Review of Literature
2. REVIEW OF LITERATURE

In 1984 AIDS was described as a disease clinically characterized by fatal opportunistic infections and malignancies (Gottlieb et al., 1981; Masur et al., 1981). A few years later the causative retrovirus was isolated and denominated first as HTLV-III/LAV, later as HIV-1 (Barré et al., 1983, Gallo et al., 1984).

HIV is transmitted primarily via unprotected sexual intercourse, contaminated blood transfusions, hypodermic needles, and from mother to child during pregnancy (in utero), delivery (intrapartam), or breastfeeding (postpartam). The most frequent mode of transmission of HIV is through sexual contact with an infected person. The majority of all transmissions occur through heterosexual contacts (i.e. sexual contacts between people of the opposite sex) (William, 2007).

2.1 Epidemiology- HIV/AIDS Global scenario

AIDS continues to be a major global health priority. Although important progress has been achieved in preventing new HIV infections and in lowering the annual number of AIDS related deaths, the number of people living with HIV continues to increase.

An estimated 34.0 million (31.6 million–35.2 million) people were living with HIV as of 2010; 3.4 million (3.0 million–3.8 million) of them were children under 15 years, and about 16.8 million (15.8 million–17.6 million) were women.
(Fig 2.1). In 2010, around 390,000 (340,000–450,000) children were infected with HIV, bringing to 3.4 million (3.0 million–3.8 million) the total number of children under 15 living with HIV. More than 90 per cent of these children live in sub-Saharan Africa (Fig. 2.2).

Every day, over 7,000 persons became infected with HIV and about 5,000 persons died from AIDS, mostly because of inadequate access to HIV prevention care and treatment services (Fig. 2.3) (www.unaids.org).
### Global summary of the AIDS epidemic | 2010

#### Number of people living with HIV

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>[Range]</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>34.0</td>
<td>31.6–35.2 million</td>
</tr>
<tr>
<td>Adults</td>
<td>30.1</td>
<td>28.4–31.5 million</td>
</tr>
<tr>
<td>Women</td>
<td>16.8</td>
<td>15.8–17.6 million</td>
</tr>
<tr>
<td>Children (&lt;15 years)</td>
<td>3.4</td>
<td>3.0–3.8 million</td>
</tr>
</tbody>
</table>

#### People newly infected with HIV in 2010

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>[Range]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.7</td>
<td>2.4–2.9 million</td>
</tr>
<tr>
<td>Adults</td>
<td>2.3</td>
<td>2.1–2.5 million</td>
</tr>
<tr>
<td>Children (&lt;15 years)</td>
<td>390 000</td>
<td>340 000–450 000</td>
</tr>
</tbody>
</table>

#### AIDS deaths in 2010

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>[Range]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.8</td>
<td>1.6–1.9 million</td>
</tr>
<tr>
<td>Adults</td>
<td>1.5</td>
<td>1.4–1.6 million</td>
</tr>
<tr>
<td>Children (&lt;15 years)</td>
<td>250 000</td>
<td>220 000–290 000</td>
</tr>
</tbody>
</table>

Source: UNAIDS, 2011
Figure 2.2 Children (< 15 years) estimated to be living with HIV, 2010

Children (<15 years) estimated to be living with HIV  |  2010

Total: 3.4 million [3.0 million – 3.8 million]

Source: UNAIDS, 2011
Figure 2.3 New HIV infections in children and adults

Over 7000 new HIV infections a day in 2010

- About 97% are in low and middle income countries
- About 1000 are in children under 15 years of age
- About 6000 are in adults aged 15 years and older, of whom:
  — almost 48% are among women
  — about 42% are among young people (15-24)

2.2 Epidemiology - HIV/AIDS Indian Scenario

The total number of people living with HIV/AIDS (PLHA) in India was estimated at 23.9 lakh (19.3 – 30.4 lakh) in 2009. Children less than 15 years account for 3.5 percent of all infections, while 83 percent were in age group 15-49 years. Of all HIV infections, 39 percent (9.3 lakhs) were among women.
The four high prevalence states of India (Andhra Pradesh–5 lakhs, Maharashtra–4.2 lakhs, Karnataka–2.5 lakhs, and Tamil Nadu–1.5 lakhs) had 55 percent of all HIV infections in the country.

West Bengal, Gujarat, Bihar and Uttar Pradesh were estimated to have more than one lakh PLHA each and together account for another 22 percent of HIV infections in India. The states of Punjab, Odisha, Rajasthan & Madhya Pradesh have 50,000–1 lakh HIV infections each and together account for another 12 percent of HIV infections.

Unprotected sex (87.4% heterosexual and 1.3% homosexual) was the major route of HIV transmission, followed by transmission from parent to child (5.4%) and use of infected blood and blood products (1.0%). While injecting drug use (IDU) was the predominant route of transmission in north eastern states, significant route of transmission of HIV infection in children below the age of 15 years occurs during pregnancy, during child birth or breastfeeding. (www.nacoonline.org) (Fig. 2.4)
Figure 2.4 Routes of Transmission of HIV, India, 2010-11

Source: NACO Annual Report 2010-11
http://www.performance.gov.in
2.2.1 Mother to child transmission (MTCT) of HIV-1

MTCT was the probable source of infection in 1.8% cases reported in India between 1986-2001. In the year 2003, out of 57781 AIDS cases reported to NACO, 1551 (2.68%) cases are due to MTCT (Kapoor et al., 2004). HIV prevalence in antenatal women aged from 15-24 in southern states (Andhrapradesh, Karnataka, Tamilnadu, Maharastra) fell from 1.7% to 1.1% (Kumar et al., 2006). The HIV prevalence among antenatal population has remained more than 1% in the high prevalent states except Tamil Nadu, which has shown HIV prevalence of less than 1%. The state of Mizoram was showing HIV prevalence among antenatal population as more than 1% but during 2005 it has remained less than 1%. Further analysis of the data on HIV prevalence site wise was carried out and it was observed that there were 95 sites with HIV prevalence of more than 1% and 9 such sites were in low prevalence states (www.nacoonline.org).

2.3 History of HIV-1 / AIDS

Researchers conducted a study in 1983 in which they indicated that this human retrovirus, although similar to HTLV in infecting CD4 lymphocytes, had quite distinct properties. In 1984 this virus was named as Lymph adenopathy Associated Virus (LAV), grew to substantial titer in CD4 cells (Montagnier et al., 1984). In 1984, National Cancer Institute, of USA, isolated a T lymphotropic
retrovirus from patients with signs or symptoms that frequently proceed to AIDS (pre-AIDS) and named it HTLV-111 (Popovic et al., 1984).

In January 1985 a number of more detailed reports were published concerning LAV and HTLV-III, and by March it was cleared that the viruses were the same and was the causative agent of AIDS (Marx, 1985). In May 1986, the International Committee on Taxonomy of Viruses recommended giving the AIDS virus a separate name, the HIV-1 (Coffin et al., 1986).

2.4 Structure of HIV

Human Immunodeficiency Virus is a retrovirus, 120 nm in diameter and roughly spherical. HIV-1 is composed of two copies of single-stranded RNA enclosed by a conical capsid comprising the viral protein p24. The integrated form of HIV-1, also known as the provirus, is approximately 9.8 kilobases in length (Muesing et al., 1985). Both ends of the provirus are flanked by a repeated sequence known as the long terminal repeats (LTRs). The genes of HIV are located in the central region of the proviral DNA and encode at least nine proteins and these proteins are divided into three classes:

1. The major structural proteins: Gag, Pol, and Env
2. The regulatory proteins: Tat and Rev
3. The accessory proteins: Vpu, Vpr, Vif, and Nef (Gallo et al., 1988)
2.4.1 Structural Proteins

The *gag* gene determines the core and shell of the virus. It is expressed as the precursor protein p55, which is cleaved into three proteins p15, p18 and p24.

The *env* determines the synthesis of envelope glycoproteins gp120, which is cleaved into the two envelope components which forms the surface spikes and gp41, which is the transmembrane anchoring protein.

The *pol* gene encodes for the polymerase reverse transcriptase. It is expressed as a precursor protein p100, which is cleaved into proteins p34, p53 and p64. A matrix composed of an association of the viral protein p16, surrounds the capsid of HIV-1 (Connor *et al.*, 1997) (Fig. 2.5)
Figure 2.5  HIV-1 Structure
2.4.2 Regulatory Proteins

Tat- Stands for "Trans-Activator of Transcription “Interacts with cellular protein to increase HIV replication and the progression towards AIDS (Campbell et al., 2004)

Rev- It is a 13-kD sequence-specific RNA binding protein, produced from fully spliced mRNAs, Rev acts to induce the transition from the early to the late phase of HIV gene expression (Zapp and Green, 1989; Kim et al., 1989).
2.4.3 Accessory Proteins

Nef: This is an early gene of HIV, is the first viral protein to accumulate to detectable levels in a cell following HIV-1 infection (Kim et al., 1989).

Vpr: The Vpr protein is incorporated into viral particles. Approximately 100 copies of Vpr are associated with each virion (Cohen et al., 1990).

Vpu: The two functions of Vpu, the down-modulation of CD4 and the enhancement of virion release, can be genetically separated (Schubert et al., 1996). Vpu also increases the release of HIV from the surface of an infected cell. In the absence of Vpu, large numbers of virions can be seen attached to the surface of infected cells (Klimkait et al., 1990).

Vif: It is a 23-kD polypeptide that is essential for the replication of HIV in peripheral blood lymphocytes, macrophages, and certain cell lines (Strebel et al., 1987).

2.4.4 HIV-1 replication cycle

The Human Immunodeficiency Virus Type 1 infects mainly CD4+ T-cells and macrophages. This CD4 tropism is due to the fact that HIV-1 uses this surface receptor for entry. The binding of HIV-1 Gp120 to CD4 leads to a conformational change in Gp120 that opens the binding site of Gp120 to the chemokine
coreceptor (mainly CCR5 or CXCR4). Due to the coreceptor binding the virus fuses with the cellular membrane and the nucleocapsid enters the cell while being disintegrated. In the cytosol the virion associated reverse transcriptase is activated and synthesizes the viral cDNA (Monini et al., 2004).

The viral cDNA within the preintegration complex (PIC) is transported into the nucleus. Although the exact mechanisms are not fully understood, members of the lentiviral subgroup are the only retroviruses which are able to infect non-dividing cell types (Carter and Ehrlich, 2008). In the nucleus the viral genome is integrated into the host-cell genome by the HIV-1 integrase. The first viral protein translated is Tat that binds to the TAR element of the viral promoter and enhances the transcription of the viral cDNA (He and Zhou, 2011). This leads to the production of genomic (unspliced) and messenger (spliced) RNAs which are transported into the cytoplasm by the help of the viral Rev protein (Grewe and Überla, 2010).
Translation of viral mRNA leads to the production of Gag-, GagPol- and Env- polyproteins and the accessory proteins Vif, Vpu, Vpr and Nef. In CD4+ T-cells and most cell lines assembly of viral particles takes place at the plasma...
membrane, where a lot of different cellular proteins are recruited and form the so called budding machinery. The exact mechanism is matter of intense investigations (Waki and Freed, 2010).

The virus buds through tetraspanin-enriched- microdomains fused with lipid rafts at the host’s plasma membrane where also the viral Gp120-Gp41 complex is located (Hogue *et al.*, 2011). During this process the *Gag*- and the *GagPol* polyproteins, the viral genomic RNA and the viral proteins Vif, Vpr and Nef form the immature virion. The viral envelope is derived from the plasma membrane of the host-cell, thus containing a large variety of cell surface molecules next to the viral Gp41-Gp120 proteins (Chertova *et al.*, 2006). Shortly post release of the immature viral particle the protease cleaves Gag and the *GagPol* polyproteins. By regrouping the *Gag*-subunits to the conical capsid the virus matures and becomes infectious. (Monini *et al.*, 2004; Frankel and Young, 1998; Waki and Freed, 2010).
2.5 Nevirapine

Figure 2.8 Structure of nevirapine

VIRAMUNE is the brand name for nevirapine, a non-nucleoside reverse transcriptase inhibitor (NNRTI) which acts against HIV-1. Nevirapine is structurally a member of the dipyridodiazepinone chemical class of compounds. The chemical name of nevirapine is 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido [3,2-b:2',3'-e][1,4] diazepin-6-one. Nevirapine is a white to off-white crystalline powder with the molecular weight of 266.30 and the molecular formula is \( \text{C}_{15}\text{H}_{14}\text{N}_{4}\text{O} \). VIRAMUNE tablets are for oral administration. Each tablet contains 200 mg of nevirapine and the inactive ingredients such as microcrystalline cellulose, lactose monohydrate, povidone, sodium starch

Nevirapine was the first non nucleoside reverse transcriptase inhibitor approved for use in the prevention of HIV-infection from mother to child transmission and is a widely used antiretroviral drug, the efficacy of which has been well demonstrated in numerous clinical trials (Knobel et al., 2008). Nevirapine treated infants had 47% of reduction in HIV transmission from the mothers (Guay et al., 1999). Nevirapine was found to be more effective in reducing HIV transmission in CD4 counts below 200 cells/mm$^3$ (Jackson et al., 2003b).

2.6 Side effects of nevirapine regimen

Nevirapine treated mother and infant pair were observed for side effects; none of them had any severe adverse reactions, rashes or jaundice (Mangala et al., 2011). The most frequent adverse drug reactions associated with nevirapine were rash, life-threatening Steven Johnson’s syndrome, and elevated liver transaminases (Taiwo, 2006). Severe and fatal hepatitis were observed in nevirapine use in women (Joy et al., 2005; Lyons et al., 2006).

The elevations in liver enzymes were found out in HIV positive women and also had developed nevirapine resistance which was continued to be associated
with an increased risk of virologic failure or death (Lockman et al., 2010). The rate of HIV transmission was higher in breastfed infants than in non breastfed infants (Supriya et al., 2012).

2.7 Risk factors for HIV-1 infection through breast feeding

High viral load measured during pregnancy or after delivery and low CD4/CD8 counts have been associated with an increased rate of MTCT of HIV-1 through breast feeding (John et al., 2001, Semba et al., 1999). The presence of high viral load in plasma and breast milk is associated with HIV-1 transmission. (Lewis et al., 1998; Semba et al., 1999; Pillay et al., 2000). The risk of HIV through breast feeding is high in infants less than 6 months of age (Nduati et al., 2000). A randomized clinical trial suggested that the volume of milk ingested is an important factor in breastmilk transmission of HIV (Richardson et al., 2003). The infant oral thrush before 6 months of age is a risk factor for post neonatal infection in children (Embree et al., 2000).

The breast health has also been associated with risk of transmission through breast feeding. Clinical and Sub clinical mastitis and elevated breastmilk sodium level, nipple bleeding, breast abscess are associated with risk of HIV transmission through breast feeding (Semba et al., 1999; John et al., 2001; Ekpini et al., 1997).
2.7.1 Prevention of breast milk transmission

The important goals, effective interventions to decrease HIV-1 transmission through breast feeding was exclusive breast-feeding with early weaning (Becquart et al., 2005). Decreasing breast milk viral load with antiretrovirals throughout lactation is likely to decrease infectivity during breast-feeding (Manigart et al., 2004; Shaprio et al., 2005).

2.8 Testing methods for HIV infection

The vertical transmission of HIV-1 leads to a high level of infant mortality, especially during the first 2 years of life (RoseMary et al., 1999). It is therefore necessary to make an early diagnosis of HIV-1 infection in newborns to initiate therapy of infected infants as early as possible. Virologic assays were also helpful to confirm infection in patients who had an inadequate specific antibody production in an advanced stage of disease (Nielson et al., 2000). PCR was the major scientific development of the year in 1989 (Guyer et al., 1989).

Virologic tests such as virus cultures and RNA or DNA PCR were useful in an earlier diagnosis of HIV infection, but the virus cultures were time-consuming, as they required a biosecurity laboratory and had a poor sensitivity (Fischer et al., 2004). P24 antigen detection was an alternative method to detect the presence of the virus but the sensitivity was lower than that of PCR (Nesheim et al., 1997). For these reasons PCR has been the preferred method for the diagnosis of HIV
infection in infants for many years (Fransen et al., 1994). The use of whole blood samples spotted on filter papers for the detection of HIV-1 infection by PCR was first demonstrated in 1991 (Dunn et al., 1995).

2.9 HIV-1 DNA PCR

2.9.1 Whole blood

The diagnostic gold standard laboratory test for HIV-1 infection in infants is the HIV-1 qualitative DNA PCR using whole blood with high sensitivities and specificities (Sherman et al., 2005). Detection of HIV-1 DNA by PCR is an established method for determining infection status in children born to HIV-1-seropositive mothers (Owens et al., 1996). Two different volumes of the whole blood were tested for HIV-1 infection in infants using Roche Amplicor kit and it was found out that the results were concordant in the volume of both the samples (Estelle et al., 2008). The sensitivity and specificity was found to be 100 % in HIV-1 DNA PCR and ELISA method (Lynn et al., 1999). The sensitivity and specificity of manual and automated DNA extraction using the whole blood for HIV-1 DNA PCR testing in infants were observed to be 95 % and 99.6 % respectively (Anthony et al., 2010). The Real-time DNA PCR and Roche DNA PCR results were 100% concordant for the identification of HIV-1 infection among the infants (Ngo-Giang et al., 2008). The stability of whole blood was checked in different timings of storage and it was observed that the results were
negative or indeterminate on 4 days of storage and on the 10\textsuperscript{th} day there was fungal contamination in the blood samples (Cheryl \textit{et al.}, 2005)

2.9.2 Filter papers

In 1963, the feasibility of collecting neonatal blood samples onto filter paper for phenylketonuria testing of newborns was studied (Guthrie \textit{et al.}, 1963). The successful extraction of DNA from the blood spotted on filter paper was reported (McCabe \textit{et al.}, 1987). Detection of HIV-1 DNA by PCR was an established method for determining infection status in children born to HIV-1-seropositive mothers (Owens \textit{et al.}, 1996). Multiple replicate testing of samples and specialized filter paper types have been used to improve the accuracy of the PCR testing for HIV-1 DNA (Biggar \textit{et al.}, 1997). The sensitivity and the specificity of S&S Isocode stix filter paper was 95 \% and 100 \% respectively (Mini \textit{et al.}, 2008).

The sensitivity was calculated between the filter paper and the Peripheral Blood Mononuclear Cells (PBMCs) in infants and it was found that the sensitivity of nested PCR was 90\%, and the specificity was 99\%. The results were compared according to the children’s age. The sensitivity was 100\% for children less than 4 months and 75\% for children between 12 and 26 months old (Fischer \textit{et al.}, 2004).

HIV exposed infected and uninfected infants and adult laboratory workers in Washington and also HIV positive mothers and their infants in Peru were
identified. The assay had a sensitivity of 98.4% and a specificity of 98.3% (Ingrid et al., 2001). In Senegal study they compared both RNA and DNA detection for HIV-1 infection in infants using both venipuncture-derived whole blood in tubes and dried blood spots to identify the infection in infants. The sensitivity and the specificity was 100 % in both these methods (Kebe et al., 2011). Dried blood appeared biologically stable on filter papers and HIV-1 DNA had remarkable stability in dried samples eliminating the need to maintain the specimens at cold temperatures. (Evengard et al., 1988; Evengard et al., 1989; Cassol et al., 1992)

2.9.3 Sensitivity, Specificity, PPV and NPV

Sensitivity is the ability of a test to correctly classify an individual as diseased and the specificity is the ability of a test to correctly classify an individual as disease-free. Sensitivity and specificity are inversely proportional, meaning that as the sensitivity increases, the specificity decreases and vice versa (Rajul et al., 2008).

The predictive value is the key characteristic, as it gives information about how likely it is that the test result correlates with actual disease (www.who.int). There are two predictive values that are to be taken into account: PPV (Positive predictive value) and NPV (Negative predictive value). PPV is the proportion of people with disease among all those who have been tested positive, NPV is the proportion of people without the disease among those who have been tested
negative and both are expressed as percentage. Both the PPV and NPV depend on the prevalence of the disease in the tested population (UteFeucht et al., 2012). To predict the probability of actual disease, clinicians rely on the sensitivity, specificity, PPV and NPV of diagnostic tests. The results were compared with Peripheral blood mononuclear cells and blood spotted filter papers and it showed 100% of specificity and 96 % of sensitivity (Panteleeff et al., 1999). Protein saver card 903 filter paper using pol and gag genes and the specificity was 100 % in both the genes and the sensitivity of pol gene was 100%, gag gene showed only 87% of sensitivity (Bhavna et al., 2011).

The sensitivity and the specificity were 100% when the results between whole blood and protein saver card 903 filter paper were compared (Anitha et al., 2011). There were 100% concordant results between dried blood on filter papers and whole blood PCR (Supriya et al., 2012). The comparative study was done with in-house, real-time PCR to the Roche Amplicor test and the sensitivity was 98.6%, the specificity 99.7% , the PPV 94.0% and the NPV 99.9%.(Ngo-Giang et al., 2008).

The sensitivity and specificity of the nested PCR using gag, env and pol genes were 93.7 % and 100 % respectively (QiZhang et al., 2008). Whole blood samples were directly collected on filter paper by a heel prick and nested PCR was performed using pol and env primers. The results showed the sensitivity to be 95% the specificity to be 100% (Nyambi et al., 1994).
2.10 Effect of nevirapine on liver function profiles

Liver toxicity is the most common adverse effect associated with nevirapine treatment (Gonzalez et al., 2002). Transaminase elevations were a common side-effect for HIV positive patients using nevirapine, and grade 3-4 toxicity occurred in 8-17% of patients (Martínez et al., 2001). Liver enzyme abnormalities were observed in 22.2% of HIV positive women (William et al., 2007). In HIV infected antiretroviral naive adults for whom nevirapine-based antiretroviral therapy had been initiated, hepatotoxicity was found to occur and mortality at sixth month (Kathryn et al., 2010). Single-dose nevirapine at the onset of labour has been shown to be effective in reducing MTCT and has not been associated with an increased risk for maternal toxicity (Dhayendre et al., 2003). The severe hepatotoxicity from nevirapine-containing highly active antiretroviral treatment was more common at higher CD4 counts and suggested that laboratory monitoring is necessary when administering nevirapine-containing regimens to pregnant women with CD4 counts ≥250 cells/mm³ (Jamisse et al., 2007).

The following table called Pediatric Toxicity Table (Table 2.1) was developed by the Division of Microbiology and Infectious diseases (DMID) (November, 2007) for grading the severity of the disease:
**Table 2.1 Pediatric Toxicity Table**

<table>
<thead>
<tr>
<th>Gastrointestinal</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>1.1 - &lt;1.5 ULN</td>
<td>1.5 - &lt;2.0 ULN</td>
<td>2.0 - 3.0 ULN</td>
<td>&gt; 3.0 ULN</td>
</tr>
<tr>
<td>AST (SGOT)</td>
<td>1.1 - &lt;2.0 ULN</td>
<td>2.0 - &lt;3.0 ULN</td>
<td>3.0 - 8.0 ULN</td>
<td>&gt; 8 ULN</td>
</tr>
<tr>
<td>ALT (SGPT)</td>
<td>1.1 - &lt;2.0 ULN</td>
<td>2.0 - &lt;3.0 ULN</td>
<td>3.0 - 8.0 ULN</td>
<td>&gt; 8 ULN</td>
</tr>
</tbody>
</table>

Source: http://www.niaid.nih.gov/

*ULN: Upper Limit Normal.*

The Toxicity Table was used to estimate grade of severity:

**GRADE 1:** Mild Transient or mild discomfort (< 48 hours); no medical intervention/therapy required.

**GRADE 2:** Moderate Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required.

**GRADE 3:** Severe Marked limitation in activity, some assistance usually required; medical intervention/therapy required hospitalizations possible.

**GRADE 4:** Life-threatening Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required hospitalization or hospice care probable.

**GRADE 5:** Death.
The following table (Table 2.2) was given by the US department of health and Human Services Food and Drug Administration, Center for Biologics Evaluation and Research (September 2007) which provided - Guidance for the Industry-Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine and clinical trials.

**Table 2.2 Laboratory Abnormalities**

<table>
<thead>
<tr>
<th>Serum *</th>
<th>Mild (Grade 1)</th>
<th>Moderate (Grade 2)</th>
<th>Severe (Grade 3)</th>
<th>Potentially Life Threatening (Grade 4)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (gm/dl)</td>
<td>2.8 – 3.1</td>
<td>2.5 – 2.7</td>
<td>&lt; 2.5</td>
<td>--</td>
</tr>
<tr>
<td>Total Protein (gm/dl)</td>
<td>5.5 – 6.0</td>
<td>5.0 – 5.4</td>
<td>&lt; 5.0</td>
<td>--</td>
</tr>
<tr>
<td>Alkaline phosphate (IU/L)</td>
<td>1.1 – 2.0 x ULN</td>
<td>2.1 – 3.0 x ULN</td>
<td>3.1 – 10 x ULN</td>
<td>&gt; 10 x ULN</td>
</tr>
<tr>
<td>ALT, AST (IU/L)</td>
<td>1.1 – 2.5 x ULN</td>
<td>2.6 – 5.0 x ULN</td>
<td>5.1 – 10 x ULN</td>
<td>&gt; 10 x ULN</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>1.1 – 1.5 x ULN</td>
<td>1.6 – 2.0 x ULN</td>
<td>2.0 – 3.0 x ULN</td>
<td>&gt; 3.0 x ULN</td>
</tr>
<tr>
<td>Hemoglobin (gm/dl) (Female)</td>
<td>11.0 – 12.0</td>
<td>9.5 – 10.9</td>
<td>8.0 – 9.4</td>
<td>&lt; 8.0</td>
</tr>
<tr>
<td>WBC (cell/mm$^3$)</td>
<td>2,500 – 3,500</td>
<td>1,500 – 2,499</td>
<td>1,000 – 1,499</td>
<td>&lt; 1,000</td>
</tr>
</tbody>
</table>


** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4).

***ULN”” is the upper limit of the normal range
2.11 The Course of HIV-1 Infection

The human immunodeficiency virus type 1 (HIV-1) infection is characterized by a long and often prolonged asymptomatic period after initial infection. For adults in developed countries, in the absence of antiviral therapy, the median time from initial infection to the development of AIDS is about 10 to 11 years (Touloumi and Hatzakis, 2000). Mean duration of survival after diagnosis with HIV in India was 92 months (Kumarasamy et al., 2003) while median time for progression from HIV infection to acquired immunodeficiency syndrome was 7.9 years (Hira et al., 2003).

HIV disease is a continuum of progressive damage to the immune system from the time of infection to the manifestation of severe immunologic damage. Infection with HIV-1 initiates a process that leads to progressive destruction of CD4+ T lymphocytes, the target cell preference for HIV-1 infection. A typical pattern of HIV-1 infection in vivo is characterized by three phases: the acute or primary infection, the asymptomatic, and the symptomatic phase (Touloumi and Hatzakis, 2000).
2.12 Immunological assessment

The immune status of a child or adult living with HIV can be assessed by measuring the absolute number (per mm$^3$) or percentage of CD4+ cells, and this is regarded as the standard way to assess and define the severity of HIV-related immunodeficiency. Progressive depletion of CD4+ T cells is associated with progression of HIV disease and an increased likelihood of opportunistic infections and other clinical events associated with HIV, including wasting and death. Individual counts may vary within an individual adult or adolescent and assessing the CD4 count over time is more useful. The CD4 count usually increases in response to effective combination antiretroviral therapy, although this may take many months (www.who.int).
CD4 (T) cells serve as both essential regulators and effectors of the immune response; infection with HIV induces a progressive loss of these cells. The CD8 (T8) cell marker is present on the subset of suppressor lymphocytes that control or suppress specific ongoing immunologic activity (Grant et al., 1999).

The levels of CD8+ T Cells vary throughout the course of disease. Following the resolution of acute primary infection, CD8+ T Cells generally rebound to higher than normal levels and may remain that way throughout the clinically latent stage of disease. During the late stage of HIV infection, there may be a significant reduction in the number of CD8+ T Cells (Fauci et al., 2001). The toxicities associated with nevirapine among HIV positive women with higher CD4 cell counts and all of the adverse events in the nevirapine group occurred among women with an entry CD4 cell count greater than 250 cells/mm$^3$ (Hitti et al., 2004). The correlation between CD4 cell counts were noted, 12.9% of HIV positive women had the side effects with CD4 counts < 250cells/mm$^3$ and 22.5% women had developed side effects with $\geq$ 250cells/mm$^3$ of CD4 counts (William et al., 2007).

2.13 Correlation of TLC and CD4

A retrospective study was done in Military Medical Center and they suggested that easily available potential measure of immune function was the total lymphocyte count which was generated by performing a routine Complete Blood
Count (CBC) (Stephen et al., 1993). The study was conducted in developing country setting focusing on the capability and clinical utility of TLC as a surrogate marker for CD4 count in patients on HAART and it was observed that TLC was a strong marker for direction of concomitant change in CD4 count (Mahajan et al., 2004).

While comparing the ability of TLC and hemoglobin to predict CD4 count, it was found that TLC <1,200 cells/mm$^3$ was associated with CD4 count <200 cells/mm$^3$. Algorithms that include the combination of TLC, clinical staging, and hemoglobin may be more useful in predicting low CD4 cell counts (Spacek et al., 2003). When CD4 cell counts are unavailable for HIV positive women, TLC should be combined with WHO staging to initiate ART (Gupta et al., 2007). The sensitivity and the specificity of TLC and CD4 counts were 53.2% and 52.2%, respectively (Deresse Daka and Eskindir Loha, 2008).

Association of TLC, hemoglobin and delayed-type hypersensitivity (DTH) in HIV positive women was investigated to initiate highly active antiretroviral therapy and suggested that if CD4 counts were not available, the measures of TLC and hemoglobin should be <1250 cells/ mm$^3$ and <10.6 g/dl respectively for the initiation of treatment in resource limited settings. (Anastos et al., 2004).

In an observational study of antiretroviral-naive patients over a 3-year period it was found that TLC <800 cells/mm$^3$ and TLC between 800 cells/mm$^3$
and 1,400 cells/mm$^3$ were associated with increased risk of mortality (Bedell et al., 2003). TLC was performed to monitor the immune response where CD4 count testing is not available or too expensive for routine use and has been investigated as an alternative to CD4 count in resource-limited countries and a significant correlation between TLC and CD4 count (Badri and Wood, 2003). Risk of death exceeded 15% in children <2 years of age when TLC was 3,800 cells/ mm$^3$ (Mofenson et al., 2003; Mofenson et al., 1999). The risk of death was independently related to CD4 count and viral load, in children in resource-poor countries (Taha et al., 2000).

### 2.14 WHO clinical staging of HIV/AIDS

The clinical stage was useful for assessment at baseline (first diagnosis of HIV infection) into long-term HIV care and in the follow-up of patients in care and treatment programmes. It should be used to guide decisions on the time of starting co-trimoxazole prophylaxis and other HIV-related interventions, including the time of initiating antiretroviral therapy. The clinical stages have been shown to be related to survival, prognosis and progression of clinical disease without antiretroviral therapy in adults and children (Table 2.3)

Based on WHO’s guidelines the following criteria should be initiated for antiretroviral therapy in HIV infected infants and children:
Infants: ART should be initiated for all HIV-infected infants diagnosed in the first year of life, irrespective of CD4 count or WHO clinical stage.

Children: ART should be initiated for all HIV-infected children between 12 and 24 months of age irrespective of CD4 count or WHO clinical stage. ART for all HIV-infected children between 24 and 59 months of age with CD4 count of $\leq 750$ cells/mm$^3$ or $\%$CD4+ $\leq 25$, whichever is lower, irrespective of WHO clinical stage.

Initiation of ART for all HIV-infected children more than 5 years of age with a CD4 count of $\leq 350$ cells/mm$^3$ (as in adults), should be done irrespective of WHO clinical stage.

ART should be started for all HIV-infected children with WHO clinical stages 3 and 4, irrespective of CD4 count. ART should be initiated for any child less than 18 months of age who has been given a presumptive clinical diagnosis of HIV infection (www.who.int).
Table 2.3 WHO clinical classification of established HIV infection

<table>
<thead>
<tr>
<th>HIV-ASSOCIATED SYMPTOMS</th>
<th>WHO CLINICAL STAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>1</td>
</tr>
<tr>
<td>Mild symptoms</td>
<td>2</td>
</tr>
<tr>
<td>Advanced symptoms</td>
<td>3</td>
</tr>
<tr>
<td>Severe symptoms</td>
<td>4</td>
</tr>
</tbody>
</table>

World Health Organization proposed immunological classification for established HIV infection. These proposed immunological classifications outline four bands of HIV-related immunodeficiency: no significant immunodeficiency, mild immunodeficiency, advanced immunodeficiency and severe immunodeficiency (Table 2.4).

Table 2.4 Immunological classification in infants

<table>
<thead>
<tr>
<th>HIV-ASSOCIATED IMMUNODEFICIENCY</th>
<th>AGE-RELATED CD4 VALUES</th>
<th>&lt;11 months (%CD4+)</th>
<th>12–35 months (%CD4+)</th>
<th>36–59 months (%CD4+)</th>
<th>&gt;5 years (absolute number per mm³ or %CD4+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None or not significant</td>
<td>35</td>
<td>30</td>
<td>25</td>
<td>&gt; 500</td>
<td></td>
</tr>
<tr>
<td>Advanced</td>
<td>25–29</td>
<td>20–24</td>
<td>15–19</td>
<td>200–349</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>&lt;25</td>
<td>&lt;20</td>
<td>&lt;15</td>
<td>&lt;200 or &lt;15%</td>
<td></td>
</tr>
</tbody>
</table>
TLC was used only when CD4 measurement was not available in children with WHO clinical stage 2 diseases (Table 2.5). It could not be used in asymptomatic children and TLC was also not useful for monitoring ART.

Table 2.5 Diagnosing severe immunodeficiency in children using TLC

<table>
<thead>
<tr>
<th>Classification of HIV-associated immunodeficiency</th>
<th>&lt; 11 months</th>
<th>12-35 months</th>
<th>36-59 months</th>
<th>≥ 5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC</td>
<td>&lt; 4000</td>
<td>&lt; 3000</td>
<td>&lt; 2500</td>
<td>&lt; 2000</td>
</tr>
<tr>
<td>CD4 count</td>
<td>&lt; 1500</td>
<td>&lt; 750</td>
<td>&lt; 350</td>
<td>&lt; 200</td>
</tr>
</tbody>
</table>

For low-income countries, WHO recommends ART for people who are in WHO clinical stage IV regardless of CD4, consideration of treatment for those in stage III with CD4 below 350/mm$^3$, and treatment for those with CD4 below 200/mm$^3$ regardless of clinical stage determining viral load being unnecessary (Gilks et al., 2006).
Based on National AIDS Control Organisation’s (NACO) guidelines (Table 2.6) HIV-1 positive infants should be initiated antiretroviral therapy to improve their immune system.

Table 2.6 ART in children-NACO Guidelines (NACO, 2006)

<table>
<thead>
<tr>
<th>Age Group</th>
<th>CD4 Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 11 month infants</td>
<td>CD4 &lt; 1500 cells/mm³ (&lt; 25%)</td>
</tr>
<tr>
<td>12–35 months</td>
<td>CD4 &lt; 750 cells/mm³ (&lt; 20%)</td>
</tr>
<tr>
<td>36–59 months</td>
<td>CD4 &lt; 350 cells/mm³ (15%)</td>
</tr>
<tr>
<td>&gt; 5 years old</td>
<td>Follow adult guidelines ie start ART if CD4 &lt; 350 cells/mm³ especially if symptomatic. Initiate ART before CD4 drops below 200 cells/mm³.</td>
</tr>
</tbody>
</table>

2.15 HIV as a risk factor for Anemia

Inadequate housing, poor nutrition and increased exposure to infectious disease are worsening the health of HIV positive patients and leading to a weakened immune system. HIV-infected mothers had lower hemoglobin, hematocrit and white blood cell counts than HIV negative mothers. Similarly HIV exposed infants had lower haemoglobin, hematocrit and white blood cell counts than HIV non exposed infants (Kasonde et al., 2009). The decreased nutritional
status and weight loss were more among HIV positive breast feeding mothers than in non breast feeding mothers (Papathakis et al., 2006).

Anemia was found to be a strong independent predictor of disease progression and mortality among HIV-positive women (O’Brien et al., 2005). HIV-exposed negative infants had lower hemoglobin levels, less neutrophils and monocytes, than HIV non exposed infants (Ono et al., 2008). Anemia was common in HIV infection and independently associated with disease progression and mortality. HIV strongly increased anemia risk and interpretation of hematologic indicators in infants (Melissa et al., 2006). There was a significant correlation between low maternal hemoglobin and low CD4 counts in HIV positive mothers (Noel et al., 2008).

The causes of anemia are many. Its pathophysiology involves three mechanisms: (1) decreased red blood cell (RBC) production: opportunistic infection, direct effect of HIV infection itself, myelosuppressive medications, decreased production of erythropoietin, hypogonadism; (2) increased RBC destruction: autoimmune hemolytic anemia, thrombotic microangiopathy, disseminated intravascular coagulation; and (3) ineffective RBC production: folic acid and vitamin B12 deficiencies. Nutritional deficiencies such as vitamin and iron deficiencies are common in developing countries. The degree of inflammation
and its impact on hemoglobin levels could eventually be useful as a marker of disease status and prognosis of HIV positive patients (Antelman et al., 2000)

2.16 HIV-1 RNA PCR

Both HIV RNA PCR and HIV DNA PCR have been found to be highly sensitive and specific for early diagnosis of pediatric HIV infection with a higher sensitivity of viral load technique (RNA PCR) as compared to proviral DNA PCR in younger infants (Ira, 2006). Maternal HIV-1 RNA load was found to be the strongest predictor of mother to child transmission (Arvold et al., 2007).

CD4 cell counts and RNA viral loads are the two most commonly used prognostic markers of the clinical progression of HIV infection (Hammer et al. 2006). Risk factors for transmission were lower CD4 cell count and higher viral load in breastfeeding mothers (Gray et al., 2005). There was a significant relationship between CD4 count and viral load, those with lower CD4 counts had higher viral loads, 8% of HIV positive patients had lower CD4 cell counts of < 200 cells/mm$^3$ and higher viral load of > 10,000 copies/ml (www.nwpho.org.uk). HIV viral load, CD4 cell count and CD4% vary with age and disease complications in HIV-infected children. (Shah, 2006).

2.17 Nevirapine resistance

The detection of drug resistance is important in clinical and programmatic implications for prevention of mother to child transmission and for future
treatment of both mothers and their infants in India. Women had detectable plasma concentrations of nevirapine up to three weeks after taking a single dose of nevirapine (Cressey et al., 2005; Muro et al., 2005). The estimated efficacy in preventing nevirapine resistance was approximately 86% for both mothers and infants at six weeks (www.aidsmap.com).

Babies infected with HIV and exposed to single-dose nevirapine had drug resistance at 6-8 weeks old and the viral resistance mutations were observed at 4–8 weeks postpartum of about 35% in mothers and 50% in children (Arrive et al., 2007). Reverse transcriptase (RT) sequences from plasma HIV-1 were analyzed for HIV positive mothers after 6 weeks of nevirapine dosing, K103N NVP resistance mutation was detected in 20% of the women (Jackson et al., 2000).

Nevirapine resistance was more frequent among infants who were infected in utero than among infants who were diagnosed with HIV infection after birth by 6–8 weeks of age and was still detected in some infants 6–12 months after single dose nevirapine exposure (Jessica et al., 2009). The emergence of nevirapine resistance after a single dose nevirapine was associated with higher viral loads and lower CD4 cell counts at the time of exposure and the rate of nevirapine resistance mutations was significantly higher in women with HIV-1 subtype C than in women with subtype A or D (Eshleman et al., 2001).
Single dose of nevirapine had higher rates of virologic suppression similar to those among women who were not exposed to nevirapine, and it was important to evaluate virologic responses after at least 24 months of follow-up while initiation of non-nevirapine-based regimens should be considered for women starting antiretroviral treatment within 6 months after receiving a single dose of nevirapine (Shahin et al., 2007).

The sensitivity of the OLA was compared with consensus sequencing and it was found that OLA was more rapid, sensitive, and economical than consensus sequencing. (Giovanina et al., 2004). OLA was simple to perform and did not require expensive equipment or technical expertise; it offered a practical alternative to dideoxynucleotide sequencing for the detection of HIV-1 mutations associated with high-level resistance to antiretroviral where resources are limited (Ingrid et al., 2002).

K103N and Y181C mutations were detected from 1 week to 18 months after single-dose nevirapine prophylaxis in HIV positive women and it suggested that subsequent NNRTI-based ART should not be started earlier than 12 months after single-dose nevirapine prophylaxis (Hauser et al., 2011). The P1060 study team looked at treatment outcomes among infants who were exposed to single-dose nevirapine and found that 83% of them experienced virological failure or stopped therapy (Paul et al., 2010).