural area settings and possible limited influence of intravenous drug injection practices. Subsequently, the risk behaviours that are associated as high risk to HCV infection like prostitution and homosexuality are less common (Kenya HIV County profile 2016). This region being an agricultural zone, there is a likelihood of individuals being busy on the farms hence they may not be engaged in risk behaviour or loitering in town and bars (Nyandarua County HIV/AIDS strategic plan 2016-2020).

The HIV/Syphilis co infection prevalence was found to be 3.1%. The males had a prevalence of 1.3% and females 1.8%. This is higher than the national prevalence of Kenya (1.8%), (KAIS 2007), Tanzania (2.7%) and Uganda (2.0%) (HIV sentinel surveillance report 2008 (Malawi) and Uganda HIV/AIDS indicator survey 2011). The higher prevalence may have been attributed to condomless sex engaged by those already HIV infected. The HIV infection is mostly associated with high risk of sexual behavior which can also be a potential route of transmitting syphilis. Subsequently, the sample size could have been relatively smaller and may not reflect the real prevalence of entire nation. Syphilis being an ulcerative disease may predispose persons engaging on sexual activities hence predisposing them to other sexually transmitted infections like HIV (Rampalo et al., 2001). By virtue of this region being engaged in agricultural activities, there is high trading hence mix up of people from diverse areas of Kenya. This migration
of traders could contribute to some extent transmission of this infection between the locals and the foreigners during their sex interactions. Subsequently, devolution has led to emergency of towns and urban population is steadily growing. This could be a key driver of prostitution hence contributing to higher prevalence of Syphilis. The strategic location of Kinangop SubCounty, where Engineer Hospital is situated, neighbours major towns of Nakuru County such as Naivasha with high prevalence of HIV (5.3%), improved road network and could have likely facilitated the cross county transmission of HIV which predisposes Syphilis infection (Nyandarua County HIV/AIDS strategic plan 2016-2020).

Likewise, the majorities of the participants were of primary and secondary levels of education and could have limited knowledge regarding transmission. Subsequently, these findings was lower than of cohort aged (15-64) (16.9%) (KAIS 2007), 7.5%, 5.7% in Ghana and Cameroon (Nagalo et al., 2011). Also varied in relation to WHO (2012) which indicates 3.5% in females and 3.9% in males in an African region based survey. The lower rates could be associated with the social economic activity, with most of the study participant being engaged with farming and this attributes them to be busy. The study having been done in a rural setting, there was likelihood of getting lower prevalence in relation to urban setting (Muriuki et al., 2013). The participants were likely to be economically empowered and these could limit their engagement in exchange of sexual favours. The study participants were likely to get daily wages by working in farms and these reduces their chances of involvement in prostitution to earn their living. Equally, most of the study participants were locals and by nature of their locality they could limitedly participate in prostitution and multiplicity of partners. Men had low level of
infections compared to women. This observation could be attributed to the fact that men can work as casual laborious in farms and even in odd hours contributing to a certain level of sufficiency. However, women could not work odd hours due to probably young children and this makes them poor hence making them vulnerable to engage in other relations off marriage for financial favours. Nevertheless, women also do constitute large population of this County.

5.1.2 Risk factors

The study involved various variables which could influence the the establishment of HIV co infections. Gender as a variable was found to be a risk factor significantly influencing the acquisition of HBV with \( \chi^2 = 8.822, p = 0.008 \). The males were found to be at higher risk of getting HBV as compared with females. This was likely to happen as men could have sex with more than one partner (multiplicity). Also they are likely to engage in unprotected sex increasing chances of transmission and are related with other studies (Harania et al., 2008). Subsequently, the cumulative effect of HBV exposure risk increases with time. This was found to be consistent with age of < 25 years with less co infections compared with (35 – 44) and 45-54) years and was comparable with (Shimeli et al., 2008). However, age as a variable was found to have a relationship with HBV with \( r = -0.133, p = 0.009 \). Other variables such as marital status (married), jobs (farmers) and education (primary) were found to be risk factors but could not significantly influence the co infections of HBV.
Females of ages > 45 years were found to be at increased risk of getting infected with HCV compared to males. This findings was consistent with (Kerubo et al., 2015). However, age could not significantly be defined as a risk factor $\chi^2 = 0.004$, p= 1.000. Other variables such as gender, marital status, jobs and education were also found not to be significant with their p > 0.005

None of the variables were found to be a risk factor of Syphilis with their p= > 0.005. Nevertheless, the married and single participant were more infected with syphilis. This was likely of sharing partners and activeness on single participant in search of sexual satisfaction. Subsequently, more exposures likely as single participants engage in sex for financial favours. However, marital status could not significantly be associated as a risk factor of Syphilis $\chi^2= 0.633$ and p =0.889.

5.1.3 Disease stage

The WHO 2005 report states that “Clinical staging can be used effectively without access to CD4 or other laboratory testing”. It notes that CD4 testing is useful for determining the degree of immunocompromise and where CD4 facilities are available they should be used to support decision making. Data on CD4 levels are not a prerequisite for starting ART and should only be used in conjunction with consideration of the clinical stage. The report presents CD4 levels in relation to the severity of immunosuppression. It states that for clinical purposes, long term prognosis has been shown to be related to the highest or lowest ever value of CD4. It should be noted that the immunological staging of disease reverses with successful ART (Kuyper et al., 2004). Nevertheless,WHO 2015
recommends immediate treatment initiation to all confirmed HIV positive cases regardless of WHO 2005 on disease staging and CD4 counts (Eholie et al., 2016).

The rate of HBV, HCV and syphilis co infection increased with decreasing CD4+ T cell count, especially within ages of 25 to 54 years and patients with counts <200 cells/Ml (40%) and 200–349 cells/μL (38.4%) had significantly higher rate of HBV exposure compared with those in the range 350–499 cells/μL (14.5%) and > 500 cells/μL (7.01%). This might possibly be associated with the fact that patients with a lower level of immunity are commonly at increased risk of acquiring an infection since they are immunologically suppressed.

The CD4 levels decreased with the advancement of participant’s age. These was observed almost from age 5 - 65 years. The decrease of CD4 levels with age may be associated with infections and probably poor nutritional supipliments. However, age was found to have an association with CD4 level with $\chi^2=35.096$, $p=0.028$.

The women were found to have high levels of CD4 in relation to men especially on counts 200 -349 cell/μl of blood (44.1%) while male (26.2%). This was consistent with (Maini et al., 1996) women had quite higher CD4 counts compared with males even after they have been infected with HIV. Also supported by (Lugada et al., 2004) on immunologic analysis. Males are prone to various invasions not only by viral but also bacterial infection which can cause depletion of CD4 cells and women have their high normal ranges to return to after successful treatment. Similarly, gender was significantly associated with CD4 levels $\chi^2 =12.319$, $p= 0.006$. 
Treatment had significant influence on the levels of CD4 counts $\chi^2= 34.778$, p- value 0.010. The CD4+ T cells and viral loads are among the determinants that are monitored on HIV infected patients in response to treatment. They assess the treatment response hence guide whether there is drug resistance and drug switch done.

From this study, patients were categorized as per WHO disease staging (2005). In the study, patients who had the CD4+ T cells <500 cell/µl were mostly on first line drug regimen. This confirms that most patients were responding well to treatment and had successful drug adherence (98.96%) with viral copies (LDL) below 1000/µl of blood and four (1.04%) on drug failure HDL with viral copies > 1000/µl of blood. This could be an indicator of patient strict adherence to treatment and proper management. Consequently, adherence and virologic suppression have been found related (Kuyper et al., 2004). However, the study found no association between viral load and CD4 counts $\chi^2= 0.878$, p= 0.831.

### 5.2 Conclusions

i. The study revealed moderate prevalence of co infections of HBV (3.9%), Syphilis (3.1%) and low for HCV (0.78%) among the entire population of HIV persons attending Engineer hospital comprehensive care clinic.

ii. Gender was found to be risk factor to HBV infections with males being the most affected. In addition, across age, those of 45-54, were equally most affected. However, gender, age, immune status were not associated with any risk to HCV or syphilis infections.
iii. The disease stage of the HIV infected patients at the facility was advanced with average CD4 of 227 counts /µl of blood.

5.3 Recommendations

i. There is need to incorporate screening for HBV, HCV and syphilis on HIV infected individuals attending comprehensive care clinic for better management of these co infected patients.

ii. Gender, moreso males need to be frequently screened and treated when the have predisposed themselves to risk behaviors that can lead to acquisition of HBV.

iii. There is need for patients to adhere on drugs as these improves body immune system, hence assists to cumb spread of infections.
REFERENCE


Kenya (HIV) Human Immunodeficiency Virus, County profile 2016.


Nyandarua County, Human Immunodeficiency Virus / Acquired ImmunoDeficiency Syndrome strategic plan 2016-2020.


APPENDIX I: RESEARCH APPROVAL

FROM: Dean, Graduate School
TO: Kiarii Kiibe Daniel
     C/o Microbiology Department

DATE: 28th June, 2017
REF: 156/28670/2014

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

We acknowledge receipt of your revised Research Proposal as per our recommendations raised by the Graduate School Board of 17th May, 2017 entitled “Prevalence of Hepatitis B, C and Syphilis among HIV Patients Attending Engineer Level IV Hospital Comprehensive Care Clinics, Nyandarua County, Kenya”.

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology and Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking Forms per semester. The form has been developed to replace the Progress Report Forms. The Supervision Tracking Forms are available at the University’s Website under Graduate School webpage downloads.

Thank you.

EDWIN OBUNGU
DEAN, GRADUATE SCHOOL

Chairman, Microbiology Department

Supervisors:

1. Dr. Anthony Kebara
   C/o Microbiology Department
   Kenyatta University

2. Dr. John Maingi
   C/o Microbiology Department
   Kenyatta University
APPENDIX II: RESEARCH AUTHORIZATION

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

Our Ref: 156/28670/2014

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

DATE: 21st June, 2017

Director General,
National Commission for Science, Technology
& Innovation
P.O. Box 30623-00100,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR KIARIE KIBE DANIEL – REG. NO. 156/28670/2014

I write to introduce Mr. Kiarie Kibe Daniel who is a Postgraduate Student of this University. He is registered for M.Sc. degree programme in the Department of Microbiology.

Mr. Kibe intends to conduct research for a M.Sc Proposal entitled, “Prevalence of Hepatitis B,C and Syphilis among HIV Patients Attending Engineer Level IV Hospital Comprehensive Care Clinics, Nyandarua County, Kenya”.

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL
APPENDIX III: ETHICAL APPROVAL

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
    kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

Our Ref: KU/ERC/ APPROVAL/VOL.1 (166)  Date: 17th July, 2018

KIARIE KIBE DANIEL,
P.O Box 103,
North Kinangop

Dear Daniel,

APPLICATION NUMBER: PKU/821/1887 “PREVALENCE OF HEPATITIS B,C AND
SYPHYLLIS AMONG HIV PATIENTS ATTENDING ENGINEER LEVEL IV HOSPITAL
COMPREHENSIVE CARE CLINIC, NYANDARUA, COUNTY, KENYA”

1. IDENTIFICATION OF PROTOCOL
The application before the committee is with a research topic “Prevalence Of Hepatitis B,C And
Syphilis Among HIV Patients Attending Engineer Level Iv Hospital Comprehensive Care
Clinic, Nyandarua, County, Kenya” received on 20th February, 2018 and discussed on 12th June,
2018

2. APPLICANT

Kiari Kibe Daniel

3. SITE
Nyandarua County

4. DECISION
The committee has considered the research protocol in accordance with the Kenyatta University
Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee
Guidelines and APPROVED that the research may proceed for a period of ONE year from
12th June, 2018.
5. **ADVICE/CONDITIONS**

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.

ii. Serious and unexpected adverse events related to the conduct of the study are reported to this committee immediately they occur.

iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.

iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.

If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

![Signature]

26 JUL 2018

PROF. JUDITH KIMIYWE
CHAIRPERSON, ETHICS REVIEW COMMITTEE

I …………….. accept the advice given and will fulfill the conditions therein.

Signature……………….. Dated this day of…….26.………………… 2018.

cc.

DVC-Research Innovation and Outreach
APPENDIX IV: APPROVAL LETTER FROM ENGINEER HOSPITAL

COUNTY GOVERNMENT OF NYANDARUA
DEPARTMENT OF HEALTH SERVICES
ENGINEER COUNTY HOSPITAL
Email: engineercountyhospital@gmail.com. P.O Box 103-20318, North Kinangop.

1/7/2018

Kenyatta University
Department of Biochemistry, Micro-Biology and Biotechnology
School of Pure and applied Science
P.O. BOX 43844-00100
Nairobi

REF: RESEARCH

I wish to bring to your attention that Mr. Daniel Kiarii Kibe, ID 21617311 has been granted permission to carry out research on our Comprehensive Care Clinic clients from July 2018 until when he will have achieved the required sample size.

The research will involve screening Hepatitis B, C and Syphilis among the HIV infected patients. This will lead to him being granted a Master of Science degree in Medical Microbiology from Kenyatta University.

He should accord the highest degree of confidentiality and equally allow free will participation of the study participants.

I wish to recommend him.

Thanks.

Mr. K.I. Maguta
HAO.
Engineer County Hospital
APPENDIX V: QUESTIONNAIRE

CCC NO…………………

DATE…………………

FILL THE FOLLOWING:

(i) Sex……………………m/f

(ii) Actual age in years………

(iii) Education level (tick): never went to school/primary/secondary/college or university

(iv) Occupation ………………………


(v) Current drug regimen…………………

Sign………………………..Date………………
APPENDIX VI: PARTICIPANT WRITTEN CONSENT FORM

My name is Kiarie Kibe Daniel. I am a Master of Science (Medical Microbiology) student at Kenyatta University and I am conducting a research on title “Sero-profile, risk factors of Hepatitis B, C, Syphilis and disease staging among HIV patients attending Engineer level IV hospital comprehensive care clinics, Nyandarua County, Kenya”. The information obtained will be used in evaluating whether there is need to incorporate routine screening of Hepatitis B, C and Syphilis in HIV patients and improve on patients care and treatments.

**AIM of the study:** To determine the sero profile of Hepatitis B, C and Syphilis among HIV patients at Engineer level IV hospital comprehensive care clinics. Also determines the risk factors that are associated with the co infections and determine the immune status.

**Procedure:** To participate in these particular activity you will be asked some questions and be examined by clinician before you are sent to laboratory for blood sample to be collected. The amount of blood that will be collected is 5ml venous blood, put in EDTA tubes by a medical laboratory technologist and will be used to screen for Hepatitis B, C, Syphilis and immune status determination.

**Care and protection of research participant’s:** The skin on the puncture site (vein) will be disinfected using sterile 70% alcohol pads. You will feel minimal pain, needles and syringes used in the collection of blood will be safe and have never been used by anybody else.
Protection of research participant’s confidentiality. During interview high level of confidentiality will be maintained. All participants will be coded and no names will be used for references. The interviews will be conducted in an excluded room while administering the questionnaire including specimen collection.

Community considerations: During the research period, safety measures will be adhered to. Sterile 70% alcohol swabs will be used, use of one pair of glove per patient, proper segregation and disposal of waste through incineration to avoid access of infectious materials to the community. Also there will be no charges to every participant. The general information that will be collected will also be communicated to the community and way forwards on possible prevention and where to seek care will be communicated.

Willing to participate: Participant willingness to participate in the study will be highly appreciated and taken with great concern. Note that participation in these study is voluntarily and you are free to ask any questions regarding the study.

Benefits: The study will help me to know whether Hepatitis B, C and Syphilis co-infections are common among HIV infected population and the information obtained will help to recommend whether there is need to incorporate them as mandatory screening tests during clinics visits. Also can be used by policy makers in improving patients care
Withdrawal from participation: If in any way you feel not comfortable with the study, feel free to cancel your participation and once you have withdrawn no samples will be collected.

Questions/enquiry: In case you intend to ask any question or make enquiry on anything you don’t understand about the study, kindly contact: Kiarie Kibe Daniel, mobile no: 0721103315 or email: ariekibe@yahoo.com. or the ethical review committee secretariat Kenyatta university through, secretariat. kuerc@ku.ac.ke, chairman.kuerc@ku.ac.ke.

Your participation rights enquiries should be addressed to; the secretary, institute of research science and technology p. o box 43844 GPO Nairobi, Kenya. Tel 254-20-8710901

Participant declaration

I have read and understand the purpose of the study and the information regarding my participation is very clear .I have also been granted enough time to ask questions which have been answered to my satisfaction.

NOTE: Those below 18 years parents or guardians will consent on their behalf after reading, agreeing with them where required especially those who can read, write and understand the contents of the study. For the case of those who cannot understand parent/guardian will have final say either to participate or decline.

(Tick): agree to participate/ not agree to participate.
Signature/thumbprint............................................................Date...........

Interviewer:

signature......................................Date......................................................