CHAPTER II

BIOLOGICAL ASPECTS OF HIV INFECTION

2.1 Introduction

Acquired Immuno Deficiency Syndrome (AIDS) is caused by the Human Immunodeficiency Virus (HIV), which crossed from primates into humans. Although isolated cases of infection in people might have appeared earlier, the first cases of the current epidemic probably occurred in the 1930s, and the disease spread rapidly in the 1970s. AIDS was publicly reported on 5 June 1981, in the Morbidity and Mortality Weekly Report produced by the Centers for Disease Control (CDC) in Atlanta in the USA. Doctors recorded unexpected clusters of previously extremely rare diseases such as Pneumocystis carinii, a type of pneumonia, and Kaposi’s sarcoma, a normally slow-growing tumour. These conditions manifested in exceptionally serious forms, and in a narrowly defined risk group-young homosexual men.

AIDS is an illness that damages a person’s ability to fight off disease, leaving the body open to attack from ordinarily innocuous infections and some forms of cancers. HIV is the causative agent responsible for AIDS. This virus infects certain types of white blood cells, primarily CD4 cells and monocytes/macrophages. CD4 cells and macrophages have important functions in the immune system. The disruption of the function of these cells lies at the
heart of the immunodeficiency that characterizes AIDS. HIV has ribonucleic acid (RNA) as their genetic material, and is called retroviruses. HIV also belongs to the family of viruses known as lentiviruses, which means slow-acting. In humans, lentiviruses result in diseases that develop over a long period, and thereby many are affected in the immune system and brain. HIV invade cells to reproduce. Within these cells, it produces more virus particles by converting viral RNA into DNA (deoxyribonucleic acid) in the cell and then making many RNA copies. The conversion is done through an enzyme called reverse transcriptase.

The switch over from RNA to DNA and back to RNA is significant; and it makes combating HIV difficult. Each time when it occurs, there is a possibility of errors and the virus mutating. This made more likely because reverse transcriptase lacks the normal ‘proofreading’ that occurs with DNA replication. Once formed, the copies or virus particles break out of the cell, destroying it and infecting other cells.

When a person is newly infected, they sero-convert- this means the virus has taken hold in the body and it (or its antibodies) will be detectable by an HIV test. However, there is a ‘window period’ when a person may be infected and infectious, but the virus is not yet detectable. In the period immediately after infection, a person will be having very infectious. This is of epidemiological importance. The window period is followed by the long
incubation stage. During this phase, the viruses and the cells they attack are reproducing rapidly and are being wiped out as quickly by each other. Every day up to 5% of the body's CD4 cells (about 2,000 million cells) may be destroyed by approximately 10 billion new virus particles. Eventually, the virus destroys immune cells more quickly than they can be replaced. A healthy CD4 cell count is normally over 1000 cells per mm$^3$ of blood.

The World Health Organization recognizes four stages in HIV disease progression. Stage one is asymptomatic infection, when the CD4 count is normally greater than 500 per mm$^3$ of blood. Stage two is when the count is between 350 and 499 per mm$^3$, and symptoms might include some mild weight loss, fungal infections, and herpes zoster (shingles). When the CD4 cell count falls below 350, in stage three, a person has advanced immunosuppression with opportunistic infections, fever, severe weight loss, diarrhea, and possibly TB. Stage four, AIDS, occurs when there are fewer than 200 CD4 cells per mm$^3$ of blood and the person is seriously ill with diseases such as TB which may spread beyond the lungs, Pneumocystis carinii and other pneumonias, the parasitic disease toxoplasmosis, and meningitis. A few people may experience symptoms of disease with CD4 counts above 200, while others show no symptoms with CD4 counts below 200. Generally, infections will increase in frequency, severity, and duration until the person dies. The CD4 count is one of the measures used by physicians in deciding when to begin drug therapy.
2.2 Structure of HIV

HIV is a member of lentivirus family of retroviruses. When viewed under the electron microscope, HIV appears as spherical particles that are approximately 110 nm in diameter, with knoblike projections on the surface of the virus and a cone shaped viral core. HIV particles contain two copies of an RNA genome, each of which is approximately 10,000 base pairs in length and encodes nine genes. Two different HIV species have been identified: HIV-1 and HIV-2.

The outer coat of the virus, known as the viral envelope, is composed of two layers of fatty molecules called lipids, taken from the membrane of a human cell when a newly formed virus particle buds from the cell. Each viral
particle contains 72 glycoprotein complexes, which are integrated into this lipid membrane, and are composed of trimmers of an external glycoprotein gp120 and a transmembrane spanning protein gp41. The bonding between gp120 and gp41 is only loose and therefore gp120 may be shed spontaneously within the local environment. Glycoprotein gp120 can be detected in the serum as well as within the lymphatic tissue of HIV-infected patients. The matrix protein p17 is anchored to the inside of the viral lipoprotein membrane. The p24 core antigen contains two copies of HIV-1 RNA. The HIV-1 RNA is part of a protein-nucleic acid complex, which is composed of the nucleoprotein p7 and the reverse transcriptase p66. The viral particle contains all the enzymatic equipments that are necessary for replication: a reverse transcriptase, an integrase p32 and a protease p11.

After the virus has entered the cell, the HIV reverse transcriptase enzyme converts the single-standard HIV RNA into DNA. Through a complex process, some of the viral DNA migrates into the nucleus, and the viral enzyme integrase splices the viral DNA into the host cell chromosomal DNA. Once HIV DNA becomes integrated into the human DNA, it is referred to as proviral DNA. Subsequently, the host can transcribe proviral DNA into messenger RNA (mRNA), a process controlled by the interaction of the promoters and enhancers in the viral genome with the Tat protein and cellular factors. Posttranscriptional processing of the viral mRNA takes place in the host cell nucleus. The viral mRNA is transported into the host cell cytoplasm; once
there, viral mRNA is translated into viral proteins. The late stage of viral replication involves both the assembly of the viral particles, with each viral core incorporating two copies of the viral RNA genome and the budding; and release of the virus from the surface of the cell.

2.3 Genesis of HIV

After entering a person’s body, HIV infects cells and starts to replicate in them (essentially CD4T cells and macrophages). The virus induces the body’s immune system to produce antibodies specific to HIV. The period between the acquisition of infection and production of detectable HIV antibodies is called the ‘window period’. The window period can last for 2-12 weeks. During this period, the person is highly infectious but may not test positive on common HIV antibody tests. Upto 30-50 percent of people have a recognizable acute illness at the time of infection characterized by fever, lymphadenopathy (enlargement of the lymph bodies), night sweats, skin rash, headache and cough.

HIV-infected people may remain asymptomatic for as long as 10 or more years. An HIV-infected person may take from 6 months to 10 years to develop AIDS; on an average, 50% of those infected take 8 years to progress to AIDS. People in this phase potentially play an important role in the transmission of HIV as they remain infectious but can be identified only by screening their serum for HIV antibodies. After a period of time, varying from
one individual to another, viral replication resumes and is accompanied by the
destruction of CD4 lymphocytes and other immune cells, resulting in a
progressive immunodeficiency syndrome. The progression depends on the
type of infection and different factors that may cause faster progression, such
as age less than five years or over 40 years, other infections (opportunistic
infections) and possibly heredity (genetic) factors.

Various infections, diseases and malignancies occur among HIV
infected individuals. These are correlated with the degree of immune
suppression and include tuberculosis (TB), oral hairy cell leukoplakia, oral
candidiasis, popular pruritic eruption, Pneumocystis carinii pneumonia,
cryptococcal meningitis, cytomegalovirus (CMV) retinitis and Mycobacterium
avium infection, Kaposi sarcoma, etc.

2.4 Spread or Transmission of HIV/AIDS

The risk of HIV transmission is present, if an HIV-negative person
comes into contact with the blood, semen or vaginal fluids of an HIV positive
source person. HIV is found in all body fluids of an infected person, although
in minimal quantities in sweat, tears and saliva. Exposure to blood or blood
products carries the maximum risk of infection. This is why there is so much
concern around blood safety and hygiene in health care settings, and why there
are high levels of transmission among drug users who share syringes.
However, sexual intercourse is the most common source of transmission: 75-85
percent of people are infected this way. This includes both homosexual and heterosexual intercourse, though globally heterosexual intercourse predominates. The virus can be passed from infected mothers to their infants by crossing the placenta, during the birth process, and through breast milk. Reducing the risk prior to or during birth is simple: in most resource-poor settings, the drug nevirapine is used, which lowers mother-to-child transmission from about 25% to between 8% and 17%.

2.5 Modes of Transmission

HIV infection spreads through the following four modes.

- Unprotected hetero/homo sexual contacts
- Infected blood transfusion
- Sharing of unsterile needles for drug abuse
- Perinatal transmission

Unprotected Hetero / Homo Sexual Contacts

The majority of HIV infections are acquired through unprotected sexual contacts. HIV can enter the body through the mucous membranes that line the vagina, rectum, urethra, and possibly, on rare occasions, the mouth. Damage to a mucous membrane may increase the risk of transmission of HIV, but is not necessary for transmission to occur. Almost all cases of sexually transmitted HIV have been caused by anal or vaginal intercourse without condom. In the
United States and Europe, most cases of sexually transmitted HIV infection occur during homosexual contact, which is between two people of the same gender. In Africa, Asia and other parts of the world, HIV is transmitted primarily through heterosexual contact that is between two people of opposite genders.

**Infected Blood Transfusion**

Spread of HIV through blood occurs through specific identifiable practices and in specific identifiable situations. They include blood transfusion, sharing of blood and other skin-piercing instrument without proper cleaning and sterilization. Blood taken from one person with the infection and given to another person for medical treatment, would lead to infection to the latter. The tattooing and piercing the patients caused by the health care workers like doctors and nurses are of high-risk and such things would lead to infection by HIV. Moreover HIV can be spread in health care settings through accidental needle sticks or contact with contaminated fluids.

**Sharing of Unsterile Needles for Drug Abuse**

Sharing unsterilized injection equipment that has been previously used for some one who is infected is an important route of HIV transmission in many countries with a high prevalence of intravenous drug users. HIV frequently spread among drug users who share needles or syringes that are contaminated with blood from an infected person. It is rare, however, for a
patient to give HIV to a health care worker or vice-versa by accidental sticks with contaminated needles or other medical instruments.

**Perinatal Transmission**

HIV can be transmitted from an infected woman to her fetus during pregnancy and during delivery. This is referred to as vertical or perinatal transmission. Antiretroviral therapy used at the appropriate time in pregnancy significantly reduces the risk of transmission from mother to fetus. Additionally, using certain safe methods for delivery also helps to reduce transmission. Since breast milk can transmit HIV, avoiding breastfeeding further reduces vertical transmission.

**2.6 The Different Stages of HIV Infection**

HIV infection can generally be broken down into four distinct stages

- Primary infection
- Asymptomatic infection
- Symptomatic HIV infection
- Progression from HIV to AIDS

**Primary Infection**

HIV is disseminated to the brain, central nervous system and lymphatic tissue (lymph nodes, spleen, tonsils, and adenoids). Lymphatic tissue is the major reservoir of HIV in the study.
Ten to thirty days after infection, about 80% to 90% of people develop what is called “acute retroviral syndrome” or “primary HIV infection”. This is an illness that resembles the flu and usually lasts about one to two weeks. Symptoms include fever, swollen glands, sore throat, faint rash that generally starts in the torso, sores on the mouth and sometimes around the anus, weight loss, and muscle or joint pain.

During the first two to three months of HIV infection, viral load may be high and the CD4 count drops below normal. After a few months, the CD4 count generally rises close to normal levels and viral load drops. Viral load stabilizes at about 3 to 9 months to what is known as a viral “set point”. A higher viral set point and more severe acute retroviral syndrome symptoms are considered predictors of more rapid progression to AIDS.

**Asymptomatic Infection**

Asymptomatic means without symptoms. Many people with HIV may have few or no signs or symptoms of the disease for up to 10 years. However, some people may progress much faster, seeing their CD4 cells decline within a few years and experiencing symptoms in the first few years after infection.

During asymptomatic period, the only evidence of HIV infection may come from lab tests. Blood tests may show lower-than-normal numbers of CD4 cells and moderate levels of HIV. The amount of HIV in the blood is usually called the viral load. Although the immune system is able to fight HIV,
it cannot get rid of the virus completely. Despite lack of symptoms, HIV disease is progressing. On average, CD4 cells decline at a rate of approximately sixty points per year, while viral load gradually increases.

**Symptomatic HIV Infection**

This stage is said to be ‘Early and medium stage of HIV infection’. In this third stage, the immune system becomes so damaged by HIV that the symptoms begin to appear. The symptoms, mild at first, become more severe. The infection takes advantage of the vulnerable state of the immune system, and affects almost all the systems of the body. After some years, a variety of medical symptoms may develop, often involving skin and gastrointestinal disorders. Viral load continues to rise, and the CD4 count shows a more accelerated decline about 1.5 to 2 years before development of AIDS-defining illness.

**Progression from HIV to AIDS**

HIV infection and a specific group of diseases or conditions are indicative of severe immunosuppression related to infection with the human immunodeficiency virus. This is the terminal stage of the disease; and it represents the irreversible break down of the immune mechanism; and this leads to the susceptibility of the patient to wide variety of opportunistic infections and malignancies. Without treatment, it appears that the majority of HIV infected people will develop AIDS within ten to fifteen years after being
infected, though some people who have been infected longer than this remain healthy even without treatment. Effective treatment slows or stops the progression of HIV disease to AIDS and, for most people, seems likely to extend healthy life indefinitely.

2.7 HIV Tests

Medical opinion now strongly recommends that if any one is at some risk of being HIV-infected, he or she should be tested so that the person can benefit from recent dramatic advances in medical care, if test is positive. People infected with HIV can now get special medical care before the development of any noticeable symptoms-care that has been shown to delay AIDS and extend healthy life. HIV test detects antibodies to HIV or the genetic material (DNA or RNA) of HIV in the blood or another type of sample. This determines whether an HIV infected is HIV-positive. HIV infects white blood cells called CD4+ cells, which are part of the body’s immune system that help fight infections. HIV can progress to Acquired Immuno Deficiency Syndrome (AIDS).

There are several tests that can detect the presence of antibodies or genetic material (RNA) to the HIV virus, in semen, saliva or urine. These tests include ELISA, Western Blot, Polymerase Chain Reaction (PCR) and Indirect Fluorescent Antibody (IFA).
ELISA (Enzyme Linked Immuno-Sorbent Assay) Test

HIV antibody testing provides accurate information only if it is done properly. A very sensitive test called the ELISA (Enzyme Linked Immuno-Sorbent Assay) test is used first. This test will pick up any blood sample positive for HIV. It may produce false positives (be positive in the absence of HIV infection) because it cannot tell the difference between HIV antibodies and certain other antibodies that might be present.

HIV from a laboratory source is grown in human white blood cells in a test tube. The resultant virus is chemically disrupted and then used to coat a small container or well. Serum (the cell-free portion of blood) from the person being tested is added to the coated well. If antibody to HIV is present, it will bind with the viral fragments lining the well. The serum is then washed away, leaving only the attached antibody behind. Another preparation is then added to the well. This preparation contains antibodies to human antibodies. These anti-antibodies have been chemically attached to an enzyme, and the anti-antibody-enzyme complex binds to the HIV antibody left in the well from the subject’s serum. The well is then washed again to remove any material that has not bound to HIV antibody. Finally, a substance is added that produces a visible color change. This color change is then measured with a photometer (a device that measures the color of light reflected from the well). If the color change is over a preset threshold, the result is considered positive.
Western Blot Test

The Western blot test is an antibody detection test. This test will usually eliminate false positives. If the western blot analysis is positive, the laboratory will report a positive result. HIV antibodies in the test serum are detected by their reacting to a variety of viral proteins. The viral proteins are initially separated into bands according to their molecular weight on an electrophoresis gel. These proteins are then transferred or ‘blotted’ on to a nitrocellulose paper. The paper is then incubated with the patient’s serum. HIV antibodies to specific HIV patients bind to the nitrocellulose paper at precisely the point to which the target protein migrated. Bound antibodies are detected by colorimetric techniques.

Polymerase Chain Reaction (PCR) Test

A PCR (Polymerase Chain Reaction) test can detect the genetic material of HIV rather than the antibodies to the virus, and so can identify HIV in the blood within two or three weeks of infection. If a patient is concerned regarding an acute infection, the implementation of HIV PCR is useful. The PCR is also recommended in case of a highly positive screening and negative confirmative test results. Babies born to HIV positive mothers are usually tested using a PCR test because they retain their mother’s antibodies for several months, making an antibody test inaccurate. Blood supplies in most developed countries are screened for HIV using PCR tests. However, they are not often
used to test for HIV in individuals, as they are very expensive and more complicated to administer and interpret than a standard antibody test.

**Indirect Fluorescent Antibody (IFA) Test**

A serological test is used to detect agent-specific antibodies. Both infected and uninfected cells are attached to the microscope slide. Test serum, is placed over the cells and left to allow antibody binding to antigens; unbound antibodies are then washed off the slide. A fluorescent-labeled species-specific anti-immunoglobulin antibody is added to the slide and allowed to bind to the antibodies; unbound labeled antibody is washed off; then, a fluorescent microscope is used to observe specific fluorescence. These assays are rapid, inexpensive and highly sensitive, but their interpretation is subjective and requires a skilled observer.

**AIDS Related Complex (ARC)**

Infected people during this stage begin to show constitutional signs and symptoms. ARC covers a wide range of the disease. When a specific laboratory diagnosis was not available, this stage was considered to be a significant one for diagnosis of AIDS. ARC diagnosis in a person is reported to have a continuous low grade fever, a loss of body weight (more than 10%), continuous diarrhea, loss of energy, night sweats, etc.

This kind of diagnosis of HIV/AIDS would be rather difficult, because the signs and symptoms mentioned above could occur due to poor nutrition or
heavy load of other infections. So, a physician has to request a specific laboratory diagnosis to be made when a suspicion is there that a patient might have encountered a high risk situation for AIDS.

2.8 Prevention of HIV Infections

As there is no cure for AIDS, prevention of HIV infection becomes extremely important in controlling the disease. Efforts to prevent the spread of AIDS include:

(i) Restricting sexual activity to a single partner and practicing safer sex (i.e., always using a condom). Besides avoiding the risk of HIV infection, condoms are successful in reducing other sexually transmitted diseases and unwanted pregnancies. Before engaging in a sexual relationship with someone, getting tested for HIV infection is recommended.

(ii) Avoiding needle sharing among intravenous drug users.

(iii) Donating one's blood, before planned major surgery, to prevent risk of infection from a blood transfusion, although blood supplies are extremely safe in the developed world.

(iv) Practicing universal safety precautions when handling body fluids or needles. Healthcare professionals, first responders and teachers, for example, are now trained in these precautions.
(v) Testing for HIV infection by anyone who suspects infection. If treated aggressively and early, the development of AIDS may be postponed. If HIV infection is confirmed, it is also vital to let past sexual partners know so that they can be tested and receive medical attention.

2.9 Treatment for HIV/AIDS

The good news about treatment of HIV disease is dramatic: medication is available that can prevent illness and death; and significantly extend the life of the HIV-infected people. Those who receive proper medical care at the right time in the course of infection are likely to have an essentially normal lifespan. Further, such treatment can extend healthy life for years.

However, the treatment now available does not eradicate the virus from the body, and in this sense it is not a cure. Ongoing medical monitoring and continuous use of medication are necessary. Medications have both long-term and short-term side effects. Further, treatment requires money for medical expertise, medication, and laboratory tests; it is not available to the vast majority of HIV-infected people worldwide.

The most significant advancement has been the development of various effective antiretroviral medications that are used in combination. This treatment is referred to as HAART (Highly Active Antiretroviral Therapy). The drugs halt or slow down the replication of the virus, limit damage to the immune
system, and frequently restore immune function and thus prevent opportunistic infections, the causes of illness and death in HIV disease. A tremendous increase in knowledge about the virus is opening routes for the development of new categories of antiretrovirals.

Tests that accurately track the level of virus in the body and resistance of virus to medication allow for more effective use of drugs. Improved treatment and prevention of specific opportunistic infections have also helped to lessen the illness and death from HIV disease.