

CHAPTER ONE
INTRODUCTION

Introduction

1.1 Background:

Upper Nile State is one of the ten states in the Republic of South Sudan. It is in the former Greater Upper Nile Region bordering the Republic of the Sudan in the north. According to the 2008 census, South Sudan has a population of 964337 (Government of Sudan, census of the Sudan, 2008, Khartoum).

The capital Town is Malakal with a population of 126,483 people according to the Household Survey conducted in June, 2010. South Sudan has been embroiled in a war for more than 21 years only ending in 2005 with the signing of the Comprehensive Peace Agreement (CPA). The dynamics of the HIV epidemic during the period of conflict and the great movement of people and social changes that have followed the coming of peace remain largely unexplored. Many factors occurred during the war which claimed 2.5 million inhabitants and would be relevant to the epidemiology of HIV in South Sudan.

The Republic of South Sudan covers approximately 640,000 square kilometers (km²), and lies between 25° to 30° east longitude and 4° to 12° north latitude. It borders Ethiopia to the East, Kenya and Uganda to the South, the Democratic Republic of Congo to the South West and the Central African Republic to the West, and therefore, it lies within the Meningococcal belt of the African Continent. South Sudan is divided into ten states, 79 counties and 514 administrative Payams and 2,159 Bomas. The latter comprise clusters of households or villages which form the smallest formal administrative units. South Sudan has widely contrasting terrain with vast low lying plains that easily flood during the long rainy season between April and November of each year and Mountainous area to the north and to the west that easily drain after rains. South Sudan is traversed by many rivers and streams. Significantly large areas of the country are swampy marshlands which become flooded in the rainy season. This forms a rich ecosystem for a number of human microorganisms and vectors that cause serious diseases (MOH, 2011).

The population of South Sudan is estimated at 964337 and expected to increase to 12 million by 2014 owing to high rate of natural growth and the return of refugees from neighboring countries and internally displaced populations located in Sudan. There are 300 ethnic groups in South Sudan. Centrally, eastwards and to significant proportion of the south, the predominant culture is nomadic pastoralist, but there are significant sedentary farming groups. Moreover, sedentary practices are increasingly emerging with resettlement after a protracted war (MOH, 2011).

South Sudan has wide variation in cultural beliefs and traditional practices on one hand a rapid transition through affluence, a factor that has significant health implications. South Sudan is one of the poorest countries in the world, although prospects of oil revenue promise future economic improvement. With few exceptions, population density is low, presenting some serious constraints in the distribution of health care personnel and commodities (MOH, 2011).

1.2 Human Immunodeficiency Virus:

Human Immunodeficiency Virus (HIV) is a retro virus (member of the genus Lenti virus), part of the Retroviridae family that causes Acquired Immunodeficiency Syndrome (AIDS), a condition in which the immune system begins to fail, leading to life – threatening opportunistic infections. Infection with HIV occurs by transfer of blood, semen, vaginal fluid and pre-ejaculate or breast milk. HIV infection in humans is considered pandemic by World Health Organization (WHO). From its discovery in 1981 to 2006, AIDS killed more than 25 million people. HIV infects about 0.6% of the world's population (JUNP,2006).

In many regions of the world, new HIV infections are concentrated among young people (15 – 24 years of age), which accounted for 40 per cent of new HIV infections in 2006. Sub-Saharan Africa continues to bear the brunt of the global epidemic. Two thirds (63%) of all adults and children with HIV globally live in this region, and almost 72 per cent of all adults and children death due to AIDS in 2006 occurred in this region, with its epicenter in Southern Africa. About 32 per cent of all people with HIV globally live in Southern Africa and 34 per cent of all deaths due to AIDS in 2006 occurred there. Overall Sub-Saharan Africa is home to an estimated 24.7 million adults and children infected with HIV in 2006 (WHO and UNAIDS, 2006).

In 2009, there were approximately 2.2 million new infections in adults; nearly 1million of them were in East-South Africa (UNAIDS, 2009). Globally, approximately 370,000 babies were born with HIV in 2009; 57% or 210,000 were in East-South Africa (UNAIDS, 2009).

The HIV indicator in South Sudan shows that the HIV prevalence estimate for the country is 3.1% making the estimated people living with HIV/AIDS (PLWHA) to be 155,000. The HIV epidemic is likely to grow worse due to existence of several factors that favor the transmission of the disease (AIDS) in South Sudan. These include the lack of access to HIV prevention and care

service, lack of awareness among the communities, polygamy, wife inheritance and traditional practices (UNAIDS, 2008; SSAC, 2007; MOH, 2009; SSAC, 2011).

South Sudan is described as having a low generalized epidemic with an average HIV prevalence rate of 3% among pregnant women (MoH/SSAC, 2010). The prevalence shows wide disparities in geographical locations with some areas as high as 7.2% in Western Equatoria State (WES), prevalence rate in Eastern Equatoria State (EES) is 3.3% in adult population (Ante-Natal Clinics surveillance report, 2009), in Jonglei State (JS) the number of people tested from September 2010 to August 2011 for HIV/AIDS were 3,472 (female: 58.3%, male: 41.7% and positive clients were 103 (male: 51.2% and female: 48.8%) (SAC Jonglei State, 2011) in Upper Nile State the number of people tested for HIV/AIDS were 899 (255 males and 644 females), those tested positive were 27 (10.5%) males and 68 (10.5) females and low in Northern Bahr Ghazal State (NB) and Warrap State 0.7% (Annual Stakeholders' Forum 28th – 30th Sep, 2011). Areas along the borders of Uganda, Kenya and The Democratic Republic of Congo (DRC) as well as areas along major transport corridors are believed to have significantly higher prevalence rates. With peace prevailing and mobility restored, South Sudan is therefore likely to experience a rapid increase in HIV prevalence that can reach as high as nearly 6 percent by 2015 (UNAIDS 2010). It is estimated that there are 116,000 people living with HIV/AIDS in South Sudan, of which 46,500 are in need of treatment. This is more devastating than the civil war itself which claimed 2.5 million lives over a period of twenty one years. Unless current interventions are scaled-up and sustained, the epidemic will threaten or even reverse, some of the important, hard, won gains such as CPA, health and education (MOH, 2010).

The region south of the Sahara within which South Sudan belongs has been badly hit. The UNAIDS's statistics point out that in 2007, 25.5 million people in the Southern part of the Sub-Saharan Africa were positive and an approximately 1.7 million children have been orphaned by AIDS (UNAIDS, 2010).

Key drivers of the epidemic include knowledge about HIV and AIDS which is extremely low; result from Sudan Household Survey 2006; showed that only 9.8% of Southern Sudanese (South Sudanese) women aged 15-24 years were knowledgeable about the three ways of preventing transmission of HIV and AIDS. The problem was much greater in some of the states such as Jonglei (25%), Warrap (28%), and Northern Bahr El-Ghazal (35%)(SHHS, 2006).

High population movements, including the return of four (4) million displaced South Sudanese (returning home from African countries were the Uganda, Kenya and Ethiopia hardest hit by HIV/AIDS), businessmen, truck drivers, amid the situation of opening of roads and hence, exposing rural areas to increased risk of HIV infection.

Poverty, low school enrollment especially among girls; In 2003, Southern Sudanese children had the world's lowest primary school gross enrolment ratio (20%), lowest primary school completion rate (2 %), and lowest ratio of female-to-male enrolment (35 %). The adult literacy rate was 24 percent (for adult females: 12 percent), and the youth literacy rate was 31 percent. High risk behaviors including the practice of multiple concurrent sexual partners in stable and casual relationships; along with institutionalized powerlessness among women and girls that obviate safer sex practices, put people especially women and girls at increased risk of HIV transmission;

High levels of AIDS related stigma, discrimination and denial, coupled with limited coverage of formal health care systems, contribute to reduced access to HIV prevention, treatment, care and support services for people at risk and those infected and affected by HIV and AIDS (UNICEF, 2004).

In Sudan, Sudan National AIDS Programme (SNAP) reported that there were 173 HIV cases during 2005, 249 cases in 2004 and 916 in 2003 with overall 6.83% prevalence rate (SNAP, 2005).

The National Health Information Centre (NHIC), the data centre for Federal Ministry of Health, reported that there were 512 HIV cases during 2004 (NHIC, 2004).

The Republic of South Sudan shares borders with countries reported to have high rates of HIV/AIDS in Uganda 6.5%, Kenya 6.3%, Ethiopia 1.1%, Democratic Republic of Congo 3.4%, and Central African Republic 4.9% (Southern Sudan ANC Surveillance, 2009).

In Southern and Eastern Africa, several studies on occupationally distinct populations have been conducted to determine rates of HIV seroconversion and to identify associated risk factors. For example, among male factory workers in Harare, Zimbabwe during 1993 to 1995, incidence of HIV was 2.9 per 100 person-years, and factors that include reported genital ulcers, multiple

sexual partners and being married but separated from their spouses were each significantly associated with seroconversion (Grosskurth *et al.*, 1995). In Dar es Salaam, Tanzania, incidence of HIV during 1994 to 1998 was about 2.0 per person-years (Bakari *et al.*, 2000).

The UNAIDS report of 2008 gave the estimate of people living with HIV and AIDS by the end of 2007 to be 33 million with 22 million (66.7%) of these living in Sub-Saharan Africa. The distribution of the epidemic in Africa is variable with the Southern Africa countries (South Africa, Botswana, Swaziland, Lesotho, Zimbabwe, Malawi and Zambia) bearing the greatest brunt of the epidemic followed by East and Central Africa. The epidemic in Western Africa is less severe than the rest of Africa (Kwesgabo, 2008).

Sentinel surveillance among pregnant women attending antenatal clinics (ANCs) has been the main source of information on HIV trends in Sub-Saharan Africa. These data have also been used to generate national HIV and AIDS estimates. New technologies and resources have allowed many countries to conduct national population based surveys that include HIV prevalence measurement, as an additional source of information on the epidemic (UNAIDS and WHO, 2003).

A STD in either the HIV-negative or HIV-positive partner facilitates the transmission of HIV. The risk of transmission is 8 to 10 times higher. If aSTD, such as syphilis, chancroid or herpes, causes ulceration in the genital or perineal region of the uninfected partner, it becomes far easier for HIV to pass into his or her tissues. STDs cause inflammation. T-cells and monocytes / macrophages, get concentrated in the genital area. In a person already infected with HIV, some of these key cells of the immune system will be carrying the virus which magnifies the risk of transmission to the uninfected partner. As for HIV-infected people, they are more infectious to others in the very early stages, before antibody production during the “window period” and when the infection is well advanced, because levels of virus in the blood is higher than at other times (Park, 2007).

Annually, Sexual Transmitted Diseases (STDs) such as syphilis, gonorrhea and trichomoniasis are significant public health problem in many parts of Africa (Over, 1993). STDs cause substantial morbidity and mortality, and increase the risk of acquiring HIV infection among pregnant women, pose additional problem because of adverse pregnancy outcomes and higher

infant morbidity and mortality. STD treatment, partner reduction and consistent condom use are appropriate preventive measures that have been shown to reduce sexual transmission of HIV and Syphilis (Wasserheit, 1992). Better treatment of STD has reduced the incidence of HIV infection by 42% in Tanzania (Grosskurth *et al.*, 1995).

In the developed world, syphilis infections declined throughout the 1980s and 1990s due to widespread use of antibiotics and the effect of the Human Immunodeficiency Virus (HIV) epidemic. Since the year 2000, rates of syphilis have been increasing again in the United States of America (USA), United Kingdom (UK), Australia and Europe. Much of the increase has occurred among men who have sex with men and is attributed to increased rates of unsafe sexual practices (Kevin *et al.*, 2001; Christopher *et al.*, 2005).

Gonorrhea is a very common infectious disease. The center for Disease Control (CDC) estimates that more than 700,000 people in the United States get new gonorrheal infections each year. Only about half of these infections are reported to CDC. In 2004, 330,132 cases of gonorrhea were reported to the CDC. After the implementation of a national gonorrhea control program in the mid-1970s, the national gonorrhea rate declined from 1975 to 1997. After a small increase in 1998, the gonorrhea rate has decreased slightly since 1999. In 2004, the rate of reported gonorrheal infections was 113.5 per 100,000 persons (CDC, 2004).

Gonorrhoea is one of the most frequently reported infectious diseases in the United States. The causal agent, *Neisseria gonorrhoeae*, a Gram-negative diplococcus, is frequently observed inside polymorphonuclear leukocytes of clinical samples obtained from infected patients. *N. gonorrhoeae* is usually transmitted during sexual contact through an infected birth canal. It does not survive long outside the human body because it is highly sensitive to dehydration (Harvey *et al.*, 2007).

The American Social Health Association (ASHA) estimates trichomoniasis affects 7.4 million previously unaffected Americans each year and is the most frequently presenting new infection of the common sexually transmitted diseases (CDC, 2004). While trichomoniasis is usually passed sexually, it may be picked up from contact with damp or moist object such as towels, wet clothing, or a toilet seat, if the genital area gets in contact with these damp or moist objects. Unlike most STDs, the parasite can live for about an hour on damp towels, washcloths and

bathing suits. If someone uses these towels or washcloths or puts on the bathing suit, the disease may be passed on that way. The good news is that trichomoniasis is curable but often goes undiagnosed because symptoms may not be noticed or even experienced (CDC, 2004). 6175 pregnant women tested for syphilis in the 24 Ante-Natal Clinics sentinel sites in 10 states of Southern Sudan (the former of South Sudan) with 9.9% sero-positive, and 5913 pregnant women tested for HIV in the ANC sentinel sites with 3% ANC Surveillance, 2009).

Syphilis is a sexually transmitted disease caused by the spirochetal bacterium *Treponema pallidum* subspecies *pallidum*. The route of transmission of syphilis is almost always through sexual contact, although there are examples of congenital syphilis via transmission from mother to child in utero. Enormous evidences are available indicating that syphilis increases the risk of HIV infection (Ribud, 2005).

In South Sudan sexually transmitted diseases are a common cause of illness as seen in health facilities. Moreover, recent reports from complaints 10 were confirmed to have syphilis (8%). Another organization that supports health clinics around Yei has reported an average of 600 clients coming for STI services per month (UNAIDS and WHO, 2003).

Candidiasis (candidosis) is caused by the yeast *Candida albicans*, and other *Candida* species, which are normal body flora found in the skin, mouth, vagina, and intestines. Although considered yeast, *C. albicans* is dimorphic, and can form a true mycelium. Infections occur when competing bacterial flora are eliminated, for example, by antibiotics, allowing the yeast to overgrow. Candida infections have various manifestations depending on the site. For example, oral candidiasis (thrush) presents as raised, white plaques on the oral mucosa, tongue, or gums. The plaques can become confluent and ulcerated and spread to the throat. Most HIV- positive individuals eventually develop oral candidiasis, which often spreads to the esophagus. The later condition is considered an indicator of full-blown AIDS. Vaginal candidiasis presents an itching and burning pain of the vulva and vagina, accompanied by a thick or white discharge. HIV-positive females often experience recurrent vaginal candidiasis (Harvey *et al.*, 2007).

1.3 Rationale:

To my best knowledge, the prevalence of HIV and STDs among pregnant women and voluntary counseling and testing attendants who have not been investigated in Malakal, Upper Nile State (UNS) is apparently high. HIV is a major health problem in the State. Therefore, it is more important to carry out this study to evaluate the seroprevalence of HIV and STDs among pregnant women and voluntary counseling and testing attendants in Malakal town; and to determine the possible risk factors that may have allow the rapid spread of HIV among the population, and trying to set a reliable plan for active prevention and control against these fatal diseases.

1.4 Objectives:

1.4.1 General Objective:

To estimate the seroprevalence of HIV and STDs among pregnant women and voluntary counseling and testing attendants in Malakal Town, Upper Nile State

1.4.2 Specific Objectives:

1. To determine HIV and Syphilis antibodies by using serological techniques.
2. To assess the behavior of the target group (about condoms use, abstinence and faithfulness).
3. To determine risk factors associated with disease transmission.
4. To assess the attitudes of pregnant women towards blood test for HIV/AIDS and other STDs.

CHAPTER TWO
LITERATURE REVIEW

Literature Review

2.1 History of AIDS:

It was not known or clear in which year and place (origin) did the virus enter the human body. But it was said to have been probably transferred to human in Africa between 1884 and 1924, Haiti in about 1966 and, it entered the USA in around 1970s. African doctors saw a rise in opportunistic infections, but western scientists and doctors remained ignorant of the growing epidemic. Therefore, below is the timeline showing how the disease AIDS was noticed in the world (Tortora *et al.*, 2006).

In 1981, there was an increase in rare lung infection called pneumocystis carinii pneumonia (PCP) and other opportunistic infections among gay men (homosexuals) and injecting drug users in California, New York and Los Angeles in the USA. Epidemic was investigated and announcement was made by Center for Disease Control (CDC) that these infections were caused by HIV. In the same year, the United Kingdom (UK) announced its occurrence (Gottied and Jeffries, 1989; Hymes and Greene, 1981).

In 1982, the same infections were reported among Haemophilic and Haitians in the USA. The infections were referred to kaposi's sarcoma (benign cancer), lymphadenopathy (swollen gland), gay compromise syndrome, etc because AIDS was not known. Also the cases were reported in several European countries.

In July 1982, the acronym AIDS was suggested in Washington DC, USA based on its definition as an Acquired Immuno-deficiency Syndrome (AIDS), was seconded by doctors as appropriate because disease is not inherited, but resulted in a deficiency within the immune system. This definition was adopted by World Health Organization (WHO) to conduct surveillance of AIDS in Africa (Gottied and Jeffries, 1989; Hymes and Greene, 1981).

In 1983, heterosexual transmission was reported among women with no other risk factors. Experts became more confident that the cause of AIDS is an infection (Gottied and Jeffries, 1989; Hymes and Greene, 1981).

In 1984, the Center for Diseases Control found that man (human) was a link between number of different cases and named him as patient or zero, meaning "out of California". This announcement was made by Darrow (Gottied and Jeffries, 1989; Hymes and Greene, 1981).

In April 1984, the USA Health and Human Services Secretary, Margaret Heckler announced that Dr. Robert Gallo of National Institute had isolated the virus which caused AIDS and named it as Human T cell Lymphotropic Virus-3 (HTLV- III). This brought two viruses into contradiction to which one was responsible for infection, ie HTLV-3 and Lymphadenopathy Associated Virus (LAV) (Gottied and Jeffries, 1989; Hymes and Greene, 1981).

In 1985, CDC removed Haitians from the AIDS list since they were suspected to have brought disease in USA. In March 1985, LAV and HTLV- III were discovered as the same virus (Gottied and Jeffries, 1989; Hymes and Greene, 1981).

In early 1986, the name of the virus had itself become a political football as the French Insisted on Lymphadenopathy Association Virus (LAV) while Gallo's group used Human Tcell Lymphotropic Virus (HTLV- III).

In May the same year, International Committee of Taxonomy ruled it to be called as Human Immuno-deficiency Virus (HIV) and dropped two disputed viruses (Gottied and Jeffries, 1989; Hymes and Greene, 1981).

AIDS had many different names in Africa. For instance, it is called Slim in Uganda and Tanzania, VIGS in South Africa and many other countries (Gottied and Jeffries, 1989; Hymes and Greene, 1981).

2.2 The Origin of AIDS:

HIV is now believed to have arisen by the mutation of a virus that had been endemic in wildlife in some areas of central Africa. Genetic studies of the virus have led to the conclusion that HIV-2 (a type of HIV that is weakly contagious and not found often outside of West Africa) is a mutation of a Simian Immunodeficiency Virus (SIV). Mangabey monkeys in West Africa are naturally and harmlessly infected with this SIV. More recently, studies show that HIV-1 (the primarily HIV found worldwide in humans) is genetically related to another SIV that is carried by chimpanzees in Central Africa. The chimpanzee virus is probably a hybrid of two mangabey monkey SIVs. Chimpanzees are predators and eat other monkeys that presumably carried two versions of HIV. These SIV infections apparently crossed over relatively recently (well into the twentieth century) into the human population, known to eat "bushmeat". Mathematical models of

the supposed evolution of HIV, by Robert Gallo of the Los Alamos National Laboratory, calculated that the virus probably made the transition to human around 1930. The disease may have smoldered with little notice as long as transmission was limited to small villages where rates of sexual promiscuity were lower. The virus could not have killed or incapacitated its hosts quickly; otherwise, it could not have been maintained in the village population. With the sudden end of European Colonialism, the Social Structure of Sub-Saharan Africa was disrupted. The population became urbanized; the developments that result from urbanization, such as an increase in prostitution and the growth of highway transmission, which contributed to an increase in sexual promiscuity are believed to be responsible for the spread of the disease. The earliest documented case of AIDS is from a patient in Leopoldville, Belgian Congo (now Kinshasa, capital of the Democratic Republic of the Congo). This man died in 1959; preserved samples of his blood contain antibodies to HIV. In the Western world, the first confirmed case of AIDS was the death of a Norwegian sailor in 1976, who probably was infected in 1961 or 1962 by contacts in Western Africa and second was found in tissue sample from an American teenager who died around 1969 in St. Louis (Tortora *et al.*, 2006).

In February 1999, a group of researchers led by Paul Sharp of Nottingham University and Beatrice Hahn of Alabama University made a discovery during the course of 10 years, long study about the origin of the virus. They announced and claimed that the sample proved that chimpanzees were the source of HIV-1 because HIV-1 is related to a type of Simian Immuno-deficiency Virus (SIV) which was almost identified in a captive chimpanzee known as pan troglodytes troglodytes, common in West and Central Africa.

In sample investigated by researchers in 1940 and 1945 also identified HIV-2 which corresponded to Simian Immuno-deficiency Virus found in the Sooty mangabey monkey called white collared monkey in Western Africa. HIV-2 strain is a non-rapid cause of AIDS. In Africa, it was first identified in Kinshasa and was believed to be introduced into the Democratic Republic of Congo from Cameroon and Central Africa Republic by trade men along the river. It was identified in 1983 in Uganda among businessmen and identified in Sudan in 1986 among the war veterans and business men (Gottied and Jeffries, 1989; Hymes and Greene, 1981).

In May 2006, the same group of researchers who first identified pan troglodytes troglodytes strain narrowed the origin and announced that Cameroon is the origin of HIV-1. This came after

the analyses of 599 samples of chimps droppings of which 12 samples gave accurate results that the same with HIV (Gottied and Jeffries, 1989; Hymes and Greene, 1981).

Transfer Theories of HIV from Chimpanzees to Human: The hunters' theory; the most commonly accepted theory is that of hunters. In this scenario, SIV Chimpanzee was transferred to human as a result of chimpanzees being killed and eaten or their blood got into cuts or wounds of the hunters.

The oral polio vaccine theory: in his book,"The River", the Journalist Edward Hooper suggested that HIV could be traced to testing of an oral polio vaccine called chat. Chat has been given to many people in Belgium, Congo, Rwanda, Burundi, etc. In 1950s the chat/polio vaccine was cultivated in kidney of local chimpanzees infected with SIV Chimpanzees so, this theory considers polio vaccine as a source of infection.

The colonialism theory: this is a recent theory and was imposed in 2000 by Jim Moore, an American Specialist in Primate Behaviour, who published his finding in the Journal AIDS Research and Human Retrovirus. He stated that many Africans were forced to overwork and confined in camps where food was scarce, sanitation was poor and other harsh conditions. These factors weakened their immune system gave chance to SIV to invade their bodies and changed within their bodies to Human Immuno-deficiency Virus (Gottied and Jeffries, 1989; Hymes and Greene, 1981).

The conspiracy theory: In this theory, HIV is widely believed to be man-made disease. This was revealed in survey carried out in USA in which number of Africans Americans significantly believed that HIV was manufactured as part of biological war programme to wipe-out large number of blacks and homosexual men (Gottied and Jeffries, 1989; Hymes and Greene, 1981).

In the USA, the first case was reported in 1981. The earliest cases of AIDS were seen in large urban centers, such as Los Angeles, San Francisco, and New York City. Clusters of young, male, homosexual patients exhibited a puzzling complex of symptoms, including severe pneumonia caused by pneumocystis jirovei (harmless organism), kaposi sarcoma, sudden weight loss, swollen lymph nodes, and general suppression of immune function. This came to be known as acquired immune deficiency syndrome (AIDS). Early attempts at understanding the diseases focused on the possibility of immune suppression induced by chronic injectable drug use (IDU)

or infection. Soon, however, cases were reported in non-homosexual, non-IDU patients who had received blood or blood products by transfusion. By 1984, AIDS was recognized as an infectious disease caused by a virus, and eventually HIV was isolated from AIDS patients. In the decade after this initial recognition, AIDS killed more United States citizens than the Korean and Vietnam wars combined (Harvey *et al.*, 2007).

2.3 The Structure of HIV:

HIV is an enveloped particle. The viral envelope, formed from the host cell membrane, contains spikes knobs. These consist of a trans membrane protein, TM (fusion protein, also called gp41), which is linked to a surface protein, and attachment protein (gp120) that binds to a cell receptor during infection. Host cell proteins, including the major histocompatibility complex class-2 proteins, are also found in the envelope. The virion has a corn-shaped, icosahedral core containing the major capsid protein (CA also called p24). Between the capsid and the envelope is an outer matrix protein (p17), which directs entry of the double-stranded DNA provirus into the nucleus, and is later essential for the process of virus assembly. There are two identical copies of the positive sense, single-stranded RNA genome in the capsid (that is, unlike other viruses, retroviruses are diploid). The RNA is tightly complexed with a basic protein (p17), in a nucleocapsid structure that differs in morphology among the different retrovirus genera. Also found within the capsid are the enzyme reverse transcriptase and integrase (which are required for viral DNA synthesis and integration into the host cell chromosome) and protease (essential for viral assembly). A host cellular tRNA is hydrogen-bonded at the 5' end of each viral RNA molecules, where it functions as a prime for initiation of reverse transcription. The first phase of HIV replication, which includes viral entry, reverse transcriptase, and integration of the virus into the host genome, is accomplished by proteins provided by the virus. The second phase of replication, which includes the synthesis and processing of viral genome, mRNAs, and structural proteins, uses the host cell machinery for transcription and protein synthesis. The end result of HIV replication in most cell types is cell death (Harvey *et al.*, 2007; Tortora *et al.*, 2006).

Attachment to a specific cell surface receptor is accomplished via the SU fragment of the env gene product on the surface of the HIV, which preferentially binds to a CD4 molecule. Thus, the virus infects helper T cells, lymphocytes, monocytes, and dendritic cells, which contain this protein in their cell membranes (Harvey *et al.*, 2007; Tortora *et al.*, 2006).

Entry of virus into the cell, an additional co-receptor a chemokine receptor is required for entry of the viral core into the cell. Macrophages and T cells express different chemokine receptors that fulfill this function. Binding to a co-receptor activates the viral Tm (fusion) glycoprotein, triggering fusion between the viral envelope and the cell membrane (Harvey *et al.*, 2007; Tortora *et al.*, 2006).

Reverse transcription of viral RNA, after entering the host cell, the HIV RNA is not translated. It is transcribed into DNA by reverse transcriptase an RNA directed DNA polymerase that enters host cells as part of the viral nucleocapsid. A host cellular transfer RNA (tRNA) is hydrogen-bonded to specific site on each viral RNA molecule, where it functions as a primer for initiation of reverse transcription. This process takes place in the cytoplasm, within the core structure. The viral reverse transcriptase first synthesizes a DNA-RNA hybrid molecule, then its Rnase activity degrades the parental RNA molecule while synthesizing the second of DNA. This process results in duplication of the ends to form the long terminal repeats (LTR). The resulting linear molecule of double-stranded DNA is the provirus. LTRs at either end of the provirus contain promoter and enhancer sequences that control expression of the viral DNA (Harvey *et al.*, 2007; Tortora *et al.*, 2006).

Integration of the provirus into host cell DNA, the provirus still associated with virion core components is transported to the nucleus with the aid of MA (matrix protein). There, viral integrase cleaves the chromosomal DNA and covalently inserts the provirus; the integrated provirus thus becomes a stable part of the cell genome. The insertion is random with respect to the site of integration in the recipient DNA. Thus, HIV has two genomic forms; namely single-stranded RNA present in the extra-cellular virus, and proviral double-stranded DNA within the cell (Harvey *et al.*, 2007; Tortora *et al.*, 2006).

Transcription and translation of integrated viral DNA sequences, the provirus is transcribed into a full-length mRNA by the cell RNA polymerase II. The genome-length mRNA has at least three functions; some copies will be the genomes of progeny virus, and are transported to the cytoplasm in preparation for viral assembly; some copies are translated to produce the virion gag proteins. Further, by reading post the stop codon at the end of the gag gene about one of twenty times, a gag-pol poly protein is produced. This is the source of the viral reverse transcriptase and integrase that will be incorporated into virion; still other copies of viral RNA are sliced, creating

new translatable sequences. In all retroviruses, one of the spliced mRNAs is translated into the envelope proteins. In the complex viruses, such as HIV and other viruses, additional spliced molecules produce accessory proteins that are important in regulating transcription and other aspects of replication (Harvey *et al.*, 2007; Tortora *et al.*, 2006).

Assembly and maturation of infectious progeny, these pathways differ from most other enveloped viruses. The envpoly protein is processed and transported to the plasma membrane by the usual cellular route through the Golgi and cleaved into SU and TM molecules by a host cell protease. Assembly begins as the genomes and uncleaved gag and gag-pol poly proteins associate with the TM-modified plasma membrane. As the virion buds from the surface, viral protease is activated and cleaves the poly proteins into their component proteins, which then assemble into the mature virion (Harvey *et al.*, 2007; Tortora *et al.*, 2006).

2.4 Incidence and Epidemiology of HIV/AIDS and STDs:

HIV/AIDS is a global pandemic. As of 2010, approximately 34 million people have HIV worldwide. Of these, approximately 16.8 million are women and 3.4 million are less than 15 years old (UNAIDS, 2011).

Approximately 25 million people have died from AIDS. An estimated 5 million are becoming infected every year. It is the leading cause of death in Sub-Saharan Africa. As the disease becomes established in the huge populations of Asia, especially China and India, the incidence of HIV could exceed more than a million new cases a year. Eastern Europe, Russia, and Central Asia are also areas reporting a steep rise in HIV infections. In Western Europe and the United States the mortality from AIDS has decreased because of the availability of effective antiviral drug. It is projected that 100 million HIV-infected individuals in the world; more than 90% of these will be in developing countries. Deaths from HIV-related causes in 2010 probably exceeded 8 million people.

In the United States, Canada, Western Europe, Australia, Northern Africa, and certain parts of South America, HIV has primarily affected injecting drug users (IDU) and homosexual and bisexual males. In Western Europe and North America, the incidence of heterosexual spread has increased, and women are being infected in at least the same rate as men. The CDC estimates that about 40,000 Americans become infected each year (Tortora *et al.*, 2006).

Certain racial/ethnic populations in the United States have higher rates of HIV infections. In one study, the Hispanic and non-Hispanic black populations constituted 21% of the total population, but accounted for 84% of the heterosexually-acquired HIV infections. African-American women and youths now account for about two-thirds of AIDS cases (Tortora *et al.*, 2006).

In Asia the epidemic is being spread by IDUs, commercial sex workers, and by young heterosexual males. The numbers of persons infected are expected to eventually be huge because of the immense populations, but the percentage of population infected is not expected to approach that of Sub-Saharan Africa (Tortora *et al.*, 2006).

Syphilis is believed to have infected 12 million people in 1999, with greater than 90% of cases in the developing world. It affects between 700,000 and 1.6 million pregnancies a year, resulting in spontaneous abortions, stillbirths, and congenital syphilis. In sub-Saharan Africa, syphilis contributes to approximately 20% of per natal deaths. Rates are proportionally higher among intravenous drug users, those who are infected with HIV and men who have sex with men. In the United States, rates of syphilis as of 2007 were six times greater in men than women, while they were nearly equal in 1997. African Americans accounted for almost half of all cases in 2010 (Kent and Romanelli, 2008; Stamm, 2010; Mullooly and Higgins, 2010).

Syphilis was very common in Europe during the 18th and 19th centuries. In the developed world during the early 20th century, infections declined rapidly with the widespread use of antibiotics, until the 1980s and 1990s. Since the year 2000, rates of syphilis have been increasing in the USA, Canada, the UK, Australia and Europe, primarily among men who have sex with men. Rates of syphilis among American women have, however, remained stable during this time, and rates among UK women have increased, but at a rate less than that of men. Increased rates among heterosexuals have occurred in China and Russia since the 1990s. This has been attributed to unsafe sexual practices, such as sexual promiscuity, prostitution, and decreasing use of barrier protection.

Untreated, it has a mortality of 8% to 58%, with a greater death rate in males. The symptoms of syphilis have become less severe over the 19th and 20th centuries, in part due to widespread availability of effective treatment and partly due to decreasing virulence of the spirochetes. With early treatment, few complications result. Syphilis increases the risk of HIV transmission by two

to five times, and co-infection is common, 30–60% in a number of urban centers, (Kent and Romanelli, 2008; Stamm, 2010; Mullooly and Higgins, 2010).

Gonorrhea is a common infectious disease. WHO estimates that 62 million cases of gonorrhea appear each year. In the United Kingdom 196 per 100,000 males 20 to 24 years old and 133 per 100,000 females 16 to 19 years old were diagnosed in 2005. The CDC estimates that more than 700,000 people in the United States get new gonorrheal infections each year. Only about half of these infections are reported to CDC. In 2004, 330,132 cases of gonorrhea were reported to the CDC. After the implementation of a national gonorrhea control program in the mid-1970s, the national gonorrhea rate declined from 1975 to 1997. After a small increase in 1998, the gonorrhea rate has decreased slightly since 1999. In 2004, the rate of reported gonorrheal infections was 113.5 per 100,000 persons (CDC, 2008; Vickerman *et al.*, 2005)

In the US, it is the second most common bacterial sexually transmitted infections after *Chlamydia*. According to the CDC, "Overall, African Americans are most affected by gonorrhea. Blacks accounted for 69% of all gonorrhea cases in 2010(CDC, 2010).

Vaginal infections by yeast like fungi of the genus *Candida albicans* are responsible for millions of physicians' office visits every year. By the time they reach the age of 25, an estimated half of college women will have had at least one physician-diagnosed episode. Nonprescription antifungal therapies to treat these infections are among the best-selling over the counter products in the United States. *Candida albicans* is the most common species, causing 85 - 90% of cases. *Candida albicans* often grows on mucous membranes of the mouth, intestinal tract, and genitourinary tract. Infections are usually a result of opportunistic overgrowth when the competing micro biota is suppressed by antibiotics or other factors. *Candida albicans* is the cause of oral candidiasis, or thrush. It is also responsible for vulvo vaginal candidiasis, which is the most common cause of vaginitis. About 75% of all women experience at least one episode (Tortora *et al.*, 2006).

Globally trichomoniasis affects approximately 152 million people as of 2010 (2.2% of the population). It is more common in women (2.7%) than males (1.4%). The American Social Health Association estimates trichomoniasis affects 7.4 million previously unaffected Americans

each year and is the most frequently presenting new infection of the common sexually transmitted diseases (Vos, 2012; Associated Press, 2007).

2.5 Transmission:

2.5.1 HIV:

HIV is found in all body fluids such as semen, blood, tears, sweat, saliva, urine, and pre-ejaculated fluid, vaginal fluids and breast milk. However, the fluids containing the high concentration of HIV that can transmit the virus are; blood, semen and pre-ejaculated fluids and breast milk HIV transmission can take place via different modes, described as follows:

HIV is transmitted primarily via unprotected sexual intercourse (whether vaginally, anally or orally), contaminated blood transfusion and hypodermic needles and from mother to child during pregnancy, delivery, or breastfeeding (WHO, 2000 and Warren, 2002).

Sexual contact: HIV is present in both semen and vaginal secretions, is transmitted primarily as cell-associated virus in the course of either homosexual or heterosexual contact. Disruption of mucosal surfaces by sexually transmitted diseases, particularly those such as syphilis and chancroid that result in genital ulcerations, may greatly facilitate HIV infection (Ivan, 2006, Harvey *et al.*, 2007).

Sub-Saharan Africa is more heavily affected by HIV and AIDS than any other region of the world. An estimated 22.9 million people are living with HIV in the region around two-thirds of the global total. In 2010 around 1.2 million people died from AIDS in Sub-Saharan Africa and 1.9 million people became infected with HIV. Since the beginning of the epidemic, 14.8 million children have lost one or both parents to HIV and AIDS (UNAIDS, 2010).

Heterosexual practices among youth mark a major route of HIV transmission in this group, as evidenced by Kaaya and colleagues who found that, both males and females youth scholars in Sub-Saharan Africa engage in early sexual intimacy, half of which practice unprotected sexual intercourse with multiple partners (Kaaya *et al.*, 2002).

In South-East Asia Region (SEAR), the number of reported cases continued to increase and is likely to do so well into the early part of 21st century. As of Dec. 2006, an estimated 7.2 million people are living with HIV and AIDS in this region. The potential for continued spread of HIV and AIDS in Asia and Western Pacific is real, and requires determined and sustained prevention efforts. Several countries have already experienced intense epidemic in certain population e.g., of all HIV positive cases in SEAR about 49 per cent are commercial sex workers and their clients, injectable drug users about 22 per cent and a small but significant proportion of infection, about 5 per cent, is in men who have sex with men (UNAIDS and WHO, 2006).

Blood Transfusion: Since blood carries a high concentration of HIV, it should always be tested because if infected, this blood and its products should not be provided to someone (Park, 2006);

Sharing unsterilized metal equipment's; Sharing sharp metal equipment's such as razors, piercing equipment, and injecting equipment like needles, syringes and tattooing that are not sterilized and or have been used before by infected people can increase the risk of HIV/AIDS transmission (Warren, 2002);

Maternal-Fetalor Mother to Child Transmission: HIV may pass from an infected mother to her fetus, through the placenta or to her infant during delivery or by breast-feeding. In the absence of any intervention, rates of this form of transmission can vary from 15-30 per cent without breast-feeding, and reaches as high as 45 per cent with prolonged breast-feeding. Transmission during the per partum period accounts for one-third to two-thirds of overall numbers infected, depending on whether breast-feeding transmission occurs or not, and this period has, therefore, become a focus of prevention efforts. The risk of infection is higher if the mother is newly infected, or if she has already developed AIDS (WHO, 2004);

Organ Transplant: Transmission of HIV occurs when the organ is taken from an infected person to someone whom the transplant is done. Hence infection with the HIV (Warren, 2002); through kissing it has been found in others fluids like saliva. This can happen when there are mouth sores or wounds (Warren, 2002; Park, 2007).

Risk of HIV-1 transmission by type of exposure and proportion of global infection; sexual intercourse 90%, blood transfusion 5-10%, injection drug use 5-10%, perinatal 2-3%, needle stick 0.01% (Nielsen, 2005).

HIV is not transmitted through casual contact with an infected person at home , in a workplace, in society; through casual contact with an infected person at home , in a workplace, in society; playing sports; working together; coughing, sneezing or breathing the same air; sharing food, eating or drinking; sharing utensils or towels; sharing toilets or showers; using public swimming pools; getting a mosquito or insect bite; using a public phone; visiting a health facility; shaking hands or hugging; donating blood after testing (Warren, 2002; Park, 2007).

2.5.2 Syphilis:

Syphilis is caused by spirochetal bacterium *Treponema pallidum* subspecies *pallidum*. It is transmitted mostly through sexual contact, although there are examples of congenital syphilis via transmission from mother to child in uterus or at birth. In fact, the disease was dubbed the “Great Imitator” because it was often confused with other diseases, particularly in its tertiary stage (CDC, 2004).

Prevention is abstinence from any and all types of sexual activity or intimate physical contact with an infected person is very effective at reducing the transmission of syphilis. Many microbes that cause sexually- transmitted infections are transmitted only through exposure to body fluids such as semen or blood; by contrast, the bacterium *T.pallidum* readily crosses both cut or intact mucosa and cut skin, including body parts that cannot be protected by a condom. Proper and consistent use of a latex condom substantially reduces, but does not completely eliminate, the spread of syphilis through sexual contact (CDC, 2004).

2.5.3 Gonorrhea:

Gonorrhea is a common sexual transmitted infection caused by the bacterium *Neisseria gonorrhoea* (also called Gonococcus, which is often abbreviated as ‘GC’ by clinicians). In the USA, its incidence is second to *Chlamydia* among bacterial STDs (CDC-STD Surveillance-Gonorrhea, 2008). In both men and women if gonorrhea is left untreated, it may spread throughout the body, affecting joints and even heart valve (CDC Fact Sheet, 2008).

The infection is transmitted from one person to another through vaginal, oral, or anal sexual relation, though transmission rarely occurs with safe sex practices of condom usage with lubrication. The incubation period is 2 to 30 days with most symptoms occurring between 4-6 days after being infected ([http:// www.gonorrhea-symptoms.com/ STD/](http://www.gonorrhea-symptoms.com/STD/)). Men have a 20% risk of getting the infection from a single act of vaginal intercourse with gonorrhea. The risk for men who have sex with men is higher (Howard Brown Health Center, 2009). Women have a 60-80% risk of getting the infections from a single act of vaginal intercourse with a man infected with gonorrhea (National Institute of Allergy and Infectious Diseases, 2001). An infected mother may transmit gonorrhea to her newborn during childbirth, a condition known as ophthalmia neonatorum (Kumar *et al*, 2007).

A small number of people may be asymptomatic for a lifetime. Between 30% and 60% of people with gonorrhea are asymptomatic or have subclinical disease. In males, symptoms include a yellowish discharge from the penis, associated with painful, and sometimes frequent, urination. Symptoms can develop from two to thirty days after infection. A few percent of infected men have no symptoms. The infection may move into the prostate, seminal vesicles, causing pain and fever. Untreated, gonorrhea can lead to sterility. It is not unusual for men to have asymptomatic gonorrhea. Men may complain of pain on urinating and examination may show a reddened external urethral meatus. Ascending infection may involve the testicles, or prostate gland, causing symptoms such as pain or swelling (YT van, 1999).

More than half of women with gonorrhea show no symptoms, or symptoms mild enough to be ignored. Women may complain of vaginal discharge, difficult urination (dysuria), projectile urination, off-cycle menstrual bleeding, or bleeding after sexual intercourse. The cervix may appear anywhere from normal to the extreme of marked cervical inflammation with pus. Early symptoms may include a discharge from the vagina, discomfort in the lower abdomen, irritation of the genitals, pain or burning during urination and abnormal bleeding. Less advanced symptoms, which may indicate development of pelvic inflammatory disease (PID), include cramps and pain, bleeding between menstrual periods, vomiting, or fever. Women who leave these symptoms untreated may develop severe complications. The infection will usually spread to the uterus, fallopian tubes, and ovaries, causing pelvic inflammatory disease (YT van, 1999).

In the United Kingdom, the majority of patients with gonorrhoea are treated in dedicated sexual health clinics. The current recommendation is for ceftriaxone or cefixime as first-line therapy; no resistance to either drug has yet been reported in the UK. Levels of spectinomycin resistance in the UK are less than 1%, which would make it a good choice in theory, but intramuscular spectinomycin injection is very painful (Health Protection Agency, 2005).

2.5.4 Trichomoniasis:

Trichomoniasis, sometimes referred to as “trich” or “fishy fanny syndrome” because of the smell associated there-with, is a common cause of vaginitis. It is caused by the single-celled protozoan parasite *Trichomonas vaginalis* by producing mechanical stress on host cells and then ingesting cell fragments after death. Trichomoniasis is primarily an infection of the urogenital tract; the most common site of infection is the urethra and the vagina in women (Middlej and Benchimol, 2010).

A draft sequence of the genome was published on January 12, 2007 in the Journal of Science confirming that the genome *Trichomonas* has at least 26,000 genes, a similar number to the human genome (Physorg. com. Jan, 2007). *Trichomonas* can be transmitted through sexual intercourse. In many instances, however, a history compatible with sexual transmission cannot be documented. While trichomoniasis is usually passed sexually, it may be picked up from contact with damp or moist objects such as towels, wet clothing, or a toilet seat, if the genital area gets in contact with these damp and moist objects. Unlike most STDs, the parasite can live for about an hour on damp towels, washcloths and bathing suits. If someone uses these towels or washcloths or puts on the bathing suits, the disease may be passed on that way (Associated Press, 2007).

Typically, only women experience symptoms associated with *Trichomonas* infection. Symptoms include inflammation of the cervix (cervicitis), urethra (urethritis) and vagina (vaginitis) which may produce an itching or burning sensation. Discomfort may increase during intercourse and urination. There may also be a yellow-green, itchy, frothy foul-smelling vaginal discharge. In rare cases, lower abdominal pain can occur and symptoms usually appear in women within 5 to 28 days of exposure. In many cases women may hold the parasite for some years without any signs (dormant). While symptoms are most common in women, some men may temporarily

exhibit symptoms such as an irritation inside the penis, mild discharge, or slight burning after urination or ejaculation (ASHA, 2012).

2.5.5 Candidiasis:

Candidiasis is caused by *C. albicans*, which is a normal constituent of the human flora, a commensal of the skin and the gastrointestinal and genitourinary tracts, is responsible for the majority of *Candida* bloodstream infections (candidemia). It is transmitted through un-protected intercourse, direct contact with skin lesions, kissing, sharing of objects such as clothes, utensils, towels and toilet seats (Denfert and Hube, 2007).

2.6 The Effectiveness and Pathogenicity of HIV:

The spikes enable the virus to attach to the CD4 receptor on the host cells. CD4 receptors are found on helper T cells. Macrophages and dendritic cells are the main targets of HIV infection. Certain co-receptors are also required. The two best known chemokine co-receptors, originally called fusion, are named CCR5 and CXCR4. Attachment of the virus is followed by entry into the host cell. In the host, viral RNA is released and transcribed into DNA by the enzyme reverse transcriptase. This viral DNA then becomes integrated into the chromosomal DNA of the host cell. The DNA may control the production of an active infection in which new viruses bud from the host cell. Alternatively, this integrated DNA may not produce new HIV but remains hidden in the host cell's chromosome as a provirus. HIV produced by a host cell is not necessarily released from the cell, but may remain latent virion in vacuoles within the cell. In fact, a subset of the HIV-infected cells, instead of being killed they become long-lived memory T cells in which the reservoir of latent HIV can persist for decades. This ability of the virus to remain as a provirus or latent virus within host cells shelters it from the immune system. Another way HIV evades the immune system is by cell-cell fusion, by which the virus moves from an infected cell to an adjacent uninfected cell (Tortora *et al.*, 2006).

The pathology of HIV disease results from either tissue destruction by the virus itself or host's response to virus infected cells. In addition, HIV can induce an immunodeficiency state that leads to opportunistic diseases that are rare in the absence of HIV infection. The progression from HIV infection to AIDS develops in 50% of HIV-infected individuals in an average of ten

years, and if untreated, it is uniformly fatal generally within two years of diagnosis. However, there is a significant fraction (about 10%) of HIV-infected individuals who have not developed AIDS after twenty years. Development from HIV infection to end-stage AIDS progresses through several phases (Taylor, 1996; Ivan, 2006; Harvey *et al.*, 2007). Initial infection, after the acquisition of HIV and the initially infected cells are generally macrophages within the genital tract. From this initial localized infection, HIV disseminates via the blood, and virus may then localize in dendritic cells throughout the lymphoid tissue from the surface of follicular dendritic cells. HIV can then infect CD4⁺ lymphocytes moving through the germinal centers of lymph nodes. This process creates a reservoir of chronically HIV-infected cells within the lymphatic tissue of the body.

Acute phase viremia, several weeks after the initial infection with HIV, one third to two thirds of individuals experience an acute disease syndrome (also referred to as the primary infection) similar to infectious mononucleosis. During this period, there is a high level of virus replication occurring in CD4⁺ cells. Large amounts of virus and capsid protein (CA antigen) are present in the blood, and circulating antibody appears in one to ten weeks after the initial infection. A constant level of virus and virus-infected cells are maintained by a combination of replacement of the CD4⁺ cells killed by HIV infection with cells newly produced in lymphoid organs and the subsequent infection of these new cells with progeny virus. Lymph nodes also become infected during this time; they later serve as the sites of virus persistence during the asymptomatic period. Latent period, the acute phase viremia is eventually reduced significantly with appearance of a HIV-specific cytotoxic T-lymphocyte response, followed by a humoral antibody response. A clinically asymptomatic or latent period lasting from months to many years follows the acute infection. During this latent period, the majority (90%) of HIV pro-viruses' are transcriptionally silent, so that only 10% of the cells containing integrated HIV DNA also contain viral mRNA or viral proteins. There are transient peaks of viremia that are often correlated with other pathogens or by immunization. Although there is continuous loss of those CD4⁺ cells in which HIV is replicating, active replacement through stem cell multiplication compensates for this loss, and the CD4⁺ count declines only slowly over a period of years. In addition, the host immune response is still sufficiently effective to maintain a relatively stable, low level of virus production. It has been estimated that 10¹¹ virion and 10¹¹ CD4⁺ T cells are produced each day. Virus isolated during this period is also less cytopathic for CD4⁺ cells and replicates more slowly

than does that isolated later during symptomatic AIDS. Despite the nearly normal levels of CD4⁺ cells, however, impairment of T-cell responses to specific antigens is evident. The infection remains relatively clinically asymptomatic as long as the immune system is functional. During this period whose length is variable, but lasts on average about ten years, there are multiple, non-specific conditions, such as persistent, generalized lymphadenopathy (swollen lymph nodes), diarrhea, chronic fever, night sweats, and weight loss. The more common opportunistic infections such as herpes zoster and candidiasis may occur repeatedly during this period, as well as when patients progress to AIDS. The CD4⁺ cell count remain normal or gradually declines, but is greater than 200/ul. The progression from asymptomatic infection to AIDS is not sudden, but in fact occur as a continuum of clinical states. A number of virologic and immunologic changes occur that affect the rate of this progression. For example, co-infection with a number of the herpes viruses, such as human herpes viruses, can trans activate transcription from the silent HIV provirus, increasing HIV replication. Any stimulation of an immune response causing activation of resting T cells also activates HIV replication. Not only does this increase the number of infected CD4⁺ cells, but it also increases the opportunity to create generations of virus mutants. Eventually, a more highly cytocidal, more rapidly multiplying variant appears. In addition, these variants are often highly syncytium-inducing, promoting fusion between infected and previously by un-infected cells. T-cell precursors in the lymphoid organ are also infected and killed, so the capacity to generate new CD4⁺ cells are gradually lost. The capacity to contain the infection is further compromised by the appearance of HIV mutants with altered antigenic specificity, which is not recognized by the existing humoral antibody or cytotoxic T lymphocytes. The eventual result of these accumulating, interaction factors are increasingly rapid decline in CD4⁺ count (falling below 200/ml), accompanied by loss of immune capacity (Taylor, 1996; Ivan, 2006; Harvey *et al.*, 2007).

Nearly all systems of the body can be affected as a result of HIV infection, either by HIV itself or by opportunistic organisms. The weakening immune system leads to many complications including malignancies. Cell types other than CD4⁺ lymphocytes can be infected by HIV. Infection of these cells produces some of the additional manifestations of end-stage disease. Chief among these are infected cells of the monocyte-macrophage lineage, which are not killed as rapidly as CD4⁺ T cells and can transport the virus into other organs. For example, macrophages are the HIV-infected cells present in brain of patients with AIDS encephalopathy,

which typically evolves over a period of one year, with gradual deterioration resulting in severe dementia. Virus has also been found in langerhans cells in the skin, dendritic cells in lymph nodes, and monocytes in bone marrow, but their significance in the disease process is not clear. The eye is another site affected by HIV infection itself, which produces focal areas of ischemia in the retina. HIV infection of blood progenitors in the bone marrow leads to the anemia seen in most AIDS patients.

Multiple recurrent bouts of infections with fungi, bacteria, and viruses occur as the CD4⁺ cell count declines. For example, the nervous system can be the site of opportunistic infections with *Toxoplasma*, *Cryptococcus* and *Mycobacterium*. The eye cannot only be infected with HIV, but also with opportunistic agents, the most prominent of which is cytomegalovirus (CMV) a cause of retinal destruction. The lungs are also primarily affected by opportunistic infections, P.Jirovecci pneumonia being one of the most common. Mycobacterial infections are also a common problem in the lungs for example, currently 30% of AIDS patients die from tuberculosis. Serious gastrointestinal tract illnesses are due to opportunistic pathogens, but these may be in concert with HIV infection. Protozoal parasitic diseases, as well as infections with gram-negative enteric bacteria are other sources of gastrointestinal disorders. The immune deficiency also provides the opportunity for latent herpes viruses infections to recur repeatedly or become chronic and spread extensively. Mucocutaneous candidiasis (for example, oral, esophageal, or vaginal) is an ongoing problem in AIDS patients as well. In fact, vaginal candidiasis is the most frequent reason HIV-infected females seek medical attention. A number of malignancies commonly arise in HIV-infected patients. The most characteristic neoplasm present in AIDS patients is Kaposi Sarcoma (KS), which involves skin, mucus membranes, and deep viscera. Various lymphomas, including those of the CNS, are also common. These are probably the result of the immune compromise and not HIV itself. KS has been associated with human herpes virus type 8 or (HHV-8). In AIDS patients, body cavity lymphomas are also usually associated with Epstein-Barr Virus (Harvey *et al.*, 2007).

The causative agent of syphilis is *Treponema pallidum*, thin and tightly coiled. Stain poorly with the usual bacterial stains. (The bacterial name is derived from the Greek words for twisted thread and pale) (Harvey *et al.*, 2007). *Treponema pallidum* subspecies *pallidum* is a spiral-shaped, Gram-negative, highly mobile bacterium. Three other human diseases are caused

by related *Treponema pallidum*, including yaws (subspecies *pertenue*), pinta (subspecies *carateum*) and bejel (subspecies *endemicum*). Unlike subtype *pallidum*, they do not cause neurological disease. Humans are the only known natural reservoir for subspecies *pallidum*. It is unable to survive without a host for more than a few days. This is due to its small genome (1.14 MDa) failing to encode the metabolic pathways necessary to make most of its macronutrients. It has a slow doubling time of greater than 30 hours (Kent and Romanelli, 2008; Eccleston *et al.*, 2008).

2.7 Signs and Symptoms:

According to the Center for Disease Control and Prevention (CDC) signs and symptoms have four stages or acute infection or window period that include; stage one when a person is first infected with HIV virus and when the body starts producing antibodies against the virus before the antibody becomes detectable. It is often accompanied by a short flu like illness for few days. It might includes fever, sore throat, chills, night sweats, rashes which about 70% of people experience a few weeks after initial infection with the virus, most HIV- infected people have no symptoms for the first five years or so. They look healthy and feel well although right from the start they can transmit the virus to others. Once infected, people are infected for life. Scientists have not found as yet, a way of curing them, or making them non-infectious to others. HIV antibodies usually take between 2 to 12 weeks to appear in the blood-stream, though they have been known to take longer. The period before antibodies are produced is the “ window period “ during which, although the person is particularly infectious because of the high concentration of virus in the blood, he or she will test negative on the standard antibody blood test. Though the body’s immune system reacts to the invasion of HIV by producing antibodies, these do not inactivate the virus in the usual way (Lawrence *et al*; 2004, Harvey *et al*; 2007); stage two clinically asymptomatic and lasts for an average of ten years, antibodies are detectable in the blood, fast decline of the immune system and the virus rapidly replicates with persistent generalized lymphadenopathy; stage three or symptomatic HIV infection. Once the immune system is damaged, many people will begin to experience some mild symptoms includes skin rashes, fatigues, slight weight loss, night sweats, thrush in the mouth during this phase, there is usually a progressive loss of CD4+ T cell in lymphoid tissues and destruction of the lymphoid tissues. Eventually, the blood CD4+ T cell count begins to decline, and when the count falls

below 200 per mm³ (the normal being about 1500 cells per mm³), patients become susceptible to infections and are said to be suffering from AIDS (Abbas and Lichtman, 2001); stage four includes progression from HIV to AIDS. In this stage, as the immune system becomes more and more damaged leading eventually to an AIDS diagnosis and people may experience opportunistic infections e.g., tuberculosis (TB), candidiasis, syphilis, malaria, pneumonia, meningitis, skin infections and severe diarrhea (Taylor, 1996; Ivan, 2006).

Window period; when a person gets infected with HIV, the test will not immediately show that the person is HIV-positive. There is a period of three to six weeks (some times as long as three months) that the body is still able to fight the HIV virus. This period of time that passes while the test is still negative is called the "window period". It is important to understand that the person can still pass on HIV virus during these weeks, even if the tests are negative. Hence, you cannot tell exactly when a person got infected (Lawrence *et al*; 2004, Harvey *et al*; 2007).

Syphilis can present in one of four different stages; primary, secondary, latent, and tertiary, and may also occur congenitally. It was referred to as "the great imitator" by Sir William Osler due to its varied presentations. Primary syphilis is typically acquired by direct sexual contact with the infectious lesions of another person. Approximately 3 to 90 days after the initial exposure (average 21 days) a skin lesion, called a chancre, appears at the point of contact. This is classically (40% of the time) a single, firm, painless, non-itchy skin ulceration with a clean base and sharp borders between 0.3 and 3.0 cm in size. The lesion, however, may take on almost any form. In the classic form, it evolves from a macule to a papule and finally to an erosion or ulcer. Occasionally, multiple lesions may be present (40%) with multiple lesions more common when co-infected with HIV. Lesions may be painful or tender (30%), and they may occur outside of the genitals (2–7%). The most common location in women is the cervix (44%), the penis in heterosexual men (99%), and anally and rectally relatively commonly in men who have sex with men (34%). Lymph node enlargement frequently (80%) occurs around the area of infection occurring seven to 10 days after chancre formation. The lesion may persist for three to six weeks without treatment.

Secondary syphilis occurs approximately four to ten weeks after the primary infection. While secondary disease is known for the many different ways it can manifest, symptoms most

commonly involve the skin, mucous membranes, and lymph nodes. There may be a symmetrical, reddish-pink, non-itchy rash on the trunk and extremities, including the palms and soles. The rash may become maculopapular or pustular. It may form flat, broad, whitish, wart-like lesions known as condylomata on mucous membranes. All of these lesions harbor bacteria and are infectious. Other symptoms may include fever, sore throat, malaise, weight loss, hair loss, and headache. Rare manifestations include hepatitis, kidney disease, arthritis, periostitis, optic neuritis, and interstitial keratitis. The acute symptoms usually resolve after three to six weeks; however, about 25% of people may present with a recurrence of secondary symptoms. Many people who present with secondary syphilis (40–85% of women, 20–65% of men) do not report previously having had the classic chancre of primary syphilis.

Latent syphilis is defined as having serologic proof of infection without symptoms of disease. It is further described as either early (less than 1 year after secondary syphilis) or late (more than 1 year after secondary syphilis) in the United States. The United Kingdom uses a cut-off of two years for early and late latent syphilis. Early latent syphilis may have a relapse of symptoms. Late latent syphilis is asymptomatic, and not as contagious as early latent syphilis.

Tertiary syphilis may occur approximately 3 to 15 years after the initial infection, and may be divided into three different forms: gummatous syphilis (15%), late neuro-syphilis (6.5%), and cardiovascular syphilis (10%). Without treatment, a third of infected people develop tertiary disease. People with tertiary syphilis are not infectious (Kent and Romanelli, 2008; Eccleston *et al.*, 2008)

Clinical significance of gonorrhea most often colonizes the mucus membrane of the genitourinary tract or rectum. There the organisms may cause a localized infection with the production of pus, or may lead to tissue invasion, chronic inflammation, and fibrosis. A higher production of females than males are generally asymptomatic, these individuals act as the reservoir for maintaining and transmitting gonococcal infections. Genitourinary tract infections, symptoms of gonococcal infection are more acute and easier to diagnose in males. The patient typically presents with a yellow, purulent urethral discharge and painful urination. In females, infection occurs in the endocervix and extends to the urethra and vagina. A greenish-yellow cervical discharge is common, often accompanied by intermenstrual bleeding. The disease may

progress to the uterus, causing inflammation of the fallopian tubes, pelvic inflammatory disease, and fibrosis. Infertility occurs in approximately 20% of women. Rectal infections, prevalence in male homosexuals, are characterized by constipation, painful defecation, and purulent discharge. Ophthalmia neonatorum is an infection of the conjunctival sac that is acquired by a newborn during passage through the birth canal of a mother infected with gonococcus. If untreated, acute conjunctivitis may lead to blindness (Harvey *et al.*, 2007).

Candidiasis (candidosis) is caused by the yeast *Candida albicans*, and other *Candida* species, which are normal body flora found in the skin, mouth, vagina, and intestines. Although considered yeast, *C. albicans* is dimorphic, and can form a true mycelium. Infections occur when competing bacterial flora are eliminated, for example, by antibiotics, allowing the yeast to overgrow. *Candida* infections have various manifestations depending on the site. For example, oral candidiasis (thrush) presents as raised, white plaques on the oral mucosa, tongue, or gums. The plaques can become confluent and ulcerated and spread to the throat. Most HIV-positive individuals eventually develop oral candidiasis, which often spreads to the esophagus. The latter condition is considered an indicator of full-blown AIDS. Vaginal candidiasis presents as itching and burning pain of the vulva and vagina, accompanied by a thick or white discharge. HIV-positive females often experience recurrent vaginal candidiasis (Harvey *et al.*, 2007).

2.8 Laboratory Diagnosis:

Most people infected with HIV eventually develop AIDS. These individuals mostly die from opportunistic infections or malignancies associated with progressive failure of the immune system (Lwan, 2004).

Early diagnosis is important because it may help infected people to live long, be healthier, simple and accurate screening tests that detect antibodies to HIV are done. Tests may be done on blood or saliva. If screening test results are positive, they are confirmed by more accurate and specific test such as the Western Blot. Often these tests are not positive in the first weeks up to two months after initial infection because antibodies to HIV are not yet being produced. Specific tests include:

Enzyme-Linked Immunosorbent Assay (ELISA): this screening test is often used to detect HIV antibodies, but it requires complex equipment (Tortora *et al.*, 2006). In this diagnostic technique, antibody specific for an antigen of interest is bound to the walls of a plastic micro-titer well. Patient's serum is then incubated in the wells, and any antigen in the serum is bound by the antibody on the well walls. The wells are then washed, and a second antibody is added, this one also specific for the antigen, but recognizing epitopes different from those bound by the first antibody after incubation, the wells are again washed, removing any unattached antibody. Attached to the second antibody is an enzyme, which, then presented with its substrate, produces a coloured product, the intensity of the colour produced being proportional to the amount of bound antigen. ELISAs can also be used to detect or quantitate antibody in a patient's serum. In this instance, the wells are coated with antigen specific for the antibody in question. The patient's serum is allowed to react with the bound antigen, the wells are washed, and a secondary antibody conjugated to a colour product-producing enzyme is added to the well. After a final washing, substrate for the bound enzyme is added to the well, and the intensity of the coloured product can be measured by using spectrophotometer (Harvey *et al.*, 2007, Tortora *et al.*, 2006).

Western Blot: this test is usually done to confirm the diagnosis when screening test results are positive. It is more difficult to do than screening tests, but is more accurate; it is based on detecting specific antibody to viral core protein (p24) and envelop glycoprotein (g41) (Lawrence *et al.*, 2004).

Immunochromatographic Test (ICT): rapid serological test, utilizes conjugate and multiple recombinant HIV proteins to selectively detect antibodies to HIV-1 and HIV-2 in serum specimen (Tortora *et al.*, 2006).

Also several laboratory markers are available to provide prognostic information and guide therapy decisions. The most widely used marker is the absolute CD4 lymphocyte count. As the count decrease, the risk of opportunistic infection increase. People with healthy immune system usually have more than 950 CD4 cells/ μ of blood. The number falls over the course of HIV infection. People with AIDS usually have CD4 cell count below 200 (USA makes CD cell count below 200 in an HIV-infected person a definition of AIDS). The trend of the count is much more important than any single reading. The frequency of performance of counts depends on the patient's health system. Patients whose count is substantially above the threshold for antiviral therapy (500 cells/ μ) should have count performed every three months. This is necessary for evaluating efficacy of antiviral therapy and for initiation P.carinii prophylactic therapy, when the count falls below 200 cells/ μ (Lawrence *et al.*, 2004).

In the 20th century that effective tests and treatments for syphilis were developed. Microcopy of fluid from the primary or secondary lesion using dark field illumination can diagnose treponemal disease with high accuracy. As there are other treponemes that may be confused with *T. pallidum*, care must be taken in evaluating with microscopy to correlate symptoms with the correct disease (Pickering, 2006).

At Present-day syphilis screening tests, such as the Rapid Plasma Reagin (RPR) and Venereal Disease Research Laboratory (VDRL) tests are cheap and fast but not completely specific, as many other conditions can cause a positive result. These tests are routinely used to screen blood donors. It can be noted that the spirochete that causes syphilis does not survive the conditions used to store blood, and the number of transfusion transmitted cases of syphilis is minuscule, but the test is used to identify donors that might have contracted HIV from high risk sexual activity(Pickering, 2006).

Trichomoniasis is diagnosed by visually observing the trichomonads via a microscope. In women, the doctor collects the specimen during a pelvic examination by inserting a speculum into the vagina and then using a cotton-tipped applicator to collect the sample. The sample is

then placed onto a microscopic slide and sent to a laboratory to be analyzed. An examination in the presence of trichomoniasis pistaulas may also reveal small red ulcerations on the vaginal wall or cervix (ASHA, 2012).

Diagnosis of a yeast infection is done either via microscopic examination or culturing. For identification by light microscopy, a scraping or swab of the affected area is placed on a microscope slide. A single drop of 10% potassium hydroxide (KOH) solution is then added to the specimen. The KOH dissolves the skin cells, but leaves the *Candida* cells intact, permitting visualization of pseudo hyphae and budding yeast cells typical of many *Candida* species. For the culturing method, a sterile swab is rubbed on the infected skin surface. The swab is then streaked on a culture medium. The culture is incubated at 37°C for several days, to allow development of yeast or bacterial colonies. The characteristics (such as morphology and colour) of the colonies may allow initial diagnosis of the organism causing disease symptoms (Srikumar and Nagaraja, 2010).

2.9 Prevention and Control:

Western HIV/AIDS Intervention in Africa Setting is one of the most important issues facing health professionals about adolescents and the HIV epidemic in developing countries is whether the currently available risk reduction interventions that are effective in western setting are also effective in other cultures (Fitzgerld *et al.*, 1999; Magnussen *et al.*, 2004). To date there has not been any rigorous assessment in the non-western settings. Lack of funds and technical expertise are cited as reasons for the absence of rigorous evaluation. While potentially affordable, such interventions may not be effective in non-western settings since they are based on western concepts of decision making which might not be applicable in other cultures (Fitzgerld *et al.*, 1999; Magnussen *et al.*, 2004). Some successful interventions in the western settings have been based on social cognitive behavioral theories such as social learning theory and the theory of reasoned action (Witte *et al.*, 1998), which were developed in the western setting but have received little assessment in the other cultures (Merson *et al.*, 2000). These interventions have characteristically emphasized negotiations and communication practices based on assumptions regarding the rights of partners in a relationship. This may not be applicable in all settings especially in the African setting where the man has an upper hand in almost every decision made. Based on the western concept of ideal sexual behavior monogamy has been advocated to curb

the spread of HIV. The battle ground in the African and Asian countries may be quite different. There are sexual behaviors rooted in tribal traditions may prove to be obstacles to AIDS control in cultures where marital fidelity must be viewed outside the Judeo Christian model of monogamy. For example in the Zambian tradition, when a man dies, his many relatives must have sex with the widow to cleanse her from the ghosts of her husband (Sakala *et al.*, 1996). Similar practices can be found in the NillelotsLuo community of Kenya where wife inheritance is still widely practiced among brothers of the deceased (Luginaah *et al.*, 2005). HIV prevalence in the area where this community lives is estimated to be the highest in the country followed by high prevalence in the Kenyan's capital Nairobi (Luginaah *et al.*, 2005; Kenya Democratic Health Survey, 2003). Among senior women in these communities there is a strong resistance against any move to eradicate this practice. Though eradication would reduce the risk of HIV transmission it would also, reduce the opportunity of widows to remarry and thus the material prospects of both widows and their children (Sakala *et al.*, 1996).

There is no HIV success story without the mention Uganda which was one the hardest hit by the HIV rates as high as 30% in the early 1990s to an estimated 5% in 2001. Funds have been granted generously to promote abstinence (Genuis and Genuis, 2005). Some people would erroneously congratulate public health propagandists for their good work in changing people's sexual behavior, leading to a decline in HIV incidences in Uganda. However, some scholars have argued that there were reports of declining HIV incidences in 1994 in some rural Uganda locales where there had not even been adequate treatment for traditionally sexually transmitted diseases, let alone any condom or abstinence indoctrination programmes (Brody, 2004; Pettifor *et al.*, 2005).

There is however, no guarantee that the use of condoms will give full protection. One should also avoid the use of shared razors and tooth-brushes. Intravenous drug users should be informed that the sharing of needles and syringes involves special risk. Women suffering from AIDS or who are at high risk of infection should avoid becoming pregnant, since infection can be transmitted to the unborn or newborn. Educational materials and guidelines for prevention should be made widely available. All mass media channels should be involved in educating the people on AIDS (Lawrence *et al.*, 2006).

Promotion of being faithful to one trusted partner has worked for some developed countries, but for some reasons this has not been fully realized in developing countries, though some incidences in reduction of number of partners have been seen in a number of studies (Kinsman *et al.*, 2001; Gallant *et al.*, 2004; Pettifor *et al.*, 2005).

People in high-risk groups should be urged to refrain from donating blood, body organs, sperms or other tissues. All blood should be screened for HIV-1 and HIV-2 before transfusion. Transmission of infection to haemophiliacs can be reduced by introducing heat treatment of factors VIII and IX. Strict sterilization practices should be ensured in hospitals and clinics. Pre-sterilized disposable syringes and needles should be used as far as possible. One should avoid injections unless they are absolutely necessary (Lawrence *et al.*, 2006).

HIV from mother to child can be prevented almost entirely by anti-retroviral drug prophylaxis, elective caesarian section before onset of labour and rupture of membranes, and by refraining from breast-feeding. However, in economically poor countries, elective caesarian section is not a safe option. A substantial efficacy of triple combination of drugs has been shown in industrialized countries, where the rate of transmission is now below 2% in the absence of breast-feeding (WHO, 2004).

As of 2010, there is no vaccine effective for syphilis prevention. Abstinence from intimate physical contact with an infected person is effective at reducing the transmission of syphilis, as is the proper use of a latex condom. Condom use, however, does not completely eliminate the risk. Thus, the Center for Diseases Control and Prevention recommends a long-term, mutually monogamous relationship with an uninfected partner and the avoidance of substances such as alcohol and other drugs that increase risky sexual behavior. Congenital syphilis in the newborn can be prevented by screening mothers during early pregnancy and treating those who are infected. The United States Preventive Services Task Force (USPSTF) strongly recommends universal screening of all pregnant women, while the World Health Organization recommends all women be tested at their first antenatal visit and again in the third trimester. If they are positive, they recommend their partners also be treated. Congenital syphilis is, however, still common in the developing world, as many women do not receive antenatal care at all, and the antenatal care others do receive does not include screening, and it still occasionally occurs in the developed world, as those most likely to acquire syphilis (through drug use, etc.) are least likely to receive

care during pregnancy. A number of measures to increase access to testing appear effective at reducing rates of congenital syphilis in low- to middle-income countries (Kent and Romanelli, 2008; CDC, 2010; Hawkes *et al.*, 2011).

The risk of gonorrhoea infection can be reduced significantly by using condoms correctly and by having a mutually monogamous relationship with an uninfected person. It may also be reduced by avoiding sexual intercourse (CDC, 2012).

Use of male condoms may help prevent the spread of trichomoniasis, although careful studies have never been done that focus on how to prevent this infection. Infection with Trichomoniasis through water is unlikely because *Trichomonas vaginalis* dies in water after 45–60 minutes, in thermal water after 30 minutes to 3 hours and in [diluted urine](#) after 5–6 hours (Rob *et al.*, 2008; American Social Health Association, 2008).

CHAPTER THREE
MATERIALS AND METHODS

3. MATERIALS AND METHODS

3. Methods:

3.1 Study Design:

This study is a cross-sectional descriptive study.

3.2 Study Area:

The study was conducted in Malakal Town which is the capital of Upper Nile State. It is situated at the eastern side of the Nile. It has three payams which include central payam, southern payam and northern payam. Malakal Town comprises about four to five tribes which are the Shiluk (Chollo), Dinka, Nuer, Burun (Maban) and some other tribes. It has population of 126000 according to the household survey conducted in 2010.

3.3 Study Duration:

The study was conducted during the period from February 2012 to February 2014.

3.4 Study Population:

Pregnant women and voluntary counseling and testing attendants in Malakal town were invited to be enrolled in research to identify epidemiologic and biologic determinants of HIV and STDs infections. 2000 participants, 1200 were pregnant women and 800 voluntary counseling and testing attendants were included from age 18-24 years old. Voluntary counseling and testing attendants were from both sexes. The selection of participants was based mainly on attendance. Informed consent was obtained after proper counseling.

3.4.1 Exclusion:

Children below the age of 17 years, elderly people above 50 years and those who refused were excluded.

3.5 Sample Size:

A total of 2000 subjects were recruited in this study.

3.6 Data Collection:

Data was conducted by using questionnaire and interview. Information required included age, sex, residence, pregnant duration, married and single.

3.7 Specimen Collection:

A sample of venous blood 2 - 3 milliliters was collected or drawn from each individual by vacutainer tube and sera were separated in less than two hours. Cervical swabs were taken and urine was taken in a sterilized container.

3.8 Specimen Processing:

Specimen processing was conducted through various methods which included serology test, culture method and Gram's stain.

3.8.1 The Diagnosis of HIV was done by using three screening tests and one confirmatory test as follows:

3.7.1.1 Uni-Gold HIV (TRINITY BIOTECH, Ireland):

Principles:

Recombinant proteins representing the immune dominant regions of the envelope proteins of the HIV-1 or HIV-2, glycoprotein gp41, gp120 (HIV-1) and glycoprotein gp36 (HIV-2) respectively are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region.

During testing 2 drops of serum, plasma or whole blood is applied to the sample port, followed by 2 drops of wash buffer and allowed to react. Antibodies of any immunoglobulin class, specific to the recombinant HIV-1 or HIV-2 proteins will react with the colloidal gold linked antigens.

The antibody protein colloidal gold complex chromatographically along the membrane to the test and control regions of the test device.

A positive reaction is visualized by pink or red band in the test region of the device.

A negative reaction occurs in the absence of human immunoglobulin antibodies to HIV in the analyzed specimen. Consequently no visually detectable band develops in the test region of the device.

Excess conjugate forms a second pink or red band in the control region of the device. The appearance of this band indicates proper performance of the reagents in the kit.

Test Procedure:

1. μ Uni-Gol test device was removed from their protective wrappers.
2. Each test was labeled with the appropriate patient information.
3. Disposable pipette was used to add the specimens and reagents.
4. Two drops of serum were added (60 μ).
5. Then 2 drops of wash reagent were added to sample port.
6. Result was read after 10 minutes.
7. A line of any intensity forming in the test region, plus a line forming in the control region indicates a positive result.
8. A line in the control region only indicates a negative result.

Evaluation performed by WHO, sensitivity was 100% and specificity was 99.70%.

3.7.1.2 Determine HIV (Inverness Medical Japan Co., Ltd.):

Principle:

Determine HIV-1/2 is an immunochromatographic test for the qualitative detection of antibodies to HIV-1/2. Sample is added to the sample pad. As the sample migrates through conjugate pad, it reconstitutes and mixes with selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site.

If antibodies to HIV-1/2 are present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window, forming a red line at the patient window site.

If antibodies to HIV-1/2 are absent, the antigen-selenium colloid flow past the patient window and no red line is formed at the patient window site.

To insure assay validity, a procedural control bar is incorporated in the assay device.

Test Procedure:

1. Protective foil cover was removed from test wrapper.
2. Fifty 50µl applied onto the test pad.
3. Result was read after 15 minutes.

Positive; red bar appear in both the control window and the patient window of the strip. Any visible red colour in the patient window should be interpreted as positive.

Negative; one red bar appears in the control window of the strip and no red bar appears in the patient window of the strip.

Invalid; if there is no red bar in the control window of the strip and even if a red bar appears in the patient window of the strip, the result is invalid and should be repeated.

3.7.1.3 Indirect ELISA (Behring Diagnostika; Behringwerke AG, Frankfurt, Germany):

Indirect ELISA Test Procedure:

1. 10 µl of serum was diluted in 1.0 ml buffer and mixed well by vortexing.
2. 100 µl of diluted patient sample were added into microplate wells according to pipetting protocol and incubated for 30 minutes at room temperature (+ 18 c° to +25 c°).
3. Wells were washed 3 times using 300 µl of wash buffer and were left for 30 to 60 seconds after that wash buffer was removed with absorbent paper.
4. 100 µl of enzyme conjugate (peroxidase-labeled anti-human IgG) were added into each of the micro plate wells and incubated for 30 minutes at room temperature (+ 18 c°bis +25 c°).

5. Wells were washed 3 times using by dispensing and aspirating of 300 µl of wash buffer and were then left for 30 to 60 seconds after that the remaining wash buffer was removed with absorbent paper
6. 100 µl of substrate solution were added into each of the micro plate well and incubated for 15 minutes at room temperature protect from direct sunlight.
7. 100 µl of stop solution were then added into each well.
8. Photometric measurement of the colour intensity was done at a wavelength of 450 nm and reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution.
9. Result was calculated as follows:

Extinction of the control or patient's sample/ Extinction of sample 2= Ratio

10. Interpreting result as follows:

Ratio <1.0: Negative

Ratio ≥ 1.0: Positive

3.7.1.4 HIV by Western Blot for Confirmation (Biorad, Richmond, CA, USA):

Fifty of lysate was put to protein assay and equal volume of 2x laemli buffer was added, boiled at 100c° for 5 minutes and then stored at -20c°. Centrifuged at 16000xg in micro centrifuge for 5 minutes and equal amount of protein loaded into the wells of SDS-GAGE, and then run for 1-2 hours at 100V. Stained at room temperature for 1hour and incubated with primary antibody in 5% or 2% blocking solution and then washed in TBST to remove excess reagent and covered in plastic wrap and then read at dark room in colorimetric detection. The stained bands then indicate the proteins to which the patient's serum contains antibody according to Gilda and Gomes (2013).

3.8 Diagnosis of syphilis (Macrovue; Becton Dickinson, Cockeysville, MD, USA):

Rapid Plasma Reagin Test Procedure:

1. RPR card was labeled with patient and control information.

2. Drop (0.05ml) was added with dropper.
3. Positive and were added.
4. The sample was spread smoothly across the circle area using the paddle of the dispenser.
5. Mixed well and a drop of antigen was added.
6. Card was placed on an automatic rotator and covered to maintain humidity for 8 minutes.
7. Result was read immediately microscopically in the state under a high intensity light source.

None-reactive; no clumping or slight roughness.

Reactive; any degree of clumping

3.8.3 Diagnosis of Gonorrhoea done by.

3.8.3.1 Gram's Stain

Gram's stain was applied according to Ochei and Kolhatkar (2000). The slides (smears), were placed on the staining rack and were flooded with crystal violet stain (base stain), for 1 minute, then the stain was washed off with distilled water. The smears were covered with Lugol's iodine (mordant) 1-2 seconds, and then rinsed in distilled water. The smears were counter stained and safranin for 1 minute rinsed in water, blotted with filter paper was allowed to air-dry. The prepared slides were examined microscopically with oil immersion lens objective. Bacteria coloured violet was labeled as Gram's positive and red-coloured bacteria were labeled Gram's negative.

3.8.3.2 Culture Method:

In the laboratory, the cervical swab was inoculated in Modified New York City (MNYC) medium and incubated at 37°C overnight. The plate was examined after 24-48 hours incubation. Colonies with cultural characteristics and cells with microscopic features similar to *Neisseria gonorrhoea* (small 1mm, grey and smooth colonies of Gram negative diplococci) after 24 hours, and after 48

hours the colonies are larger (1.5-2.5mm) with crenate margin and opaque raised centre were especially selected for purification. Gram's stain was prepared and examined from the *Neisseria gonorrhoea* colonies. Pure cultures of *Neisseria gonorrhoea*-like colonies were differentiated by using standard biochemical reaction of isolates, the medium used is recorded (Young, 1978).

3.8.3.3 Purification of Isolates:

The resulting growth was checked for purity by Gram's stain and was examined microscopically.

3.8.3.4 Identification of the Isolated Bacteria:

(a) Primary Identification

Gram's staining to see the shape, arrangement and Gram's reaction were performed for primary identification. Bacterial smears were prepared by emulsifying small inoculums of the bacterial culture in a drop of normal saline and spreading it onto a clean glass slide (15-20mm). The smears were allowed to dry on air and then fixed by gentle heating.

3.8.4 Biochemical Tests:

The test was performed by adding 0.1ml of a heavy saline suspension of test organism to each of four test tubes containing glucose, lactose, maltose and sucrose carbohydrate disc and a fifth tube containing a carbohydrate-free disc (include as a negative control). All the tubes were incubated in a water bath at 37c° and examined at 30 minute intervals for up to 5hours for a change in colour from red to yellow or red to yellow-orange, indicating carbohydrate utilization. The organism was identified as *N. gonorrhoea* based on positive oxidation of carbohydrate, positive glucose, negative maltose, negative fructose and negative sucrose

Biochemical Testing:

The result of biochemical examination of *Neisseria gonorrhoea* isolate was illustrated in below Table.

Characteristic	<i>N. Gonorrhoea</i>
Oxidation of Carbohydrates	+
Glucose	+

Maltose	-
Fructose	-
Sucrose	-

3.8.5 CD4 Count Method:

Hundred μ of peripheral blood was prepared in a separation buffer and placed in 12x75mm, 5ml tube and then 20ul of Magni-sort Anti-Human was added, mixed well by pulse vortexing 5 times and incubated at room temperature for 10 minutes. Washed with separation buffer and centrifuged at 300xg for 5 minutes and then supernatant was discarded and 27ul of magni-sort was added also, mixed well 5 times and then incubated at room temperature for 10 minutes. Volume was brought to 2.5ml with cell separation buffer mixed well 3 times and the tube was inserted into magneto, incubated at room temperature for 5 minutes. Tube was removed from magneto, washed and transferred into 15ml conical tube. CD14-depleted cells coated and then centrifuged at 300xg for 5 minutes and transferred to a new, 12x15mm, 5ml tube. Anti-Human CD4 Biotin per 100ul of cells was added, mixed well 5 times and then incubated at room temperature for 10 minutes. Volume was brought to 2.5ml with separation buffer, mixed well by pipetting 3 times and then the tube was inserted into magneto until the bottom and then incubated at room temperature for 5 minutes. Supernatant was discarded and washed 3 times and then the tube containing CD4⁺ T cell was removed from magneto and then 1ml of cell separation buffer was added. The slide of the tube was washed by pipetting the buffer down the sides and then result was read as follows, 50 cells / mm³, 100 cells / mm³, 120 cells / mm³, 140 cells / mm³, 150 cells / mm³, etc.

3.8.6 Wet Preparation of Fresh Urine:

Three loopfuls of well-mixed fresh urine were placed on a slide, and covered with cover glass. Preparation was examined using the 10 \times and 40 \times objectives with the condenser iris closed sufficient to give good contrast, and then *Trichomonas vaginalis* was seen motile.

3.9 Ethical Consideration:

It was obtained from the Ministry of Health in Juba.

3.10 Data Analysis:

Data analysis was conducted manually by using tables, pie charts and chi-square.

CHAPTER FOUR

RESULTS

Results

In this study a total of 2000 individuals (1200 pregnant women and 800 voluntary counseling and test attendants) were recruited to participate in this study.

The most prevalent STD among pregnant women was syphilis (0.6%) followed by HIV (0.5%) infection and candidiasis (0.3%) each (Table 2).

Among the HIV infected pregnant women (4) co-infection with syphilis was found in 3 (75%), with candidiasis in 2 (50%) and with trichomoniasis in 2 (50%), Table 3.

One third of HIV infected VCT Attendants (5) were co-infected with syphilis (Table.4).

The most frequent STD in age group 18 – 20 years, was syphilis (2.0%) followed by candidiasis (1.0%) while was the most frequent STD in the age group 21 – 24 years was HIV (0.37%), Table 5.

In females the most frequent STD was syphilis (0.6%) followed by HIV (0.5%), while was the most frequent STD in males was syphilis (1.0%) followed by HIV (0.5%), Table 6.

The rate of infection by syphilis, HIV and candidiasis was higher in Malakia (1.0%, 0.8% and 0.8%) respectively, followed by Jalaba and Mudiria (0.4%) each (Table 7).

All the investigated STDs were higher among the illiterates (Table 8).

The rate of syphilis and HIV was higher among the married participants compared to the unmarried (single) (Table 9).

The pattern of CD4⁺ count among the participants positive for the investigated STD is shown in Table (10) which shows that all HIV patients (10) have CD4⁺ count below 200/mm³ and patients with trichomoniasis (3) have CD4⁺ count also between 50 - 100/mm³ below (Table 10).

Table (1).The percentage of the STDs investigated in the studied population (n = 2000).

Type of STD:	Positive for Disease	
	Frequency	Percentage %
HIV	10	0.5
Syphilis	14	0.7
Candidiasis	6	0.3
Trichomoniasis	3	0.15

Gonorrhoea	1	0.05

Table (2).Distribution of the different STDs among pregnant women and VCT Attendants (n= 2000):

Type of STD:	Pregnant Women:			VCT Attendants:		
	Total	Positive	%	Total	Positive	%
NO:						
HIV	1200	4	0.3	800	6	0.75
Syphilis	1200	8	0.6	800	6	0.75
Candidiasis	1200	4	0.3	800	2	0.25
Trichomoniasis	1200	2	0.16	800	1	0.125

Gonorrhoea	1200	1	0.08	800	0	0.0

Figure 1: Reasons stated by the condom users (n= 500).

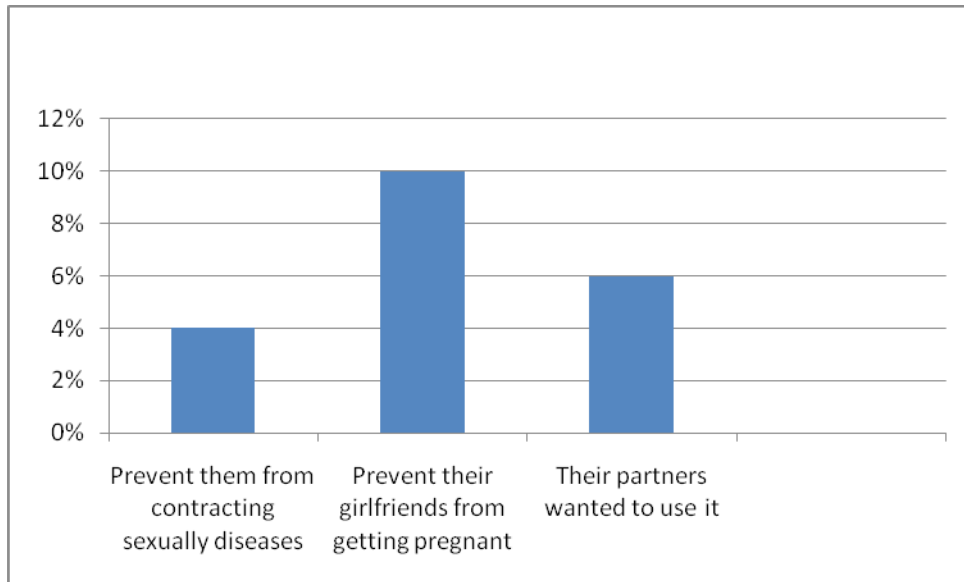


Figure 2: Reasons stated by the non-condom users (n= 500).

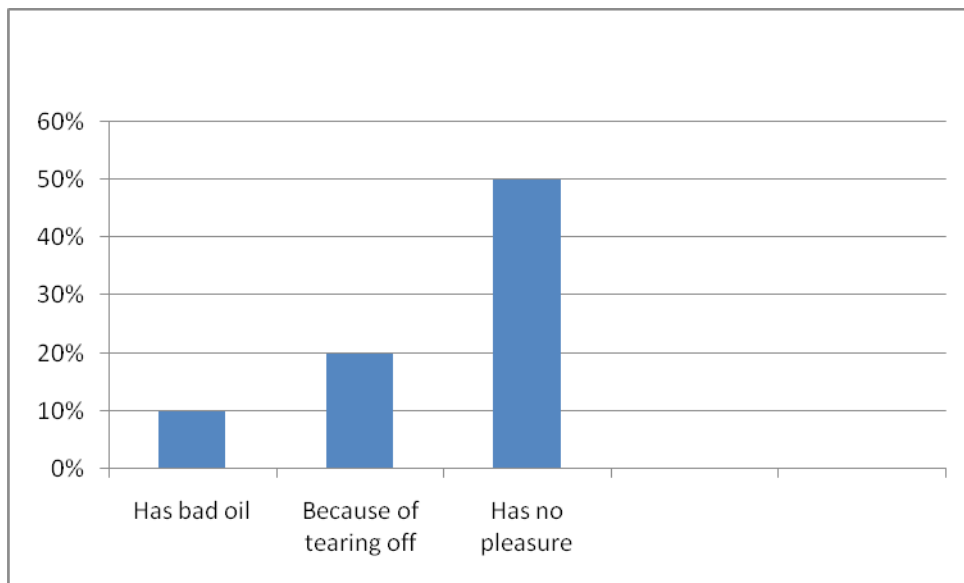


Figure 3: Reasons stated for the inability to abstain (n= 500).

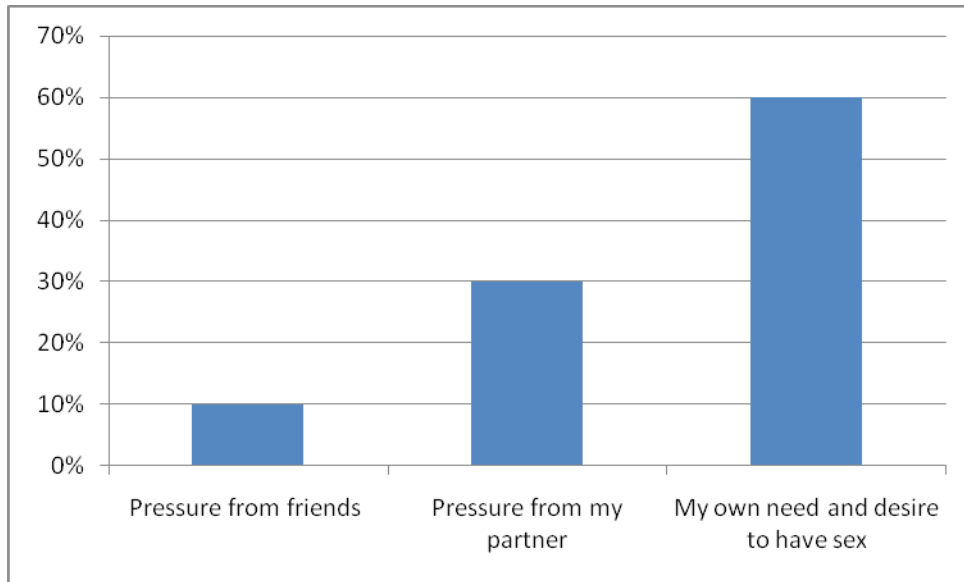


Figure 4: Reasons stated for not being faithful (n= 500).

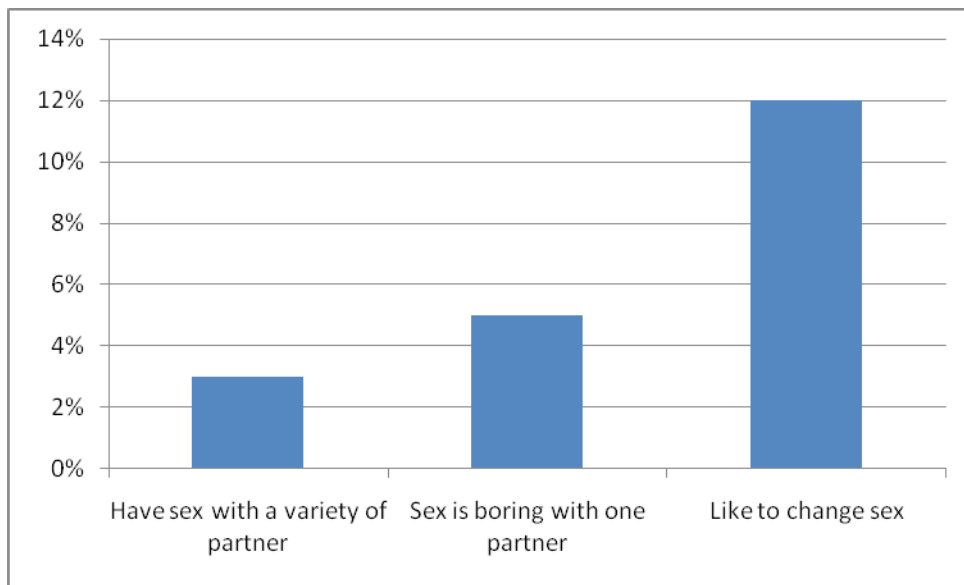


Table (3).Co-infection of HIV Positive with other STDs among pregnant women (n=4):

Type of STD:	Number:	%
Syphilis	3	75
Candidiasis	2	50
Trichomoniasis	2	50

Table (4). Co-infection of HIV Positive with other STDs among VCT Attendants (n= 6):

Type of STD:	Number:	%
Syphilis	2	33.3

Candidiasis	2	100
Trichomociasis	1	100

Table (5) .Distribution of the different STDs among the study group according to age (n= 2000):

Type of STD:	Age: 18 – 20 years.			Age: 21 – 24 years.			
	NO:	Total	Positive	% +ve	Total	Positive	% +ve
HIV		400	2	0.5	1600	8	0.5
Syphilis		400	8	2.0	1600	6	0.37
Candidiasis		400	4	1.0	1600	2	0.12
Trichomoniasis		400	2	0.5%	1600	1	0.06
Gonorrhoea		400	1	0.25%	800	0	0.0

Table (6). Distribution of the different STDs among the study group according to sex (n= 2000):

Type of STD:	Female:			Male:			
	NO:	Total	Positive	% +ve	Total	Positive	% +ve
HIV		1600	8	0.5	400	2	0.5
Syphilis		1600	10	0.6	400	4	1.0

Candidiasis	1600	6	0.37	400	0	0.0
Trichomoniasis	1600	3	0.18	400	0	0.0
Gonorrhoea	1600	1	0.06	400	0	0.0

Table (7).Distribution of the different STDs among the study group according to residence (n= 2000):

Type of STD:	Malakia:			Jalaba:			Mudiria&Assoa:			Lwakat:			
	NO:	Total	+ve	%	Total	+ve	%	Total	+ve	%	Total	+ve	%
HIV		500	4	0.8	500	2	0.4	700	3	0.4	300	1	0.3
Syphilis		500	8	1.6	500	3	0.6	700	1	0.1	300	2	0.6
Candidiasis		500	4	0.8	500	0	0.0	700	1	0.1	300	1	0.3
Trichomoniasis		500	1	0.2	500	1	0.2	700	1	0.1	300	1	0.3
Gonorrhoea		500	0	0.0	500	0	0.0	700	0	0.0	300	1	0.3

Table (8).Distribution of the different STD among the study group according to educational background (n= 2000):

Type of STD:	Literate:			Illiterate:			
	NO:	Total	Literate	%	Total	Illiterate	%
HIV		1500	2	0.1	500	8	1.6
Syphilis		1500	6	0.4	500	8	1.6
Candidiasis		1500	2	0.1	500	4	0.8
Trichomoniasis		1500	1	0.06	500	2	0.4

Gonorrhoea	1500	0	0.0	500	1	0.2
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Table (9).Distribution of the different STD among the study group according to marital status (2000):

Type of STD: NO:	Married & Widow:			Single:		
	Total	Married & Widow	%	Total	Single	%
HIV	1600	9	0.5	400	1	0.25
Syphilis	1600	12	0.75	400	2	0.5
Candidiasis	1600	5	0.3	400	1	0.25
Trichomoniasis	1600	3	0.1	400	0	0.0
Gonorrhoea	1600	0	0.0	400	1	0.25

Table (10).Distribution of CD4 count in participants tested positive for HIV with other STDs (n= 34):

Disease:	CD4 Count	CD4 Count	CD4 Count	CD4 Count
	50 – 100	110 – 150	160 – 200	Above 200
HIV	4	5	1	0
Syphilis	0	2	3	9
Candidiasis	0	1	2	3
Trichomoniasis	3	0	0	0
Gonorrhoea	0	0	0	1

CHAPTER FIVE
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

In this study the overall seroprevalence of HIV among the studied population was found to be (0.5%). This is in agreement with what was reported by JUNP and UNAIDS (JUNP, 2006 and UNAIDS, 2012). But very much lower than what was reported in UNS by SSAC (2010).

There was no different in rate of infection with HIV among the two age groups which both revealed seroprevalence of 0.5%.

Also there was no sex variation regarding rate of HIV infection as both sex showed seroprevalence of 0.5%.

The rate of HIV infection was higher in Malakia, this can be explained by high percentage of foreigners from the nearby countries with high prevalence of HIV infection.

Illiterates were found to be more affected by HIV than the educated participants. This is due to the fact that illiterates are not aware about HIV and other STDs.

The married group of participants has a higher rate of HIV infection compared to unmarried (single). This can be explained by the fact that married individuals were practicing sex to multiple partners if we consider the tradition of inheritance of wife. In fact four of the HIV

infected participants were inherited wives. Two studies done in Kenya revealed similar results (Luginaah *et al.*, (2005) and Sakalaet *al.*, (1996).

The overall seroprevalence of syphilis was found to be 0.7% among pregnant women and VCT Attendants. This is very much lower than what was obtained in the 10 states of South Sudan by ANC Surveillance (2009) and (Stamm, 2010; Mullooly and Higgins, 2010).

The rate of infection with syphilis among the group of 18 – 20 is higher 2.0%.

There was sex variation regarding rate of syphilis as males showed seroprvalence of 1.0% compared to 0.6% females. This is in agreement with studies done in USA, Canada, the UK and Australia by (Kent and Romanelli, 2008; Stamm, 2010). This can be explained that males were having multi partners.

In Malakia the rate of syphilis was found higher 1.6%. This can be explained by high

Percentage of foreigners, sex workers and inherited wives.

Illiterates were found to be more affected by syphilis than the educated participants. This is due to the fact that illiterates are not aware about syphilis and other STDs.

The married group of participants has a higher rate of syphilis infection compared to unmarried (single). This can be explained by the fact that married individuals were having unsafe sex with multiple partners.

The prevalence of candidiasis was found to be 0.3% among pregnant women and VCT Attendants. This is in agreement with study done by (Park, 2007; Ribud, 2005; Harvey *et al.*, 2007). But very much lower than what was reported by Tortora *et al.*, (2006).

The rate of candidiasis among the two age groups was observed higher in the age group of 18 – 20 years.

There was sex variation regarding rate of candidiasis infection as female revealed prevalence of 0.37%.

The rate of candidiasis infection was higher in Malakia. This can be explained that there is association with other STDs due to unsafe sex with multiple partners.

The married group has a higher rate of candidiasis infection than unmarried (single) group of participants.

Illiterates were found to be more affected by candidiasis than the literate participants. This is due to the fact that illiterates are not acknowledgeable about candidiasis and other STDs.

Prevalence of trichomoniasis was found to be 0.15% among pregnant women and VCT Attendants. This is in agreement with what was reported by (Park, 2007; Ribud, 2005; Harvey *et*

al., 2007). But very much lower than what was reported in USA by (Vos, 2012; Associated Press, 2007).

The rate of infection with *T.vaginalis* among two age groups showed prevalence of 0.5% in the age group 18 – 20 years.

Females showed higher rate of infection with *T.vaginalis*. This is in agreement with studies done in USA by (Vos, 2012; Associated Press, 2007).

Higher rate of trichomoniasis was in Lwakat. This can be explained by lack of personal hygiene and unsafe sex measures.

Illiterates were found to be more affected by trichomoniasis than literates. This is due to lack of knowledge about the disease.

Married group of participants have a higher rate of infection than unmarried (single) individuals. This can be explained by the fact that married practiced sex more than singles.

The overall prevalence of gonorrhoea was to be 0.05% among pregnant women and VCT Attendants. This is in agreement with studies done by (Park, 2007; Ribud, 2005; Harvey *et al.*, 2007). But very much lower than what was reported in USA by CDC (2008).

Only one participant (0.25%) was found to have *N.gonorrhoeae* and she was a female in the age group of 18 – 20 years.

That participant with gonorrhoea was from Lwakat.

The only case was found to be an illiterate married woman.

The overall prevalence of the studied different STDs among pregnant women were HIV (0.3%), syphilis (0.6%), candidiasis (0.6%), trichomoniasis (0.16%) and gonorrhoea (0.08%). This can be explained by bad habits like inheritance of wife, removal of lower teeth and multiple sex partners, unsafe sex or lack of personal hygiene.

Con-infection of HIV positive with other STDs was as follows; syphilis (75%), candidiasis (50%) and trichomoniasis (50%). This is in agreement with what was reported by Over (1993).

Prevalence of the studied different STDs among VCT Attendants was HIV (0.75%), syphilis (0.75%), candidiasis (0.25%) and trichomoniasis (0.125%). This can be explained by bad habits like inheritance of wife, removal of lower teeth and multiple sex partners, unsafe sex or lack of personal hygiene.

Con-infection of HIV positive with other STDs was as follows; syphilis (33.3%), candidiasis (33.3%) and trichomoniasis (100%).

CD4⁺ count was found to be less than 200 cells / mm³ in all HIV positive participants and 3 of the participants with trichomoniasis. This result is expected in HIV patient, but no reason can be stated regarding trichomoniasis patient.

Knowledge about risk factors of 500 participants was assessed by such as condom use, abstinence and faithfulness, but none of those using condoms was found to be positive for any of the sexually diseases tested for. Concerning condom, 100 (20%) participants were found to be condom users and those at risk were 400 (80%). Of those who reported condom use, 4 (4%) stated that they used condoms to protect themselves from contracting sexually diseases, 10 (10%) used it to prevent their girlfriends from getting pregnant, 6 (6%) used it because their partners wanted to use it and of those reported non-condom use, 40 (10%) said it has bad oil, 200 (50%) said it has no pleasure and 80 (20%) because of tearing off. This is in agreement with report by Brody (2004); however most of the respondents think that condom does not give full protection which is in agreement with report by Lawrence *et al.*, (2006).

Feelings about abstinence, 200 (40%) thought that it was possible to abstain while 300 (60%) did not think it was possible to abstain and they are considered at risk. The reasons given for the inability to abstain are pressures from friends were 30 (10%), pressures from my partner were 90 (30%) and my own need and desire to have sex were 180 (60%). This is in agreement with report by Genuis and Genuis (2005).

Feelings about being faithful, when asked if it was possible to be faithful to one trusted partner 400 (80%) thought this was possible while 100 (20%) did not think it was possible and they are also at risk. The reasons given for not being faithful are as stated three (3%) said this was because they like to have sex with a variety of partners, five (5%) reported that because sex is boring with one partner while twelve (12%) reported that because they would like to change sex.

The information obtained from this study reflect that the studied population lack basic information about the sexually transmitted diseases, more over bad traditions are being practiced.

5.2 Conclusions

The overall prevalence of the studied STDs was HIV (0.5%), syphilis (0.7%), *Candida* (0.3%), *T.vaginalis* (0.15%) and *Gonorrhoea* (0.05%) among pregnant women and VCT Attendants.

Among pregnant women the prevalence of different STDs was HIV (0.3%), syphilis (0.6%), *Candida* (0.3%), *T.vaginalis* (0.16%) and *Gonorrhoea* (0.08%).

Among VCT Attendants the prevalence of different STDs was HIV (0.75%), syphilis (0.75%), *Candida* (0.25%) and *T.vaginalis* (0.125%).

Co-infections of HIV positive with other STDs among pregnant women was (75%) with syphilis, (50%) with candidiasis and (50%) with trichomoniasis.

Co-infections of HIV positive with other STDs among VCT Attendants was as follows syphilis (33.3%), *Candida* (100%) and *T.vaginalis* (100%).

CD4⁺ count was very low among all HIV and Trichomoniasis positive cases.

None of the participants who use condom was found positive for any of the investigated STD.

5.3 Recommendations

1. Further studies with large sample size and different communities are recommended.
2. Health education campaigns encouraging safe sex, abstaining, faithfulness and the use of condoms consistently and correctly.
3. Measures that aim to combat bad habits and traditions like wife inheritance and other malpractices such as removal of teeth, tattooing and scarification of forehead, ears and lips are recommended.
4. Training of people living with HIV and AIDS on positive living to create supportive environment
5. Involvement of youth in and out of school for positive prevention through health education.

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APPENDICES

Appendix (1)

Materials:

(a) Enzyme-Linked Immunosorbent Assay (ELISA) made in Germany

Contents:

- Well of micro-titration plate;
- Enzyme labelled antihuman globulin;
- Hydrolyzed substrate
- Buffer preparation contents :

1. Bicarbonate/carbonate coating buffer:

3.03g Na_2CO_3

6.0g NaHCO_3

1000ml distilled water

pH 9.6

2. PBS:

1.16g Na₂ HPO₄

0.1g KCL

0.1g K₃PO₄

4.0g NaCL (500 ml distilled water)

pH 7.4

3.Blocking Solution:

Commonly used blocking agents are 1% BSA, serum, non-fat dry milk, casein, gelatin in PBS

4.Wash Solution:

Usually PBS or Tris –buffered saline (pH 7.4) with detergent such as 0.05% (v/v) Tween 20 (TBST)

5.Antibody Dilution Buffer:

Primary and secondary antibody should be diluted in 1x blocking solution to reduce non-specific binding.

Indirect ELISA Protocol:

1. Dilute antigen to a final concentration of 1-2 or Bicarbonate/carbonate coating buffer.Coat the wells of a PVC microtiter plate with the antigen by pipeting 50ul of the antigen dilution in the top well of the plate.

2. Dilute down the plate as required. Seal the plate and incubate overnight at 4°C or 2 h at room.
3. Wash plate 3 times with PBS.
4. Block the remaining protein-binding sites in the coated wells by adding 200 ul blocking buffer, 5% non fat dry milk/PBS, per well. Alternative block reagents include BlockACE or BSA.
5. Cover the plate with an adhesive plastic and incubate for at least 2 hrs at room temperature or, if more convenient, overnight at 4°C.
6. Wash the plate 3 times with PBS.
7. Add 100 ul of diluted primary antibody to each well.
8. Cover the plate with an adhesive plastic and incubate for 2 hrs at room temperature.
9. Wash the plate 4 times with PBS.
10. Add 100 ul of conjugated secondary antibody, diluted at the optimal concentration in blocking buffer immediately before use.
11. Cover the plate with an adhesive plastic and incubate for 1- 2 hrs at room temperature.
12. Wash the plate 5 times with PBS.
13. Dispense 100 ul (or 50ul) of the substrate solution per well with a multichannel pipette
14. After sufficient colour development add 50-100ul of stop solution to the wells.
15. Record the absorbance at 450 nm on a plate reader within 30 minutes of stopping the reaction.

(b) Western Blot (Bio-Rad, Richmond, CA, USA)

(c) Immunochromatographic Test (ICT), Determine, Japan

Contents:

- Determine HIV-1/2 test card;
- HIV- 1/2 recombinant antigen and peptide coated;
- Pipette;
- Chase Buffer

(d) Immunochromatographic Test (ICT), Uni-Golg, Ireland

Contents:

- Test device with recombinant HIV proteins, recombinant HIV proteins as test zone, and a control line;
- Wash reagent;
- Pipette;
- HIV- 1/2 recombinant antigen and peptide coated;
- Chase Buffer

(e) Immunochromatographic Test (ICT) made in Republic of South Korea

Contents:

- Test device;
- Pipette;
- Diluent

(f) Rapid Plasma Reagin (RPR) made in USA

(g) CD4 Cell Count Machine or Partec Flow Cytometry instrument

(Partec Cyflow, Germany) this includes as follows:

- Partec test tube (Code No. 04-2000);
- CD4 % easy count kit;
- Count check beads green;
- Cleaning solution for flow systems;
- Decontamination solution for flow systems (contain proteolytic enzymes);
- EDTA;
- Micropipette and pipette tips;
- Research pipette 10 ml;
- Research pipette 20 ml;
- Research pipette 100-1000 ml;

(h) Media: Modified New York City (MNYC) Medium was obtained from Oxford, London. It is a selective medium used to isolate *Neisseria gonorrhoeae*.

Formula (per litre):

GC agar base (Difco or Oxoid)	18g
Distilled water	446ml
Yeast extract powder	0.5g
Sterile saponinlyzed blood	50ml
Sterile glucose, 10% w/v	5ml
LCAT antibiotic supplement	10ml

Preparation: The medium was used at a concentration 18.5g in every 100ml of distilled water. It was mixed and dissolved by boiling; then sterile by autoclaving at 121°C for 15 minutes and cooled to 50-55°C for PH adjustment (7.0-7.4) at room temperature. It was distributed into petri dishes under aseptic conditions (Monica, 1984).

- (i) Potassium hydroxide 10%

To make 50ml:

Potassium hydroxide (KOH)	10g
Distilled water	50ml

1. Weigh the potassium hydroxide pellets. Transfer to a clean bottle.

Caution: Potassium hydroxide is a highly corrosive deliquescent chemical, therefore handle with great care and make sure the stock bottle of chemical is tightly stopper after use

2. Add the water, and mix until the chemical is fully dissolved.
3. Label the bottle and mark it Corrosive. Store at room temperature. The reagent is stable for up to 2 years.

Appendix (2)

My name is Simon Deng NyicarKak; I am currently studying at Sudan University of Science and Technology (SUST) taking a course in HIV and STDs. This research is for a fulfillment of my PhD course. The information you provide in this questionnaire will be used for academic purposes only. Your name and identity will remain confidential and therefore do not write your name in any part of this questionnaire. Be honest with your responses in-order to gather accurate data. Your co-operation and honesty will be highly appreciated.

Questionnaire:

Age:

- a. 14 – 18 year old
- b. 19 – 24 year old
- c. Above

Sex:

Female ()

Male ()

Marital Status:

Single ()

Married ()

Educational Background:

Illiterate ()

Literate ()

Widow:

Yes ()

No ()

Use Condom:

Yes ()

No ()

Had you been given blood before?

Yes ()

No ()

Had you been infected with syphilis?

Yes ()

No ()

Had you been infected with gonorrhea?

Yes ()

No ()

Had you been infected with trichomonas?

Yes ()

No ()

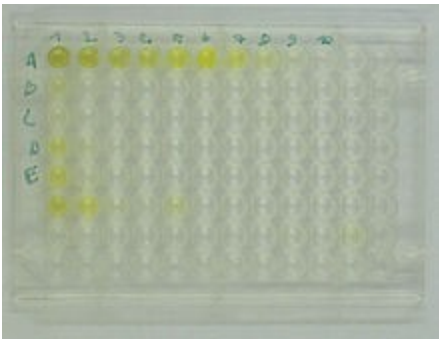
Had you been infected with candidiasis?

Yes ()

No ()

Appendix (3)

ELISA Micro titer Plate



This shows positive Determine



POSITIVE



NEGATIVE



Test Result: **REACTIVE**



Test Result: **NON-REACTIVE**



Test Result: **INVALID**

This shows positive Uni-Gol

