PREVALENCE OF HSV-2, SYPHILIS AND HEPATITIS B IN HIV-1 INDIVIDUALS IN SELECTED HEALTH FACILITIES IN NAIROBI, KENYA

BY
KHAYOTA GRACE N. TAGO (B.ED SC.)
REGISTRATION NUMBER: 156/CE/15668/05

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (MICROBIOLOGY) OF KENYATTA UNIVERSITY

JUNE 2012
DECLARATION

THIS THESIS IS MY ORIGINAL WORK AND HAS NOT BEEN PRESENTED FOR A DEGREE IN ANY OTHER UNIVERSITY

KHAYOTA GRACE N. TAGO: I56/CE/15668/05

DEPARTMENT OF PLANT AND MICROBIAL SCIENCES

Signature……………………… Date……………………

WE CONFIRM THAT THE CANDIDATE UNDER OUR SUPERVISION CARRIED OUT THE WORK REPORTED IN THIS THESIS

SUPERVISORS:

PROF. PAUL OKEMO Signature……………………… Date……………………

DEPARTMENT OF PLANT AND MICROBIAL SCIENCES

KENYATTA UNIVERSITY

DR. SAMOEL KHAMADI Signature……………………… Date……………………

KENYA MEDICAL RESEARCH INSTITUTE (KEMRI)
DEDICATION

I dedicate this work to my husband Vincent and children David and Mercy Tago. I also dedicate this work to my mother Dinah, my siblings and to the memory of my late father William N. Khayota.
ACKNOWLEDGEMENTS

The successful completion of this work was possible due to the assistance of a number of individuals and institutions. I shall always be indebted to the Kenya Medical Research Institute for allowing me to carry out my project in the Institute’s laboratories.

I am especially indebted to my supervisors, Prof. Paul Okemo for his assistance and advice throughout the program. Dr. Samoel Khamadi for his support and assistance during the research project at KEMRI. Both offered invaluable guidance and encouragement.

I am grateful to other faculty members of the Plant and Microbial Sciences including Dr. Ethel Monda, Dr. Grace Gatheri, Dr. Cheruyot Joseph Mafura for their encouragement.

I acknowledge the staff at KEMRI for allowing me access and assistance in the use of the equipment in the CD4 and HIV laboratories. I am especially grateful to Ms. Glennah Kerubo for her assistance with the technical aspects in the labs. T. Shikokoti of AHS, thank you.

Special gratitude to my husband Vincent Juma Tago, son David Tago and daughter Mercy Nasike Tago for their overwhelming support and encouragement. To my brothers and sisters especially Dr. Mourice Khayota and Dr. Beatrice Khayota for their undying faith in my abilities. To my mother Dinah Khayota; a woman whose strength I am still to find in any other for her constant reminder of what should be done. To my greatest fan my late father, William Khayota who passed on at the height of my data collection. He dedicated his life to guiding his children in achieving their best.

My friends, colleagues and students of Alliance H. School have always been a source of encouragement. My Christian friends have been of great help when the going was not easy.

Above all God has granted me great mercy and His grace has been sufficient for me throughout the course.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATIONS</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
<td>xii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xiv</td>
</tr>
</tbody>
</table>

## CHAPTER ONE: INTRODUCTION

1.1 Background

1.2 Problem statement

1.3 Objectives

1.3.1 General objective

1.3.2 Specific objectives

## CHAPTER TWO: LITRATURE REVIEW

2.1 The Human Immunodeficiency Virus

2.1.1 Structure of HIV

2.1.2 Genome organization

2.1.3 Infection by HIV

2.1.4 Transmission of HIV

2.1.5 Diagnostic tests for HIV
2.4.3 Clinical manifestation of HBV.................................................................31
2.4.4 Diagnosis of HBV.............................................................................31
2.4.5 HBV and HIV-1 co-infection............................................................32

CHAPTER THREE: MATERIALS AND METHODS......................................36
3.1 Study sites.........................................................................................36
3.2 Sample size.......................................................................................38
3.3 Sampling techniques .........................................................................38
3.4 Sample collection...............................................................................39
3.5 Laboratory procedures.......................................................................38
3.5.1 CD4 Enumeration using BD FACSCalibur....................................38
3.5.2 HSV-2 testing................................................................................40
3.5.3 Syphilis testing using the QuickTest™ Syphilis Serum/Plasma/Whole blood strip.................................................................42
3.5.4 HBV testing....................................................................................41
3.5.4.1 Confirmation of HBV using DRG ELISA kit for HBsAg..........42
3.6 Data management.............................................................................42

CHAPTER FOUR: RESULTS....................................................................45
4.1 Bio-data of individuals sampled.....................................................45
4.1.1 Gender..........................................................................................45
4.1.2 Ages of the respondents...............................................................46
4.1.3 Gender/Age correlation...............................................................46
4.2 CD4 status of sampled population................................................46
4.2.2 Mean CD4 counts.......................................................................47
4.2.3 CD4 counts and age groups

4.3 HSV-2 infection among HIV-1 positive individuals

4.3.2 Gender and HSV-2 infection

4.3.3 Ages of respondents and HSV-2 infection

4.3.4 Comparison HSV-2 and CD4 counts

4.3.4.2 HSV-2 infections among HIV-1 positive (CD4<250µL) samples

4.3.4.3 HSV-2 infections among HIV-1 positive (CD4>250µL) samples

4.3.4.4 HSV – 2/HIV – 1 co-infection rates between samples with CD4 counts < 250 µL and those with CD4 >250 µL

4.4 Syphilis and HIV-1 co-infection

4.4.2 Gender and Syphilis infection

4.4.3 Syphilis infections and ages of the respondents

4.4.4 Comparison between Syphilis infection to CD4 counts

4.5 HBV and HIV-1 co-infection

4.5.2 Gender and HBV infection

4.5.3 Ages of the respondents and HBV infections

4.5.4 Comparison between HBV to CD4 counts

4.6 Comparison between Herpes simplex Virus, syphilis and Hepatitis B infection

4.6.2 Comparison between HSV-2 to Syphilis infection

4.6.3 Comparison between HSV-2 and HBV infection

4.6.4 Co-infection of Syphilis and HBV

CHAPTER FIVE: DISCUSSION CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction
5.1.2 Bio-Data of the individuals sampled.................................................................62
5.1.2.1 Gender and HIV-1 infection........................................................................62
5.1.2.2 Ages of respondents..................................................................................62
5.1.2.3 Gender/Age correlation..........................................................................63
5.1.3 CD4 status of the sampled population.........................................................63
5.1.4 HSV-2 infections among HIV-1 positive individuals.................................64
5.1.4.2 Gender and HSV-2 infection......................................................................65
5.1.4.3 HSV-2 infections and CD4 counts among HIV-1 positive samples..........66
5.1.5 Syphilis and HIV-1co-infections .................................................................66
5.1.6 HBV and HIV-1co-infections ......................................................................67
5.1.7 Comparison between Herpes simplex virus, syphilis and Hepatitis B infection....69
5.2 Conclusions ......................................................................................................69
5.3 Recommendations..........................................................................................71

REFERENCES .....................................................................................................72
LIST OF FIGURES

Figure 1: Structure of HIV........................................................................................................................................8
Figure 2: The HIV replication cycle..........................................................................................................................10
Figure 3: Map of Counties of Kenya..........................................................................................................................36
Figure 4: A detailed map of the Nairobi county showing the study sites.................................................................37
Figure 5: Age distribution of the sampled population.................................................................................................46
Figure 6: Mean CD4 counts in the health centres........................................................................................................48
Figure 7: CD4 Count and age groups..........................................................................................................................49
Figure 8: Ages of the respondents and HSV-2 infections.............................................................................................51
Figure 9: Distribution of syphilis infection in the health facilities...................................................................................54
Figure 10: Ages of the respondents and Syphilis infections..........................................................................................55
Figure 11: Distribution of HBV in the health facilities..................................................................................................56
Figure 12: Ages of the respondents and HBV infections..............................................................................................57

LIST OF TABLES

Table 1: Gender distribution of the sampled population............................................................................................45
Table 2: Distribution of the sampled population CD4 status in the centres.................................................................47
Table 3: HSV – 2 infections among HIV – 1 positive individuals..................................................................................49
Table 4: Gender and HSV-2 infections........................................................................................................................50
Table 5: HSV-2 and CD4 counts...................................................................................................................................51
Table 6: HSV–2 infections among HIV–1 positive (CD4 < 250/µL) samples...............................................................52
Table 7: HSV – 2 infection among HIV – 1 positive (CD4 > 250/µL) samples..............................................................53
Table 8: Gender and Syphilis infections.........................................................................................................................54
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>CD4 status and their percentage infection by syphilis</td>
<td>56</td>
</tr>
<tr>
<td>10</td>
<td>Gender and HBV infections</td>
<td>57</td>
</tr>
<tr>
<td>11</td>
<td>HBV and CD4 counts</td>
<td>58</td>
</tr>
<tr>
<td>12</td>
<td>HSV-2 and syphilis coinfection</td>
<td>59</td>
</tr>
<tr>
<td>13</td>
<td>HSV-2 and HBV infection</td>
<td>59</td>
</tr>
<tr>
<td>14</td>
<td>HBV and syphilis infection</td>
<td>60</td>
</tr>
</tbody>
</table>
ABBREVIATIONS

AIDS       Acquired immunodeficiency syndrome
anti-HBe   Anti-hepatitis B core antigen
CD4       T4 Helper HIV receptor cells
CDC        Centre for disease control
CSF        Cerebrospinal fluid
ELISA      Enzyme-linked immunosorbent assay
FTA-ABS    Fluorescent treponemal antibody absorption test
HAART      Highly active antiretroviral therapy
HBV        Hepatitis B Virus
HBeAg      Hepatitis B e-antigen
HBsAg      Hepatitis B surface antigen
HIV        Human immunodeficiency virus
HSV-1      Herpes simplex virus type 1
HSV-2      Herpes simplex virus type 2
KEMRI      Kenya Medical Research Institute
KDHS       Kenya’s Demographic and Health Survey
NASCOP     Kenya National AIDS and STI Control Programme
NHANES     National Health and Nutrition Examination Survey
NNRTIs     non-nucleoside reverse transcriptase inhibitors
PEPFAR     The U.S. President’s Emergency Plan for AIDS Relief (PEPFAR)
PCR        Polymerase Chain Reaction
RPR        Rapid plasma reagin
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>STD</td>
<td>Sexually transmitted diseases</td>
</tr>
<tr>
<td>STI</td>
<td>Sexually transmitted infections</td>
</tr>
<tr>
<td>TPI</td>
<td><em>Treponema pallidum</em> immobilization reaction</td>
</tr>
<tr>
<td>UNAIDS</td>
<td>United Nations Programme on HIV/AIDS</td>
</tr>
<tr>
<td>VDRL</td>
<td>Venereal Disease Research Laboratory</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organisation</td>
</tr>
</tbody>
</table>
ABSTRACT

HIV infection continues to be among the leading causes of global mortality. Genital ulcer disease increases the risk of HIV infection. Ulcerative STDs such as Herpes simplex virus type 2 (HSV-2) and syphilis cause breaks in the skin or mucous membranes, disrupt barriers that provide protection against HIV-1 infections. It has been shown that HSV-2 is the single most important sexually transmitted infection that is fueling the HIV epidemic in Africa. There is also an estimated two to fivefold increased risk of acquiring HIV infection when syphilis is present. Globally, co-infection with Hepatitis B Virus (HBV) and HIV-1 is becoming common and a growing public health concern because both viruses share similar transmission routes. Prevalence rates of HSV-2, HBV and syphilis in HIV-1 infected patients in Kenya are scanty. In this study, we sought to determine the prevalence of HSV-2, HBV and syphilis in the same HIV-1 infected persons in four health facilities in Nairobi. CD4 counts were determined using BD FACSCalibur, HSV-2 infection rates using HSV-2 ELISA tests; Syphilis tests were done using Ultra Rapid Test while the HBV test involved using the Hepanostika® HBsAg Ultra kit. Results obtained from the study will provide additional avenues into the fight against HIV infections. Data generated in this study was analysed using statistical package INSTAT. This study found the prevalence of HSV in the sampled population to be 54.2%. The statistical strongly indicated close association between HIV-1 and HSV-2 infections amongst the sampled population. There was indication that being HSV-2 positive did not necessarily affect ones CD4 count. This study showed low prevalence of syphilis and HBV at 2.2% and 4.5% respectively indicating that syphilis and HBV may not be fuelling the HIV-1 pandemic in the sampled population. There was no sample that tested positive for all the three infections (HSV-2, Syphilis and HBV) nor any sample tested positive for both HBV and Syphilis. This indicated that infection by any one of the three STIs (HSV-2, Syphilis and HBV) does not necessarily predispose one to higher risk of getting infected by any of the other two STIs.
CHAPTER 1

INTRODUCTION

1.1 Background

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS). HIV infection is considered pandemic by the World Health Organization (WHO). As of 2010 UNAIDS and WHO estimated approximately 34 million people have HIV with an annual mortality of three million. This makes it one of the most destructive pandemics in recorded history (UNAIDS 2011).

The HIV-1 epidemic is much higher in Africa than in the western countries. Many parts in Africa have greater incidence of HIV-1 infection and transmission than comparative rates in Europe and North America and this is having a devastating effect on the continent (Rockstroh et al., 2008). Sub-Saharan Africa is the region most affected. In 2010, an estimated 68% (22.9 million) of all HIV cases and 66% of all deaths (1.2 million) occurred in this region (UNAIDS 2011). The impact of HIV/AIDS, and other sexually transmitted infections (STIs), on society in Africa is immense, placing great strain on resources and undermining already fragile economies. Transmission rates of HIV-1 are higher and this is in part associated with other STIs acting as cofactors. In Africa, STIs are highly prevalent and it is important to understand their relationship with HIV-1 (Mayaud, 2007).

Sexually transmitted infections (STI), especially those causing genital ulcers, are associated with increased sexual transmission of HIV-1 (Wald and Link, 2002). Genital ulcerations and inflammation caused by STDs are implicated as cofactors for acquiring or transmitting HIV-1 infections (Royce et al., 1997).
Infections with *Herpes simplex* virus type 2 (HSV-2) is a major cause of genital ulcers worldwide and has been found to enhance HIV acquisition (Dickerson *et al.*, 1996). Recent studies have highlighted the synergy between HIV-1 and HSV-2 (Corey *et al.*, 2004, Wald and Link, 2002). The trajectories of HIV-1 and HSV-2 incidence and prevalence contrast with the decline of bacterial STIs in sub-Saharan Africa (Mayaud, 2007). Approximately 45 million people in the US have HSV-2 infection with 1 million new cases each year (Corey *et al.*, 2004).

A study among fishermen along Lake Victoria in Kenya recorded an HSV-2 prevalence of 63.9% among heterosexual men who were HIV-1 positive (Ng’ayo *et al.*, 2008). The epidemiological overlap between HSV-2 and HIV-1 is substantially larger than that of bacterial STIs with HIV-1. This is supported by the epidemiological data that indicates that globally HIV-1 and HSV-2 have overlapping prevalence patterns. It is estimated that 50–90% of HIV-1 infected individuals are co-infected with HSV-2 compared with approximately 20% of HIV-negative individuals. The prevalence of HSV-2 shedding is 4–5 times greater in HIV-1 infected individuals than in HIV-2 infected individuals (Clark *et al.*, 2008).

HSV-2 appears to be more prevalent in Africa than previously believed, though few data are available, as many people do not present in the hospital. In a study of the effects of STIs on HIV incidence a baseline seroprevalence rate of 72.7% was detected among HIV-negative women in Kenya (Kaul *et al.*, 2004), while a 43.5% incidence rate was reported in Uganda (Wawwer *et al.*, 1999). HSV-2 has been shown to have an increasingly important role in HIV spread in African populations. Results suggest that HSV-2 is responsible for a large proportion of new HIV infections in Africa (Freeman *et al.*, 2007), highlighting the potential impact of herpes control for HIV prevention.
On the other hand, there are over 12 million cases of syphilis globally with 155,000 deaths each year (Scherbaum et al., 2005). The incidence and prevalence of syphilis differ due to region, ethnic factors, gender and socio-economic factors. Reports indicate that syphilis incidence has been rising in the U.S. leading some epidemiologists to suspect that similar increases in HIV-1 infection may not be far behind (Fenton et al., 2001).

In the developed world, syphilis infections were in decline until the 1980s and 1990s due to widespread use of antibiotics. Since the year 2000, rates of syphilis have been increasing in the US, UK, Australia and Europe primarily among men who have sex with men (Stamm 2010) This is attributed to unsafe sexual practices Oral sex has been implicated in 14% of syphilis infections in a large U.S. city (CDC 2004).

In the United States, health officials reported over 36,000 cases of syphilis in 2006, including 9,756 cases of primary and secondary syphilis persons who were 20 to 39 years of age (CDC 2006). The incidence of primary and secondary syphilis was highest in women, 20 to 24 years of age and in men, 35 to 39 years of age. Although 1990 marked a peak in the U.S. incidence of syphilis since the introduction of penicillin, the new millennium began with more promising trends. An all-time low case rate was recorded in 2000, prompting the CDC to develop a plan to eliminate syphilis in the U.S (CDC, 2006).

Epidemiological studies demonstrate that syphilis, particularly genital ulcers associated with primary syphilis, are associated with an increased risk of HIV-1 acquisition (Darrow et al., 1997). Case reports suggest that coexisting HIV-1 infection may alter the natural history of syphilis. Notable are those presenting with multiple or deeper chancres and overlap of primary- and secondary-stage features of syphilis in co-infected patients (Rolfs et al., 1997).
The genital ulcers caused by syphilis can bleed easily, and when they come into contact with oral and rectal mucosa during sex, increase the infectiousness of and susceptibility to HIV-1 (CDC, 2006). In a study among patients seen in an STD clinic, patients with HIV-1 infection were more likely than HIV-1 negative individuals to present with signs and symptoms of secondary syphilis. They were more likely to have chancre still present at the time of secondary syphilis diagnosis (Hutchinson and Hook, 1994).

Globally an estimated 350–400 million people are chronically infected with Hepatitis B Virus (HBV) (Lee, 1999, Burnett et al., 2005). Out of the 400 million chronic carriers of HBV, 50 million are estimated to reside in sub-Saharan Africa (Apurva et al., 2007). Although HBV prevalence varies widely across the continent, hepatitis B surface antigen (HBsAg) positivity is estimated at 8-20%, while 70-95% has previously been exposed to infection. In Kenya, HBV is probably highly prevalent as it is the main cause of liver cancer (Okoth, 1990).

HIV and HBV infections are two major viral infections worldwide (Umolu et al., 2005). Co-infection with HBV and HIV-1 is becoming common and a growing public health concern because both viruses share similar transmission routes (Otedo, 2004, Sheng et al., 2007, Shimelis et al., 2008). In HIV-1-infected individuals, HBV infection prevalence is approximately ten times higher than in the general population (Pereira et al., 2006). Individuals with HIV-1 who contract acute hepatitis B are more likely to develop chronic hepatitis B than individuals who contract acute hepatitis B without HIV-1. Chronic HBV infection appears to have its natural progression modified by HIV-1 co-infection, with significantly increased morbidity and mortality attributable to liver disease in co-infected patients, compared with those with HBV alone (Shimelis et al., 2008).
Decreased clearance rates of HBsAg and HBeAg, reactivation of HBV, higher levels of HBV-DNA, and lower ALT levels are some of the most important findings that differentiate HIV-1-HBV co-infected individuals from those infected only with HBV (Sheng et al., 2007).

1.2 Problem Statement

HIV-1 infection in humans is one of the most destructive pandemics in recorded history. The impact of HIV and AIDS, on society in Africa is immense, placing great strain on resources and undermining already fragile economies. Ulcerative STDs that cause sores, ulcers, or breaks in the skin or mucous membranes, disrupt barriers that provide protection against HIV-1 infections. Infection with HSV-2, syphilis and HBV has been shown to increase the risk of HIV-1 acquisition. These infections lead to proliferation of CD4 cells which enhances HIV-1 replication. On the other hand, as the body’s immunity is lowered, STI infections are enhanced and severity increased. Understanding the proportion of new HIV-1 infections attributable to these STIs and the role they play in fanning the HIV-1 pandemic in Kenya will provide insight into current epidemic dynamics. Knowledge of this may be used in designing and evaluating future HIV-1 interventions.

1.3 Objectives

1.3.1 General objective

To determine the prevalence of HSV-2, Syphilis and Hepatitis B among HIV-1 positive individuals seeking CD4 testing services at selected health facilities in Nairobi.
1.3.2 Specific Objectives

1. To determine HSV-2 prevalence among HIV-1 positive immunocompromised persons from selected health facilities in Nairobi with CD4 counts above and below 250/µL.

2. To compare HSV-2/HIV-1 co-infection rates between individuals with CD4 counts <250/µL and those with CD4 counts >250/µL.

3. To determine the prevalence of syphilis and hepatitis B among these HIV positive immuno-compromised persons.

4. To determine the co-relation between HIV-1, Herpes simplex Virus, syphilis and Hepatitis B.
CHAPTER TWO

LITERATURE REVIEW

2.1 The Human immunodeficiency virus

Human immunodeficiency virus (HIV) is a lentivirus that causes acquired immunodeficiency syndrome (AIDS). This is an RNA virus that belongs to a family of viruses known as retroviruses. The name comes from the fact that these viruses can convert their RNA into a DNA copy using an enzyme known as reverse transcriptase (Taylor et al., 2004). HIV is a highly variable virus which mutates very readily. This means there are many different strains of HIV, even within the body of a single infected person.

HIV infects vital cells in the human immune system such as helper T cells (specifically CD4+ T cells). HIV infection leads to low levels of CD4+ T cells through three main mechanisms: First, direct viral killing of infected cells; second, increased rates of apoptosis in infected cells; and third, killing of infected CD4+ T cells by CD8 cytotoxic lymphocytes that recognize infected cells. When CD4+ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections (Cunningham et al., 2010).

There are two types of HIV: HIV-1 and HIV-2. Both types are transmitted by sexual contact, through blood, and from mother to child, and they appear to cause clinically indistinguishable AIDS. However, it seems that HIV-2 is less easily transmitted, and the period between initial infection and illness is longer in the case of HIV-2. The predominant virus worldwide is HIV-1 (CDC, 2006). The HIV-2 type is relatively uncommon and is concentrated in West Africa and rarely found elsewhere. Generally when people refer to HIV without specifying the type of virus they will be referring to HIV-1 (Taylor et al., 2004).
2.1.1 Structure of HIV

HIV is approximately 120 nm in diameter (120 billionths of a meter; about 60 times smaller than a red blood cell) and roughly spherical. The virus comprises a coat of fatty material known as the viral envelope on the exterior. About 72 little spikes, formed from the proteins gp120 and gp41, project from this coat. A layer called the matrix lies just below the viral envelope and is made from the protein p17. The viral core (or capsid) is usually bullet-shaped and is made from the protein p24. Inside the core are three enzymes required for HIV replication called reverse transcriptase, integrase and protease. The HIV’s genetic material is held within the core, consisting of two identical strands of RNA (Figure 1).

![Figure 1: Structure of HIV](http://wwwpp.uwrf.edu/~kk00/hivvector/hivvector.htm 2008)

2.1.2 Genome organization

There has been extensive research on the genome and proteins of HIV since the discovery of the virus in 1983.
It is a well known fact that no two HIV genomes are the same, not even from the same person, causing some to speculate that HIV is a "quasispecies" of a virus (http://en.allexperts.com/e/h/hi/hiv_structure_and_genome.htm 2009)

2.1.3 Infection by HIV

The HIV can only survive and be transmitted in body fluids. HIV can also only replicate inside human cells and can be passed from person to person through body fluids which include sexual fluids, blood and breast milk. Once inside the body, the viral particle bumps into a cell that carries on its surface a special protein called CD4.

The spikes on the surface of the virus particle stick to the CD4 and allow viral envelope to fuse with the cell membrane, or enter the lymphocyte by endocytosis. The viral RNA of the HIV particle is then injected into the cell cytoplasm, leaving the envelope behind outside the host cell (http://www.tthhivclinic.com/overview_asympt.htm 2008).

The HIV enzyme reverse transcriptase converts the viral RNA into DNA, which is compatible with human genetic material in a process called reverse transcription. The DNA formed is spliced into the human DNA in the host cell’s nucleus by the HIV enzyme integrase, thus it becomes a permanent part of the cell genome and every time the human cell divides, so does the viral DNA (Taylor et al., 2004). Once integrated, the HIV DNA is known as a provirus and it may lie dormant within a cell for at least six years. This is the stage called the latency period, but may be revived to treat human genes in much the same way as HIV genes when the cell becomes activated. Through transcription it converts the genes into messenger RNA among them complete copies of HIV genetic material. It utilises human enzymes in this process. The messenger RNA is transported outside the nucleus, and is used for producing new HIV proteins and enzymes during the process of translation (Taylor et al., 2004).
The HIV genetic material together with newly made HIV proteins and enzymes are assembled forming new viral particles, which are then released from the cell through budding. The enzyme protease plays a vital role at this stage of the HIV life cycle. Long strands of protein are chopped up into smaller pieces, which are used to construct mature viral cores. These are ready to infect another cell and repeat the process all over again. The virus spreads quickly through the human body and the person can pass HIV on to others in their bodily fluids.

**Figure 2:** The HIV replication cycle

The CD4 count is used in combination with the viral load test, which measures the level of HIV in the blood, to determine the outlook of the disease. The count indicates how strong one's immune system is, how far HIV disease has advanced (the stage of the disease), and helps predict the risk of complications and debilitating infections. The CD4 count is most useful when it is compared with the count obtained from an earlier test (http://www.labtestsonline.org/understanding/analytes/cd4/test.html 2010).

CD4 antigens on T4 cells is the major receptor for both HIV-1 and HIV-2. T4 cells are, not surprisingly, the major cell type that is infected by the virus causing immuno-suppression. Infected CD4+ T4 helper cells become targets for HIV-specific CD8+ killer cells but also die from a variety of other causes including lyses.

During the early acute infection stage, mostly mucosal CD4+ T4 cells are lost, while during chronic infection which may last many years, CD4+ T4 cells generally proliferate and die as a result of immune activation and other factors. The overall consequence is the inability of the body to fight opportunistic infections (http://pathmicro.med.sc.edu/lecture/hiv7.htm 2009).

2.1.4 Transmission of HIV

HIV can only be transmitted in certain ways. Outside the body fluids, HIV can barely survive for a few minutes. It thrives in blood and other body fluids such as semen and vaginal fluids. To be infected with HIV, there is need to allow some body fluid from an infected person to get inside the body of one who is not infected. The virus can enter the body via contact with the bloodstream or by passing through delicate mucous membranes, such as inside the vagina, rectum or urethra (CDC, 2003). The most common ways of transmission include having unprotected sexual intercourse with an infected partner, blood transfusion using infected blood.
Others include injecting drugs using a needle or syringe that has been used by someone who is infected and as a baby of an infected mother, during pregnancy, labour or delivery, or through breastfeeding.

Others include infection in health-care settings and through cuts with HIV contaminated instruments. There are ongoing debates on whether HIV can be transmitted through kissing, sneezing, coughing, sharing utensils, or through insect bites (CDC, 2003).

2.1.5 Diagnostic Tests for HIV

Initial tests for HIV are usually conducted using the EIA (or ELISA) antibody test or a rapid antibody test. EIA tests which can detect either one or both types of HIV have been available for a number of years.

According to the US Centres for Disease Control and Prevention, current HIV-1 EIAs "can accurately identify infections with nearly all non-B subtypes and many infections with group O HIV subtypes" (MMWR, 2001).

Rapid tests which can produce a result in less than an hour - are becoming increasingly popular. Most modern rapid HIV-1 tests are capable of detecting all the major subtypes of group M. Rapid tests which can detect HIV-2 are also now available (Phillips, 2000).

2.1.6 Prevalence of HIV

The number of people living with HIV has risen from around 8 million in 1990 to 33-40 million today (UNAIDS, 2009). So far more than 25 million people have died of AIDS since 1981 (UNAIDS, 2006). There are over 9.5 million people in immediate need of life-saving AIDS drugs in the developing countries. Of these, only 4 million (42%) are receiving the drugs (http://www.avert.org/worldstats.htm).
Global estimates of HIV/AIDS statistics indicated that by the end of 2008, there were about 33.4 million people living with HIV/AIDS; of these, 31.3 million were adults, while women accounted for 50% of all adults living with HIV-1. In the year 2008, more than two and a half million adults and children became infected with HIV-1 worldwide. According to the same findings, 2.0 million deaths were attributed to AIDS that year (UNAIDS, 2009).

Regional analysis of HIV prevalence amongst adults (ages 15–49) in 2008 indicated that of the 33-40 million infected cases, 22.7 million are based in Africa. There were 1.4 million deaths in 2008 in Africa alone. In Western and Central Europe 850,000 people had HIV/AIDS with 13,000 deaths that year. North America had 1.4 million people infected with HIV and 25,000 deaths in 2008 (UNAIDS, 2009).

Although Africa is inhabited by just over 14.7% of the world's population, it is estimated to have more than 67% of people living with HIV and accounted for 75% of all AIDS deaths in 2007. Currently, Africa has over 14 million AIDS orphans (UNAIDS, 2008). Even with such alarming statistics in Africa, North Africa accounts for less than 300,000 cases of people living with HIV/AIDS. Sub-Saharan Africa is more heavily affected by HIV and AIDS than any other region of the world with a staggering 22.4 million adults and children living with HIV at the end of 2008.

At the end of 2007, estimates of people living with HIV/AIDS and the number of deaths from AIDS in individual sub-Saharan Africa countries indicated great diversities in numbers. While countries such as Comoros had as little as less than 200 people living with HIV/AIDS, South Africa had a staggering 5.7 million cases. West Africa has been less affected by HIV and AIDS as compared to East and Southern Africa. Apart from Nigeria, all the other countries with more than one million cases of HIV/AIDS are in East and Southern Africa.
With 2.6m HIV-1 cases, prevalence in Nigeria is considered low compared to the rest of Africa due to its large population (prevalence rate of 3.1%). The national adult HIV-1 prevalence rate in three southern African countries now exceeds 20%. These countries are Botswana (23.9%), Lesotho (23.2%) and Swaziland (26.1%).

In 2003, Kenya’s Demographic and Health Survey (KDHS), found an overall prevalence rate of 6.7% among adults 15 to 49 years of age (4.6% in men and 8.7% in women). The prevalence dramatically reduced to about 6.1 percent in 2005. However by July 2008, the HIV-1 prevalence in Kenya was at 7.0 up from the previously declining index of 6.0%. It is thought that the previous decline was partially due to an increase in awareness education, access to antiretroviral therapy (ART) and high death rates leading to significant behavioural change, (UNGASS, 2008).

### 2.1.7 Impacts of the AIDS epidemic

The most obvious effect of HIV/AIDS crisis has been illness and death, but the impact of the epidemic has not been confined to the health sector alone: households, schools, workplaces and economies have also been badly affected. Africa's HIV/AIDS epidemic has had important effects on society, economics and politics in the continent (Whiteside and Barnett 2003).

#### 2.1.7.1 The effect on healthcare

In all affected countries, the epidemic is putting strains on the health sector. As the epidemic develops, the demand for care for those living with HIV rises. According to AIDS Newsletter1986, AIDS epidemic is adding additional pressure on the health sector. As the epidemic matures, the demand for care for those living with HIV rises, as does the number of health care workers affected (AIDS Newsletter, 1986).
Cost of HIV treatment

It was estimated in 2009 that to achieve universal access targets an investment of $7 billion was required for treatment and care out of the $25 billion total needed to achieve all targets in low and middle-income countries (UNAIDS 2009). However, of this $25 billion, only $15.9 billion was made available in 2009 and by the end of 2010 this figure had decreased further, so that only $15 billion was available for the AIDS response (UNAIDS 2011). In a global economic downturn, the prospect of greater funding for AIDS seems unlikely. The Global Fund, which provides antiretroviral treatment to 2.5 million people worldwide, received $11.3 billion for three years in 2010 out of the $20 billion it calculated it needed over this period. Funding from the US government, mainly through PEPFAR, was reduced from 2009-2010 for the first time since its creation in 2003. The PEPFAR budget for antiretroviral treatment was also reduced and funds for ARV drugs fell by 17 percent (International AIDS Society 2010). One fifth (all in sub-Saharan Africa) said they had already felt the impact of the crisis on their treatment programmes by 2009. Moreover, the percentage of countries where antiretroviral treatment programmes were adversely affected by reduced external funding rose from 11 percent to 21 percent from July 2008 to July 2009 (UNAIDS 2009). Treatment expansion will be difficult in countries with a high HIV prevalence but low government spending on HIV/AIDS as they are heavily reliant on foreign funding (UNAIDS 2009).

2.1.8 Management of HIV/AIDS

With no cure, most treatments are limited to relieving symptoms. Present research is concentrated on restoration of the damaged immune system of the victims, developing drugs that will stop or slow down the growth of the virus and treatment of opportunistic infections resulting from HIV infection as well as developing a vaccine against the virus (Taylor et al., 2004).
Highly Active Antiretroviral Therapy (HAART) has significantly reduced the rate of HIV and AIDS-related morbidity and mortality. There are many antiretroviral drugs and over 25 formulations approved by the US Food and Drug Administration (FDA) for the treatment of HIV-infected persons, including 7 nucleotide and nucleoside reverse transcriptase inhibitors (NRTIs), 3 non-nucleoside reverse transcriptase inhibitors (NNRTIs), 9 protease inhibitors (PIs) and 1 fusion inhibitor (EIs). Through combining drugs targeting different HIV-1 replication steps, HAART delays clinical progression by suppressing viral replication, measured by a substantial reduction in HIV RNA, and allowing immune reconstitution, measured in most studies by increase in the CD4 cells count (Jing et al., 2005).

2.2 Herpes simplex virus

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are large double-stranded DNA viruses of the *Herpesviridae* family. HSV-1 and HSV-2 share 83% sequence homology of their protein-coding regions and the structure of their genomes are alike, but can be distinguished serologically (Dolan et al., 1998). These viruses cause lifelong infections with intermittent clinical and subclinical viral reactivation and shedding from mucosal surfaces.

Herpes simplex virus (HSV) infection is a common cause of ulcerative mucocutaneous disease in both immunocompetent and immunocompromised individuals causing cold sores. Classically, HSV-1 is acquired in childhood and causes oralabial ulcers around the lips and mouth; whereas HSV-2 is transmitted sexually and causes anogenital ulcers (painful blisters on the genitals and in the pubic area, thighs, and buttocks) (Coyle et al., 2003). However, both oral infection with HSV-2 and particularly genital infection with HSV-1 are on the increase likely as a result of oral-genital sexual practices. Both types form acute initial outbreaks, go dormant, and reactivate. For most victims, frequent outbreaks are clear signs of stress or immunosuppression. Both types are equally dangerous for infants (Scoular et al., 2002).
2.2.1 Prevalence of HSV-2.

HSV-2 has a seroprevalence in adults reaching 70% in developed countries and 100% in developing countries. An estimated one million people are newly infected with the Herpes Simplex Virus (HSV) each year. However in the past decade, USA, Canada, and several European countries have seen an upsurge in genital HSV-1 infections, which now account for at least half of first episodes of genital herpes (Scoular et al., 2002; Roberts et al., 2003; Coyle et al., 2003; Manavi et al., 2004).

The National Health and Nutrition Examination Survey (NHANES) is a large ongoing population-based study in the United States. The body has been carrying out research on the seroprevalences of HSV-1 and HSV-2. In 1991 NHANES III, the seroprevalences of HSV-1 and HSV-2 were 68% and 22%, respectively (Fleming et al., 1997). Both infections were more common among women and non-white participants. Poverty, less education, and markers of sexual behaviour (earlier sexual debut, higher number of sexual partners) were also associated with HSV infection.

Compared with earlier NHANES surveys, the seroprevalence of HSV-2 had increased 30% since the 1970s, although analyses of the recent NHANES IV survey data (1999-2004) demonstrated flattening of HSV-2 seroprevalence during the 1990s, with declining seroprevalence among youth (Xu et al., 2006) It is estimated that 50 million persons in the United States have genital HSV infection. Similar HSV prevalence has been reported in Europe, and even higher seroprevalences have been seen in many parts of the developing world (Corey and Handsfield, 2000).

The prevalence of HSV-2 varies from 7–40% in pregnant women (Cowan et al., 2003), to 60–95% in people infected with HIV and female sex workers worldwide (Hashido et al., 1998). In Kenya, a study among fishermen along Lake Victoria recorded a HSV-2 prevalence of 63.9%
among heterosexual men (Ng’ayo et al., 2008).

2.2.2 Transmission of HSV-2

HSV-2 infection considered an epidemic in the making is the major worldwide cause of sexually transmitted genital ulcers and is a significant opportunistic infection (OI) in HIV-I infected individuals, often resulting in frequent reactivation of latent HSV-2 and severe, long-lasting genital lesions (Posavad et al., 1997). HSV-2 is mainly transmitted by sexual contact with women are more susceptible to HSV-2 infection than men (Langenberg et al., 1999; Mertz et al., 1992). Approximately one out of five women (14 to 49 years of age) is infected while in men about one out of nine men (14 to 49 years of age) are infected. Transmission from an infected male to his female partner is more likely than from an infected female to her male partner (Langenberg et al., 1999).

Most people infected with HSV-2 are not aware of their infection although in some cases if the signs and symptoms occur during the first outbreak, they can be quite pronounced.

The first outbreak usually occurs within two weeks after the virus is transmitted, and the sores typically heal within two to four weeks. In the United States only 10–25% of people with HSV-2 antibodies were found to be aware that they had genital herpes (Fleming et al., 1997). Most HSV-2 transmissions commonly occur among those who are unaware that they are infected (Mertz et al., 1992).

2.2.3 Diagnosis for HSV-2

Clinical range of manifestations HSV-2 infection is often not appreciated by patients and clinicians (Langenberg et al., 1999). Additionally, the diagnosis might be missed because laboratory tests for HSV-2 are not done routinely, even in people tested for other STIs.
2.2.4 Clinical manifestations of HSV-2

Both HSV-1 and HSV-2 cause genital herpes and affect individuals and public health. Although new genital HSV-1 infection is seen infrequently in people with HSV-1 antibodies, those with genital HSV-1 infection remain at risk of genital HSV-2 acquisition (Sucato et al., 1998).

Symptoms of genital herpes include itching, burning in the genital area, discomfort when urinating, and a watery vaginal or urethral discharge. A weeping vesicular eruption in the vagina or on the penis is an early sign. The early symptoms of herpes include a burning, tingling or itching sensation in the genital area. This symptom can sometimes be mistaken for a yeast infection in women. Pain or burning around the buttocks along with flu-like symptoms which include a fever and headache, and depression or irritability are also symptoms of herpes. Other genital herpes symptoms can include painful urination, vaginal discharge and muscle aches. Lymph nodes, specifically those located near the genial area, may become swollen and tender (http://signsandsymptomsofherpes.com/2007)

Immunocompetent people with genital HSV-2 infection can have frequent, painful, and recurrent genital lesions associated with much psychosocial distress (Dolan et al., 1998). Vertical transmission from an infected mother to her baby at delivery can result in disseminated infection, central nervous system complications, or death of the infant, even if treatment is started immediately (Corey et al., 1999).

The immunogenetic determinants of disease severity are unknown, and disease activity varies from asymptomatic to life threatening, with most people having mild disease. While previous infection with HSV-1 could offer a small margin of protection against HSV-2 infection, HSV-2 infection seems to protect against HSV-1 acquisition (Looker and Garnett, 2005).
Pre-existing HSV-1 immunity protects against symptomatic acquisition of HSV-2. Re-infection with different HSV-1 or HSV-2 strains has been recorded; however, this seems to be infrequent (Roest et al., 2004).

2.2.5 HSV-2 management

The importance of HSV-2 in Africa has been reinforced by evidence that both men and women have high HSV-2 antibody levels (Corey et al., 2004). Despite the strength of this epidemiological evidence, only minimal treatment is available for genital herpes throughout most of the African continent despite treatment for other STIs being readily available. Herpes is often referred to as an incurable STI and although this is also the case for HIV, the availability of Highly Active Antiretroviral Therapy (HAART) has reduced mortality and now made HIV-1 a manageable condition in most cases. This progress in HIV-1 treatment suggests that the perceived logistical difficulties with drug management of HSV-2 infections could be resolved in a similar way.

At the individual level, patients presenting with recurrent genital herpes can be treated in three main ways: first, symptomatically or syndromically in those presenting with ulcers. However for those with recurrences, this strategy equates to provider-initiated episodic treatment but for those with recurrences lasting more than 48 hours after presentation, the delay is too great to have much impact. Secondly, there is patient-initiated episodic therapy that involves patients treating themselves at the earliest symptom or sign that an outbreak of genital herpes is noticed. This strategy is often advised if patients have six or more episodes per year. The third way entails taking treatment continuously and whilst it is the most effective way of suppressing recurrences, the cost implications are enormous.
The WHO approach to treating STIs in developing countries has been to promote syndromic management by treating symptoms and signs based on the organisms most commonly responsible for each syndrome or condition. This approach was useful in poor countries where laboratory diagnostic tests might be too costly or cause delays in treatment. It worked well initially in those African countries where the accuracy of clinical diagnosis of genital ulceration was low. Although the WHO treatment of algorithm genital ulcer syndrome has long been heralded as a success (Nahmias et al., 1990), with the emergence of herpes as the most frequent cause of genital ulcer disease further assessment of its effectiveness is needed.

2.2.6 HSV-2 and HIV-1 co-infection

HSV-2 infection is a significant opportunistic infection (OI) in HIV-I infected individuals, often resulting in frequent reactivation of latent HSV-2 and severe, long-lasting genital lesions (Posavad et al., 1997). Over the past two decades, HSV-2 infection has also been linked to a three times higher risk of sexually acquired HIV-1 (Freeman et al., 2006). HSV-2 has been shown to have an increasingly important role in HIV spread in African populations.

The proportion of incident HIV-1 attributable to HSV-2 infection increases with maturity of the HIV-1 epidemic. Results suggest that HSV-2 is responsible for a large proportion of new HIV infections in Africa, (Freeman et al., 2007) highlighting the potential impact of herpes control for HIV prevention. Mucosal disruption caused by genital ulcers favours HIV acquisition by providing a ready portal of entry (Corey et al., 2004). Moreover, HSV-2 reactivation results in mucosal infiltration with activated CD4-bearing lymphocytes, the target cells for HIV-1 attachment.

It is estimated that 50–90% of HIV-1 individuals are co-infected with HSV-2 compared with approximately 20% of HIV-negative (HIV-2) individuals (Stamm et al., 1988).
The prevalence of HSV-2 shedding is 4–5 times greater in HIV-1 positive individuals than in HIV-2 positive individuals a likely increase in HSV transmission (Augenbraun, et al., 1995).

Infection with HSV-2 is a major health problem for HIV-1 individuals. Many studies show that HSV-2 infection increases the subsequent risk of HIV-1 acquisition by about three times in both men and women (Schacker et al., 1998). The role of HSV-2 in the elevation of the HIV-1 pandemic is heightened in frequent often unrecognized reactivations resulting in genital ulcerations and mucosal disruptions.

Genital HSV-2 reactivations have been implicated in increasing the efficiency of HIV transmission. Laboratory data support mucosal interactions between HSV-2 and HIV-1, including the ability of co-infection of the same cell. It involves up-regulation of HIV-1 replication and trans-activation of the HIV-1 long terminal repeats by HSV-2 proteins (Moriuchi et al., 2000).

CD4+ lymphocytes, HIV-1 target cells, have been detected in herpetic lesions, which could increase HIV-1 susceptibility during sexual exposure. In-vitro experiments show that HSV-2 increases HIV-1 transcription. This explains the high levels of HIV-1 RNA seen in herpetic lesions and in the plasma, thus supporting higher HIV-1 re-infectivity in dually infected subjects. In addition, HIV-1 infection has been shown to substantially increase mucosal HSV-2 shedding. Moreover, an increase in acyclovir-resistant strains of HSV-2 is complicating the treatment of HSV-2 infection in HIV-1 individuals (Englund et al., 1990) thus, the high seroprevalence of HSV-2, the high rate of HSV-2 shedding and frequent HSV disease, the presence of HIV in herpetic lesions, and the increase in acyclovir-resistant HSV strains make HSV infection a major health problem for HIV-1 individuals (Wald et al., 2001).

New evidence (currently in preparation) from Burkina Faso and Peru suggests that HSV-2 therapy can reduce the frequency and quantity of HIV shedding as well as plasma HIV loads.
The mechanism(s) involved in the worsening of HSV disease in HIV-1 individuals is not known. Since individuals with depressed T cell function suffer more serious HSV-2 related illnesses than immunocompetent individuals or patients with Ig defects, the cellular arm of the adaptive immune system has been implicated in preventing and resolving HSV-2 reactivations (Yasukawa and Zarling, 1984, Koelle, et al., 1994).

Although CD4 and CD8 T cells specific for HSV are present in blood and in genital herpes lesions, the relative importance of HSV-specific cellular immune responses compared with other components of the immune response in controlling HSV disease expression is unclear (Yasukawa and Zarling, 1984, Koelle et al., 1994).

HIV infection and subsequent immunosuppression in individuals provide a unique model system to study critical immune functions necessary for control and resolution of human HSV infections. In addition, the study of HSV-specific immune responses in HIV-1 individuals provides an opportunity to study the mechanisms involved in the emergence of a clinically relevant opportunistic infection (Posavad et al., 1997)

2.3 Syphilis Infection

Syphilis is a sexually transmitted infection caused by Treponema pallidum; a specific type of motile spirochete bacteria. T. pallidum has one of the smallest bacterial genomes at 1.14 million base pairs (Mb) and has limited metabolic capabilities, reflecting its adaptation through genome reduction to the rich environment of mammalian tissue.

2.3.1 Transmission of syphilis

Syphilis is generally acquired by close sexual contact, entering the host via breaches in squamous or columnar epithelium although there are examples of congenital syphilis via transmission from mother to child in utero.
2.3.2 Clinical disease

Syphilis is characterized by four stages: primary, secondary, latent, and tertiary. After becoming infected with syphilis, there is an incubation period of 9 to 90 days (the average being around 21 days) before the first signs and symptoms of the disease appear. Each stage of syphilis has characteristic signs and symptoms but any particular sign or symptom may or may not be present (CDC, 2007).

2.3.2.1 Primary stage

Primary syphilis is typically acquired via direct sexual contact with the infectious lesions of a person with syphilis (Pickering, 2006). The primary stage of syphilis is usually marked by the appearance of a single sore (called a chancre), but there may be multiple sores. The chancre is usually firm, round, small, and painless at the spot where syphilis entered the body. The chancre lasts 3 to 6 weeks, and it heals without treatment. As a result, many patients do not seek medical care immediately and the infection progresses to the secondary stage. Syphilis can not be contracted through toilet seats, daily activities, hot tubs, or sharing eating utensils or clothing (CDC, 2007).

2.3.2.2 Secondary stage

Secondary syphilis occurs approximately 1–6 months after the primary infection. There are many different manifestations of secondary disease. There may be a symmetrical reddish-pink non-itchy rash on the trunk and extremities (Dylewski and Duong, 2007).

The rash can involve the palms of the hands and the soles of the feet. In moist areas of the body, the rash becomes flat, broad and whitish lesions known as condylomata lata. Mucous patches may also appear on the genitals or in the mouth. All of these lesions are infectious and harbor active treponeme organisms.
A patient with syphilis is most contagious when he or she has secondary syphilis. In addition to rashes, symptoms of secondary syphilis may include fever, swollen lymph glands, sore throat, patchy hair loss, headaches, weight loss, muscle aches, and fatigue. The signs and symptoms of secondary syphilis will resolve with or without treatment (CDC, 2006).

### 2.3.2.3 Latent stage

Latent syphilis is defined as having serologic proof of infection without signs or symptoms of disease (Pickering, 2006). The latent (hidden) stage of syphilis begins when primary and secondary symptoms disappear without treatment. The infected person will continue to have syphilis even though there are no signs or symptoms; infection remains in the body for 10-20 years (CDC 2006). Approximately 50% of those infected with latent syphilis will progress into late stage syphilis, 25% will stay in the latent stage, and 25% will make a full recovery.

### 2.3.2.4 Tertiary stage

Tertiary syphilis usually occurs 1–10 years after the initial infection, though in some cases it may take up to 50 years. This stage is characterized by the formation of gummas which are soft, tumor-like balls of inflammation known as granulomas. The granulomas are chronic and represent an inability of the immune system to completely clear the organism (Clark and Danbolt 1964). In the late stages of syphilis, the disease may subsequently damage the internal organs seriously enough to cause death. These include the brain, nerves, eyes, heart, blood vessels, liver, bones, and joints.

Signs and symptoms of the late stage of syphilis include difficulty coordinating muscle movements, paralysis, numbness, gradual blindness, and dementia (CDC, 2006). The more severe manifestations include neurosyphilis and cardiovascular syphilis. In a study of untreated syphilis, 10% of patients developed cardiovascular syphilis, 16% had gumma formation, and 7% had
neurosyphilis (Clark and Danbolt, 1964). General paresis, otherwise known as general paresis of the insane, is a severe manifestation of neurosyphilis. It is a chronic dementia that ultimately results in death in as early as 2–3 years. In general, patients have progressive personality changes, memory loss and poor judgment. In more rare instances, they can have psychosis, depression or mania. Imaging of the brain usually shows atrophy.

2.3.3 Diagnosis of syphilis

It is not sufficient to make a syphilis diagnosis based on symptoms alone. This is because signs of syphilis might be absent, disappear without treatment, or be confused with those of other diseases (http://syphilis.emedtv.com/syphilis/syphilis-diagnosis.html).

Making laboratory testing is an important aspect of diagnosis of syphilis. Syphilis has several clinical manifestations and many unsuspected cases are discovered by laboratory testing. The etiological agent, Treponema pallidum, cannot be cultured, and there is no single optimal alternative test. Several serological and alternative tests are currently available and relate their results to the likely corresponding clinical stage of the disease (Ratnam 2005).

The doctor usually uses three approaches of its signs and symptoms; microscopic identification of syphilis bacteria by taking a scraping from the surface of the ulcer or chancre, and examines it under a special "darkfield" microscope to detect the organism itself.

He will then carry out blood tests to detect syphilis and decide upon the stage of infection. The blood-screening tests most often used to detect evidence of syphilis are the VDRL (Venereal Disease Research Laboratory) test and the RPR (rapid plasma reagin) test. Two blood tests are usually used as they may give false- negative results because they may not show signs of
infection despite its presence for up to three months after infection. (http://womenshealth.about.com/cs/syphilis/a/syphilisdiagnos.htm).

The doctor administers a confirmatory blood test when the initial test is positive. These tests include the fluorescent treponemal antibody-absorption (FTA-ABS) test that can accurately detect 70 to 90 percent of cases. Another specific test is the *T. pallidum* hemagglutination assay (TPHA). These tests detect syphilis antibodies (http://womenshealth.about.com/cs/syphilis/a/syphilisdiagnos.htm 2010)

**2.3.4 Syphilis co-infection with HIV-1**

The immune system plays an important role in protecting against syphilis. Impairment of both cell-mediated and humoral immunity by HIV may limit the host's defences against *Treponema pallidum*, thereby altering the clinical manifestations or natural course of syphilis infection (Bowen *et al.*, 1985). In animal models, selective impairment of cell-mediated immunity shortens the incubation time of syphilis, increases the number and spreading of lesions, and slows healing time (Pacha *et al.*, 1979). There is anecdotal evidence that the incidence of neurosyphilis is higher in HIV-1 patients, and some have recommended that all HIV-1 positive patients with syphilis should have a lumbar puncture to look for asymptomatic neurosyphilis (Walter *et al.*, 2006).

Like many acute infections in the HIV-1 infected patients, early syphilis may decrease CD4+ T-cell counts (CD4 cell counts) and increase HIV-1 RNA in plasma and semen.

Molecular pathogenetic mechanisms to explain the role of the spirochete in facilitating HIV-1 transmission may include up-regulation of gene expression, such as that of the CCR5 co-receptor used in HIV-1 entry. HIV-1-induced meningeal inflammation may facilitate penetration of spirochetes into the central nervous system (CNS) and thus contribute to the development of symptomatic neurosyphilis. Most HIV-1 infected patients with *T. pallidum* infection present with
typical dermatologic clinical features of primary and secondary disease, such as chancres and
diffuse maculopapular rashes (Rolfs et al., 1997, Hook et al., 1992, Hook et al., 1989,
Hutchinson et al., 1994). The genital ulcers caused by syphilis can bleed easily, and when they
come into contact with oral and rectal mucosa during sex, increase the infectiousness of and
susceptibility to HIV-1 (CDC 2006).

In a study among patients seen in an STD clinic, however, patients with HIV-1 infection were
more likely than HIV-1 negative individuals to present with signs and symptoms of secondary
syphilis and were more likely to have chancres still present at the time of secondary syphilis
diagnosis (Hutchinson et al., 1994). In a multicenter study of STD clinic, patients with early
syphilis, HIV-infected patients were more likely to present with multiple chancres, but the size of
the chancres, characteristics of the skin rash, and duration of the chancres or rash before
presentation did not differ according to HIV-1 status (Rolfs et al., 1997).

2.4 Hepatitis B virus Infection

HBV infection is one of the commonest infections in the world (Lai et al., 2003). It is caused by
HBV, which is a partially double-stranded DNA virus belonging to the Hepadnaviridae family.
Despite the current availability of an effective vaccine, almost one million people worldwide still
die each year from HBV related diseases (CDC, 2005).

HBV enters the liver via the bloodstream, and replication occurs only in liver tissue. The intact,
infectious virus is 42–47 nm in diameter and circulates in the blood in concentrations as high as
108 virions per ml (Shepard et al., 2006). The inner core of the virus contains hepatitis B core
antigen, hepatitis B e antigen (HBeAg), a partially double-stranded 3,200-nucleotide DNA
molecule, and DNA polymerase with reverse transcriptase activity (Lee, 1999).
Hepatitis B surface antigen (HBsAg) is found both on the surface of the virus and as self-assembling, non-infectious spherical or tubular particles (Shepard et al., 2006).

2.4.1 Prevalence of HBV

According to the recent World Health Organization (WHO) estimate, two billion people worldwide have serologic evidence of past or present HBV infection, and 350 million are chronically infected and at risk for HBV-related liver disease (Shepard et al., 2006). Approximately one third of all cases of cirrhosis and half of all cases of hepatocellular carcinoma can be attributed to chronic HBV infections. HBV is estimated to be responsible for 500,000–700,000 deaths each year (Shimelis et al., 2008).

The prevalence of HBV infection varies from country to country, and region to region based on environmental factors and host characteristics (Candan et al., 2002). The prevalence of HBsAg positivity in the Far East, the middle East, Africa and parts of South America ranges from 8 to 15% (Andre et al., 2000). Past reviews of HBV epidemiological studies conducted in sub-Saharan countries in the last three decades have shown that HBV infection is highly endemic in sub-Saharan Africa (Burnett et al., 2005).

It has been estimated that HBsAg carrier rates in the region range from 9.6% in South Africa to 20.6% in the Democratic Republic of the Congo (DRC), while HBV exposure rates range from 56.2% in Kenya to 91% in Senegal.

In Nigeria and Namibia, the prevalence is as high as 35% and 75% respectively (Ajayi et al., 2007). In Kenya, a study done on circulating HIV-1 sub types in voluntary blood donors co-infected with HBV, HCV, syphilis and HTLV-1 in Western Kenya, shows the HBV prevalence to be 2.5% among this population (Makokha et al., 2004).
In another study done on HBV, HIV co-infection in Kisumu district hospital shows that 47% of the patients screened had HBV infection (Otedo, 2004).

### 2.4.2 Transmission of HBV

HBV is transmitted by percutaneous or mucosal exposure to infected blood or other body fluids (Shepard et al., 2006). HBV is present in blood, saliva, semen, vaginal secretions, and menstrual blood of infected individuals. Because HBV is resistant to breakdown outside the body, it is easily transmitted through contact with infected body fluids (Wright, 2006).

Perinatal vertical transmission is the most common mode of transmission worldwide (Richman et al., 2002). High levels of virus in serum (HBV DNA and HBeAg positivity) have been associated with increased risk of transmission by needle stick exposure and by vertical routes (Wright, 2006). Infants born to HBeAg positive mothers who have high levels of viral replications (>80 pg/ml) have 70 to 90% risk of perinatal acquisition. On the other hand the risk of mother-to-child transmission from HBeAg negative mothers is substantially lower (10 to 40%) (Richman et al., 2002).

In Kenya, vertical transmission is not a common route of hepatitis virus transmission as most expectant mothers are HBeAg negative and are therefore less infectious (Okoth et al., 1990). Heterosexual sex now accounts for the majority of HBV infections (Richman et al., 2002). In heterosexuals the risk factors associated with infections are: duration of sexual activity, number of sexual partners, a history of sexually transmitted diseases, and positive serologic test results for syphilis. Sexual partners of injection drug users, prostitutes, and clients of prostitutes are at particularly high risk of infections (Richman et al., 2002).
2.4.3 Clinical manifestations of HBV

HBV infection may result in subclinical or asymptomatic infection, acute self-limited hepatitis, or fulminant hepatitis requiring liver transplantation (Shepard et al., 2006). Persons infected with HBV may also develop chronic HBV infection, which can lead to cirrhosis or hepatocellular carcinoma. The likelihood that newly infected persons will develop chronic HBV infection is dependent on their age at the time of infection (Shepard et al., 2006).

More than 90% of infected infants, 25–50% of children infected between one and five years of age, and 6–10% of acutely infected older children and adults develop chronic infection (i.e., they are HBsAg-positive but negative for immunoglobulin M antibodies to hepatitis B core-antigen). Immunosuppressed persons (e.g., hemodialysis patients and persons with HIV infection) are also at higher risk of developing chronic infection.

2.4.4 Diagnosis of HBV

The specimen of choice for the diagnosis of HBV infection is blood. Definitive diagnosis of HBV is based on serologic findings, as symptoms are not specific to HBV infection. Serological tests for viral antigens and antibodies are typically used for diagnostic screening and can be performed on either serum or plasma. It is accomplished by testing for a series of serological markers of HBV and by additional testing to exclude alternative etiological agents such as hepatitis A and C viruses. Serological tests are used to distinguish acute, self-limited infections from chronic HBV infections and to monitor vaccine-induced immunity (Krajden et al., 2005).

The diagnosis of acute hepatitis B generally rests upon the finding of hepatitis B surface antigen (HBsAg) and IgM antibody to hepatitis B core (anti-HBc) in the serum of a patient with clinical and biochemical evidence of acute hepatitis. The first serological marker to be detectable in the serum is HBsAg which appears during the incubation period and rapidly rises in titre.
All persons who are HBsAg+ are considered infectious. IgM anti-HBc and IgG anti-HBc are also markers of acute infection. The presence of HBeAg indicates higher infectivity. HBeAg positivity is associated with very high titers (108-9) of circulating virions (Lee, 1999).

In chronic carriers the conversion from HBeAg to anti-HBe may signal resolution of hepatocellular disease. Anti-HBs become detectable during convalescence after the disappearance of HBsAg (among those who clear infection). Chronic carriers have persistently detectable HBsAg and IgG anti-HBc (Mahoney, 1999). Such individuals often have little or no evidence of acute liver disease when initially infected. HBsAg may persist in high titers with chronic infection. Spontaneous conversion with development of protective surface antibody and clearance of antigens occur about 1% per year for HBsAg+ (Sharon et al., 2003). Nucleic acid testing for HBV-DNA is increasingly being used to quantify HBV viral load and measure the effectiveness of therapeutic agents (Krajden et al., 2005).

2.4.5 HIV-1 and Hepatitis B Co-Infection

Human immunodeficiency virus (HIV) and HBV infections are two major viral infections worldwide (Umolu et al., 2005). Co-infection with HBV and HIV-1 is becoming common and a growing public health concern because both viruses share similar transmission routes (Otedo, 2004, Sheng et al., 2007, Shimelis et al., 2008).

In HIV-1-infected individuals, HBV infection prevalence is approximately ten times higher than in the general population (Pereira, 2006). Individuals with HIV-1 who contract acute hepatitis B are more likely to develop chronic hepatitis B than individuals who contract acute hepatitis B without HIV-1. Chronic HBV infection appears to have its natural progression modified by HIV-
co-infection, with significantly increased morbidity and mortality attributable to liver disease in co-infected patients, compared with those with HBV alone (Shimelis et al., 2008). Decreased clearance rates of HBsAg and HBeAg, reactivation of HBV, higher levels of HBV-DNA, and lower ALT levels are some of the most important findings that differentiate HIV-1-HBV co-infected individuals from those infected only with HBV (Sheng et al., 2007).

Although a number of prevalence studies of HIV/HBV co-infection have been performed in Africa, conflicting results have been observed with both higher and lower rates of HBV being reported in HIV-positive patients (Apurva et al., 2007).

Studies done in Zambia on pregnant women showed a slight non-significant increase in HBsAg in the HIV-1 positive group (7.1% as opposed to 5.4% in the HIV-1 negative group), but of interest was the high prevalence of HBeAg positivity in the HBsAg positive/ HIV-1-positive women (25% as opposed to 8.5% in the HIV-1 negative women), suggesting a much higher rate of infectivity (Burnett et al., 2005).

In Kenya, in a study done on HIV-positive patients attending the HIV clinic at the Aga Khan University in Nairobi, a total of 23 (6%) were found to be co-infected with HBV (Harania et al., 2008). Another study done in Kisumu District Hospital in patients who presented with jaundice showed a 53% (177) rate of co-infection between HIV-1 and HBV and 43% had monoinfections (Otedo, 2004).

In another study, data shows 32 (78%) out of 41 patients with AIDS had serological evidence of exposure to HBV (Ezegbudo et al., 2004). These studies give evidence that both viral infections are endemic in this region.

HIV-1 co-infection accelerates the progression of HBV and increases the risk of cirrhosis, end stage liver disease and death from liver disease especially in patients with a low CD4 cell count.
or concomitant alcohol use (Koziel and Peters, 2007). Histological assays of a series of 132 homosexual men with chronic hepatitis B, of which 65 were HIV-1 co-infected, showed a higher prevalence of liver cirrhosis in HBV/HIV-1 co-infected patients (Hoffman et al., 2006). There is evidence that HIV-1-infected individuals who are subsequently infected with HBV are more likely to have a high HBV replication rate increasing the risk of HBV transmission, and are more likely to be HBeAg-positive for a much longer time (Burnett et al., 2005). The treatment of HIV-1 using HAART has been reported to result in hepatotoxicity and reactivation of HBV in co-infected individuals (Burnett et al., 2005).

Guidelines for the adequate management of chronic hepatitis B in HIV-1-coinfected individuals have recently been released, and several reviews have updated the knowledge on this topic, providing useful information about how to manage HBV/HIV-1-coinfected patients (Soriano et al., 2006). Four main variables should guide the selection of patients to be treated for HBV and of the drug(s) of choice: transaminase levels, serum HBV DNA viral loads, presence of serum HBeAg and liver fibrosis staging (Burnett et al., 2005).

Given that chronic hepatitis B patients with elevated transaminase levels tend to have liver damage, they should generally be considered primary candidates for treatment especially in the context of HIV-1 co-infection, since HBV-related liver damage tends to progress faster in HIV-1-coinfected patients than in HBV-mono-infected patients (Burnett et al., 2005). Four drugs have been approved so far for the treatment of chronic hepatitis B: interferon alpha (standard or pegylated), lamivudine, adefovir and, more recently, entecavir. However, other drugs with anti-HBV activity such as tenofovir and emtricitabine are already approved for the treatment of HIV-1 infection and therefore are frequently used in co-infected patients as anti-HBV agents (Nunez, 2003). IFN treatment does not lead to resistance, but it is appropriate only for a narrow group of HIV-1-HBV-coinfected persons: those with high CD4+ T cell counts, HBeAg-positive
patients, and those with high ALT levels and low HBV DNA levels (Sterling, 2003). Another option for HIV-1-HBV-coinfected persons with preserved immune function is to defer HBV treatment until combination antiretroviral therapy is needed (Osborn et al., 2007). All HBV patients should avoid or limit alcohol intake.

Current guidelines recommend ART for HIV-infected patients who require treatment for HBV infection, regardless of CD4+ count, simplifying the treatment regimen can be achieved by offering at least two agents with dual activity against HIV and HBV. Vaccination against hepatitis A (HAV infection may cause fulminant liver failure). Counseling about risk for transmission to household, sexual, and needle-sharing contacts (contacts should be vaccinated). Conclusively, in HIV-1-HBV-coinfected patients, antiretrovirals should be selected and monitored to minimize the risk of HBV and HIV-1 resistance (NIH and CDC2009).
CHAPTER THREE
MATERIALS AND METHODS

The study was approved by KEMRI under Protocol No: SSC 1447.

3.1 Study sites

The map below shows the 47 counties of Kenya numbered 1 to 47 (Figure 3).

Figure 3: Map of Counties of Kenya (http://en.wikipedia.org/wiki/Counties_of_Kenya2010)
This study involved four health facilities Kangemi, Ngaira, Pumwani and Waithaka in Nairobi County the capital city of Kenya (Figure 4).

Figure 4: A map of the Nairobi county showing the study sites (http://www.museums.or.ke 2009)

Two of the four health centres: Kangemi and Waithaka are located on the outskirts of Nairobi and are high density urban slum areas, lacking in basic amenities and with few health facilities. Families around these health facilities are among severely disadvantaged households struggling to survive. Pumwani is located in the lower middle-class residential east lands of Nairobi. Ngaira however, is located in the Nairobi’s central business district and also serves low income earners (Figure 5).
3.2 Sample size:

In a serological study carried out among 250 fishermen along the shores of Lake Victoria in Kisumu district, Kenya, the HSV-2 seroprevalence was 63.9% (Ngayo et al., 2008). In multivariate analysis, fishermen were more likely to be infected with HSV-2 if they were HIV positive. Using this prevalence to calculate our sample size, then the minimum sample size to be used in this study was as shown below:

\[ N = \frac{Z^2 \cdot p \cdot (1-p)}{D^2} \]

Where:

\[ N \] = Minimum sample size required
\[ Z \] = 1.96 standard
\[ p \] = Postulated prevalence (0.64).
\[ D \] = 0.05 the inverse of 95% confidence limit (the allowable error)

\[ N = \frac{1.96^2 \cdot 0.64 \cdot (1 - 0.64)}{0.05^2} = 354 \text{ samples} \]

Therefore a minimum of 354 samples was used to detect HSV-2, syphilis and HBV infection in selected health facilities in Nairobi.

3.3 Sampling techniques

In this study sampling was done using Non-probability Sampling technique and specifically Purposive sampling where the sample was chosen from a limited number of possible subjects. Samples were collected from individuals who were 18 years and above and who had signed the consents only.
3.4 Sample collection

Samples were collected from HIV-1 positive patients aged 18 years and above only seeking HIV-1 and CD4 testing services at Kangemi, Ngaira, Pumwani and Waithaka health centres. All the patients involved in this study had previously been tested for and found to be HIV-1 positive and were receiving treatment for HIV-1. Informed consent was obtained from the participants before the study commenced. Individual consent was sought from each participant. All study participants were informed concerning the study verbally and a write-up given to each one of them to ensure that they had all the information needed to make an informed choice. This included a complete description of the aims of the study; infectious agents that were being screened; potential benefits and risks; blood collection procedures and assurance of confidentiality of any information given as well as test results.

Samples comprising four millilitres of whole blood were collected from each participant by trained personnel from the four health centres. World Health Organization standard operating procedures for sample collection in field based studies (http://www.searo.who.int/en/Section10/Section17/Section53/Section482_1779.htm 2010) were followed. Blood was drawn by a sterile needle from a vein in the arm and collected in green top CD4 stabilization tubes EDTA anticoagulant. The specimens were labelled appropriately using the patients’ name (for future references); code numbered then transported to the KEMRI HIV-CD4 laboratories immediately.

3.5 Laboratory procedures

3.5.1 CD4 Enumeration using BD FACS Calibur

CD4 enumeration was done using Becton Dickinson FACS Calibur flow cytometer. Flow cytometry (FCM) is an automated process that allows enumeration of cells in a single file in a
liquid media. Up to 10,000 cells can be counted per second using this method. FCM was used to automatically determine and analyze both absolute and percentage lymphocyte subset values of CD4.

After the initial equipment start up, the whole-blood specimens in the labelled tubes were allowed to mix on the rotator for 10min. A 12x75 mm falcon tube was labelled with each specimen accession number and a 1 to indicate the tube containing the CD4 markers. A second 12x75 falcon tube was similarly labelled with each specimen accession number and a 2 to indicate the tube containing CD8 markers. 50 μl of mixed whole blood were added to each tube as per the specimen accession numbers. 20 μl of CD45/CD3/CD4 monoclonal were added to each tube labelled 1 while 20μl of CD45/CD3/CD8 monoclonal were added to each tube labelled 2 and both sets of tubes vortexed for five sec.

The samples were allowed to stain in the dark for 15min after which 450 μl of 1x FACS lysing solution were added to each tube capped and vortexed for five sec. The tubes were once more placed in darkness to lyse for 15 min.

The tubes were then kept in the dark at 2-8°C for a maximum period of 24 hrs during which period analysis was done by running on the FACS Calibur instrument. The instrument was then cleaned following the laid down procedure and shut down. The computer print-out indicated the CD4 and CD8 readings for each specimen analysed. Serum was extracted from the remaining blood and stored at -20°C to be used in other tests later.

3.5.2 HSV-2 testing

An Enzyme-linked immunosorbent assay (ELISA) was carried out for the detection of HSV -2 antibodies using Kalon HSV type 2 ELISA Kit of Kalon Biological Ltd Unit Guildford, United Kingdom (http://www.kalonbio.co.uk/Hsv2Ifu.pdf 2009).
The assay is based on the indirect ELISA principle using a recombinant type 2 antigen (gG2) that had been modified to eliminate reactivity arising from type 1 infections whilst retaining the natural antigen characteristics of HSV-2.

Polystyrene microtitre plate wells were supplied by Kalon Biological Ltd. pre-coated with a recombinant modified gG2 antigen of HSV-2. Serum samples were diluted by dispensing 200 μL of the Assay Diluent (buffered saline with protein stabiliser, surfactant and 0.01% Thiomersal) into each well. About 10 μL of each test specimen was then dispensed into their designated wells containing the diluent and mixed thoroughly. These were then sealed in plastic bags and incubated at 37°C for 30 min during which reactive antibody was captured.

A wash step involving filling and aspirating the microtitre wells with 350 μL of fresh working strength wash solution of buffered saline and surfactant was done a total of four times. The residual wash solution was then tapped out using paper towelling. The surface of the well was probed for IgG class antibodies by incubating at 37°C for 30 min with an enzyme conjugated anti-IgG tracer.

Following a second wash step, enzyme substrate and a TMB chromogenic reagent were added to the wells and incubated at 25°C for 30 min. The enzyme incubation was halted by addition of acid stop solution (0.5mol/L sulphuric acid) into each well, which also had the effect of both changing and enhancing the amount of colour produced. This was measured in a photometer. The optical density was proportional to the amount of anti-HSV2 IgG present in the original sample. This was compared with a cut-off calibrator which was run in each assay and has been designed to distinguish between non-specific binding and a true positive reaction. Score results with an optical density greater than the Cut-off (1.1) were recorded as positive while those with an less than the Cut-off 0.9 were recorded as negative.
Results between these values that is (0.9) < Cut-off > 1.1 were equivocal and were repeated to confirm the status.

3.5.3 Syphilis testing using The QuickTest™ Syphilis Serum/ Plasma/Whole Blood Strip

**Blood Strip**

The Syphilis Ultra Rapid Test Strip (QuickTest™ Syphilis Serum/ Plasma/Whole Blood Strip) manufactured by Orgenics of Yavne 70650, Israel was used to determine the presence of syphilis antibodies. This is a qualitative membrane strip based immunoassay for the detection of *Treponema pallidum* antibodies (IgG and IgM) in whole blood serum or plasma. In this test procedure, recombinant syphilis antigen was immobilized in the test line region of the strip. After a specimen (serum) was added to the specimen pad it reacted with syphilis antigen coated particles that had been applied to the specimen pad. The mixture migrated chromatographically along the length of the test strip and interacted with the immobilized syphilis antigen. This double antigen test format can detect both IgG and IgM in the specimens. For specimens containing *Treponema pallidum* a red line appeared in the test line region indicating a positive result; those without *Treponema pallidum* did not have a red line appearing in this region indicating a negative result.

To serve as a control, a pink line appeared in the control line region indicating that the proper volume of the specimen was added and membrane wicking had occurred.

3.5.4 HBV testing

An Enzyme immunoassay procedure was carried out for the detection of HBsAg in the samples using the Hepanostika® HBsAg Ultra kit. The kit is based on the “sandwich” ELISA principle. Upon completion of the assay, the development of colour indicated the presence of HBsAg, while no colour or low colour development suggested the absence of HBsAg.
The wells of microelisa strips are coated with anti-HBs (murine and human monoclonals). During the screening, 25 µl of specimen diluents were pipetted into the assigned wells. 100 µl of undiluted sample or control was added into the assigned wells. Three negative and one positive control were included in each strip holder. The plate was incubated at 37°C for 60 min after which 50 µl of conjugate solution was pipetted into each well. Incubation was done at 37°C for 60 min. The plate was then washed using the phosphate buffer provided. One hundred microlitres of tetramethylbenzidin (TMB) substrate was then added into each well. The plate was covered and incubated in a dark place for 30 min at 37°C for colour development. Finally, 100 µl of stop solution (1M sulphuric acid) was added into each well. The absorbance values of the test samples were then read at 450 nm using micro plate reader.

### 3.5.4.2 Confirmation of HBV using DRG ELISA kit for HBsAg

A confirmation for the presence of HBsAg was done using the DRG ELISA kit from Biocare Diagnostics Ltd. of Zhuhai, China. The kit is an in vitro enzyme linked immunosorbent assay for the detection of HBsAg in human serum or plasma samples. The solid phase of multi wells was coated with anti- HBsAg antibodies (primary antibody). 50 µl of serum or plasma sample (containing HBsAg) and 50 µl of enzyme (horseradish peroxidase, HRP) conjugated antibody (secondary antibody, HRP-conjugated; “enzyme conjugate”) were added to the coated wells. This was followed by a one hour incubation period that allowed a complex to form between the primary antibody (anti-HBsAg), the antigen (HBsAg) and the HRP-conjugated antibody. After a final washing step to remove unbound components, 50 µl of the substrate was added to the wells which, after an incubation period of 30 min resulted in the formation of a coloured product (blue colour). After the addition of stop solution a yellow coloured product was formed. The presence of HBsAg was then detected at 450nm in an ELISA reader. Samples that turned positive by Hepanostika® HBsAg Ultra kit and DRG ELISA kit for HBsAg were recorded as positive.
3.6 Data management and analysis

Generated data was recorded in research workbook and entered in a computer using MS excel. Compact discs (CD) were used to back up the data. Data was analysed using statistical package INSTAT. This was used to establish the measures of central tendency (Mean, Frequency and percentages), Analysis of Variance (ANOVA) was carried out to establish mean differences in the sites for CD4 counts, HSV-2 and HBV Optical Density. In cases where there were noted significant differences, mean separation was done using Tukeys HSD test on the samples. The data presentation was made in graphs, charts and figures.
CHAPTER FOUR

4 RESULTS

4.1 Bio-Data of the individuals sampled

4.1.1 Gender

In the sampled population (n = 402), 261 (64.9%) females and 141 (35.1%) males participated in the study. This showed a ratio of 1: 2 (male: female) (Table 1)

Table 1: Gender distribution of the sampled population

<table>
<thead>
<tr>
<th>Gender</th>
<th>Kangemi</th>
<th>Ngaira</th>
<th>Pumwani</th>
<th>Waithaka</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>121</td>
<td>76</td>
<td>41</td>
<td>23</td>
<td>261</td>
</tr>
<tr>
<td>Male</td>
<td>50</td>
<td>68</td>
<td>21</td>
<td>2</td>
<td>141</td>
</tr>
<tr>
<td>Total</td>
<td>171</td>
<td>144</td>
<td>62</td>
<td>25</td>
<td>402</td>
</tr>
</tbody>
</table>

In Kangemi, 70.76% (N=121) of the sampled individuals were females while 29.23% (N=50) were males. Ngaira had 52.77% (N=76) females and 47.22% (N=68) males, Pumwani 66.12% (N=41) females and 33.87% (N=21) males, while Waithaka had 92% (N=23) females and 8% (N=2) males. It is only in Ngaira that female and males numbers are comparable but in the other three centers Kangemi, Pumwani and Waithaka, the numbers of females sampled was significantly high, nearly 70% or above of the sampled population.
4.1.2 Ages of the respondents

Most of the sampled population in this study was between 31 – 40 years of age. 34.58% of the sampled individuals were 21 – 30 years and 42.04% were between 31 – 40 years of age. Fewer individuals sampled were below 20 years (0.75%) and above 60 years (0.49%). There is however, a sudden upsurge in the numbers from below 20 years through 30 years of age distorting the normal trend in distribution (Figure 5).

![Age distribution of the sampled population.](image)

**Figure 5:** Age distribution of the sampled population.

4.1.3 Gender/Age correlation

The sampled population were in different age groups. Mean ages of the individuals were; female (33.17 years ± 0.50) and males (38.56 years ± 0.71). Males in the sampled population were slightly older than the females.
4.2 CD4 status of the sampled population

In the total population sampled, 37.8% (n = 152) of the persons had CD4 counts less than 250/µL while 62.2% (N = 250) had their CD4 counts more than 250/µL (Table 2). Considering the respective health facilities, distribution of CD4 status in the four health centres showed that, there were more persons from Ngaira having CD4 < 250 /µL. However there were more persons from Kangemi having CD4 > 250 /µL than in the other centres. (Table 2)

**Table 2:** Distribution of the sampled population CD4 status in the centres

<table>
<thead>
<tr>
<th>CD4 Status of Respondents</th>
<th>Kangemi</th>
<th>Ngaira</th>
<th>Pumwani</th>
<th>Waithaka</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;250/µL</td>
<td>106(61.99%)</td>
<td>84(58.33%)</td>
<td>40(64.51%)</td>
<td>20(80%)</td>
<td>250(62.1%)</td>
</tr>
<tr>
<td>&lt;250/µL</td>
<td>65(38.01%)</td>
<td>60(41.66%)</td>
<td>22(35.48%)</td>
<td>5(20%)</td>
<td>152(37.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>171</td>
<td>144</td>
<td>62</td>
<td>25</td>
<td>402(100%)</td>
</tr>
</tbody>
</table>

Of the sampled individuals, 61.98% (N=106) in Kangemi had CD4 counts more than 250/µL while 38.02% (N=65) had CD4 counts less than 250/µL. Ngaira had 58.33% (N=84) with CD4 counts more than 250/µL and 41.67% (N=60) had CD4 counts less than 250/µL. Pumwani 64.51% (N=40) had CD4 counts more than 250/µL and 35.49% (N=22) had CD4 counts less than 250/µL, while Waithaka had 80% (N=20) with CD4 counts more than 250/µL and 20 (N=5) had CD4 counts less than 250/µL. It is only in Ngaira that the population with CD4 higher and less than 250/µL were comparable but in the other three centres Kangemi, Pumwani and Waithaka the numbers of the sampled population with CD4 counts more than 250/µL sampled are significantly high.
4.2.2 Mean CD4 counts

Considering the health centers sampled, it was established that mean CD4 counts were not significantly different in the four health centres sampled ($F = 1.3$, $P = 0.268$, $P > 0.05$). However, mean CD4 counts of samples from Waithaka (mean CD4 counts, $419.16 \pm 47.42$) were higher than mean CD4 counts in samples from the other centres. The lowest mean CD4 counts were recorded in samples from Ngaira (mean counts $347.3 \pm 21.74$) (Figure 6).

![CD4 Counts](image-url)

**Figure 6:** Mean CD4 counts in the health centres

4.2.3 CD4 Count and age groups

Of the sampled population, majority of the individuals with CD4 $<250$ and $>250/\mu$L are between the ages of 31-40 years. None of the respondents below the age of 20 and above 60 years had CD4 counts below $250/\mu$L. There is no significant difference in the various age-groups between individuals with CD4 $<250/\mu$L and those with CD4$>250/\mu$L (Figure 7).
Figure 7: CD4 Count and age groups

4.3 HSV – 2 infections among HIV – 1 positive individuals

HSV-2 infection is significantly high among HIV-1 positive sampled persons. Of the sampled population, more than half (54.23% (N=218) were found to be HSV-2 positive while 44.77% (N=184) were HSV-2 negative (Table 3).

Table 3: HSV – 2 infections among HIV – 1 positive individuals

<table>
<thead>
<tr>
<th></th>
<th>Kangemi</th>
<th>Ngaira</th>
<th>Pumwani</th>
<th>Waithaka</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2 Positive</td>
<td>93(54.38%)</td>
<td>69(47.91%)</td>
<td>38(61.29%)</td>
<td>18(72%)</td>
<td>218(54.23%)</td>
</tr>
<tr>
<td>HSV-2 Negative</td>
<td>78(45.62%)</td>
<td>75(52.08%)</td>
<td>24(38.70%)</td>
<td>7(28%)</td>
<td>184(45.77%)</td>
</tr>
<tr>
<td>Total</td>
<td>171(100%)</td>
<td>144(100%)</td>
<td>62(100%)</td>
<td>25(100%)</td>
<td>402(100%)</td>
</tr>
</tbody>
</table>
HSV-2 was recorded in all the centres and in higher numbers unlike Syphilis and HBV among the sampled population. Apart from Ngaira with 38% HSV-2 positive, other three centres recorded over 50% of their population positive for HSV-2. Waithaka had the highest percentage (72%) testing positive for HSV-2 (Figure 9).

### 4.3.2 Gender and HSV-2 infection

Infection of HSV-2 was not significantly different in the gender of the sampled population. 54.0% of the female respondents tested HSV-2 positive while 54.6% of the male tested positive; however, 64.68% of the respondents who tested positive for HSV-2 were females while 35.32% were males (Table 4).

#### Table 4: Gender and HSV-2 infections

<table>
<thead>
<tr>
<th>Gender</th>
<th>HSV-2 NEG</th>
<th>HSV-2 POS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>120(46.0%)</td>
<td>141(54.0%)</td>
</tr>
<tr>
<td>Male</td>
<td>64(45.4%)</td>
<td>77(54.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>184</td>
<td>218</td>
</tr>
</tbody>
</table>

### 4.3.3 Ages of the respondents and HSV-2 infection

HSV-2 infection was reported in all age groups; however, more individuals aged between 20-40 years were infected with HSV-2 compared to other age groups. HSV-2 prevalence in these age groups (21-30 and 31-40) was over 50% of the age groups compared to the other age groups which had infection rates below 50% (Figure 8).
Figure 8: Ages of the respondents and HSV-2 infections

4.3.4 Comparison between HSV-2 to CD4 counts

Infection with HSV-2 in the patients was found to be significantly higher ($\chi^2 = 1.256$) in those having CD4 counts >250/µL. 64.7% of patients who tested positive for HSV-2 had their CD4 counts > 250/µL (Table 5).

Table 5: HSV-2 and CD4 counts

<table>
<thead>
<tr>
<th></th>
<th>CD4 &lt; 250/µL</th>
<th>CD4 &gt;250/µL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2 negative</td>
<td>75 (40.8%)</td>
<td>109 (59.2%)</td>
<td>184 (100%)</td>
</tr>
<tr>
<td>HSV-2 positive</td>
<td>77 (35.3%)</td>
<td>141 (64.7%)</td>
<td>218 (100%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>152</td>
<td>250</td>
<td>402 (100%)</td>
</tr>
</tbody>
</table>

4.3.4.1 HSV – 2 infections among HIV – 1 positive (CD4 < 250/µL) samples.

Amongst one hundred and fifty two samples studied with CD4 < 250/µL, 49.5% (N = 75) of the samples were HSV-2 negative and 50.7% (N = 77) were HSV-2 positive. In Samples from Kangemi, 50.8% (N = 33) were HSV-2 positive, 49.2% (N = 32) were negative. In samples from
Ngaira 46.7% (N = 28) were HSV-2 positive, 53.3% (N = 32) were negative. Pumwani had the highest percentage (63.6% N = 14) of the samples testing positive while 36.4% (N = 8) were negative. Samples from Waithaka had 40.0% (N = 2) HSV-2 positive and 60% (N = 3) HSV-2 negative (Table 6).

**Table 6**: HSV–2 infections among HIV–1 positive (CD4 < 250/µL) samples.

<table>
<thead>
<tr>
<th></th>
<th>Kangemi</th>
<th>Ngaira</th>
<th>Pumwani</th>
<th>Waithaka</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2 Positive</td>
<td>33 (50.8%)</td>
<td>28 (46.7%)</td>
<td>14 (63.6%)</td>
<td>2 (40.0%)</td>
<td>77 (50.7%)</td>
</tr>
<tr>
<td>HSV-2 Negative</td>
<td>32 (49.2%)</td>
<td>32 (53.3%)</td>
<td>8 (36.4%)</td>
<td>3 (60.0%)</td>
<td>75 (49.5%)</td>
</tr>
</tbody>
</table>

**4.3.4.2 HSV – 2 infection among HIV – 1 positive (CD4 > 250/µL) samples**

Of the two hundred and fifty samples with CD4 > 250µL, 56.4% (N = 141) were HSV – 2 positive while 43.6% (N = 109) were HSV-2 negative. Samples from Kangemi had 56.6% (N = 60) HSV-2 positive and 43.4% (N = 46) negative; samples from Ngaira had 48.8% (N = 41) positive and 51.2% (N = 43) HSV – 2 negative; those from Pumwani had 60.0% (N = 24) positive and 40.0% (N = 16) HSV – 2 negative; many 80.0% (N = 16) of the samples from Waithaka were positive while 20.0% (N = 4) HSV – 2 negative (Table 7).
Table 7: HSV – 2 infection among HIV – 1 positive (CD4 > 250/µL) samples

<table>
<thead>
<tr>
<th></th>
<th>Kangemi</th>
<th>Ngaira</th>
<th>Pumwani</th>
<th>Waithaka</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2 Positive</td>
<td>60 (56.6%)</td>
<td>41 (48.8%)</td>
<td>24 (60.0%)</td>
<td>16 (80.0%)</td>
<td>141 (56.4%)</td>
</tr>
<tr>
<td>HSV-2 Negative</td>
<td>46 (43.4%)</td>
<td>43 (51.2%)</td>
<td>16 (40.0%)</td>
<td>4 (20.0%)</td>
<td>109 (43.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>84</td>
<td>40</td>
<td>20</td>
<td>250 (100%)</td>
</tr>
</tbody>
</table>

In this group of individuals (CD4 > 250 µL), HSV – 2 Positive samples were similarly found to be more in samples from female individuals, 63.8% (N = 90) than in male individuals 36.2% (N = 51).

4.3.4.3 HSV – 2/HIV – 1 co-infection rates between samples with CD4 counts < 250 µL and those with CD4 >250 µL.

There was no significant difference in HSV-2 prevalence in samples with CD4 < 250 µL to those having CD4 > 250 µL (P > 0.05) (fig. 4.5) Mean HSV-2 optical density for samples having CD4 < 250 µL (1.01) was lower than mean counts for samples having CD 4 > 250 µL

4.4 Syphilis and HIV-1 co-infection

From the sampled population, 97.8% (N = 393) were not infected by syphilis while 2.2% (N = 9) were having syphilis.

In the four sampled sites, Syphilis was notably absent in samples from Pumwani. However in samples from Kangemi (3), Ngaira (5) and Waithaka (1) were positive for syphilis. Distribution
of Syphilis in the centres showed that a higher percentage of samples from Waithaka tested positive (4.0%) Samples from Ngaira (3.5%) and those from Kangemi (1.8%) were positive (Figure 9).

![Figure 9: Distribution of syphilis infection in the health facilities.]

### 4.4.2 Gender and Syphilis infection

The syphilis infection was not significantly different in the gender. Slightly more males (2.8%) respondents were infected than female (1.9%) respondents (Table 8).

<table>
<thead>
<tr>
<th>Gender</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>256 (98.1%)</td>
<td>5 (1.9%)</td>
</tr>
<tr>
<td>Male</td>
<td>137 (97.2%)</td>
<td>4 (2.8%)</td>
</tr>
</tbody>
</table>
4.4.3 Syphilis infections and ages of the respondents

Infection of the patients by syphilis did not significantly have any relationship to the patients’ age; however, the few respondents who tested positive for Syphilis were between the ages of 21-50 years (Figure 10)

![Age groups (years)](image)

**Figure 10:** Ages of the respondents and Syphilis infections

4.4.4 Comparison between Syphilis infection to CD4 counts

There was no significant relationship of CD4 counts to infection by syphilis (r = 0.026, P = 0.602). This study found 98.0% of patients having CD4 <250 µL / were negative for syphilis test whereas 2.0% of those having CD4 < 250/ µL tested positive for syphilis. More of the samples who had syphilis (66.7%) also had CD4 > 250/µL (Table 9). Samples which had infection by syphilis had mean CD4 counts, 480.44 ± 90.2. Those that tested negative of Syphilis had CD4 counts of 382.69 ±15.45.
Table 9: CD4 status and their percentage infection by syphilis.

<table>
<thead>
<tr>
<th>CD4 Status</th>
<th>Syphilis NEG</th>
<th>Syphilis POS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;250/µL</td>
<td>149 (37.9%)</td>
<td>3 (33.3%)</td>
<td>152</td>
</tr>
<tr>
<td>&gt;250/µL</td>
<td>244 (62.1%)</td>
<td>6 (66.7%)</td>
<td>250</td>
</tr>
<tr>
<td>Total</td>
<td>393 (100%)</td>
<td>9 (100%)</td>
<td>402</td>
</tr>
</tbody>
</table>

4.5 HBV and HIV-1 co-infection

For HBV testing, it was established that 4.5% ($N = 18$) of the samples were positive while 95.5% ($n = 384$) were negative. HBV was found in samples from all the four sites. In samples from Kangemi 8 (4.7%), Ngaira 7 (4.9%), Pumwani 2 (3.2%) and Waithaka 1 (4.0%) samples were positive. In the respective centres, distribution of HBV showed that a higher percentage of samples from Ngaira tested positive (4.9%) (Fig.7). Sample from Kangemi (4.7%), Waithaka (4.0%) and those from Pumwani (3.2%) were positive (figure 11).

![Figure 11: Distribution of HBV in the health facilities.](image-url)
4.5.2 Gender and HBV infection

Infection by HBV did not significantly differ in males to females. However, slightly more male respondents (6.4%) were infected than females (3.4%). 93.6% males tested negative while in female 96.6% tested negative for HBV (Table 10).

Table 10: Gender and HBV infections

<table>
<thead>
<tr>
<th>Gender</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>Female</td>
<td>252(96.6%)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>132(93.6%)</td>
</tr>
</tbody>
</table>

4.5.3 Ages of the respondents and HBV infections

Infection with HBV was observed in the age groups between 21-50 years. None of the respondents aged below 20 years and above 50 years tested positive for HBV (Figure 12).

Figure 12: Ages of the respondents and HBV infections
4.5.4 Comparison between HBV to CD4 counts

The study established that there was no significant correlation of HBV infection to the CD4 counts in individuals. 95.4% of the individuals with CD4 <250/µL were HBV negative while 4.6% were infected by HBV. 95.6% of individuals CD4 >250 µL were HBV negative while 4.4% were infected with HBV (Figure 11). More of the positive samples (61.1%) were noted to have CD4 > 250µL (Table 11).

Table 11: HBV and CD4 counts

<table>
<thead>
<tr>
<th>CD 4</th>
<th>HBV negative</th>
<th>HBV positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;250</td>
<td>145 (95.4%)</td>
<td>7 (4.6%)</td>
<td>152 (100%)</td>
</tr>
<tr>
<td>&gt;250</td>
<td>239 (95.6%)</td>
<td>11 (4.4%)</td>
<td>250 (100%)</td>
</tr>
</tbody>
</table>

4.6 Comparison between Herpes simplex Virus, syphilis and Hepatitis B infection.

In the sampled population there was no sample that tested positive for all the three infections (HSV-2, Syphilis and HBV).

4.6.2 Comparison between HSV-2 to Syphilis infection

Infection of the patients by HSV-2 was not significantly associated to infection by syphilis. 98.2% of the patients infected by HSV-2 were negative for syphilis infection. Similarly, 97.3% of those who were not infected by HSV-2 were not infected by syphilis (Table 12).
Table 12: HSV-2 and syphilis coinfection

<table>
<thead>
<tr>
<th></th>
<th>Syphilis negative</th>
<th>Syphilis positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2 Negative</td>
<td>179 (97.3%)</td>
<td>5 (2.7%)</td>
<td>184 (100%)</td>
</tr>
<tr>
<td>HSV-2 positive</td>
<td>214 (98.2%)</td>
<td>4 (1.8%)</td>
<td>218 (100%)</td>
</tr>
</tbody>
</table>

4.6.3 Comparison between HSV-2 and HBV infection

Infection by HSV-2 had no significant relationship to infection with HBV. 96.8% of the patients infected by HSV-2 were negative for HBV test. However, 94.0% of those who were negative for HSV-2 were also negative for HBV (Table 13).

Table 13: HSV-2 and HBV infection

<table>
<thead>
<tr>
<th></th>
<th>HBV Negative</th>
<th>HBV Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2 Negative</td>
<td>173 (9.0%)</td>
<td>11 (6.0%)</td>
<td>184 (100%)</td>
</tr>
<tr>
<td>HSV-2 positive</td>
<td>211 (96.8%)</td>
<td>7 (3.2%)</td>
<td>218 (100%)</td>
</tr>
</tbody>
</table>

4.6.4 Co-infection of Syphilis and HBV

The study established that there was no sample which tested positive for both HBV and Syphilis. All of the individuals infected by HBV tested negative for syphilis. At the same time There was a significant association in infection by syphilis to infection by HBV ($\chi^2 = .432$, df = 1, P = 0.000) (Table 14).
Table 14: HBV and syphilis infection

<table>
<thead>
<tr>
<th></th>
<th>Syphilis negative</th>
<th>Syphilis positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV Negative</td>
<td>375 (97.7 %)</td>
<td>9 (2.3%)</td>
<td>384 (100%)</td>
</tr>
<tr>
<td>HBV positive</td>
<td>18 (100%)</td>
<td>0 (0%)</td>
<td>18 (100%)</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Introduction

Sexually transmitted infections (STIs) represent a major public health problem in developing countries including Kenya. HIV-1 infection is one of the most destructive pandemics in recorded history has been spreading at an alarming rate over the years, with the current prevalence rate of HIV-1 in Kenya being 8% (KAIS, 2008). The impact of HIV/ AIDS, on society in Africa is placing great strain on resources on fragile economies. There are about 9.5 million people in immediate need of life-saving AIDS drugs in the developing and transitional countries. Of these, only 4 million (42%) are receiving the drugs (http://www.avert.org/worldstats.htm 2009).

Ulcerative sexually transmitted illnesses like Herpes simplex Type 2, Syphilis and Hepatitis B, which cause breaks in the skin or mucous membranes, reduce barriers that provide protection against HIV-1 infections. These then increase the risk of HIV-1 acquisition. Understanding the proportion of these STIs among new HIV-1 infections and the role they play in fanning the HIV-1 pandemic, provides an insight into current epidemic dynamics in Kenya. Knowledge of this may be used in designing and evaluating future HIV-1 interventions.

This study sought to get the prevalence of HSV-2, Syphilis and Hepatitis B among HIV-1 positive individuals seeking CD4 testing services at selected health facilities in Nairobi. It also compared the CD4 counts of the individuals who were HSV-2 and HIV-1 co-infected. Co-infections between HIV-1, Herpes simplex Virus, syphilis and Hepatitis were also determined.
5.1.2 Bio-Data of the individuals sampled

5.1.2.1 Gender and HIV-1 infection

This study involved use of blood samples collected from HIV-1 positive patients attending various selected health facilities in Nairobi. There were more women (64.9%) than men (35.1%) sampled in this study. These findings are in line with many studies done earlier in Kenya and globally. Reports by NASCOP in 2010 showed that women (8%) were more likely to be HIV-1 infected than men (4.3%), while the rate of HIV-1 infection was at 6.3% for the total Kenyan population. This was in line with reports from street dwellers in Gondar city in Ethiopia which showed the proportion of women found to be HIV-1 positive to be at 11.8%, twice higher than males (5.8%) (Feleke et al., 2006). According to UNAIDS 2009, the AIDS epidemic has had a unique impact on women, which has been exacerbated by their role within society and their biological vulnerability to HIV-1 infection. Biologically, women are twice more likely to become infected with HIV-1 through unprotected heterosexual intercourse than men. Females are more vulnerable to infections through mucosal membranes of the genitals during unprotected sexual intercourse. They expose comparatively a larger surface area of the mucosal membranes than the male counterpart. Due to this fact, it may also be possible that females show symptoms much earlier and to a greater magnitude than males hence present themselves to the health clinics in greater numbers than males. In many countries women are less likely to be able to negotiate condom use and are more likely to be subjected to non-consensual sex (UNAIDS, 2009).

5.1.2.2 Ages of the respondents

Age specific HIV-1 infection prevalence was particularly higher in the ages between 20-40 years with an age limits of 31-40 years (42%) and 21-30 years (34.5%).
This is in line with a study done in Ethiopia which showed the prevalence to be higher in 26-35 years among military recruits in rural Ethiopia (Feleke et al., 2006). This observation is worrisome since the most productive and economically viable age group of the populations is worst hit (Tessema et al., 2010). The study also established that there was a sudden upsurge in the numbers from below 20 years through 30 years of age distorting the normal trend in distribution.

5.1.2.3 Gender/Age correlation

Gender mean ages of the individuals were; female (33.17 years ± 0.50) and males (38.56 years ± 0.71). This indicated that males in the sampled population were slightly older than the females. This may indicate that more girls engage in sexual activities at a much earlier age than boys.

5.1.3 CD4 status of the sampled population

The CD4 count indicates immune status of an individual and prognosis of the disease and helps predict the risk of complications and debilitating infections. The findings of this study showed that 62.2% of the patients had CD4 counts to be >250 cells per µL while 37.8% had CD4 counts of less than 250 cells per µL. Generally there was a high mean CD4 count of 419 cells per µL. However, this difference was statistically not significant (P= 0.268). The introduction of Highly Active Antiretroviral Therapy (HAART) has led to significant drop in the rate of HIV-1 and AIDS-related morbidity and mortality.

HAART delays clinical progression by suppressing viral replication, measured by a substantial reduction in HIV-1 RNA, and allowing immune reconstitution, measured in most studies by increasing in CD4 cells count (Jing et al., 2005). The sampled population was of HIV-1 positive individuals under ARV treatment.

5.1.4 HSV – 2 infections among HIV – 1 positive individuals
Infections with *Herpes simplex* virus type 2 (HSV-2) is a major cause of genital ulcers worldwide and has been implicated as an important co-factor for HIV-1 infection (Dickerson *et al.*, 1996). Studies have indicated synergy between HIV-1 and HSV-2 where HSV-2 has been linked to a three times higher risk of sexually acquired HIV-1 (Freeman *et al.*, 2006). Mucosal disruption caused by genital ulcers has been found to favour HIV-1 acquisition by providing a ready portal of entry (Corey *et al.*, 2004). HSV-2 reactivation results in mucosal infiltration with activated CD4-bearing lymphocytes, the target cells for HIV-1 attachment. Moreover, HSV-2 infection is a significant opportunistic infection in HIV-1 infected individuals (Posavad *et al.*, 1997). Clinical manifestations and interaction of HIV-1 and HSV-2 suggest a vicious cycle of positive feedback for the advancement and re-infection for the two diseases. It is thus important that prevalence of HSV-2 among HIV-1 positive individuals was established.

HSV-2 was recorded in all the centres, and in higher numbers unlike Syphilis and HBV among the sampled population. The prevalence of HSV in this study was found to be 54.2%.

Ngaira was the only health centre with HSV-2 prevalence below 50%. Ngaira is located in the Nairobi’s central business district and serves low income earners but whose lifestyles are slightly better than that of the respondents from Waithaka and Kangemi. Kangemi and Waithaka are located on the outskirts of Nairobi and are high density urban slum areas, lacking in basic amenities and with few health facilities. This has contributed to the higher HSV-2 prevalence among HIV-1 positive individuals in these two health facilities. Epidemiological data indicates that HIV-1 and HSV-2 have overlapping prevalence patterns. It is estimated that 50–90% of HIV-1 infected individuals are co-infected with HSV-2 (Clark *et al.*, 2008). However reports from Pune, India, showed a prevalence of 22.6% of HSV-2 in patients infected with HIV-1 (Reynolds et al, 2003). Other reports show that most people infected with HSV-2 are not aware of their
infection (http://www.onlinedatingmagazine.com/STDs/herpes.html) posing a great risk of transmission to uninfected partners (Reynolds, 2009).

5.1.4.2 Gender and HSV-2 infection

This study established that HSV-2 infection versus gender showed comparable percentages of 54% for both males and female. It was also noted that of the individuals positive for HSV-2, prevalence was highest in the samples obtained from female participants (64.68%).

These results are in line with a report on the epidemiology of HSV-2 infection and its association with HIV-1 infection in four urban populations which indicated an HSV-2 prevalence of over 50% among women and over 25% among men, with notable high rates of infection among young women aged 15-19 years in Kisumu and Ndola (39% and 23%, respectively, among women(Weiss et al., 2001). These observations were generally higher as compared to findings from among patients attending an STD clinic in Jawaharlal Nehru Medical Hospital (JLN hospital) Ajmer India, which found a coinfection rate of HSV in HIV-1 positives to be 42.9% (Peters et al., 2004). This study also found out that a large proportion of the HSV-2 positives (40.7%) to be females of child bearing age. This was in line with findings in India (Peters et al. 2004).

The high rate of co-infection between HIV-1 and HSV-2 might be due to the fact that these pathogens share common modes of transmission and risk groups (Weiss et al, 2001).

5.1.4.3 HSV–2 infections and CD4 counts among HIV–1 positive samples.

In this study it was found that individuals having a CD4 count of more than 250cell/µL had an HSV-2 positivity of 56.4% as compared to those individuals having a CD4 count of less than
250 cell/µL which had 50.6% positivity. There is indication that being infected with HSV-2 does not necessarily affect one's CD4 count; similarly having a CD4 count above or below 250/µL does not necessarily predispose one to HSV-2 acquisition.

5.1.5 Syphilis-HIV-1 co-infection

HIV-1 and syphilis are often seen as co-infections since they share a common mode of transmission. Infection with Syphilis leads to formation of genital ulcers. That can bleed easily and when they come into contact with oral and rectal mucosa during sex, increase the infectiousness of and susceptibility to HIV-1 (CDC, 2006). It has been shown that early syphilis may decrease CD4+ T-cell counts (CD4 cell counts) and increase viral load in plasma and semen. T. pallidum’s role in facilitating HIV-1 transmission may include up-regulation of gene expression, such as that of the CCR5 co-receptor used in HIV-1 entry (Hook et al., 1992, Hutchinson et al., 1994 Rolfs et al., 1997). It is therefore important to establish the prevalence of Syphilis which is a in factors fuelling HIV-1 infection.

Prevalence of syphilis was 2.2% in the sampled population, the rate that was also slightly higher in males than females. It also showed that infection by syphilis was only noted in individuals between the ages of 21-50 years. These findings deferred from those detected in a study conducted in Pumwani maternity hospital among pregnant women which showed the prevalence to be 3% (Temmerman et al., 2000, Feleke et al., 2006). However, compared to previous studies in Ethiopia, the prevalence of syphilis has decreased progressively from 3.9% in 2003 to 1.9% in 2004, 0.1% in 2005 and 0.2% in 2006 and 2007 (Tessema et al., 2010). Contrary to these findings, reports of syphilis prevalence in HIV-1-infected individuals in other countries show prevalences of 59.7% Argentina (Griemberg et al., 2006), 12.8% in Tanzania (Tessema et al., 2010) and 8.3% in military recruits in the U.S (Kasutto and Sax, 2003).
Syphilis has infected man for centuries and a lot of research has taken place until it is under control and treatable even in developing countries like Kenya. This may explain the low prevalence rates as compared to HSV-2.

5.1.6 HBV and HIV-1 coinfection

The HBV pathogen is usually present in blood, saliva, semen, vaginal secretions, and menstrual blood of infected individuals, infection of which causes liver cirrhosis and hepatocellular carcinoma. HBV is resistant to breakdown outside the body hence it is easily transmitted through contact with infected body fluids (Wright, 2006). It can be transmitted by percutaneous or mucosal exposure to infected blood or other body fluids ((Shepard et al., 2006). Human immunodeficiency virus (HIV) and HBV infections are two major viral infections worldwide (Umolu et al., 2005). Though HBV and HIV-1 do share similar transmission routes, information on the actual role of HBV in HIV-1 infection is not well established. Since co-infection with HBV and HIV-1 is becoming common and a growing public health concern (Otedo, 2004, Sheng et al., 2007, Shimelis et al., 2008), it is important for HBV prevalence among HIV-1 positive individuals to be established to provide useful information on the management of HIV-1 infection.

The 4.5% prevalence of HBV in the sampled population was slightly higher than 2.5% from reports of a study in Kenya, done on circulating HIV-1 sub types in voluntary blood donors co-infected with HBV in Western Kenya (Makokha et al., 2004).

The findings of this study are however contrary to a study done on HBV, HIV co-infection in Kisumu district hospital showing that 47% of the patients screened had HBV infection (Otedo, 2004).
A similar study, shows 32 (78%) out of 41 patients with AIDS had serological evidence of exposure to HBV (Ezegbudo et al., 2004). A growing body of evidence indicates that HIV-1 positive individuals are more likely to be infected with HBV than HIV-1 negative individuals, possibly as result of shared risk factors (Burnett et al., 2005). The study also established that infection by HBV was slightly higher in males than females.

Two studies from Nigeria also showed an increased prevalence of HBsAg in HIV-1 infected patients (25.9% in 490 positive patients versus 14.9% in 175 HIV-1 negative blood donors (Apurva et al., 2007). However, another study carried out at Aga Khan University Hospital in Nairobi on the prevalence of HIV-1 and hepatitis B co-infection amongst HIV-1 patients indicated that 6% of the patients were found to be co-infected with HBV. The study further concluded that HIV-1 positive patients in Kenya have a low rate of co-infection with HBV although their study was limited due to the sample size used (Harania et al., 2008). This study also established that infection by HBV was only noted in individuals between the ages of 21-50 years.

The low prevalence of HBV noted in this study could be due to the introduction of guidelines for the adequate management of chronic hepatitis B in HIV-1 co-infected individuals. The low prevalence rates recorded here could also be due to the spirited campaigns carried out by the Kenyan government in 2001. These included increasing awareness of the nature of the HBV disease and mobilizing medical personnel to carry out large scale vaccination programs against the HBV.

Several reviews have also updated the knowledge on this topic, providing useful information about how to manage HBV/HIV-1-1-coinfected patients (Soriano et al., 2006).
Although a number of prevalence studies on HIV-1/HBV co-infection have been performed within Kenya and in Africa, conflicting results have been observed with both higher and lower rates of HBV being reported in HIV-1-positive patients (Apurva et al., 2007). Unfortunately to date, data on co-infection from Africa is very limited and consequently the scope and impact of the problem as well as priorities for intervention are poorly understood.

5.1.7 Comparison between Herpes simplex Virus, syphilis and Hepatitis B infection.

In the sampled population, there was no sample that tested positive for all the three infections (HSV-2, Syphilis and HBV). Infection of the patients by HSV-2 was not significantly associated to infection by syphilis. The study also established that there was no sample which tested positive for both HBV and Syphilis. These findings indicate that infection by any one of the three STIs (HSV-2, Syphilis and HBV) does not necessarily predispose one to higher risk of getting infected by any of the other two STIs.

5.2 CONCLUSIONS

This study concluded that biologically, women are twice more likely to become infected with HIV-1 through unprotected heterosexual intercourse than men.

The study also established that there was a sudden upsurge in the numbers from below 20 years through 30 years of age of the sampled population distorting the normal distribution trend. This indicates that there are many more individuals getting infected with HIV-1 soon after their high school than any other age groups

HSV-2 prevalence was higher than any of the other STIs investigated. The statistical conclusions strongly indicated close association between HIV-1 and HSV-2 infections. The high rate of co-
infection between HIV-1 and HSV-2 might be due to the fact that these pathogens share common modes of transmission and risk groups.

There is indication that being HSV-2 positive does not necessarily affect ones CD4 count; similarly having a CD4 count above or below 250/µL does not necessarily predispose one to getting infected with HSV-2. This may mean that the HSV-2 prevalence is irrespective of the CD4 count. Distribution of CD4 status in the four health centers showed that, a higher percentage of persons from Ngaira had CD4< 250, indicating that this was the worst hit population in terms of AIDS disease.

The results of this study showed that a small percentage (2.2%) of individuals are co-infected by both HIV-1 and syphilis; indicating that of the population sampled, syphilis may not be playing a significant role in fuelling the HIV-1 pandemic.

The study concluded that HIV-1 positive patients in Kenya have a low rate of co-infection with HBV (4.5%). The low prevalence of HBV noted in this study could be due to the introduction of guidelines for the adequate management of chronic hepatitis B in HIV-1 co-infected individuals. This could also be due to the spirited campaigns carried out by the Kenyan government in 2001. These included increasing awareness of the nature of the HBV disease and mobilizing medical personnel to carry out large scale vaccination programs against the HBV. HBV may not be playing a significant role in fuelling the HIV-1 pandemic.

The study concluded that infection by any one of the three STIs (HSV-2, Syphilis and HBV) does not necessarily predispose one to higher risk of getting infected by any of the other two.
5.3 RECOMMENDATIONS

This study targeted informed HIV-1 positive individuals who were already receiving CD4 testing services, leaving out a high percentage of the Kenyan population who do not access medical services yet are HIV-1 infected, getting infected or in the process of infecting others. It is also important that deliberate and effective measures be put in place by the ministry of health to access everyone.

Majority of studies have examined low risk populations, pregnant women and blood donors which may underestimate the prevalence of co-infection. Studies should also be done in high risk populations and the general population at large to get a clearer picture of the rates of HIV-1 and STIs infection in Kenya

Since the rate of HSV-2 infections is high, it is important that routine screening for this STI and HIV-1 infection be carried out concurrently. There is need for the ministry of health to provide measures of effective management of HIV-1/HSV-2 co-infections so as to avoid high mortality due to both viruses. It is also important that safe and effective vaccines be made available to all health facilities.

In addition there is need for more research to be done on HIV-1, HSV, syphilis, and HBV co-infections so as to get a clear picture of the prevalence of these infections and also to avoid incidences of conflicting results.

There is need to research on why HIV-1/HSV-2, HIV-1/syphilis, HIV-1/HBV co-infected individuals have higher CD4 counts than HIV-1 positive individuals who are not co-infected.

It is also important that the search for a cure or vaccine for HIV-1 should also be intensified.
REFERENCES


3. Andre, F., Hepatitis B epidemiology in Asia, the Middle East and Africa. *Vaccine* 2000; 18 (Suppl. 1); S20-S22.


55. *Morbidity and Mortality Weekly Report* Revised Guidelines for HIV Counseling, Testing, and Referral", 9 November 2001;50(RR19);1-58,


65. Pereira R., Roldão de Almeida A., Duarte Hg Mussi A., Azevedo V., Silva C. and Dutra Souto F. Hepatitis B Virus infection in HIV-1-positive population in Brazil: Results of a survey in the state of Mato Grosso and a comparative analysis with other regions of Brazil. *BMC Infectious Diseases* 2006. 6, 34.


67. Phillips S, Granade C, Pau C-P 1 "Diagnosis of Human Immunodeficiency Virus Type 1 Infection with Different Subtypes Using Rapid Tests" by, *Clinical and Diagnostic Laboratory Immunology*, 2000.


87. Stamm E, Handsfield H, Rompalo M, Ashley L, Roberts L, Corey L. The association between


90. Sucato G, Wald A, Wakabayashi E, Vieira J, Corey L. Evidence of latency and reactivation of both herpes simplex virus (HSV-1) and HSV-2 in the genital region. *J Infect Dis* 1998; 177: 1069–72


GlobalReport/2011


110. Wright, T. Introduction to Chronic Hepatitis B virus infection. *The American Journal of Gastroenterology*. 2006; 101; S1


120. http://womenshealth.about.com/cs/syphilis/a/syphilisdiagnos.htm2010
123. http://www.britishlivertrust.org.uk/home/health-professionals/literature-for-professionals/a-
130. http://www.searo.who.int/en/Section10/Section17/Section53/Section482_1779.htm 2010